Morphological and Molecular Characterization of Cultivated Guinea Yam Accessions and their Wild Relatives (*Dioscorea cayenensis* Lam. complex) from South and Southwest Ethiopia.

Candidate: Wendawek Abebe Mengesha

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in partial fulfilment of the requirement for the Degree of Doctor of Philosophy in Biology (Applied Genetics Stream).

March, 2008

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Abstract

Yams (*Dioscorea* species L.) are among the most important of the tuber crops mainly cultivated in the tropics. They are also an important source of diosgenein, a starting material for the industrial production of sex hormones and steroidal drugs with pharmaceutical properties. Despite their cultural and economic importance, there are taxonomic confusions in the groups called Guinea yams that belong to the *D. cayenensis* complex. Identification of living or dried specimens using the currently used classification scheme can be extremely difficult. Establishing the taxonomic identity and understanding the systematic and genetic relationships among the accessions of Guinea yams and their wild relatives is vital to the conservation and management of the crop. Therefore, the major objectives of this study were to evaluate the existing taxonomy and to determine the amount and distribution of genetic variation within *Dioscorea cayenensis* complex in Ethiopia. Collections of plant material were conducted between the months of July and September 2005 and 2006 from different localities in South and Southwestern parts of Ethiopia.

Morphometric analyses were carried out based on a similarity matrix constructed using 26 morphological characters on 40 accessions of *Dioscorea cayenensis* complex . The results of the cluster and ordination analyses revealed that the wild and cultivated Guinea yams are closely related. None of the UPGMA clusters entirely contained those accessions considered as discrete taxa according to the existing classification system.

The three primer combinations used in the Amplified Fragment Length Polymorphism (AFLP) analyses generated 158 scorable bands, with an overall polymorphism of 78%. Ordination and cluster analyses of AFLP data failed to produce any clear species boundary between the species within *D. cayenensis* complex. The average genetic similarity between the accessions ranged from 60 % to 100 %. The first, second and third principal coordinates axes cumulatively account 77.5 % of the total variation. AFLP analyses also revealed a higher genetic divergence among cultivated Guinea yams accessions of the Sheko cultivars.

Estimates of population parameters using microsatellite or simple sequence repeat (SSR) markers were made by studying 7 loci. The total number of alleles amplified for the 7 loci were found to be 60, with an average of 8.6 alleles per locus. Analyses of the data indicated that Guinea yams and their wild relatives in the study area displayed a tremendous genetic diversity. The wild forms exhibited greater allelic diversity than the cultigens. Contrary to what is expected in vegetatively propagated crops, none of the seven loci studied showed a significant excess of heterozygotes. The levels of heterozgosity found in the study group were, in most cases lower than expected. Analyses of the taxonomic status using microsattellite data also revealed comparable results with both morphometry and AFLP. The accessions tended to group based on their geographical origin rather than their supposed taxonomic identity.

In the present studies, the phenograms and scatter plots based on morphological, AFLP and microsatellite markers failed to produce a clear partitioning of the study individuals studied into discrete taxa according to the existing classification system. Therefore, we believe that at least the wild or managed populations and cultivated Guinea yams of South and Southwest Ethiopia form a single taxonomic entity. It also appears that the Sheko population displayed the greatest genetic diversity. From a conservation perspective, it is important that both the range of cultivars and the diversity within them is protected both *in-situ* in the Sheko region, and perhaps also in *ex-situ* in selected areas in gardens. Future studies must be undertaken at the population scale and in a broad range of ecosystems, so as to take the diversity of each of the yams currently regarded as distinct species into account.

Key words: Guinea yams, morphometry, AFLP, microsatellite, taxonomic status, Genetic diversity.

1. Introduction

Yams rank as the world's fourth most important tuber crop in economic terms (Mignouna *et al.,* 2005), after potatoes (*Solanum tuberosum* L.*)*, Cassava (*Manihot esculenta* Crantz), and Sweet potatoes (*Ipomoea batatas* (L.) Poir.). Yams are cultivated in most tropical countries, but especially in West Africa, which produces over 90% of the world's output (Mignouna and Dansi, 2003) and they are the staple carbohydrate source of millions. Worldwide at least 50-60 species of *Dioscorea* of the more than 600 known (Govaerts & Wilkin, 2007) are recognized to be cultivated or wild-harvested, for food or pharmaceutical purposes (Coursey, 1967; Craufurd *et al.,* 2001)

The African domesticates known as Guinea yams (*D. cayenensis* Lam.-*D. rotundata* Poir. complex) are one of the most important, preferred and widely planted tuber crops in the tropics (Mignouna and Dansi, 2003), although *D. alata* L., a cultigen of Asian origin, is also widely grown in Africa. They are the major source of carbohydrate in the"yam zone" of West Africa (Coursey, 1967). Elsewhere in Africa, there are pockets of extensive yam cultivation amid a widespread wild or semi-wild plants harvested as famine food when cereal crops run short or fail. Due to their importance in the diet of people in Africa, Guinea yams have been the subject of many studies by researchers in a number of disciplines including systematics, plant breeding, pathology and genetics (see e.g. Coursey, 1967). The primary goal of these researchers has been to look for the ancestral species from which the cultivated yams have originated, both to understand the patterns of variation and processes of domestication, and to apply this knowledge to yam breeding and improvement. Guinea yams have been proposed to result from a process of domestication of wild yams of *Dioscorea* sect. *Enantiophyllum* Uline by African farmers (Mignouna and Dansi, 2003; Dumont *et al.,* 2006). Although currently practiced by relatively few farmers, yam domestication is still ongoing, especially in places like Southwest Ethiopia.

However, there is still no agreement over the systematics or relationships within the species complex; the number of species and the names which should be applied to them have never been adequately clarified and determined. i.e., the delimitation of the *D. cayenensis-D. rotundata* complex and the relationships within the complex are still far from clear. Identification of living or dried specimens in the *D. cayenensis.-D. rotundata*

complex using the current taxonomic literature is extremely difficult, as was experienced when trying to name Ethiopian material.

The taxonomy and evolution of the Guinea yams remain controversial, partly because of the continuous variation in morphological descriptors observed in the cultivated and wild species. These species exhibit considerable morphological polymorphism, high plasticity and predominant vegetative reproduction (Terauchi *et al.,* 1992). For example recent studies made on Guinea yams from Southwest Ethiopia have indicated that leaf shapes within an individual plant vary considerably (Hildebrand, 2003). There are also reports which indicate that wild individuals identified as *Dioscorea praehensilis* Benth. or *D. abyssinica* Hochst. ex Kunth or *D. burkilliana* Miège can directly "become" *D. rotundata* or *D. cayenensis* following domestication without any genetic change (Mignouna *et al.,* 2005).

Conclusions drawn from earlier studies of relationships among Guinea yams based on morphometry, chemotaxonomy, cytology, isozyme and molecular analysis were not consistent (Ramser *et al.,* 1997). For example, a numerical taxonomic analysis of 97 cultivars of Guinea yams (*D. rotundata* and *D. cayenensis* accessions), based on 75 morphological descriptors resulted in a phenetic tree with two main trunks interconnected with many anastomosing branches. The authors suggested that all the 97 cultivars investigated belong to a single highly evolved species (Martin and Rhodes, 1978). In contrast, a similar study by Onyilagha and Lowe (1985) on 22 cultivars of *D. rotundata* and *D. cayenensis* clearly separated the accessions as two distinct species. Using RFLP (Restriction Fragment Length Polymorphism) markers Terauchi *et al.,* (1992) failed to evidently discriminate between the various species of Guinea yams. The authors proposed that species referred to Guinea yams are all closely related. However, recent studies by Scarcelli *et al.* (2005) using AFLP distinguished three groups of Guinea yams belonging to *D. cayenensis-D. rotundata* complex, *D. abyssinica* and *D. praehensilis* in Benin. Furthermore, cluster analysis based on RAPD (Randomly Amplified Polymorphic DNA) and double stringency PCR (DS-PCR) data discriminate the cultivars classified as *D. cayenensis* from *D. rotundata* (Mignouna *et al.,* 2005). The authors proposed that *D. cayenensis* should be considered as a taxon separate from *D. rotundata.*

Establishing the taxonomic identity of germplasm and understanding the systematic relationships among crops are vital to the management of genetic resources and the utilization of accessions (Bretting and Widrlechner, 1995). Yam cultivation systems in Ethiopia and East Africa have not been studied as well as their West African counterparts. Recent studies conducted in South and Southwest Ethiopia have revealed that the local community has a strong tradition in cultivating and domesticating various species of yams with wide genetic bases (Sebsebe Demissew *et al.,* 2003; Hildebrand *et al.,* 2002). However, this excellent local knowledge of yam cultivation and domestication is beginning to deteriorate as farming practice in the area reorients towards cash crops and coffee plantation (Hildebrand *et al.,* 2002).

The major objectives of this study were to evaluate the current taxonomic classification of Guinea yams and to determine the amount and distribution of genetic variation in the Ethiopian materials collected from south and southwestern part of the country, as a prerequisite to devise a sound conservation strategy. Both morphological and molecular (AFLP and Microsatellite) data were gathered using cultivated accessions of Guinea yams and their wild relatives collected from South and Southwest Ethiopia. We adopted the "*D. cayenensis* complex" (used by some authors e.g. Hildebrand *et al.,* 2002) as a provisional name for the set of sub-Saharan yam species whose relationships are currently being examined: *D. cayenensis, D. rotundata* (in some literature both species recognized as *D. cayenensis-D. rotundata* complex)*, D. abyssinica,* and *D. praehensilis,* plus *D. sagittifolia* Pax, which occurs rarely in Ethiopia (Miège and Sebsebe Demissew, 1997) and was not seen in the areas studied.

2. Background

2.1 Brief description of the family Dioscoreaceae

Members of the family Dioscoreaceae probably appeared 165 to 130 million years ago, i.e. during the Cretaceous period, together with other primitive angiosperms (Coursey, 1976). Recent studies based on analyses of rbcL sequence data reported 116 million years as the stem node age of the family Dioscoreaceae (Jansen and Bremer, 2004). The genetic isolation of the African Dioscoreaceae probably date back to Miocene (60-20 millions years ago) when the desertification of what is now South Western Asia occurred (Coursey, 1976). The diversification of *Dioscorea* section *Enantiophyllum* in Africa took place during the last 40 millions years. All representative members of this section occur in mainland Africa, but not in Madagascar (except the introduced *D. minutiflora* Engl.) (Burkill and Perier de la Bathie, 1950), which was separated from the African continent, around 40 million years ago. Hence, the evolution of African yams of the section *Enantiophyllum*, appears to be relatively recent, which may explain the incomplete speciation and the large reserve of variability some of them still appear to have.

The family Dioscoreaceae is classified under the monocotyledons. However, some features in yams such as the presence of a second non-emergent cotyledon and reticulate-venation of the leaves are similar to those of certain dicotyledonous plants (Purseglove, 1972). This has led to the suggestion that the genus *Dioscorea* might have been derived from plant forms that occurred before the differentiation of monocots and dicots (Degras, 1993), or that it was part of the first diverging lineage of monocot evolution (Dahlgren *et al*., 1995). However, molecular analyses have confirmed that it is nested well within the monocots in the Dioscoreales (Chase *et al.,* 1993; Caddick *et al.,* 2002a).

According to Méige and Sebsebe Demissew (1997) the family includes about seven genera (B*orderea* Méige., *Dioscorea* L., *Epipetrum* Phil., *Testudinaria* Salisb. ex Burch, *Rajania* L., *Stenomeris* Planch. and *Tamus* L.) with the greatest diversity occurring in Central and South America, Indo-Malaysia, Micronesia and Madagascar. Representatives also occur in Eastern Europe and Africa, but here the diversity is relatively low. Of the seven genera recognized only one, *Dioscorea,* is represented in tropical Africa (Sebsebe Demissew *et al.,* 2003). A more recent classification (Caddick *et al.,* 2002a) recognized

only 4 genera (*Dioscorea, Stenomeris, Tacca* J.R. & G. Frost (previously Taccaceae) and *Trichopus* Gaertn.) within the family. The dioecious Dioscoreaceae genera; *Borderea, Epipetrum, Nanarepenta, Rajania, Tamus* and *Testudinaria* Salisb. ex Burch. were shown to be nested within *Dioscorea* and are therefore proposed sunk into it (Caddick *et al.,* 2002a; Caddick *et al.,* 2002b).

Species of Dioscoreaceae are most frequently encountered as climbers which perennate by rhizomes or tubers in forest margins and more open habitats (Wilkin, 2001).

2.2 The genus *Dioscorea*

The genus includes more than 600 species (Govaerts and Wilkin, 2007). In Ethiopia and Eritrea ca 11 species of *Dioscorea* are recognized so far (*D. quartiniana* A. Rich*, D. dumetorum* Pax.*, D. cochleari-apiculata* De Wild.*, D. gillettii* Milne-Redh.*, D. bulbifera* L.., *D. schimperiana* Kunth.*, D. alata, D. abyssinica, D. cayenensis-D. rotundata* complex*, D. sagittifolia* Pax. and *D. praehensilis*) (Sebsebe Demissew *et al.,* 2003). One of the species, *D. gillettii.,* is a near-endemic occurring in Southeast Ethiopia and Northern Kenya bordering Ethiopia. The remaining species are widespread in sub-Saharan Africa. Some of the species, such as *D. cayenensis-D. rotundata* complex and *D. abyssinica* occur both in the wild and in cultivations, and others such as *D. quartiniana*, *D. dumetorum, D. cochleari-apiculata* and *D. schimperiana* occur only in the wild (Sebsebe Demissew *et al.,* 2003). *Dioscorea alata* has never been found in the wild. It is an introduction from Southeast Asia and may have developed from crosses and domestication involving the Asian species *D. hamiltonii* Hook. f. and *D. persimilis* Prain and Burkil (Purseglove, 1972). *Dioscorea bulbifera* is common in the wild in Asia and Africa, and the different forms of *D. bulbifera* have been named as separate species by some authors (Coursey, 1967).

In tropical Africa the genus *Dioscorea* includes twining or climbing herbs, often prickly below or sometimes unarmed. The flowers are small and unisexual, the plants are dioecious with an extremely irregular production of male and female flowers, which are pollinated by insects. The male inflorescences are spicate, racemose, or rarely cymose, axillary or forming panicles at the ends of leafless branches. Male flowers have campanulate to spreading tepals and six stamens, either all fertile or three reduced to

staminoides. The female inflorescences are spicate and axillary. Female flowers have tepals similar to the male ones. The capsules are triangular or deeply three-lobed in cross-section dehiscing with three valves, and with 1-2 seeds in each locule (Méige and Sebsebe Demissew, 1997). The seeds are most often winged and usually go through a dormancy period of three to four months before germination can occur. In the cultivated forms seed production is rare, so they are vegetatively propagated using the basal nodal region of the tuber or the bulbils (Degras, 1993). The leaves are petiolate, often cordate at the base, and entire to lobed (except for *D. dumetorum* and *D. quartiniana* which have trifoliate or pentafoliate leaves), and of an arrangement either opposite or alternate with axillary buds (Degras, 1993).

Members of the genus *Dioscorea* have usually a thin twining stem which allows the plants to climb. The direction of the stem twining (clockwise or anticlockwise) is used as one of the major taxonomic characters, to classify the species within the genus into different sections (Table 1).

Dioscorea schimperiana and the members of the *D. cayenensis* complex usually produce a single annual tuber, which varies in size, shape and weight, depending on species/cultivar and growing conditions. The colour of the tuber flesh also varies between species and cultivars. Some members of the genus, such as *D. alata* also produce bulbils (aerial tubers) on the leaf axils which could weigh from ca 20 to 100g. Others such as *D. bulbifera* produce a corresponding structure which can weigh up to one kg (Degras, 1993).

Tuber morphology, stem twining direction, dioecy, and fruit/seed wing shape are among the most important characters in the systematics of *Dioscorea*. The first taxonomic treatments of *Dioscorea* involving a large number of species were those of Kunth (1850) and Uline (1898). Knuth recognized ca. 600 species and divided them into four subgenera based on seed wing position, and then into 60 sections. However, later it was proposed that many of Knuth's infrageneric taxa are clearly

para- or even polyphyletic. The taxonomic ideas of Knuth were to some extent refined and improved by Burkill (1960). In his infrageneric classification of the Old World taxa, based on seed characters, underground organ morphology, and morphology and development of male inflorescence, Burkill avoided the rank of subgenus, and divided some 220 species into 23 sections (Fig. 1). Since 1960, the genus has been the subject of piecemeal floristic studies (e.g., Méige 1968; Milne-Redhead, 1975; Tellez and Schubert, 1994; N'Kounkou. 1993; Méige and Sebsebe Demissew, 1997; Ding and Gilbert, 2000). The only complete taxonomic treatment was that of Huber (1998), in which the Knuth/Burkill system of classification was recapitulated, with all of the dioecious taxa of Dioscoreaceae included in subfamily Dioscoreoideae as "genera and genus-equivalent sections" (in Wilkin *et al.*, 2005)

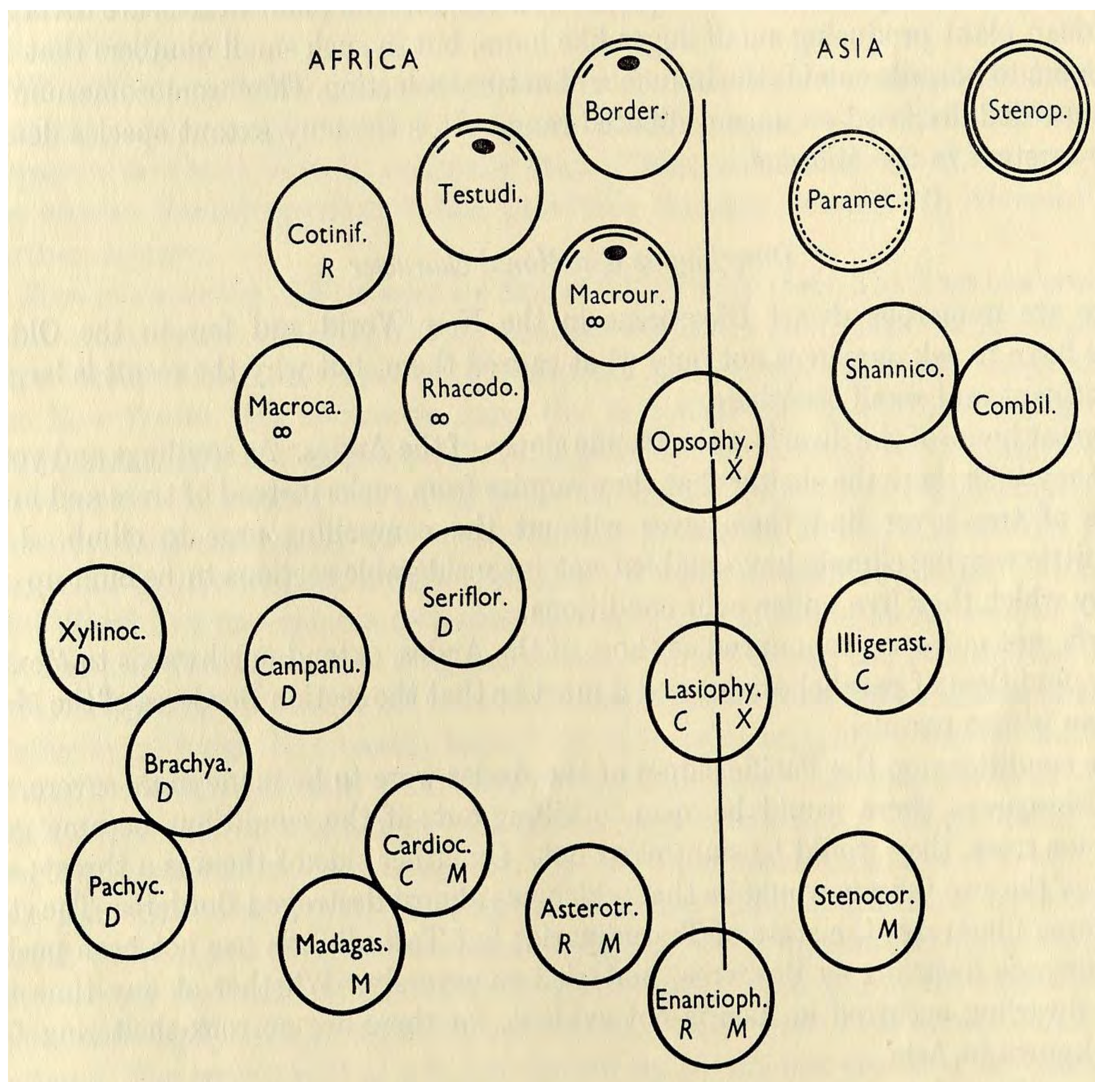


Figure 1. Relationships of sections of *Dioscorea* (Burkhill, 1960)

Some of the characteristics and representative examples of the major sections of the genus *Dioscorea* are presented in Table 1 (Alexander and Coursey, 1969). The section *Enantiophyllum* is the largest in terms of the number of species

Table 1. The major sections of the genus Dioscorea based on morphological characteristics of the species (Alexander and Coursey, 1969)

Sections	Major characteristics	Representative species
Enantiophyllum	• usually single tuber • twine to the right • winged stems •occasional bulbils	*D. alata* *D. cayenensis* *D. rotundata* *D. preahensilis* *D. abyssinica*
Lasiphyton	•cluster of medium sized	*D. hispida*. Dennst.

	tubers • twine to the left • large thorns on stems	*D. dumetorum* *D. pentaphylla* L. *D. quartiniana*
Opsophyton	•aerial bulbils • twine to the left	*D. bulbifera*
Combilium	•large number of individually small tubers • twine to the left	*D. esculenta* (Lour.) Burkill
Macroura	•small and very toxic bulbils • twine to the right	*D. sansibarensis*. Pax.
Macrgynodium	• small tubers • twine to the left • spineless stem	*D. trifida* L.f.

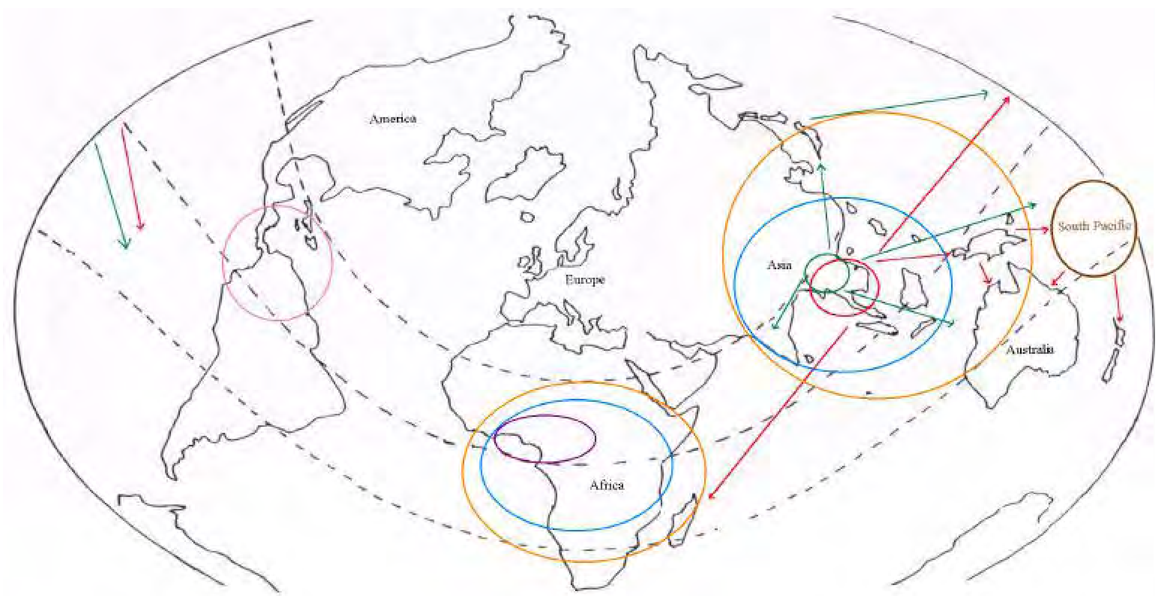
2.3. Origin and distribution of yams

The generic name (*Dioscorea*) of yams and the family name Dioscoreaceae was given in honour of Dioscorides, the 1st century Greek botanist and physician, who used yams for medicine, as well as for food. The common name "yam" was a derivation of a word from a West African language ("nyami"), picked up by Portuguese slave traders. While the Portuguese were watching indigenous people digging up the yam tuber, they asked what they were used for. Failing to understand the question fully, they replied that it was "something to eat", nyami in the local dialect (Guinea). This became "inhame" in Portuguese, then "igname" in French, and "yam" in English (www.innvista.com/health/foods/vegetables/yams.htm).

Burkill (1960) thought that the family Dioscoreaceae arose in part of Laurasia which is now in Southeast Asia between 160-130 million years ago (Burkhill, 1960). From the Far East, the genus *Dioscorea* had spread worldwide by the end of the Cretaceous period, approximately 75 million years ago (Alexander and Coursey, 1969). However, the major cultivated *Dioscorea* species appear to have originated from tropical areas in three separate continents: Africa (the *D. cayenensis* complex), South East Asia and south Pacific (*D. alata* and *D. esculenta* (Lour.) Burkill), and South America (*D. trifida* L.f.) (Coursey, 1976; Dergas, 1993) (Fig. 2). *Dioscorea rotundata* and *D. cayenensis* are believed to have originated in eastern Nigeria and from land tracts adjoining the Niger and Benin rivers in West Africa (Coursey, 1967). These species were slowly brought eastwards reaching as far

as East Africa. There was little or no cultivation of the African species in Asia. In India, however, *D. rotundata* has been recently introduced from Africa by the International Institute of Tropical Agriculture (IITA)**.** The Asiatic yam, *D. alata* is believed to have originated in tropical Myanmar and Thailand. *Dioscorea alata* probably spread from Southeast Asia to India and across the Pacific Ocean to reach the east coast of Africa about 2000 years ago. Later during the time of the slave trade, both *D. alata* and *D. rotundata/D.cayenensis* were taken from West Africa to the Caribbean and the Americas where they are now established as important food crops (Craufurd *et al.,* 2001). *Dioscorea bulbifera,* characterized mainly by the production of bulbils, is native to both Asia and Africa, where the wild forms still exist. The cush-cush yam (*D. trifida*) is the only yam of tropical American origin to have attained significance as food crop; its production is currently restricted to the West Indies (Onwueme and Charles, 1994).

In prehistoric times the most widely distributed of the starchy crops were various species of the genus *Dioscorea* (Degras, 1993). The past importance of yams to many communities is shown by the part they still play in socio-religious events (Alexander and Coursey, 1969). Some *Dioscorea* species are mentioned as supplying medical ingredients in the earliest Chinese medical documents (before 2000 BC) (Coursey, 1967). The suitability of yams as food on ships greatly facilitated their distribution through out the world (Coursey, 1967).



_______ *D. alata* _______ *D. dumetorum* _______ *D. trifida* _______ *D. esculenta*.
_______ *D. cayenensis/D. rotundata* _________ *D. bulbifera*

Figure 2. Origin and distribution of *Dioscorea* species (Degras, 1993)

2.4 Domestication of yams

Domestication is a set of practices that are applied to edible (or potentially edible) wild plants that involve their adaptation to the environmental conditions of agriculture. This is achieved by changing the genetic equilibrium of the initial populations and enhancing their valuable traits through selection. Many cultivars used for domestication purposes are clones of edible wild forms and a few putative wild forms are probably feral plants that have escaped from cultivation. Some cultivars are also clones of hybrids between wild forms and feral or cultivated plants (Lebot *et al.,* 2005)

According to Lebot *et al.,* (2005) the practice of domestication of tuber crops, such as yams, could be summarized as:

1. Selection of wild genotypes: the domesticator identifies a morphotype and tests its "chemotype". If it seems acceptable after chewing the flesh of the underground organ, a propagule is collected.
2. Improvement of the environment: the soil where the propagule is planted is well prepared. Unlike the wild plants, the clone is planted into a considerably modified environment. This improved environment contributes directly to the ennobled development of the underground organs.
3. Rejuvenation of the plant: From a perennial and/or herbaceous wild form, regular vegetative propagation induces a rejuvenation process which leads to an annual cultivar. Farmers uproot their plants as soon as there is a consumable yield.

According to Coursey (1976) the pre-forest zone extending across Nigeria and Benin was the first place for domestication of Guinea yams. However, it is not known exactly where they were first domesticated for cropping under savannah agriculture conditions. Different arguments indicate that domestication of yams in West Africa is a recent phenomenon (Dumont *et al.,* 2006).

The starting material for the domestication of Guinea yams are populations of wild morphotypes that have well developed vegetative organs, sexual vigour, and a small relatively bitter tuber which is difficult to harvest because it is often long and sometimes branched or protected by spiny crown roots (Dumont *et al.,* 2006). The physico-chemical characteristics of their tubers are the most useful traits which are selected and domesticated.

In fact, the major differences between cultivated and wild forms are not morphological, but rather chemical (Lebot *et al.,* 2005). The tuber material or propagules of these wild yams are usually collected either in the bush (most often near the village) or in the forest during hunting. These clones are planted into a considerably modified environment. This improved environment contributes directly to some phenotypic modifications including the ennobled development of the underground organs (Lebot *et al.,* 2005). Farmers in Benin try to obtain the desired phenotypic modification in tuber form, size and taste by maintaining and planting the predomesticated tuber in their home garden for at least three years (Scarcelli *et al.,* 2006b). During this period the aerial architecture of the plant is substantially modified. i.e., stem internodes become shorter, with a concomitant equivalent reduction in stem length. Changes mainly occur on the lower parts of the stem. The reduction of primary branches at the stem base leads to the development of a large number of secondary branches that are quickly covered with thick foliage. These transformations condense the mass of the aerial vegetative organs, thus reducing or eliminating the need for staking (Dumont *et al.,* 2006). The tuber becomes shorter and thicker, with fewer roots. The selected wild forms produce a tuber morphologically identical or similar to the cultivated forms after 3-6 years of cultivation (Scarcelli *et al.,* 2006a). A study in southwest Ethiopia reported that wild growing yams transplanted to domestic gardens retain their wild traits for up to 4 years, before they begin to take on traits of the cultivated forms (Hildebrand, 2003). There are also reports which indicate that the domesticated plants return to their wild state if they are abandoned (Dumont *et al.,* 2006).

The mechanism underlying the phenotypic modifications observed during the process of domestication is unknown. A phenotype is the result of the interaction of the genotype and the environment. A change in the latter can lead to profound modifications in the phenotype. A genotype can thus have various phenotypes. Transferring wild yams to a cropping environment subject them to a drastic change of habitat, and these changes might induce phenotypic modifications observed during the process of domestication (Dumont *et al.,* 2006). Different authors consider phenotypic plasticity, epigenetic modifications (Tostain *et al.,* 2003) or somatic mutations (e.g. Scarcelli *et al.,* 2006a) as possible explanation for the changes encountered during the domestication practice in yams.

Tubers of the predomesticate obtained after 3-6 years of cultivation in the home garden are evaluated for their agronomic quality and then multiplied if the farmers are satisfied with

the evaluation (Scarcelli *et al.,* 2006b). The aerial morphological traits of the predomesticate are not of particular importance for selection. If they are used, it is for identifying a familiar morphotype which is known to present underground organs with an acceptable chemotype. Since farmers propagate yams vegetatively, they are likely to select genotypes that allocate more resources to tuber development than to sexual reproduction. Thus, a decrease in flowering ability could also be considered as one domestication syndrome trait (Scarcelli *et al.,* 2006a). The farmer also take into consideration some physiological traits, such as production period, storage, seed potential, and sometimes the ability to produce large number of tubers. More general criteria relating to the plant's biological plasticity and yield potential are also taken into account for selection (Dumont *et al.,* 2006).

During the process of domestication some farmers collect different tubers with different genotypes and put them simultaneously into the domestication process. Consequently after a while, farmers are unable to identify each clone separately. This practice leads to the cultivation of different clones from unknown origin under the same cultivar. When the process of domestication is considered as completed by the farmers, they generally mix the tubers obtained through the process with those cultivars that are found to be alike. It is only when the shape of the newly domesticated yams tuber does not correspond to existing varieties that the farmers give them a new name (Chaír *et al.*, 2005).

The process of domestication, in yams could be described as the adaptation of spontaneous (wild) plants to cultivation constraints without any genetic changes (Scarcelli *et al.,* 2006b). Since only vegetative propagation is used in yams no genetic changes are expected during the process of domestication. The genotypes are not modified and so there is no barrier to sexual reproduction. There is a possibility that reciprocal gene flow would occur between the cultivated yams and their wild relatives (Dumont *et al.,* 2006). According to Mignouna and Dansi (2003), although *D. praehensilis* is the most exploited (in Benin), three species of wild yams namely: *D. abyssinica, D. burkilliana* and *D. praehensilis* are used for domestication purpose. Although, *D. burkilliana* is not found in Ethiopia, Hildebrand *et al.* (2003) have reported the same findings regarding the species most commonly used for domestication purposes in Ethiopia. Since the wild yams, *D. abyssinica* and *D. preahensilis,* are principally the result of sexual reproduction (Ayensu and Coursey, 1972),

sexual reproduction indirectly contributes to the evolutionary dynamics of yams through the domestication of wild species of the *D.cayenensis* complex (Scarcelli *et al.,* 2006a).

2.5. Economic importance of Yams

Worldwide 50-60 species of *Dioscorea* are known to be cultivated or at least gathered for food or pharmaceutical purposes. There are however, ca 12 species of economic significance as food (Coursey, 1967). The most important of these are: *D. rotundata* (White Guinea yam), *D. alata* (Water yam, Winged yam or Greater yam), *D. cayenensis* (Yellow yam or Yellow Guinea yam), *D. esculenta* (Lesser yam, Potato yam or Chinese yam), *D. dumetorum* (Bitter yam or Trifoliate yam), *D. bulbifera* (Aerial potato yam), *D. trifida* (Cush-cush yam), *D. opposita* Thunb. also known as *D. batatas* Decaisne. (Cinnamon yam), *D. nummularia* Lam.*,* *D. pentaphylla* L., and *D. hispida* (Craufurd *et al.,* 2001).

Yams are an important staple food and source of carbohydrate for millions of people in tropics and subtropics (Craufurd *et al.,* 2001; Hochu *et al.* 2006). They are also important medicinally and have ritual and socio-cultural significance (Craufurd *et al.,* 2001). A study by IFPRI (International Food Policy Research Institute, Washington) indicated that production of food yams increased by 183% between 1983 and 1996 (in Dumont *et al.,* 2006). The field performance of yams lags behind demographic growth and the supply has been increased mainly through an expansion of the cultivation area (Tschannen *et al.,* 2005). According to the 2005 report by FAO, Africa accounts for nearly 96% of the world's yam production. Almost all of the African output is confined to West Africa, with *D. rotundata/ D. cayenensis* yams, representing nearly 91% of all yams cultivated (FAO, 2005; Dumont *et al.,* 2006). The total production of yam in Ethiopia was estimated to be 277 metric tons from an area of ca 68000 ha, corresponding to a yield of about 4 tons per hectare (FAO, 2005) (Table. 2).

Table 2. Mean annual production of yam for the period 1990 to 2005 (United Nations Food and Agricultural Organization (FAO) (2005)

	Area harvested (x1000 ha)	yield (Kg/ha)	total production (x1000 MT)
World	**3572**	**9694**	**34355**
Africa	**3418**	**9708**	**32874**
West Africa	**3149**	**10088**	**31388**
Ethiopia*	**68**	**4065**	**277**

* **Figures are mean values for the years 1992 to 2005**

In West Africa tubers of food yams are consumed in different ways, they are processed into pounded yam, boiled yam, roasted or grilled yam, fried yam slices, yam balls, yam chips or yam flakes. Fresh yam tubers could also be peeled, chipped, dried and milled into flour that can be used to prepare dough (Mahalakshmi *et al.,* 2007). In Ethiopia tubers are mostly consumed boiled without processing. In some areas however, boiled yam tubers are pounded and mixed with butter and fermented milk before they are served. The practice of roasting tubers has also been reported in some localities (Muluneh Tamiru, 2006).

The tubers of food yams, which have a high capacity to store food reserves are regarded mainly as a source of carbohydrate, some species are nearly as rich in protein as rice or maize (Hahn *et al.,* 1987). Typically, yam tubers contain 65 to 81% moisture, 16 to 31% carbohydrate, 1.4 to 3.5 % protein, 0.03 to 1.2 % lipid, (all % of fresh weight) and important quantities of amino acids (aspartic acid, glutamic acid alanine and phenylalanine), minerals (Calcium, Phosphorus and Magnesium) and vitamins (Ascorbic acid, Beta carotene, Thiamine and Riboflavin) (Table 3). Recent study by Muluneh Tamiru (2006) revealed that the starch content of yam tubers collected from South Ethiopia ranged from 65.2% to 76.6% of the dry matter, while protein content varied between 6.4% and 13.4%.

Table 3. Range of nutritional values of yams (nutrients in 100 g of edible tuber) (FAO, 2005)

Nutrient	Unit	Composition
Calories	calories	71-135
Moisture	(%)	65-81
Protein	(g)	1.4-3.5
Fat	(g)	0.2-0.4
Carbohydrate	(g)	16.4-31.8
Fiber	(g)	0.1-0.4
Ash	(g)	0.6-1.7
Calcium	(mg)	12-69
Phosphorus	(mg)	17-61
Iron	(mg)	0.7-5.2
Sodium	(mg)	8-12
Potassium	(mg)	294-397
Beta carotene	(mg)	0.0-0.1
Thiamine	(mg)	0.01-0.11
Riboflavin	(mg)	0.01-0.04
Niacin	(mg)	0.3-0.8
Ascorbic acid	(mg)	4-18

Yam tubers have been suggested to have nutritional superiority compared to other tropical root crops. They are reported as a good source of essential dietary nutrients. A few yam species (mainly the wild forms), however, produce toxic compounds that can cause serious health complications. In some species poisonous substances, such as oxalic acid, are found just beneath the skin of the tubers, and could be destroyed by peeling and boiling (http://www.kew.org/information). In general the tubers with toxic compounds usually taste bitter and cause vomiting and diarrhoea when large amount are ingested without proper processing or even eaten raw. In some species of yam (*D. dumetorum* and *D. hispida*), the toxic component has been reported as dioscorine, a toxic alkaloid that could triggers fatal paralysis of the nervous system, when even fragments of the tuber are ingested. Similarly, histamine was reported as principal allergen in some plants of the family Dioscoreaceae, causing mild inflammation and itching. The bitter substances have been reported to be saponins (in many species of yams) and furanoid-norditerpene group compounds (in some) (Rajabhandari and Kawabata, 2005).

Yams are not cultivated exclusively for their role as food alone; they are also a rich source of diosgenin such as sapogenins, the primary precursor of corticosteriods and anabolic

drugs (Purseglove, 1985; O'Hair, 1990; Twyford *et al.,* 1990; Craufurd *et al.,* 2001). Diosgenin is a steroidal sapogenin which is the starting compound for the synthesis of sex hormones and steroidal drugs with pharmaceutical properties. Diosgenin is known from several species of *Dioscorea* (Adam *et al.,* 2002). Approximately 50 species of *Dioscorea* are considered to have medicinal value. Among these 50 species only 5 to 7 species (*D. composita* Hemsl., *Dioscorea deltoidea* Wall., *Dioscorea elephantipes* Engl., *Dioscorea floribunda* Mart & Gall., *Dioscorea sylvatica* Ecklon.) are found cultivated in Asia and Central America providing diosogenin, which accounts two-thirds of steroid production (Niño *et al.,* 2006).

Over 50 different steriodal saponins have been discovered and characterized so far. Most species of *Dioscorea* contain steroid saponins and sapogenins, such as diosgenin. Diosgenin is used in the in the synthesis of many steroids which are on the market as anti-inflammatory, androgenic, estrogenic and contraceptive drugs. Several pharmacological *in vitro* and *in vivo* assays allowed researchers to characterize various pharmacologically active steroid saponins in *Dioscorea* species having cytotoxic, immunomodulating, antimicrobial, anabolizing, hormonal, anti-osteoportic, anti-inflammatory and anti-allergic activities (Sautour *et al.,* 2007). Some species of *Dioscorea* are used in traditional Chinese Medicine as anticancer agents, cardiocerebrovascular, gastrophaty-protective, curative agent and anti-rheumatism agents (Sautour *et al.,* 2007).

2.6 Cultivation of yams

All cultivars of root crops are vegetatively propagated and they share a narrow within clone genetic base. Unlike most crops, root crops are not cultivated for the characteristics of their sexual organs (fruits and seed), and their flowering is erratic. They have variable ploidy levels, are predominantly allogamous, highly heterozygous. They are usually cultivated for the interesting chemical compositions of their underground organs. Some of these biological characters are not specific to root crops but these species present all of them together (Craufurd *et al.,* 2001).

Yam tubers have traditionally been classified as root tubers rather than stem tubers. However, they are modified stems which develop from the hypocotyle, i.e. a short region of meristematic cells below the cotyledons (Conlan *et al.,* 1995; Craufurd *et al.,* 2001).

Cultivated yams are propagated vegetativelly from whole tubers (seed yams), large pieces of tubers (setts) or increasingly, from minisetts. They can also be propagated from true-seeds though this practice is largely limited to breeding programmes (Craufurd *et al.,* 2001). The sett cut from apical tuber parts emerge earlier and yield better than setts cut from the lower part of the tuber. The key sett characteristics that play an important role in yam crops are its size and its physiological condition, which could in turn be influenced by storage conditions. Although the storage period varies largely because of climatic and cultural conditions, the generally quoted vegetation period of 7 to 12 months suggests storage of seed tubers from 0 to 5 months. Biologically this period corresponds to the post harvest component of the dormant period, which allows the yam to overcome periods unfavourable to growth (Tschannen *et al.,* 2005).

Cultivated yams are grown as annuals with tubers being planted between February (in the humid forest) and April (in the savanna area). In West Africa flowering usually occurs between June and September. Harvesting can take place 180 days after planting, i.e., in August in the humid forest agroecological zone, but mostly it is carried out when the shoot senesces at about 180 to 270 days later in the savanna and humid forest, respectively (i.e., October and November). The harvest season is usually celebrated with special rituals with *D. alata* having the most important social significance (Alexander and Coursey, 1969). After harvest, tubers are sun dried to prevent fungal infections. They are commonly stored in barns, which provide good ventilation, and protection from termite attack and flooding (Degras, 1993). Yam tubers can also be stored well in a dry, dark, cool, and ventilated place such as storage huts. Tubers are stored for several months for consumption, and for provision of planting materials for the following season (Coursey, 1967).

Harvested tubers remain dormant (incapable of developing an internal or external shoot bud) for 30 to 150 days, depending on the date of harvest and growing and storage conditions (Ile *et al.,* 2006). Dormancy in yam tubers prevents precocious sprouting, prolongs storability and maintains food quality (Ile *et al.,* 2006). Dormant yam tubers, uniquely and in contrast to potatoes (*Solanum tuberosum*) do not have apical buds. Instead, dormant yam tubers have meristematic cells below the surface of the tuber (Ile *et al.,* 2006). Once the dormancy period is over, sprouting tubers are planted at the start of the rainy season. The new yam plant draws on material of the mother tuber until the eighth

week (Tschannen *et al.,* 2005). The plants are usually grown in ridges or mounds with stakes or live support, which allow the vines to climb. Yams are frequently grown with other types of plant species. Yam intercropping with grain legume is a common practice, because it is an economical method for weed management (Coursey, 1967; Onwueme, 1988).

Yams exhibit the sigmoidal growth pattern that is common to most annual plants. This is a period of slow growth during establishment followed by a phase of rapid exponential growth as the canopy reaches its maximum area and finally a declining growth rates as the canopy senesces. In brief, following the breaking of dormancy (sprouting), the following four distinct phases of development are commonly recognized (Craufurd *et al.,* 2001):

1. Tuber germination and sprout emergence: dormancy ends when the tubers germinate and the growing shoots or vines emerge. The duration of this phase is typically between 30 and 50 days, but can be protracted if the conditions are unfavourable.
2. Canopy establishment and tuber initiation: typically, this phase lasts between 20 and 70 days. The vines elongate, cataphylls and then true leaves are initiated and expand and the plant becomes autotrophic.
3. Maximum canopy development and maximum tuber growth rate: this third phase is the most critical period for growth of the yam tuber; it is characterized by maximum canopy development and tuber growth rate. It has a typical duration of 60 to 90 days. During this period plant growth is highly plastic in response to both positive and negative elements, such as management inputs, weeds, fertilizers and pests.
4. Canopy senescence and tuber maturity: during this phase of development leaves senesce and dry matter accumulation declines. Tubers attain their maximum volume and weight. The combined duration of phase 3 and 4 varies from 80 to more than 150 days.

2.7 Reproductive biology of Guinea yams

Guinea yams are usually dioecious, rarely monoecious individuals have also been observed. Monoecious individuals are often encountered when plants grown from seeds (Hamon, 1987; Zoundjihèkpon, 1993). For centuries, cultivated yams have as mentioned been vegetatively propagated from tubers of local cultivars. This continued vegetative propagation and lack of hybridization have precluded the possibility of genetic improvement of yams through breeding programs (Senou *et al.,* 1992). In West Africa no direct use of seeds by farmers has been reported for the main cultivated species (*D. rotundata*). The farmers use tubers from the previous harvest. The two wild relatives, *D. abyssinica* and *D. praehensilis,* mainly reproduce by sexual means, an insect called Thrips (*Larothrips dentipes*) being the major pollinator. Other species such as, *Acantolepsis* spp., *Chirothrips* spp. and *Haplothrips* spp. may also be involved in the pollination (Scarcelli *et al.,* 2006). According to Senou *et al.* (1992), insects belonging to five different families were found entering the open, receptive flowers, and their presence on yams coincides with the duration of the flowering period.

Flowers, especially the male ones, are small, difficult to handle (e.g. in crossing experiments) and often have sticky pollen (Zoundjihékpon *et al.,* 1994). Mature male flowers start opening in the morning and reach peak number late in the afternoon. Up to five male flowers open daily depending on the spike length. Female flowers open acropetally any time after attaining maximum size and remain open or partially closed after pollination. Usually 2-3 female flowers open per spike daily (Akoroda, 1983).

Studies from different countries regarding variation in sex ratio revealed some differences. For example the study by Dansi *et al.,* (1999) indicated that the two sexes are almost equally represented in Benin. A higher male to female ratio has been reported from the Ivory Coast (Hamon, 1987), whereas the reverse has been reported from Togo (Kassmada, 1982 in Dumont *et al.,* 2006). According to Dumont *et al.* (2006) sex might not be fully genetically determined and might be influenced by yet unknown environmental factors. Sex reversal has also been reported in juvenile hybrids of early-maturing Ivorian cultivars (Zoundjihèkpon, 1993).

Based on the success of controlled crosses in wild and cultivated yams, pollen obtained from the wild forms was found to be more efficient compared to the cultivated forms (Zoundjihèkpon *et al.,* 1994). The authors suggested a high quantity of relatively fertile pollen produced by the wild forms, as a possible reason to explain the observed results. The success of cross-pollination not only requires the presence of pollen on the stigma but also the absence of genetic incompatibility and sterility (Zoundjihèkpon *et al.,* 1997)

Adequate knowledge of the floral biology of a crop is a prerequisite for overcoming the morphological and genetic barrier to successful hybridization (Akoroda, 1983). Cultivated Guinea yams have a highly variable flowering capacity which might be associated with different genetic and environmental factors. Some of the environmental factors are related to climate (rainfall quantity and distribution) and agronomic conditions, such as soil fertility, planting density, staking practice, weed control etc. Photoperiod is also an important factor for flowering. The photosensitivity of *D. rotundata* has been demonstrated by Okezie *et al.* (1993). Some studies link flowering and fruiting abilities of the different cultivars to the maturity period. Thus, the late maturing cultivars were reported to exhibit less fertility compared to the early maturing cultivars (Dumont *et al.,* 2006). The maturation period has also been linked to the fertility of cultivated Guinea yams (Zoundjihèkpon, 1993). According to the results of this study, fertility was found to be low in late maturing *D. rotundata* cultivars, with several male cultivars producing abnormally small pollen grains with low germination rate. The author attributed the low fertility to lack or deficiency in the mechanism controlling the tepal opening during floral development.

Efforts to improve yam production are hampered by the low rate of flowering, the very low rate of fruit setting and poor seed germination (Senou *et al.,* 1992). With hand pollination Akorda (1983) was able to increase fruit set in Guinea yams threefold and proposed that low fruit set is primarily due to the less efficient mechanism of pollination by insects. Some have attributed low fruiting in *D. rotundata* to pistil sterility, while others suggested poor pollen release or poor pollen germination (Zoundjhekpon *et al.,* 1997). Poor reproductive capacity in yams has often been attributed to the polyploid nature of the crop (Egesi *et al.,* 2002).

Earlier studies on flowering pattern in *D. rotundata* indicated that there is difference in the time of flower initiation and maturation in male and female clones (Ayensu and Coursey,

1972; Akoroda, 1983; Senou *et al.,* 1992). According to the results of these studies staminate plants flowered earlier than pistilate flowers. However, an overlap in the timing of flower opening has been reported by Zoundjhèkpon *et al.* (1997).

2.8 Cytogenetic studies.

At present, yams are widely regarded as being polyploids. The basic chromosome number for polyploid yams has been reported to be x = 9 or x = 10. All the Asian *Dioscorea*, 52% of the African species, and 13% of the American species have the basic chromosome number x = 10, whereas most of the American clones display a basic chromosome number of x = 9 (Essad, 1984). However, recent data have challenged the previous report and revealed new basic chromosome numbers for yams, x = 6 (Segarra-Moragues *et al.,* 2004) and x = 20 (Scarcelli *et al.,* 2005; Bousalem *et al.,* 2006). These results should lead us to reconsider the ploidy level of some species of yams, including Guinea yams (Bousalem *et al.,* 2006).

The existence of various ploidy levels and the lack of diploid relatives to the cultivated polyploid yams have complicated the study on yams (Bousalem *et al.,* 2006). Reports about the ploidy level in the wild species are rare and often contradictory. Thus using flowcytometry Hamon (1992) reported that *D. abyssinica, D. mangenotiana* Méige and *D. praehensilis* have the same DNA content. However, Essad (1984) described the former as tetraploid and both *D. mangenotiana* and *D. praehensilis* as octoploids. According to Zoundjihèkpon (1993), *D. mangenotiana* and *D. burkilliana* were considered to be hexaploid and tetraploid, respectively. Using the conventional methods of chromosome counting and flow cytometry, Gamiette *et al.* (1999) depicted the ploidy of levels of Guinea yams (including their wild relatives) fitted in a 4x, 6x and 8x ploidy series. These authors also proposed that *D. cayenensis*-*D. rotundata* cultigens and their wild relatives might belong to the same gene pool as most of the clones tested have the same DNA content. The ploidy levels described by Gamiette *et al.* (1999) were later supported by Dansi *et al.* (2001).

The results of segregation analyses for three enzyme systems in the progeny from a controlled cross between two cultivars of yams from Ivory Coast matched the theoretical 1-2-1 segregation expected in diploid individuals from a cross involving heterozygote

parents (Zoundjihèkpon, 1993). The author described the cultivars as tetraploids segregating like diploids. Another study involving population of F_1 crosses between two presumed heterozygous parents of *D. rotundata* by Mignouna *et al.* (2002a) produced a similar result. The authors proposed that *D. rotundata* genome is an allotetraploid with 2n = 4x = 40. Although both studies revealed the segregation pattern expected in diploids, the authors did not use these results to challenge the ploidy status of the cultivated Guinea yams. By monitoring enzymatic traits in the progeny of monoecious clones of a cultivar in Benin, Daïnou *et al.* (2002) were the first to propose the diploid status of *D. rotundata* (2n = 40). Later the analysis of the segregation pattern of two isozyme loci and six microsattellite markers in the progeny of a self-fertilized monoecious plant by Scarcelii *et al.* (2005) supported diplody of *D. rotundata* (2n = 40). The latter authors also suggested both *D. abyssinica* and *D. praehensilis* to be diploids with 2n = 40.

2.9. Taxonomic and phylogenetic relationships between the species in the study group

The taxonomy of the species *Dioscorea cayenensis* and *D. rotundata* has been the subject of much confusion (Coursey, 1967). They were first described as separate species: *D. cayenensis* by Lamarck (1789) based on specimens from French Guiana (hence the name Cayenne) and *D. rotundata* by Poiret (1813) based on samples from Puerto Rico long before their African origin was established. Grisebach (1864) reduced *D. rotundata* to subspecfic status within *D. cayenensis* (Onyilagha and Lowe, 1985). This was accepted by Prain and Burkill (1919) and subsequently maintained by Francophone writers such as Chevalier (1936) and Méige (1952, 1968). Burkill (1921) restored *D. rotundata* to species status, and this was accepted by Hutchinson and Dalziel (1936). Chevalier (1936) created a new taxon; subsection *Cayenensis* Chev., under the section *Enantiophyllum* Uline, which includes all the Guinea yams and their wild relatives (Terauchi *et al.*, 1992). A numerical taxonomic analysis by Martin and Rhodes (1978) on 97 cultivars of Guinea yams (*D. rotundata -D. cayenensis* complex accessions), based on 75 morphological descriptors, resulted in a phenetic tree with two main trunks interconnected with many anastomosing branches. The authors suggested that all the 97 cultivars investigated belong to a single highly evolved species. Based on an agro-botanical study of the West African cultivars, Akoroda and Chheda (1983) proposed that *D. cayenensis* and *D. rotundata* should be considered as distinct species and referred the taxonomic confusion to the existence of

some intermediate forms which are presumably hybrids. This idea was later supported by Onyilagha and Lowe (1985). Using 76 morphological and ecological characters on eight cultivars, the authors conducted the study for three successive years.

The concept of the *D. cayenensis* species complex, first proposed by Ayensu and Coursey (1972) was discussed in 1978 at a seminar on yams conducted in Cameroon. This concept was later supported by Hamon (1987) as a way of pooling all West African cultivated yams that are not bulbiferous and have entire leaves under the same name (Dumont *et al.*, 2006). Some authors use *D. cayenensis* complex as a provisional name for the set of sub-Saharan yam species whose taxonomic relations are currently being examined: *D. cayenensis, D. rotundata, D. abyssinica, D. praehensilis* and *D. sagittifolia* (Wilkin and Caddick, 2000; Wilkin, 2001; Wilkin *et al.*, in prep). Member species are indigenous to Ethiopia and occur all over sub-Saharan Africa from 500 m to 1800 m altitude, especially in seasonally hot and moist areas (Miège and Sebsebe Demissew, 1997).

2.9.1. The Cultivated Guinea Yams: *D. rotundata* and *D. cayenensis*

According to Dumont *et al.* (2006), there has been considerable confusion regarding the yams *D. rotundata* and *D. cayenensis.* In general, in English speaking West Africa, particularly Nigeria, they are known as white yams and yellow yams respectively, and pooled under the term Guinea yams. Farmers in French speaking Africa, on the other hand do not make a clear distinction between *D. rotundata* and *D. cayenensis* where the generic name is accordingly used for all the cultivated yams. For the African farmer, a yam cultivar is identified by its common name, which often contains technical or historical information. Yam cultivars are best differentiated on the basis of their tuber traits like colour or taste of the flesh. The characteristics of the vegetative organs are sometimes, but not always, also used as distinctive markers. Farmers, in general, define yam cultivars by sets of technical criteria consisting of agronomic requirements, harvesting time, cooking quality and storage life (Dumont *et al.,* 2006).

Dioscorea cayenensis and *D. rotundata* are believed to be domesticated from wild African *Dioscorea* species of the section *Enantiophylum*. The two taxa differ to some extent with respect to some traits. But none of the studies conducted so far have clearly established the

identity of each taxon as a separate species (Dumont *et al.*, 2006). It is difficult to consistently differentiate the two taxa using morphology. This is because the original diagnoses are not complete enough to define them precisely and many forms are intermediate between the two (Méige and Sebsebe Demissew, 1997). However, *D. rotundata* could be described as a group of cultivated yams of African origin, with a short annual vegetative cycle (6-8 months), tubers with a long dormancy period (3-5 months) and with slightly to non-pigmented creamy or white flesh (Dumont *et al.,* 2006). It is harvested twice a year, prefers a short rainy season, has ovate leaves and 4, 8 or 12 vascular bundles (Coursey, 1967; Méige, 1968; Ayensu, 1970; Hamon and Toure, 1990a). In *Dioscorea cayenensis* the vegetative cycle ranges from 8 to 12 months and the tuber flesh is usually yellow (Terauchi *et al.,* 1992). It is harvested annually, prefers a long rainy season, has orbicular leaves, and 8 vascular bundles (Coursey, 1967; Méige, 1968; Ayensu, 1970; Hamon and Toure, 1990a).

Hamon (1987) suggested that *D. cayenensis* might be the product of interspecific hybridization and emphasized the likely involvement of *D. burkilliana.* Other authors claimed that *D. cayenensis* is phyletically close to or the same as the domesticated form of *D. burkilliana* (Akoroda and Chheda, 1983; Onyilgha and Lowe, 1985; Mignouna *et al.*, 1998; Dansi *et al.,* 2000). According to Terauchi *et al.* (1992), *D. rotundata* yams are usually cultivated in both the savanna and rainforest zone, whereas *D. cayenensis* is restricted to the rainforest zone.

Conclusions drawn from earlier studies of relationships among members of the *D. cayenensis* complex based on morphometry, chemotaxonomy, cytology, isozyme and molecular analyses, have not been consistent (Ramser *et al.,* 1997). In the last two decades different studies using various techniques have been conducted. Based on the results from RFLP markers and analyses of chloroplast and ribosomal DNA, Terauchi *et al.* (1992) proposed the species name *D. rotundata* to encompass all the cultivated Guinea yams. Using isozyme analyses Hamon *et al.* (1997) supported the idea of Terauchi *et al.* (1992). However, using different molecular techniques (RAPD, MP-PCR and RAMPO and cp-DNA sequencing) on 42 accessions of cultivated Guinea yams and their wild relatives, Ramser *et al.* (1997) were able to differentiate *D. rotundata* from *D. cayenensis,* supporting the idea that both should be treated as separate species. Based on analyses of isozymic and morphological characters, Dansi *et al.* (2000) argued that *D. rotundata* and *D.*

cayenensis represent different genetic entities. Morphological and isozyme analyses by Mignouna *et al.* (2002b) and Mignouna and Dansi (2003) distinguished between *D. cayenensis* and *D. rotundata* cultivars, but they failed to demonstrate that they are distinct species. Recent studies by Scarcelli *et al.* (2006) using AFLP data delimitated three groups of Guinea yams (and their wild relatives) from Benin belonging to *D. cayenensis-D. rotundata complex, D. abyssinica* and *D. praehensilis,* respectively. Cluster analysis of molecular data, generated by using RAPD and DS-PCR discriminated the cultivars classified as *D. cayenensis* from *D. rotundata* (Mignouna *et al.,* 2005), and these authors proposed that *D. cayenensis* should be considered as a taxon separate from *D. rotundata.*

Molecular markers have also been used to characterize Guinea yams at cultivar levels. Isozyme marker analysis of *D. rotundata* from Ivory Coast and Benin indicated that the cultivars in both countries appear to have the same genetic structure (Dumont *et al.,* 2006)

2.9.2. Wild Yams: *Dioscorea praehensilis* and *Dioscorea abyssinica*

Evolution within the section *Enantiophyllum* has produced *D. alata* in Asia and *D. cayenensis* and *D. rotundata* in Africa. These domestication products, with their high cultivar diversity, account for virtually all yam production worldwide. The wild species of this section, such as *D. abyssinica* and *D. praehensilis,* are considered to be the major source of the variability (Dumont *et al.,* 2006). It is hard to accurately define the morphological boundaries between *D. abyssinica* and *D. praehensilis*. Moreover, in West Africa the latter is often regarded as the same species as *D. lecardii*, which in turn is poorly separated from *D. sagittifolia* (Dumont *et al.,* 2006).

Most of the criteria set for the separation of *D. abyssinica* from *D. praehensilis* are based on taxonomic studies of dried herbarium plant material. There are little or no detailed reports regarding studies on these wild species at population level. Therefore, the range of variability of the two species and any taxonomic and genetic relationships between them still remain obscure. Nevertheless, both have several traits in common. They usually produce one tuber, and tubers and aerial vegetative parts are renewed annually. Both reproduce sexually and they are propagated mainly by seeds, in contrast to the cultivated yams, which are vegetatively propagated (Scarcelli *et al.,* 2006a). Studies conducted in West Africa have indicated that most of the wild plants belonging to *D. abyssinica* and *D.*

praehensilis are male plants (Dumont *et al.,* 2006). This phenomenon has been explained in terms of the biological characteristics and population structure of wild *Dioscorea* species. In the wild, the plants are often found very scattered and they are pollinated by insects such as thrips (*Larothrips dentipes*) (Zoundjihèkpon, 1993). The population size of these insects is largely determined by climatic conditions. Therefore, as a means of ensuring survival, the wild plants need to produce surplus pollen. The female plants in the wild usually produce several dozen to several thousand flowers, each with six ovules, so each plant can be pollinated by a large number of male parents. The products of these fertilizations are half sibs as they all have the same maternal genetic heritage. Thus the less represented sex has reproductive advantage, as it has higher probability of passing its genes (Dumont *et al.* 2006).

Dioscorea abyssinica and *D. praehensilis* also share some ecological and morphological characters. Firstly, their preferred ecosystem is regenerating plant environments. Both species are dependent on fallows or windfall areas, where they grow while the climax plant population become re-established. Secondly, the aerial architecture of *D. abyssinica* and *D. praehensilis* is typical of wild yams overall. The stems grow to a considerable height before branching, as they need to rise above the supporting shrub vegetation before exposing leaves and flowers. Lastly, both species are extremely polymorphic; the variability being linked to the age of the plant, which can be viewed in two levels: 1. Variation associated with the annual renewal of the vegetative organs and tubers. Some traits vary during the annual vegetative cycle, for example leaf shape and size. 2. Variation associated to the genotype age, which corresponds to the number of annual vegetative cycles since the plant first grew from seed. Some morphological traits are modified as a result of inter-annual variations. For example, in West Africa, the elongated leaves and vine colour at the leaf base are linked to the juvenility of the genotype. The morphological diversity of a genotype is generally reduced with ageing (Dumont *et al.,* 2006).

Dioscorea praehensilis has a wide geographical range in Africa, being found throughout the Western, Central and Eastern parts of the continent to as far South to Zimbabwe. This species is regarded as a forest yam in West Africa. According to Dumont *et al.,* (2006), it grows abundantly in post fire regeneration areas within dense communities of semi-deciduous trees. It is common in the bimodal rainfall zone, but under drier climatic conditions it takes refuges in the rare remnants of mesophyll forests that have survived the

combined effects of annual fire and anthropogenic pressure. *Dioscorea liebrechtsiana*, which ranges from central Africa to Cameroon, is morphologically very close to *D. praehensilis.* Some authors (e.g. Wilkin, 2001) found no difference between them.

No comprehensive studies are conducted on the genetic diversity of *D. praehensilis,* except a few studies that have been undertaken in West Africa. The study conducted by Tostain *et al.* (2002), which involved 46 accessions of *D. praehensilis* revealed that grouping of the genotypes correlated to their geographical location.

Dioscorea abyssinica has long been regarded as the same species as *D. togoensis* (Miège 1952). Based on morphological characters Miège (1982) proposed that *D. abyssinica, D. lecardii,* and *D. sagittifolia* are morphologically so similar that their status as distinct species is questionable.

Dioscorea abyssinica grows mainly north of the Equator in sub-Saharan Africa in the climatic belt roughly ranging from latitudes 8^0 to 12^0 N. *Dioscorea abyssinica* is a savanna yam appearing to prefer a unimodal rainfall regime (Dumont *et al.,* 2006). In West Africa, *D. abyssinica* is distributed throughout the area where *D. rotundata* yams are domesticated (Miège, 1968)**.** In Ethiopia *D. abyssinica* is found widely distributed in the southern, western and northern part of the country in woodlands or wooded grasslands between 1000 m and 1800 m above sea level (Miège and Sebsebe Demissew, 1997).

Several scientific studies have been conducted on the genetic diversity of *D. abyssinica.* Ramser *et al.* (1997) placed *D. abyssinica* in an intermediate position among *D. praehensilis, D. liebrechtsiana* and *D. rotundata,* on the basis of four types of molecular markers. Another study by Tostain *et al.* (2002), using AFLP, has shown genetic continuity between *D. abyssinica* and *D. praehensilis.* A study in Benin (Dansi *et al.,* 1999) indicated that a variety domesticated from wild *D. praehensilis* yams genetically resembled *D. abyssinica.* The authors proposed that wild yams identified as *D. abyssinica* might actually be *D. praehensilis* adapted to the forest-savanna transition or an escape from cultivated fields in the form of seeds. Furthermore, study on *D. abyssinica* materials from Benin, Togo and Guinea using AFLP, revealed a geographically structured genetic diversity (Tostain *et al.,* 2002).

2.9.3 Phylogenetic relationships between wild and cultivated species

The phylogenetic relationships between cultivated and wild yams have long been the focus of scientific investigations. According to Dumont *et al.* (2006)Chevalier linked one cultivar of *D. rotundata* from Benin first (Chevalier, 1920) to *D. praehensilis* and later (Chevalier, 1936) to *D. lecardii*. Burkill (1939) proposed that *D. rotundata* is derived from *D. abyssinica* or from another wild yam of the same type (possibly *D. lecardii*). Miège (1952) proposed D. *abyssinica*, *D. sagittifolia*, *D. preahensilis, D. liebrechstiana, D. mangenotiana* and *D. lecardii* as possible ancestors of Guinea yams*,* whereas Coursey (1976) suggested *D. praehensilis* as the possible predecessor for the cultivated Guinea yams.

Studies based on morphology, ecology and chemotaxonomy have suggested a polyphyletic origin of Guinea yams involving one or multiple hybridization events (Ramser *et al.,* 1997). Several scientific works (Hamon, 1987; Terauchi *et al.*, 1992; Zoundjihèkpon, 1993; Ramser et al, 1997; Dansi *et al.*, 1999; Tostain *et al* , 2002) based on different molecular markers have supported that the wild yams *D. praehensilis* and *D. abyssinica* are possible ancestors of the cultivated Guinea yams *(*Dumont *et al.* 2006), but they may be part of a single biological species as discussed above.

Terauchi *et al.* (1992) proposed *D. abyssinica, D. liebrechtsiana*, *D. praehensilis* or their hybrids as possible predecessors for *D. rotundata* (all characterized by annual replacement of tubers and stems). According to those authors *D. cayenensis* is possibly an interspecific hybrid between *D. rotundata, D. praehensilis*, *D. liebrechtsiana* or *D. abyssinica* (as possible female parents) and the perennial species *D. minutiflora*, *D. burkilliana*. or *D. smilacifolia* De Wild. (as possible male parents). Morphological and ecological data support the hypotheses of the hybrid nature of *D. cayenensis.* It has a longer growth period (8-12) months, some cultivars have perennial nature, with relatively a large corm, thick and flat leaves, its main habitat being in the rain forest zone (Terauchi *et al.,* 1992).

Terauchi *et al.* (1992) used the RFLP technique to analyze chloroplast DNA and ribosomal DNA, but failed to separate *D. rotundata* and *D. cayenensis* from their putative wild parents. Similar results were obtained by Chaïr *et al.* (2005) with chloroplast DNA data and accessions from Benin. However, Chaïr *et al.* (2005) found a unique haplotype for

some of the accessions of *D. abyssinica*. The authors proposed that cultivars sharing identical haplotypes with the cultivated Guinea yams (*D. cayenensis-D. rotundata* complex) might be considered as morphotypes within the complex. They might be escapes found in forests or ancient forest bushes, originating from either seed germination or sprouting from remains of tuber fragments after harvesting. Morphotypes with a different haplotype might represent the true wild type of *D. abyssinica.* Tostain *et al.* (2002) and Scarcelli *et al.* (2006a) used AFLP to compare wild (*D. praehensilis, D. abyssinica*) and cultivated (*D. cayenensis-D. rotundata* complex) Guinea yams that had been domesticated in the past or were in the course of domestication. According to the authors, cluster and ordination analyses partially separated the wild forms from the cultivated Guinea yams. Further more, the degree of relatedness of the wild to the cultivated forms was found to depend on the geographical distance between the wild and the cultivated yams.

Successful interspecific hybridization between wild and cultivated Guinea yams has been carried out at the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria (Mignouna *et al.,* 2005). There are some genetic evidence for spontaneous hybridizations between wild yams and cultivated yams (Scarcelli *et al.,* 2006b). The sympatric situation of wild and cultivated species, field introduction of wild plants during domestication, and to some extent synchronization of flowering time could favour inter-specific hybridization in nature (Scarcelli *et al.,* 2006a).

2.10 Studies on yams in Ethiopia

Ethiopia is considered to be the center of origin for *D. abyssinica*, which is found widely distributed in the savanna region of West Africa (Coursey 1967). Generally the country is regarded as an isolated center of yam cultivation outside the yam belt of West Africa (Norman *et al.,* 1995).

Recent studies made on *Dioscorea* species in South Ethiopia (Muluneh Tamiru, 2006) and Southwest Ethiopia (Hildebrand, 2003; Sebsebe Demisew *et al.,* 2003) have revealed that people in the study area have a strong tradition in cultivating and domesticating various yam cultivars with a wide genetic base. However, this excellent knowledge of yam cultivation and domestication is as mentioned beginning to deteriorate as farming reorients towards cash crops and coffee plantations (Hildebrand *et al.,* 2002). These studies reported

a total of 60 named cultivars (landraces) in the study area of which the majority have limited distribution and abundance. The number of landraces per farmer in southern Ethiopia ranged from 1 to 6 (Muluneh Tamiru, 2006), even if the local classification not always is consistent.

Yam is exclusively cultivated by subsistence farmers in the densely populated areas of Southern, Southwestern and Western parts of Ethiopia, where it has considerable importance in the local livelihood. Yam tubers are the preferred food product for honored guests, and traditional meals made of yams are served during the main traditional and religious festivals. Accordingly, farmers sell yam tubers at relatively high prices compared to other root and tuber crops. Hence, yam is important not only for household food security, but also as a source of cash income (Muluneh Tamiru, 2006).

According to Muluneh Tamiru (2006), farmers in the southern part of Ethiopia recognize two major categories of yams which differ in their maturation period. The early maturing group consists of male yams, which grow vigorously and tolerate drought. These are the most popular, as they fit well into the local subsistence agriculture and, are the preferred choice for yam production. The late maturing group are all female plants. They grow less vigorously and produce poorly under suboptimal conditions. Within each group, morphological attributes such as stem color, presence or absence of spines, leaf color, leaf shape, and tuber flesh color are the major criteria for identifying individual landraces.

There are very few reports dealing with aspects of yam production in Ethiopia (Muluneh Tamiru, 2006). Yam requires 7 to 11 months from planting to harvesting. The supply of fresh tubers to local markets in Ethiopia is limited to the periods from May to September. The planting of yams usually starts in October (in most parts of Southern Ethiopia), November and December (in South Western and Western part of the country) (Muluneh Tamiru, 2006; Hildebrand 2003). Factors such as soil moisture content, intensity of the dry season and anticipated harvesting time are considered in timing of field planting. There is no formal seed supply system nor do farmers specialize in producing yam planting materials. Farmers mostly rely on seed tubers saved from the preceding cropping season. Some partly meet their demand for seed tubers through purchases from local markets or exchanges with neighbors. At the end of each cropping cycle, healthy tubers are selected and stored in shallow pits under shade for one to three months or until required for field

planting. For single-harvested landraces that normally produce a single tuber per plant, the head region (proximal end) of each tuber is retained while the remaining part is consumed. With the double-harvested landraces, a single plant produces many tubers used as for propagation (Muluneh Tamiru, 2006). The decision as to type and number of cultivars to be planted on a farm, is mainly influenced by environmental factors (such as altitude), time of maturation and market demand. Selection for desirable agro-morphological traits, as well as socio-cultural factors, appear to be the major forces behind the dynamics of yam diversity in South Ethiopia (Muluneh Tamiru, 2006)

The genetic structure and diversity of both cultivated and wild yams in Ethiopia is poorly understood (Hildebrand *et al.,* 2003). The studies by Hildebrand *et al.,* (2003) and Muluneh Tamiru (2006) revealed that there are a large numbers of cultivars grown in small farm holds in south and southwest Ethiopia. According to Hildebrand (2003) wild and domestic yams appear to vary significantly in time and space of their availability. Wild yams are harvested from September to February, whereas the cultivated forms from June to October. Wild yams thrive in lowland wooded grasslands, whereas cultivated yams do better in upland settings with more rainfall.

A study conducted on 48 accessions of Ethiopian materials (Muluneh Tamiru, 2006) from South Ethiopia and 8 cultivars of *D. cayenensis* and *D. rotundata* from West Africa based on AFLP, revealed that although the Ethiopian materials are genetically closer to the West African *D. cayenensis* and *D. rotundata* (compared to their genetic distance to *D. bulbifera* and *D. alata*), they show some degree of distinctiveness. The author suggested that the distinctiveness of the Ethiopian materials may represent a divergent evolutionary pathway isolated from the widely known center of diversity in West Africa. Based on the results of morphometric analyses, Muluneh Tamiru (2006) divided the Ethiopian accessions into six groups with two major clusters that mainly differ in their maturity time.

2.11. Statement of the problem and objectives of the study

2.11.1 Statement of the problem

The genus *Dioscorea* includes ca 600 species (Govaerts and Wilkin, 2007), occurring mainly in the Old and New World tropics; the highest levels of species diversity per unit area occur in mainland tropical Asia, Madagascar, South Africa, the Caribbean, Mexico and central South America. There are ca 11 species are found in Ethiopia and Eritrea (Sebsebe Demissew *et al.,* 2003). Among the species of *Dioscorea* section *Enantiophylum*, there is a high degree of phenotypic plasticity in morphology and identification based on morphological characters is sometimes difficult (Lay *et al.,* 2001). The currently used classification scheme which relies on identification keys constructed mainly using vegetative and male inflorescence characters do not delimit species boundaries consistently between *D. cayenensis, D. rotundata, D. abyssinica, D. praehensilis* and *D. sagittifolia* (Wilkin, 2001; Sebsebe Demisew *et al.* 2003). For example recent studies made on *Dioscorea* species of Southwest Ethiopia have indicated that leaves of members of the *D. cayenensis* complex vary considerably in shape within one individual plant (Hildebrand, 2003).

Yam cultivation systems in Ethiopia and East Africa have not been studied as well as their West African counterparts, and the delimitation of the *D. cayenensis* complex and relationships within it are still far from clear. Studies of both morphological and molecular patterns of variations in the wild and cultivated *D. cayenensis* complex in Ethiopia may shed new light on the classification and domestication history of this complex. In particular, such research may test whether the current species boundaries, which are based on some rather cryptic characters, have any grounding in biological fact (Hildebrand *et al,.* 2002).

The study conducted in Southwest Ethiopia has also revealed that the local community has a strong tradition in cultivating and domesticating various species of yams with a wide genetic base. However, the removal of vegetation cover by human activities (for agricultural expansion and settlement) undoubtedly reduces the genetic base of these important cultivated and semi-cultivated crops leading to genetic erosion (Hildebrand *et al.* 2002; Sebsebe Demisew *et al.,* 2003).

Plant genetic resources are one of the most valuable assets to mankind. Protection and conservation of these resources for future generations, therefore, assume great significance. Reliable information on the distribution of genetic variation is a prerequisite for sound selection, breeding and conservation programs. Genetic variation of a species or population can be assessed by measuring morphological and quantitative characters in the field or by studying molecular markers in the laboratory. The uses of DNA based markers are increasingly playing an important role in conservation and use of plant genetic resource (Rao, 2004). Thus determination of the extent of variation at the genetic level within and among populations is of value in guiding genetic conservation activities, which are aimed at maintaining genetic diversity, and molecular marker data have been widely used in taxonomic evaluations particularly in the identification of genotypes (Ford-Lyold, 2001). Good taxonomy is fundamental to conservation and crop improvement programs.

The usefulness, reliability and potential of molecular markers in identification, assessment of genetic diversity and establishment of genetic relatedness have been well documented (Graham *et al.,* 1996). Reports on identification or characterization of cultivars of *Dioscorea* species from West Africa using the isozyme electrophoresis method and the morphological methods have been published (Twyford *et al,.* 1990; Lebot *et al.* 1998; Dansi *et al.* 2000; Mignouna *et al.* 2002b). Recently molecular based techniques, such as the randomly amplified polymorphic DNA (RAPD), AFLP, and microsatellites or simple sequence repeats (SSRs) have been recognized as powerful and efficient tools to detect genetic diversity and assess phylogenetic relationships (Lay *et al.* 2001; Mignouna *et al.,* 2003).

A comparative study by Mignouna *et al.* (2003) using three different molecular techniques indicated that AFLP showed the highest efficiency in detecting polymorphism and revealing genetic relationships that most closely reflected the morphological classification. AFLP has also been used to asses the genetic relationships between *D. alata* and other edible *Dioscorea* species from different section including Guinea yams. The study revealed that members of the sections *Enantiophyllum* are distinguished from each other and are genetically distant from species of other sections. They also proposed that AFLP can be used to characterize *Dioscorea* species at a varietal level (Malapa *et al.,* 2005).

2.11.2. Objectives of the study

2.11.2.1 General objectives

The main objectives of this study are:

- To evaluate and improve the taxonomy and species delimitation within the *D. cayenensis* complex by using both morphological and molecular techniques.
- To investigate the amount and distribution of genetic variation among and within populations of the *D. cayenensis* complex, as a prerequisite for devising conservation strategy.

2.11.2.2 Specific objectives

- To determine the taxonomic status of the speices within in the *D. cayenensis* complex in Ethiopia
- To determine the genetic diversity of the wild and cultivated species within the *D. cayenensis* complex.
- To determine level of population differentiation and population structure among the populations of the *D. cayenensis* complex in Ethiopia.
- To identify sites or areas where priority should be given for *in-situ* conservation of Guinea yams in Ethiopia.

3. Materials and Methods

3.1 Morphometry

3.1.1 The plant material

The plant material used for morphometry consisted of 40 dried herbarium specimens of wild and cultivated accessions of the *D. cayenensis* complex, collected in different localities in South and Southwestern parts of Ethiopia (Table 4) from a field trip conducted in August 2006. Collections were first named using the folk taxonomy as the field identification and formal taxonomic identification to species level was made later using the voucher specimens at Royal Botanic Gardens, Kew. D. *cayenensis* and *D. rotundata* were treated as a single species (under the former earlier name) because they are indistinguishable based on the morphology of the above-ground organs. The major morphological characters used for identification of the specimens to species level are presented in appendix 1.Voucher specimens were housed at the Royal Botanic Gardens, Kew, London, UK. During the field work, flowers from the live specimens of each of the individual plants were collected and preserved in 70 % alcohol.

Table 4. List of accessions of cultivated and wild yams sampled in the field and used for mormphometric analysis

Serial No	Species name	Accession number	Place of collection, locality
1	***D. abyssinica***	**Daby-20**	Bench- Magi zone Sheko area , Selalea locality,13 km along the Mizan- Sheko road
2	***D. abyssinica***	**Daby-22**	Bench- Magi zone Sheko area , Selalea locality,13 km along the Mizan- Sheko road
3	***D. abyssinica***	**Daby-59**	Areka agricultural institute, Welaita zone, at the outskirt of Areka town
4	***D. abyssinica***	**Daby-69**	Areka agricultural institute, Welaita zone, at the outskirt of Areka town
5	***D. abyssinica***	**Daby-77**	Welayita zone, Sodo Zuria district, Wejekerea locality, 3 km along the Sodo- Areka road
6	***D. abyssinica***	**Daby-8**	Bench- Magi zone Sheko area , Selalea locality,13 km along the mizan- sheko road
7	***D .bulbifera***	**Dbul-28**	Ilubabor, 28 km along the Bedelea-Metu road near dedesa state farm
8	***D. cayenensis***	**Dcay-1**	Bench- Magi zone Temenja yadj area ,25 km along the Chena-mizan road
9	***D. cayenensis***	**Dcay-17**	Bench- Magi zone Sheko area , Shekoka locality, 8-10 km along the mizan- sheko road
10	***D. cayenensis***	**Dcay-18**	Bench- Magi zone Sheko area , Selalea locality,13 km along the mizan- sheko road
11	***D. cayenensis***	**Dcay-19**	Bench- Magi zone Sheko area , Selalea locality,13 km along the mizan- sheko road
12	***D. cayenensis***	**Dcay-21**	Bench- Magi zone Sheko area , Selalea locality,13 km along the mizan- sheko road
13	***D. cayenensis***	**Dcay-35**	Wellega, Nedjo area 5 km along Nedjo-Ghmbi road

14	***D. cayenensis***	**Dcay-38**	Wellega, Nedjo area 5 km along Nedjo-Ghmbi road
15	***D. cayenensis***	**Dcay-47**	Gedeo, Kocherea district, Hama locality
16	***D. cayenensis***	**Dcay-48**	Gedeo, Kocherea district, Hama locality
17	***D. cayenensis***	**Dcay-49**	Gedeo, Yirgachefea district, Konga locality
18	***D. cayenensis***	**Dcay-50**	Gedeo, Yirgachefea district, Konga locality
19	***D. cayenensis***	**Dcay-56**	Sidama, Aleta wondo district, Debi locality
20	***D. cayenensis***	**Dcay-57**	Sidama, Aleta wondo district, Debi locality
21	***D. cayenensis***	**Dcay-61**	Areka agricultural institute, Welaita zone, at the outskirt of Areka town
22	***D. cayenensis***	**Dcay-62**	Areka agricultural institute, Welaita zone, at the outskirt of Areka town
23	***D. cayenensis***	**Dcay-67**	Areka agricultural institute, Welaita zone, at the outskirt of Areka town
24	***D. cayenensis***	**Dcay-68**	Areka agricultural institute, Welaita zone, at the outskirt of Areka town
25	***D. cayenensis***	**Dcay-7**	Bench- Magi zone sheko area , 13 km along the mizan- sheko road
26	***D. cayenensis***	**Dcay-70**	Areka agricultural institute, Welaita zone, at the outskirt of Areka town
27	***D. cayenensis***	**Dcay-71**	Areka agricultural institute, Welaita zone, at the outskirt of Areka town
28	***D. cayenensis***	**Dcay-73**	Areka agricultural institute, Welaita zone, at the outskirt of Areka town
29	***D. cayenensis***	**Dcay-74**	Areka agricultural institute, Welaita zone, at the outskirt of Areka town
30	***D. cayenensis***	**Dcay-75**	Areka agricultural institute, Welaita zone, at the outskirt of Areka town
31	***D. cayenensis***	**Dcay-76**	Welayita zone, Sodo Zuria district, Wejekerea locality, 3 km along the Sodo- Areka road
32	***D. praehensilis***	**Dprh-13**	Bench- Magi zone Sheko area , 13 km along the mizan- sheko road 8 km along the Mizan-Sheko road
33	***D. praehensilis***	**Dprh-16**	Bench- Magi zone Sheko area , Shekoka locality, 8-10 km along the mizan- sheko road.
34	***D. praehensilis***	**Dprh-25**	Ilubabor, Yayu area, ca 13 km along the road from Yayu to Bedele
35	***D. praehensilis***	**Dprh-26**	Ilubabor, Yayu area, ca 25 km along the road from Yayu to Bedele
36	***D. praehensilis***	**Dprh-27**	Ilubabor, Yayu area, ca 25 km along the road from Yayu to Bedele
37	***D. praehensilis***	**Dprh-30**	Wellega, ca 28 kms along the Nekemt-Ghimbi road
38	***D. praehensilis***	**Dprh-31**	Wellega, ca 28 kms along the Nekemt-Ghimbi road
39	***D. praehensilis***	**Dprh-32**	Wellega, ca 28 kms along the Nekemt-Ghimbi road
40	***D. praehensilis***	**Dprh-53**	Sidama, zone, at the outskirts of Dilla town, ca 3 km form Dilla town, near the main road from Dilla to Awassa

3.1.2 Morphological characters

Leaf and floral characters were used in the morphometric analyses. Two mature leaves (one in few individuals) from each of the individuals were randomly selected to be used for measurements using an ordinary ruler and a protractor. Measurements of floral characteristics were made using only the male inflorescence (because more than 75 % of

the specimens are male); female flowers and fruits are too infrequent in populations to obtain a sufficiently large sample. Two male flowers from the same inflorescence were picked randomly. As the flowers are very small in size, measurement of floral characters was made by using a binocular microscope (American Optical Corporation Mod 570) with a magnification power of 60X. The morphological characters used in this study are listed in Table 5 (see Fig. 3 for illustration of leaf characters).

Table 5. List of morphological characters used in morphometric analysis.

Leaf characters	Male floral characters
1. Blade length (L) 2. Tip base width (TBW) 3. Basal width (through point of petiole insertion) (BW) 4. Sinus base width (SBW) (between apices of auricles either side of basal sinus). 5. Width at ¼ length from the base (14W). 6. Width at ½ length from the base (12W) 7. Width at ¾ length from the base (34W) 8. Distance from midrib to first vein at ¼ W(M1) 9. Distance from midrib to second vein at ¼ W (M2) 10. Distance from midrib to margin at ¼ W (MM) 11. Ratio of BW to SBW 12. Ratio of 14W to 12W 13. Ratio of 14W to 34W 14. Ratio of L to BW 15. Ratio of L to 14W 16. Ratio of L to 12W 17. Ratio of L to 34W 18. Ratio of M2-M1 to MM 19. Ratio of MM-M2 to MM 20. Ratio of MM-M1 to MM	1. Outer tepal length (OTL) 2. Outer tepal width (OTW) 3. Inner tepal length (ITL) 4. Inner tepal width (ITW) 5. Anther length (AL) 6. Anther width (AW) 7. Filament length (FL)

3.1.3 Data collection and analysis

Only quantitative characters were used in the morphometric analyses. The leaf measurements were log transformed before they were used in the final data analysis. These characters were also used to estimate leaf shape parameters by transforming them into ratios (Table 5).

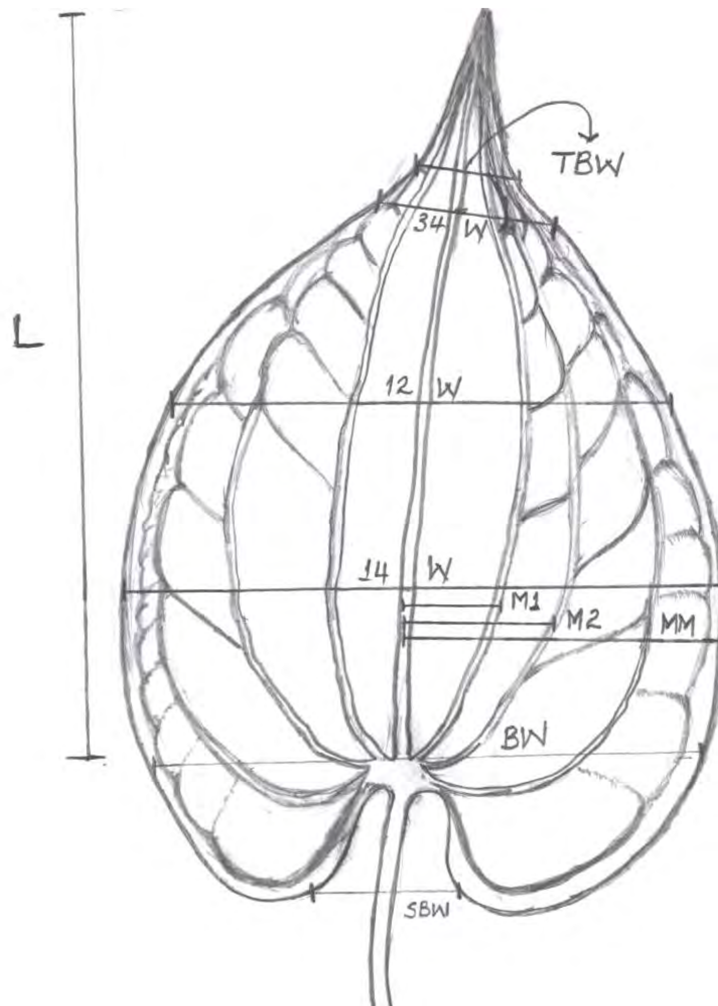


Figure 3. Illustration of a typical *Dioscorea* leaf showing the measurements of leaf characters

A data matrix was constructed using 68 individuals and 26 attributes. Each individual used in the study was considered as one OTU (operational taxonomic unit). The data matrices were subjected to multivariate analyses such as cluster analysis (Unweighed Pair Group method of Analysis, UPGMA, Sneath and Sokal, 1973) and ordination analysis (Principal Component Analysis, PCA) using NTSYSpc version 2.2 (Rohlf 2004). For the cluster analysis (UPGMA,), the SIMQUAL module with simple matching coefficients as a measure of similarity was used to construct a similarity matrix. Phenograms were constructed by using the SHAHN option. The same similarity matrix was also used for PCA.

3.2 Amplified Fragment Length Polymorphism (AFLP)

3.2.1. The plant material

The plant material of *D. cayenensis* complex consisted of 43 accessions belonging to 16 farmers' varieties (Table 6). Three accessions of *D. schimperiana* and *D. bulbifera* were included for comparative purposes. The plant materials were collected in July 2005 from a fieldtrip conducted in Sheko and its environs (Sh population, see Fig 4) of Southwest Ethiopia. Collections and identification to species level was done using the same procedure as used in morphometric analyses. The voucher specimens, listed in Table 6, have been deposited at the herbarium ETH (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

Table 6. List of accessions of cultivated and wild yams used in AFLP analysis, giving their Sheko name, collection site and identification (all collected from Bench-Maji zone expept the first two).

Serial No	Species name	Accession number	Local (Sheko) name	site of collection
1	*D. schmperiana*	14333A	-	90 km along the road from Nekemte to Bure, Oromia region
2	*D. praehensilis*	1577B	-	142 km along the road from Chagni to Mankush, Beneshangul Gumuz
3	*D. cayenensis*	CA1	Addis Kachi	Sheko district, Gaizika village.
4	*D. cayenensis*	CBD1	Banda	Ediget Behibret School yard , Mizan Teri town
5	*D. cayenensis*	CBD2		
6	*D. cayenensis*	CBD5		
7	*D. abyssinica*	CC1	Chebja	Sheko district, Mehal Sheko, Serer village and from outskirts of Sheko town near the health center.
8	*D. cayenensis*	CC10		
9	*D. abyssinica*	CC2		
10	*D. abyssinica*	CC6		
11	*D. abyssinica*	CC8		
12	*D. cayenensis*	CD2	Don	Sheko district, Gaizika village.
13	*D. cayenensis*	CD3		
14	*D. abyssinica*	CDB3	Donbai	Sheko district, Mehal Sheko, Serer village.
15	*D. praehensilis*	CDB4		
16	*D. cayenensis*	CDK1	Dizu kechi	Sheko district, Gaizika village and outskirts of Sheko town near the health center.
17	*D. cayenensis*	CDK10		
18	*D. cayenensis*	CDK12		
19	*D. cayenensis*	CDK13		
20	*D. cayenensis*	CDK14	Dizu kechi	Bench District, Gabuka Village
21	*D. cayenensis*	CDK16		
22	*D. cayenensis*	CDK3	Dizu kechi	Sheko district, Gaizika village and outskirts of Sheko town near the health center.
23	*D. cayenensis*	CDK6		
24	*D. cayenensis*	CDK7		

25	*D. abyssinica*	CDK9		
26	*D. cayenensis*	CE1	Esintie	Sheko District, outskirts of Sheko town near the health center.
27	*D. cayenensis*	CE2		
28	*D. cayenensis*	CE3		
29	*D. cayenensis*	CE4		
30	*D. abyssinica*	CKR2	Kerkebat	Bench District, Gabuka Village
31	*D. cayenensis*	CS1	Surkachi	Sheko district, Mehal Sheko, Serer village
32	*D. cayenensis*	CS2		
33	*D. cayenensis*	CS4		
34	*D. abyssinica*	CSKB2	Surkechibai	Sheko District, outskirts of Sheko town near the health center.
35	*D. cayenensis*	CSKB4		
36	*D. cayenensis*	CT1	Torbai	Sheko District, outskirts of Sheko town near the health center.
37	*D. abyssinica*	CT3		
38	*D. cayenensis*	CTS1	Tsanu	Sheko district, Mehal Sheko, Serer village.
39	*D. cayenensis*	CTS2		
40	*D. abyssinica*	CTs4		
41	*D. praehensilis*	WC8	Chebja (Wild)	Sheko District, Gaizika village, Onta
42	*D. praehensilis*	WK4	Kakeb (wild)	Sheko District, Gaizika village, Onta
43	*D. praehensilis*	WK5		
44	*D. cayenensis*	WY3	Yasint (wild)	Bench District, near Woshikit School.
45	*D.bubifera*	D.bul	Ama	Bench district, 10 km from Kitea town near forest coffee plantation
46	*D. schimperiana*	D.sch	-	Bench district, 10 km from Kitea town near forest coffee plantation

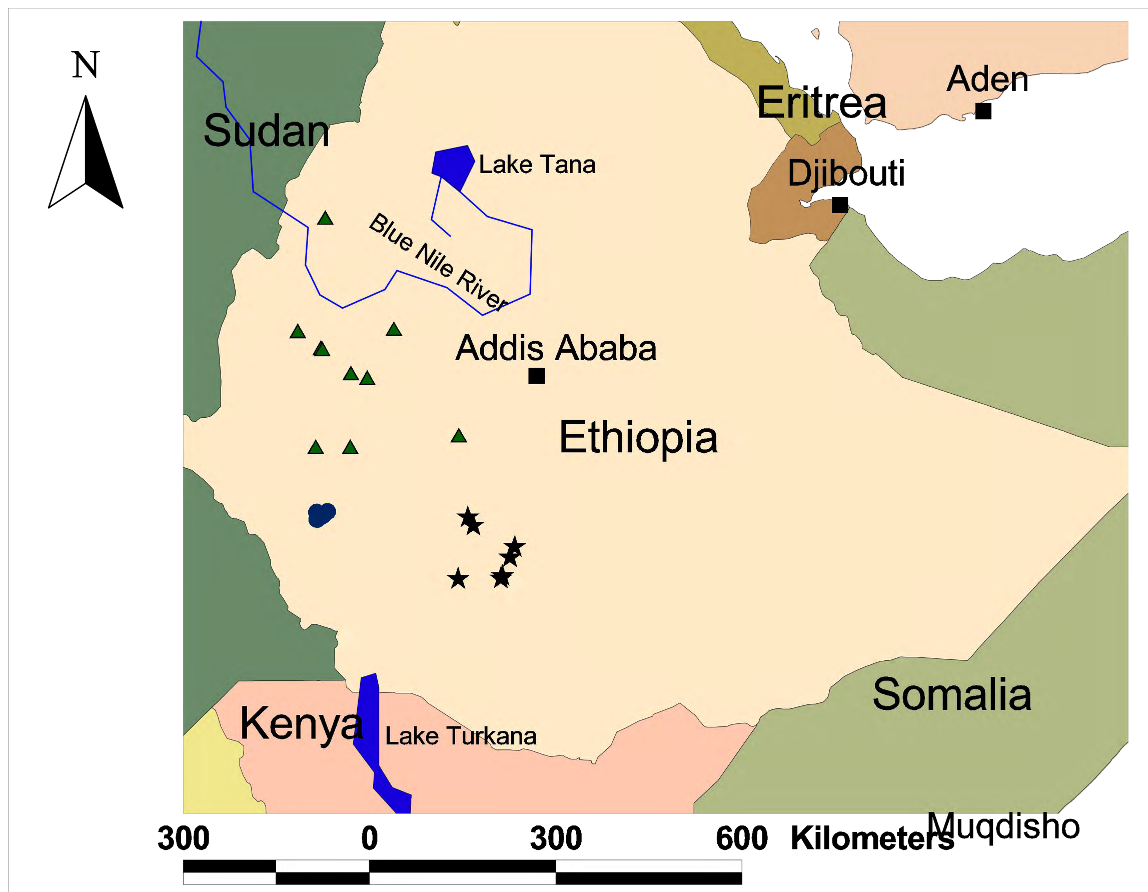


Figure 4. Map of Ethiopia showing the collection sites of *Dioscorea* accessions used in this study. (▲= Or population, all collected from Oromia region ● = Sh population from Benchi-Maji zone of SNNPRS (Southern Nations, Nationalities and Peoples Regional State) and ★= Sn population, from SNNPRS excluding Bench-Maji zone).

3.2.2. DNA extraction and purification

Total DNA was extracted from silica gel dried leaf materials using a modified CTAB procedure from Doyle and Doyle (1987). 10 ml of isolation buffer (100 mM Tris HCl pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% CTAB) was preheated in 50 ml of Blue Cap tubes to 65 °C. Dried leaf material (0.1- 0.3 g) was ground into powder using a preheated mortar and pestle. While grinding, small portions of the buffer were added until uniform slurry was obtained. Further extraction of total DNA was carried out by adding equal volume of SEVAG (24:1 chloroform: isoamayl alcohol) and rocking the mixture for about 30 minutes. This was followed by spinning the tubes at 8000 rpm at 25 °C for 10 minutes. The aqueous top phase containing the DNA was removed using Pasteur pipettes and transferred to 50 ml

Yellow Cap tubes. Then about twice the volume of ethanol (absolute) cooled to -20 ºC was added to the extract by mixing it gently to precipitate the DNA. The mixture was stored over night at -20 ºC. The next day DNA pellets were collected by centrifuging the mixture at 3200 rpm for 5 minutes. The pellets were allowed to dry in a fume hood at room temperature for about 2-3 hours. Finally the dried pellets were resuspended in 500 µl of TE buffer (10 mM Tris HCl pH 8, 0.25 mM EDTA) and stored at -20 ºC until use.

Purification of nuclear DNA was carried out using spine column chromatography. Thus, about 600 µl of binding buffer (NT buffer, 140 mM NaCl, 6 mM KCl, 1mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM glucose) and 120 µl of the DNA extract were added into a mini spin column (NucleoSpin®). The mixture was centrifuged at 12000 rpm for one minute followed by addition of 750 µl of wash buffer (PB buffer, 0.15 M Na_2HPO_4 (anhydrous) and 0.04 M NaH_2PO_4). After spinning the mixture at 12000 rpm for 1 minute, 50 µl of elution buffer (TE buffer, 10 mM Tris base, 1 mM EDTA•Na_2 , pH 7.5) was added and the mixture was allowed to stand for 30 minutes at room temperature. Finally, the dissolved DNA was collected in 0.5 ml Eppendorf tubes and stored at -20 ºC. The quality of the DNA was visually assessed by electrophoresis on a 1% agarose gel. The DNA concentration was quantified using a spectrophotometer (Eppendorf Biophotometer) at 260 nm wave length, according to the manufacturer's instructions.

3.2.3. AFLP analysis

AFLP analysis was performed according to the method of Vos *et al.* (1995), with a slight modification in the restriction and ligation of the genomic DNA. Briefly, 0.5 µg DNA was cleaved by restriction enzymes and ligated simultaneously in a mixture containing 10X T4 ligase buffer, 0.5 M NaCl, 1 mg/ml BSA, 50 pmol MseI adaptor pair, 5 pmol EcoRI adaptor pairs (Applied Biosystems AFLP®) , 1 U MseI, 5U EcoRI and 1 U T4 ligase . The mixture was incubated in a PCR machine (Applied Biosystems GeneAmp® PCR System 9700) for a period of 2 hours at 37 ºC. The products of the restriction ligation were diluted with 95.5 ml of TE buffer and subsequently used for preselective amplification. Preselective amplification was performed via 20 PCR cycles (94 ºC for 30s, 56 ºC for 30 s, 72 ºC for 1 min) using 7.5 µl Core mix (Applied Biosystems AFLP®) and 0.5 µl preselective primers for small genomes (Applied Biosystems AFLP®). The efficiency of

both the restriction and the preselective amplification reactions was assessed by visualizing the banding pattern on a 1.5 % agarose gel. The products of preselective amplification were diluted and subsequently used for selective amplification. Three selective primer combinations were used; the selective primers sequences are given in Table 7. The products of selective amplification were then diluted with 10 µl formamide and 0.2 µl Rox size standard, denatured and loaded on ABI Prism 3100 Genetic Analyzer for fragment analysis.

Table 7. Selective primer sequences used in the AFLP analyses.

Primer combinations		Labeled with
Mse	**EcoRI**	
5'-GATGAGTCCTGAGTAACAC-3'	**5'-GACTGCGTACCAATTCTT-3'**	**FAM**
5'-GATGAGTCCTGAGTAACAC-3'	**5'-GACTGCGTACCAATTCAG -3'**	**JOE**
5’-GATGAGTCCTGAGTAACTT-3'	**5'-GAC TGCGTACCAATTCAT-3'**	**NED**

3.2.4. Data collection and statistical analysis

The presence/absence of unequivocally scorable bands was transformed in to a binary character matrix (1 for presence and 0 for absence of a band at a particular position). For cluster analysis and Principal Coordinate Analysis (PCO), pair wise distance matrices were compiled by the NTYSYSpc 2.2 software packages, using the DICE coefficient of similarity. A dendrogram was constructed by UPGMA. The genetic diversity of the populations and genetic divergence among the taxa were estimated by comparing the frequency of rare fragments and the percentage of polymorphism.

3.3. Microsatellites

3.3.1. Plant material

Plant materials for the microsatellite analyses consisted of 58 accessions of wild and cultivated yams collected during the months July, August and September of 2005 and 2006, from a field trip conducted to South and Southwest Ethiopia (Fig 4). As in the case of AFLP studies, the accessions were first named using the folk taxonomy and formal taxonomic identification to species level was made later using the voucher specimens at Royal Botanic Gardens, Kew. All the accessions used for microsatellite analyses belong to the *D. cayenensis* complex (*D. abyssinica, D. praehensilis* and *D. rotundata-D. cayenensis*).

3.3.2. DNA extraction and microsatellite markers

Total DNA extraction and purification nuclear DNA was carried out using the same procedure as for the AFLP study. Variable microsatellite loci previously identified for *Dioscorea* species by Tostain *et al.* (2006) were surveyed and 9 loci with strong, unambiguous banding patterns were selected for use in this study (Table 8). These loci are all composed of six different dinucleotide repeats (GT, TG, AC, GA, CT, and AG) with repeat motifs ranging from 8-23.

3.3.3. PCR amplification

Polymerase chain reactions (PCRs) were carried out in a total volume of 10 µl , containing 50 ng of genomic DNA, 0.5 µl forward primer (10 pmol/ µl), 1 µl reverse primer (10 pmol/ µl), 1 µl M 13 primer with dye (2 pmol/ µl), 1 µl 10x NH_4 reaction buffer (160mM $(NH_4)_2SO_4$, 670mM Tris-HCl (pH 8.8 at 25°C), 0.1% Tween-20), 0.2 µl $MgCl_2$ (25 mM), 0.1 µl dNTPs (100 mM), and 0.1 µl Biotaq™ DNA polymerase (5 u/µl, Bioline). PCR was performed on a GenAmp® PCR system 9700 thermocycler (AB, Applied Biosystems). The PCR program involved denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 sec, 51 °C (annealing temperature) for 1 min and 72 °C for 1 min, with a final extension step at 72 °C for 8 min.

Table 8. Characteristics of the 9 microsatellite loci used in this study with number of alleles and allele size range observed in the study population (where F = forward primer sequence and R = reverse primer sequence)

Locus	*Primer sequences (5'-3')*	*Repeat motif*	*Size (bp)*	*No. of alleles*	*Allele size range*
Da1A01	**F: TATAATCGGCCAGAGG** **R: TGTTGGAAGCATAGAGAA**	**(GT)8**	**204**	**8**	**212–260**
Da1D08	**F: GATGCTATGAACACAACTAA** **R: TTTGACAGTGAGAATGGA**	**(CA)8**	**309**	**12**	**335-400**
Da1F08	**F: AATGCTTCGTAATCCAAC** **R: CTATAAGGAATTGGTGCC**	**(TG)13**	**177**	**11**	**165-220**
Da3G04	**F: CACGGCTTGACCTATC** **R: TTATTCAGGGCTGGTG**	**(AC)12**	**305**	**5**	**285-340**
Dab2C05	**F: CCCATGCTTGTAGTTGT** **R: TGCTCACCTCTTTACTTG**	**(GA)19**	**190**	**13**	**175-220**
Dab2D06	**F: TGTAAGATGCCCACATT** **R: TCTCAGGCTTCAGGG**	**(CT)19**	**174**	**7**	**170-205**
Dab2E07	**F: TTGAACCTTGACTTTGGT** **R: GAGTTCCTGTCCTTGGT**	**(CT)23**	**152**	**7**	**105-150**
Dpr3D06	**F: ATAGGAAGGCAATCAGG** **R: ACCCATCGTCTTACCC**	**(GA)15**	**151**	**7**	**160-190**
Dpr3F04	**F: AGACTCTTGCTCATGT** **R: GCCTTGTTACTTTATTC**	**(AG)15**	**128**	**5**	**120-145**

3.3.4. Detection and analysis of PCR products

Detection of amplification products was carried out by running the samples on a 1.5% agarose gel containing 4 µl Ethidium Bromide solution. The procedure was as follows: 5 µl of the PCR products were mixed with 5 µl of loading buffer (0.025 g Bromophenol blue and 40 g Sucrose in 100 Milli Q water), the mixture was loaded on to the gel which was subjected to electrophoresis in 1 X TBE buffer (45 mM Tris base, 45 mM Boric acid, 1 mM EDTA pH 8.0). The bands were revealed on a radiography film (Fig 5). The PCR products were then diluted with 10 µl formamide and 0.2 µl of Rox size standard, denatured and loaded on to ABI Prism 3100 Genetic Analyzer for fragment analysis.

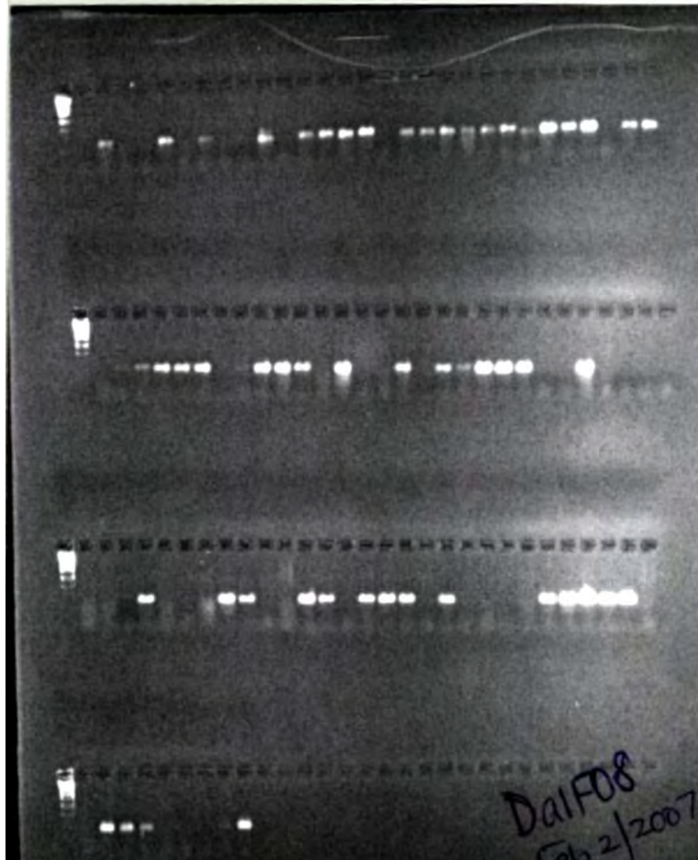
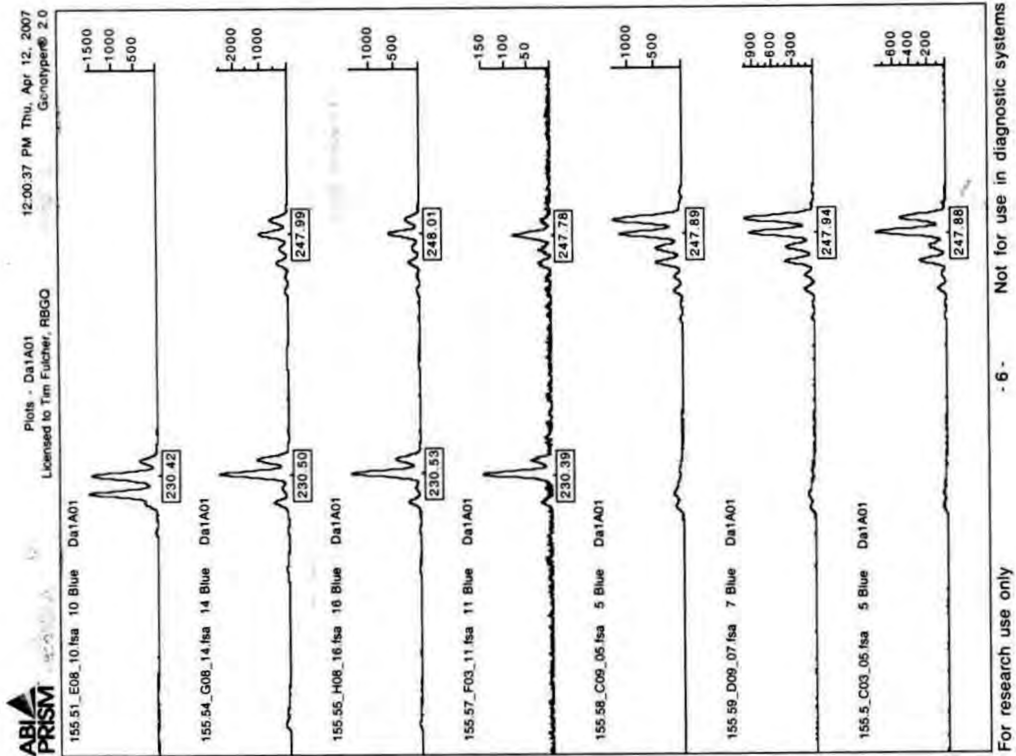


Figure 5. The PCR products as revealed by electrophoreses on 1.5 % agarose gel

3.3.5 Data collection and analysis

3.3.5.1 Data collection.

Using GENESCAN and GENOTYPER 3.6 software (Applied Biosystems), loci were initially scored as codominant marker data. Presence/absence data were then generated form the codominant marker data matrices. Both types of data matrices were subjected to various multivariate analyses using different softwares (see below). For parameters related to population genetic analyses such as, estimation of the level of population differentiation we used only 7 of the 9 loci as the alleles for two of the loci (DA1D08 and Dpr3F04) failed to be amplified for the some of the study individuals (hence, to avoid inclusion of too many missing data in the analyses). For each of the loci studied the genotyping data observed produced only one or two alleles per sample (Fig 6).



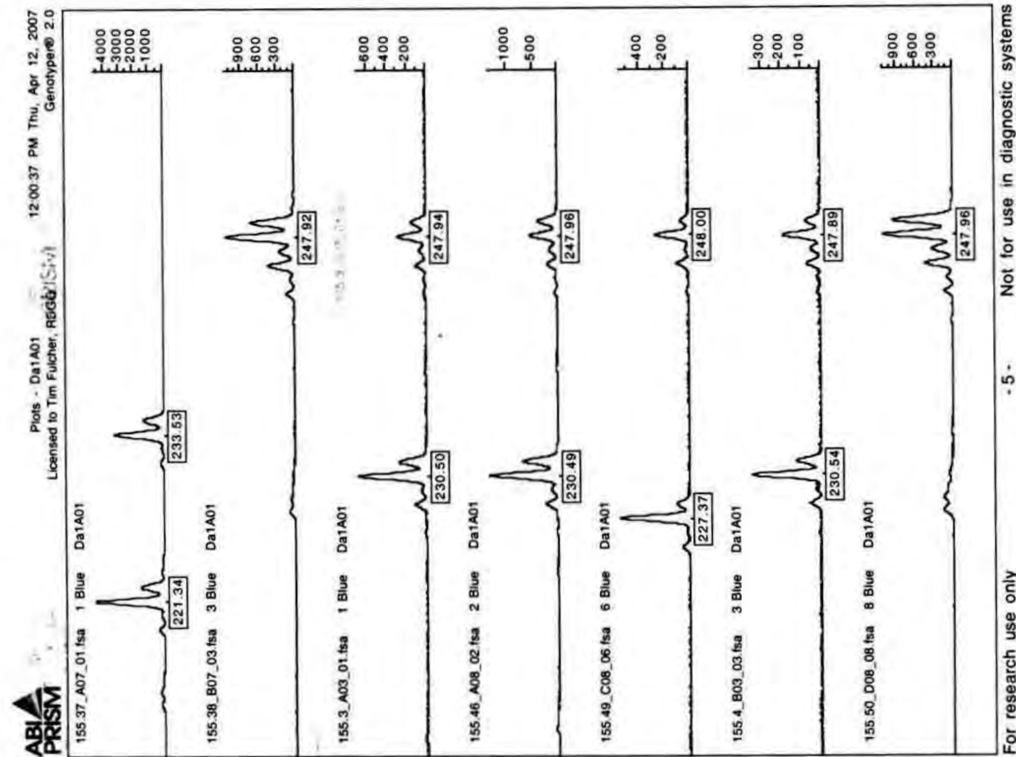


Figure 6. Microsatellite electropherogram for the locus Da1A01 as revealed by GENESCAN and GENOTYPER 3.6 software.

3.3.5.2 Taxonomic relationships between the taxa

In order to examine the relationships among the three taxa, distance trees were inferred from the presence/absence data matrix constructed using 58 individuals and 91 attributes (alleles) from the 9 loci studied. The similarity matrix generated was subjected to multivariate analyses such as, UPGMA and ordination analysis (PCO) using NTSYSpc version 2.2. A dendrogram was constructed by UPGMA using the option DICE coefficient as a measure of similarity. The same similarity matrix was also used for PCO analyses.

3.3.5.3 Genetic diversity

Three populations (Or, Sn and Sh) were defined based on their geographical location (Refer to map-Fig 4). Genetic polymorphism for each population was assessed by calculating the number of alleles per locus (A), allelic richness (R), the observed heterozygosity (H_o) and the expected heterozygosity (H_e) using the programs GENEPOP version 3.1 (Raymound and Rousset, 1995), Microsatellite analysis (MSA) (Dieringer and Schlotter, 2003) and FSTAT 2.9.3.2 (Goudet, 2001). The average expected heterozgosity (H_e) within a population is the best general measure of genetic variation. There are a variety of characteristics of average heterozygosity that makes it a valuable for measuring genetic variation. It can be used for genes of different ploidy level and in organisms with different reproductive systems. It is also a good measure of the response of a population to natural selection. It can further provide the inbreeding coefficients of individuals. The total number of alleles at a locus has also been used as a measure of genetic variation. This is a valuable complementary measure of genetic variation because it is more sensitive to the loss of genetic variation due to small population size, than heterozygosity. Accordingly, it is an important measure of the long term evolutionary potential of populations. However, unlike heterozygosity, it is highly dependent on sample size. Therefore, comparisons among samples are not meaningful unless sample sizes are similar because of the presence of many low frequency alleles in natural populations. This problem can be avoided by using allelic richness, which is a measure of allelic diversity that takes into account sample size. This measure uses a rarefaction method to estimate the allelic richness at a locus for fixed sample size, usually the smallest sample size if a series of populations are sampled. The effective number of alleles, defined as the number of alleles that, if equally frequent, would infer the observed hetrozygosity, is also used to describe genetic variation at a locus.

However, this parameter provides no more information about the number of alleles present at a locus than does heterozygosity (Allendorf and Luikart, 2007).

For each population-locus combination, departure from Hardy-Weinberg expectation was assessed using exact tests (Guo and Thompson, 1992), with unbiased p-values estimated through a Markov-chain method (Guo and Thompson, 1992). A global test across loci and populations was constructed using Fisher's method (Raymound and Rousset, 1995). The comparisons of genetic diversity and population structure of wild and cultivated accessions of the *D. cayenensis* complex were also carried out using some of the population genetic parameters listed above.

3.3.5.4 Population structure

The level of population differentiation among the three subpopulations (Or, Sn and Sh) mentioned above were estimated using unbiased estimated p-value for a log likelihood (G) base exact test (Goudet, *et al.,* 1996). Genetic differentiation was quantified using F-statistics (Weir and Cockerham, 1984) by the computer program FSTAT2.9.3.2 (Goudet, 2001). Genetic relationships among the populations were assessed using distance trees inferred from allelic frequency data. The distance matrix based on proportion of shared alleles (Dps) was generated using the program MSA (Dieringer and Schlotter, 2003). The distance matrix was then imported into PHYLIP computer package version 3.66 (Felsenstein, 1995) from which a neighbor joining phenogram was generated using the program NEIGHBOR. The distance tree was then viewed using TREEVIEW.

4. Results

4.1 Morphometry

Figures 7 and 8 show the results of the cluster and ordination analyses based on the data matrix generated from morphological characters. In the UPGMA analysis two of the *Dioscorea bulbifera* accession included for comparisons represented branches outside the study group. Within the study group the cultivated and wild accessions of *D. cayenensis* complex were intermixed within the different phenon group (Fig.7) indicating that they are closely related. Accordingly none of the clusters contained entirely those accessions treated as the same species according to the existing species concepts. The first split within the study group separates a single accession of *D. abyssinica* from the rest of the group. However, all the remaining accessions in the study group are found scattered in the phenogram. On the next level two accessions of *D. praehensilis* are separated from the rest, but again several accessions of *D. praehensilis* are found scattered on the succeeding level. The two phenons on the succeeding levels are separated by a very short distance and their internal structure show no pattern in relation to the predelimitated taxa

The principal component analysis (PCA) indicated that the first 23 axes totally account for 100% of the observed variation between the accessions. The Eigen values of the first, second and third components correspond to 51, 26 and 9% of the variability, respectively. Accordingly, the first three axes explain cumulatively 86% of the total diversity within *Dioscorea* species included in this study. A plot showing the first and second axes clustered all three species of the *D. cayenensis* complex into one group (Fig 8). The two individuals belonging to *D. bulbifera* were found to be well separated from the rest of the group. This has also been demonstrated by the three dimensional plot of the first three PCA axes accounting 86% of the total variation (Fig 9).

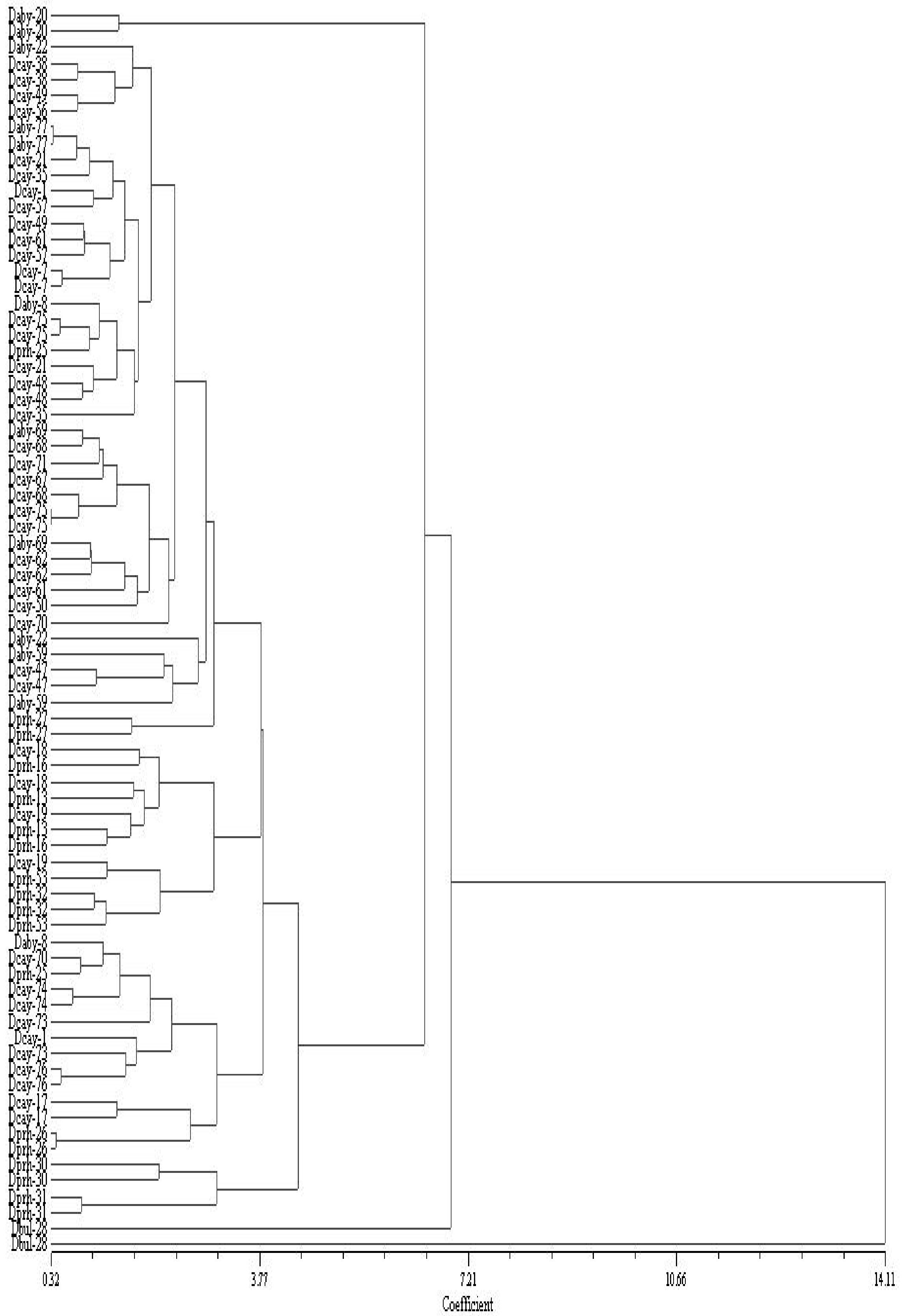


Figure 7. UPGMA cluster derived from a similarity matrix of 40 accessions of *Dioscorea* based on morphological characters (Dcay, Daby, Dprh and Dbul refer to *D. cayenesis, D. abyssinica, D. praehensilis* and *D. bulbifera* respectively)

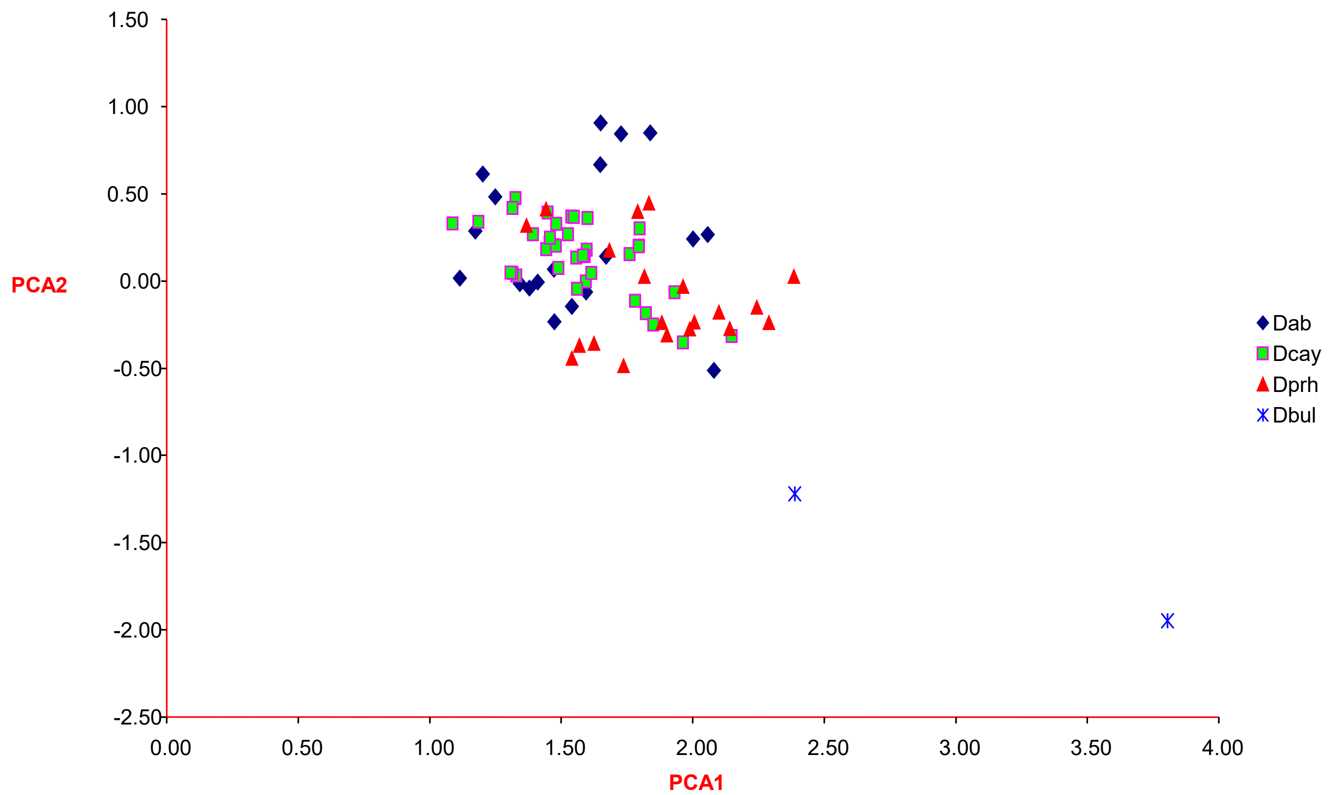


Fig 8. Figure 8. Scatter plot showing the first and second axis of the PCA based on morphological data for 40 individuals of *Dioscorea* (Dcay, Dab, Dprh and Dbul refer to *D. cayenesis, D. abyssinica, D. praehensilis* and *D. bulbifera,* respectively

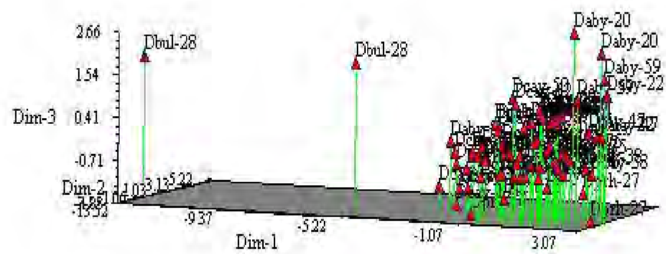


Figure 9. 3-D plot of the first three axes obtained by principal PCA analysis of morphological data among 40 individuals *Dioscorea* species (Dcay, Daby, Dprh and Dbul refer to *D. cayenesis, D. abyssinica, D. praehensilis* and *D. bulbifera*, respectively).

4.2 AFLP Analyses

4.2.1 Overall genetic structure of the populations

From the three primer combinations used a total of 245 different fragments ranging from 53 bp to 496 bp were generated. Although, amplification using the three primer pairs generated a total of 245 fragments, only 158 of the AFLP fragments were scored for the purpose of data analysis. Thus, 87 fragments were discarded from the analysis because they either showed weak signal or because they were not reproducible for some of the study individuals. Out of the selected 158 fragments 35 (22%) were found to be monomorphic and 123 (78%) were found to be polymorphic. The number of fragments generated per individual (and used for data analysis) ranged from 39 to 74. Altogether, five fragments were found to be common to all the individuals belonging to the cultivated and wild accessions of the *D. cayenensis* complex. These fragments could potentially be used as diagnostic markers for the group.

4.2.2 Taxonomic delimitation and genetic relationships among the taxa

The results of the cluster and ordination analyses failed to produce any partitioning among the taxa under study (Figs 10-13). The UPGMA tree shows no patterns of clustering of the accessions into groups in relation to the existing species concepts (Fig. 10). The UPGMA tree produced two major clusters, the level of similarity between these two clusters being 32%. The first split separated all the wild and cultivated accessions of *D. cayenensis* complex (sharing 60-100% of the average genetic similarity) from *D. bulbifera* and *D. schmeperiana*. The phenogram indicates that the genetic distance between individuals of Guinea yam accessions and their wild relatives varies from 0 to 40%. Two accessions were found to display identical AFLP profiles (Dcay13 and Dcay25) with 100% similarity. However, these two accessions might not necessarily represent a single genetic entity due to the dominant nature of AFLP markers. In the study group, the first split separates one accession of *D. abyssinica* from the rest. On the next level five accessions of Guinea yams identified as *D. cayenensis* are separated from the rest, however, several accessions of the same species are found scattered on the succeeding levels.

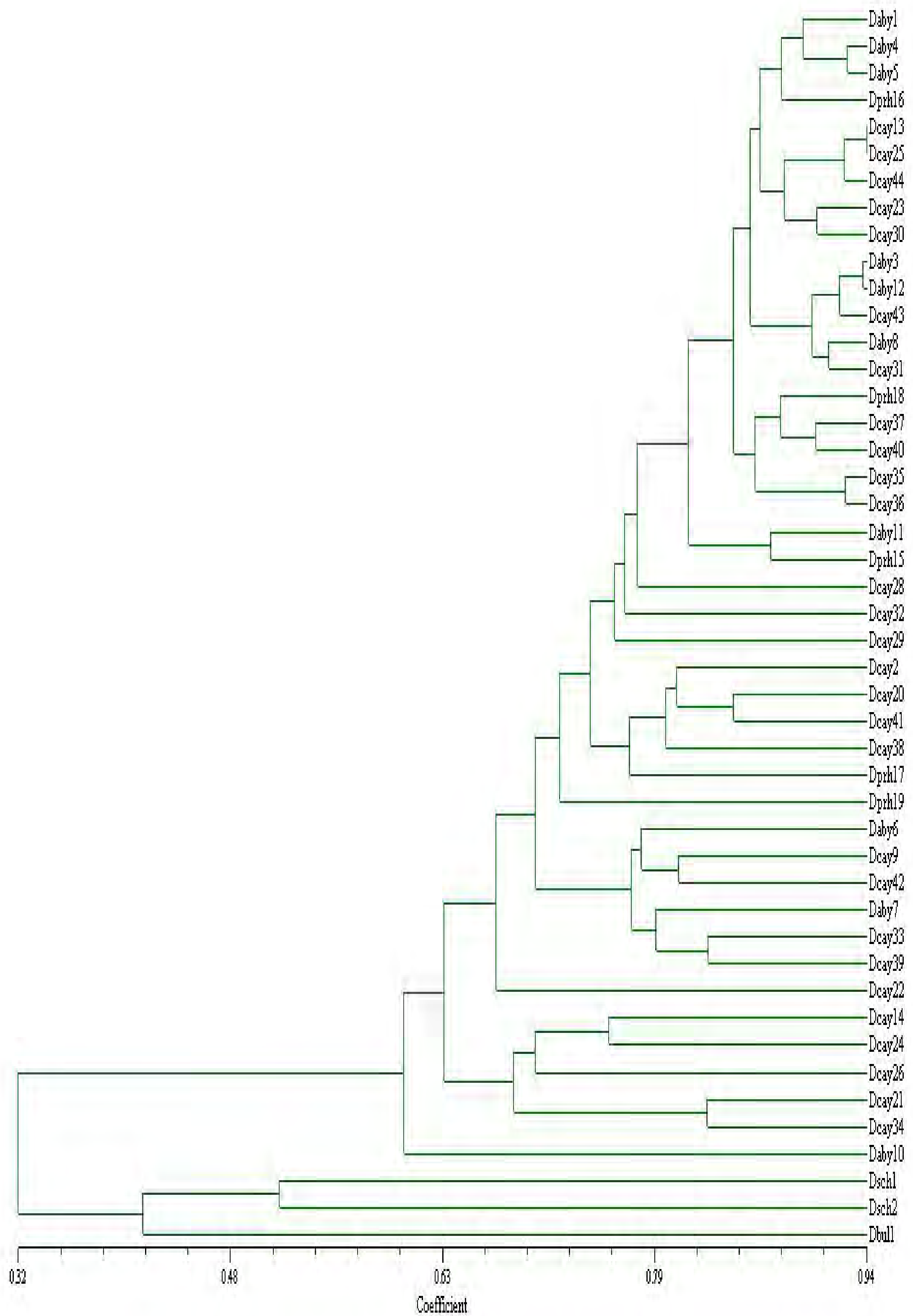


Figure 10. UPGMA cluster derived from a similarity matrix of 48 accessions of the *Dioscorea cayenensis* complex from SW Ethiopia using AFLP markers (Daby, Dsch, Dcay, Dprh and Dbul, refer to *D abyssinica, D. schemperiana, D. cayenensis, D. praehensilis* and *D. bulbifera,* respectively).

Principal Coordinates Analysis (PCO) revealed that the first, second and third principal coordinates axes account for 70%, 4% and 3% of the total variation, respectively. A plot showing the first and second axes (Fig 11.) produced a scatter plot where the three species within the *D. cayenensis* complex again were intermixed. These results are supported by the three dimensional plot of the first three PCO axis which cumulatively account 77 % of the total variation (Fig 12). As in the UPGMA analysis the species identified as *D. schimperiana* and *D. bulbifera* are well separated from the accessions belonging to the *D. cayenensis* complex. Another PCO plot excluding this two species produced a more dispersed plot where no structuring can be elucidated (Fig 13).

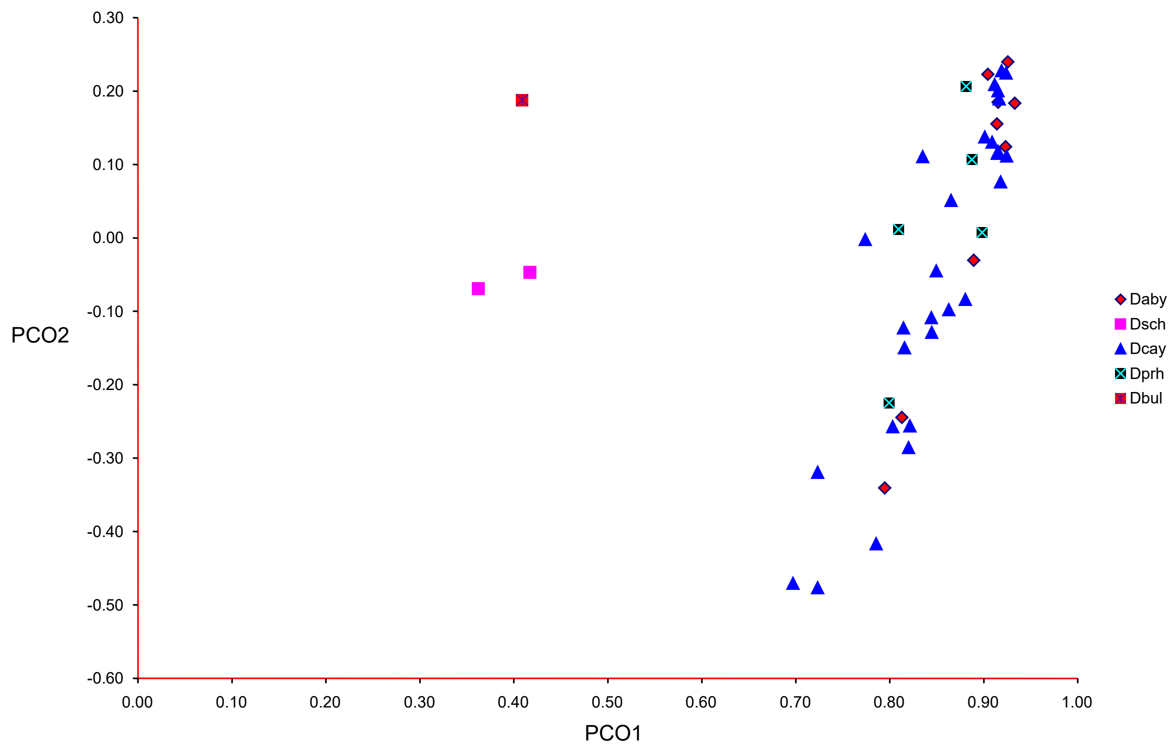


Figure 11. Scatter plot showing the first and second axis of principal coordinate analysis of AFLP data for 46 individuals of *Dioscorea* (Daby, Dsch, Dcay, Dprh and Dbul, refer to *D abyssinica, D. schemperiana, D. cayenensis, D. praehensilis* and *D. bulbifera,* respectively).

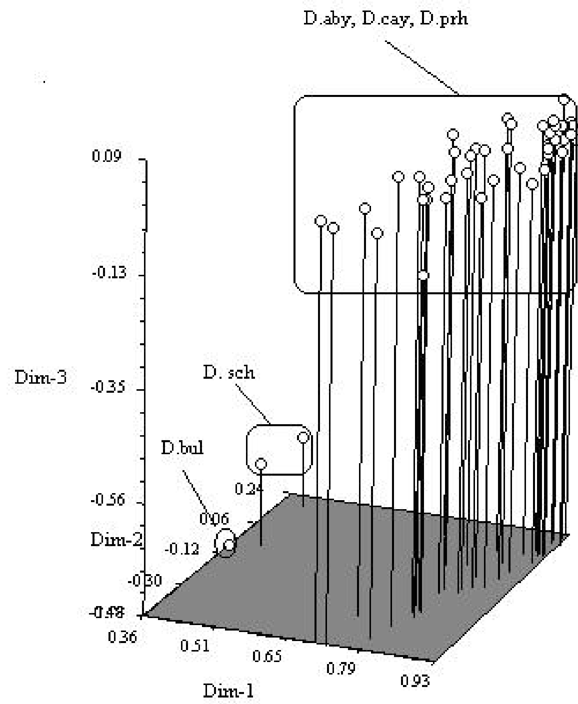


Figure 12. Three principal axes of variation obtained by principal coordinate analysis of AFLP data among 46 individuals of the *Dioscorea cayenensis* complex from SW Ethiopia (Daby, Dsch, Dcay, Dprh and Dbul, refer to *D abyssinica, D. schemperiana, D. cayenensis, D. praehensilis* and *D. bulbifera,* respectively).

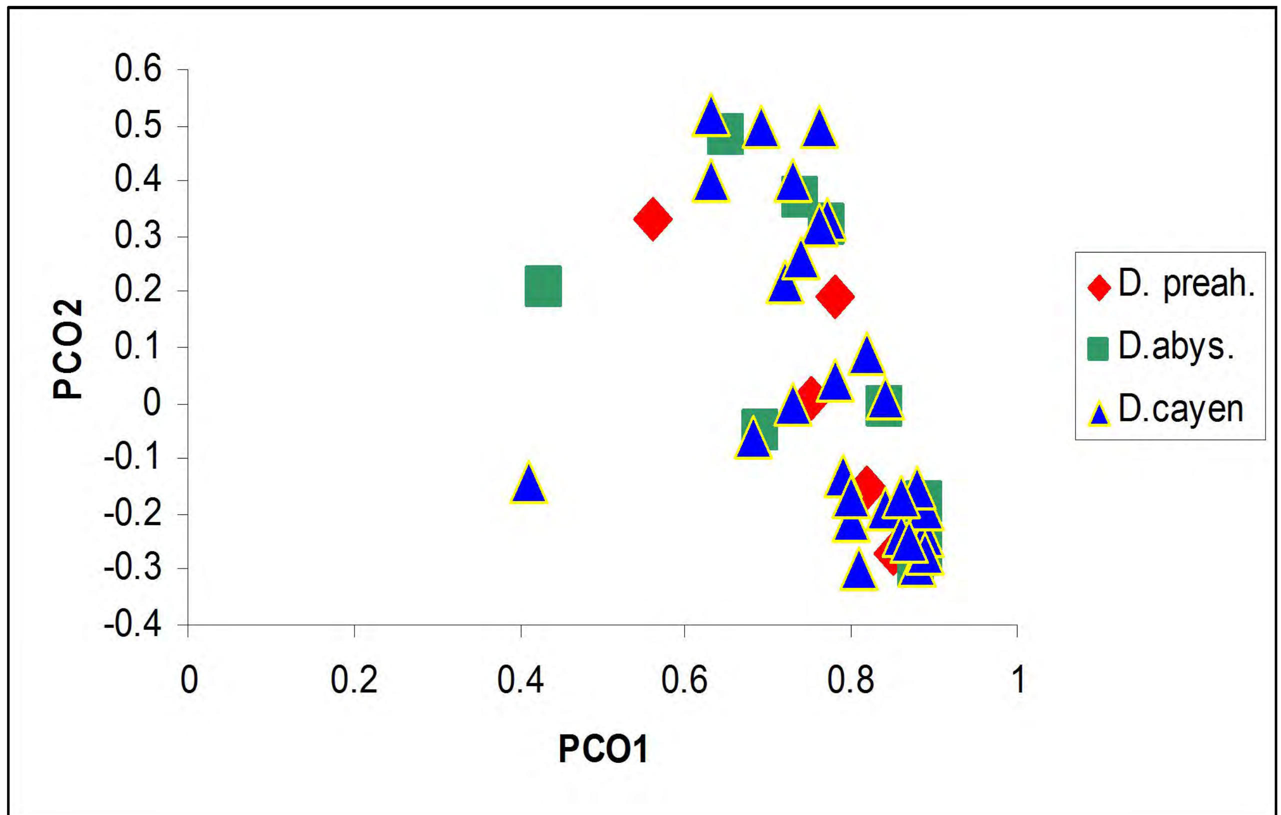


Figure 13. Scatter plot showing the first and second coordinate axis of principal coordinate analysis of AFLP data from 43 individuals of *Dioscorea* excluding *D. bulbifera* and *D. schemperiana* (Daby, Dcay and Dprh refer to *D abyssinica, D. cayenensis,* and *D. praehensilis,* respectively.

As far as their genetic relationships are concerned, no diagnostic fragments were recorded for any of the taxa in the study group. However, some fragments were found to be common to all individuals of some pairs of taxa, while they are absent in some members of the other taxon. For example, in addition to the 5 fragments shared with all memebers of the study population all individuals of *D. abyssinica* and *D. praehensilis* share five more fragments, while both *D. abyssinica* and *D. praehensilis* share only one more each with *D. cayenensis*. This might be associated to the fact that both the former are wild s or at least managed species native to the Sheko region, whereas the cultivated *D. cayenensis* may have been transported or traded there from elsewhere.

Used as estimate of the diversity, the percentage of AFLP fragments (% Ptax) that are polymorphic within each taxon were calculated. The lowest % Ptax was recorded for *D. praehensilis* (59 %) and the highest (86 %) for *D. cayenensis* (Table 9).

Table 9. Percentage of polymorphic fragments, total number of fragments analysed per individual and per population in the three taxa.

Name of the species	% of Polymorphic fragment	Total number of fragments per an individual	Total number of fragments per taxon.
Dioscorea cayenensis	**86**	**39-73**	**106**
Dioscorea abyssinica	**64**	**54-73**	**97**
Dioscorea praehensilis	**59**	**46-74**	**93**

The distribution of rare and frequent fragments in each of the taxa was also compared as an estimate of genetic divergence among individuals within the taxa, as an indirect way of estimating genetic diversity. The results indicated that the rarest and the most frequent fragments for the *D. cayenensis* accessions were found to be at lower frequencies (less than 0.3) compared to *D. abyssinica* and *D. praehensilis.* This indicates that higher genetic divergence was observed among accessions of *D. cayenensis* (Fig. 14). However, the sample size for both *D. abyssinica* and *D. praehensilis* is small. A common consequence of small sample size is that low frequency polymorphism may remain undetected, so increasing the number of individuals sampled per each taxa usually leads to better assessment of the overall genetic structure of the group.

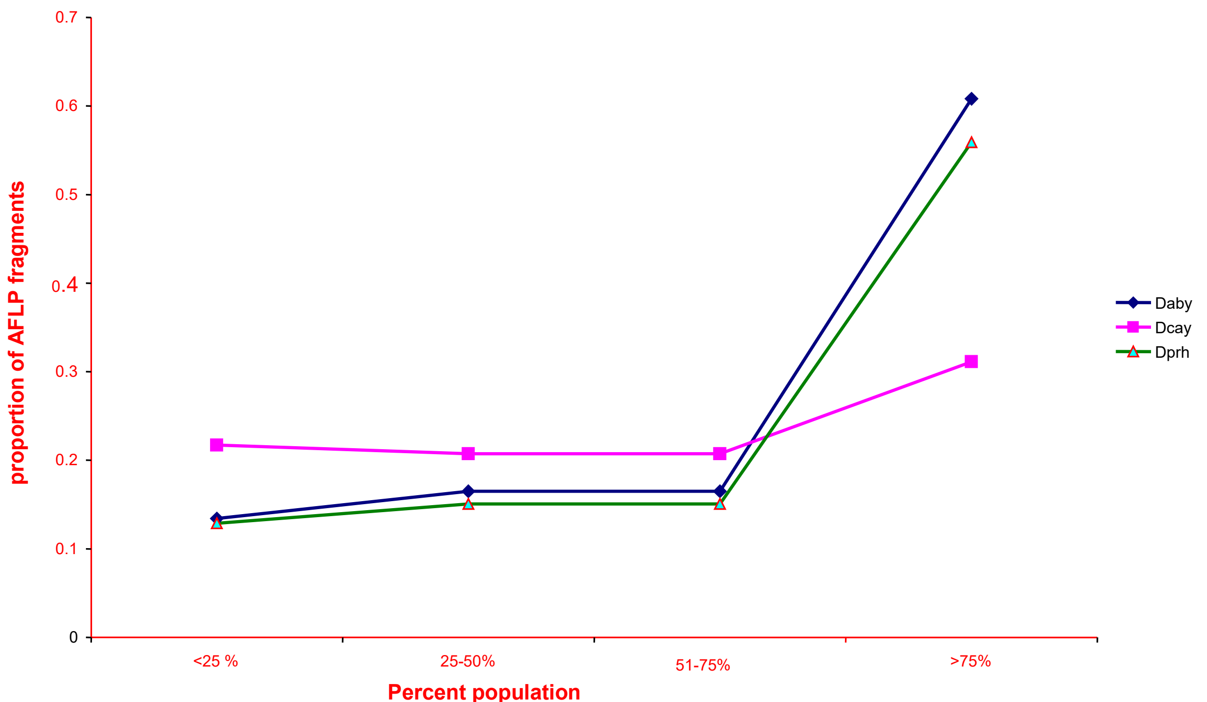


Figure 14. Distribution of rare and frequent AFLP fragments in each of the taxon as an estimate of genetic divergence among 46 individuals of *Dioscorea cayenensis* complex from SW Ethiopia (Daby, Dcay and Dprh refer to *D abyssinica, D. cayenensis* and *D. praehensilis,* respectively).

4. 3. Microsatellite Markers

4.3.1 Genetic diversity and population structure of *Dioscorea* species

4.3.1.1. Allelic variation at microsatellite loci

All the microsatellite (SSRs) used in the study are based on dinucleotide repeats, with allele size ranging from 120 to 329 bp. The smallest difference between the highest and the lowest allele size length was 12 bp at locus Da3Ga4 and the highest difference was found to be 31 bp at locus Da1F08. When the alleles detected at each locus were sorted in ascending order by their size, 62 % of adjacent alleles differed by one dinucleotide repeat unit. However, 6 % of the adjacent alleles were separated by one base pair and 32 % by more than two base pairs.

A total of 60 different alleles were recorded for the 7 loci studied, with the mean number of alleles per locus equalling 9.2 (Table 11). Out of the 60 different alleles 27 were found to be private alleles present only in one of the three population samples (16 alleles for Sh, 9 for Or and 2 for Sn populations). The frequency of such private alleles ranges from 0.01 to 0.048. On the other hand the allelic frequency in the study population was found to be between 0.009 (at locus Da1F08) and 0.93 (at locus Da3G04).

At all the loci, a higher number of alleles were detected in the Sh population (44 alleles) followed by Or (37 alleles) and Sn populations (31) (Table 10). Altogether 18 alleles were found to be shared among the three populations in the study group. When the shared number of alleles are compared, the Sh population shared 22 alleles with Or and 23 alleles with the Sn population, whereas the Or and Sn population shared 18 alleles.

Table 10. The observed number of alleles per locus and population

Population	Dba2D06	Da3G04	Da1F08	Dpr3D06	Da1A01	Dab2E07	Dab2C05	total
or	5	1	8	5	3	6	9	37
sh	6	5	7	3	6	8	9	44
sn	4	1	6	5	5	3	7	31
Total	7	5	11	7	8	9	13	60

Six of the seven loci studied (except Da3G04) were polymorphic across all the three populations sampled, with the number of alleles per locus ranging from 5 (at Da3G04) to

13 (at Dab2C05). At most of the SSR loci, alleles were detected at lower frequencies. Thus, 60 % of the alleles in all the loci were found to be at frequencies less than 0.1. The distribution of observed allele size (number of bp) at each locus and among the three populations were irregular except for loci Da3G04 and Da1F08, which showed unimodality (Fig 15).

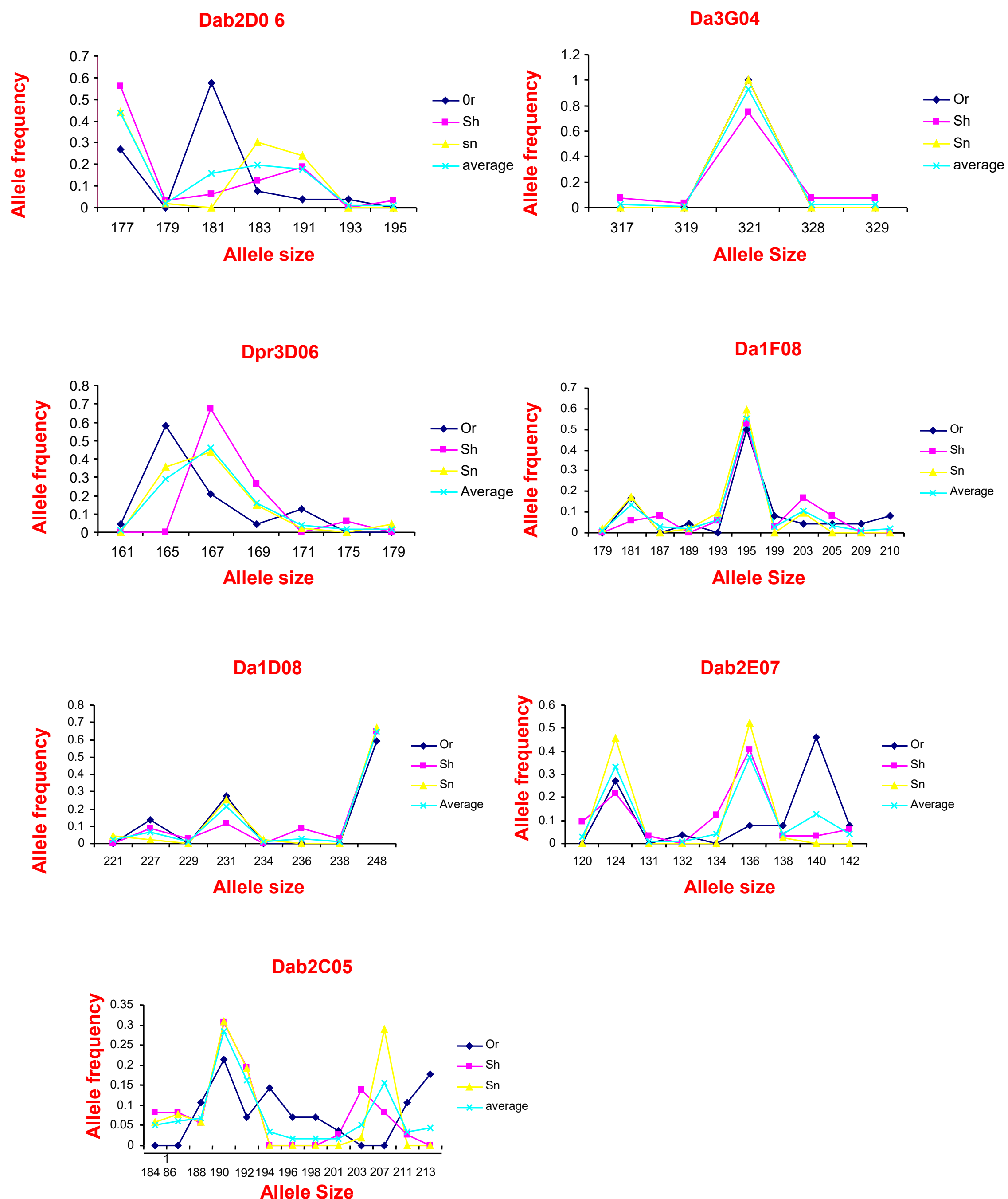


Figure 15. The distribution of observed allele size (number of bp) at each locus and among the three populations

The Sh population displayed the highest level of allelic diversity in 5 of the 7 loci studied, compared to Or (2 loci) and Sn population (none). However, for one locus (Dpr3D06) the Sh population showed the lowest level of allelic diversity compared to both Or and Sn populations (Fig 16). Pooling together all the 7 loci the Sh population displayed the highest level of allelic diversity followed by the Or populations.

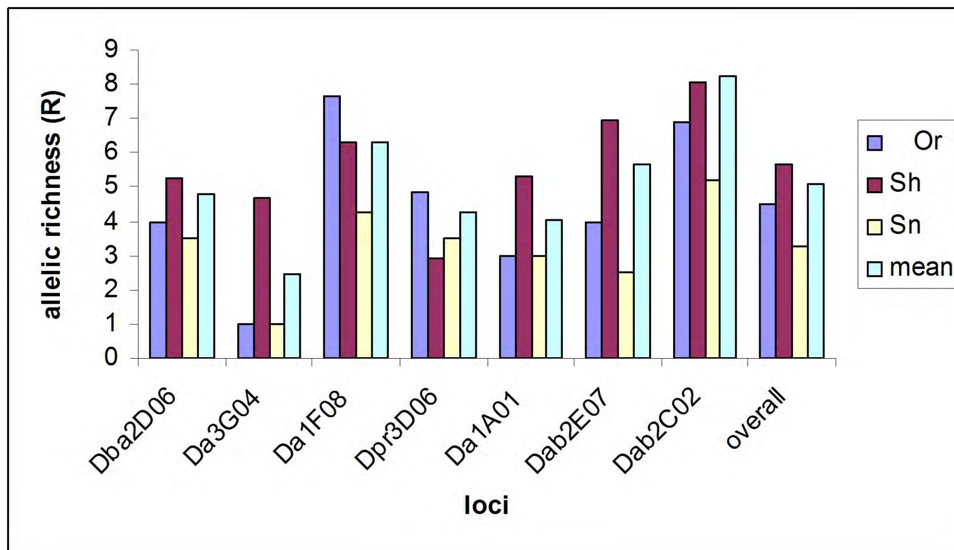


Figure 16. Allelic diversity per locus for each population based on the measure of allelic richness (R).

4.3.1.2 Genetic variation within population

Genetic diversity parameters based on allelic frequencies are shown in Table 11. In individual populations the mean number of alleles per locus (A) varied from 4.43 (Sn population) to 6.29 (Sh population) with an average of 6.09. While effective number of alleles per locus (A_e) varied from 5.86 (Sn population) to 6.45 (Sh population) with an average of 6.09. Allelic richness (R) which is a measure of allelic diversity taking into account sample size, ranges from 3.28 (Sn population) to 5.65 (Sh population) with an average of 4.47. The observed heterozygosity (Ho) ranged from 0.457 to 0.507 with an average of 0.481. The average expected heterozgosity (H_e) equalled 0.590 and varied from 0.539 to 0.636. All the above results indicated that the Sh population displayed greater allelic or genetic diversity compared to both the Or and the Sn populations. When pooling together all the 7 loci, the mean number of alleles per locus, effective number of alleles per

locus, allelic richness, and expected and observed heterozygosity for the meta population were found to be 9.20, 8.57, 5.1, 0.49 and 0.64, respectively.

The percentage of polymorphic loci (P) in the three populations studied ranges from 85.7% to 100% corresponding to a mean polymorphism of 90.47%. The Sh population displayed the highest level of polymorphism (100%), while both the Sn and the Or population possessed one monomorphic locus (Da3Ga4) with an overall allelic polymorphism of 85.7%. One of the loci (Da3Ga4) was found to be fixed for the Or and Sn populations.

Table 11. Allelic variability at the seven SSR loci in the study populations (N=population size, A= mean number of alleles per locus, A_e=effective number of alleles per locus, R=allelic richness, P= percentage polymorphic loci and H_o, H_e , average observed and expected heterozygosity respectively)

Pop	N	A	A_e	R	P	H_o	H_e
Or	14	5.29	5.96	4.49	85.71	0.457	0.595
Sh	18	6.29	6.45	5.65	100	0.480	0.636
Sn	26	4.43	5.86	3.28	85.71	0.507	0.539
Mean	**19.3**	**5.34**	**6.09**	**4.472**	**90.47**	**0.481**	**0.590**
Meta pop	**58**	**8.57**	**9.20**	**5.1**	**90.47**	**0.490**	**0.640**

Comparison of the genetic within population diversity of the three populations based on the average expected heterozgosity indicated the highest level of diversity for the Sh population (He = 0.636), while the lowest value corresponds to the Sn population (He = 0.539). In relation to the other populations the Sh population was collected from localities in close geographical vicinity.

4.3.1.3 Population genetic structure

The genetic analyses revealed moderate differentiation among the three populations (Or, Sn, Sh). The F_{ST} value which reflect the proportion of the observed genetic variation that can be explained by partitioning among populations, ranged from 0.005 for locus DA1F08 to 0.16 for locus Dpr3DO6, with an average value of 0.088. This showed that only 8.8% of the genetic diversity is found among the three populations, indicating a moderate

differentiation among them. In other words the heterozygosity in the entire population of the *Dioscorea cayenensis* complex sampled in South and Southwest Ethiopia decreased by ca 8.8% as a result of the partition among the three populations. Although, the F_{ST} value recorded for these populations was found to be small, it is highly significant ($P = 0.0001$).

Wright's F- statistic for each locus is summarized in Table 12. Altogether 5 of the 7 loci showed a statistically significant F_{ST} values ranging from 0.035 to 0.15 ($P \leq 0.004$). Two of the loci (Dab2D06 and Dpr3D06) possessed the highest F_{ST} value (0.15 and 0.16, respectively). Thus the level of heterozygosity in the entire population of the study group was found to be lower than we would expect (if the whole population is panmictic) by 15% (Dab2D06) and 16% (Dpr3D06) due to the partitioning between the three populations. These values are highly significant ($P \leq 0.0001$) and with respect to these two loci the three populations are highly differentiated. Loci Da1A01 and DA1F08 exhibited the lowest F_{ST} values (0.001 and 0.005, respectively) thus, contributing small share (0.1 and 0.5%, respectively) in reducing of the level of heterozygosity in the entire population as a result of subdivision. Accordingly, with respect to these loci the there is no differentiation among the three populations.

Pairwise comparison of genetic differentiation among the three populations indicated that the Sh and the Sn populations are genetically closest with an F_{ST} of only 4%, whereas Or population differ from both (Sh and Sn) with F_{ST} values of 13 and 12%, respectively. All the three pairwise comparisons are highly significant ($P \leq 0.0003$).

Table 12. Relative measurements of genetic differentiation among populations in the study group

Locus	global F_{ST}	P-value:	global F_{IT}	global F_{IS}
Dab2D06	0.151	0.0001	0.103	-0.056
Da3Ga4	0.120	0.0001	0.859	0.840
Da1F08	0.005	0.233	-0.022	-0.027
Dpr3D06	0.164	0.0001	0.242	0.092
Da1A01	0.001	0.2757	-0.070	-0.071
Dab2E07	0.139	0.0001	0.570	0.500
Dab2C05	0.036	0.0035	0.326	0.300
Over all loci	0.088	0.0001	0.235	0.161

4.3.1.4. Genotypic structure and deviation from Hardy-Weinberg equilibrium

Global tests for the departure from Hardy-Weinberg equilibrium showed a statistically significant deviation in the study populations ($P \leq 0.0001$). The departure from Hardy-Weinberg equilibrium was primarily due to heterozygote deficit. F_{IT} is the over all inbreeding coefficient of an individual relative to the whole set of populations, while F_{IS} is the inbreeding coefficient relative to its own population. The global F_{IT} value 0.235 indicated that overall, there is a heterozygote deficit in the study populations. Among the study population, 3 of the 7 loci showed a significant deficit in heterozygotes (DA3G04, Dab2E07 and Dab2C05, $P \leq 0.0003$) and 3 loci showed excess of the heterozygotes (negative F_{IS} value) relative to Hardy-Weinberg expectation (Tables 12 and 13), with the average F_{IS} value equalling 0.16. None of the 7 loci showed a significant excess of heterozygotes.

Results from multi locus tests for deviation from Hardy-Weinberg equilibrium expectations showed that the Sh and Or populations exhibit a significant deficit of heterozygotes (F_{IS} = 0.22 and 0.17 respectively, $P \leq 0.0001$). However, for the Sn population the results of the test for heterozygote deficit was not found to be significant (F_{IS} = 0.007 P = 0.49). Only 0.7% of the Sn population deviates from Hardy-Weinberg expectations compared to the 17% in Or and 22% in Sh.

Table 13. Expected and observed heterozygosity (H_e and H_o) and Fixation indexes (F_{IS}) per locus and population at the seven microsatellite loci.

Locus/Pop.	Or	Sh	Sn	*Mean/ F_{IS} global*
Dab2D06				
H_o (H_e)	**0.46 (0.60)**	**0.69 (0.64)**	**0.8 (0.67)**	***0.65 (0.64)***
F_{IS}	**0.29**	**-0.065**	**-0.24**	***-0.056***
Da3Ga4				
H_o (H_e)	**0 (0)**	**0. 07 (0.45)**	**0 (0)**	***0.02 (0.15)***
F_{IS}	**NA**	**0.84**	**NA**	***0.84***
Da1F08				
H_o (H_e)	**0.83 (0.73)**	**0.72 (0.69)**	**0.58 (0.61)**	***0.71 (0.67)***
F_{IS}	**-0.15**	**-0.07**	**-0.002**	***-0.027***
Dpr3D06				
H_o (H_e)	**0.5 (0.62)**	**0.3 (0.48)**	**0.75 (0.67)**	***0.51 (0.59)***
F_{IS}	**0.21**	**0.39**	**-0.26**	***0.092***
Da1A01				
H_o (H_e)	**0.45(0.58)**	**0.65 (0.57)**	**0.58 (0.50)**	***0. 56 (0.55)***
F_{IS}	**0.23**	**-0.17**	**-0.24**	***-0.071***
Dab2E07				
H_o (H_e)	**0.30 (0.72)**	**0.56 (0.78)**	**0.18 (0.53)**	***0.35 (0.67)***
F_{IS}	**0.42**	**0.29**	**0.65**	***0.5***
Dab2C05				
H_o (H_e)	**0.64 (0.89)**	**0.44 (0.85)**	**0.65 (0.79)**	***0.58 (0.84)***
F_{IS}	**0.14**	**0.45**	**0.15**	***0.3***
Mean				
H_o (H_e)	***0.46 (0.60)***	***0.49 (0.64)***	***0.51 (0.54)***	***0.48 (0.59)***
F_{IS}	***0.17***	***0.22***	***0.007***	***0.161***

4.3.1.5. Genetic relationships among the three subpopulations

Genetic relationships among the populations were assessed using distance trees inferred from allelic frequency data. The distance matrix based on proportion of shared alleles (Dps) was used to generate a neighbor joining phenogram (Fig 17). The results indicated that there is no clear partitioning among the three subpopulations. However, in some of the clusters individuals collected from same locality tend to cluster together. For example the individuals or00 8, or009, or011, or012 and or013 and the cluster containing sn002, sn020, sn009, sn001, sn018 and sn006 are collected from geographically close areas, the former along the Nedjo Ghimbi road and the latter from the Areka area.

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Figure 17. Neighbour joining (NJ) tree inferred from allelic frequency data of microsatellite data for 59 individuals of *Dioscorea* species (Sn, sh and or refer to the three populations defined based on their geographical areas) (N.. B. Or003=sn003)

4.3.2. Genetic relationships of the wild and cultivated Guinea yam species

For most of the seven loci examined a higher number of alleles were detected in the cultivated accessions compared to the wild forms. Considering the entire set of germplasm under study, 17 alleles were present only in the cultivated accessions and 8 alleles were found to be unique to the wild forms. Altogether, 36 alleles were shared between the cultivated and wild accessions. However, based on the measure of allelic richness (R), the wild forms exhibited a greater diversity in all the 7 loci studied (Fig. 18)

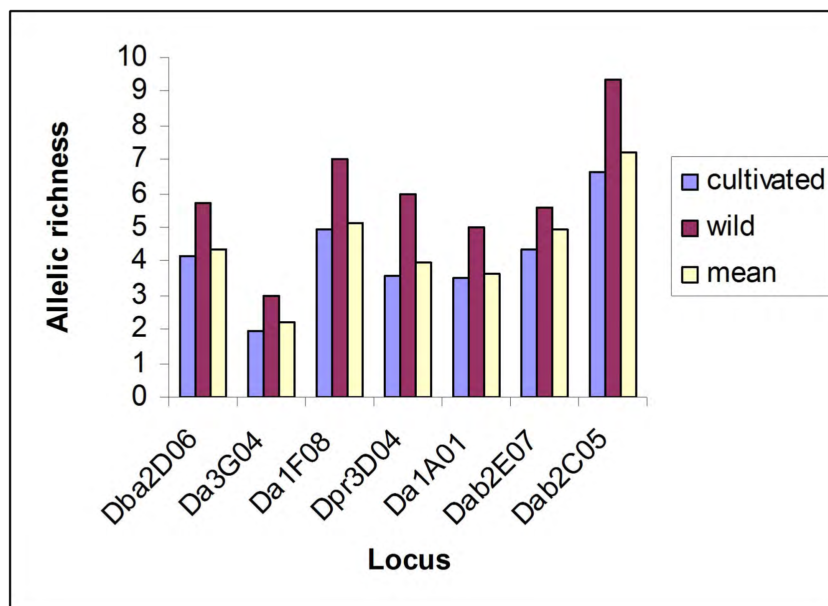

Figure 18. A comparison of allelic diversity per locus in wild and cultivated accessions of SW Ethiopian the *D. cayenensis* complex based on the measure of allelic richness

Using expected heterozygosity as a measure of genetic diversity, the wild accessions of *Dioscorea cayenensis* complex displayed a greater diversity (H_e = 0.79) compared to the cultivated forms (H_e = 0.60). The same result was obtained when comparison was made at a locus level. Thus, in all the 7 loci studied the wild forms displayed greater gene diversity (Table 14).

Table 14. Gene diversity (expected level of heterozygosity) per locus and population of wild and cultivated accessions.

Growth habit	Loci							
	Dba2D06	Da3G04	Da1F08	Dpr3D06	Da1A01	Dab2E07	Dab2C05	mean
Cultivated	0.72	0.14	0.63	0.64	0.52	0.72	0.84	0.60
Wild	0.82	0.41	0.85	0.86	0.73	0.86	0.97	0.79
mean	0.77	0.28	0.74	0.75	0.63	0.79	0.91	0.69

The F_{ST} value which reflects, the proportion of the observed genetic variation that can be explained by partitioning between populations was found to be low (F_{ST} = 0.03) but significant (P = 0.006) demonstrating little differentiation between the wild and cultivated species sampled in the study sites.

4.3.3. Taxonomic relationships among the three taxa inferred from microsatellite data.

The UPGMA tree shows no patterns of clustering of the accessions into groups that could be interpreted in terms of the existing species concepts (Fig 19.). Instead the distance tree grouped the accessions broadly according to their geographical region in which they were collected. The average genetic similarity among the Guinea yam accessions and their wild relatives used in this study ranged from 77% to 100%. The first split of UPGMA tree separated one wild accession (DprhsH71) from the rest. The second and third split contained two accessions each identified as different species. Although, they are different species the accessions in each phenon belong to the same population (the first to the Sh and the second to the Or populations). Similarly, the succeeding levels group the accessions based on mainly their geographical location rather than taxonomic identity Altogether ten duplication groups with identical allelic profiles in all the loci studied were obtained from the cluster analysis (Fig 19.). Most of the duplicates observed include accessions collected from the same geographical area. In one of the duplicates two accessions identified as different species were found to exhibit the same profile (Dcaysh58 and Dprhsh33). They were found to be cultivated accessions collected from the same farm in the Sheko locality. The farmer who owns them identified the accessions as two different cultivars.

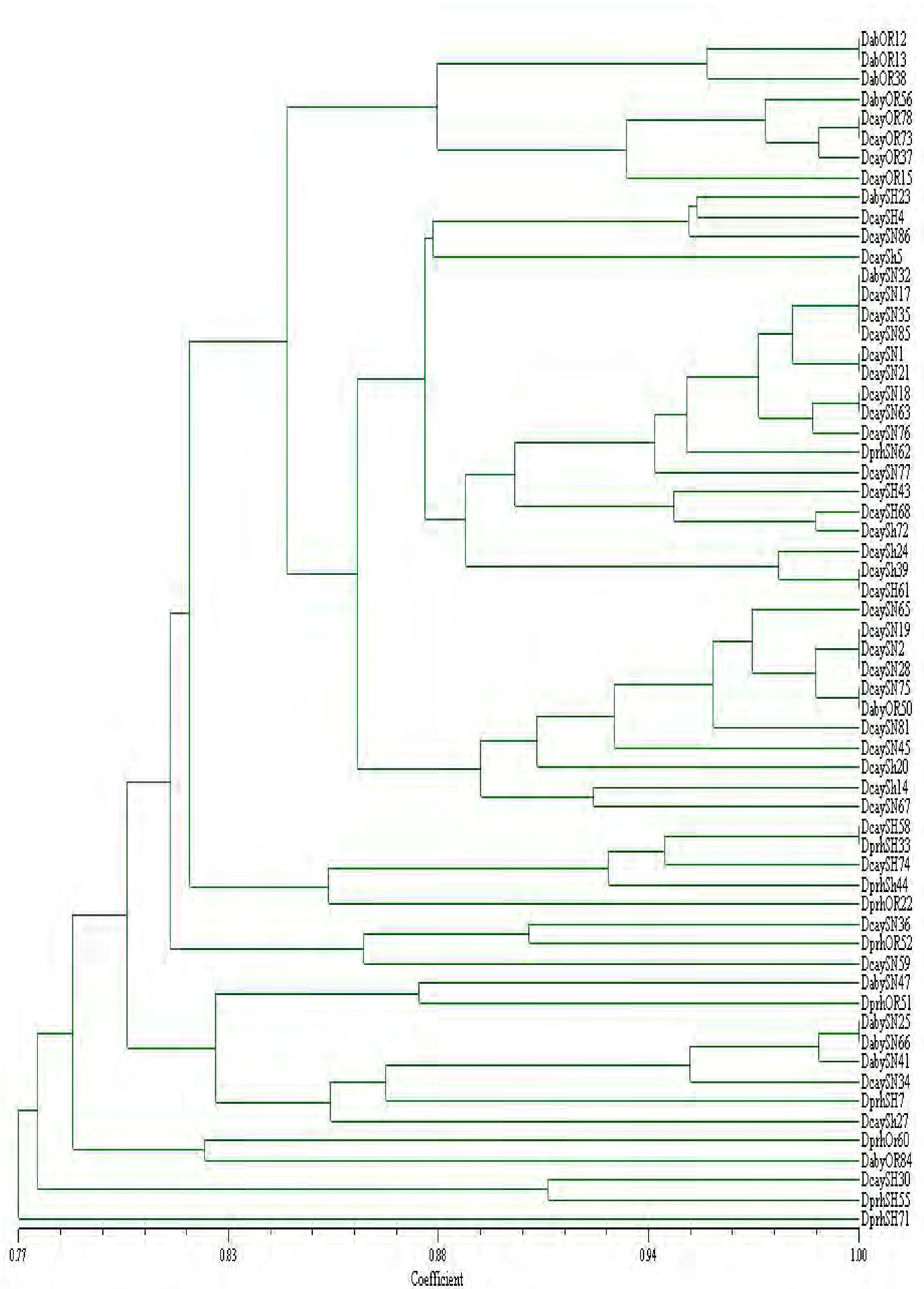

Figure 19. A dendrogram derived from a similarity matrix of 61 accessions of *Dioscorea* using microsatellite markers [labels indicate species name and geographical location or the population where that particular accession belongs, for example DabyOR refers to *D. abyssinica* belonging to Or populations (Note: DabyOR50=DabySH50)].

Associations among the 59 *Dioscorea cayenensis* complex accessions revealed by principal coordinate analysis (PCO) calculated from the microsatellite based similarity matrix are presented in Figure 18. The first principal coordinate axis (PCO1) and the second axis (PCO2) accounted 83.8% and 2.8% of the total variation, respectively. Neither of the two axes separated the three taxa into distinct groups (Fig. 20).

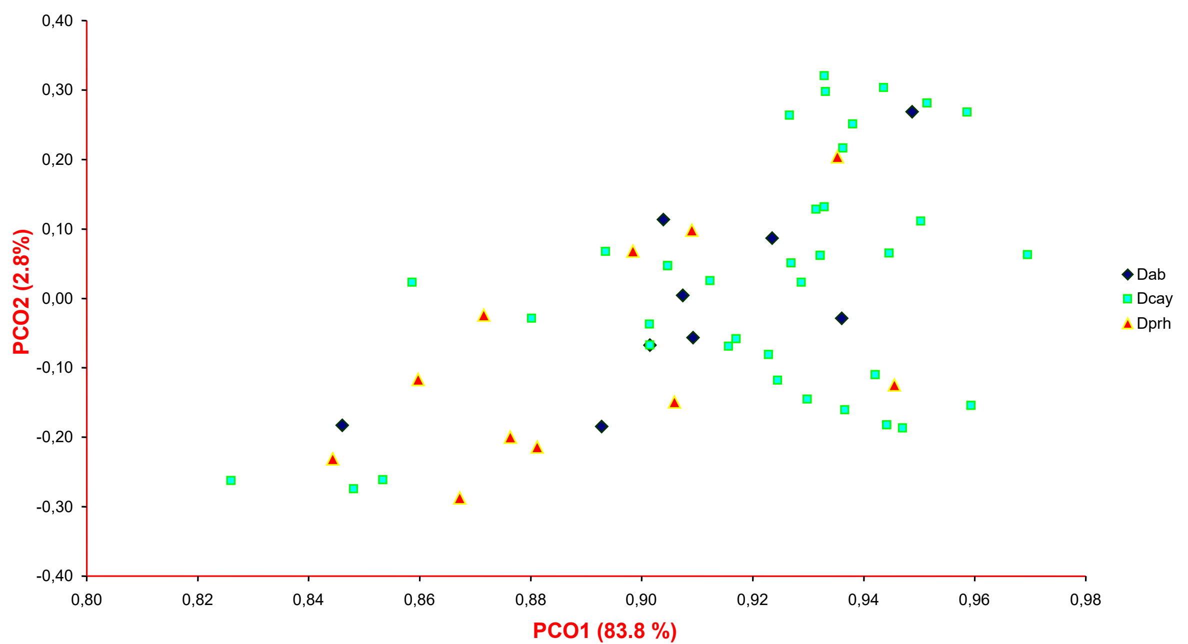

Figure 20. Scatter plot showing the first and second axis of Principal Coordinate Analysis of microsatellite data for 61 individuals of the *D. cayenensis* complex in the study population (Dab, Dcay and Dprh refer to *D. abyssinica, D. cayenensis* and *D. praehensilis*, respectively).

5. Discussion

5.1 Taxonomic relationships in the study group: one species, different species or a species complex?

Relatively few studies have been conducted to understand the taxonomic relationships among the various species of yams (Hamon and Toure 1990b; Terauchi *et al.* 1992, Mignouna and Dansi, 2003; Mignouna *et al.* 2005; Scarcelli *et al.* 2006a). Different authors consider Guinea yams (and their wild relatives) to be represented either by one or two species or even by a species complex (Ramser *et al.* 1997). In the present study the phenogram and PCO/PCA scatter plot based on morphological, AFLP and microsatellite markers failed to produce a clear partitioning of the individuals into discrete taxa that could be interpreted according to the existing classification system. Other clear taxonomic structures were neither revealed.

Our results stand in agreement with Martine and Rhodes (1978), Terauchi *et al.* (1992), and Hamon *et al.* (1997), who all emphasized the lack of genetic structure within the different species within *D. cayenensis* complex. However, our results are in contrast with Akoroda and Chheda (1983), Ramser *et al.* (1997), Dansi *et al.* (2000), Mignouna *et al.* (2005) and Scarcelli *et al.* (2006a) who claimed that the different species of Guinea yams represent different genetic entities. All the mentioned publications are based on West African materials. These studies argued that isozyme and chloroplast DNA analyses by Terauchi *et al.* (1992) and Hamon *et al.* (1997) did not show significant differentiation between the putative species, probably because of the low levels of polymorphism these markers exhibit.

Ramser *et al.* (1997), Mignouna *et al.* (2005) and Scarcelli *et al.* (2006a) used PCR based methods such as RAPD and AFLP and proposed that the species of Guinea yams should be treated as separate taxa. The discrepancy between our results and that of Ramser *et al.* (1997); Mignouna *et al.* 2005; Scarcelli *et al.* (2006a) could be viewed in terms of sample size used or validity of sampling, the difficulty in determining taxonomic status based on morphology, differences in the methodology employed and handling of the data during the analyses. Ramser *et al.* (1997) and Mignouna *et al.* (2005) basically used RAPD markers which solely depend on comigrating bands for estimating relatedness among taxa. The

general validity of this approach has been challenged, because certain percentage of comigrating bands may in fact be non-homologous, producing a random background noise that can influence the result (Allendorf and Luikart, 2007). The algorithm used to calculate genetic distance based on RAPD markers may vastly over or under estimate true similarity. Thus the assumption of 100% similarity at a locus or allele where a band is shared between two accessions is not necessarily valid. The problems are even worse in a polyploid genome, where the marker may be present in any of several dosage states. Thus, a dominant marker present at a single, double, triple or quadruple dose in one accession and in the same possible range of dosage state in another accession will result in similarity estimates of 100 % where as the actual similarity estimated could be as little as 25 % (Mignouna *et al.,* 2003). Further more, Mignouna *et al.* (2005) used accessions with 12 duplication groups with identical RAPD profiles (comprising more than 50 % of the individuals in the study group). This might indicate that most of the accessions used in the study represent clones with identical within population genetic background.

Ramser *et al.* (1997) employed a small sample size representing a wide range of geographical area, with all the plants grown at ITTA. Since cultivated *Dioscorea* species are propagated vegetatively, each of the individuals collected from wide range of geographical area are expected to show different pattern of heterozygosity and hence appear as genetically distinct groups. According to Ramser *et al.,* (1997), in view of the 500-2500 agricultural varieties that probably exist, a conclusive definition of the taxonomical status of Guinea yams would be difficult without analysing the majority of these varieties.

Scarecelli *et al.* (2006) employed a different approach to study the taxonomic status of Guinea yams and their wild relatives. Thus, they grouped the different plants under cultivation into two classes: predomesticates and cultivated varieties. Those considered as predomesticates were accessions which were under cultivation, but in the course of domestication. In the cluster and PCO analysis, this group was found to cluster with any of the three species identified as *D. cayenensis-D. rotundata*, *D abyssinica*, and *D. praehensilis*. In this study individual plants showing intermediate characteristics and accessions that did not fit into their morphological identification were also discarded from the analyses. Such an approach would clearly bias the study towards detecting discrete taxa. Furthermore, the authors scored 91 AFLP markers from 4 primer combinations (in contrast

to 158 markers from 3 primer combinations in our analysis). Some of the discrepancies observed could also be attributed to the difficulties in determining the taxonomic status of the germplasm on the basis of morphology. There are some reports which indicate that individuals with identical morphotype might display different genotypes (Mignouna and Dansi, 2003; Mignouna *et al.*, 2003). In our study we encountered the converse, i.e. two individuals (Dcaysh58 and Dpr3sh53) with different morphotypes displayed identical microsatellite allelic profiles.

Based on the results from morphometric and molecular markers (AFLP and microsatellite) analyses, the variation in the study group was found to be continuous. Therefore, at least the wild or managed populations and cultivated plants of South and Southwestern Ethiopia, the *D. cayenensis* complex is a single taxonomic entity. In a book summarizing the studies on West African Guinea yams, Dumont *et al.,* (2006) reached a similar conclusion and proposed that *D. praehensilis, D abyssinica* and *D rotundata-D.cayenensis* should be regarded as a single taxonomic entity. There are no discrete morphological characters to separate the "species" in the study group into distinct taxa at specific or subspecific rank. If regarded as a single species or species complex the correct name will have to be *D. cayenensis* (Lamark, 1789). Morphological, AFLP and microsatellite markers used in this study indicated no consistent genetic differentiation as far as three taxa are concerned. Rather a largely geographically structured clustering pattern was observed, when using the microsatellite markers. A comparative study on Ethiopian materials and a few West African accessions by Muluneh Tamiru (2006), based on AFLP revealed a similar result. Thus, the Ethiopian accessions were found to be genetically close to each other than they were to their putative conspecific West African population. However, they showed some degree of distinctiveness compared to the West African accessions (Muluneh Tamiru 2006). The author suggested that the distinctiveness of the Ethiopian Guinea yams may represent a divergent evolutionary path way isolated from the widely known centre of diversity in West Africa.

5.2. Total genetic diversity and level of polymorphism.

Both the molecular markers (AFLP and microsatellites) used in this study detected a high degree of intraspecific variation and a low degree of interspecific variation. Comparable results have been reported in most of the studies on Guinea yams of West Africa (e.g.

Ramser *et al.* 1997; Scarecelli *et al.,* 2006). A higher level of polymorphism was obtained for both AFLP (78 %) and microsatellite (90.47 %) markers. The level of polymorphism detected by AFLP in this study was found to be less than the reports from previous studies on Giunea yams from West Africa by Mignouna *et al.* (2003) (90.2 %) and Scarcelii *et al.* (2006) (94.9 %) using the same marker. In both studies however, 4 pairs of primer combinations were employed compared to 3 pairs of primer combinations, used in this study. Furthermore, in our study, AFLP analyses were carried out using accessions collected from the Sheko area only. On the other hand, the level of polymorphism determined by microsatellite markers (90.47 %) was found to be greater than in previous reports, 80.5 % was reported by Mignouna *et al.* (2003) which were based on analyses of 9 loci.

AFLP analyses revealed a high degree of similarities among the *D. cayenensis* complex accessions from Sheko area (coefficients of similarity ranging from 0.6 to 0.94). This indicates that the individuals included in this study are genetically closely related. The values of the coefficient of similarity from AFLP analyses are comparable with those obtained from West African accessions of Guinea yams by Mignouna *et al.,* (2005). Compared to the wild forms, the AFLP markers used also revealed a high genetic divergence between the accessions in the cultivated forms. This might indicate that the farmers in the study area (Sheko and its environs) cultivate different cultivars of yams with a broad genetic basis. Similar results were also reported by Mignouna *et al.,* (2005) for *D. rotundata* accessions from Nigeria. The author associated the observed high diversity in the cultivated forms with the availability of the wild yams with cropping potential, different selection pressures, successive domestication and somatic mutations. Although, high genetic divergence was obtained between the accessions of cultivated Guinea yams, comparison of wild yams and cultivated Guinea yams using SSR markers based on the measure of allelic richness indicated that the wild yams exhibited the greatest allelic diversity. Wild yams are, therefore, important for yam breeding because they could act as reservoirs of useful genes for agronomic characteristics such as yield, storability, tolerance to drought and weeds, organoleptic qualities, and tolerance to pest and diseases.

The total number of alleles amplified for the 7 microsatellite markers was found to be 60, with an average of 8.6 alleles per locus. A similar study by Tostain *et al.,* (2005) on 156 accessions of *Dioscorea* species from Benin using 17 SSR markers revealed a total of 124

alleles with an average of 7.3 alleles per locus. In our study the observed heterozygosity values were found to vary from 0.15 to 0.67 with an average of 0.48. A similar study by Tostain *et al.* (2005) reported an average observed heterozgosity value of 0.58 (0.0 to 0.94). The average expected heterozgosity (H_e) within a population is the best general measure of genetic variation. The expected heterozygosity for the accessions within *D. cayenensis* complex used in this study varied between 0.018 (for Da3Ga4) and 0.86 (Dab2C05) with an overall value of 0.64 for the metapopulation. This indicates that there is considerable genetic diversity in the wild and cultivated accessions of *Dioscorea* species from Ethiopia. Although, the species in the study group have long been considered as polyploidys, the genotyping data observed produced only one or two alleles per sample for each of the loci studied. This might indicate that all the accessions in the study group are actually diploids. Such results have also been reported by Scarcelli *et al.* (2006) form studies on West African accessions of Guinea yams and their wild relatives.

5.3 Level of heterozygosity

Out crossing plants with dioecious floral morphology are expected to have a high level of genetic heterozygosity within populations (Avise, 1994). In principle the *Dioscorea* species evaluated in this study fit into this group of plants but in contrast to the theory, the observed number of heterozygotes (H_o) was less than the expected (H_e) in 11 of the 21 locus specific comparisons. The F_{IS} values for these locus specific comparisons were found to be greater than zero, and according to Gibbs *et al.* (1997) this could be associated with non-random association of alleles in the three populations tested (Gibbs *et al.,* 1997).

Studies on *Dioscorea* species form Benin by Tostain *et al.* (2005), revealed a significant excess of heterozygotes in 9 of the 15 polymorphic loci studied. In our study, however, none of the seven loci showed a significant excess of heterozygotes. The levels of heterozgosity found in the study group were, in most cases lower than expected. The deficit of heterozygotes relative to Hardy-Weinberg proportions for microsatellite markers could be explained by a) presence of an unrecognized genetic structure within populations (Wahulund effect) b) inbreeding, that is the tendency for related individuals to mate. c) Presence of null allele such that many apparent homozygotes are, in reality heterozygotes

The most general cause an excess of homozygotes is non-random mating or population subdivision. The presence of multiple demes within a single population sample will produce an excess of homozygotes at all loci for which the demes differ in allelic frequency. Inbreeding within a single deme will produce a similar genotypic effect. However, large proportion of the study individuals are cultivated accessions of Guinea yams, which are propagated vegetatively.

The best way to discriminate between non-random mating (either inbreeding or including multiple populations in a single sample) and null alleles to explain an excess of homozygotes is to examine if the effect appears to be locus-specific or population-specific. All loci that differ in allele frequency between demes will have a tendency to show an excess of homozygotes (Allendorf and Luikart, 2007). In our study out of the 21 (7 loci x 3 subpopulations) possible tests, heterozygote deficiency was detected in 11 of the tests (5 positive F_{IS} value out of the 7 possible for the Or population, 4 for the Sh population and 2 for the Sn population). It seems that there is an unrecognized population structure in both the Or and the Sh populations, i.e. both populations might include more than one deme. This may also be demonstrated by the observed allelic frequencies in all loci within and among populations. Fine scale differentiation within and among the study populations could be reflected in a substantial difference between observed and expected heterozygosity. In our study 60 % of the observed alleles in all loci were found to be at frequencies lower than 0.1 (most of the alleles are rare alleles). Such a high proportion of rare alleles COULD BE a good indicator of high genetic divergence among the accessions in each population. This may explain the significant difference in the observed and expected heterozygosity within the study populations. And hence unrecognized (fine scale) genetic differentiations within the study populations might have been the cause of the observed heterozygote deficiency.

A homozygote excess due to null alleles should be locus-specific. When the 7 loci used in our study are compared 3 (Da3Ga4, Dab2E07, and Dab2C05) of them showed a significant excess of homozygotes for all the three different populations studied, whereas, 3 other loci (Dab2D06, Da1F08, and Da1A01) displayed an excess of heterozygotes (but not significant) relative to Hardy-Weinbergs expectation, in at least one of the study populations. Further comparison of the F_{IS} values for each of the 60 alleles at a locus level indicated that at least one allele from each of the 7 loci studied showed excess of the

heterozygote or homozygote deficit (negative F_{IS} value). This demonstrates that null alleles might be ruled out as a possible explanation for the observed deficit of heterozygotes in the study population. However, Tostain *et al.* (2005) associated the significant excess of homozygotes estimated at loci Da3G04 for Guinea yams of West Africa, with the presence of null alleles.

5.4. Population structure

Understanding the patterns of genetic differentiation among populations is crucial for protecting species and developing effective conservation plans. In addition developing priorities for conservation of a species requires an understanding of adaptive genetic differentiation among populations. Perhaps most importantly, an understanding of population genetic structure is essential for identifying units to be conserved (Allendorf and Luikart, 2007).

Methods of quantifying genetic differentiation from microsatellite data are an area of significant debate (Goldstein and Pollock, 1997). Some measures have been developed specifically for these markers, which takes into account variation in allele size under the stepwise mutation model (SMM) (Kimura and Ohta, 1978). In microsatellites, a majority of mutations may be caused by slipped strand mispairing during replication resulting in small gains or losses of repeat copy number rather than in large changes. This type of mutation behaviour is better explained by SMM. The basic idea behind SMM is that mutations predominantly differ from their previous state by the change of a single repeat unit. This type of mutational process results in a unimodal distribution of allele size (Weising *et al.,* 2005). Hence, under the SMM model alleles of similar size are assumed to be more closely related to each other than those of very different size. The theoretical framework for allozyme-based population genetics assumes that any new allele created by mutation is unrelated to ancestral alleles (Infinite allele model, IAM). On the basis of the SMM, Goldstein *et al.* 1995 and Slatkin, (1995) independently proposed a method to evaluate the genetic distance between microsatellite loci that includes allelic repeat scores. Goldstein *et al.* (1995) showed that these distances are a linear function of time. As a result, SMM based measures of population differentiation are expected to be most accurate for populations (taxa) that diverged long enough ago that current genetic differentiation reflects mutations accumulated since divergence (Goldstein and Pollock, 1997). In contrast

traditional measures based on the IAM should be more appropriate for intraspecific comparison (Weising *et al.,* 2005). In this study all the measures used to quantify population differentiation are based on the IAM model.

The genetic structure of a population has been characterized as the non-random distribution of alleles and genotypes in space. The presence of variability within species (among populations and also between individuals within populations) is essential for their ability to survive and to successfully respond to environmental changes. In all the comparisons made in our study a low mean F_{ST} (but significant) has been observed, indicating that the majority of microsatellite diversity in the *Dioscorea cayenensis* complex populations under study was found within rather than among populations. Pairwise comparison of the three subpopulations (Or, Sh and Sn) defined based on their geographical location indicated that the Sh and Sn populations are genetically close to each other, both compared to the Or population. This could probably be associated with gene flow mediated by exchange of planting materials between farmers. Gene flow reduces the genetic differences between populations and increases the genetic variation within populations (Allendorf and Luikart, 2007). Gene flow among populations is the cohesive force that holds together geographically separated populations into a single evolutionary unit. Comparison of populations of the cultivated Guinea yams and their wild relatives also resulted an F_{ST} value of only 3 %, even lower than the F_{ST} values obtained by pairwise comparisons of the three subpopulations defined based on geographical location, but highly significant ($P = 0.006$). This indicates that there is some degree of differentiation between the wild and cultivated accession of *Dioscorea cayenensis* complex populations under study. Gene flow between wild and cultivated species is mainly manifested by the still ongoing domestication practice by farmers in the study area. Spontaneous Gene flow between wild and cultivated Guinea yams has also been reported based on studies of West African material (Scarcelli *et al.,* 2006b). In general, the observed high allelic diversity and heterozygosity within the study populations and low F_{ST} estimates might suggest that genetic drift has not yet had a major influence on the *Dioscorea cayenensis* complex populations in the study area. In addition, as a crop plant selection by humans might have played a great role in shaping the genetic structure of *Dioscorea cayenensis* complex accessions under study.

5.5 The implications for yam domestication and conservation in Ethiopia

Cultivated yams of the *D. cayenensis* complex are vegetatively propagated. In West Africa, yam fields in traditional agroecosystems are seeded with tuber fragments from the previous harvest (e.g. Scarcelli *et al.* 2006a). No direct seed use by farmers has been reported. In contrast, wild yam species reproduce sexually (Ayensu & Coursey, 1972; Coursey 1976; Hildebrand *et al.,* 2002, pers. obs.). In South West Ethiopia, it is common practice for farmers to collect "wild" or managed yam tubers in the forest as food and to plant them under trees in their home garden (Hildebrand *et al.,* 2002). This process means that domestication is a constantly repeating process. If these or other yams in cultivation produce sexual organs, then gene exchange between wild and cultivated plants is possible (Hildebrand *et al.,* 2002, pers. obs). Indeed, the results above suggest that it is likely that it is a regular occurrence. It is probable that wild and cultivated plants of the *D. cayenensis* complex represent a spectrum based on degrees of management rather than distinct taxa. The morphological diversity encountered (tuber colour, time taken to mature, leaf shape, number and size of male inflorescences) is a result of differing degrees of domestication, human selection and the local environment. A good example of the ability of these plants to vary under human selection is possession of a spiny root. This has been thought to be a key character of *D. praehensilis*, especially in West Africa, although it is usually spineless in East Africa. This difference led Milne-Redhead (1975) to separate the non-spiny forms as the species *D. odoratissima* Pax. However, as we have been told by farmers (also reported by Hildebrand *et al.,* 2002) that "wild" plants having spiny roots lose this character and become spineless within a few years of being taken into a garden context. We have also encountered that a cultivar which was collected in the field as a non-spiny individual, but produced massive root spines when it was planted in a glasshouse (in Oslo). It is probable that other morphological features which farmers would select against such as stem spines, leaf shape and inflorescence number can be changed just as quickly when the environment or management regime changed. This suggests that a considerable proportion of such variation may be phenotypic. It would be expected that tuber characters (shape, colour, taste) would be stabilised by positive human selection in cultivars, in contrast to the characters mentioned above.

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One of the main aims of this study was to consider the conservation of yams in South and South Western Ethiopia, because they are in danger of being replaced by cash crops. From a conservation perspective, it appears that the vernacular names should be viewed as corresponding with cultivars. It is important that both the range of cultivars and the diversity within them is protected both *in situ*, and perhaps also in a local garden as *ex-situ*. The greater genetic divergence (from AFLP analysis) encountered in the cultivated forms over the "wild" plants of the Sh population suggests that there is indeed diversity which it will be important to protect to ensure local food security. However, the managed forest plants are also an important source of diversity and need protection, ideally by conserving the native vegetation of the Sheko region. The results of microsatellite data analyses also confirmed that the Sh population (collected from relatively a small geographical area) displayed a higher allelic diversity.

6. Conclusions and recommendations

6.1 Conclusions

In general, this study has revealed that:

1. There is no clear taxonomic boundaries among cultivated and wild accessions of *D. cayenensis* complex included in this study.
2. There is a considerable genetic diversity within the *D. cayenensis* complex in country with the Sheko cultivars (Sh population) displaying the greatest diversity, where as the Sn displayed the least. However, unlike both Or and Sh populations, the Sn population are at Hardy-Weinberg equilibrium.
3. Pairwise comparison of the different subpopulation (defined based on their geographical isolation and growth habit) indicated that there is some degree of genetic differentiation among the study population.
4. The wild yams exhibited the greatest allelic diversity (as measured by allelic richness) in all the loci studied compared to the cultivated forms and hence through domestication they contribute to the gene pool of cultivated Guinea yams.
5. High genetic divergence was observed between the cultivated accessions of Guinea yams collected from Sheko area. Thus, the farmers in the Sheko area posses different cultivars with a wide genetic basis.
6. Contrary to what is expected in vegetatively propagated crops, none of the 7 microsatellite loci displayed a significant excess of heterozygote.
7. The Sh population displayed the highest allelic diversity in 5 of the 7 SSR loci studied with the over all allelic polymorphism of 100%. The Sn and Or population displayed a fixed allele for one SSR locus (Da3Ga4), with an overall allelic polymorphism of 87.5%.
8. Results of SSR analysis indicated that, the difference in the observed and expected heterozygosity in Sh population highly significant compared to both Or and Sn populations.

6.2. Recommendations

1. Studies must be undertaken at the population scale and in a broad range of geographical regions, so as to take the diversity within each member of the Guinea yams.
2. Comparative studies involving Ethiopian and West African material must be undertaken to clarify the taxonomic confusion and observed differences in genetic structure.
3. Conservation activities aiming to conserve *Dioscorea* species in the country should primarily focus on the Sheko accessions, as they exhibit the highest genetic diversity.

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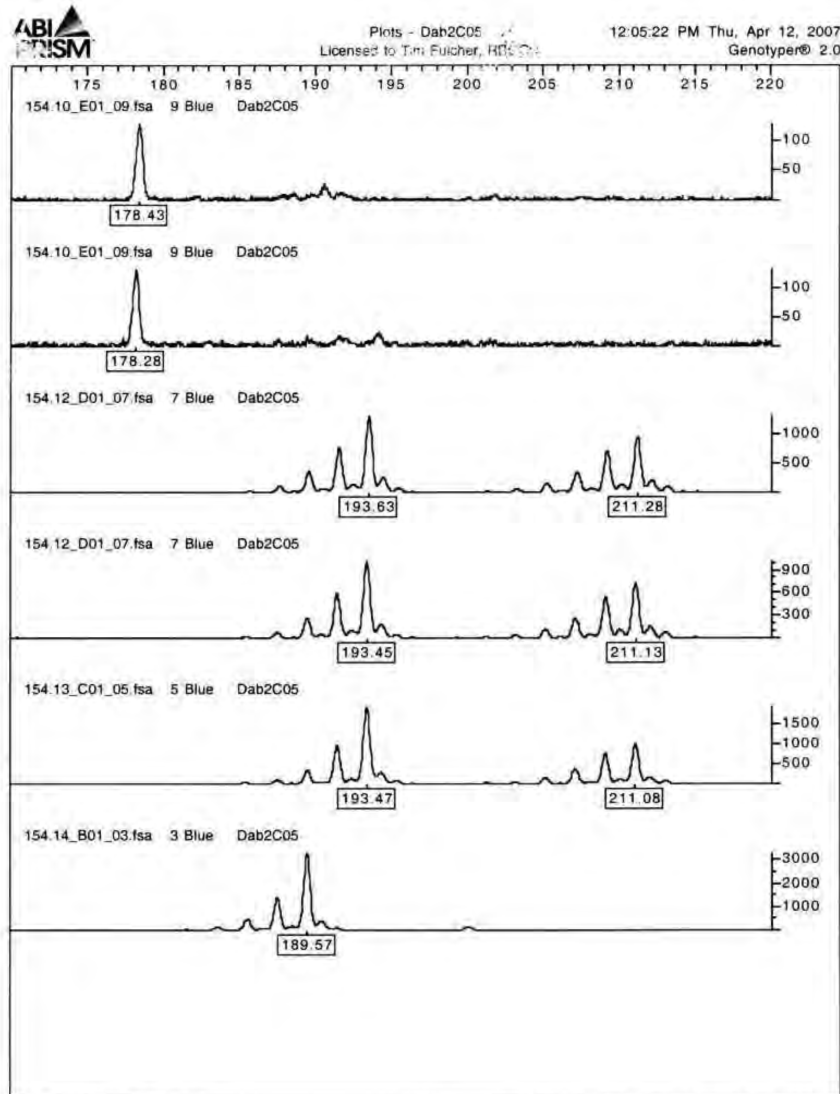
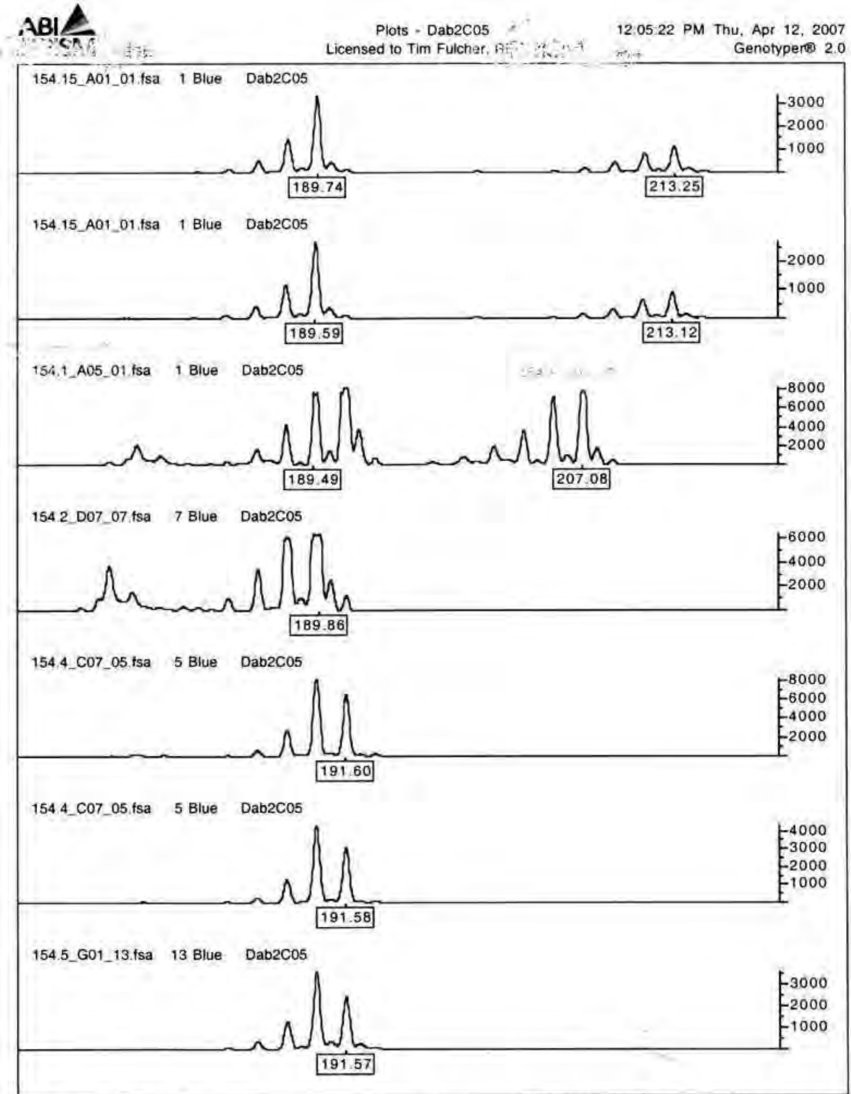
8. Appendices

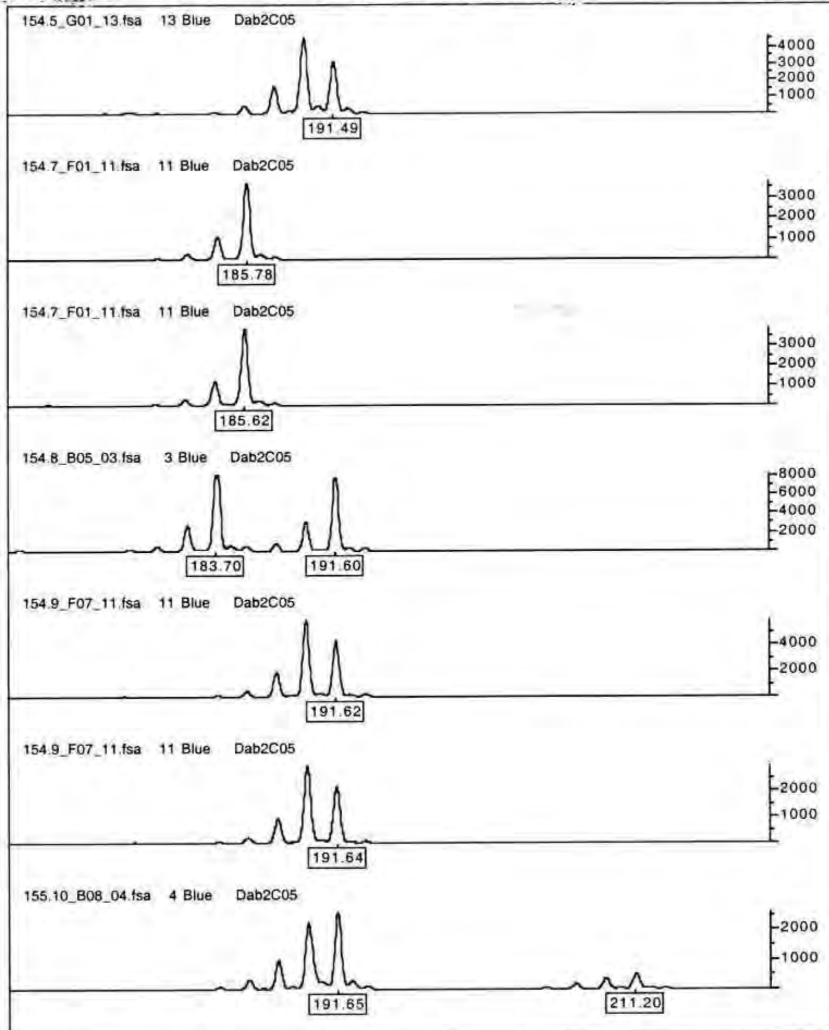
Appendix 1. List of the major diagnostic morphological characters used to identify the voucher specimens (Compiled by Dr Paul Wilkin)

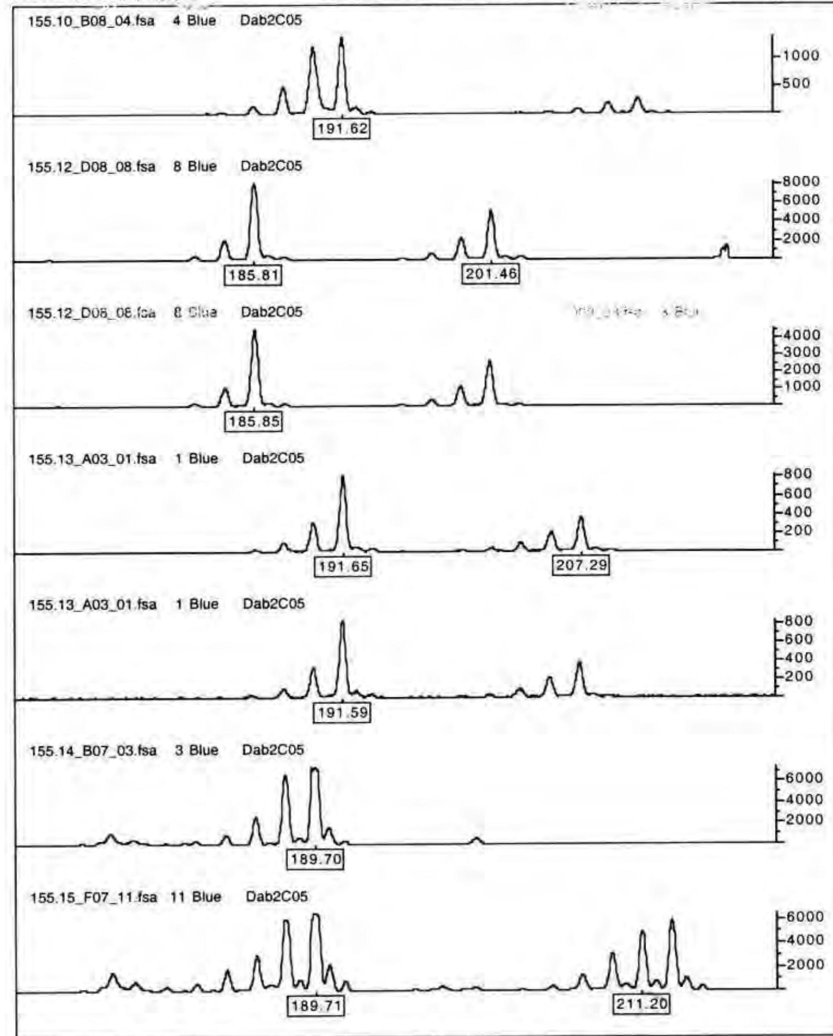
Characters	*D. rotundata/D.caynensis*	*D. abyssinica*	*D. praehensilis*
1. Thorny roots	**Absent**	**Absent**	**Present**
2. Stem prickles	**Absent or present (few to many)**	**Absent**	**Present**
3. Leaf base shape	**Deep sinus with a deltoid petiolar attachment**	**Cordate-ovate with round basal lobes**	**Shortly cordate**
4. Number of male infls/axil	**1-2 (-3)**	**2-6(-8)**	**2-6 (-8)**
5. Male **inflorescence inter-floral distance**	**Flowers at least their own diameter apart**	**Flowers at least their own diameter apart**	**Flowers less than their own diameter apart**
6. Leaf blade (L/W) ratio	**Less than 1.8**	**Less than 1.8**	**Less than 1.8**
7. Leaf texture	**Herbaceous**	**Herbaceous**	**Herbaceous**
8. Tepal texture	**Herbaceous**	**herbaceous**	**Basal half scarious**
9. Tuber form	**Variable not deeply buried**	**Cylindrical deeply buried**	**Cylindrical deeply buried**

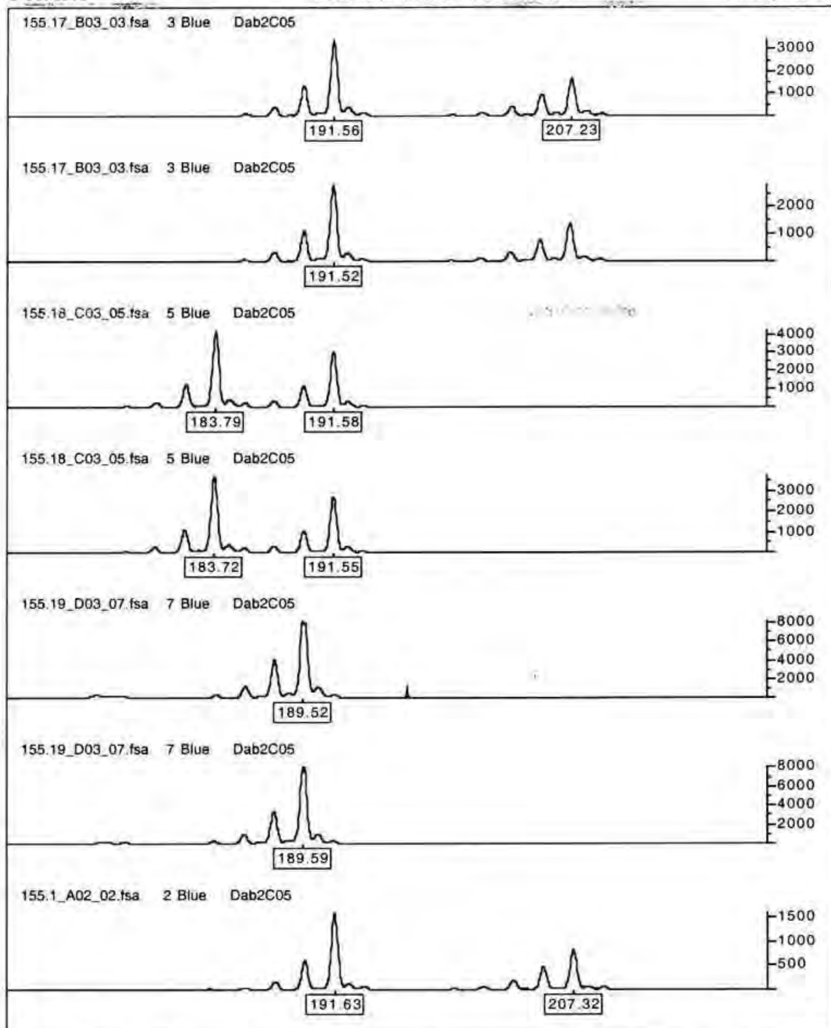
Appendix 2. Microsatellite electropherograms showing allelic distribution at each of the loci studied as revealed by GENESCAN and GENOTYPER 3.6 software.

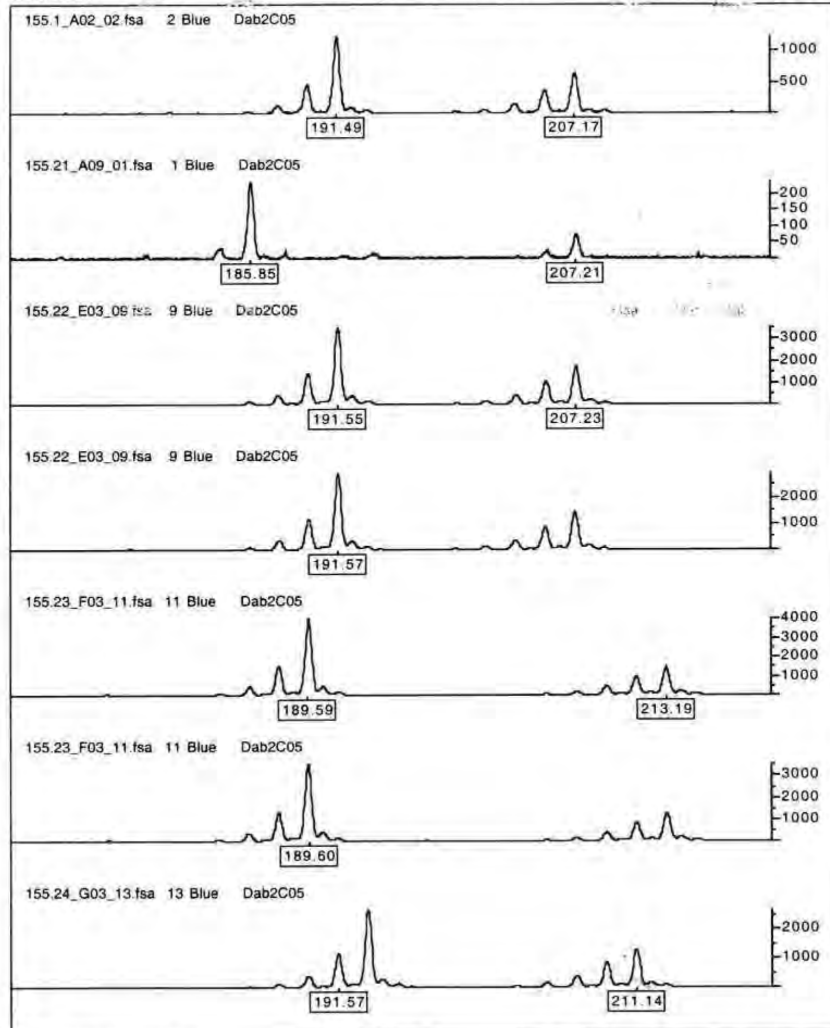





Plots - Dab2C05
Licensed to Tim Fulcher
12:05:23 PM Thu, Apr 12, 2007
Genotyper® 2.0
154.5_G01_13.fsa 13 Blue Dab2C05
191.49
154.7_F01_11.fsa 11 Blue Dab2C05
185.78
154.7_F01_11.fsa 11 Blue Dab2C05
185.62
154.8_B05_03.fsa 3 Blue Dab2C05
183.70
191.60
154.9_F07_11.fsa 11 Blue Dab2C05
191.62
154.9_F07_11.fsa 11 Blue Dab2C05
191.64
155.10_B08_04.fsa 4 Blue Dab2C05
191.65
211.20
For research use only
- 3 -
Not for use in diagnostic systems


Plots - Dab2C05
Licensed to Tim Fulcher, RBGQ
Genotyper® 2.0
155.10_B08_04.fsa 4 Blue Dab2C05
191.62
155.12_D08_08.fsa 8 Blue Dab2C05
185.81
201.46
Dab2C05
185.85
155.13_A03_01.fsa 1 Blue Dab2C05
191.65
207.29
155.13_A03_01.fsa 1 Blue Dab2C05
191.59
155.14_B07_03.fsa 3 Blue Dab2C05
189.70
155.15_F07_11.fsa 11 Blue Dab2C05
189.71
211.20
For research use only
- 4 -
Not for use in diagnostic systems


Plots - Dab2C05
Licensed to Tim Fulcher, RBGQ
Genotyper® 2.0
155.17_B03_03.fsa 3 Blue Dab2C05
191.56
207.23
155.17_B03_03.fsa 3 Blue Dab2C05
191.52
155.18_C03_05.fsa 5 Blue Dab2C05
183.79
191.58
155.18_C03_05.fsa 5 Blue Dab2C05
183.72
191.55
155.19_D03_07.fsa 7 Blue Dab2C05
189.52
155.19_D03_07.fsa 7 Blue Dab2C05
189.59
155.1_A02_02.fsa 2 Blue Dab2C05
191.63
207.32
For research use only
- 5 -
Not for use in diagnostic systems


Plots - Dab2C05
Licensed to Tim Fulcher, RBGQ
12:05:23 PM
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191.49
207.17
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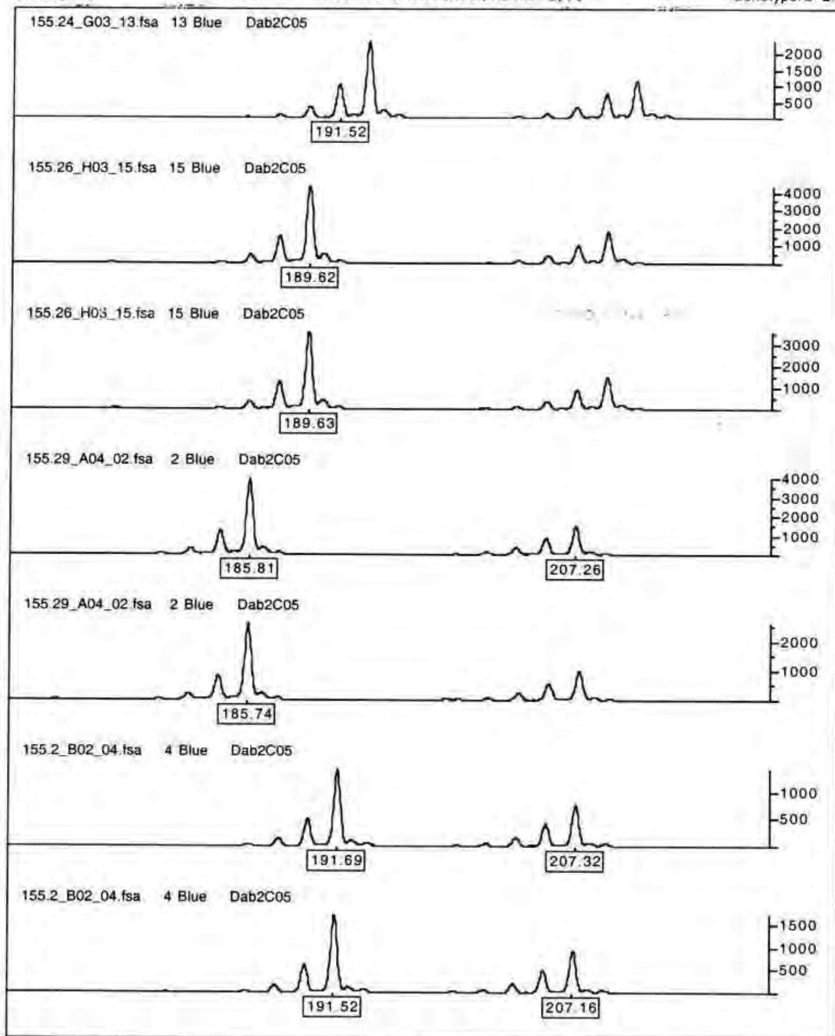
185.85
207.21
155.22_E03_09.fsa 9 Blue Dab2C05
191.55
207.23
155.22_E03_09.fsa 9 Blue Dab2C05
191.57
155.23_F03_11.fsa 11 Blue Dab2C05
189.59
213.19
155.23_F03_11.fsa 11 Blue Dab2C05
189.60
155.24_G03_13.fsa 13 Blue Dab2C05
191.57
211.14
For research use only
- 6 -
Not for use in diagnostic systems



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Licensed to Tim Fulcher, RBGQ
PM Thu, Apr 12, 2007
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2000
1000
189.63
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207.26
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500
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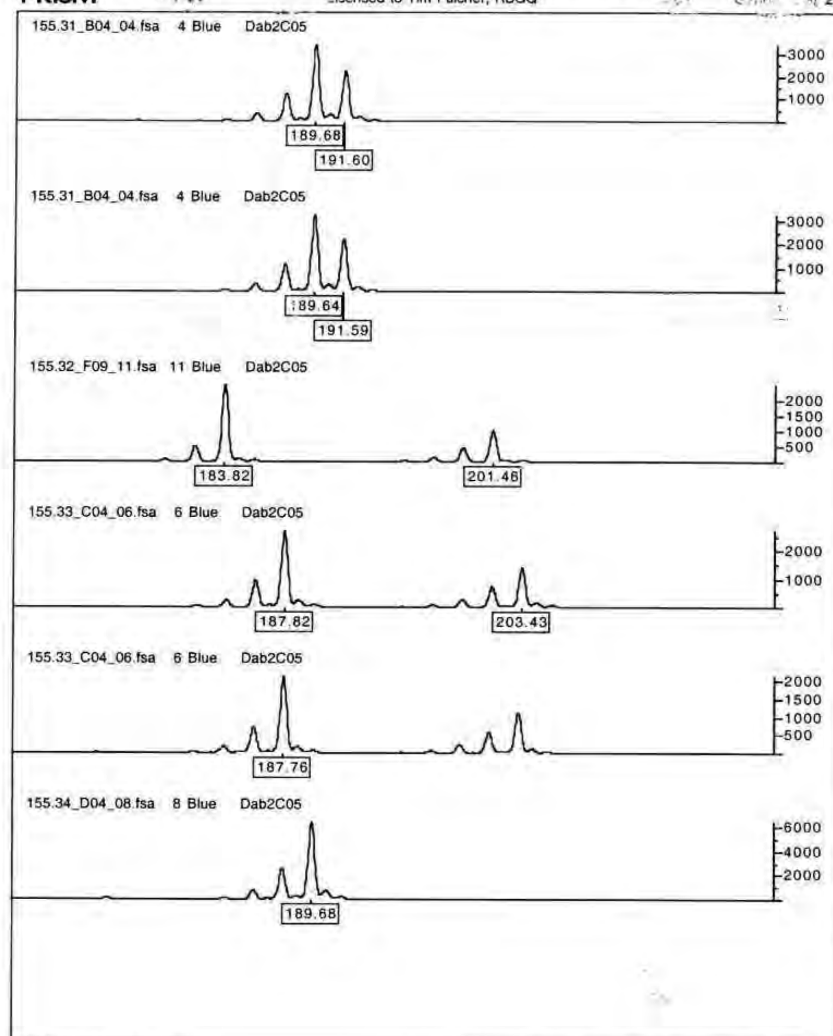
For research use only
- 7 -
Not for use in diagnostic systems



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500
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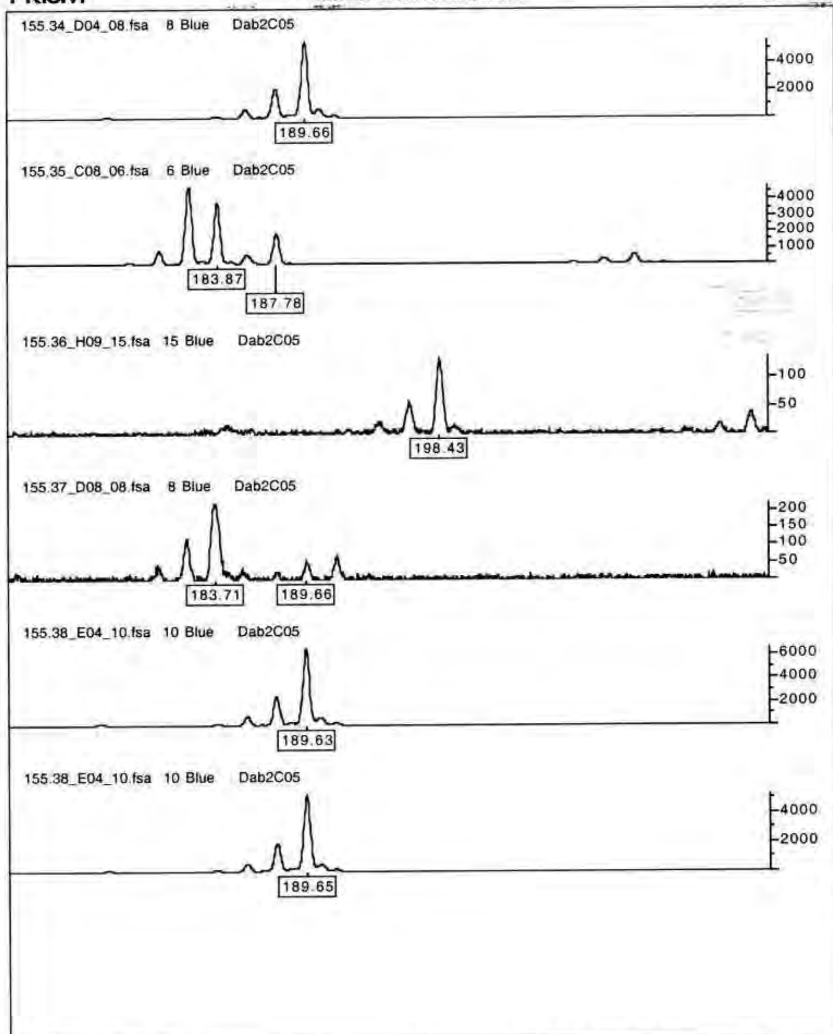
For research use only
- 8 -
Not for use in diagnostic systems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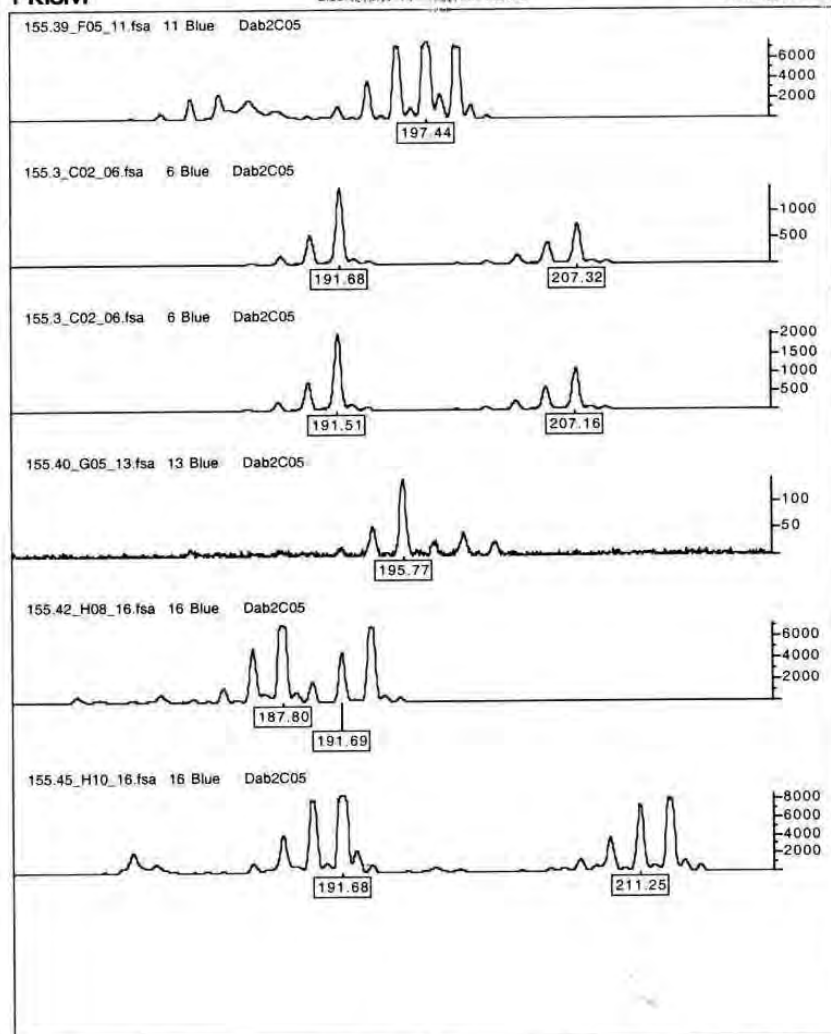
-9-
Not for use in diagnostic systems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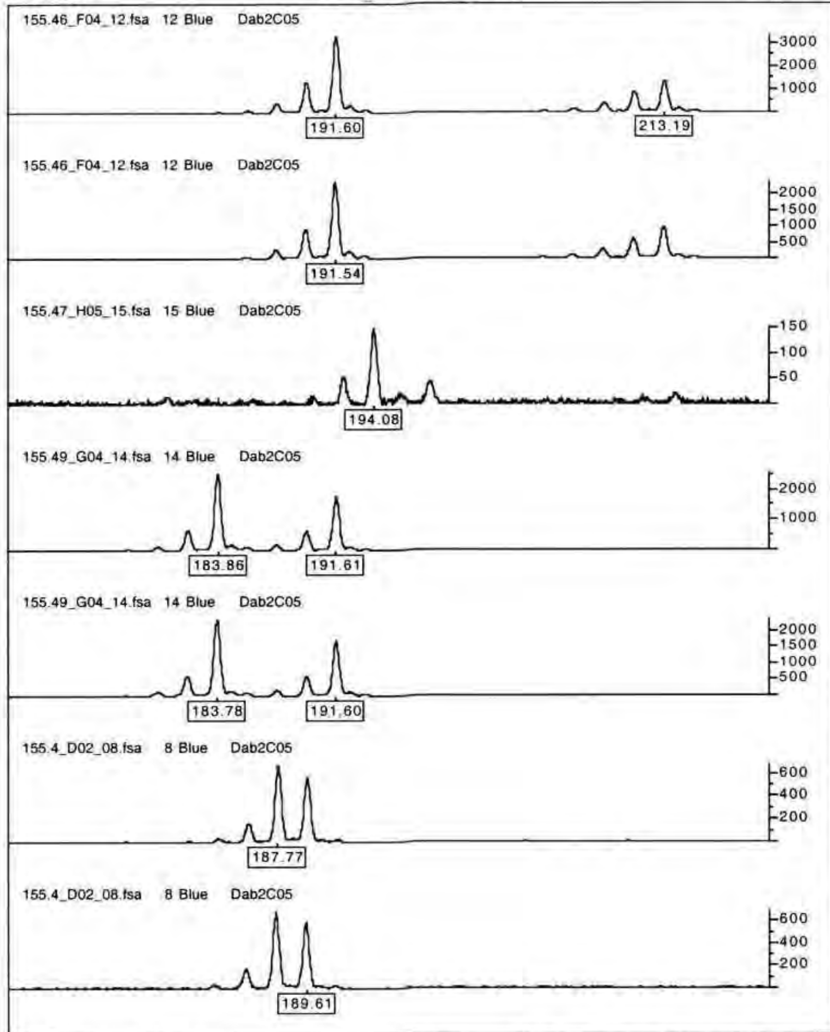
For research use only
-10-
Not for use in diagnostic systems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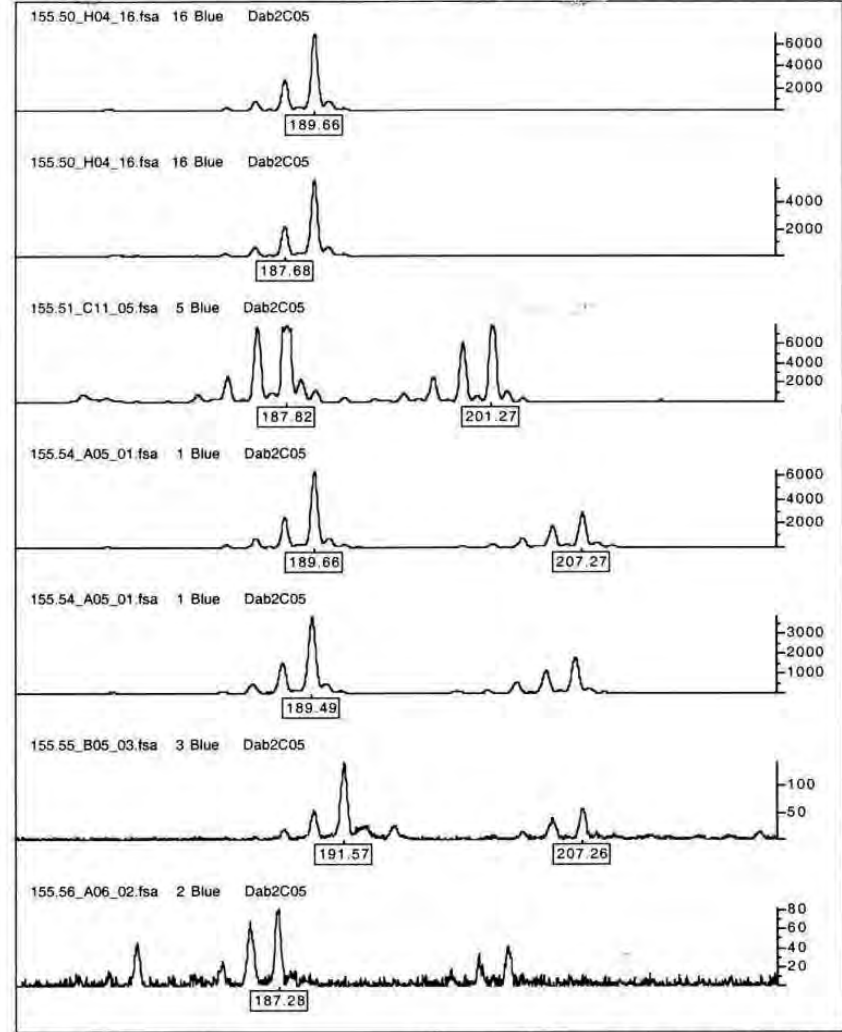
For research use only
-11-
Not for use in diagnostic systems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207.27
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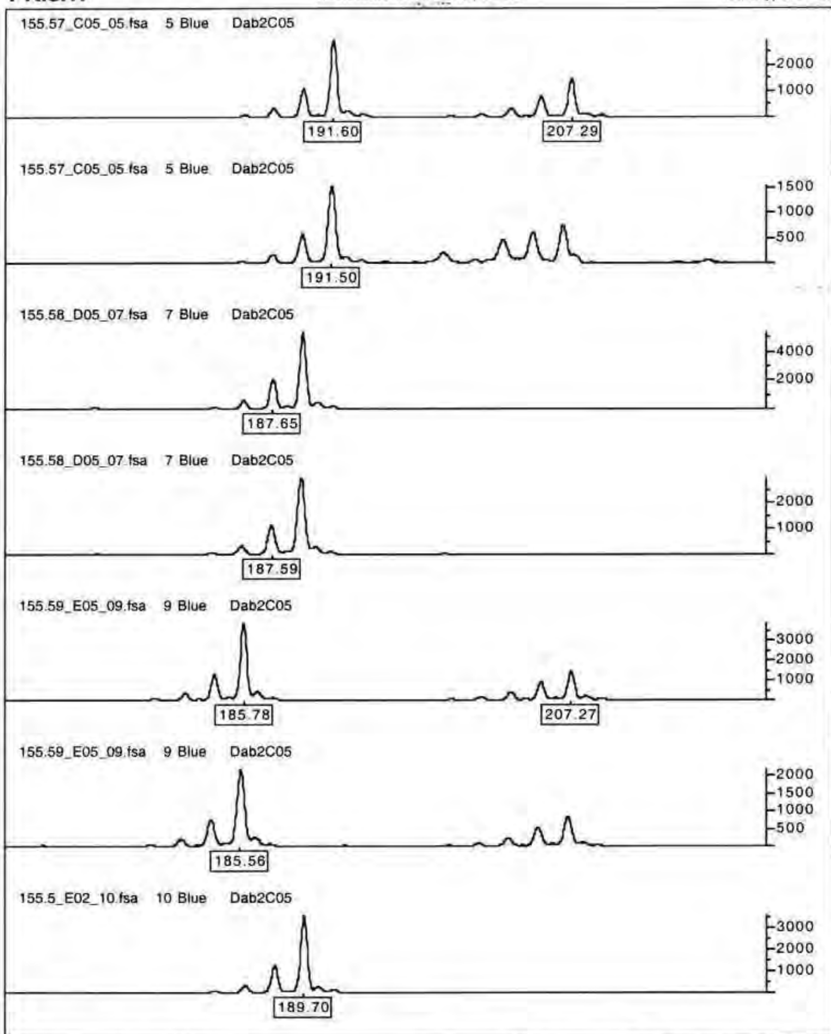
For research use only
-12-
Not for use in diagnostic systems



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155.58_D05_07.fsa 7 Blue Dab2C05
187.65
155.58_D05_07.fsa 7 Blue Dab2C05
187.59
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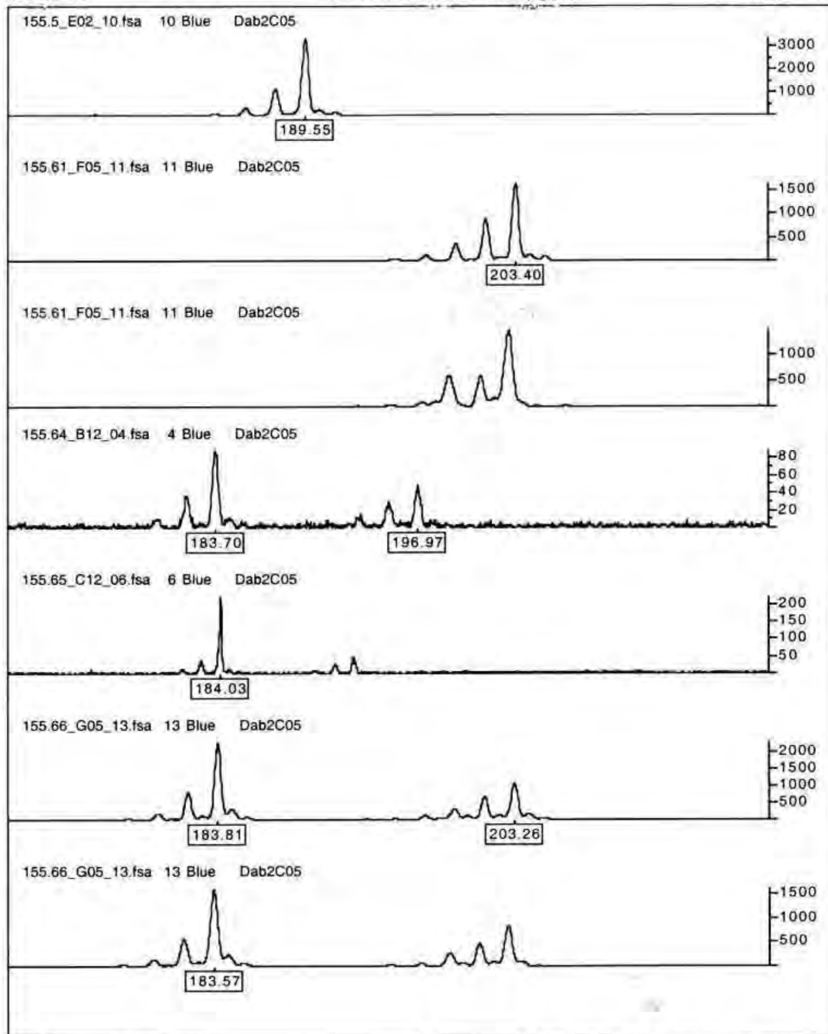
For research use only
-13-
Not for use in diagnostic systems



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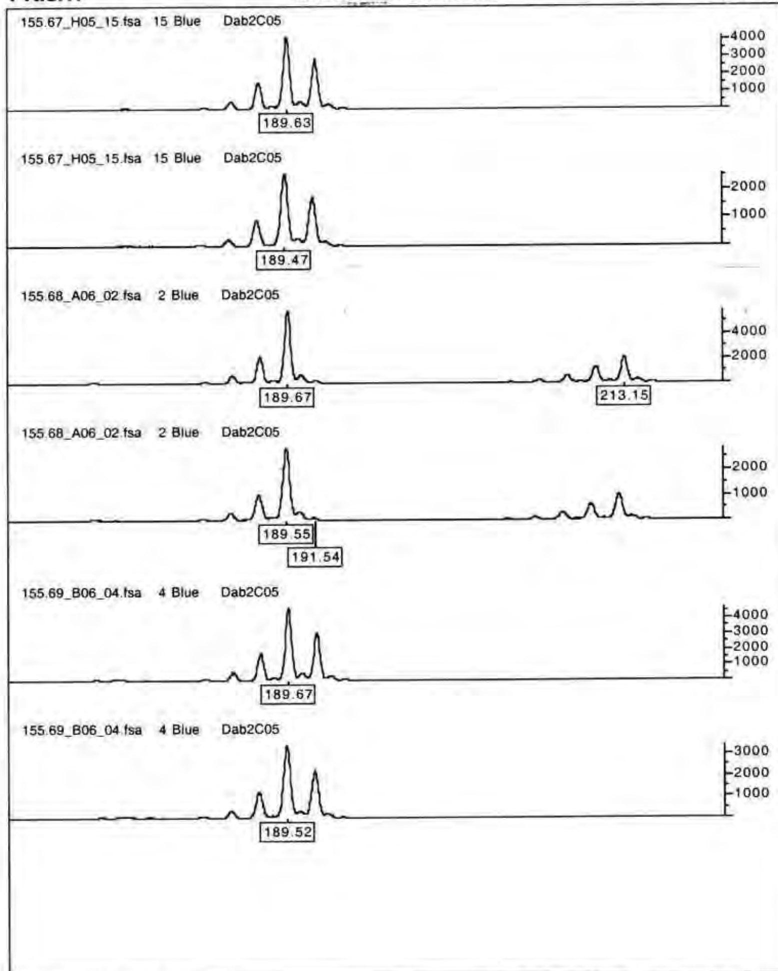
For research use only
-14-
Not for use in diagnostic systems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155.69_B06_04.fsa 4 Blue Dab2C05
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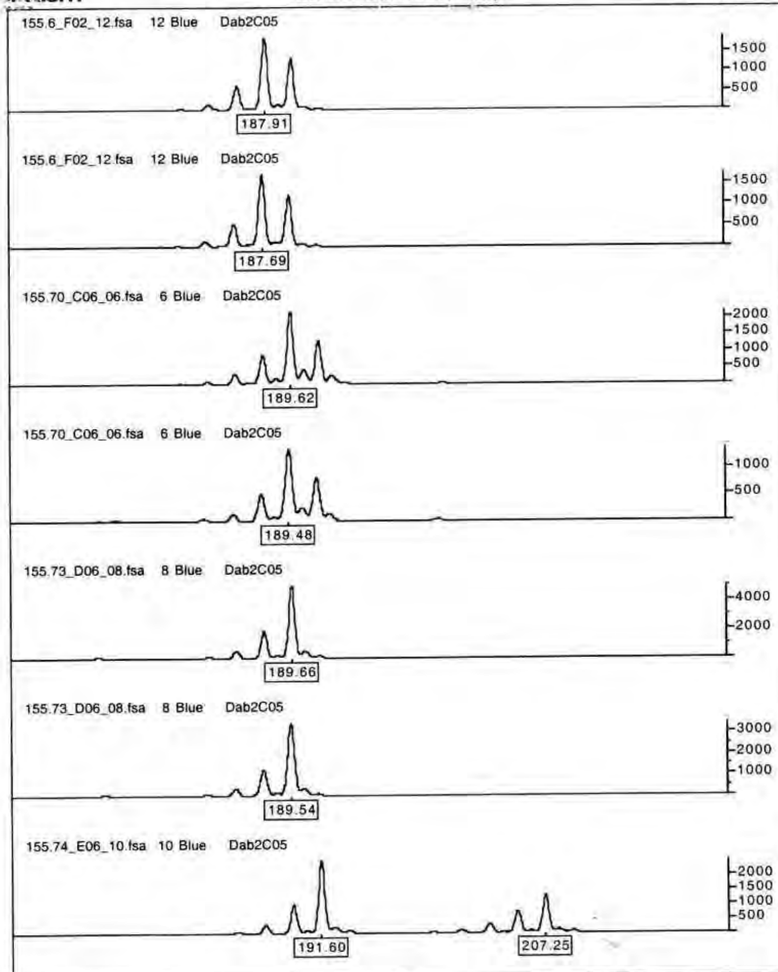
For research use only
-15-
Not for use in diagnostic systems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189.66
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191.60
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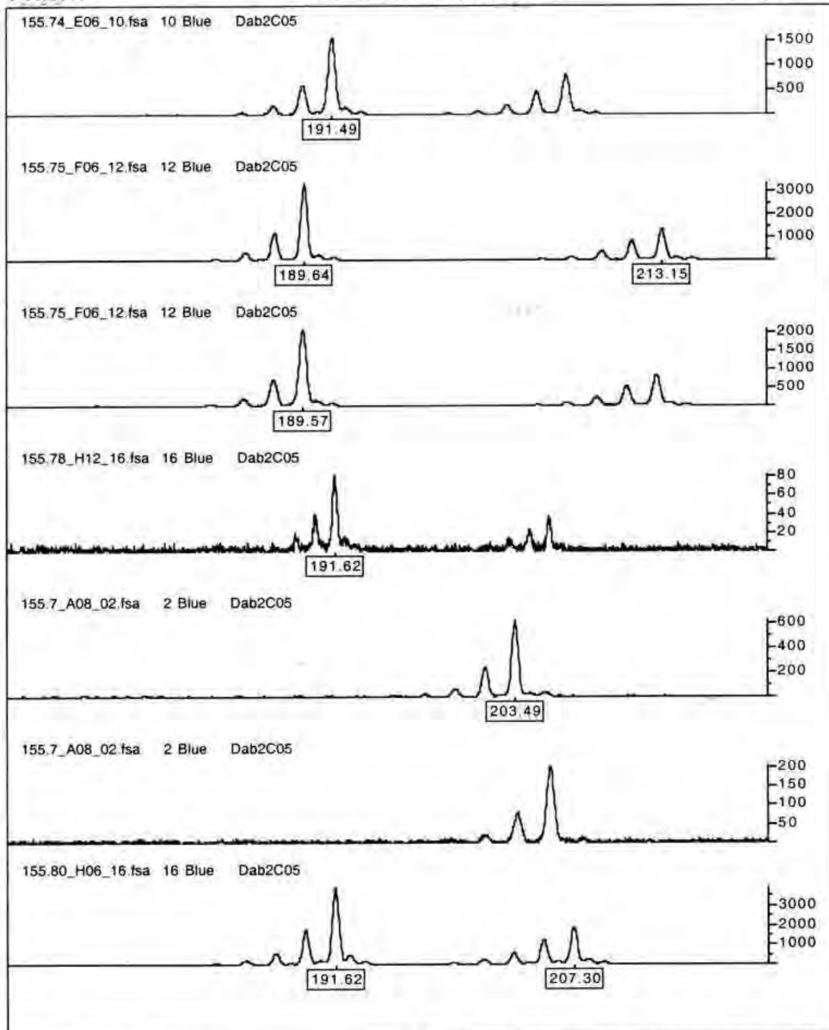
For research use only
-16-
Not for use in diagnostic systems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191.62
155.7_A08_02.fsa 2 Blue Dab2C05
203.49
155.7_A08_02.fsa 2 Blue Dab2C05
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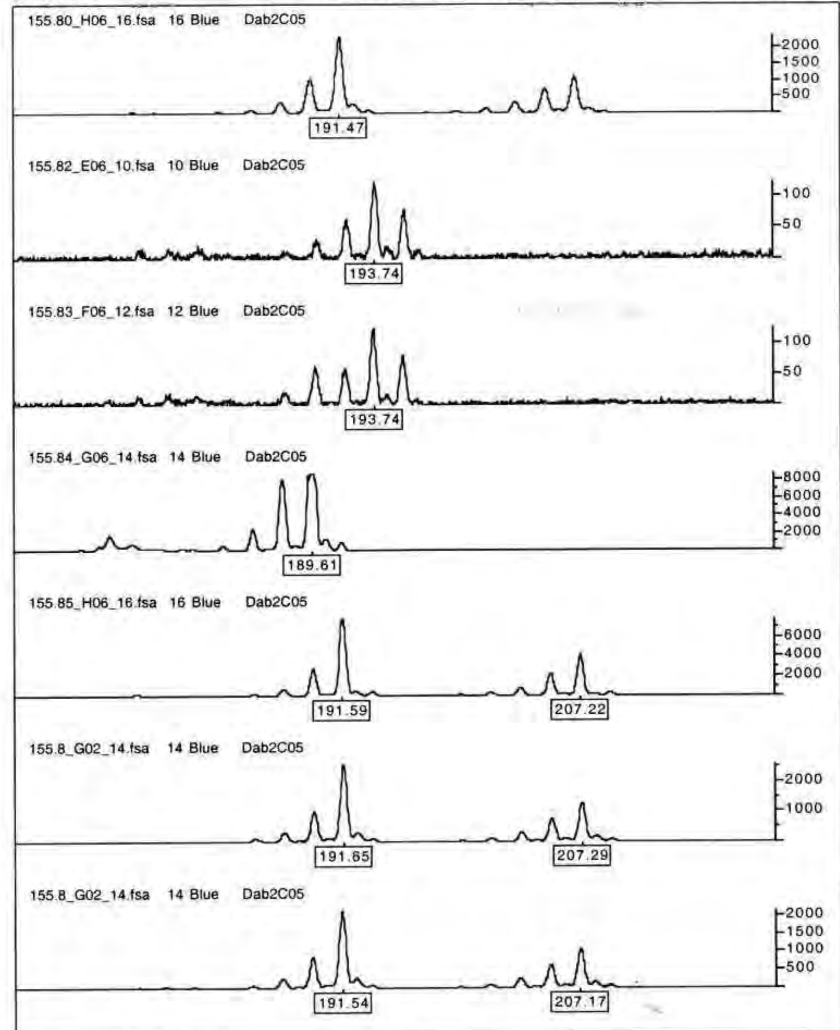
For research use only
-17-
Not for use in diagnostic systems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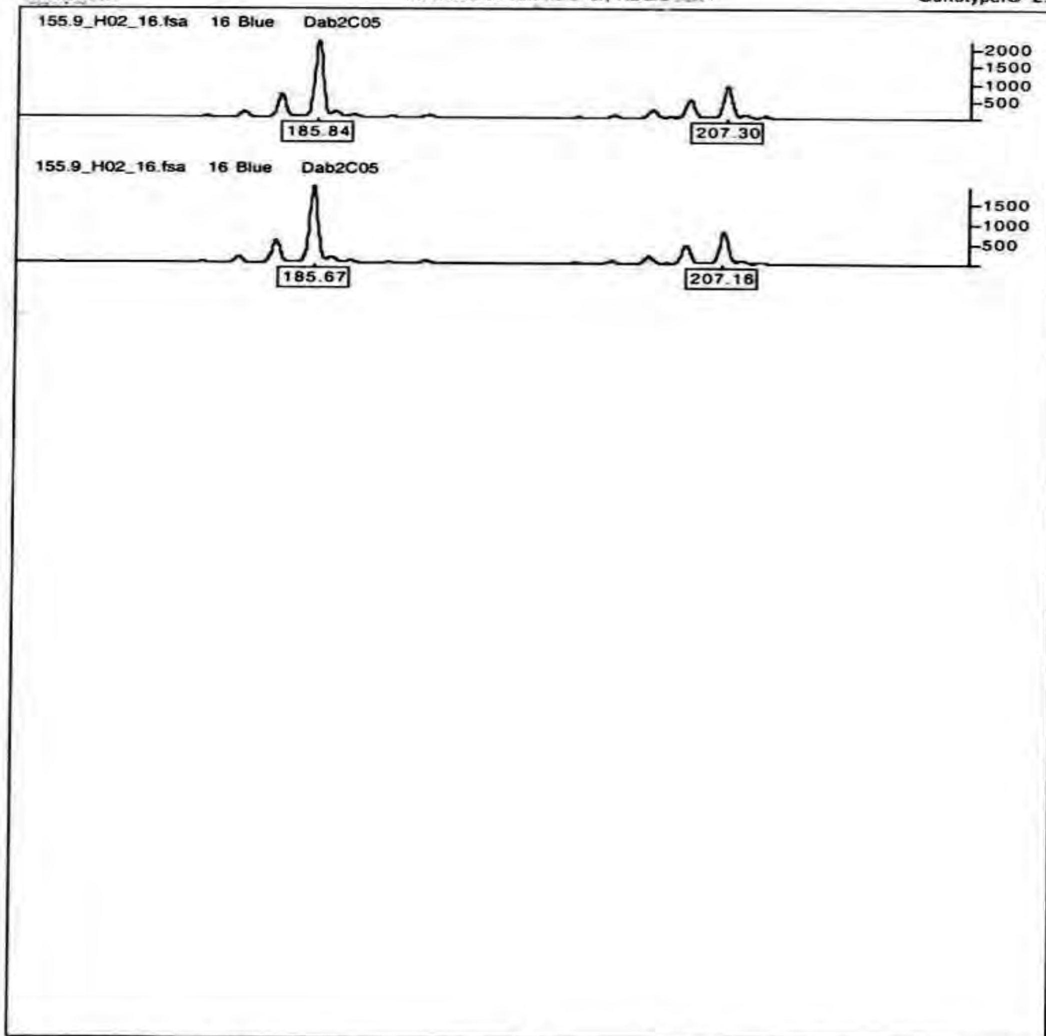
For research use only
-18-
Not for use in diagnostic systems



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500
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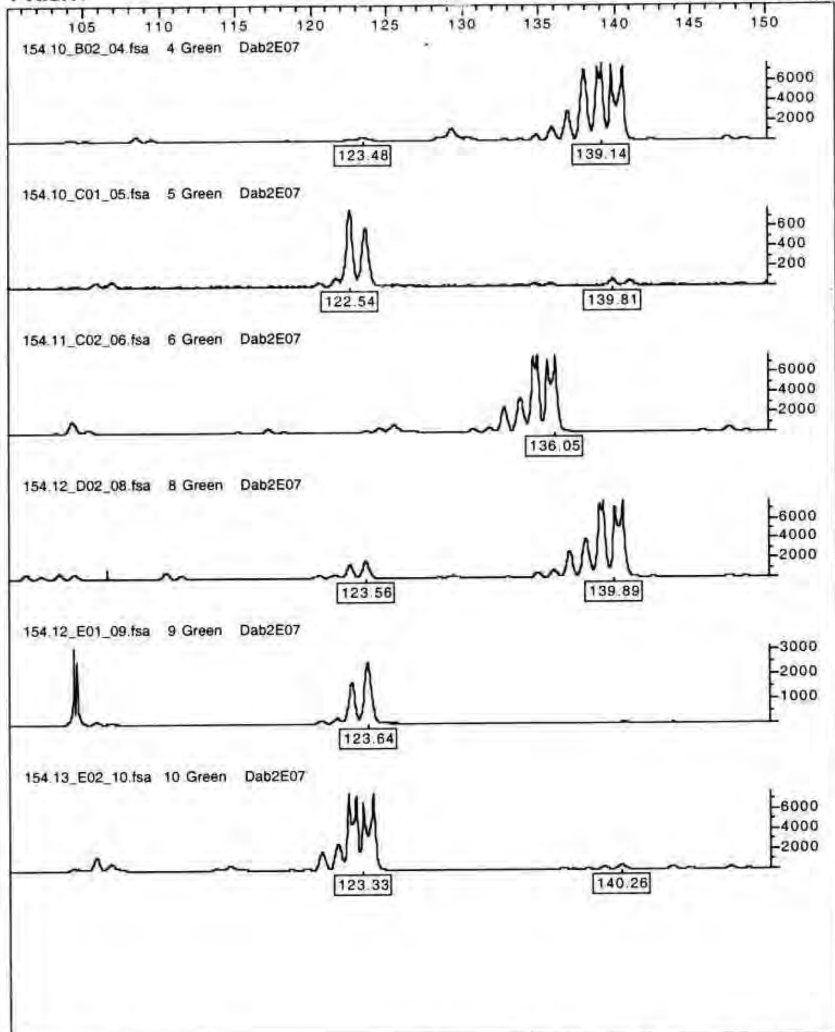
For research use only
- 1 9 -
Not for use in diagnostic systems



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136.05
154.12_D02_08.fsa 8 Green Dab2E07
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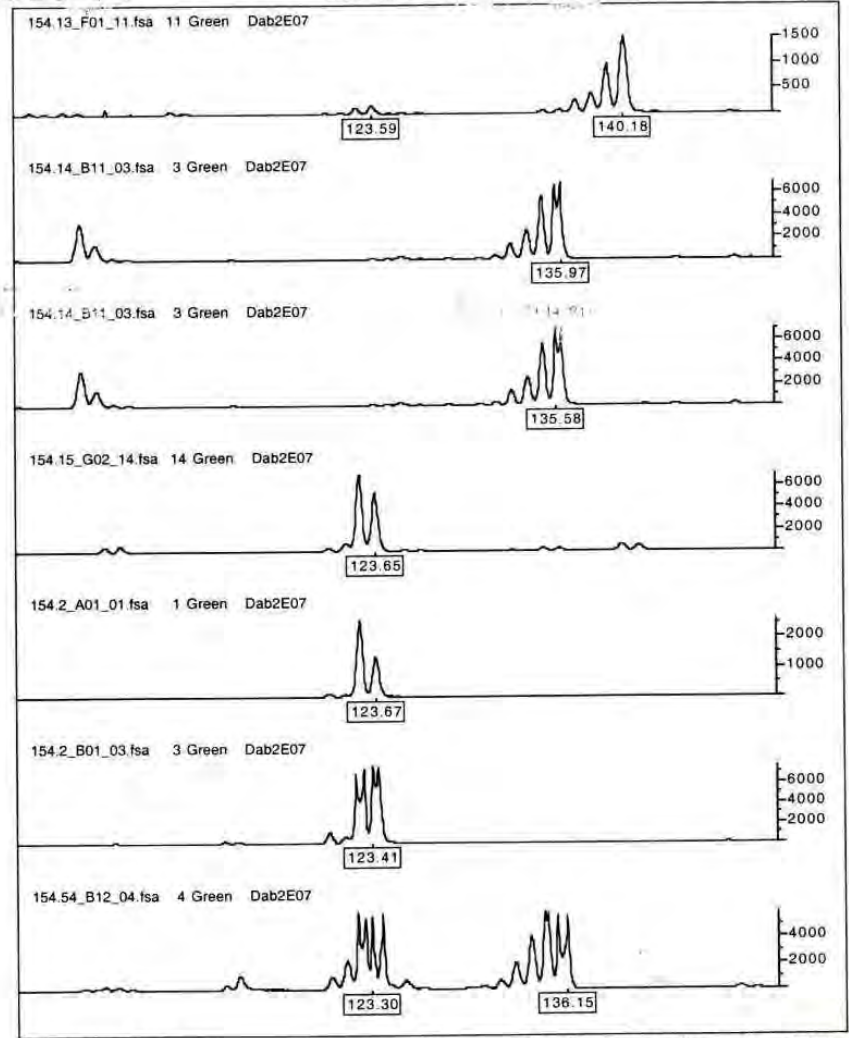
For research use only
- 1 -
Not for use in diagnostic systems



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Licensed to Tim F
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For research use only
- 2 -
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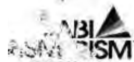

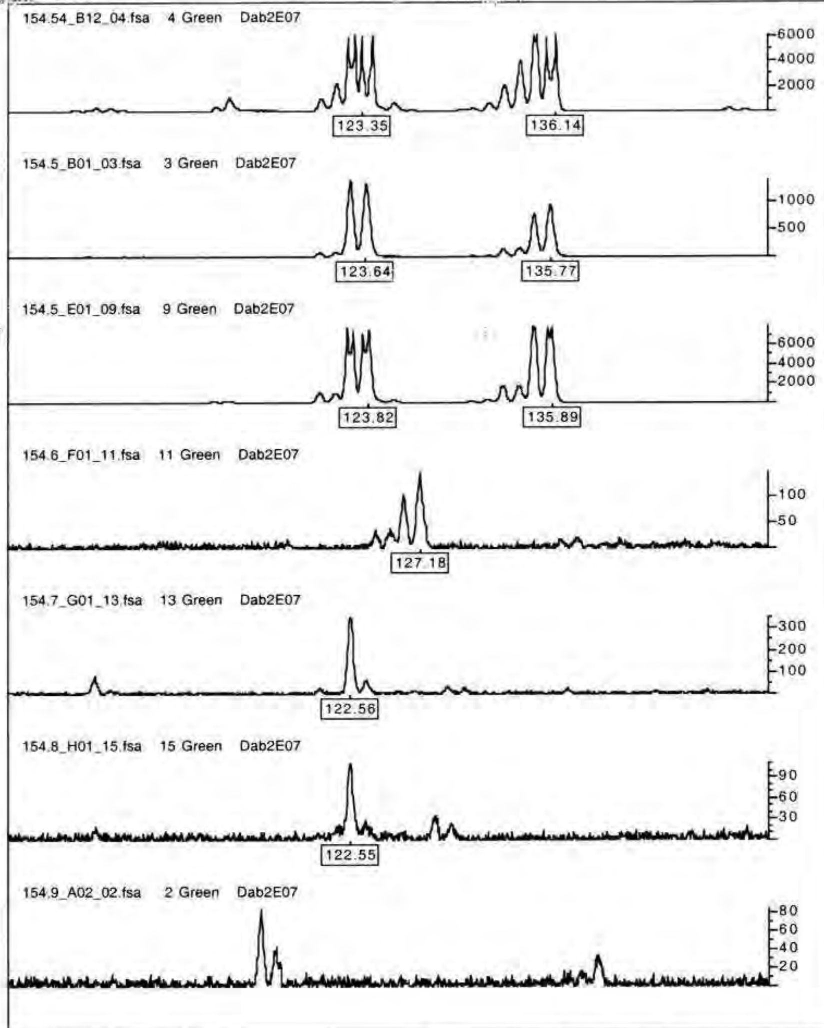

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154.7_G01_13.fsa 13 Green Dab2E07
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154.8_H01_15.fsa 15 Green Dab2E07
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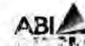
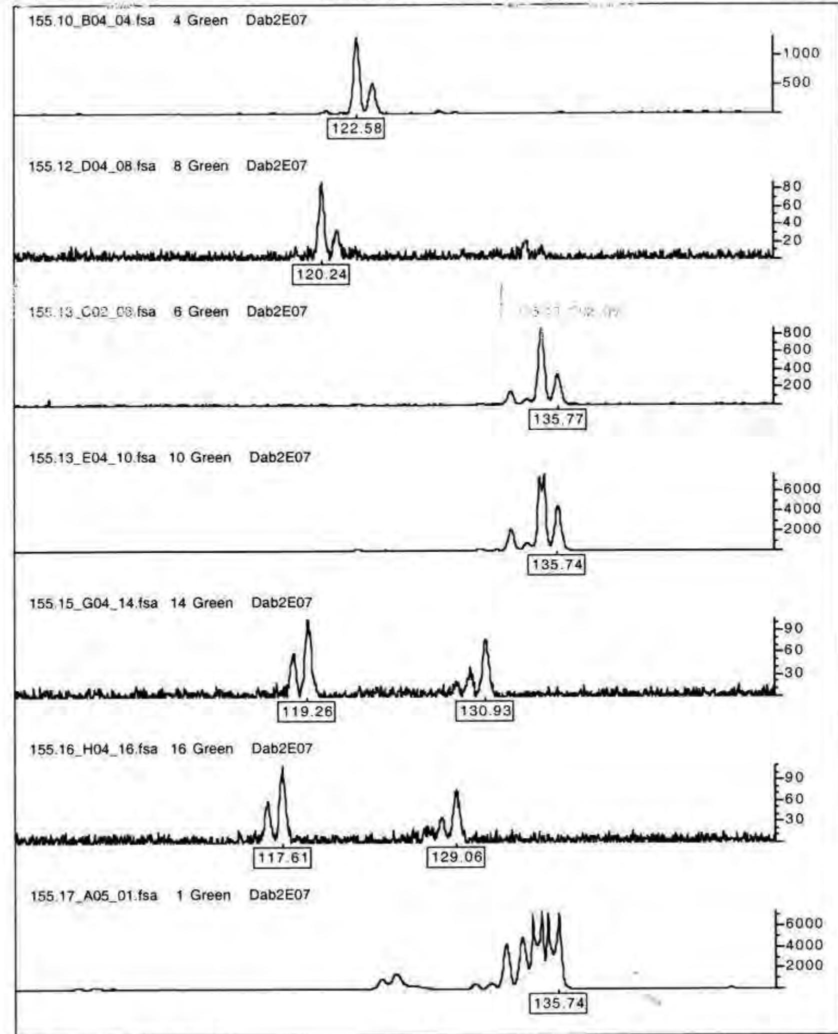
For research use only
- 3 -
Not for use in diagnostic systems





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Licensed to Tim Fulcher
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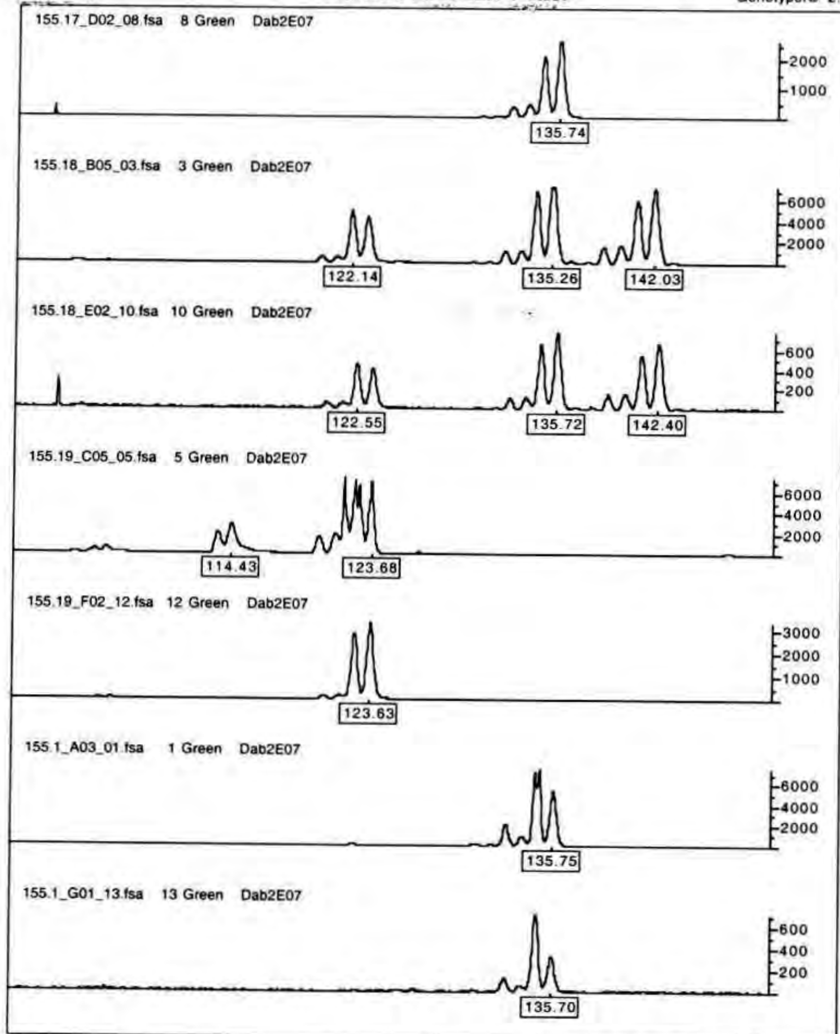
For research use only
- 4 -
Not for use in diagnostic systems



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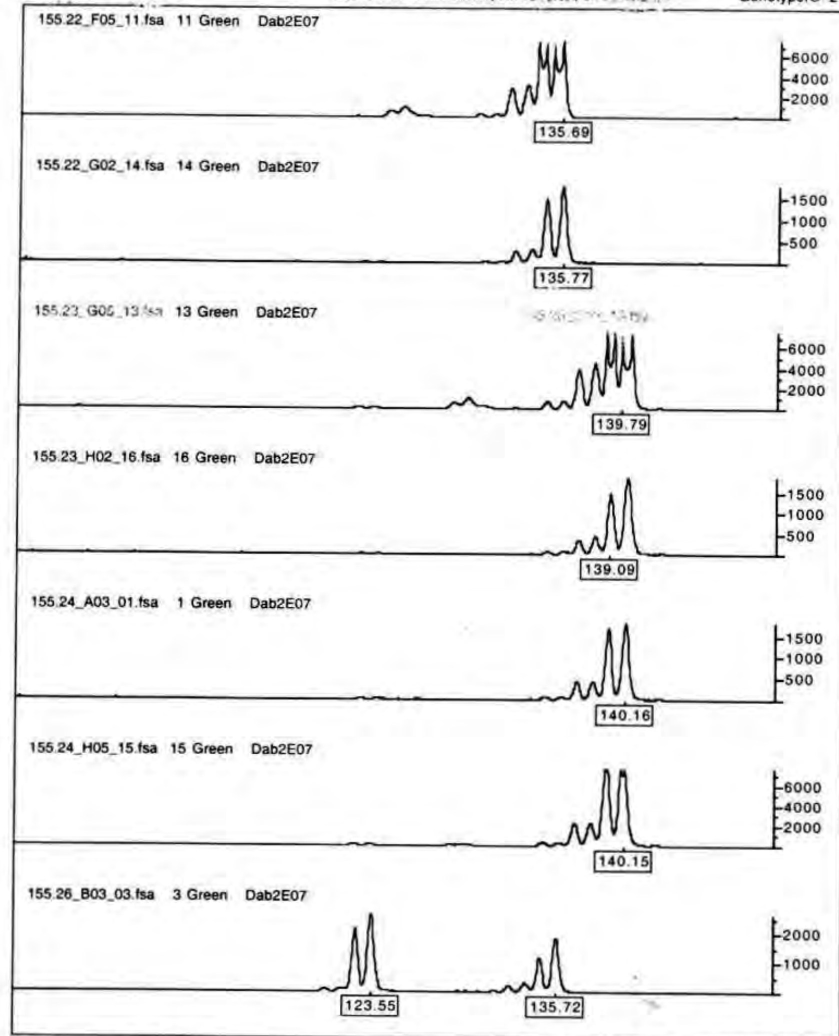
For research use only
- 5 -
Not for use in diagnostic systems



ABI
Plots - Dab2E07
Licensed to Tim Fulcher,
7:34 PM Thu, Apr 12, 2007
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13 Green Dab2E07
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155.23_H02_16.fsa 16 Green Dab2E07
139.09
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140.16
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155.26_B03_03.fsa 3 Green Dab2E07
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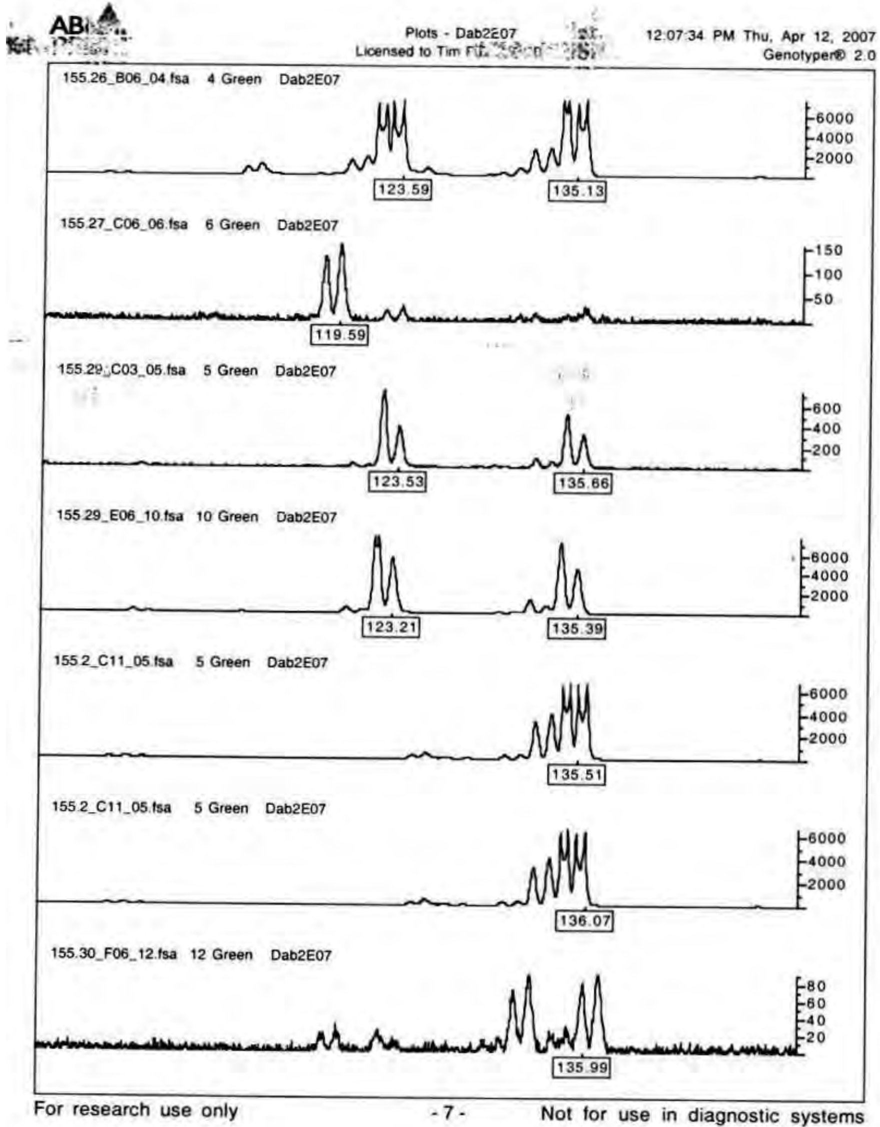
For research use only
- 6 -
Not for use in diagnostic systems



Plots - Dab2E07
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155.29_C03_05.fsa 5 Green Dab2E07
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135.66
155.29_E06_10.fsa 10 Green Dab2E07
123.21
135.39
155.2_C11_05.fsa 5 Green Dab2E07
135.51
155.2_C11_05.fsa 5 Green Dab2E07
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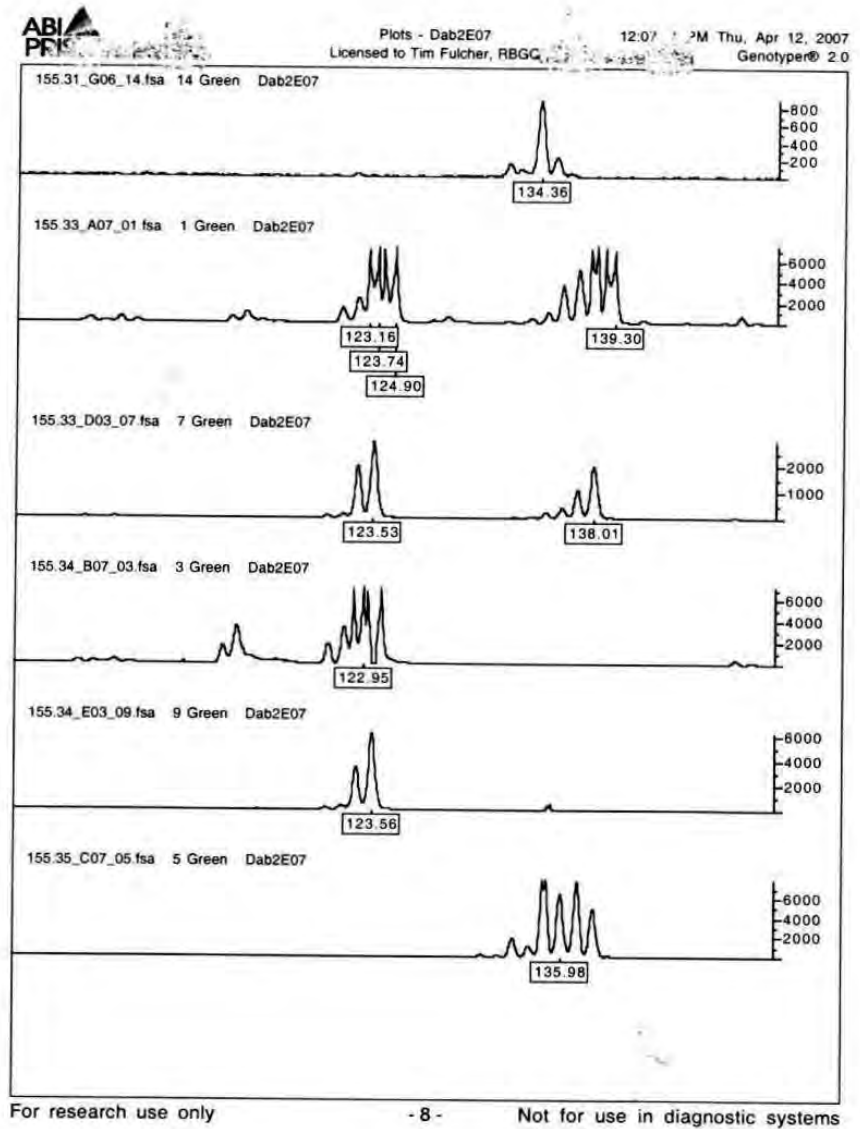
For research use only
- 7 -
Not for use in diagnostic systems



Plots - Dab2E07
Licensed to Tim Fulcher, RBGC
Thu, Apr 12, 2007
Genotyper® 2.0
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134.36
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123.16
123.74
124.90
139.30
155.33_D03_07.fsa 7 Green Dab2E07
123.53
138.01
155.34_B07_03.fsa 3 Green Dab2E07
122.95
155.34_E03_09.fsa 9 Green Dab2E07
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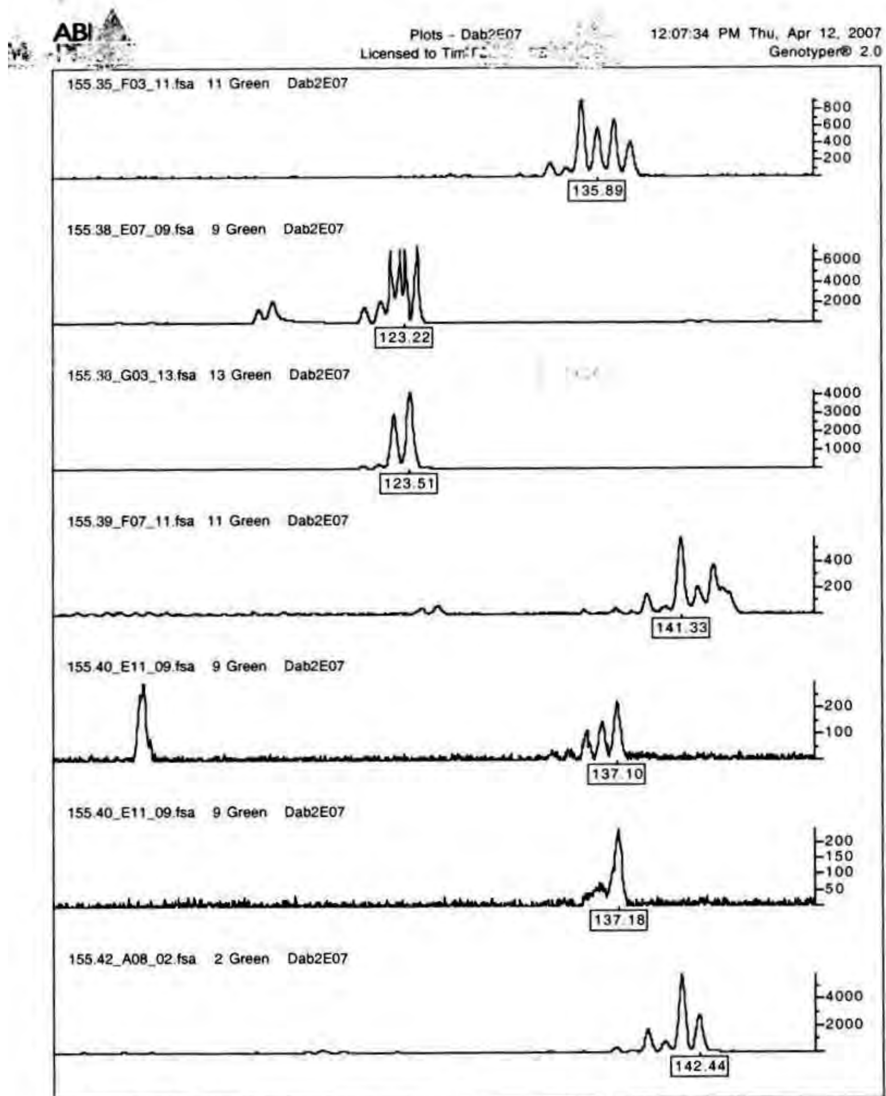
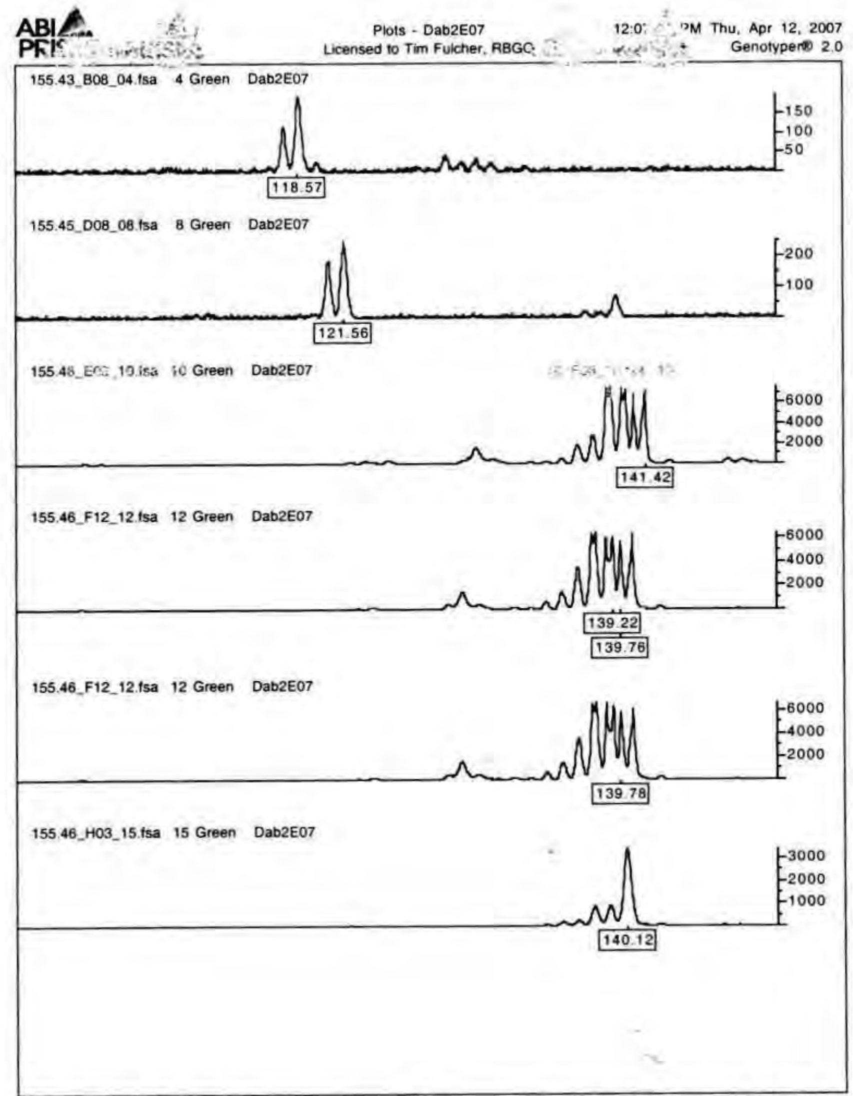
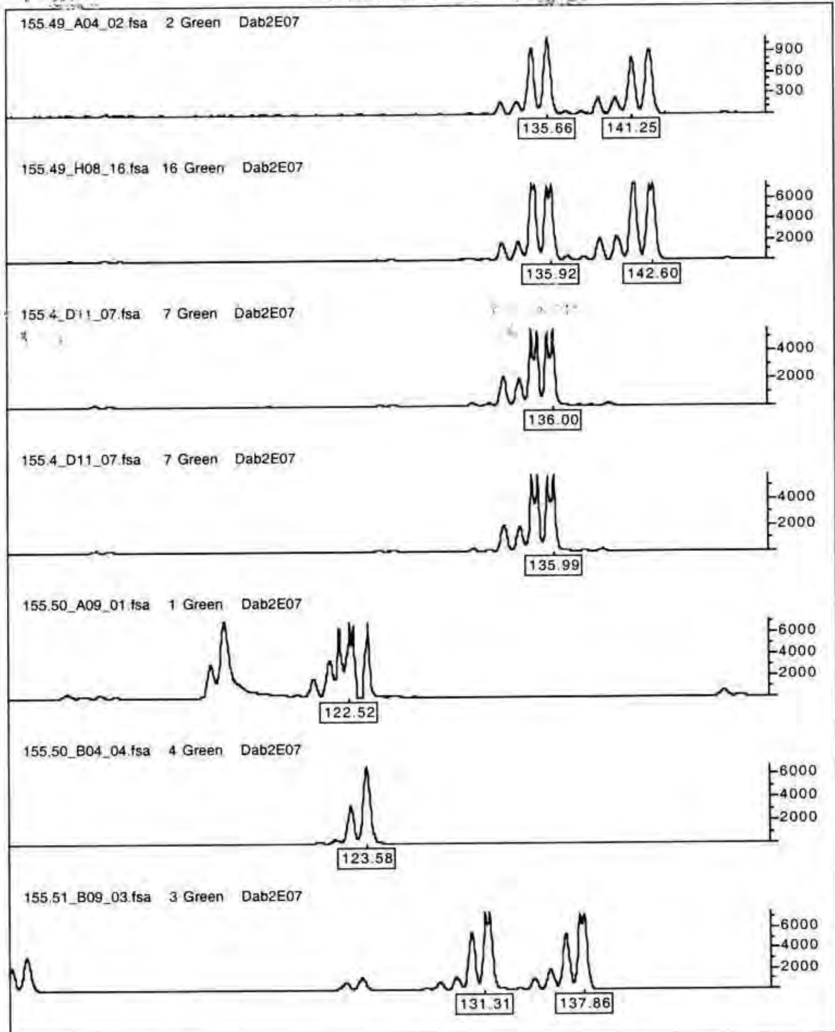
135.98
For research use only
- 8 -
Not for use in diagnostic systems



For research use only -9- Not for use in diagnostic systems



For research use only -10- Not for use in diagnostic systems


ABI PRISM
Plots - Dab2E07
Licensed to Tim Fulcher
12:07:35 PM Thu, Apr 12, 2007
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135.92
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136.00
155.4_D11_07.fsa 7 Green Dab2E07
135.99
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122.52
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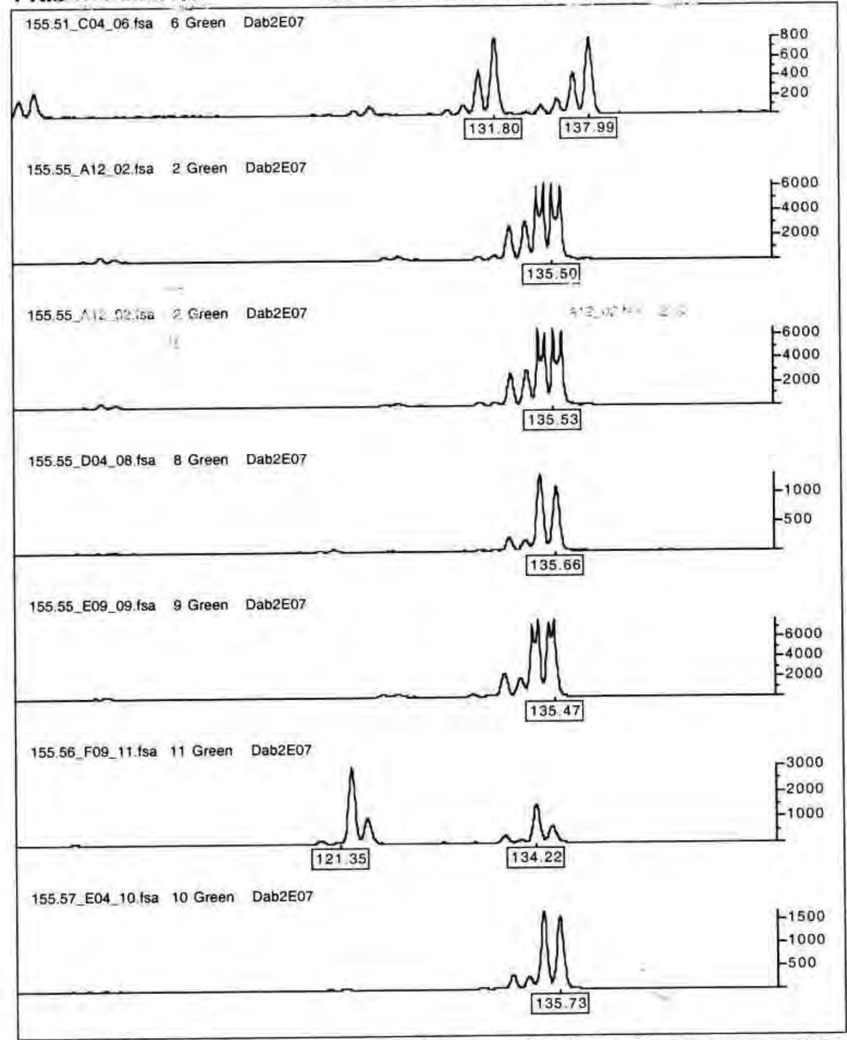
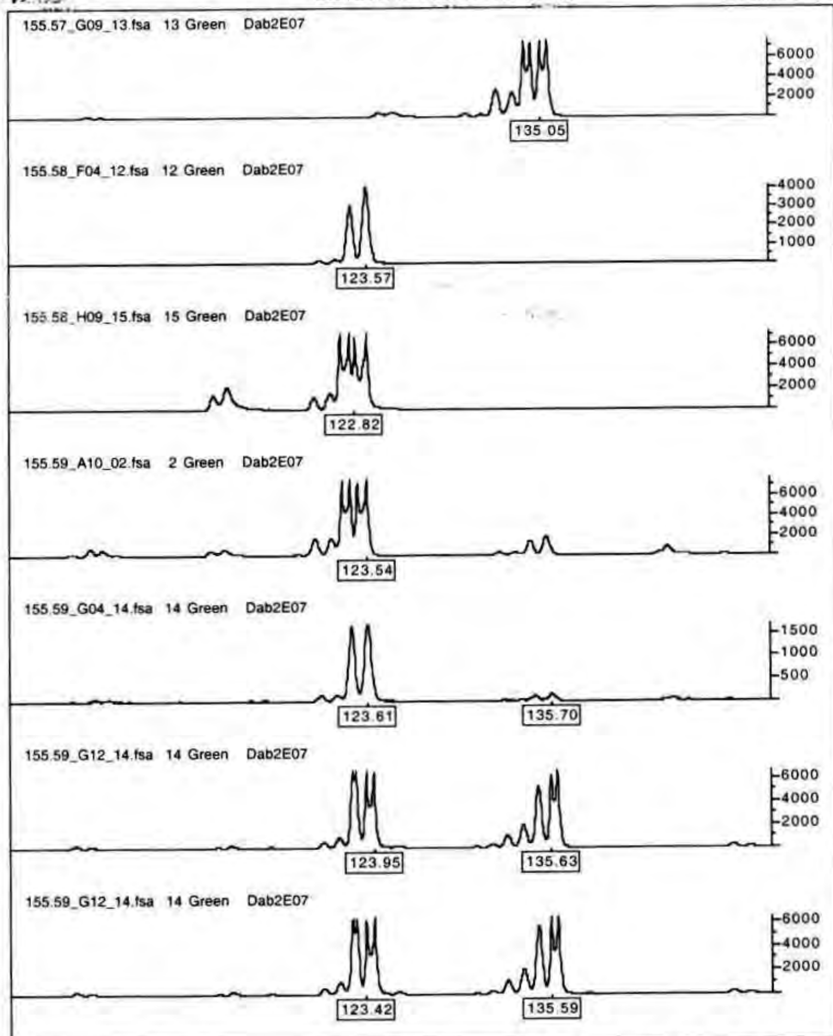
123.58
155.51_B09_03.fsa 3 Green Dab2E07
131.31
137.86
For research use only
-11-
Not for use in diagnostic systems


ABI PRISM
Plots - Dab2E07
Licensed to Tim Fulcher, RBGQ
Thu, Apr 12, 2007
Genotyper® 2.0
155.51_C04_06.fsa 6 Green Dab2E07
131.80
137.99
155.55_A12_02.fsa 2 Green Dab2E07
135.50
2 Green Dab2E07
135.53
155.55_D04_08.fsa 8 Green Dab2E07
135.66
155.55_E09_09.fsa 9 Green Dab2E07
135.47
155.56_F09_11.fsa 11 Green Dab2E07
121.35
134.22
155.57_E04_10.fsa 10 Green Dab2E07
135.73
For research use only
-12-
Not for use in diagnostic systems

Plots - Dab2E07
Licensed to Tim Ful...

12:07:35 PM Thu, Apr 12, 2007
Genotyper® 2.0



For research use only -13- Not for use in diagnostic systems

Plots - Dab2E07
Licensed to Tim Fulcher, RBGQ...

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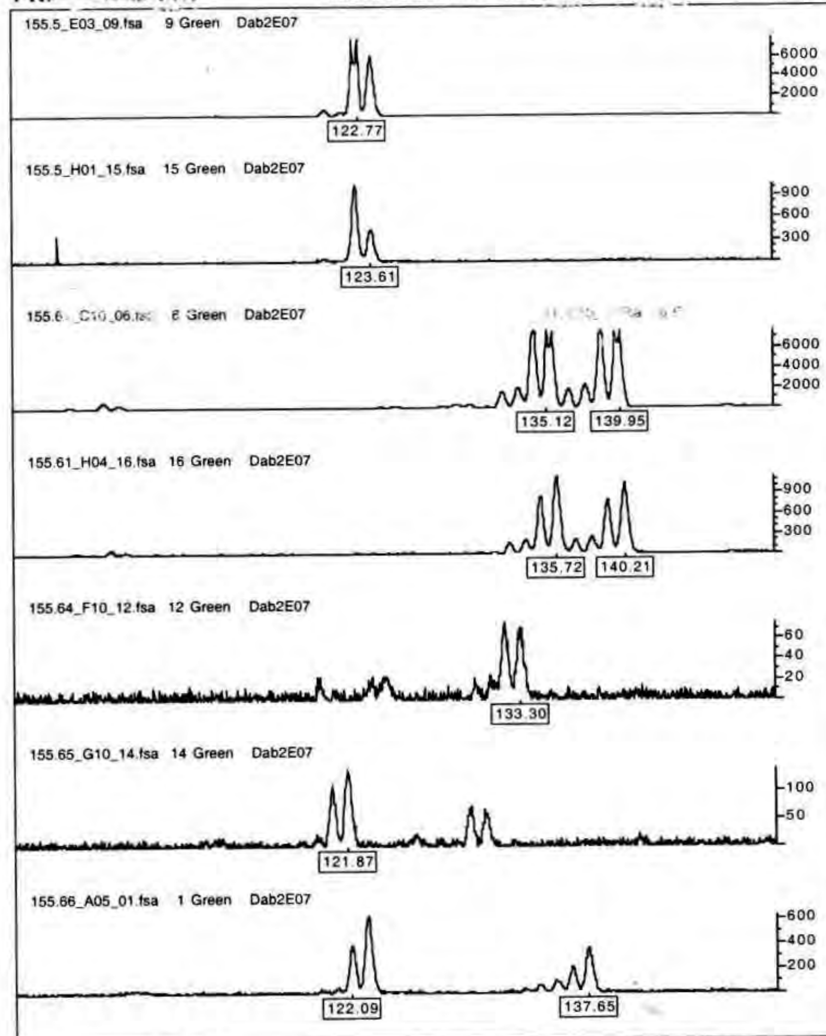
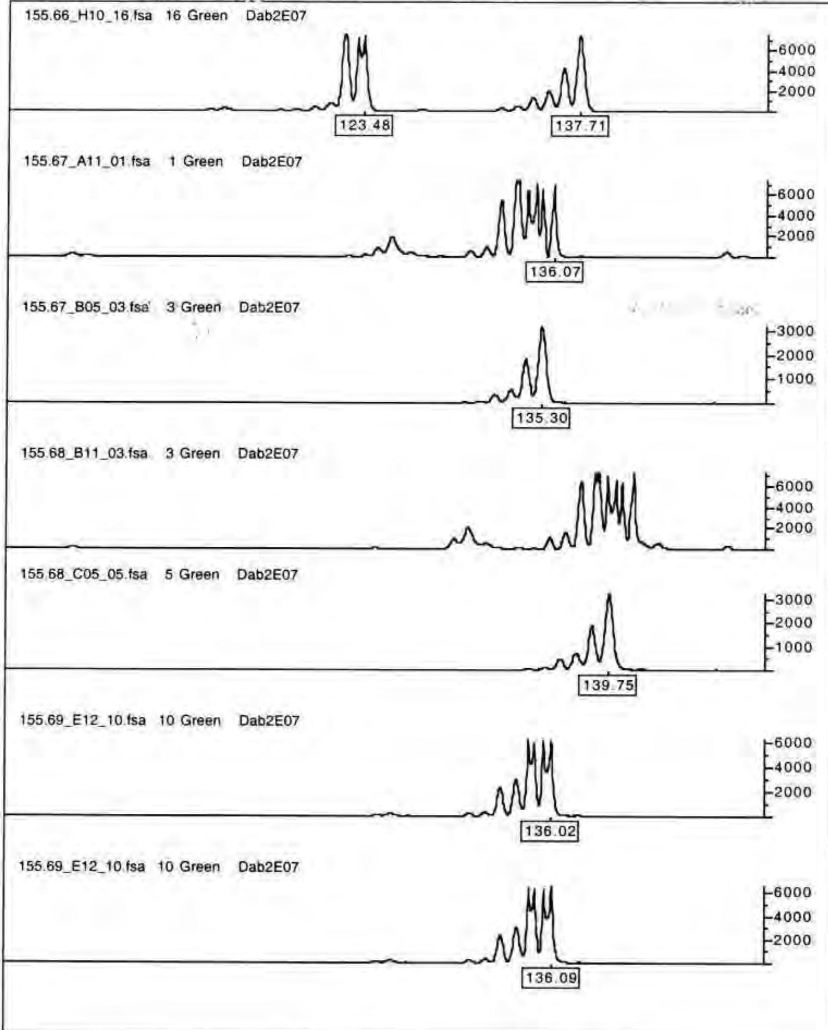
Genotyper® 2.0



For research use only -14- Not for use in diagnostic systems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Plots - Dab2E07
Licensed to Tim Fulcher, RBGQ
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136.07
155.67_B05_03.fsa 3 Green Dab2E07
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155.68_B11_03.fsa 3 Green Dab2E07
155.68_C05_05.fsa 5 Green Dab2E07
139.75
155.69_E12_10.fsa 10 Green Dab2E07
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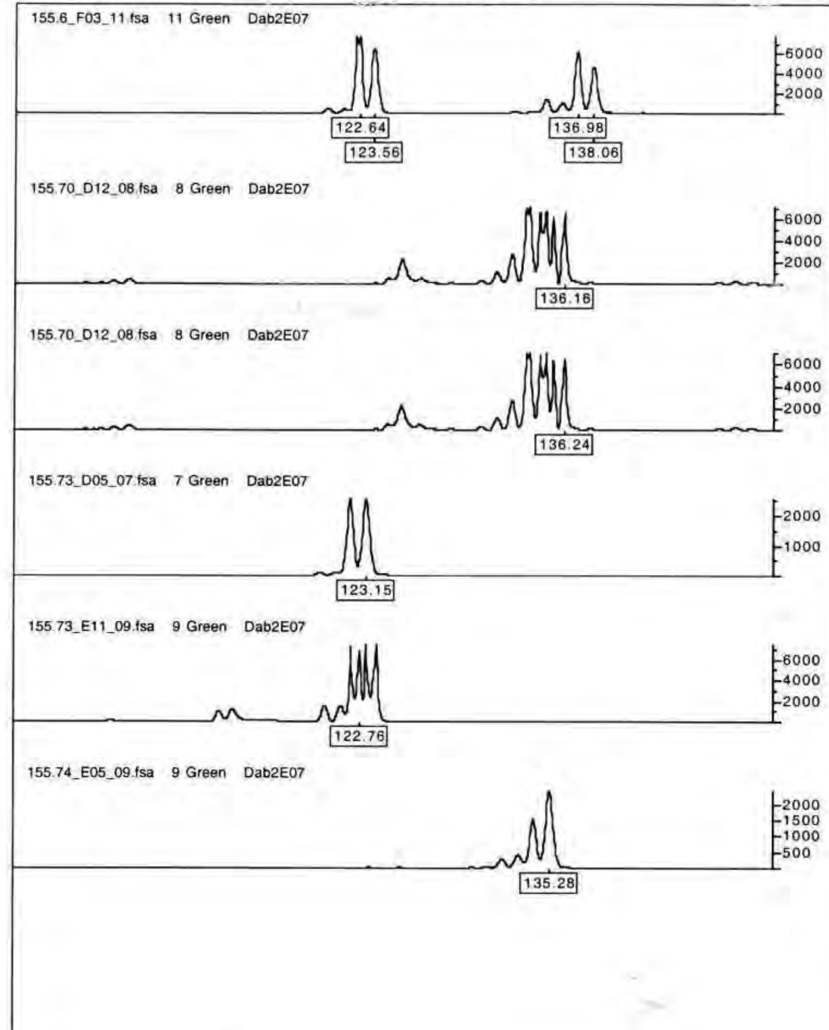
136.09
For research use only
-15-
Not for use in diagnostic systems


ABI PRISM
Tim Fulcher, RBGQ
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136.16
155.70_D12_08.fsa 8 Green Dab2E07
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155.73_D05_07.fsa 7 Green Dab2E07
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155.73_E11_09.fsa 9 Green Dab2E07
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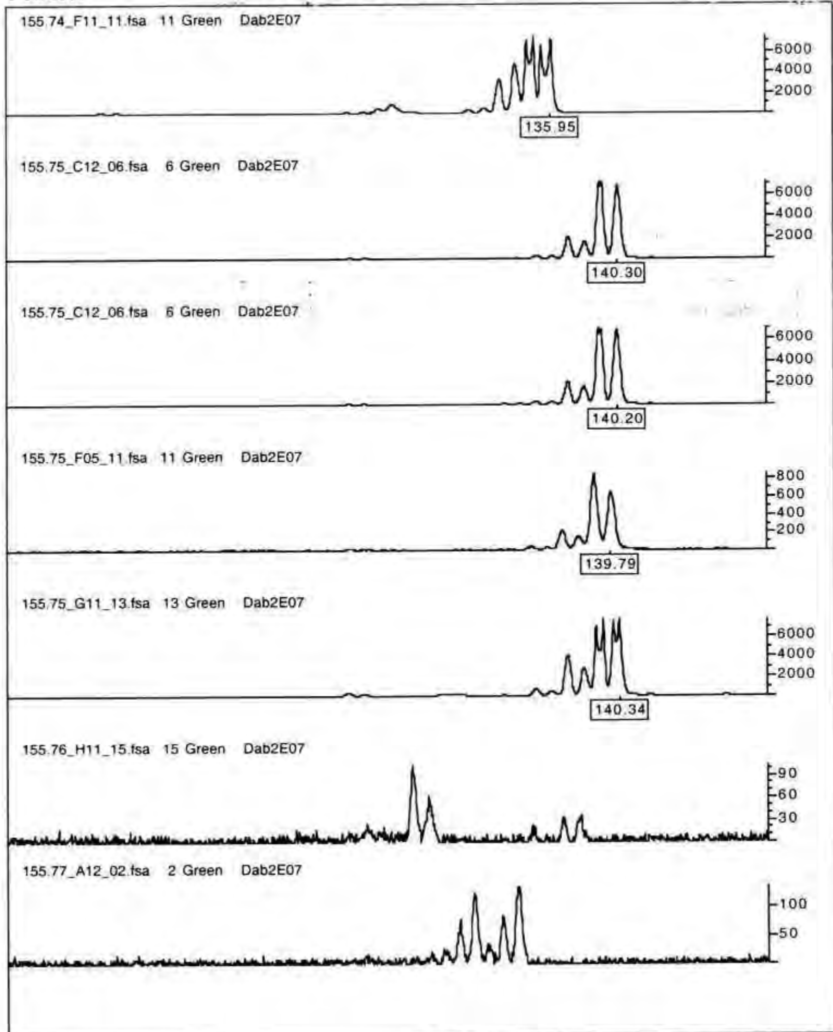
135.28
For research use only
-16-
Not for use in diagnostic systems



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155.75_F05_11.fsa 11 Green Dab2E07
139.79
155.75_G11_13.fsa 13 Green Dab2E07
140.34
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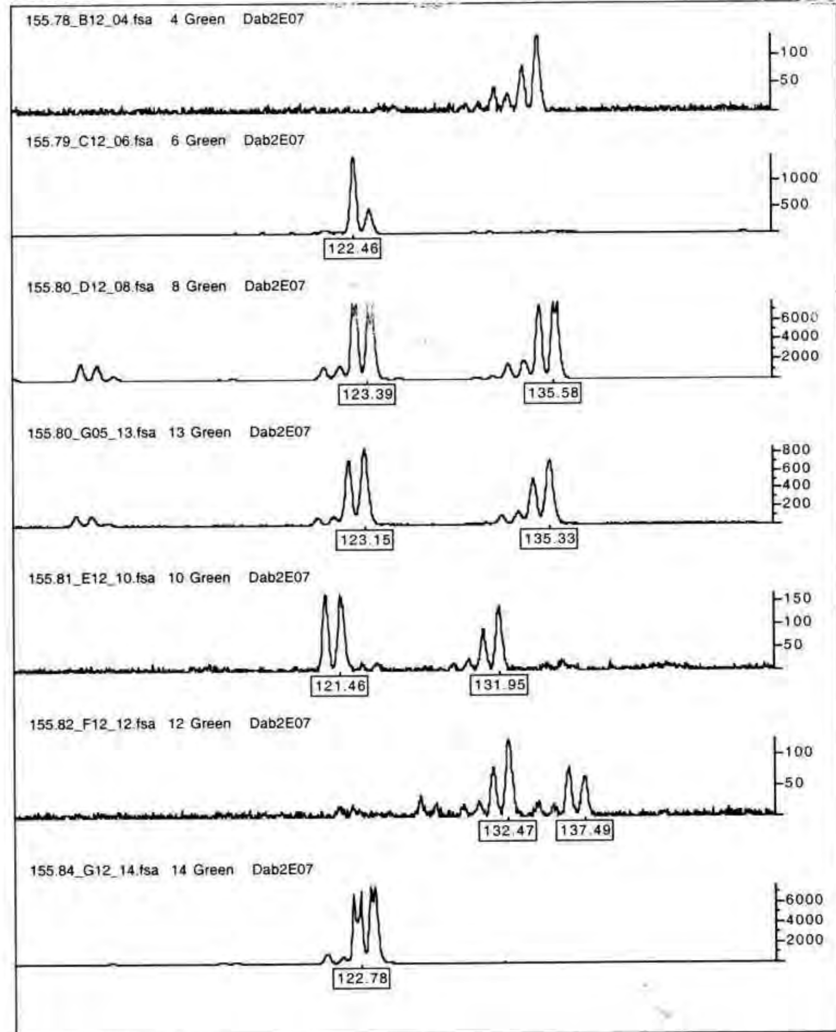
155.77_A12_02.fsa 2 Green Dab2E07
For research use only
-17-
Not for use in diagnostic systems



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122.46
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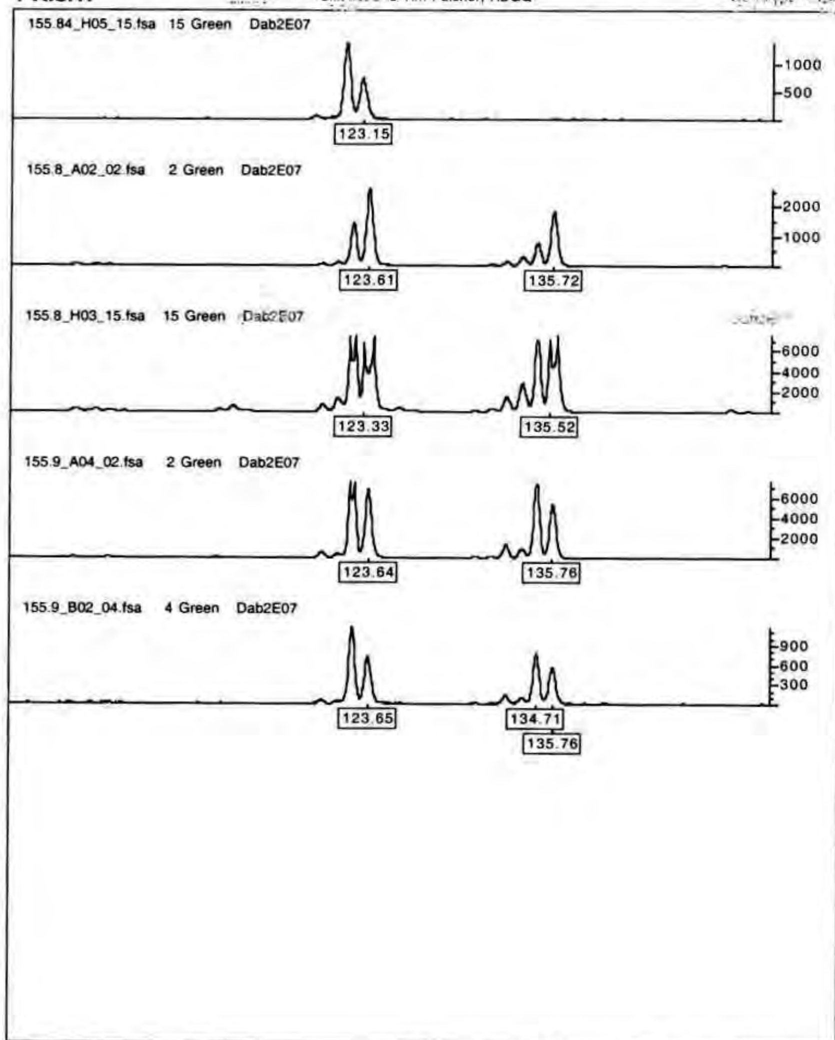
135.58
155.80_G05_13.fsa 13 Green Dab2E07
123.15
135.33
155.81_E12_10.fsa 10 Green Dab2E07
121.46
131.95
155.82_F12_12.fsa 12 Green Dab2E07
132.47
137.49
155.84_G12_14.fsa 14 Green Dab2E07
122.78
For research use only
-18-
Not for use in diagnostic systems



Plots - Dab2E07
to Tim Fulcher, RBGQ
12:07:35 PM Thu, Apr 12, 20
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123.15
155.8_A02_02.fsa 2 Green Dab2E07
2000
1000
123.61
135.72
155.8_H03_15.fsa 15 Green Dab2E07
6000
4000
2000
123.33
135.52
155.9_A04_02.fsa 2 Green Dab2E07
6000
4000
2000
123.64
135.76
155.9_B02_04.fsa 4 Green Dab2E07
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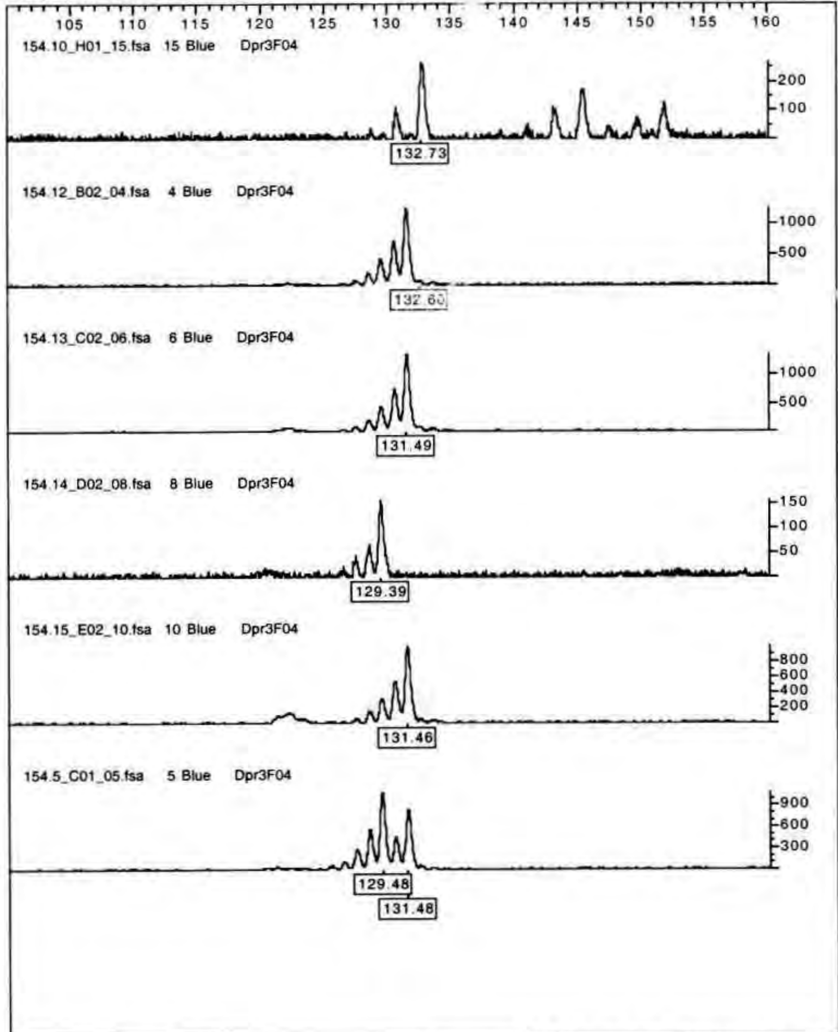
600
300
123.65
134.71
135.76
For research use only
-19-
Not for use in diagnostic systems



Plots - Dpr3F04
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Genotyper® 2.0
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132.73
154.12_B02_04.fsa 4 Blue Dpr3F04
132.60
154.13_C02_06.fsa 6 Blue Dpr3F04
131.49
154.14_D02_08.fsa 8 Blue Dpr3F04
129.39
154.15_E02_10.fsa 10 Blue Dpr3F04
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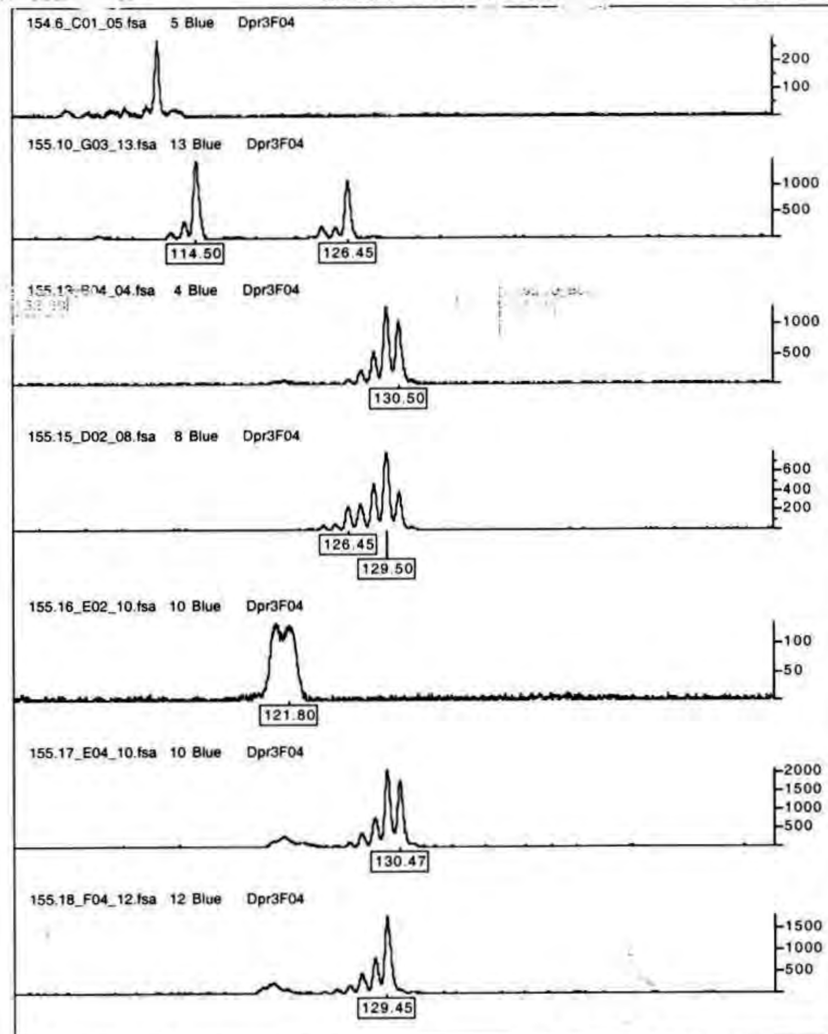
154.5_C01_05.fsa 5 Blue Dpr3F04
129.48
131.48
For research use only
- 1 -
Not for use in diagnostic systems



Plots - Dpr3Fo4
Licensed to Tim Fulch
12:09:58 PM Thu, Apr 12, 2007
Genotyper® 2.0
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155.10_G03_13.fsa 13 Blue Dpr3F04
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126.45
155.13_B04_04.fsa 4 Blue Dpr3F04
130.50
155.15_D02_08.fsa 8 Blue Dpr3F04
126.45
129.50
155.16_E02_10.fsa 10 Blue Dpr3F04
121.80
155.17_E04_10.fsa 10 Blue Dpr3F04
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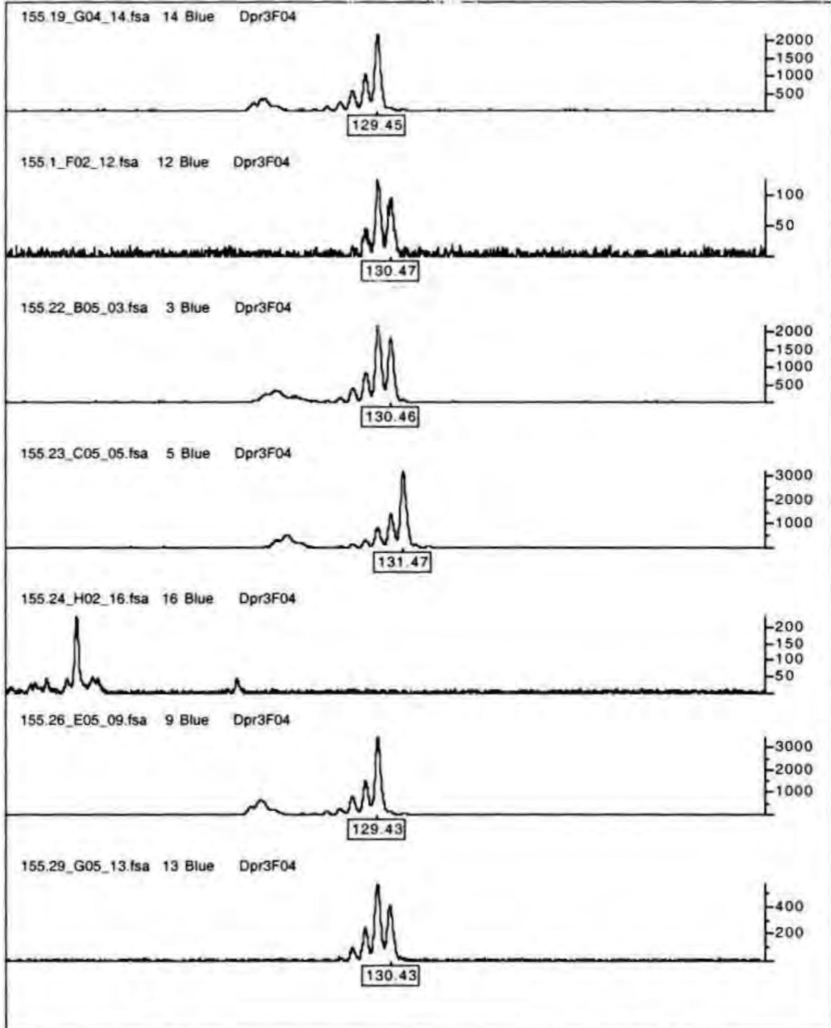
155.18_F04_12.fsa 12 Blue Dpr3F04
129.45
For research use only
- 2 -
Not for use in diagnostic systems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1000
500
129.45
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100
50
130.47
155.22_B05_03.fsa 3 Blue Dpr3F04
2000
1500
1000
500
130.46
155.23_C05_05.fsa 5 Blue Dpr3F04
3000
2000
1000
131.47
155.24_H02_16.fsa 16 Blue Dpr3F04
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150
100
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3000
2000
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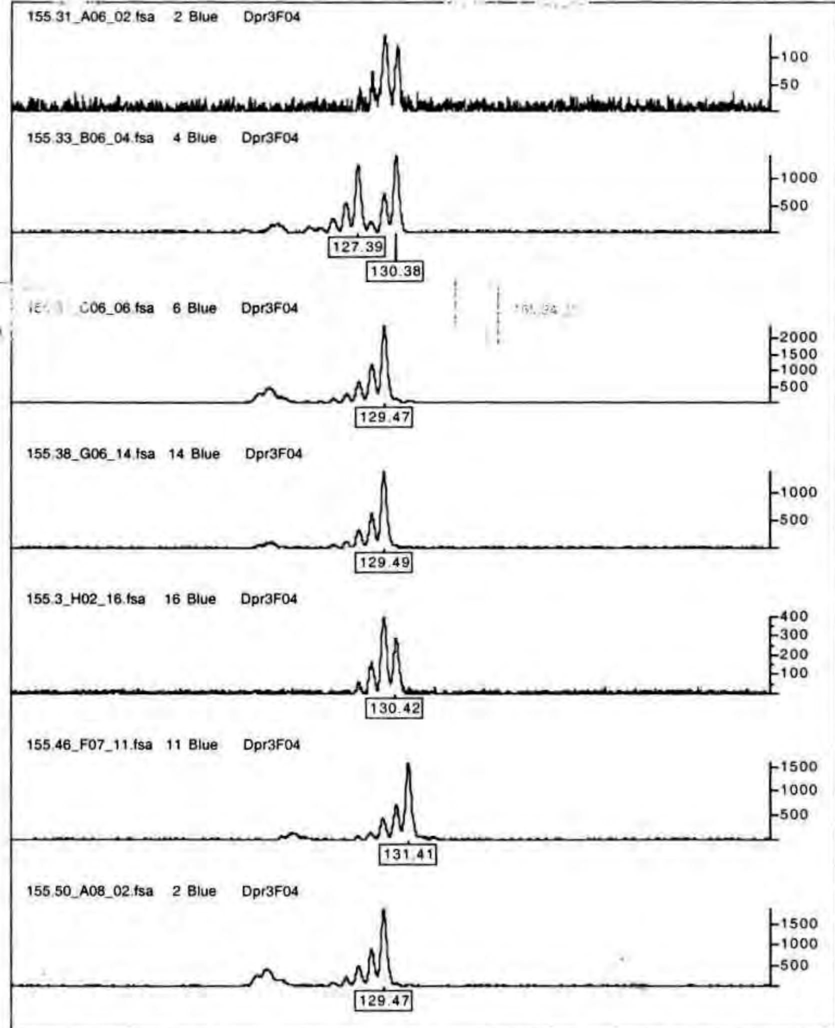
200
130.43
For research use only
- 3 -
Not for use in diagnostic systems


Plots - Dpr3F04
Licensed to Tim
12:09:58 PM Thu, Apr 12, 2007
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1000
500
127.39
130.38
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500
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155.3_H02_16.fsa 16 Blue Dpr3F04
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100
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1000
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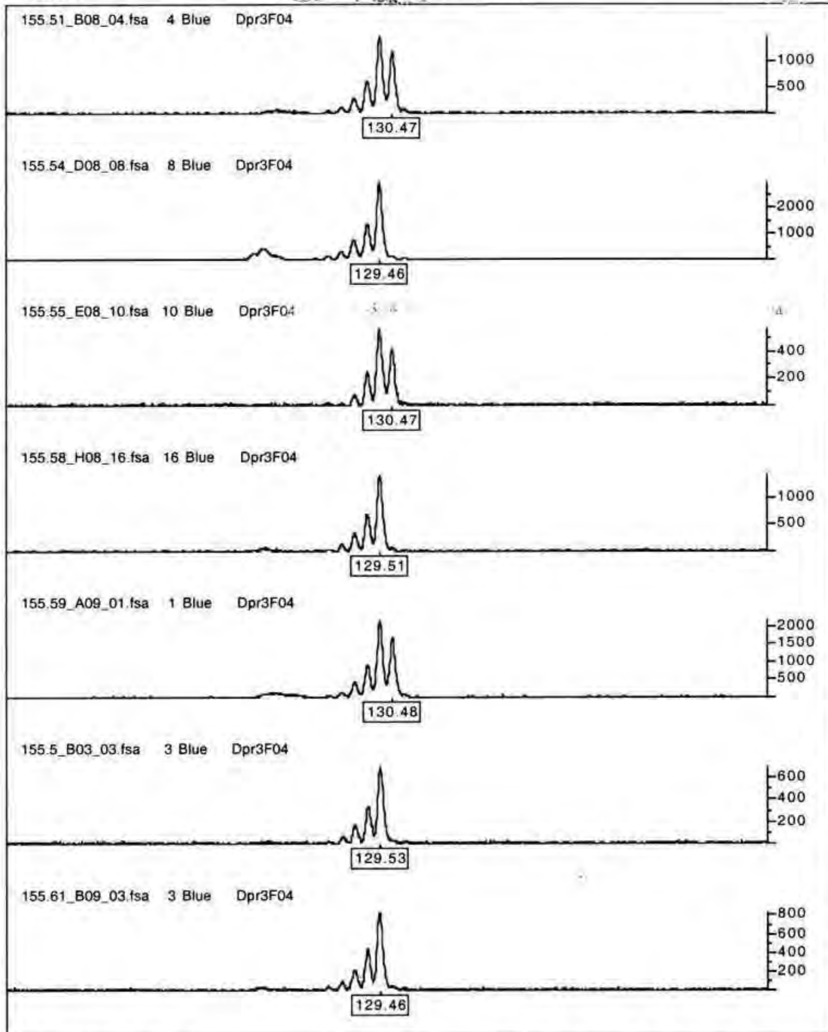
1000
500
129.47
For research use only
- 4 -
Not for use in diagnostic systems



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155.55_E08_10.fsa 10 Blue Dpr3F04
130.47
155.58_H08_16.fsa 16 Blue Dpr3F04
129.51
155.59_A09_01.fsa 1 Blue Dpr3F04
130.48
155.5_B03_03.fsa 3 Blue Dpr3F04
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155.61_B09_03.fsa 3 Blue Dpr3F04
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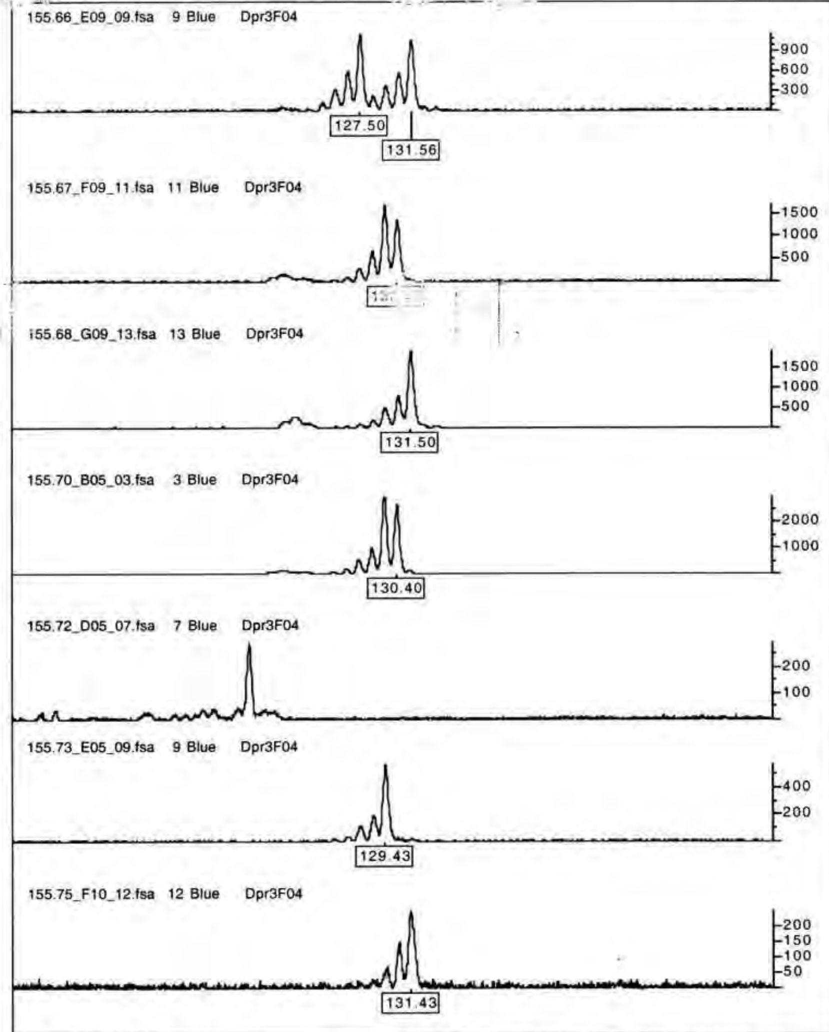
For research use only
- 5 -
Not for use in diagnostic systems



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155.68_G09_13.fsa 13 Blue Dpr3F04
131.50
155.70_B05_03.fsa 3 Blue Dpr3F04
130.40
155.72_D05_07.fsa 7 Blue Dpr3F04
155.73_E05_09.fsa 9 Blue Dpr3F04
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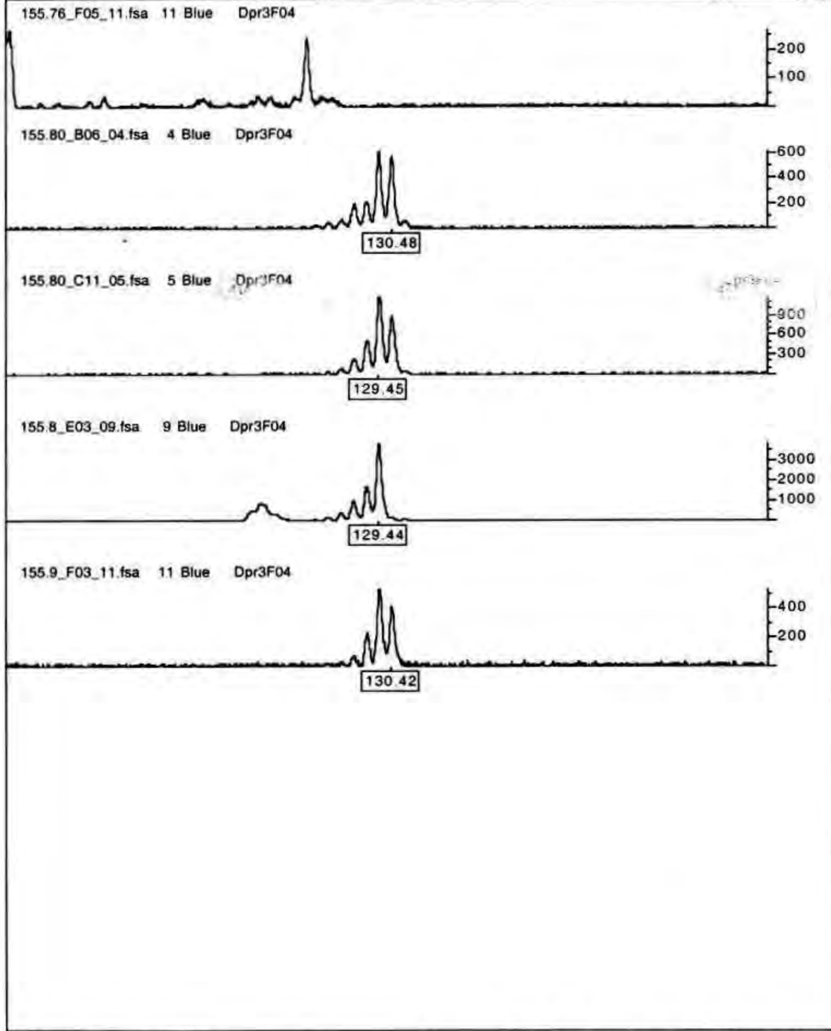
155.75_F10_12.fsa 12 Blue Dpr3F04
131.43
For research use only
- 6 -
Not for use in diagnostic systems



Plots - Dpr3Fo4
to Tim Fulcher, RBGQ
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600
400
200
130.48
155.80_C11_05.fsa 5 Blue Dpr3F04
900
600
300
129.45
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3000
2000
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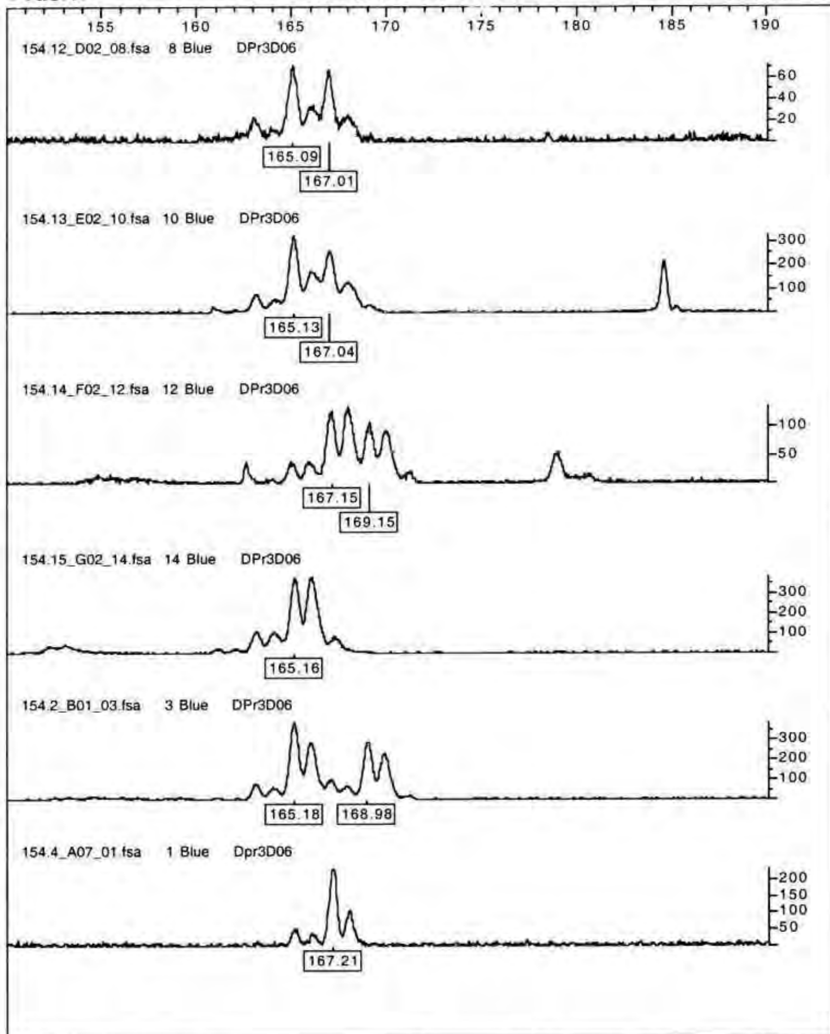
200
130.42
For research use only
- 7 -
Not for use in diagnostic systems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Genotyper® 2.0
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154.12_D02_08.fsa 8 Blue DPr3D06
165.09
167.01
154.13_E02_10.fsa 10 Blue DPr3D06
165.13
167.04
154.14_F02_12.fsa 12 Blue DPr3D06
167.15
169.15
154.15_G02_14.fsa 14 Blue DPr3D06
165.16
154.2_B01_03.fsa 3 Blue DPr3D06
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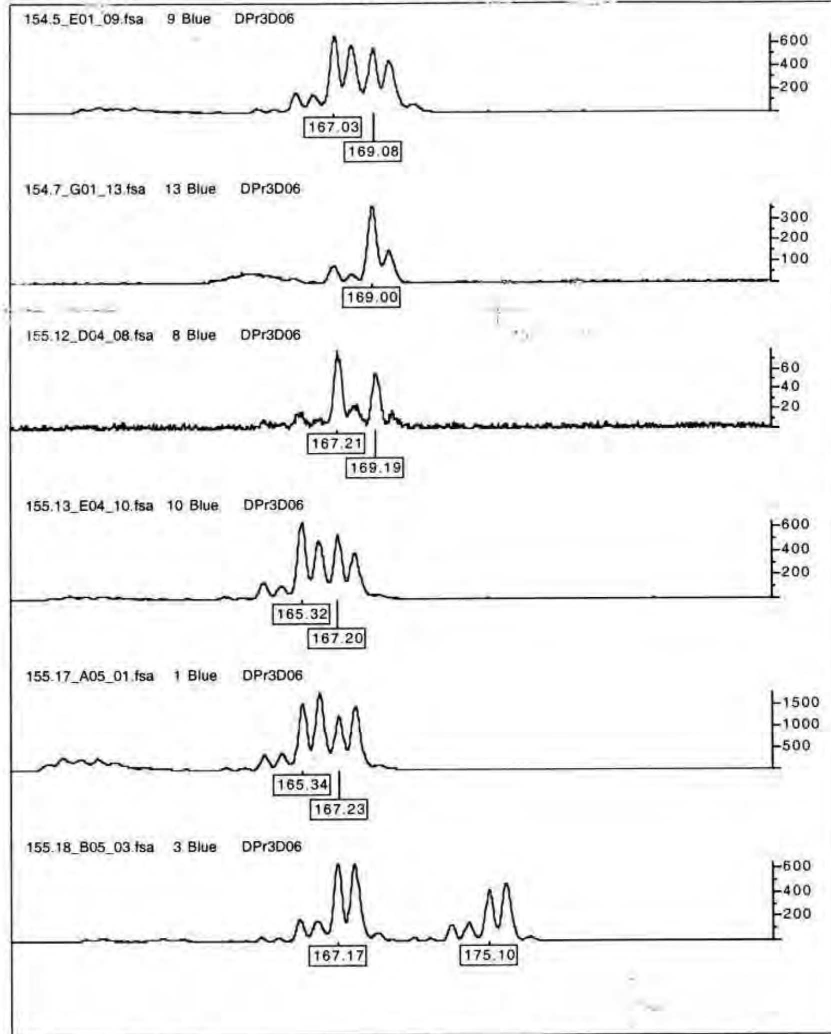
168.98
154.4_A07_01.fsa 1 Blue Dpr3D06
167.21
For research use only
- 1 -
Not for use in diagnostic systems


Plots - Dpr3D06
Licensed to Tim Fulcher
12:09:12 PM Thu, Apr 12, 2007
Genotyper® 2.0
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167.03
169.08
154.7_G01_13.fsa 13 Blue DPr3D06
169.00
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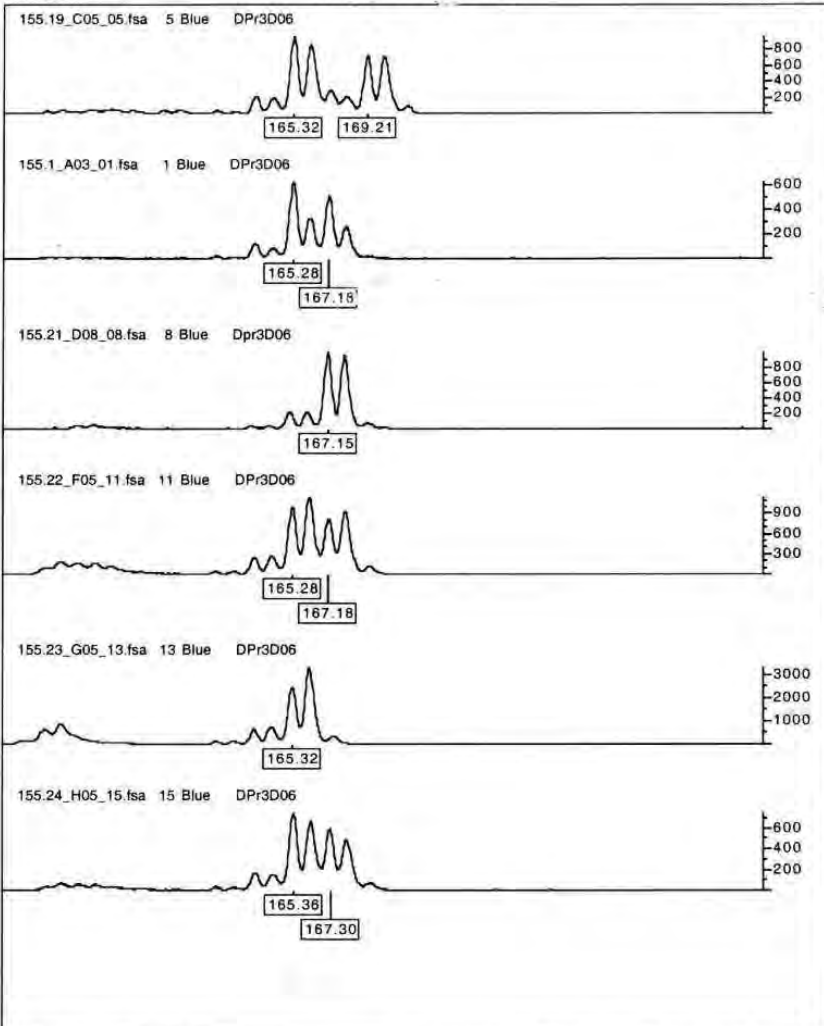
175.10
For research use only
- 2 -
Not for use in diagnostic systems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165.28
167.18
155.21_D08_08.fsa 8 Blue Dpr3D06
167.15
155.22_F05_11.fsa 11 Blue DPr3D06
165.28
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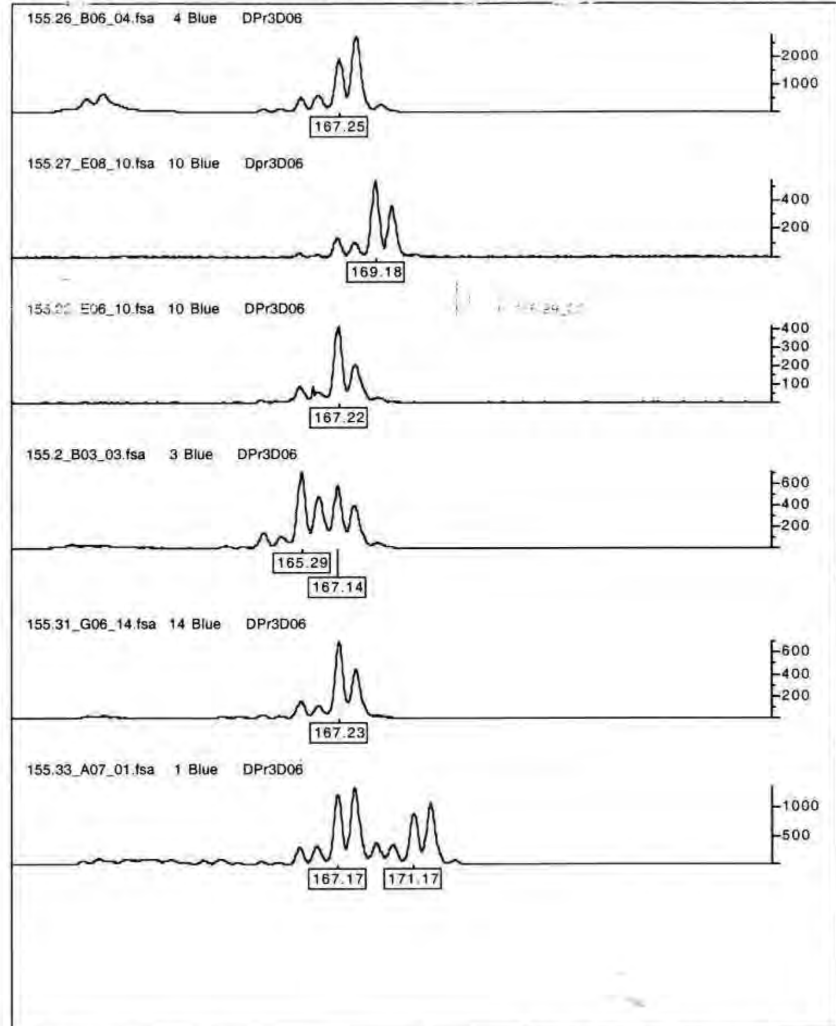
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E06_10.fsa 10 Blue DPr3D06
167.22
155.2_B03_03.fsa 3 Blue DPr3D06
165.29
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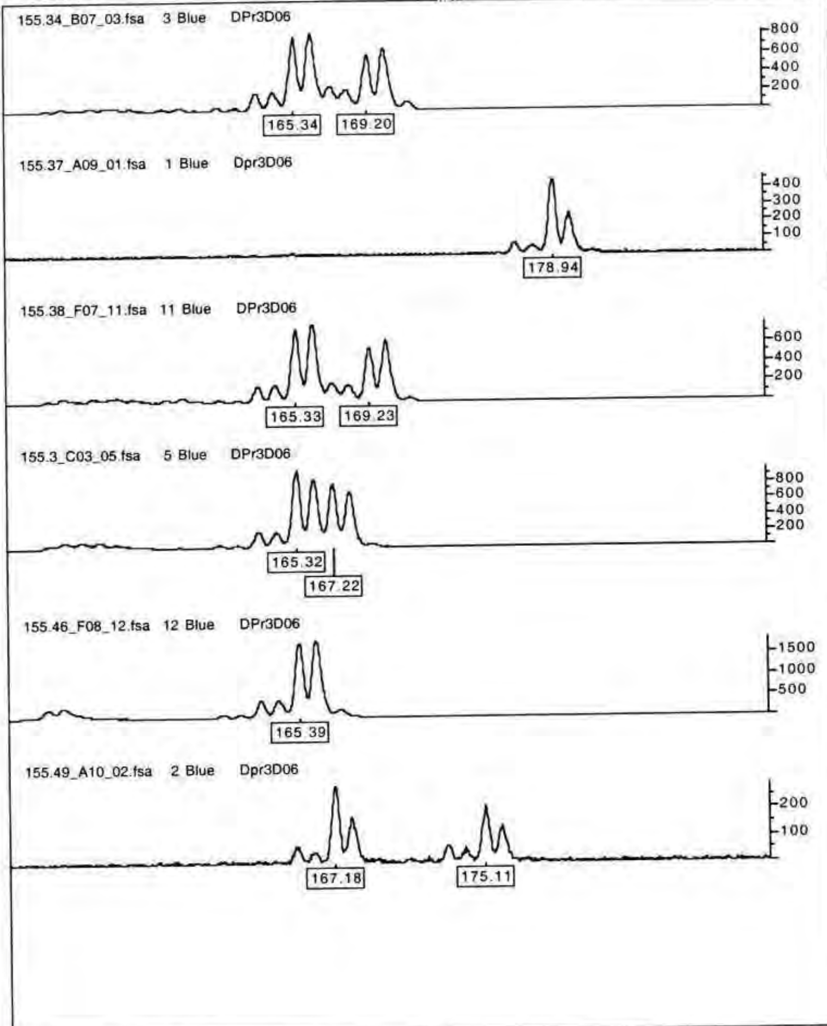
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155.38_F07_11.fsa 11 Blue DPr3D06
165.33
169.23
155.3_C03_05.fsa 5 Blue DPr3D06
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155.49_A10_02.fsa 2 Blue Dpr3D06
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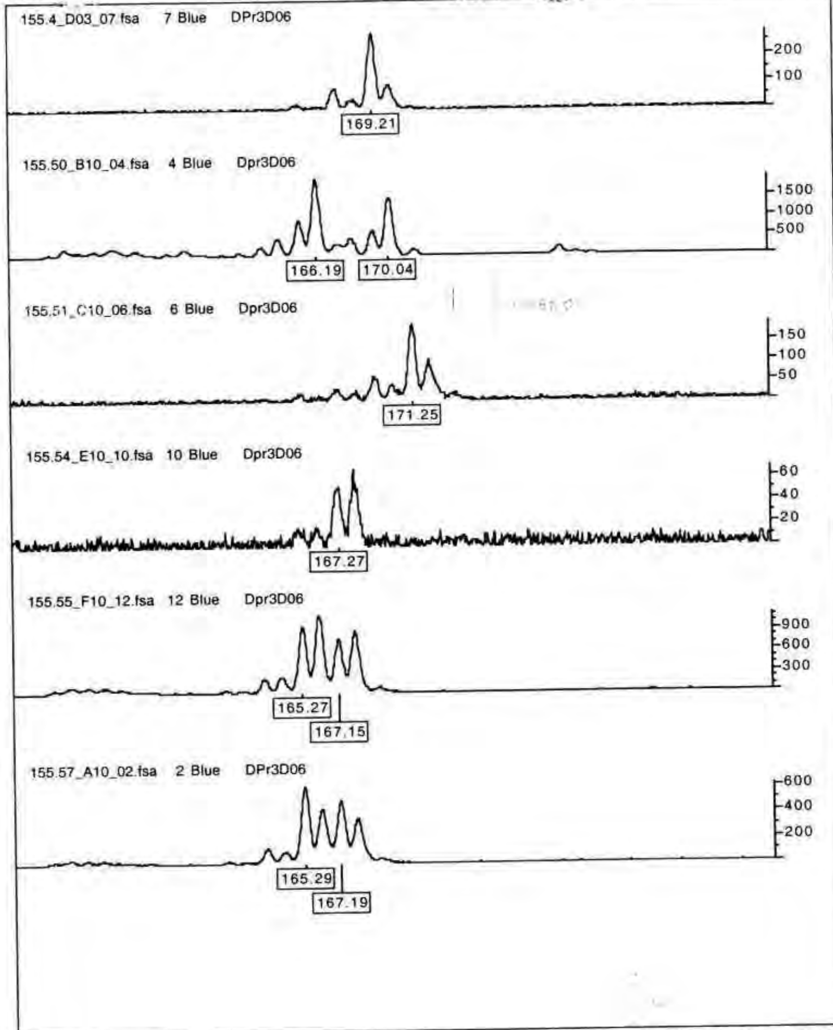
For research use only
- 5 -
Not for use in diagnostic systems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Licensed to Tim Fal
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170.04
155.51_C10_06.fsa 6 Blue Dpr3D06
171.25
155.54_E10_10.fsa 10 Blue Dpr3D06
167.27
155.55_F10_12.fsa 12 Blue Dpr3D06
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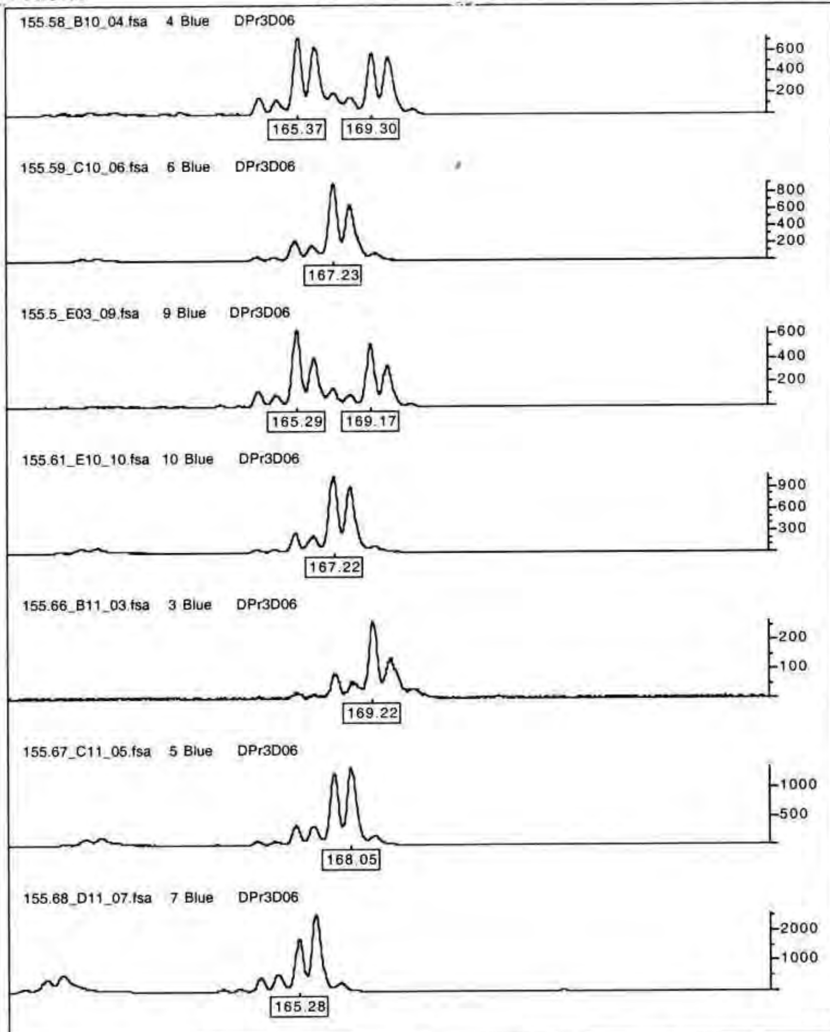
For research use only
- 6 -
Not for use in diagnostic systems



Plots - Dpr3D06
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Genotyper® 2.0
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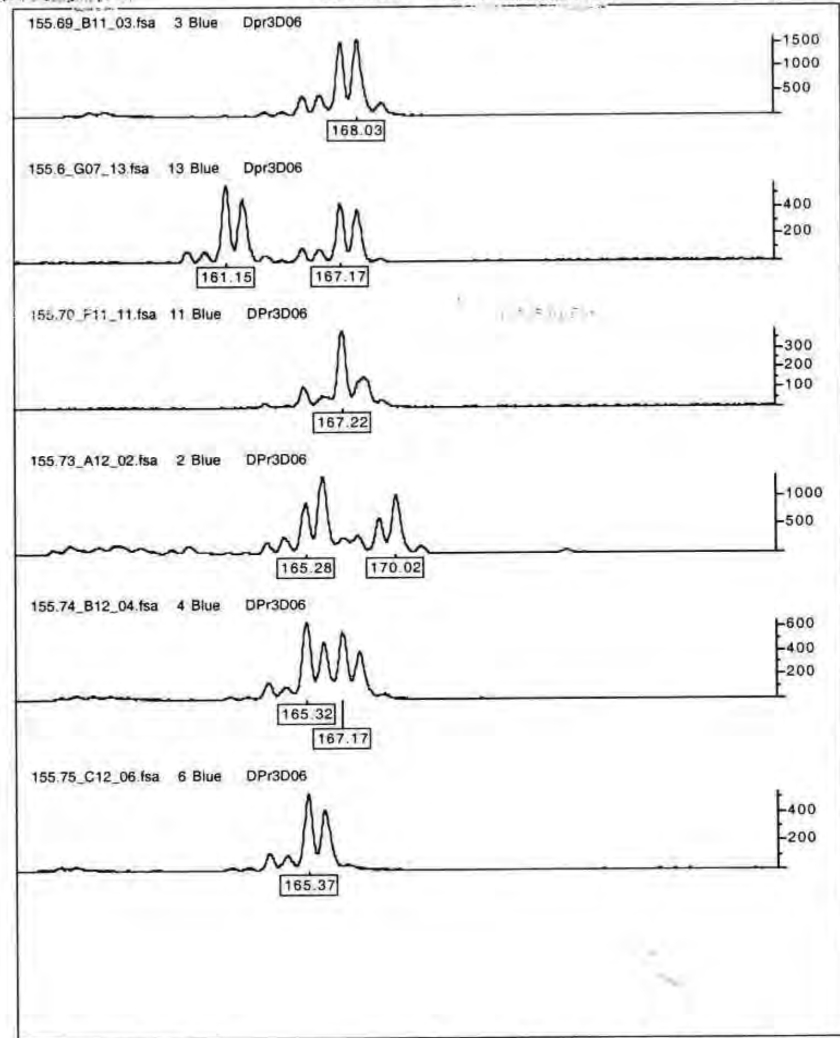
167.23
155.5_E03_09.fsa 9 Blue DPr3D06
165.29
169.17
155.61_E10_10.fsa 10 Blue DPr3D06
167.22
155.66_B11_03.fsa 3 Blue DPr3D06
169.22
155.67_C11_05.fsa 5 Blue DPr3D06
168.05
155.68_D11_07.fsa 7 Blue DPr3D06
165.28
For research use only
- 7 -
Not for use in diagnostic systems



Plots - Dpr3D06
Licensed to Tim Fulton
12:09:12 PM Thu, Apr 12, 2007
Genotyper® 2.0
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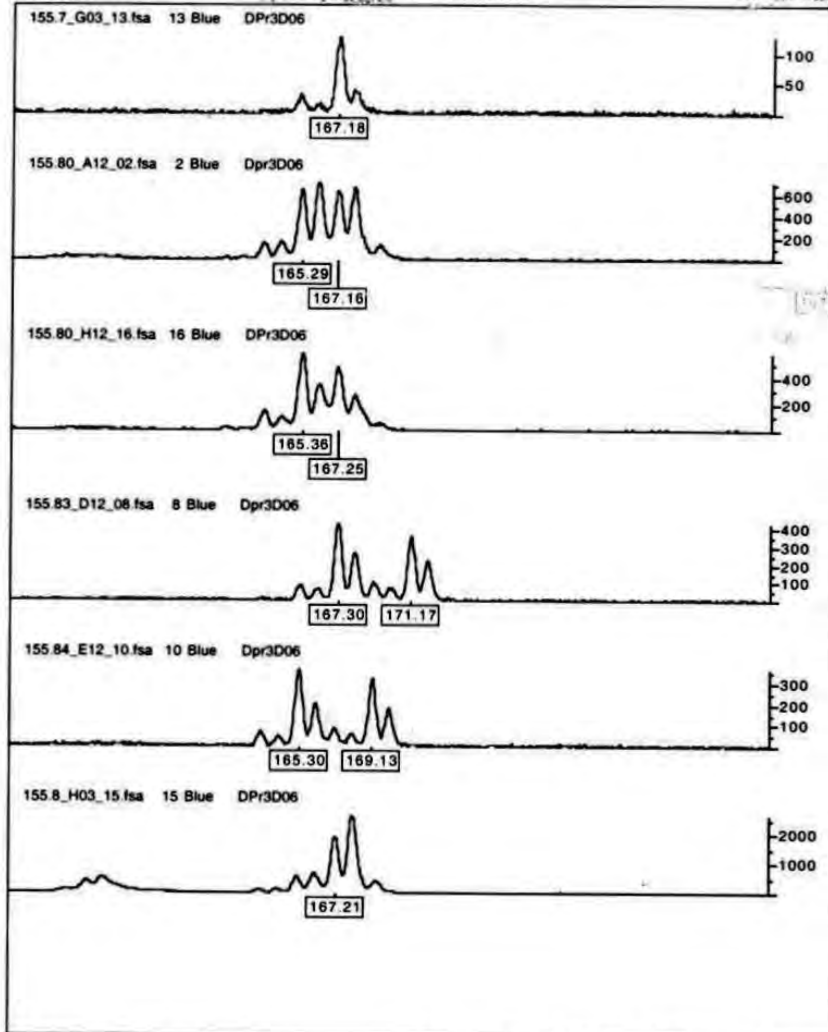
168.03
155.6_G07_13.fsa 13 Blue Dpr3D06
161.15
167.17
155.70_F11_11.fsa 11 Blue DPr3D06
167.22
155.73_A12_02.fsa 2 Blue DPr3D06
165.28
170.02
155.74_B12_04.fsa 4 Blue DPr3D06
165.32
167.17
155.75_C12_06.fsa 6 Blue DPr3D06
165.37
For research use only
- 8 -
Not for use in diagnostic systems



Plots - Dpr3D06
Tim Fulcher, RBGQ
12:09:12 PM Thu, Apr 12, 2007
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155.80_A12_02.fsa 2 Blue Dpr3D06
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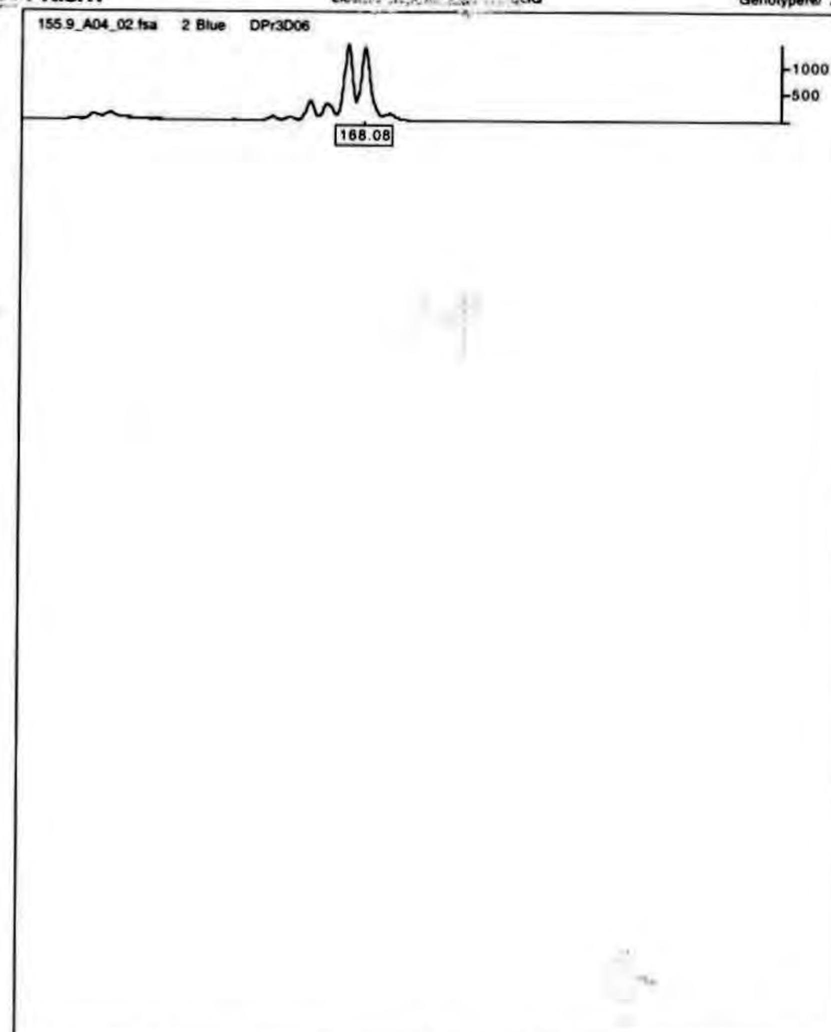
155.80_H12_16.fsa 16 Blue DPr3D06
165.36
167.25
155.83_D12_08.fsa 8 Blue Dpr3D06
167.30
171.17
155.84_E12_10.fsa 10 Blue Dpr3D06
165.30
169.13
155.8_H03_15.fsa 15 Blue DPr3D06
167.21
For research use only
-9-
Not for use in diagnostic systems



Plots - Dpr3
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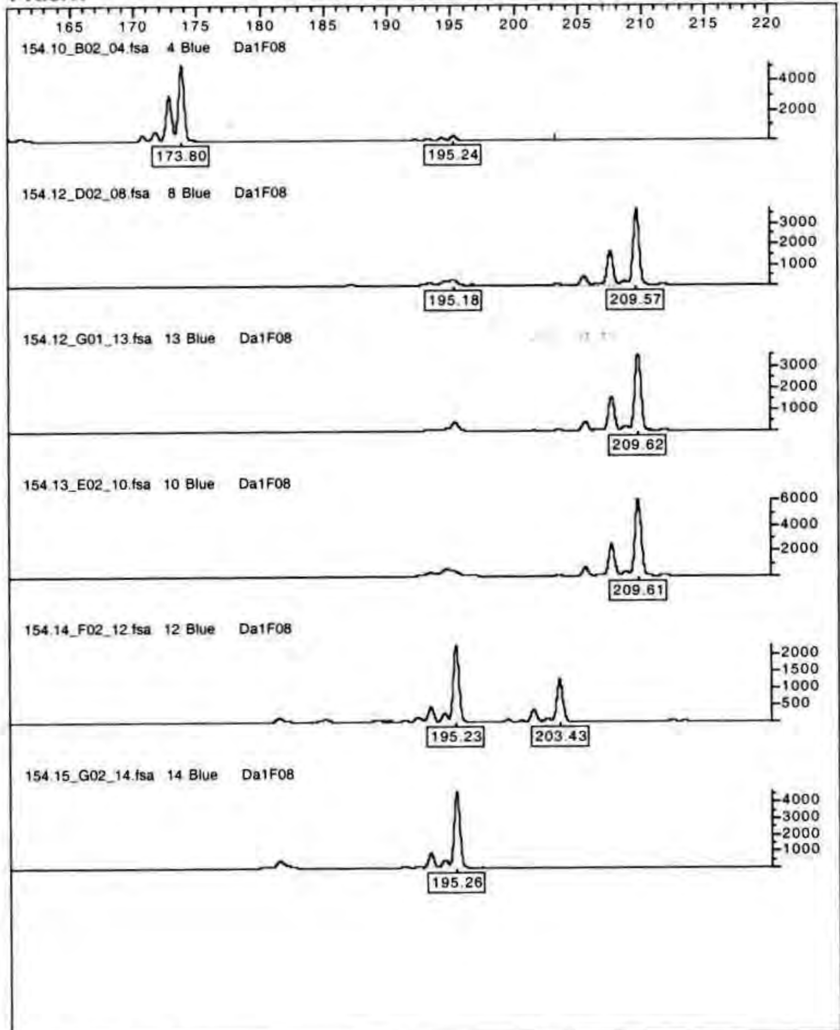
168.08
For research use only
-10-
Not for use in diagnostic systems


Plots - Da1F08
Licensed to Tim Fulcher, RBGQ
12:02:59 PM Thu, Apr 12, 2007
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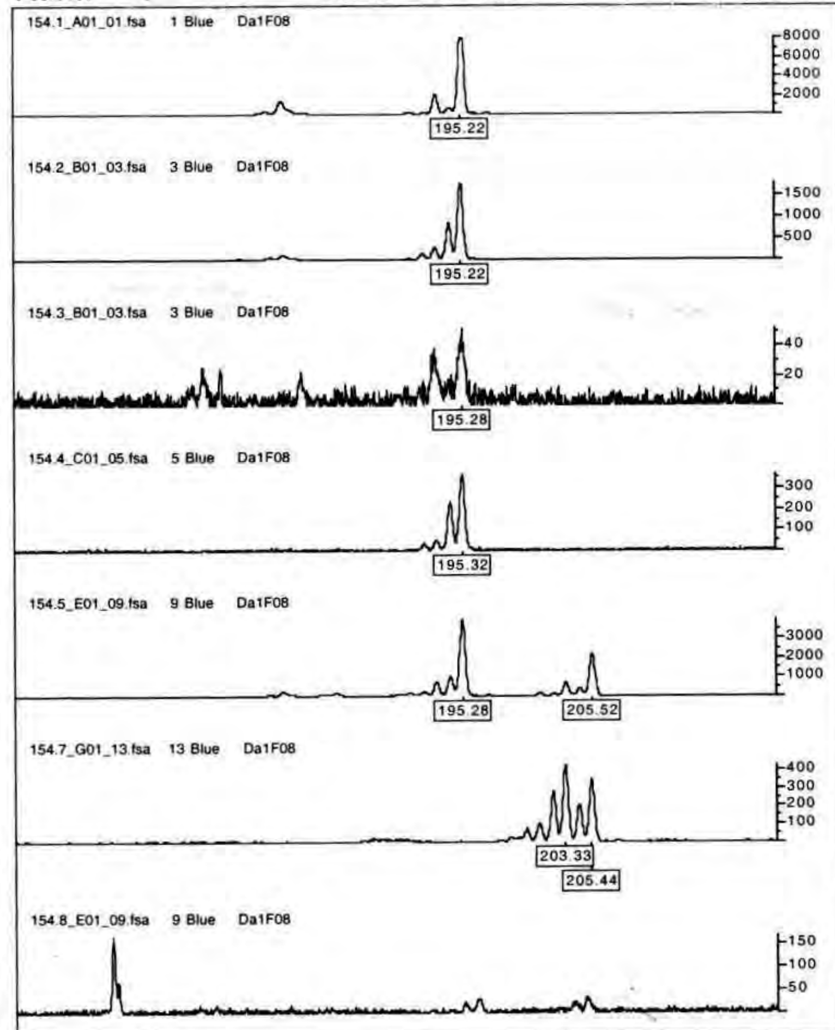
173.80
195.24
154.12_D02_08.fsa 8 Blue Da1F08
195.18
209.57
154.12_G01_13.fsa 13 Blue Da1F08
209.62
154.13_E02_10.fsa 10 Blue Da1F08
209.61
154.14_F02_12.fsa 12 Blue Da1F08
195.23
203.43
154.15_G02_14.fsa 14 Blue Da1F08
195.26
For research use only
- 1 -
Not for use in diagnostic systems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154.3_B01_03.fsa 3 Blue Da1F08
195.28
154.4_C01_05.fsa 5 Blue Da1F08
195.32
154.5_E01_09.fsa 9 Blue Da1F08
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205.52
154.7_G01_13.fsa 13 Blue Da1F08
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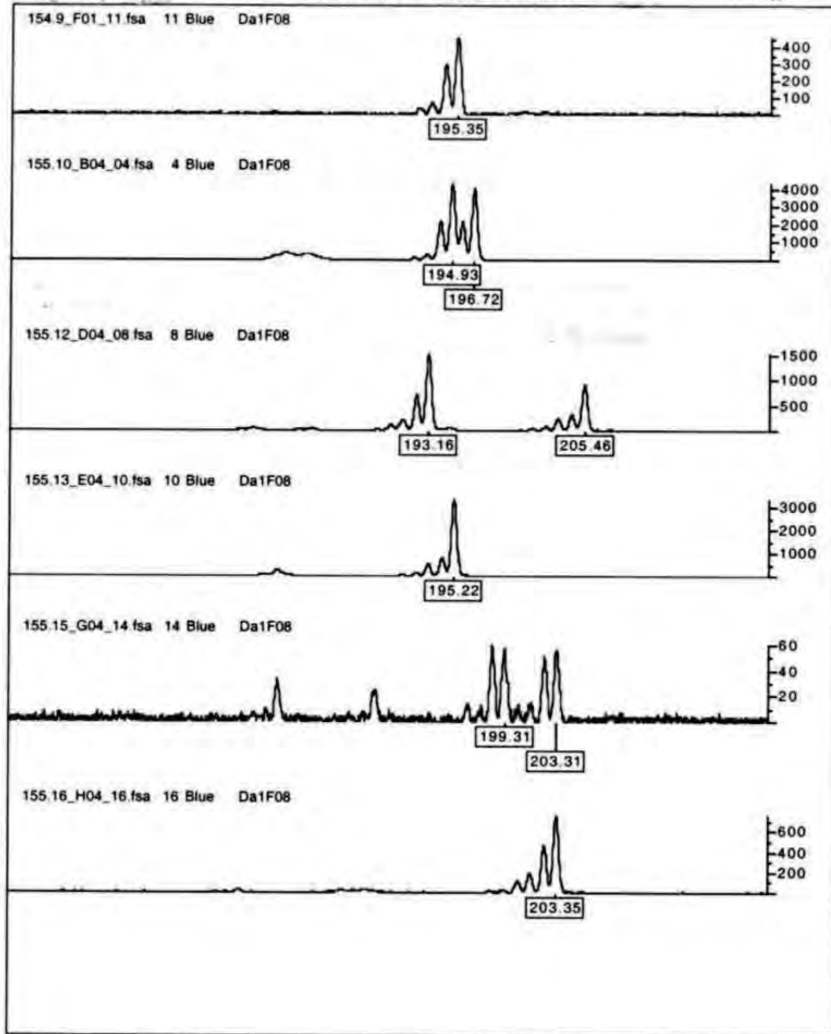
For research use only
- 2 -
Not for use in diagnostic systems



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194.93
196.72
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193.16
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155.15_G04_14.fsa 14 Blue Da1F08
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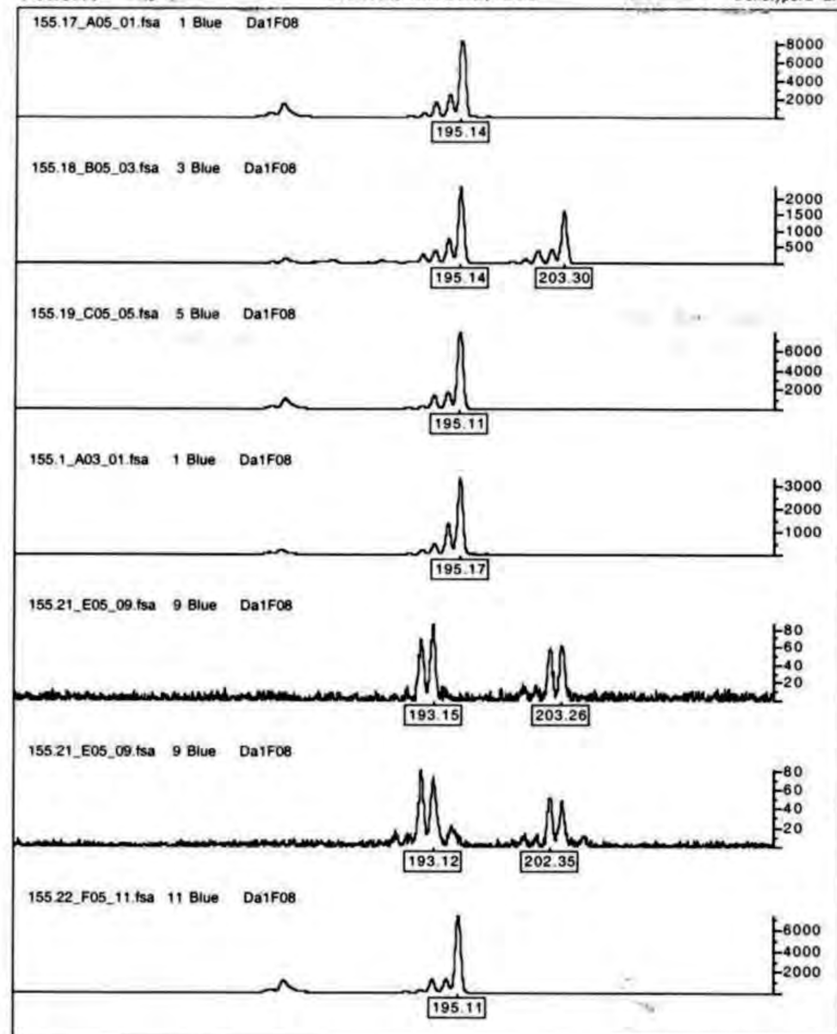
For research use only
- 3 -
Not for use in diagnostic systems



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203.30
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195.11
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195.17
155.21_E05_09.fsa 9 Blue Da1F08
193.15
203.26
155.21_E05_09.fsa 9 Blue Da1F08
193.12
202.35
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195.11
For research use only
- 4 -
Not for use in diagnostic systems

Plots - Da1F08
Licensed to Tim Fulcher, RBGQ

12:03:00 PM Thu, Apr 12, 2007
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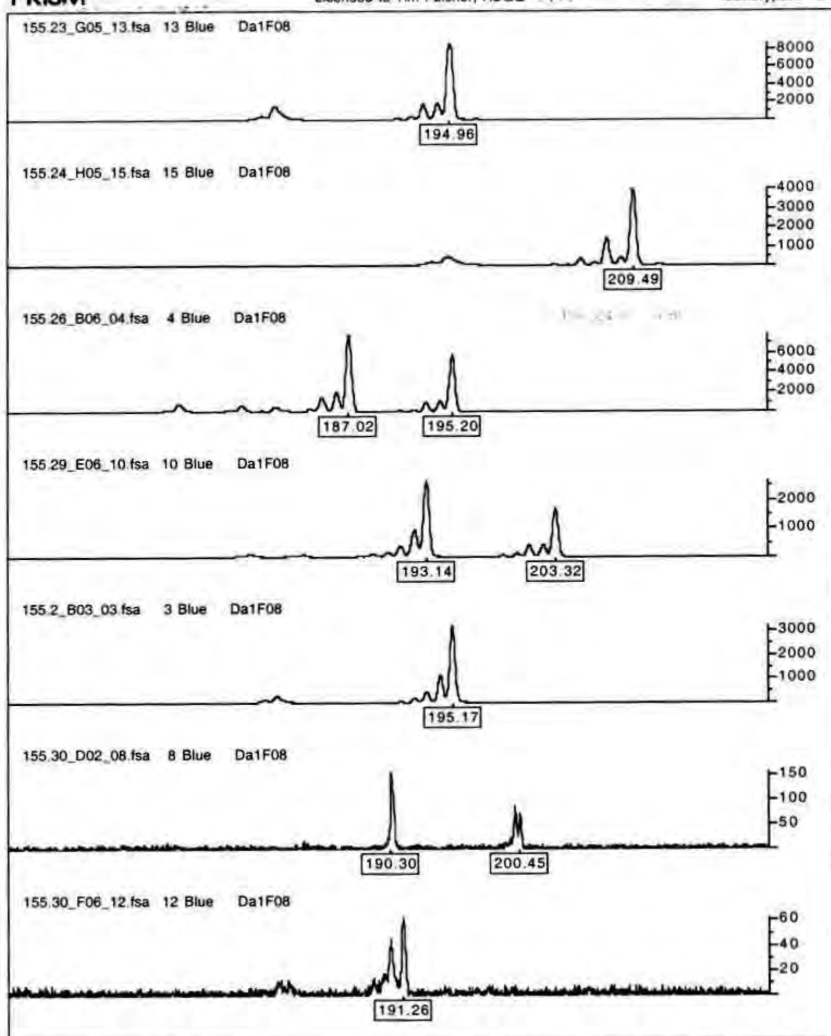

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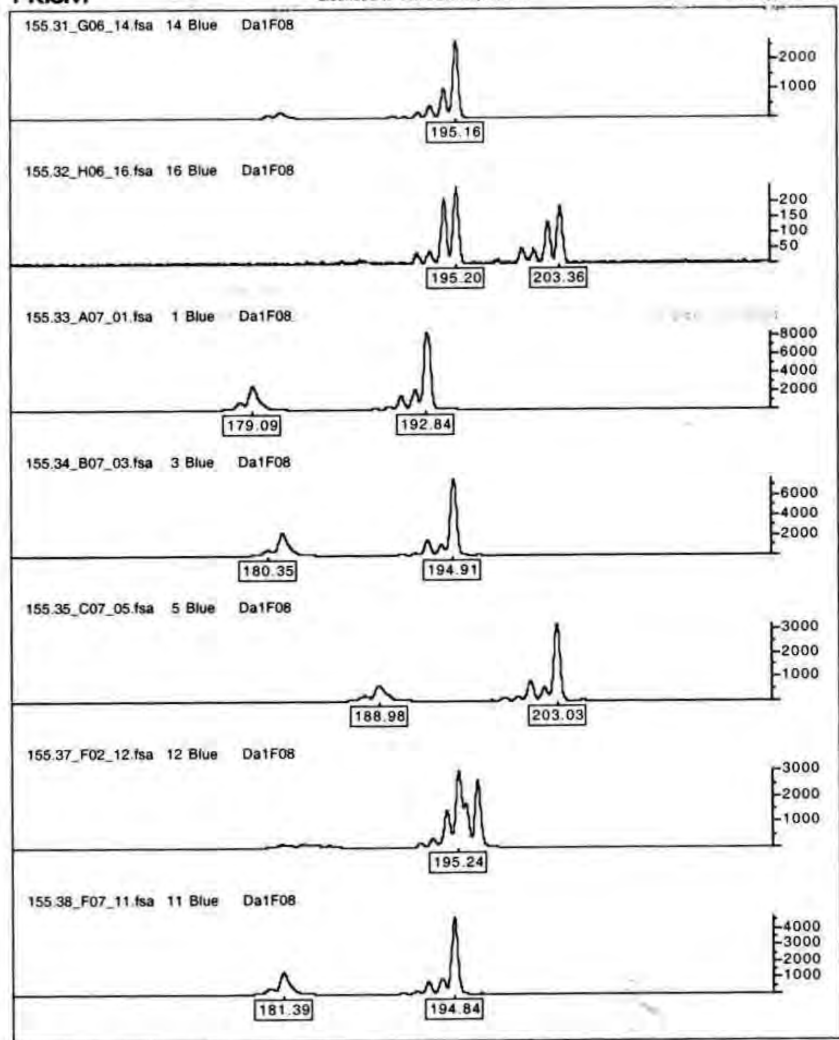
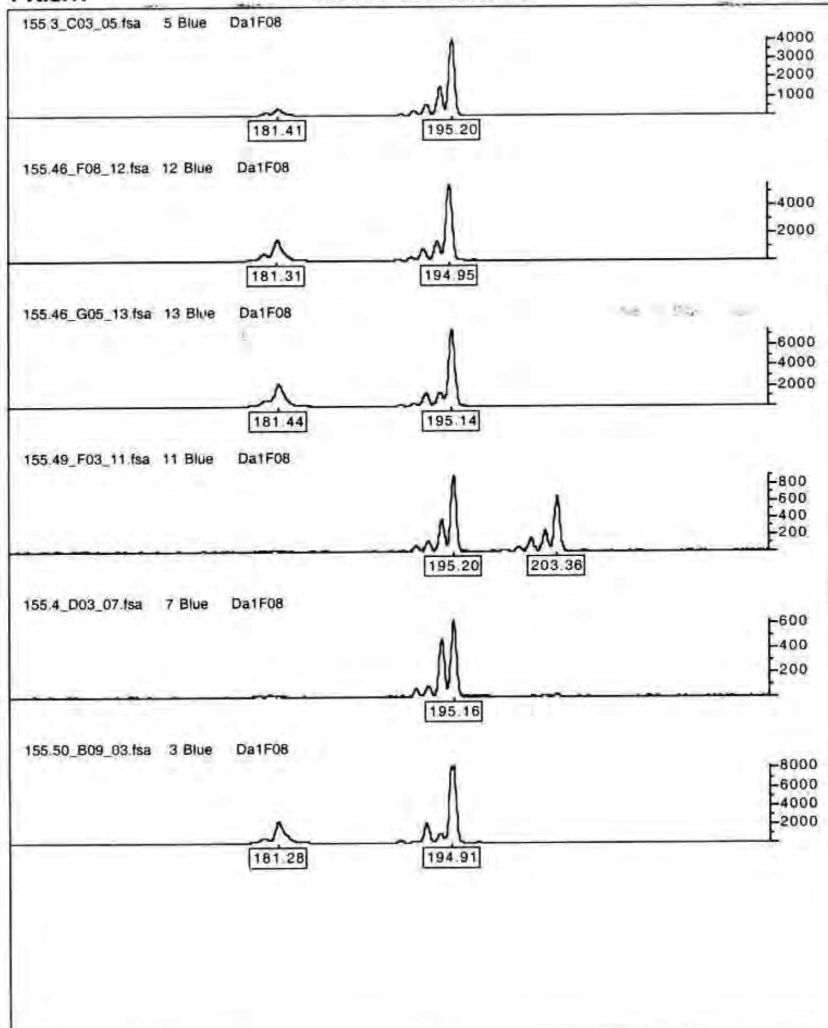


For research use only -6- Not for use in diagnostic systems



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155.49_F03_11.fsa 11 Blue Da1F08
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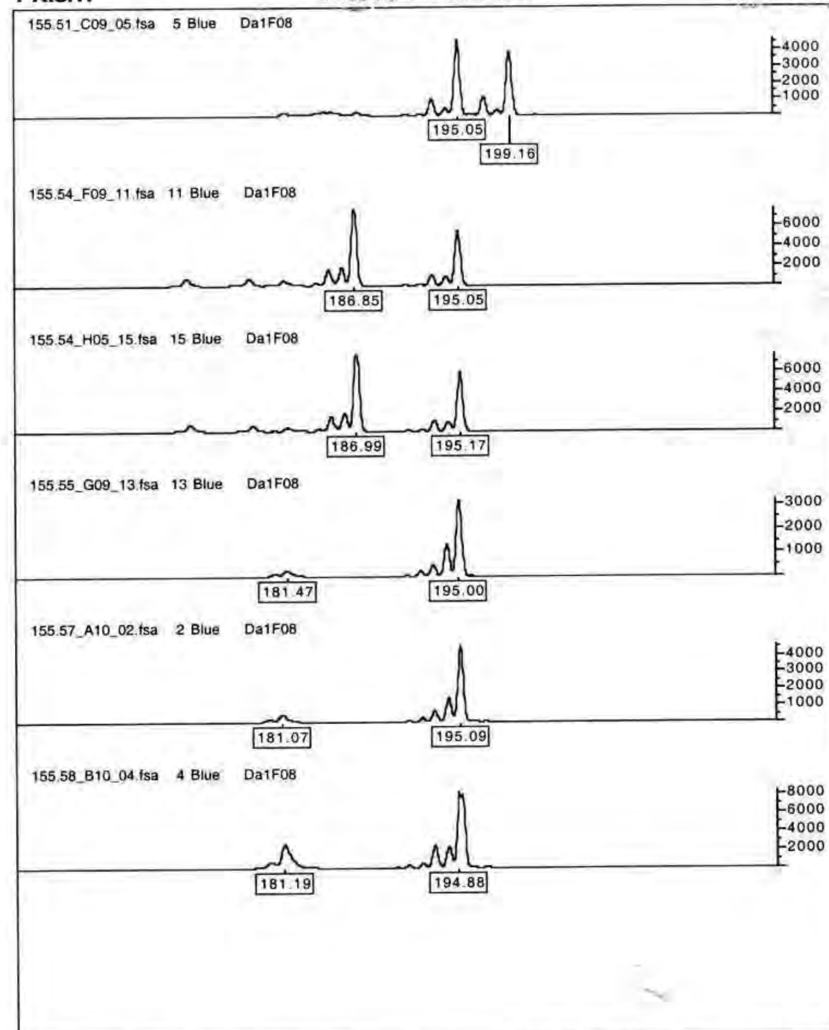
For research use only
- 7 -
Not for use in diagnostic systems



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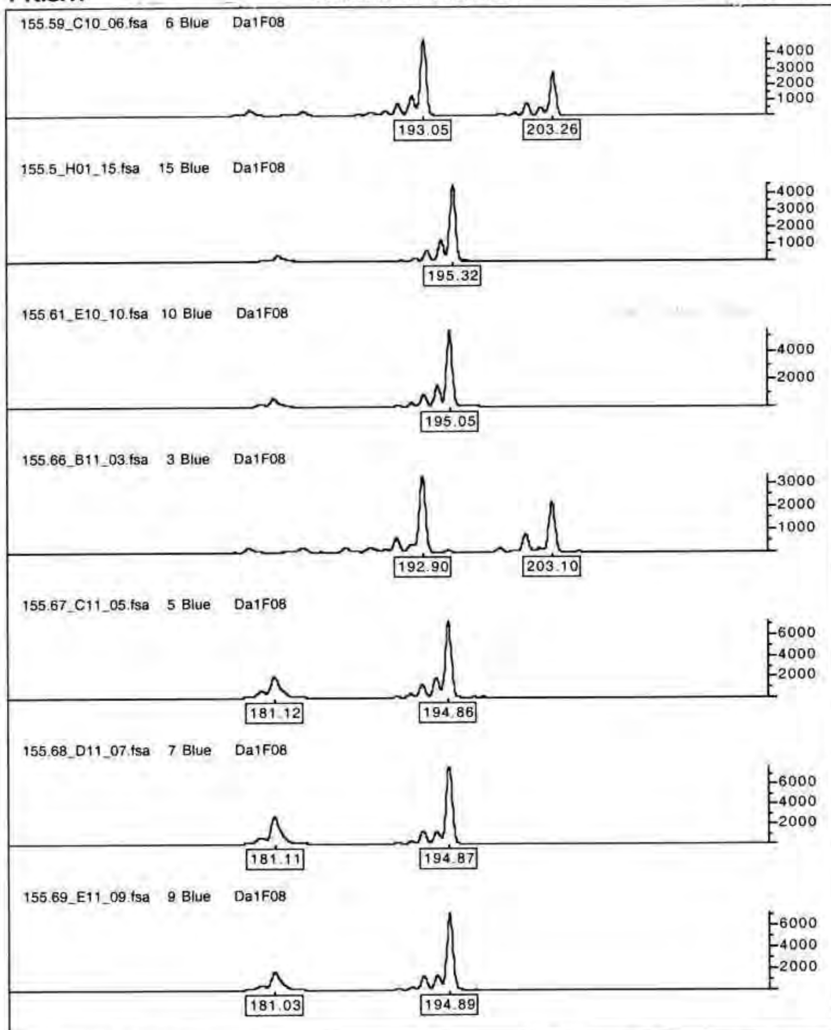
For research use only
- 8 -
Not for use in diagnostic systems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195.05
155.66_B11_03.fsa 3 Blue Da1F08
192.90
203.10
155.67_C11_05.fsa 5 Blue Da1F08
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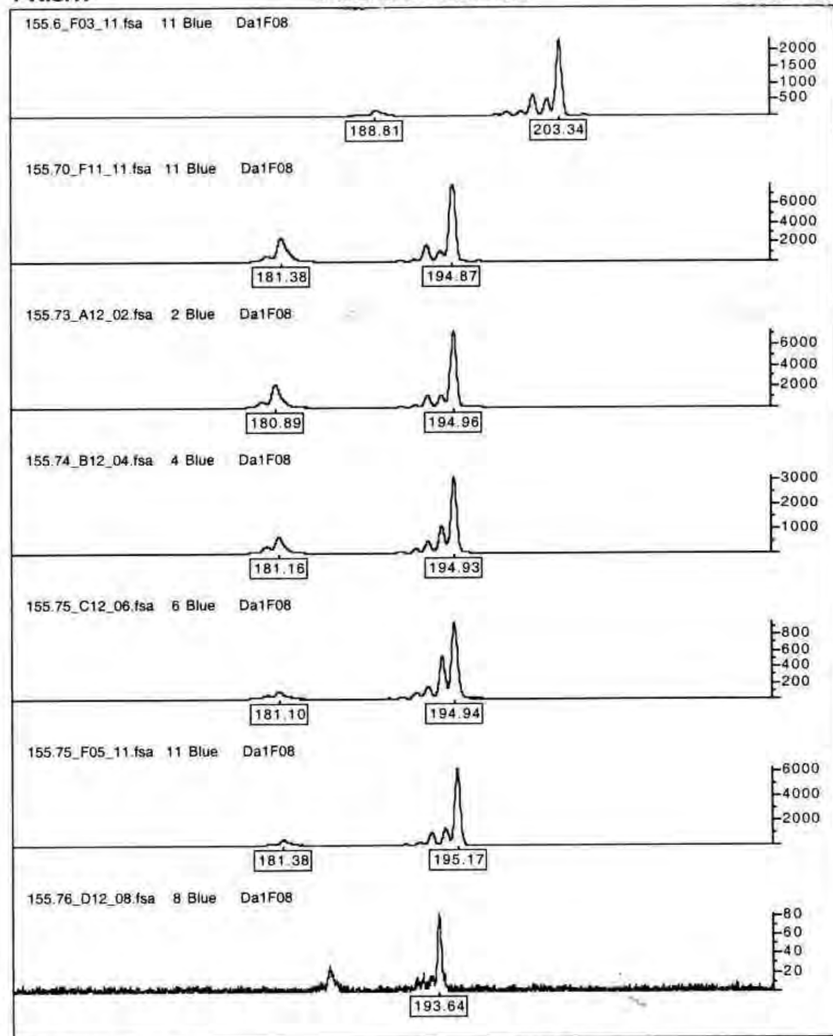
194.89
For research use only
- 9 -
Not for use in diagnostic systems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180.89
194.96
155.74_B12_04.fsa 4 Blue Da1F08
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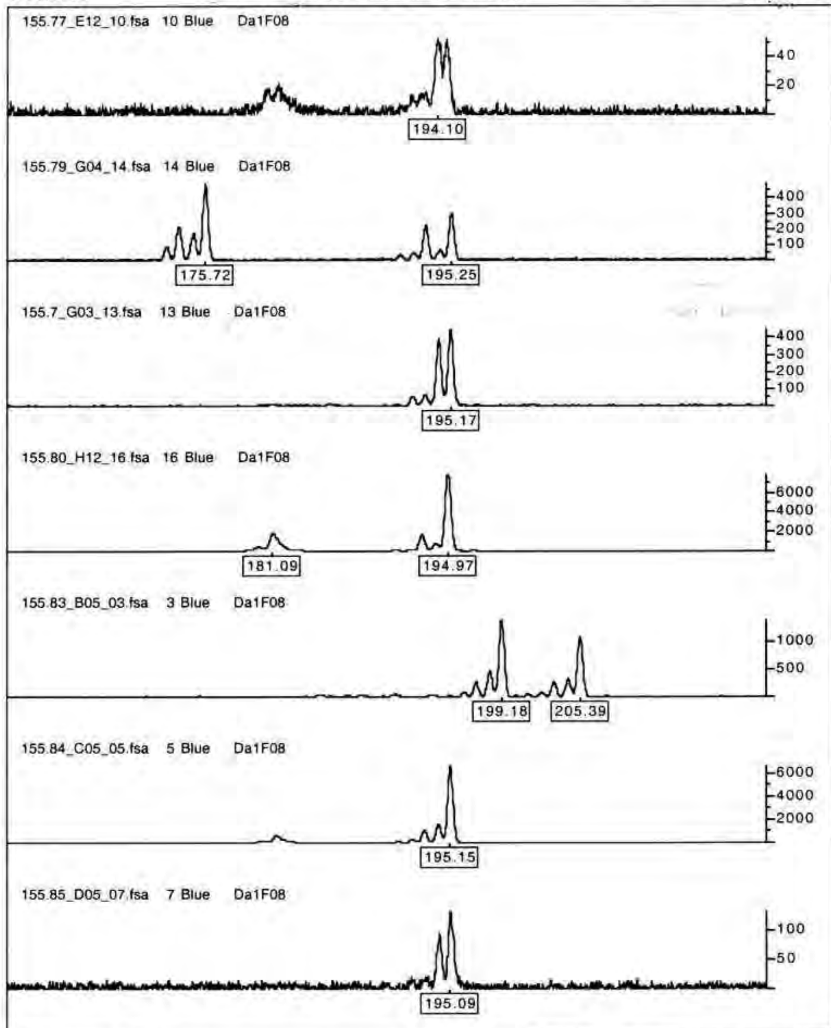
For research use only
- 10 -
Not for use in diagnostic systems



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205.39
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- 11 -
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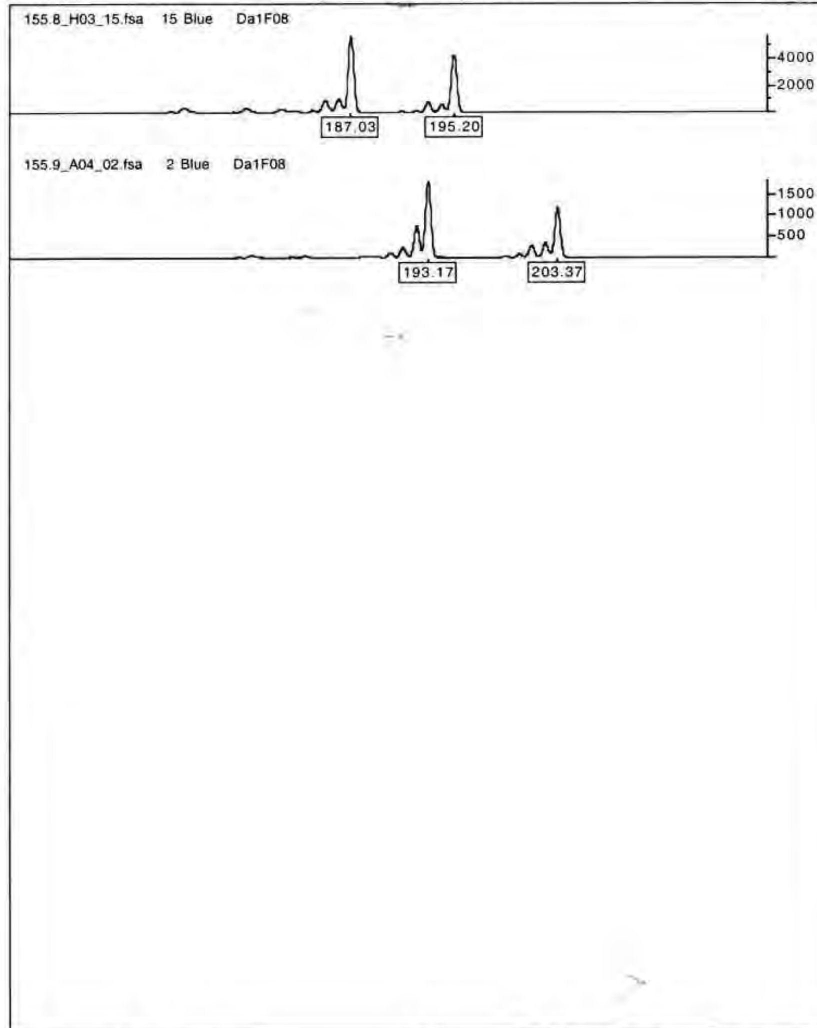

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155.9_A04_02.fsa 2 Blue Da1F08
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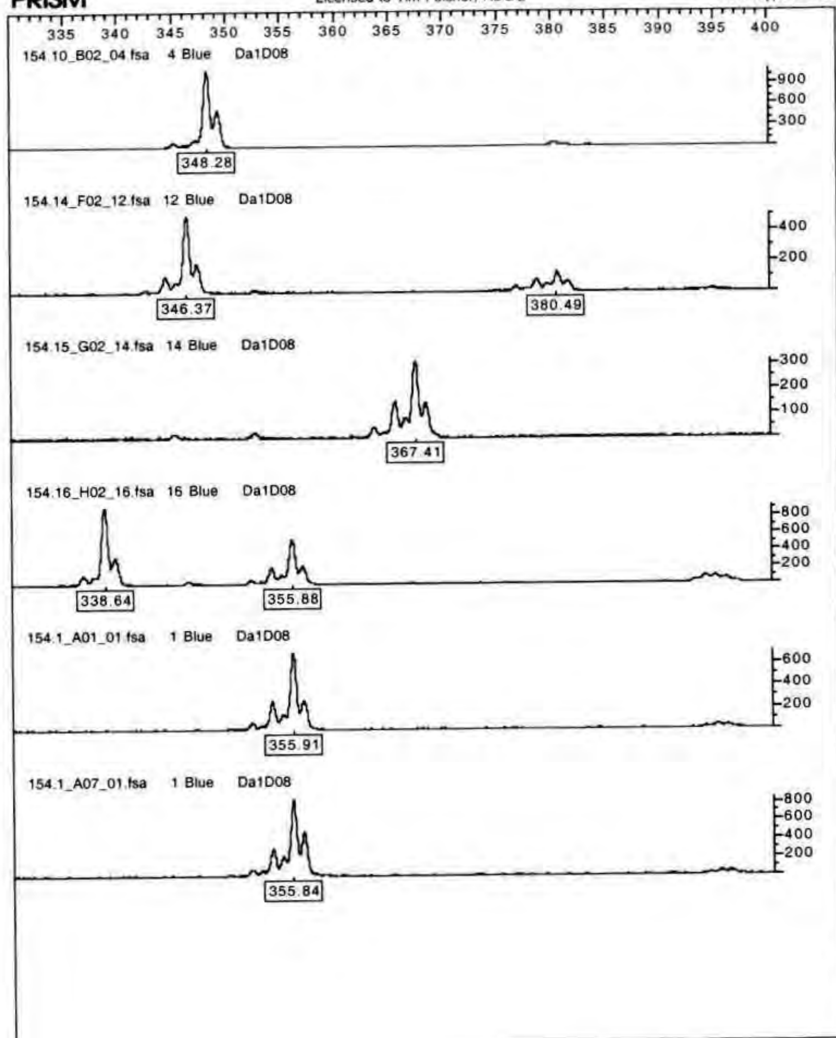
203.37
For research use only
- 12 -
Not for use in diagnostic systems



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154.14_F02_12.fsa 12 Blue Da1D08
346.37
380.49
154.15_G02_14.fsa 14 Blue Da1D08
367.41
154.16_H02_16.fsa 16 Blue Da1D08
338.64
355.88
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For research use only
- 1 -
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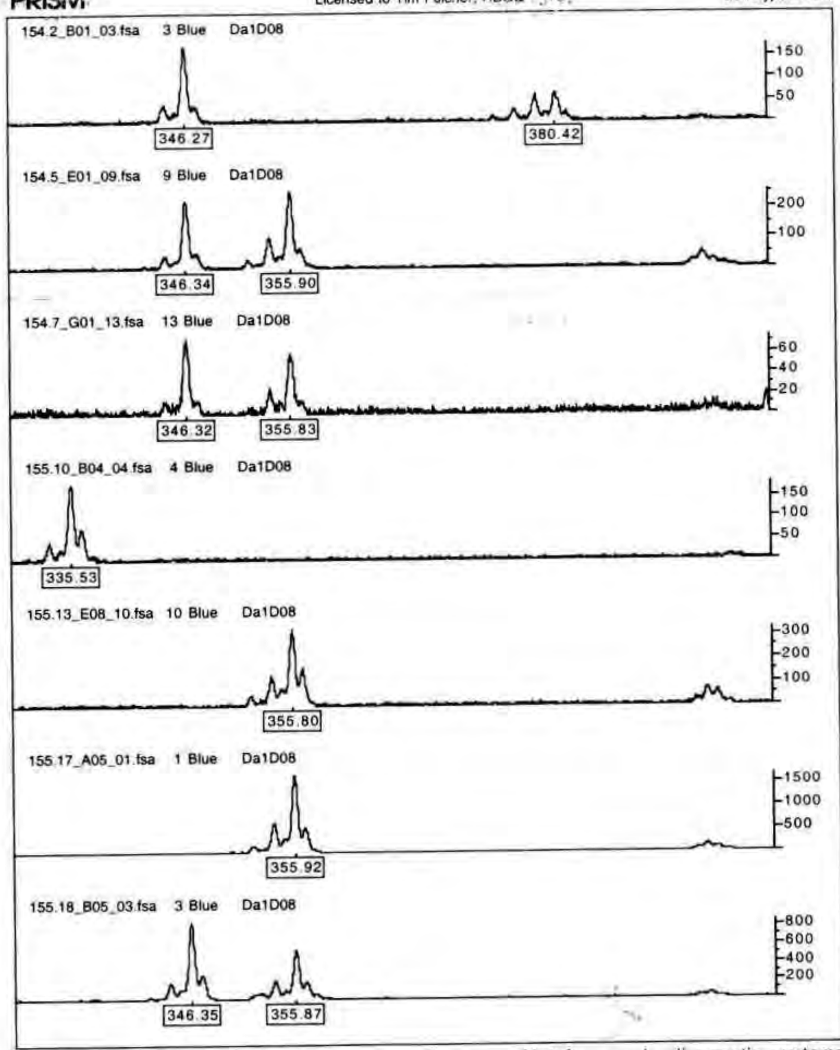

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154.7_G01_13.fsa 13 Blue Da1D08
346.32
355.83
155.10_B04_04.fsa 4 Blue Da1D08
335.53
155.13_E08_10.fsa 10 Blue Da1D08
355.80
155.17_A05_01.fsa 1 Blue Da1D08
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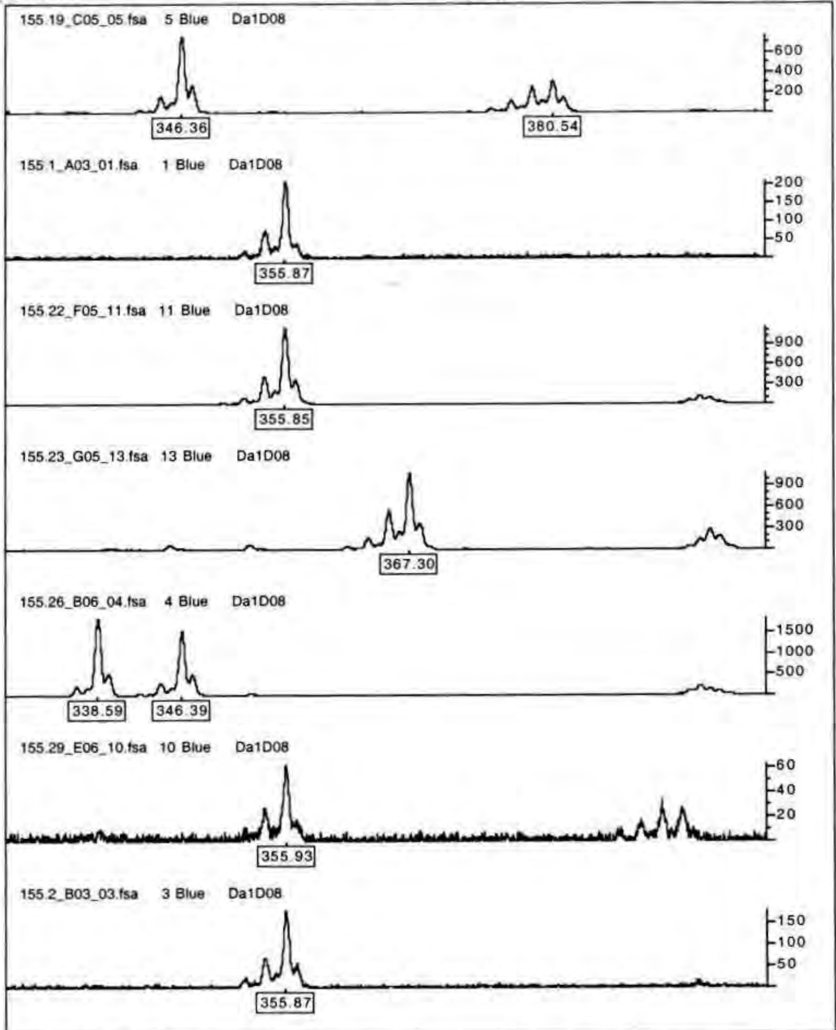
For research use only
- 2 -
Not for use in diagnostic systems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355.85
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155.26_B06_04.fsa 4 Blue Da1D08
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346.39
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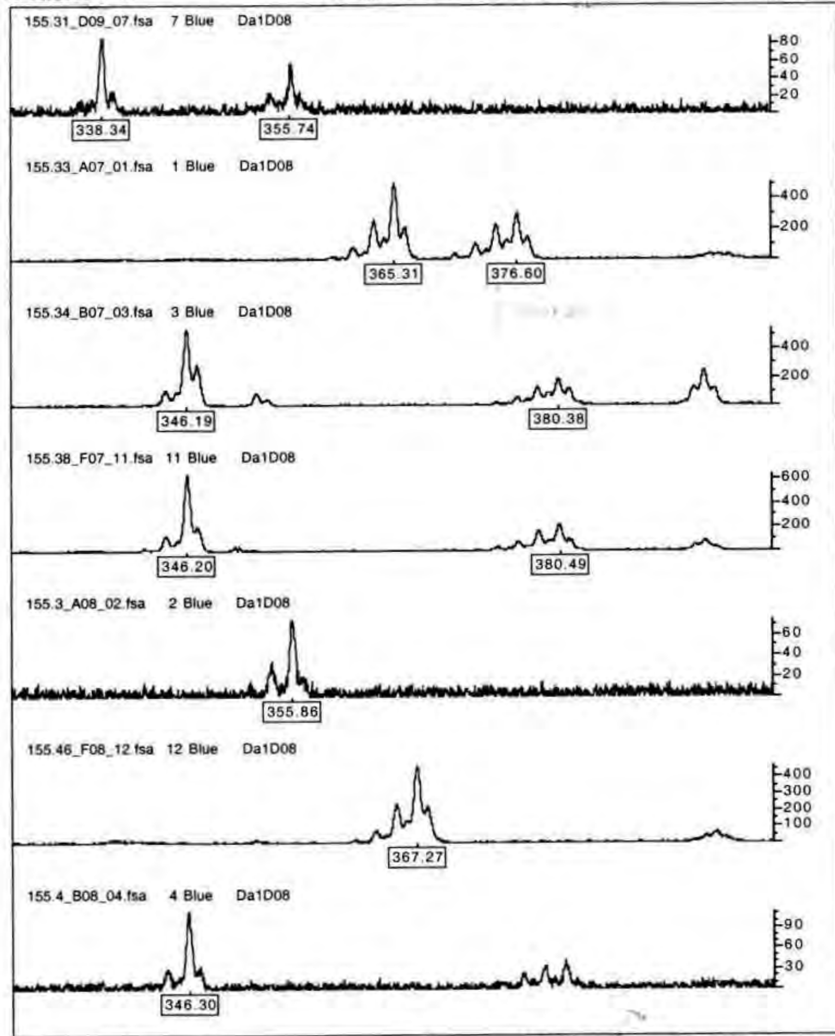
For research use only
- 3 -
Not for use in diagnostic systems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380.49
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155.46_F08_12.fsa 12 Blue Da1D08
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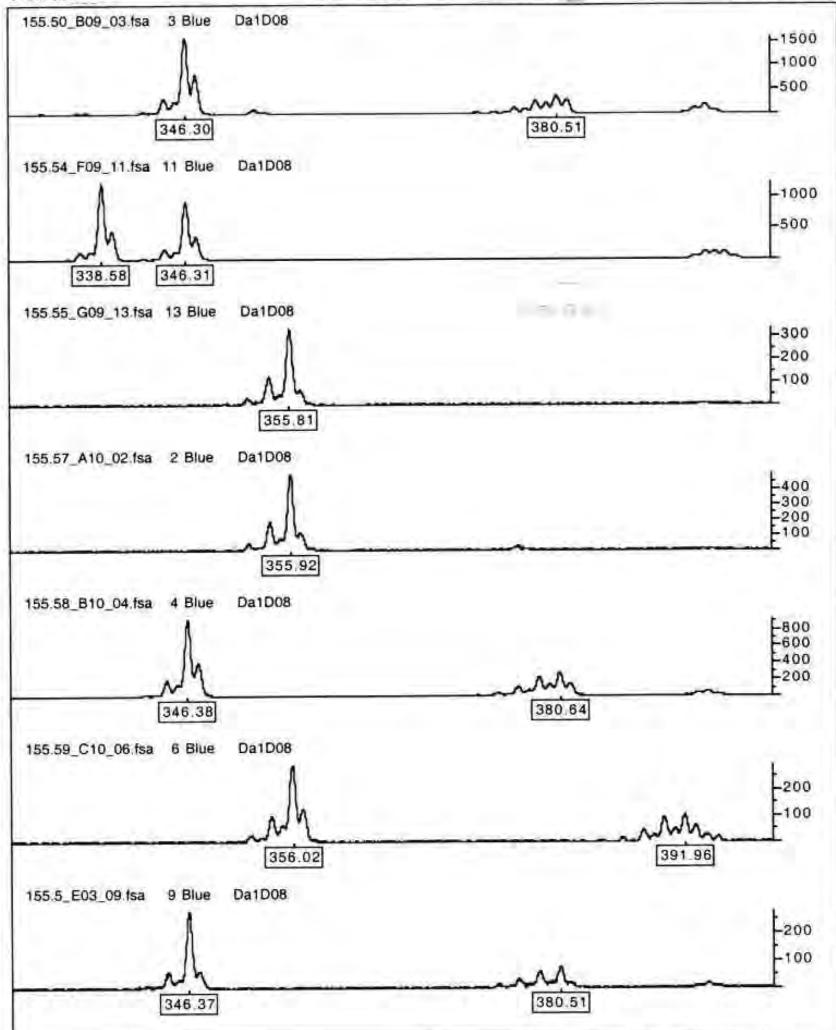
For research use only
- 4 -
Not for use in diagnostic systems



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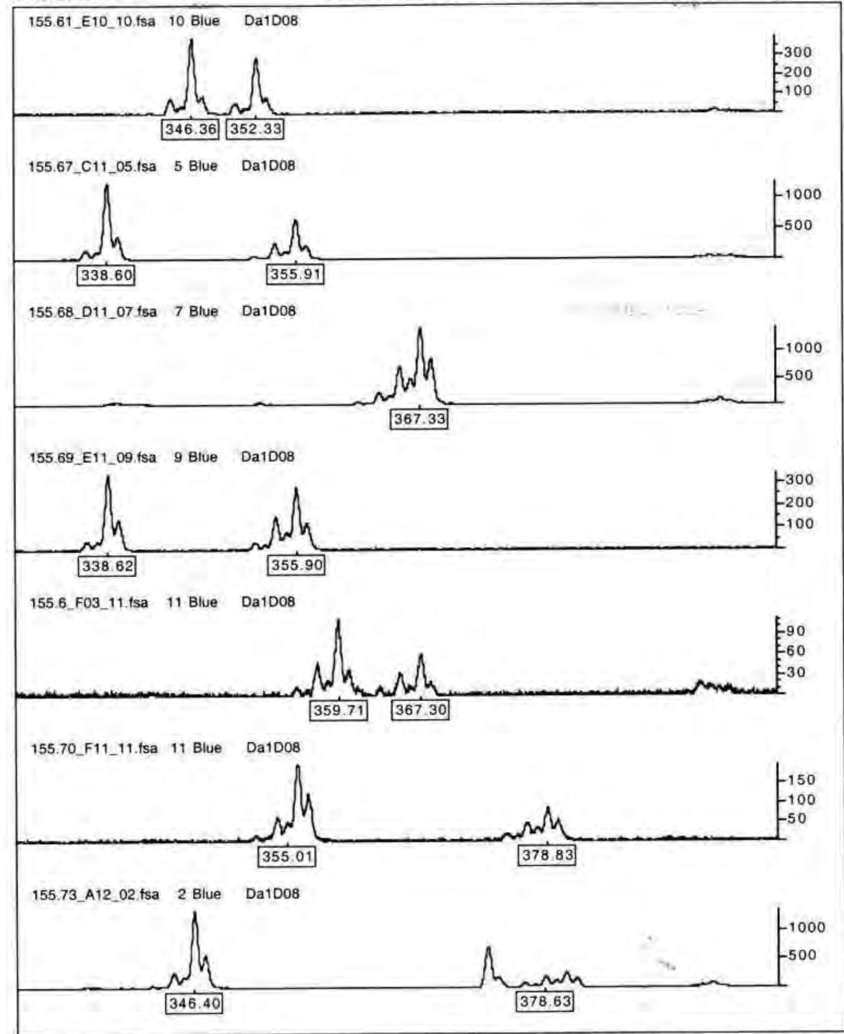
For research use only
- 5 -
Not for use in diagnostic systems



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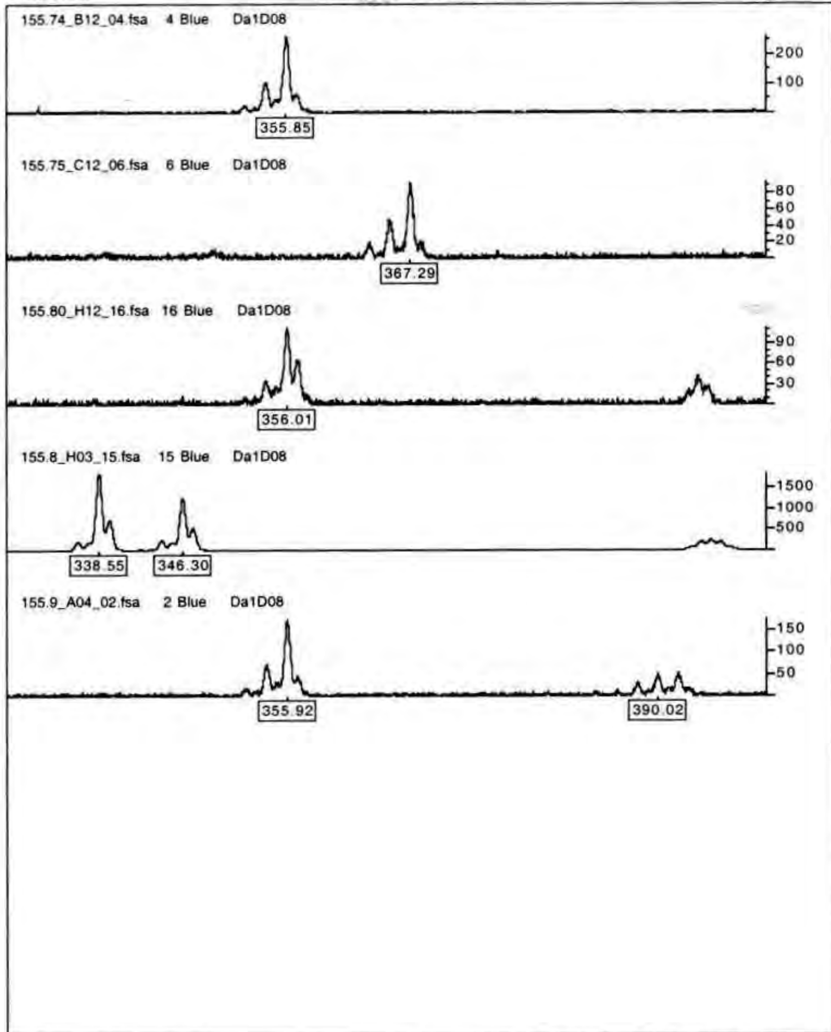
For research use only
- 6 -
Not for use in diagnostic systems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Licensed to Tim Fulcher, RBGQ
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60
40
20
367.29
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90
60
30
356.01
155.8_H03_15.fsa 15 Blue Da1D08
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1000
500
338.55
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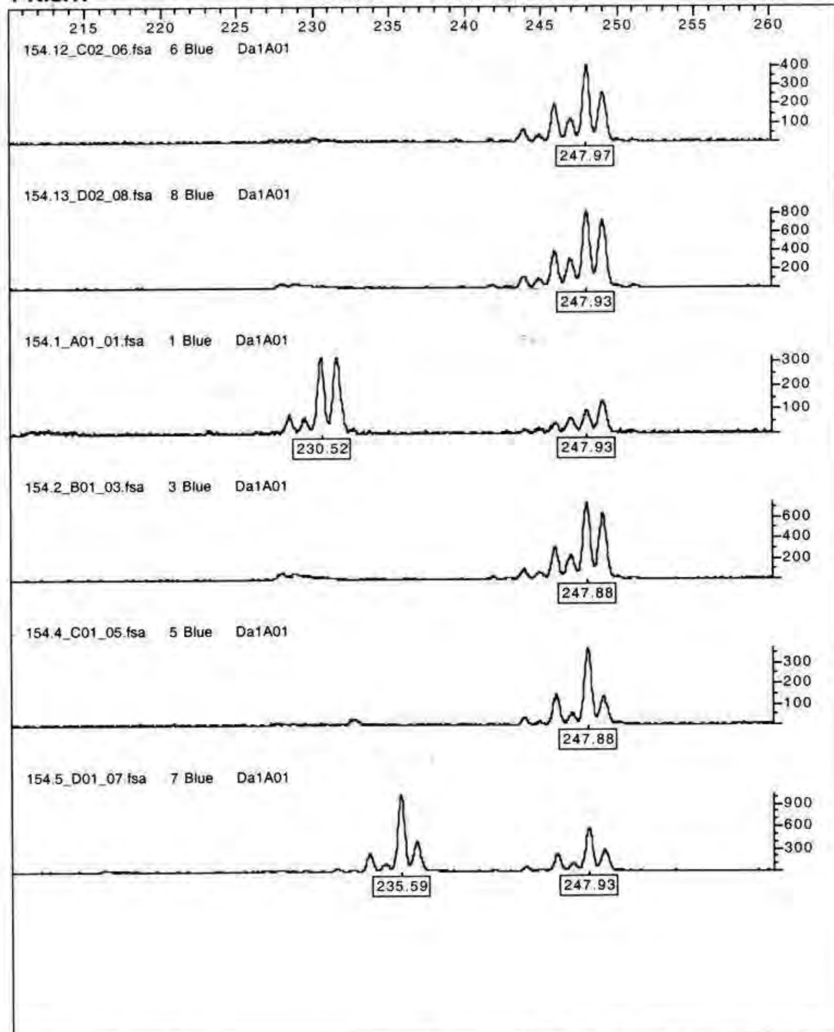
For research use only
- 7 -
Not for use in diagnostic systems



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154.13_D02_08.fsa 8 Blue Da1A01
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154.1_A01_01.fsa 1 Blue Da1A01
230.52
247.93
154.2_B01_03.fsa 3 Blue Da1A01
247.88
154.4_C01_05.fsa 5 Blue Da1A01
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154.5_D01_07.fsa 7 Blue Da1A01
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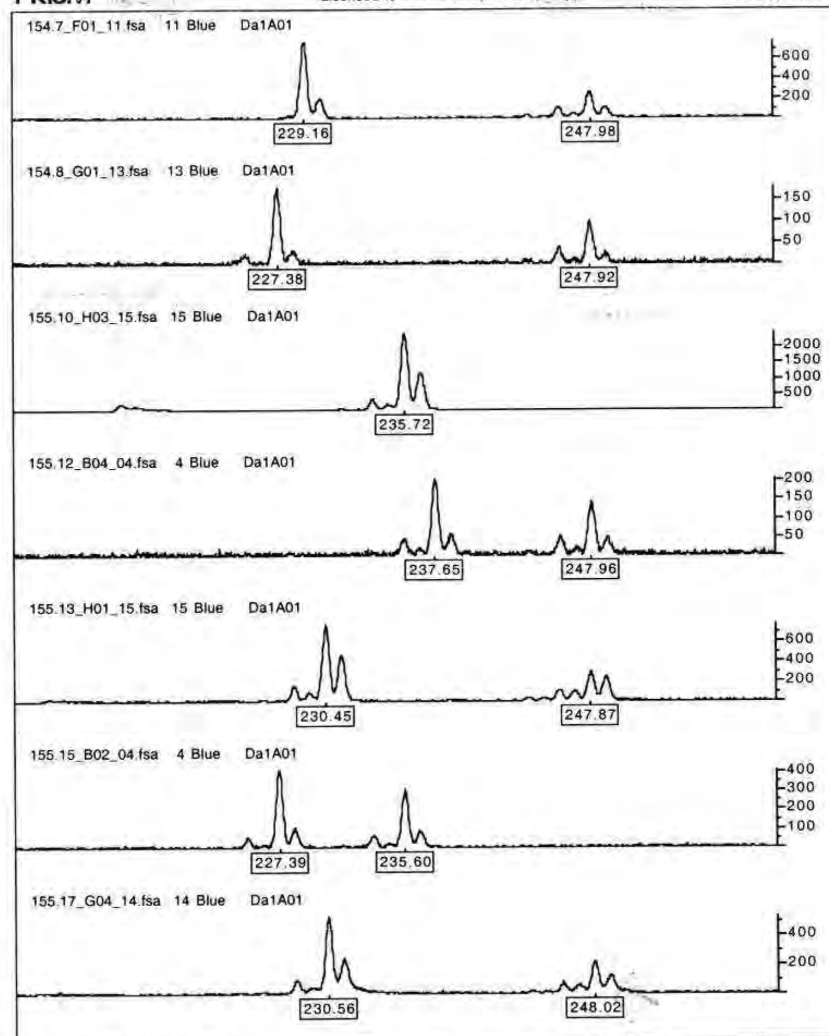
247.93
For research use only
- 1 -
Not for use in diagnostic systems



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227.38
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227.39
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155.17_G04_14.fsa 14 Blue Da1A01
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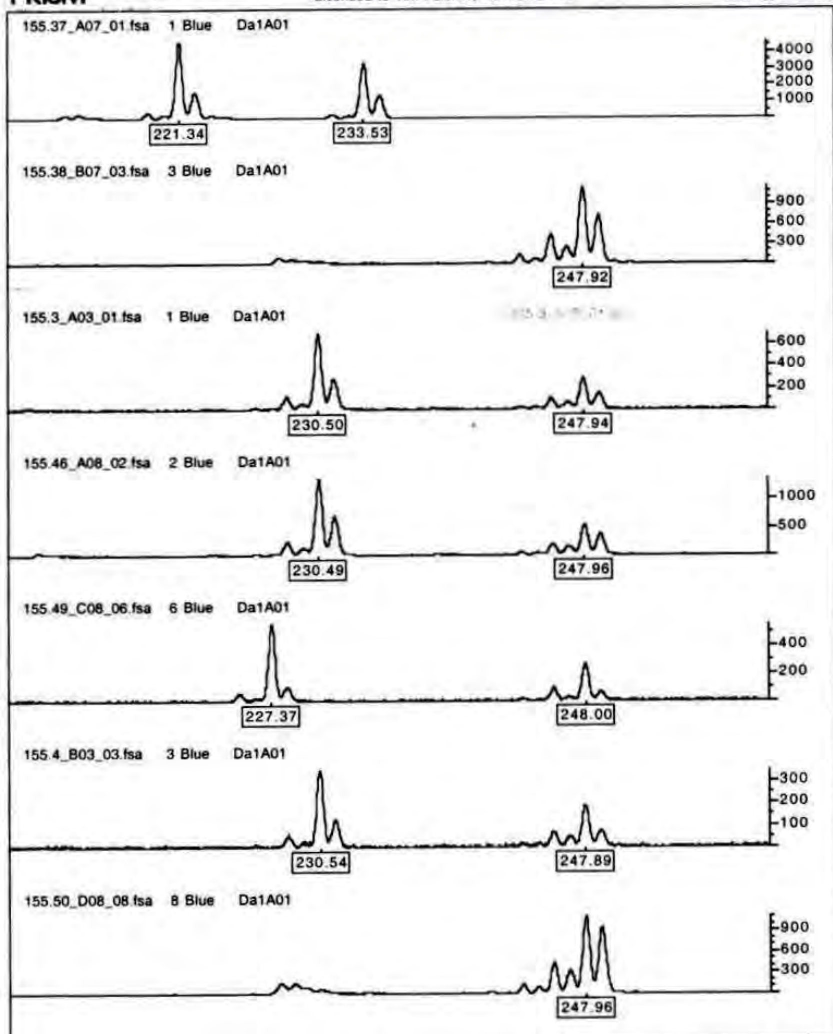
For research use only
- 2 -
Not for use in diagnostic systems

Plots - Da1A01
Licensed to Tim Fulcher, RBGO

12:00:37 PM Thu, Apr 12, 2007
Genotyper® 2.0



For research use only - 5 - Not for use in diagnostic systems

Plots - Da1A01
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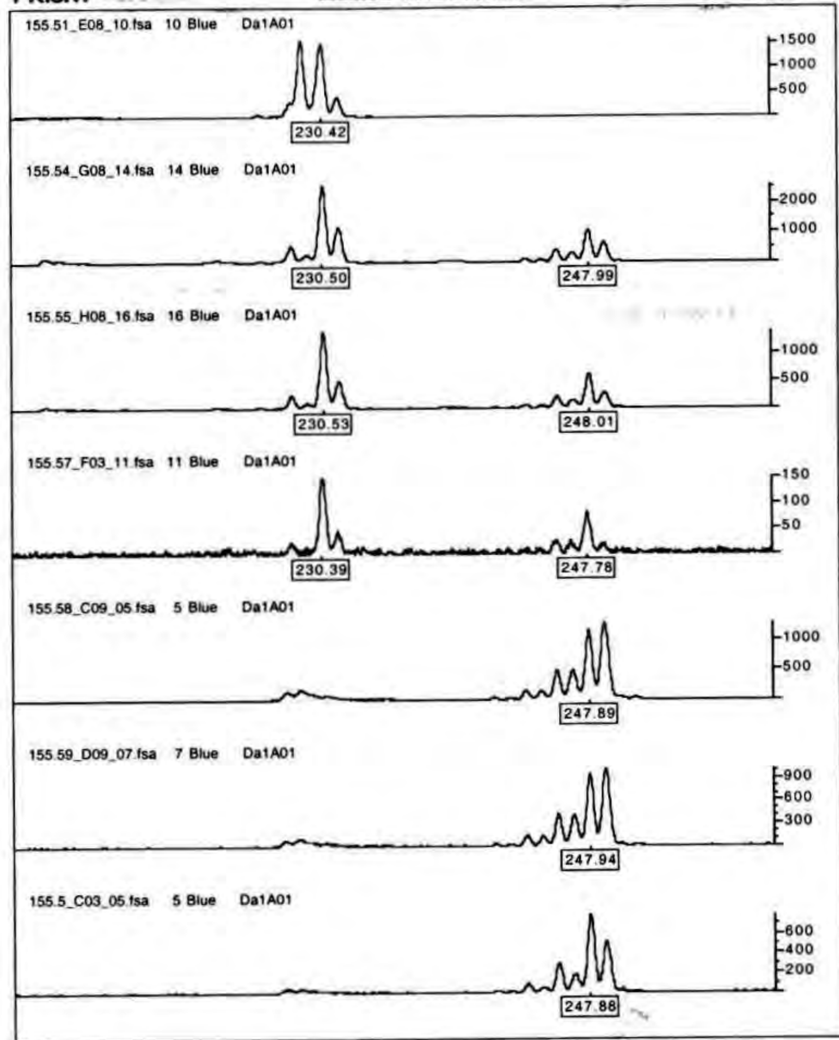
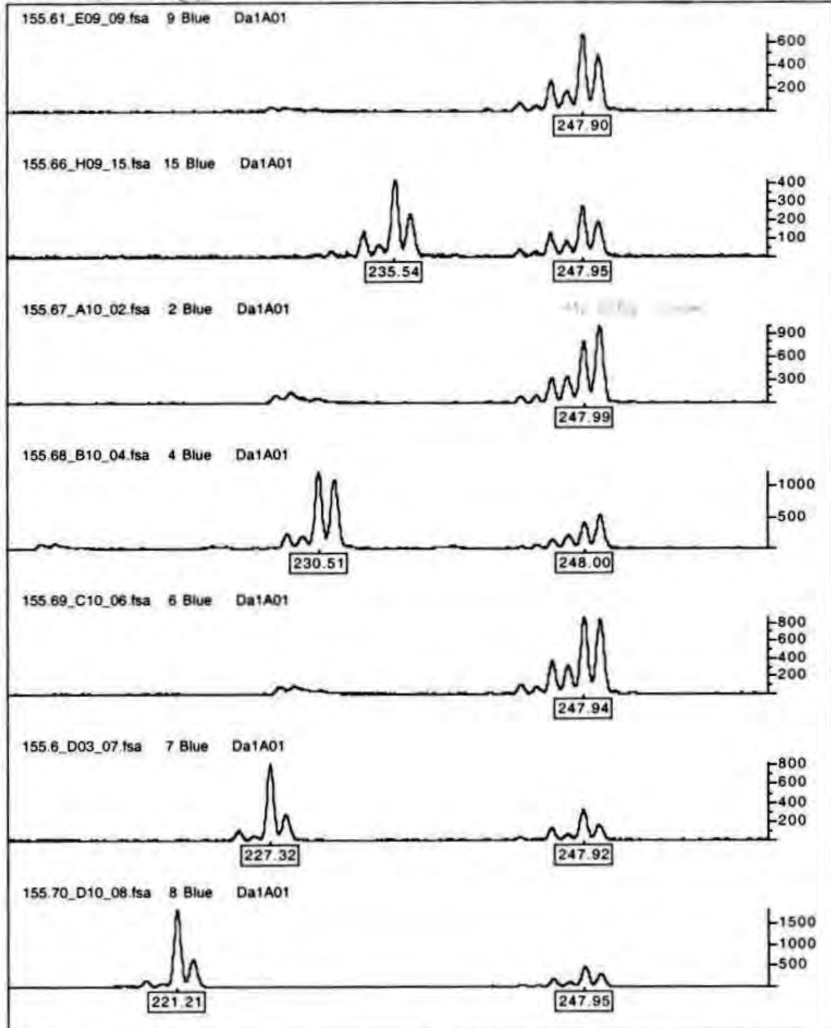


For research use only - 6 - Not for use in diagnostic systems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Licensed to Tim Fulcher, RBGQ
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Genotyper® 2.0
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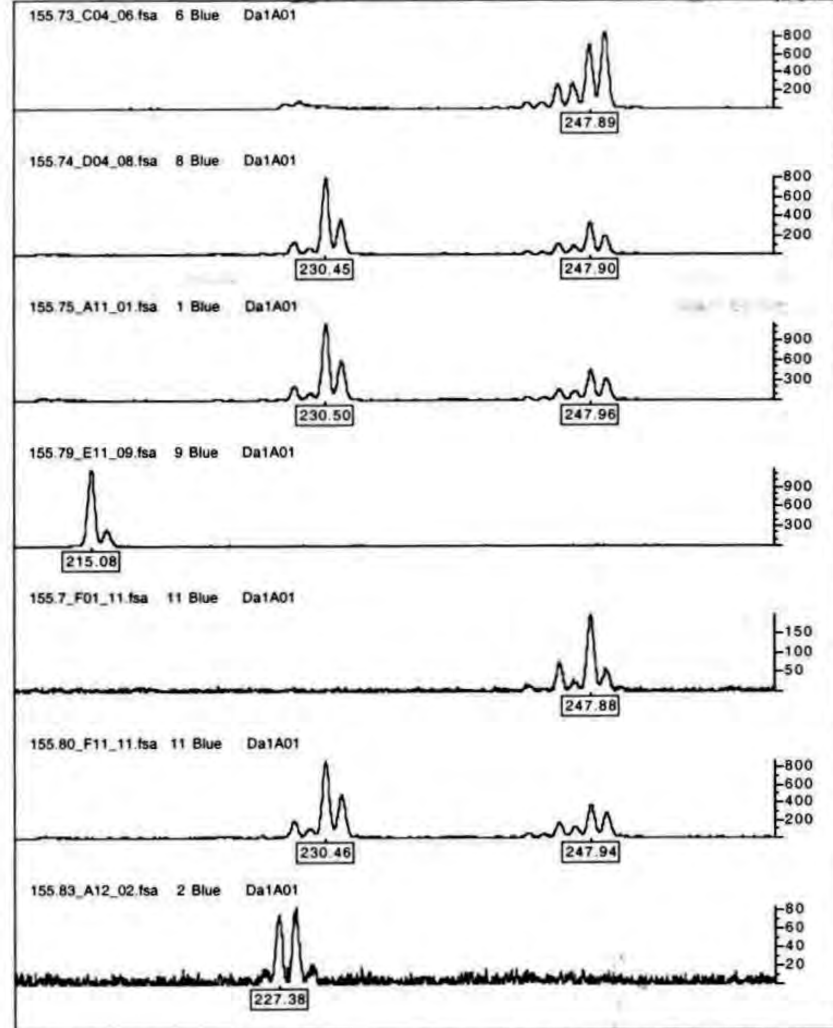
For research use only
- 7 -
Not for use in diagnostic systems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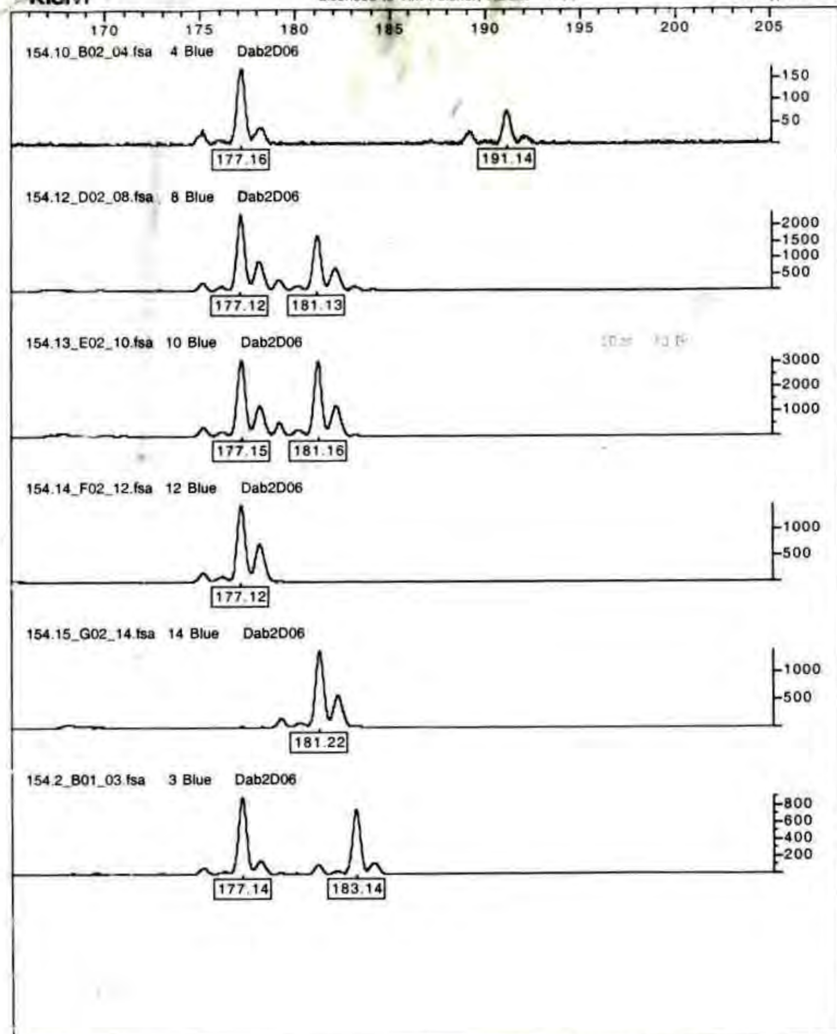
For research use only
- 8 -
Not for use in diagnostic systems



Plots - Dab2D06
Licensed to Tim Fulcher, RBGQ
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205
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191.14
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177.12
181.13
154.13_E02_10.fsa 10 Blue Dab2D06
177.15
181.16
154.14_F02_12.fsa 12 Blue Dab2D06
177.12
154.15_G02_14.fsa 14 Blue Dab2D06
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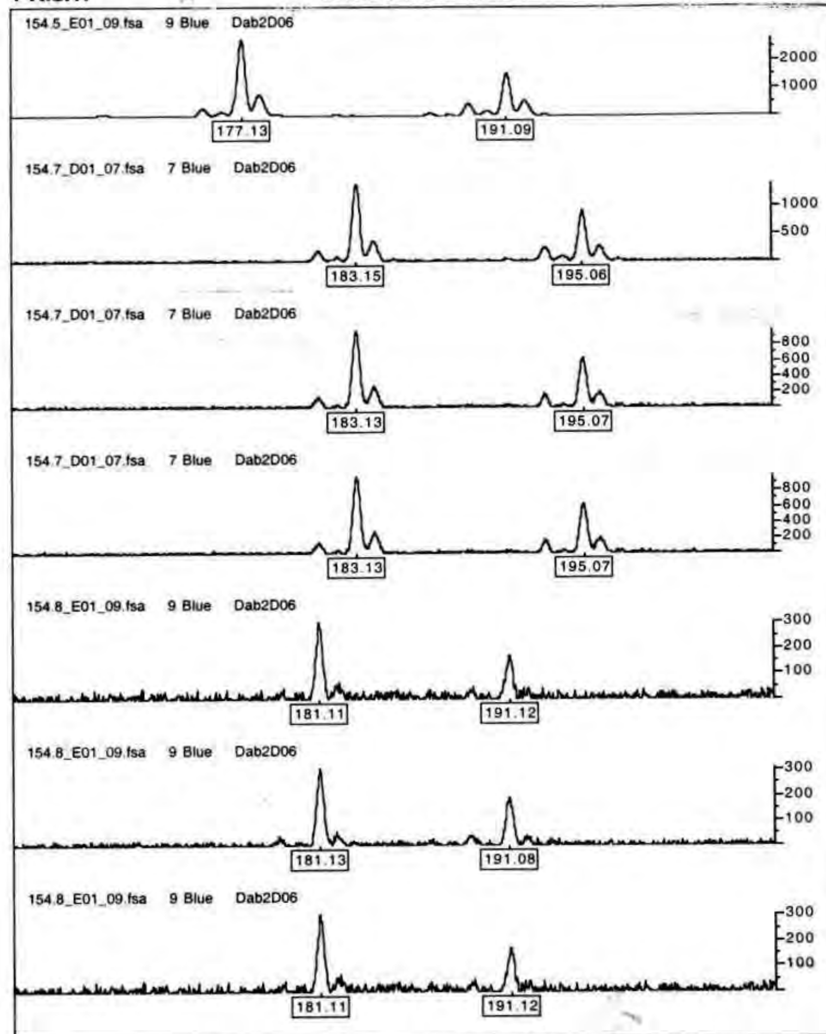
For research use only
- 1 -
Not for use in diagnostic systems



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Licensed to Tim Fulcher, RBGQ
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- 2 -
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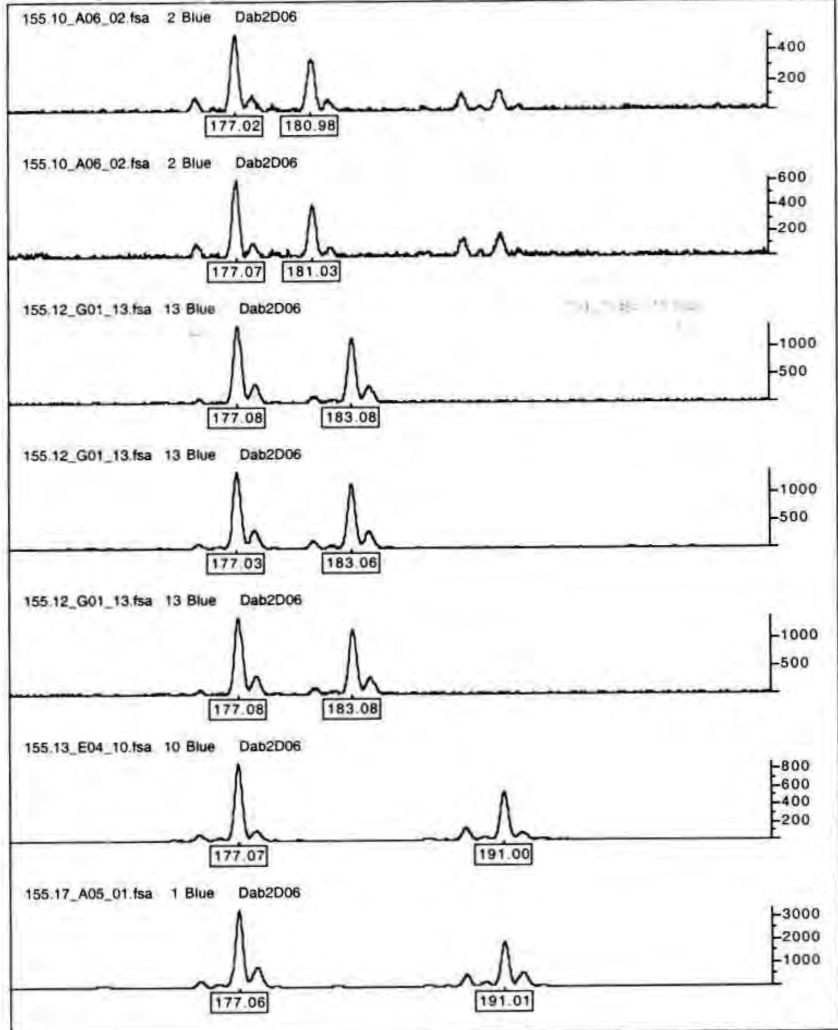

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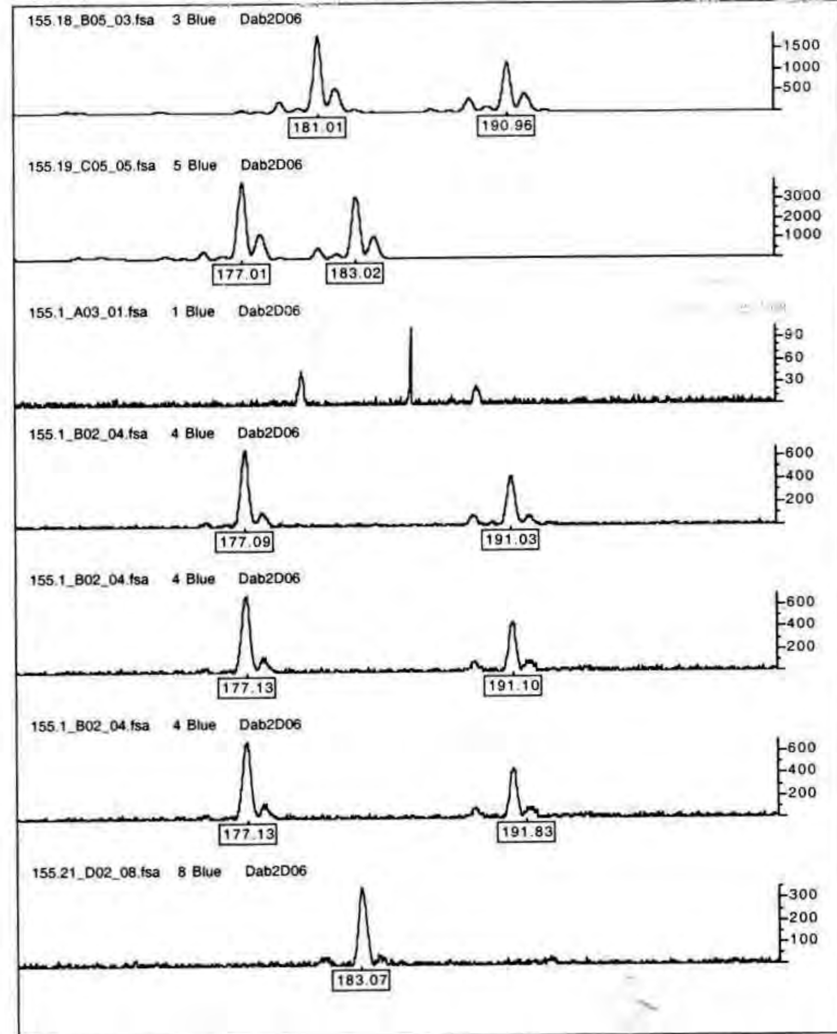

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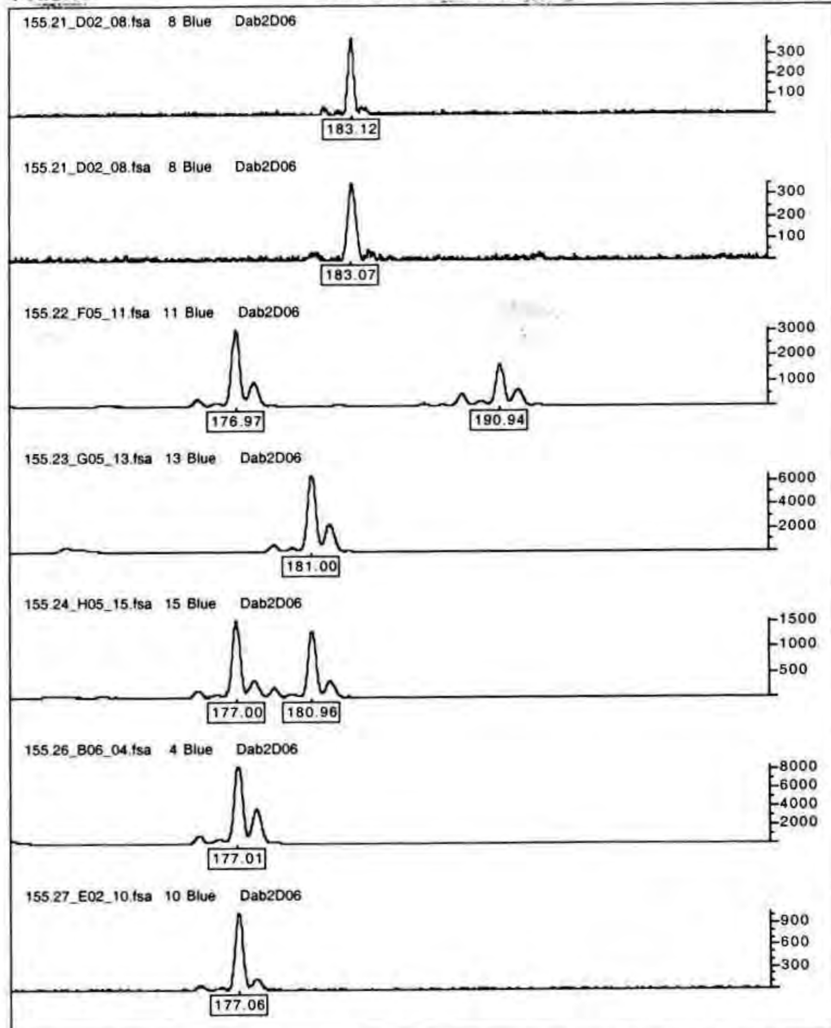

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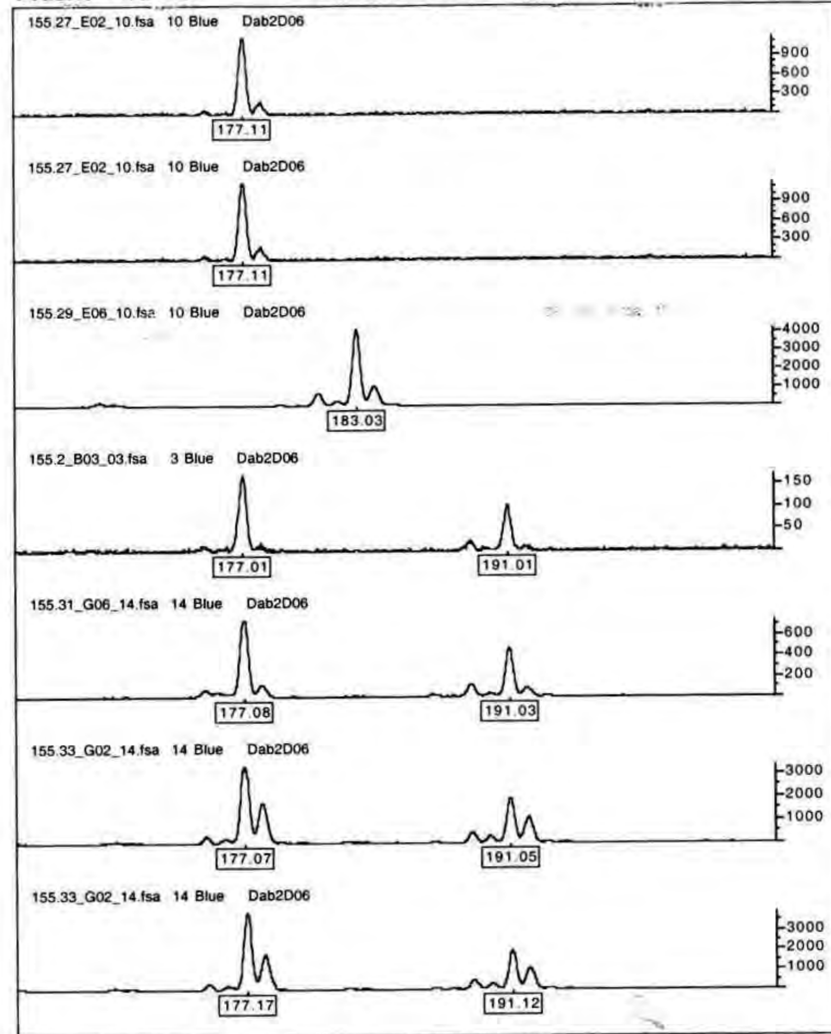

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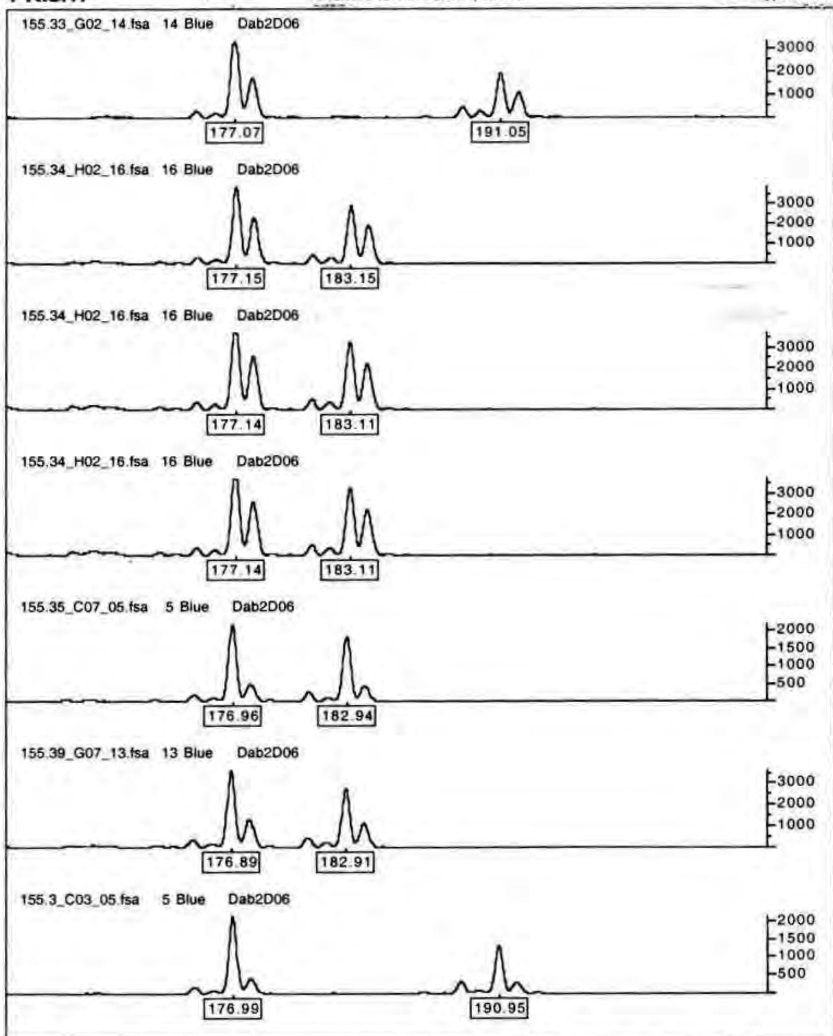

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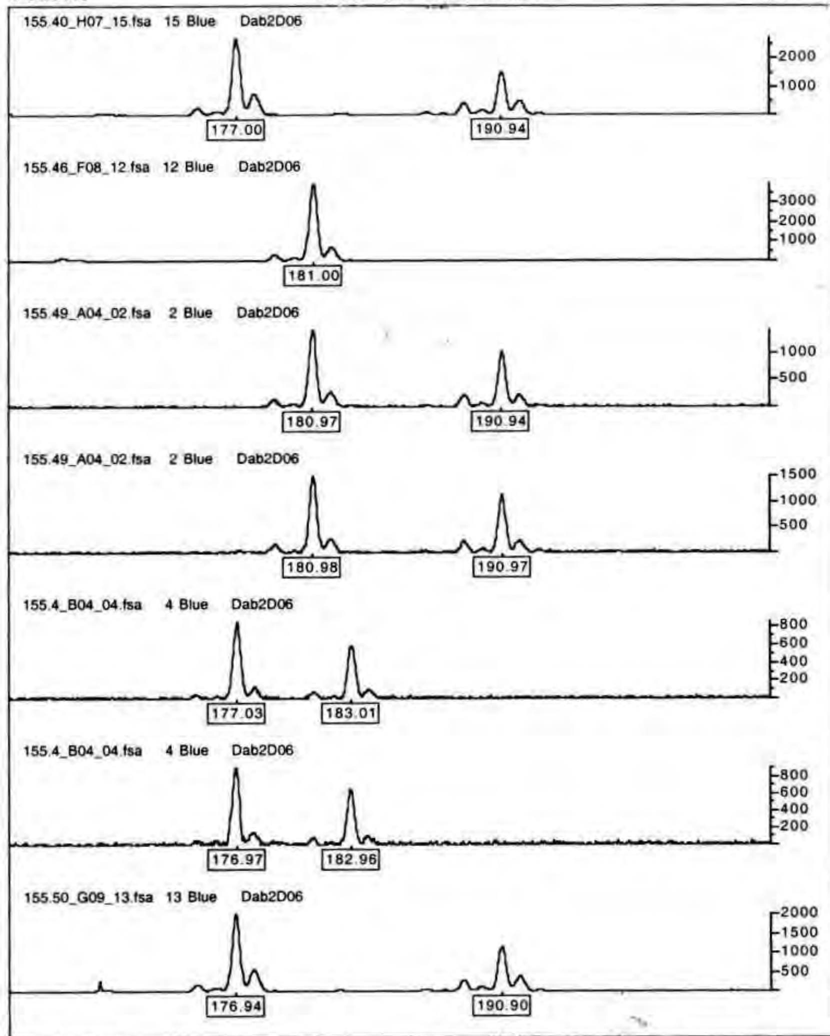
For research use only
- 7 -
Not for use in diagnostic systems



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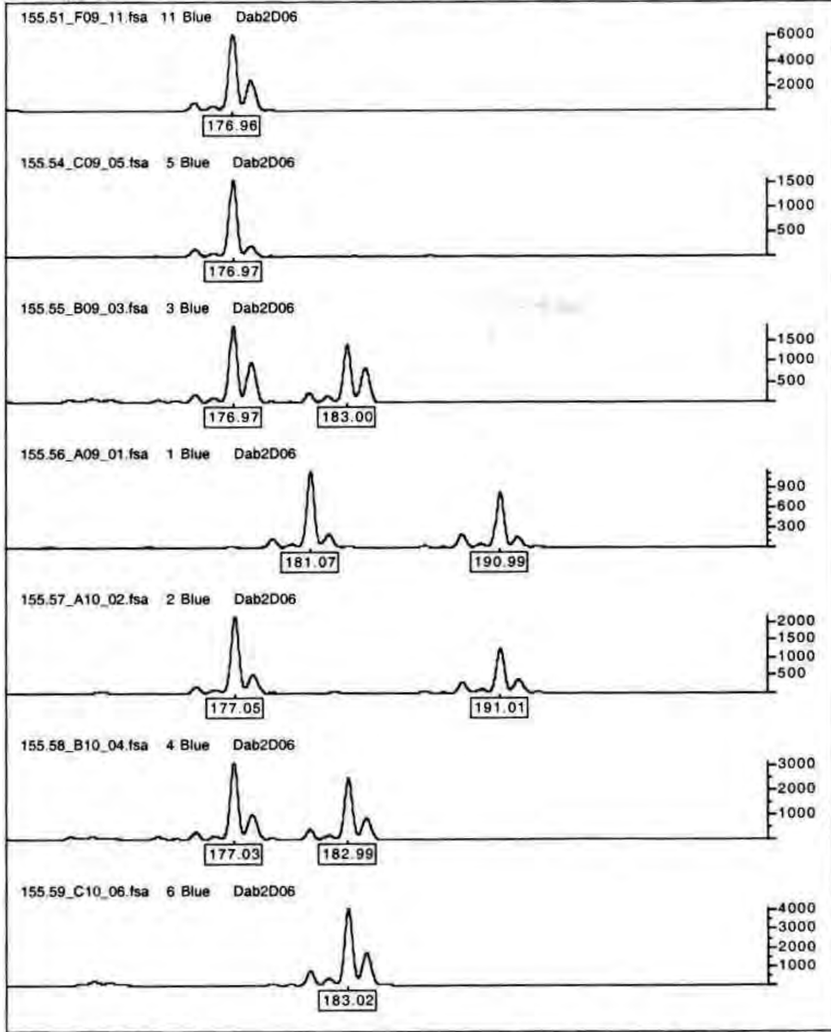
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-9-
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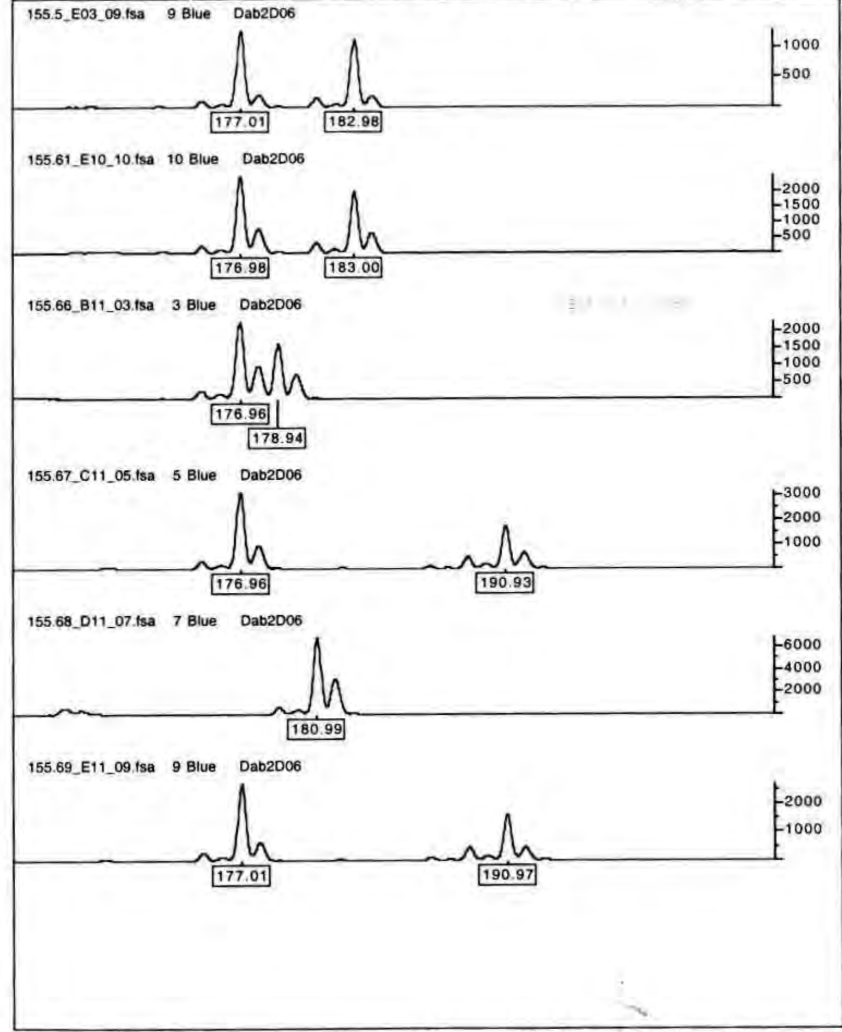
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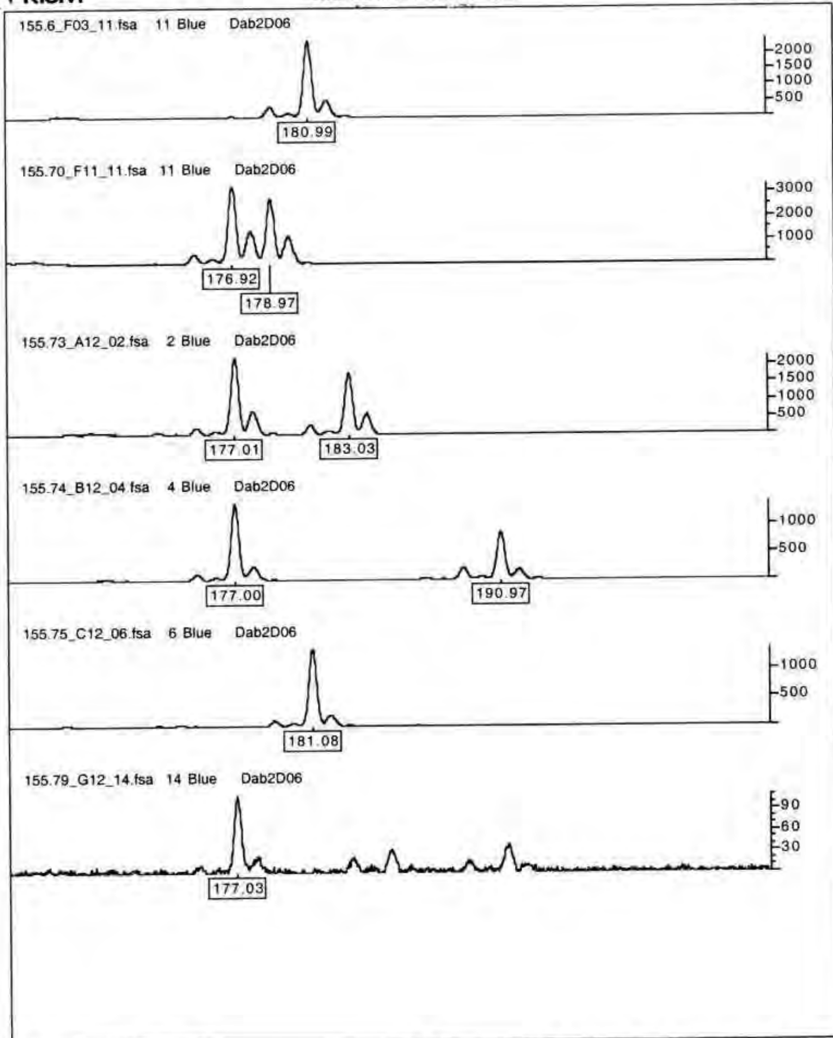
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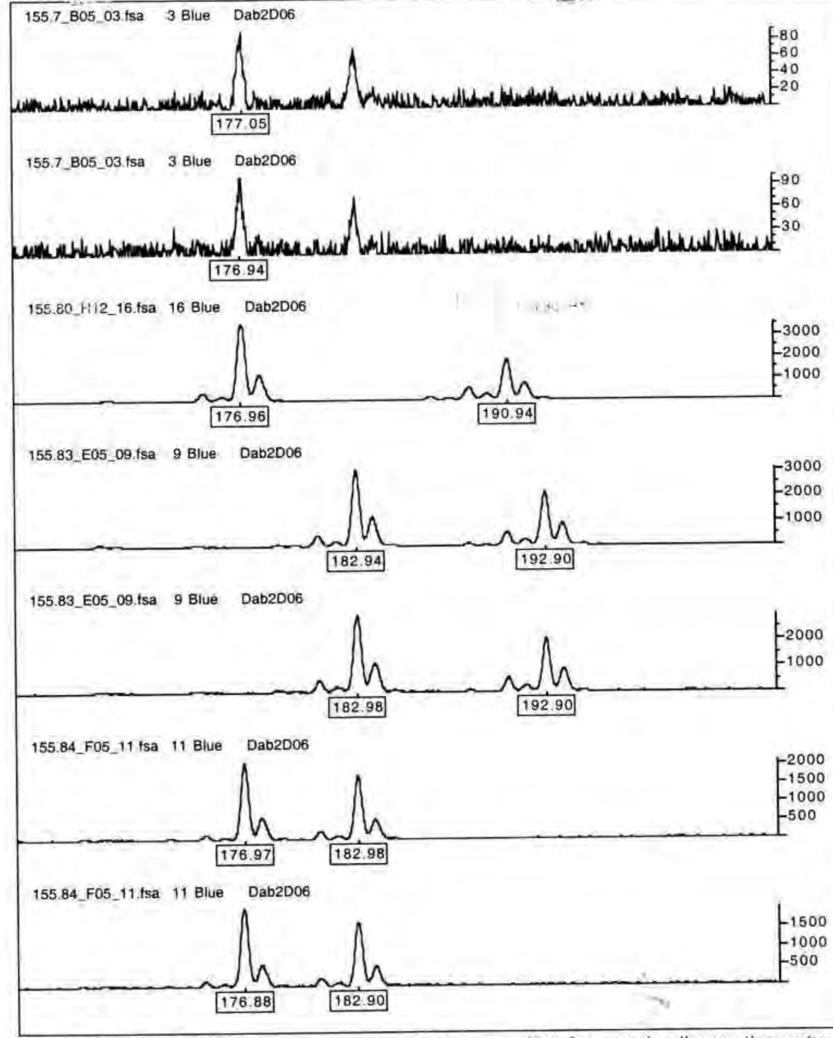
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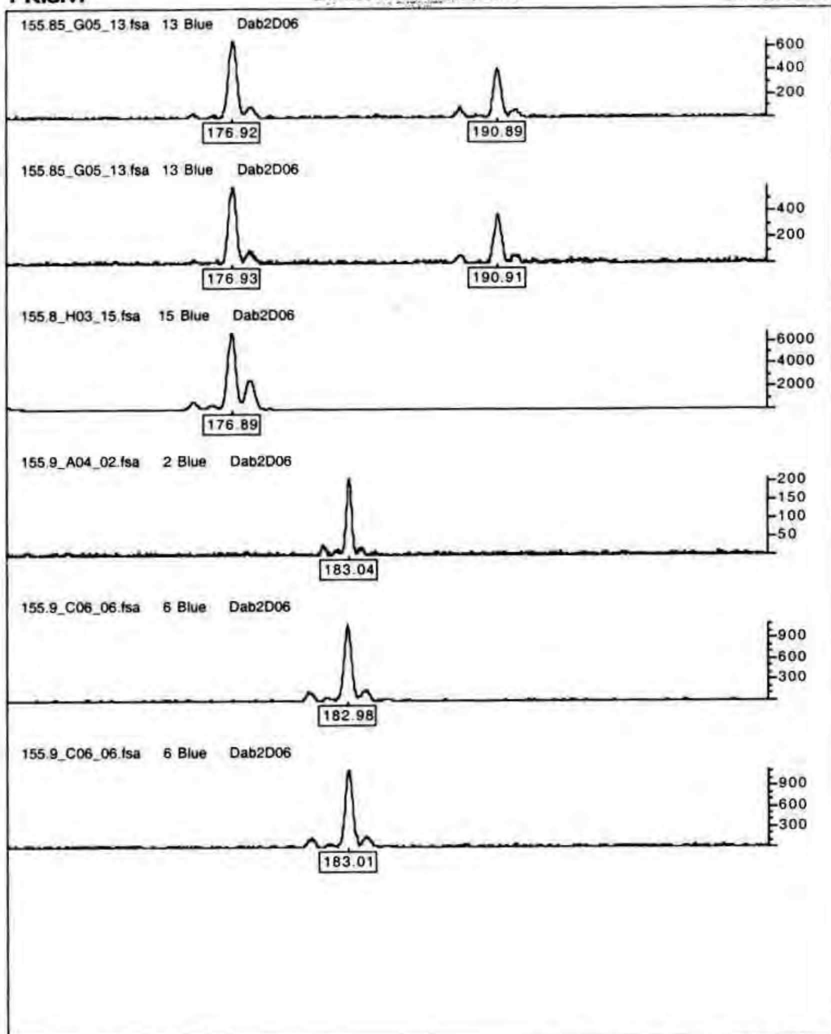
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Appendix 3. The raw data for morphometric analysis (measurements were in mm for floral and cm for leaf characters)

Acc.NO	OTL	OTW	ITL	ITW	AL	AW	FL	LEN	14W	12W	34W	Bw	L/BW	SBW	L/14W	L/12W	L/34W	M-1	M2	BW/SBW	14W/12W	12W/34W	14W/34W	M2-M1	MM-M2	MM-M1	M2-M1/M M	MM-M2/M M	MM-M1/M M
Daby-20	1.89	0.42	1.78	1.56	0.40	0.22	0.89	8.00	4.8	4	2.3	3.60	2.22	1.20	1.78	2.16	8.00	1.00	2.2	3.00	1.22	3.70	4.50	0.80	0.20	1.00	0.40	0.10	0.50
Daby-20	1.78	0.40	1.78	1.33	0.40	0.18	0.89	8.30	4.6	4.1	2.2	4.40	1.89	1.50	1.80	2.18	8.30	0.70	2	2.93	1.21	3.80	4.60	1.00	0.70	1.70	0.42	0.29	0.71
Daby-22	1.78	0.40	1.56	1.11	0.44	0.22	0.67	6.50	4.2	3.6	1.8	2.70	2.41	1.30	1.91	2.50	4.06	0.80	1.7	2.08	1.31	1.63	2.13	0.70	0.20	0.90	0.41	0.12	0.53
Daby-22	1.78	0.40	1.78	1.33	0.33	0.27	1.00	6.20	4.3	3.7	1.9	3.00	2.07	1.10	1.94	2.38	5.17	0.80	2	2.73	1.23	2.17	2.67	0.60	0.20	0.80	0.38	0.13	0.50
Daby-59	1.78	0.40	1.78	1.56	0.44	0.22	0.89	5.00	7	6.8	4.2	2.80	1.79	1.00	1.25	1.47	3.57	1.00	3.3	2.80	1.18	2.43	2.86	0.60	0.30	0.90	0.32	0.16	0.47
Daby-59	1.78	0.40	1.44	1.33	0.44	0.22	0.89	5.20	4.8	4.6	3	2.70	1.93	1.00	1.30	1.41	2.89	0.70	2.1	2.70	1.08	2.06	2.22	0.90	1.40	2.30	0.30	0.47	0.77
Daby-69	1.78	0.40	1.78	1.51	0.44	0.22	1.11	6.30	5.1	5.1	2.8	4.00	1.58	1.10	1.37	1.58	2.63	0.70	2	3.64	1.15	1.67	1.92	1.10	0.50	1.60	0.48	0.22	0.70
Daby-69	1.56	0.35	1.33	1.22	0.44	0.22	1.00	6.60	6.8	6.3	3	4.00	1.65	1.40	1.50	1.65	2.75	0.80	2.5	2.86	1.10	1.67	1.83	1.00	0.40	1.40	0.45	0.18	0.64
Daby-77	2.00	0.44	1.78	1.33	0.53	0.22	0.89	8.00	6	5.7	3.2	3.20	2.50	1.00	1.86	2.11	4.00	0.90	2	3.20	1.13	1.90	2.15	0.90	0.30	1.20	0.43	0.14	0.57
Daby-77	2.00	0.44	1.56	1.22	0.44	0.22	0.67	8.00	6.1	6	4.3	3.20	2.50	1.00	1.86	2.11	4.00	0.90	1.8	3.20	1.13	1.90	2.15	0.90	0.30	1.20	0.43	0.14	0.57
Daby-8	2.00	0.44	1.78	1.33	0.56	0.27	0.89	9.00	6.9	6.2	3.2	5.80	1.55	2.00	1.29	1.32	2.14	1.60	2.9	2.90	1.03	1.62	1.67	1.70	0.50	2.20	0.45	0.13	0.58
Daby-8	2.00	0.44	1.78	1.33	0.49	0.22	0.89	8.00	7	6.3	3.4	3.00	2.67	1.00	1.67	1.74	2.67	1.00	3.2	3.00	1.04	1.53	1.60	1.10	0.30	1.40	0.46	0.13	0.58
Dbul-28	2.11	0.47	1.78	1.22	0.44	0.22	0.89	14.00	6.8	6.2	4	15.00	0.93	3.50	0.90	1.17	2.15	2.20	2.5	4.29	1.29	1.85	2.38	2.80	2.50	5.30	0.37	0.33	0.71
Dbul-28	2.22	0.49	2.00	1.22	0.44	0.22	0.89	9.00	6.2	5.5	3.1	8.80	1.02	3.00	0.90	1.06	1.73	1.20	2.5	2.93	1.18	1.63	1.92	1.30	2.50	3.80	0.26	0.50	0.76
Dcay-1	1.78	0.40	1.56	1.31	0.44	0.22	1.11	9.50	6	6	4	4.40	2.16	1.30	1.98	2.38	4.13	1.20	1.7	3.38	1.20	1.74	2.09	1.00	0.20	1.20	0.42	0.08	0.50
Dcay-1	1.78	0.40	1.56	1.33	0.44	0.22	1.11	8.00	6	6	4	4.00	2.00	1.40	1.74	1.95	3.64	1.00	1.7	2.86	1.12	1.86	2.09	1.00	0.20	1.20	0.45	0.09	0.55
Dcay-17	1.78	0.40	1.89	1.33	0.33	0.22	0.67	12.00	4.5	3.7	1	5.00	2.40	1.50	1.74	1.94	3.75	1.50	1.8	3.33	1.11	1.94	2.16	1.40	0.60	2.00	0.40	0.17	0.57
Dcay-17	1.89	0.42	1.78	1.11	0.44	0.22	0.78	11.00	4.6	3.8	1	5.00	2.20	1.70	1.57	1.75	3.24	1.40	1.7	2.94	1.11	1.85	2.06	1.80	0.50	2.30	0.49	0.14	0.62
Dcay-18	1.89	0.42	1.78	1.11	0.44	0.27	0.89	10.00	4.5	4	2.5	5.50	1.82	1.80	1.47	1.61	2.50	1.00	1.5	3.06	1.10	1.55	1.70	1.50	0.80	2.30	0.45	0.24	0.70
Dcay-18	1.89	0.42	1.67	1.33	0.44	0.27	0.89	8.50	4.2	3.9	2	5.00	1.70	1.60	1.37	1.55	2.74	0.90	1.8	3.13	1.13	1.77	2.00	1.60	0.70	2.30	0.50	0.22	0.72
Dcay-19	1.89	0.42	1.78	1.11	0.44	0.22	0.67	8.20	3.4	2.6	1.6	4.30	1.91	1.50	1.37	1.37	2.05	0.60	1.5	2.87	1.00	1.50	1.50	1.10	1.30	2.40	0.37	0.43	0.80
Dcay-19	1.89	0.42	2.00	1.33	0.44	0.29	0.89	9.00	3.2	2.6	1.2	4.50	2.00	1.00	1.50	1.50	2.25	0.70	1.4	4.50	1.00	1.50	1.50	1.00	1.30	2.30	0.33	0.43	0.77
Dcay-21	2.22	0.49	1.56	1.33	0.44	0.22	0.89	7.50	4.8	4.4	2.8	3.80	1.97	1.00	1.67	1.88	3.00	0.60	2	3.80	1.13	1.60	1.80	0.90	0.70	1.60	0.41	0.32	0.73
Dcay-21	1.78	0.40	1.67	1.33	0.44	0.29	0.89	8.00	6.5	5.8	4.2	3.60	2.22	1.00	1.90	2.05	4.00	0.80	2.9	3.60	1.08	1.95	2.10	1.00	0.30	1.30	0.48	0.14	0.62
Dcay-35	1.67	0.37	1.56	1.07	0.44	0.22	0.78	8.80	6	5	2	3.80	2.32	1.60	1.87	1.91	3.14	1.20	2.5	2.38	1.02	1.64	1.68	0.80	0.40	1.20	0.33	0.17	0.50
Dcay-35	1.78	0.40	1.56	1.11	0.42	0.22	0.78	7.50	6.1	4.9	2	3.00	2.50	1.00	1.97	2.27	3.75	0.80	2.6	3.00	1.15	1.65	1.90	0.90	0.30	1.20	0.45	0.15	0.60
Dcay-38	1.78	0.40	1.56	1.33	0.38	0.33	0.89	7.00	4	3.6	2.2	2.50	2.80	0.90	2.33	2.26	3.50	0.80	1.8	2.78	0.97	1.55	1.50	0.70	0.10	0.80	0.44	0.06	0.50
Dcay-38	1.67	0.37	1.67	1.22	0.44	0.22	0.78	6.80	4	3.7	2.6	2.50	2.72	0.90	2.27	2.19	3.40	0.80	1.8	2.78	0.97	1.55	1.50	1.10	0.20	1.30	0.52	0.10	0.62
Dcay-47	2.22	0.49	2.00	1.11	0.44	0.22	0.89	5.50	15.5	12	6.5	2.40	2.29	0.70	1.83	1.96	3.06	0.60	5	3.43	1.07	1.56	1.67	0.80	0.20	1.00	0.50	0.13	0.63
Dcay-47	2.00	0.44	2.00	0.89	0.44	0.22	0.89	6.00	10	8.5	5.2	2.80	2.14	1.00	1.88	2.00	3.33	0.60	2.5	2.80	1.07	1.67	1.78	0.90	0.20	1.10	0.53	0.12	0.65
Dcay-48	2.00	0.44	2.00	1.11	0.44	0.36	0.78	7.80	3.8	3.2	1.7	3.50	2.23	1.00	1.63	1.66	2.60	0.80	1.9	3.50	1.02	1.57	1.60	1.00	0.70	1.70	0.40	0.28	0.68
Dcay-48	2.00	0.44	2.00	1.22	0.44	0.22	0.78	8.00	4	3.6	1.5	4.00	2.00	1.20	1.74	1.82	2.86	0.60	2	3.33	1.05	1.57	1.64	1.20	0.50	1.70	0.52	0.22	0.74
Dcay-49	2.00	0.44	1.78	1.33	0.44	0.22	0.67	7.60	4.6	4	2	3.00	2.53	1.40	1.81	1.90	3.17	0.80	1.5	2.14	1.05	1.67	1.75	0.90	0.50	1.40	0.41	0.23	0.64
Dcay-49	2.00	0.44	1.78	1.33	0.44	0.22	0.67	7.00	4.7	3.8	2	3.80	1.84	1.30	1.75	1.79	3.50	0.70	1.5	2.92	1.03	1.95	2.00	1.00	0.30	1.30	0.50	0.15	0.65
Dcay-50	1.78	0.40	1.33	1.11	0.44	0.27	0.67	7.00	5.2	5.2	4.2	4.80	1.46	1.50	1.40	1.75	2.92	0.70	1.2	3.20	1.25	1.67	2.08	0.30	1.50	1.80	0.12	0.60	0.72
Dcay-56	2.00	0.44	1.56	1.33	0.44	0.22	0.78	7.50	5.3	5.3	4	3.00	2.50	1.20	2.08	2.14	3.13	0.80	1.8	2.50	1.03	1.46	1.50	0.90	0.30	1.20	0.45	0.15	0.60
Dcay-57	2.00	0.44	1.89	1.44	0.44	0.22	0.89	8.00	4.7	4.6	2.8	3.60	2.22	1.60	1.90	2.00	3.81	0.90	2	2.25	1.05	1.90	2.00	1.00	0.40	1.40	0.43	0.17	0.61
Dcay-57	2.00	0.44	1.78	1.33	0.47	0.22	0.67	7.00	3.8	3.3	2	3.60	1.94	1.50	1.56	1.75	3.68	0.80	1.7	2.40	1.13	2.11	2.37	1.10	0.50	1.60	0.46	0.21	0.67
Dcay-61	1.78	0.40	1.56	1.33	0.40	0.18	0.67	8.00	3	3.1	2	5.00	1.60	1.50	1.45	1.63	2.67	1.10	1.5	3.33	1.12	1.63	1.83	1.00	0.40	1.40	0.40	0.16	0.56
Dcay-61	1.56	0.35	1.56	1.44	0.36	0.22	0.67	7.00	3	3.1	2	3.60	1.94	1.40	1.56	1.84	3.50	1.00	1.9	2.57	1.18	1.90	2.25	0.90	0.30	1.20	0.41	0.14	0.55
Dcay-62	1.56	0.35	1.33	1.11	0.44	0.22	0.89	6.80	3	2.8	1.8	4.70	1.45	1.50	1.36	1.55	2.62	1.00	1.4	3.13	1.14	1.69	1.92	1.10	0.60	1.70	0.41	0.22	0.63
Dcay-62	1.56	0.35	1.33	1.11	0.42	0.22	0.78	7.40	3.2	3	1.8	4.20	1.76	1.50	1.45	1.64	2.85	1.00	1.5	2.80	1.13	1.73	1.96	1.20	0.50	1.70	0.44	0.19	0.63
Dcay-67	1.78	0.40	1.56	1.11	0.44	0.33	0.89	6.20	4.8	4.7	3	4.00	1.55	1.20	1.24	1.32	1.88	1.00	1.8	3.33	1.06	1.42	1.52	1.00	0.50	1.50	0.40	0.20	0.60
Dcay-68	1.78	0.40	1.56	1.33	0.44	0.22	0.89	6.50	4.6	4.4	2.8	3.40	1.91	1.20	1.55	1.71	2.32	0.80	1.8	2.83	1.11	1.36	1.50	1.00	0.40	1.40	0.45	0.18	0.64
Dcay-68	1.78	0.40	1.33	1.33	0.33	0.22	0.67	6.50	4.2	4	2.4	4.00	1.63	1.10	1.48	1.71	2.71	0.80	1.7	3.64	1.16	1.58	1.83	1.00	0.30	1.30	0.48	0.14	0.62
Dcay-7	1.78	0.40	1.78	1.33	0.33	0.29	0.67	7.00	4	3.9	2	3.60	1.94	1.00	1.67	1.94	3.89	1.00	1.7	3.60	1.17	2.00	2.33	0.70	0.40	1.10	0.33	0.19	0.52
Dcay-7	1.78	0.40	1.78	1.33	0.33	0.33	0.67	7.00	5	4	2.4	3.70	1.89	1.00	1.63	1.89	3.68	1.00	1	3.70	1.16	1.95	2.26	1.00	0.20	1.20	0.45	0.09	0.55
Dcay-70	1.78	0.40	1.78	1.22	0.22	0.22	0.89	9.50	5.4	5.7	4.5	5.70	1.67	1.80	1.42	1.53	2.38	1.60	1.7	3.17	1.08	1.55	1.68	1.20	0.60	1.80	0.35	0.18	0.53
Dcay-70	1.78	0.40	1.78	1.22	0.44	0.22	0.67	6.70	6	6	4.2	4.00	1.68	0.80	1.46	1.72	2.91	1.00	1.7	5.00	1.18	1.70	2.00	0.90	0.40	1.30	0.39	0.17	0.57
Dcay-71	2.00	0.44	1.56	0.89	0.40	0.33	0.89	6.00	3.6	3.5	2.4	3.50	1.71	1.00	1.40	1.67	2.50	1.00	1.7	3.50	1.19	1.50	1.79	0.80	0.40	1.20	0.36	0.18	0.55

Acc.NO	OTL	OTW	ITL	ITW	AL	AW	FL	LEN	14W	12W	34W	Bw	LBW	SBW	L/14W	L/12W	L/34W	M-1	M2	BW/SBW	14W/12W	12W/34W	14W/34W	M2-M1	MM-M2	MM-M1	M2-M1/M M	MM-M2/M M	MM-M1/M M
Dcay-73	2.00	0.44	2.11	1.33	0.44	0.33	0.89	9.50	4.2	4	2.1	4.80	1.98	2.00	1.58	1.79	3.17	1.10	1.9	2.40	1.13	1.77	2.00	1.30	0.60	1.90	0.43	0.20	0.63
Dcay-73	2.11	0.47	2.11	1.11	0.44	0.33	1.11	8.60	4.5	4	1.9	4.00	2.15	1.60	1.39	1.65	2.32	1.00	1.9	2.50	1.19	1.41	1.68	1.40	0.60	2.00	0.47	0.20	0.67
Dcay-74	1.78	0.40	1.56	1.33	0.44	0.33	1.11	8.50	4	3.4	1.4	5.00	1.70	2.00	1.42	1.63	2.36	1.30	1.6	2.50	1.15	1.44	1.67	1.20	0.60	1.80	0.39	0.19	0.58
Dcay-74	1.78	0.40	1.56	1.44	0.44	0.22	0.89	9.00	4	3.7	1.8	5.00	1.80	2.00	1.36	1.53	2.31	1.40	1.6	2.50	1.12	1.51	1.69	1.20	0.80	2.00	0.35	0.24	0.59
Dcay-75	1.78	0.40	1.33	1.11	0.40	0.22	0.67	6.30	5.5	4.9	3	3.00	2.10	1.00	1.62	1.70	2.25	0.90	2.1	3.00	1.05	1.32	1.39	0.90	0.20	1.10	0.45	0.10	0.55
Dcay-75	1.78	0.40	1.33	1.33	0.33	0.22	0.67	8.00	4.5	3.8	2	3.60	2.22	1.20	1.78	2.00	2.96	1.10	1.9	3.00	1.13	1.48	1.67	0.90	0.20	1.10	0.41	0.09	0.50
Dcay-75	1.78	0.40	1.56	1.11	0.44	0.22	0.89	6.30	5	4.4	2.6	3.00	2.10	1.00	1.62	1.70	2.25	0.90	2.1	3.00	1.05	1.32	1.39	0.90	0.20	1.10	0.45	0.10	0.55
Dcay-75	1.78	0.40	1.78	1.33	0.44	0.22	0.67	8.00	5.1	4.5	2.6	3.60	2.22	1.20	1.78	2.00	2.96	1.10	2.2	3.00	1.13	1.48	1.67	0.90	0.20	1.10	0.41	0.09	0.50
Dcay-76	1.78	0.40	1.67	0.89	0.44	0.22	0.67	9.10	5	4.7	3.3	5.00	1.82	2.00	1.98	2.28	3.79	1.40	2	2.50	1.15	1.67	1.92	1.00	0.20	1.20	0.38	0.08	0.46
Dcay-76	2.00	0.44	1.56	1.22	0.22	0.22	0.67	9.10	4.2	3.8	2.8	5.00	1.82	2.00	1.98	2.28	3.79	1.40	1.8	2.50	1.15	1.67	1.92	1.00	0.20	1.20	0.38	0.08	0.46
Dprh-13	1.78	0.40	1.89	1.56	0.56	0.29	0.78	8.50	4.4	3.8	2.4	4.10	2.07	1.00	1.67	1.67	3.04	0.90	1.8	4.10	1.00	1.82	1.82	1.10	0.80	1.90	0.39	0.29	0.68
Dprh-13	2.00	0.44	1.78	1.56	0.44	0.31	0.67	9.50	4.6	4	2.4	4.70	2.02	1.20	1.40	1.51	3.17	0.90	1.8	3.92	1.08	2.10	2.27	1.60	1.10	2.70	0.44	0.31	0.75
Dprh-16	2.00	0.44	1.56	1.56	0.44	0.22	0.56	8.80	4.4	4	2.4	3.50	2.51	1.00	1.47	1.54	2.75	0.80	1.8	3.50	1.05	1.78	1.88	1.20	1.00	2.20	0.40	0.33	0.73
Dprh-16	1.67	0.37	1.78	1.44	0.44	0.22	0.67	9.50	6.7	6.2	4	4.20	2.26	1.50	1.56	1.58	2.21	0.70	2.8	2.80	1.02	1.40	1.42	1.10	1.20	2.30	0.37	0.40	0.77
Dprh-25	1.78	0.40	1.78	1.33	0.44	0.44	1.11	8.40	4.6	3.9	2.3	3.50	2.40	1.00	1.75	1.91	3.00	1.20	1.9	3.50	1.09	1.57	1.71	0.80	0.20	1.00	0.36	0.09	0.45
Dprh-25	2.00	0.44	1.89	1.33	0.44	0.22	0.89	9.50	4.3	3.6	2.4	5.50	1.73	1.50	1.46	1.64	2.26	1.50	1.8	3.67	1.12	1.38	1.55	1.40	0.50	1.90	0.41	0.15	0.56
Dprh-26	1.78	0.40	1.78	1.22	0.38	0.22	0.67	10.00	6	5.3	3	5.20	1.92	1.50	1.67	2.00	5.00	1.20	2.4	3.47	1.20	2.50	3.00	1.30	0.40	1.70	0.45	0.14	0.59
Dprh-26	1.89	0.42	1.78	1.22	0.44	0.31	0.89	10.00	6.2	5.2	3.7	5.10	1.96	1.50	1.64	2.04	5.00	1.40	2.4	3.40	1.24	2.45	3.05	1.20	0.50	1.70	0.39	0.16	0.55
Dprh-27	2.00	0.44	1.78	1.56	0.33	0.44	0.89	7.70	6	5.2	3.6	2.20	3.50	0.60	1.93	2.14	3.50	0.80	2.5	3.67	1.11	1.64	1.82	1.00	0.30	1.30	0.48	0.14	0.62
Dprh-27	1.78	0.40	1.56	1.38	0.47	0.22	0.89	7.50	6.6	5.9	3.9	1.80	4.17	0.70	1.88	2.03	2.88	0.60	2.6	2.57	1.08	1.42	1.54	1.20	0.20	1.40	0.60	0.10	0.70
Dprh-30	2.00	0.44	1.67	1.33	0.44	0.22	0.67	9.30	3.9	3.7	2.8	3.60	2.58	1.50	2.45	2.91	5.47	1.00	1.8	2.40	1.19	1.88	2.24	0.90	0.10	1.00	0.45	0.05	0.50
Dprh-30	1.89	0.42	1.56	1.33	0.42	0.22	0.67	10.50	4.5	4	2.7	3.80	2.76	1.60	2.63	2.92	7.00	1.00	2	2.38	1.11	2.40	2.67	1.00	0.10	1.10	0.48	0.05	0.52
Dprh-31	1.67	0.37	1.67	1.44	0.33	0.22	0.67	9.50	3.9	3.7	2.8	4.00	2.38	1.50	2.07	2.38	4.75	0.50	1.8	2.67	1.15	2.00	2.30	1.00	0.50	1.50	0.50	0.25	0.75
Dprh-31	2.00	0.44	1.56	1.11	0.44	0.22	0.67	9.50	4.5	4	2.7	4.00	2.38	1.60	2.02	2.50	4.75	0.50	2	2.50	1.24	1.90	2.35	1.00	0.90	1.90	0.42	0.38	0.79
Dprh-32	1.56	0.35	1.33	1.22	0.44	0.22	0.67	6.50	4.6	4	2.4	4.00	1.63	1.20	1.25	1.25	1.55	0.60	2.4	3.33	1.00	1.24	1.24	0.60	1.10	1.70	0.26	0.48	0.74
Dprh-32	1.56	0.35	1.44	1.11	0.42	0.22	0.78	6.70	4.6	4	2.4	4.10	1.63	1.00	1.26	1.26	1.68	0.80	2.4	4.10	1.00	1.33	1.33	1.00	0.90	1.90	0.37	0.33	0.70
Dprh-53	1.78	0.40	1.78	1.44	0.44	0.22	0.67	6.00	4.3	3.8	2	3.50	1.71	1.00	1.11	1.05	1.33	0.70	1.8	3.50	0.95	1.27	1.20	1.00	1.00	2.00	0.37	0.37	0.74
Dprh-53	2.00	0.44	1.78	1.44	0.47	0.22	0.67	7.40	4.3	3.8	2	4.50	1.64	1.30	1.23	1.23	1.76	0.50	1.8	3.46	1.00	1.43	1.43	1.20	1.50	2.70	0.38	0.47	0.84

Appendix 4. The raw data for AFLP analyses

Category	D. prh	D. cay	D. cay	D. cay	D. cay	D. cay	D. aby	D. aby	D. aby	D. aby	D. cay	D. cay	D. aby	D. cay	D. cay	D. cay	D. cay	D. cay	D. cay	D. cay	D. aby	D. cay	D. cay	D. cay	D. cay	D. aby	D. cay	D. cay	D. cay	Daby	Dcay	D. aby
56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
57	0	1	1	0	0	0	1	0	0	1	1	0	1	1	1	0	0	0	1	1	0	1	0	0	0	1	0	1	0	0	0	0
58	1	0	0	0	1	1	0	1	1	0	0	0	0	0	0	1	1	1	0	0	1	0	1	1	1	0	1	0	1	1	1	0
60	1	1	1	1	1	1	1	1	1	0	0	1	0	0	1	1	1	1	0	1	1	1	1	1	1	0	1	0	1	1	1	0
61	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
64	0	0	1	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	1	1	0	1	0	0	0	1	0	1	0	0	0	0
65	0	1	0	1	1	1	1	1	1	0	0	1	0	1	0	1	1	1	0	0	1	0	1	1	1	0	1	0	1	1	1	0
67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	1	0	1	1	1	0	0	1	1	0	0	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	0	0
72	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0
75	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
77	1	1	0	1	1	1	1	1	1	0	1	1	0	0	0	1	1	1	1	0	1	0	1	1	1	0	1	0	1	1	1	0
78	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0
79	0	0	1	0	0	0	1	0	0	1	1	0	1	0	1	0	0	0	0	1	0	1	1	1	1	1	0	1	0	0	0	0
80	1	1	0	1	1	1	1	1	1	0	0	1	0	1	0	1	1	1	1	0	1	0	1	1	1	0	1	0	1	1	1	0
86	0	1	0	1	1	1	1	1	1	0	1	1	0	0	0	0	1	1	0	0	1	0	1	1	1	0	0	0	1	1	1	1
87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
91	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
98	1	1	1	0	0	0	1	1	1	0	0	1	0	1	1	1	1	0	0	0	1	1	1	1	1	0	1	0	1	1	1	0
106	0	0	0	0	0	0	1	0	0	1	1	0	1	0	0	1	0	1	0	1	0	0	0	1	1	1	0	1	1	0	0	0
107	0	1	1	0	1	1	1	1	1	1	0	1	0	0	1	0	0	1	0	1	1	0	1	1	1	1	0	1	1	1	1	0
108	0	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	0
109	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0
110	0	1	0	0	0	0	1	1	1	0	1	0	0	0	1	1	0	1	0	0	0	0	0	1	1	1	0	0	1	0	0	0
111	0	0	0	1	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0	1	0	0
112	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0
123	0	0	1	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0
124	0	1	0	1	1	1	1	1	1	0	0	1	1	0	0	1	1	1	0	0	1	0	1	1	1	0	1	0	1	1	1	0
133	0	1	0	1	1	0	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	0	1
137	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
138	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
139	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
140	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
141	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
152	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1	0	1	0	1	0	1	0	1	1	1	1	1	0	0	0	1
155	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
162	1	1	1	1	0	1	0	0	0	1	1	0	1	0	1	1	1	0	1	1	0	1	0	0	0	1	1	1	1	0	0	1
163	0	0	0	0	1	0	1	1	1	0	0	1	0	0	0	0	1	1	0	0	1	0	1	1	1	0	0	0	1	1	1	0
167	0	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	0	1	1	0	1	1	1	1	1	1	1	1	0	0
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Category	D. prh	D. cay	D. cay	D. cay	D. cay	D. cay	D. aby	D. aby	D. aby	D. aby	D. cay	D. cay	D. aby	D. cay	D. cay	D. cay	D. cay	D. cay	D. cay	D. cay	D. aby	D. cay	D. cay	D. cay	D. cay	D. aby	D. cay	D. cay	D. cay	Daby	Dcay	D. aby
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Category	D. prh	D. cay	D. cay	D. cay	D. cay	D. cay	D. aby	D. aby	D. aby	D. aby	D. cay	D. cay	D. aby	D. cay	D. cay	D. cay	D. cay	D. cay	D. cay	D. cay	D. aby	D. cay	D. cay	D. cay	D. cay	D. aby	D. cay	D. cay	D. cay	Daby	Dcay	D. aby
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303	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	0	1	0	1	0	1
304	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
313	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
389	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
422	1	1	0	0	1	0	1	1	1	0	0	0	1	0	1	0	1	1	1	1	1	0	1	1	1	0	0	1	0	1	0	0
437	0	1	0	0	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	1	0	0	1	0	1	1	1	0	1
438	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0
442	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
478	0	1	0	0	1	1	1	1	1	0	1	1	0	0	1	0	1	0	0	1	1	0	1	1	1	1	0	1	0	1	0	0
496	0	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	1	1	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
73	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
74	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
76	0	0	1	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0
82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85	0	1	1	1	0	0	0	1	0	1	1	0	1	1	1	0	0	0	1	1	0	1	1	1	1	1	1	1	1	1	0	0
95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
97	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
113	0	1	0	0	1	1	1	1	1	0	0	1	0	0	1	0	1	1	0	0	1	0	1	1	1	0	1	0	1	1	1	1
119	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
120	1	0	0	0	0	1	0	1	1	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0
121	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
122	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
125	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
126	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	1	1	0	0	0	0	0	1	1	1	0	0	0	1	1	0	0
145	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Category	D. prh	D. cay	D. cay	D. cay	D. cay	D. cay	D. aby	D. aby	D. aby	D. aby	D. cay	D. cay	D. aby	D. cay	D. cay	D. cay	D. cay	D. cay	D. cay	D. cay	D. aby	D. cay	D. cay	D. cay	D. cay	D. aby	D. cay	D. cay	D. cay	Daby	Dcay	D. aby
148	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
149	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
153	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	1	1	1	0
180	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
182	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0
204	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
210	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
212	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
213	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
217	0	0	0	1	0	1	0	1	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0
229	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
277	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
54	0	1	1	0	1	1	0	1	1	0	0	1	0	0	1	1	1	1	1	0	1	0	0	0	1	1	1	0	1	0	0	1
55	1	0	1	0	1	1	1	0	1	1	1	1	1	0	1	0	1	0	1	1	1	1	1	1	1	1	0	1	1	0	0	0
58	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
77	1	0	1	0	1	1	1	1	1	0	0	1	0	0	1	0	1	1	1	0	1	0	1	1	1	1	1	0	1	1	1	1
80	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0
85	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0
87	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	1	0	0	0	0
88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0
97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	1	1	0	1	0	0	0	0
109	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
113	0	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1
114	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
129	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
132	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
140	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
159	0	1	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
160	0	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1
167	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
169	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
171	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
179	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
181	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
182	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
192	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0
195	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
210	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
221	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
227	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
236	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
237	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
242	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
256	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
257	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
266	1	0	0	1	0	1	1	1	1	0	1	0	1	1	1	0	0	0	1	0	1	0	0	0	1	0	0	0	0	1	0	0
267	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
282	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
283	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
301	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
312	1	1	0	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1
394	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
404	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
444	1	1	0	0	1	1	1	1	1	0	1	1	1	0	1	0	1	1	1	0	1	0	1	1	1	1	0	1	1	0	0	0

Category	D. cay	D. cay	D. cay	D. aby	D. cay	D. aby	D.bul.	D.shc.	D. prh	D. prh	D. prh	D. prh	D. cay
56	0	0	0	0	0	0	1	0	0	0	0	0	0
57	1	0	1	0	0	0	0	0	1	0	1	0	1
58	0	1	0	1	1	1	0	1	0	1	0	1	0
60	0	1	0	1	1	1	1	0	1	1	1	1	1
61	1	1	1	1	1	1	0	0	1	1	1	1	1
64	1	0	1	0	0	0	0	0	0	0	0	0	1
65	0	1	0	1	1	1	1	0	1	1	1	1	0
67	0	0	0	0	0	0	0	1	0	0	0	0	0
71	1	1	0	0	0	1	1	1	0	1	1	1	0
72	0	0	0	1	0	0	0	1	1	0	1	0	0
75	0	0	0	0	1	1	1	0	0	1	0	1	0
77	0	1	0	1	1	1	0	0	1	1	0	1	0
78	0	0	0	0	0	0	0	0	0	0	0	0	0
79	1	0	1	0	1	1	0	0	0	0	1	1	1
80	0	1	0	1	1	1	1	0	1	1	1	1	0
86	0	1	0	0	1	1	1	1	1	1	1	1	0
87	0	0	0	0	0	0	0	1	0	0	1	0	0
90	0	0	0	1	1	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0	0	0	1	0	0
98	1	0	0	1	1	1	0	0	1	1	1	1	1
106	0	0	0	0	1	1	0	0	0	0	0	1	1
107	0	1	1	0	1	1	0	0	1	0	1	1	0
108	1	1	1	1	1	1	0	0	1	1	1	1	0
109	0	0	0	0	0	0	0	0	0	0	0	0	1
110	0	1	0	0	1	1	0	0	1	1	0	1	0
111	0	0	0	0	1	1	0	0	0	0	0	0	0
112	1	0	0	0	0	0	0	0	0	0	0	0	1
123	0	0	1	1	0	0	0	1	1	0	0	0	1
124	0	1	0	0	1	1	1	1	0	1	1	1	0
133	1	1	1	1	1	1	0	0	1	0	0	1	1
137	0	0	0	0	0	0	0	0	0	0	0	0	0
138	0	0	0	0	0	0	0	0	0	1	0	1	0
139	0	0	0	0	0	0	1	1	0	0	0	0	0
140	0	0	0	0	0	0	1	0	0	0	0	0	0
141	0	0	0	0	0	0	1	0	0	0	0	0	0
152	1	1	1	1	1	1	1	0	1	0	1	1	1
155	0	0	0	0	0	0	0	1	0	0	0	0	0
162	0	1	1	1	0	0	0	0	1	0	1	0	1
163	0	0	0	0	1	1	0	0	0	1	1	1	0
167	0	1	0	0	1	1	0	0	1	1	1	1	1
170	0	0	0	0	0	0	1	0	0	0	0	0	0
175	1	0	1	1	1	0	0	0	1	0	1	0	1
176	1	1	1	1	1	1	0	1	1	1	1	1	1
177	0	1	0	0	1	1	1	1	1	1	0	1	1
178	1	0	0	0	0	0	0	0	0	0	1	0	1
179	0	1	1	1	1	1	1	0	1	1	1	1	0
181	0	0	0	0	0	0	0	1	0	0	0	0	0
183	0	0	0	0	0	0	1	0	0	0	0	0	0

Category	D. cay	D. cay	D. cay	D. aby	D. cay	D. aby	D.bul.	D.shc.	D. prh	D. prh	D. prh	D. prh	D. cay
184	0	0	0	0	0	0	1	0	0	0	0	0	0
185	1	1	1	1	1	1	0	0	1	1	1	1	1
186	0	0	0	0	0	0	1	0	0	0	0	0	0
188	0	0	0	0	0	0	0	1	0	0	0	0	0
193	0	0	1	0	1	1	0	1	0	1	0	1	0
206	1	1	1	0	1	1	0	0	0	0	0	0	0
216	1	0	0	0	1	1	1	0	1	1	1	1	1
219	0	0	0	0	0	0	1	0	0	0	0	0	0
221	0	0	0	0	0	0	0	1	0	0	0	0	0
235	1	1	1	1	1	1	0	0	1	1	1	1	1
236	0	0	0	0	1	1	0	0	1	1	0	1	1
237	0	1	1	0	1	1	0	0	0	0	0	0	0
239	0	0	0	0	0	0	1	0	0	0	0	0	0
241	0	0	0	0	0	0	0	0	0	0	0	0	0
242	1	1	1	1	1	1	0	0	1	1	1	1	1
243	0	0	0	0	0	0	0	0	0	0	1	0	0
244	0	1	0	1	1	0	0	0	1	1	1	0	1
245	0	1	0	0	0	1	0	0	1	1	0	1	0
246	1	1	1	1	1	1	0	1	1	1	1	1	1
256	0	0	0	0	0	0	0	1	0	0	0	0	0
258	0	0	0	0	0	0	0	1	0	0	0	0	0
261	0	0	0	0	1	1	0	0	0	0	0	0	0
281	0	0	0	0	1	0	1	0	0	1	1	1	0
283	0	0	0	0	0	0	1	0	0	0	0	0	0
300	1	0	1	1	1	1	0	0	0	0	0	0	0
301	0	0	0	0	0	0	0	0	0	1	1	1	1
302	0	0	0	0	0	0	0	0	0	0	0	0	0
307	0	0	0	0	0	0	0	0	0	0	0	1	0
309	0	0	0	0	0	0	0	0	0	0	0	0	0
310	0	0	0	0	0	0	0	0	0	0	0	0	0
311	0	1	0	0	0	0	0	0	0	0	0	0	0
312	0	0	0	0	0	0	1	0	0	0	0	0	0
313	0	0	0	0	0	0	0	1	0	0	0	0	0
321	1	1	0	1	1	1	1	0	1	1	1	1	1
322	0	0	0	0	0	0	0	1	0	0	0	0	0
326	0	0	0	0	0	0	0	0	0	0	0	1	0
327	0	0	0	0	0	0	0	0	0	0	0	0	0
350	1	0	1	0	0	0	0	0	0	0	0	0	0
351	0	1	0	1	1	1	0	0	1	1	1	1	0
357	1	1	1	0	1	1	0	0	1	1	1	1	1
366	1	1	1	1	1	1	0	0	0	0	0	1	0
369	1	1	1	1	1	1	0	0	1	1	1	1	1
385	0	1	0	1	1	1	0	0	0	1	1	1	0
388	1	1	1	1	1	1	0	0	1	1	1	1	1
53	1	1	1	1	1	1	0	0	1	0	1	1	1
56	0	1	1	1	1	1	0	0	1	1	0	1	0
59	0	0	1	1	0	0	0	0	0	1	0	1	0
64	0	0	0	0	0	0	0	0	0	0	0	0	1
65	0	1	1	1	1	1	0	1	1	0	1	1	0
66	0	0	0	0	0	0	0	0	0	1	0	0	0

Category	D. cay	D. cay	D. cay	D. aby	D. cay	D. aby	D.bul.	D.shc.	D. prh	D. prh	D. prh	D. prh	D. cay
68	0	0	0	0	0	0	0	1	0	0	0	0	0
74	0	0	1	0	0	0	0	0	1	0	1	1	0
76	1	1	1	0	0	0	1	0	0	0	1	0	1
78	0	0	0	0	0	0	0	1	0	0	0	0	0
79	0	0	1	0	0	0	0	0	0	0	0	0	0
81	0	0	1	0	0	0	0	0	0	0	0	0	0
82	1	0	0	1	1	1	0	0	1	1	0	1	0
84	0	0	0	0	0	0	1	0	0	0	0	0	0
86	0	1	1	1	1	1	1	1	1	1	1	1	1
90	0	0	0	1	1	1	0	0	1	0	0	0	0
91	0	0	0	1	1	1	1	0	1	1	0	1	0
94	0	0	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	1	0	0	0	0	0
102	1	1	1	1	1	1	1	1	1	1	1	1	1
106	1	1	1	0	0	0	0	0	1	0	1	0	1
107	0	0	0	1	1	1	0	0	0	1	0	1	0
111	0	1	0	0	0	0	0	0	0	0	1	0	1
112	0	1	1	1	1	1	0	0	1	1	0	1	0
121	0	1	0	0	0	0	1	0	0	0	0	0	1
122	1	0	1	1	1	1	0	0	1	1	1	1	0
126	0	0	0	0	0	0	0	0	0	1	0	0	0
131	0	0	0	0	0	0	1	0	0	0	0	0	0
133	0	0	0	0	0	0	1	1	0	0	0	0	0
134	0	1	1	1	1	1	0	0	1	1	1	1	1
141	0	0	0	0	0	0	0	1	0	0	0	0	0
149	0	1	1	1	1	1	0	0	1	1	1	1	0
150	0	0	0	0	0	0	0	1	0	0	0	0	0
151	1	0	1	1	1	1	0	0	1	1	0	0	0
161	0	0	1	1	1	1	1	1	1	1	0	1	0
168	0	1	1	1	1	1	0	1	1	1	1	1	0
186	0	0	1	1	1	1	0	0	1	1	0	1	0
187	0	1	1	1	1	1	0	0	1	1	1	0	0
188	0	1	1	1	0	0	0	0	1	0	0	1	0
189	0	0	0	0	0	0	0	0	0	0	0	0	0
190	1	1	1	1	1	1	0	0	1	0	1	1	1
191	0	0	0	0	0	0	0	0	0	1	0	0	0
194	0	0	0	0	0	0	0	1	0	0	0	0	0
201	0	0	0	0	0	0	0	0	0	0	0	0	0
206	0	0	0	0	0	0	0	0	0	0	1	1	0
209	0	0	0	0	0	0	1	0	0	0	0	0	0
217	0	1	0	1	1	1	0	0	1	1	1	1	0
222	0	1	1	1	1	1	0	0	1	0	1	1	1
223	0	0	0	0	0	0	0	0	0	1	0	0	0
225	0	0	1	0	0	0	0	0	0	0	0	0	0
230	0	0	0	0	0	0	1	1	0	0	0	0	0
239	0	1	1	1	1	1	1	0	1	1	1	1	1
246	0	0	0	0	0	0	0	0	0	0	0	0	0
250	0	0	0	0	0	0	0	0	0	1	0	0	0
253	0	0	0	0	0	0	0	0	0	0	0	0	0
264	0	0	0	0	0	0	0	1	0	0	0	0	0

Category	D. cay	D. cay	D. cay	D. aby	D. cay	D. aby	D.bul.	D.shc.	D. prh	D. prh	D. prh	D. prh	D. cay
284	0	0	1	1	1	1	0	0	1	1	0	1	0
285	0	0	0	0	0	0	0	0	0	0	0	0	0
287	0	0	0	0	0	0	0	0	0	0	0	0	0
295	0	1	0	0	0	0	0	0	0	0	1	0	0
296	0	0	1	1	1	1	0	0	1	1	0	1	0
297	0	0	0	0	0	0	0	0	0	0	0	0	0
303	0	1	1	1	1	1	0	0	1	1	0	1	0
304	1	0	0	0	0	0	0	0	0	0	0	0	0
313	0	0	0	0	0	0	0	0	0	0	0	0	0
389	0	0	0	0	0	0	0	0	0	0	0	0	0
422	0	1	0	1	1	1	0	0	1	0	0	1	0
437	0	1	1	1	1	1	0	0	1	1	1	0	0
438	0	0	0	0	0	0	0	0	0	0	0	1	0
442	0	0	0	0	0	0	0	0	0	0	0	0	0
478	0	1	0	1	1	1	0	0	0	0	0	0	0
496	0	1	0	0	1	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	1	1	0	0	0	0	0
54	1	1	1	1	1	1	1	1	1	1	1	1	1
59	0	0	0	0	0	0	0	0	0	0	0	0	0
70	1	1	1	1	1	1	0	1	1	1	1	1	1
73	0	0	1	0	0	0	0	1	0	0	0	0	1
74	1	1	0	0	0	0	0	1	0	0	0	0	0
76	0	0	1	0	0	0	0	1	0	0	1	0	1
82	0	0	0	0	0	0	0	1	0	0	0	0	0
85	1	0	1	0	0	0	0	1	0	0	1	1	1
95	0	0	0	0	0	0	1	1	0	0	0	0	0
97	0	0	0	0	0	0	0	0	0	0	0	0	0
113	0	1	1	1	1	1	0	1	1	1	1	1	0
119	0	0	0	1	0	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0	1	1	1	1	1	0
121	0	0	0	0	1	1	0	0	1	0	0	1	0
122	0	0	0	0	0	0	0	0	0	0	0	0	0
125	0	0	0	0	0	0	1	0	0	0	0	0	0
126	0	0	0	0	1	0	0	1	0	0	0	1	0
145	1	1	1	1	1	1	0	1	1	1	1	1	1
148	0	0	0	0	0	0	1	1	0	0	0	0	0
149	0	0	0	0	0	0	1	1	0	0	0	0	0
153	0	0	0	0	0	0	0	1	0	1	0	0	0
180	0	0	0	0	0	0	0	1	1	0	1	1	0
182	0	0	0	0	0	0	0	1	0	0	1	1	1
204	0	0	0	0	0	0	0	1	0	0	0	0	0
210	0	0	0	0	0	0	0	1	0	0	0	0	0
212	1	1	1	1	1	1	0	0	1	1	1	1	1
213	0	0	1	1	0	0	0	1	0	0	0	1	0
217	0	0	1	0	0	0	0	1	0	1	1	1	1
229	0	0	0	0	0	0	0	1	0	0	0	0	0
277	1	1	1	1	1	1	0	1	1	1	1	1	1
54	1	1	0	0	1	1	1	0	0	1	0	0	0
55	0	1	1	1	1	1	1	1	1	0	1	1	0
58	1	1	0	0	0	0	0	0	1	0	1	0	0

Category	D. cay	D. cay	D. cay	D. aby	D. cay	D. aby	D.bul.	D.shc.	D. prh	D. prh	D. prh	D. prh	D. cay
60	0	0	0	0	0	0	1	0	0	0	0	0	0
63	0	0	0	0	0	0	1	1	0	0	0	0	0
73	0	0	0	0	0	0	1	0	0	0	0	0	0
77	0	0	0	1	1	1	0	0	1	1	0	1	0
80	0	0	0	0	0	0	0	1	0	0	0	0	0
85	0	0	0	0	0	0	0	0	0	1	0	1	0
87	0	0	1	0	0	0	1	0	1	0	0	0	0
88	0	0	0	0	0	0	0	0	0	0	0	0	0
89	0	0	0	0	0	0	0	0	1	1	1	1	0
97	0	0	0	0	0	0	1	0	0	0	0	0	0
101	0	0	0	0	0	0	1	0	0	0	0	0	0
103	0	0	0	0	0	0	0	1	0	0	0	0	0
108	0	1	1	0	0	1	0	1	1	0	1	0	0
109	1	1	1	1	1	1	1	0	1	1	1	1	0
113	1	1	1	1	1	1	1	0	1	1	1	1	0
114	0	0	0	0	0	0	0	0	0	0	0	0	0
128	0	0	0	0	0	0	1	0	0	0	0	0	0
129	0	0	0	0	0	0	1	0	0	0	0	0	0
132	0	0	0	0	0	0	0	0	0	0	0	0	0
140	0	0	0	0	0	0	1	1	0	0	0	0	0
159	0	1	0	0	1	0	0	0	0	0	1	0	0
160	1	1	1	1	0	0	0	0	1	1	1	0	0
167	0	0	0	0	0	0	0	0	0	0	0	0	1
169	0	0	0	0	0	0	0	0	0	0	0	0	0
171	0	0	0	0	0	0	1	0	0	0	0	0	0
179	0	0	0	0	0	0	0	0	0	0	0	0	0
181	0	0	0	0	0	0	1	0	0	0	0	0	0
182	0	0	0	0	0	0	1	0	0	0	0	0	0
192	0	0	1	0	0	0	1	0	0	0	1	0	0
195	0	0	0	0	0	0	1	1	0	0	0	0	0
210	0	0	0	0	0	0	1	0	0	0	0	0	0
221	0	0	0	0	0	0	1	0	0	0	0	0	0
227	0	0	0	0	0	0	1	0	0	0	0	0	0
236	0	0	0	0	0	0	1	0	0	0	0	0	0
237	0	0	0	0	0	0	0	0	0	0	0	0	0
242	0	0	0	0	0	0	0	0	0	0	0	0	0
256	0	0	0	0	0	0	1	0	0	0	0	0	0
257	0	0	0	0	0	0	0	0	0	0	0	0	0
266	0	0	1	1	0	1	1	0	0	1	0	0	0
267	0	0	0	0	0	0	0	0	0	0	0	0	0
282	0	0	0	0	0	0	1	0	0	0	0	0	0
283	0	1	0	0	0	0	0	0	0	0	1	0	0
301	0	0	0	0	0	0	1	0	0	0	0	0	0
312	0	1	0	1	1	1	1	1	1	1	1	1	0
394	0	1	1	1	1	1	1	0	1	1	1	1	0
404	0	0	0	0	0	0	1	0	0	0	0	0	0
444	0	1	1	1	1	1	0	0	1	1	1	1	0

Appendix 5. Microsatellite codominant data used to estimate population genetics parameters

Species name	Extraction#	Dba2D06	Dba2D06	Da3G04	Da3G04	Da1F08	Da1F08	Dpr3D06	Dpr3D06	Da1D08	Da1D08	Dpr3F04	Dpr3F04	Da1A01	Da1A01	Dab2E07	Dab2E07	Dab2C05	Dab2C05
DabOR12	154.12	177	181	321	321	195	210	165	167			132	132	248	248	124	140	194	211
DabOR13	154.13	177	181	321	321	210	210	165	167			132	132	248	248	124	140	194	211
DabOR38	155.24	177	181	321	321	195?	209	165	167			?	?	248	248	140	140	192	211
DabyOR31	155.16					203	203					122	122			118	129		
DabyOR56	155.46	181	181	321	321	181	195	165	165	367	367	132	132	231	248	140	140	192	213
DabySH23	155.7	177	177	329	329	195	195	167	167					248	248			203	203
DabySN32	155.17	177	191	321	321	195	195	165	167	356	356	130	130	231	248	136	136	192	207
DabySN47	155.35	177	183			189	203									136	136	184	184
DabySN49	155.37					195	195	179	179					221	234			184	190
DabySN79	155.76											?	?						
Dalata246	155.34	177	183	321	321	181	195	165	169	346	380	129	129	248	248	124	124	190	190
Dalata26	155.10	177	181	308	320	195	197			336	336	115	127	236	236	124	124	192	211
DabySN25	155.9	183	183	321	321	193	203	167	167	356	390	130	130	248	248	124	136	186	207
DabySN41	155.29	183	183	321	321	193	203	167	167	356	356	130	130	248	248	124	136	186	207
DabySN42	155.30					191	199									136	136		
DabySN66	155.59	183	183	321	321	193	203	167	167	356	390	130	130	248	248	124	136	186	207
D/prhSN62	155.55	177	183	321	321	181	195	165	167	356	356	130	130	231	248	136	136	192	207
DcayOR15	154.15	181	181	321	321	195	195	165	165	367	367	132	132			124	124	190	213
DcayOR78	155.75	181	181	321	321	181	195	165	165	367	367	132	132	231	248	140	140	190	213
DcaySN65	155.58	177	183	321	321	181	195	165	169	346	380	130	130	248	248	124	124	188	188
DcayOr11	154.11															136	136		
DcayOR37	155.23	181	181	321	321	195	195	165	165	367	367	132	132	231	248	140	140	190	213
DcayOR69	155.64																	184	196
DcaySh14	154.14	177	177	321	321	195	203	167	169	346	380	132	132			136	136	190	190
DcaySh16	154.16									339	356								
DcaySh20	155.4	177	183			195	195	169	169	346	381			231	248	136	136	188	188
DcaySh24	155.8	177	177	321	321	187	195	167	167	339	346	129	129	231	248	123	136	192	207
DcaySh27	155.12	177	183			193	205	167	169					238	248	120	120	186	201
DcaySH30	155.15			317	317	199	203					127	130	227	236	120	131	190	211
DcaySh39	155.26	177	177	321	321	187	195	167	167	339	346	130	130	231	248	124	136	190	207
DcaySH4	154.4					195	195	167	167					248	248			192	192
DcaySH43	155.31	177	191	321	321	195	195	167	167	339	356	130	130	248	248	134	134	190	190
DcaySh5	154.5	177	191	321	321	195	205	167	169	346	356	129	132	236	248	124	136	192	192
DcaySH54	155.43															118	118		
DcaySh57	155.47	181	191															194	194
DcaySH58	155.49			321	321	195	203	167	175					227	248	136	142	184	192
DcaySH6	154.6											?	?			127	127		
DcaySH61	155.54	177	177	321	321	187	195	167	167	339	346	130	130	231	248	124	136	190	207
DcaySH68	155.67	177	183	328	328	195	195	167	167	346	352	129	129	248	248	136	140	203	203
DcaySh72	155.69	177	191	321	321	181	195	167	167	369	356	130	130	248	248	136	136	190	190
DcaySH74	154.8	177	191	321	321	181	195	167	167	339	356			248	248	136	136	190	190
DcaySh8	155.81	181	191											227	248	124	124	184	192
DcaySH82	154.9															120	132		
DcaySH9	154.1					195	195											192	192
DcaySN1	155.1					195	195			356	356			231	248			190	207
DcaySN17	155.2	177	191			195	195	165	167	356	356	130	130	231	248	136	136	192	207
DcaySN18	155.3	177	191	321	321	195	195	165	167	356	356			231	248	136	136	192	207
DcaySN19	154.2	177	191	321	321	181	195	165	167	356	356	130	130	231	248			192	207
DcaySN2	155.5	177	177	321	321	195	195	165	169	346	380			248	248	124	124	190	190
DcaySN21	155.13	177	183	321	321	195	195	165	169	346	380	130	130	248	248	124	124	190	190

Species name	Extraction#	Dba2D06	Dba2D06	Da3G04	Da3G04	Da1F08	Da1F08	Dpr3D06	Dpr3D06	Da1D08	Da1D08	Dpr3F04	Dpr3F04	Da1A01	Da1A01	Dab2E07	Dab2E07	Dab2C05	Dab2C05
DcaySN28	155.19	177	191	321	321	195	195	165	167	356	356	130	130	231	248	136	136	192	207
DcaySN34	155.21	177	183	321	321	195	195	165	169	346	380	129	129	248	248	124	124	190	190
DcaySN35	155.22	183	183			193	203	167	167									186	207
DcaySN36	155.33	177	191	321	321	195	195	165	167	356	356	130	130	231	248	136	136	192	207
DcaySN45	155.50	177	191	321	321	179	193	167	171	365	376	127	130	227	231	124	138	188	203
DcaySN59	155.56	177	191	321	321	181	195	165	169	346	380	130	130	248	248	124	124	190	190
DcaySN63	155.57	181	191													124	136		
DcaySN64	155.60	177	191	321	321	181	195	165	167	356	356			231	248	136	136	192	207
DcaySN67	155.70																		
DcaySN75	155.73	177	179	321	321	181	195	167	167	356	380	130	130	221	248	136	136	190	190
DcaySN76	155.74	177	183	321	321	181	195	165	169	346	380	130	130	248	248	123	123	190	190
DcaySN77	155.80	177	191	321	321	181	195	165	167	356	356			231	248	136	136	192	207
DcaySN81	155.84	177	191	321	321	181	195	165	167	356	356	130	130	231	248	124	136	190	207
DcaySN85	155.85	177	183	321	321	195	195	165	169					248	248	123	123	190	190
DcaySN86	155.61	177	191	321	321	195	195							231	248			192	207
DprhOR22	155.6	181	181	321	321	189	203	161	167	370	367			227	248	124	138	188	188
DabySh50	155.38			321	321	181	195	165	169	346	380	129	129	248	248	124	124	190	190
DprhOR29	155.14																	190	190
DprhOR40	155.27	177	177					169	169							120	120		
DprhOR48	155.36													231	248			198	198
DprhOR51	155.39	177	183													142	142	198	198
DprhOR52	155.40	177	191													136	136	196	196
DprhOR53	155.42															142	142	188	192
DprhOr60	155.51	177	177	321	321	195	199	171	171			130	130	231	231	132	138	188	201
DprhOR70	155.65																	184	184
DprhSH33	155.18	181	191	321	321	195	203	167	175	346	356	129	129	227	248	136	142	184	192
DprhSh44	155.32					195	203											184	201
DprhSH55	155.45			331	331											120	120	190	211
DprhSH7	154.7	181	195			203	205	169	169	346	356			229	248	124	124	186	186
DprhSH71	155.66	177	179	319	321	193	203	169	169			127	132	236	248	124	138	184	203
Daby84	155.83	183	193	321	321	199	205	167	171					227	227			194	194
DcayOR73	155.68	181	181	321	321	181	195	165	165	367	367	132	132	231	248	140	140	190	213
DcaySN83	155.82															132	138	194	194
Dsch3	154.3					195	195												
Dsch80	155.79	177	177	321	321	175	195							215	215	124	124		
DschSh10	154.10	177	191	302	302	173	195			348	348	132	132			124	140	178	178

Appendix 6. microsatallite data used to infer population structure

Pop.	place of coll	Dba2D06		Da3G04		Da1F08		Dpr3D06		Da1A01		Dab2E07		Dab2C05	
or1	ilubabor	181	181	321	321	189	203	161	167	227	248	124	138	188	188
or2	ilubabor			321	321	181	195	165	169	248	248	124	124	190	190
or4	Ghibe	177	183									142	142	198	198
or5	Ghibe	177	191									136	136	196	196
or6	wellega	177	181	321	321	195	210	165	167	248	248	124	140	194	211
or7	wellega	177	181	321	321	195	210	165	167	248	248	124	140	194	211
or8	wellega	181	181	321	321	195	195	165	165			124	124	190	213
or9	wellega	181	181	321	321	195	195	165	165	231	248	140	140	190	213
or10	wellega	177	181	321	321	195	209	165	167	248	248	140	140	192	211
or11	wellega	181	181	321	321	181	195	165	165	231	248	140	140	192	213
or12	wellega	181	181	321	321	181	195	165	165	231	248	140	140	190	213
or13	wellega	181	181	321	321	181	195	165	165	231	248	140	140	190	213
or14	Ghibe	183	193	321	321	199	205	167	171	227	227			194	194
or15	gojam	177	177	321	321	195	199	171	171	231	231	132	138	188	201
sh1	sheko	181	191	321	321	195	203	167	175	227	248	136	142	184	192
sh2	sheko	183	195			203	205	169	169	229	248	124	124	186	186
sh3	sheko	177	179	319	321	193	203	169	169	236	248	124	138	184	203
sh4	sheko	177	183			195	195	169	169	231	248	136	136	188	188
sh5	sheko	177	177	321	321	187	195	167	167	231	248	124	136	192	207
sh6	sheko	177	183			193	205	167	169	238	248	120	120	186	201
sh7	sheko			317	317	199	203			227	236	120	131	190	211
sh8	sheko	177	177	321	321	187	195	167	167	231	248	124	136	190	207
sh9	sheko					195	195	167	167	248	248			192	192
sh10	sheko	177	191	321	321	195	195	167	167	248	248	134	134	190	190
sh11	sheko	177	191	321	321	195	205	167	169	236	248	124	136	192	192
sh12	sheko	181	191	321	321	195	203	167	175	227	248	136	142	184	192
sh13	sheko	177	177	321	321	187	195	167	167	231	248	124	136	190	207
sh14	sheko	177	183	328	328	195	195	167	167	248	248	136	140	203	203
sh15	sheko	177	191	321	321	181	195	167	167	248	248	134	134	190	190
sh16	sheko	177	191	321	321	181	195	167	167	248	248	136	136	190	190
sh17	sheko	177	177	329	329	195	195	167	167	248	248			203	203
sh18	sheko	177	177	321	321	195	203	167	169			136	136	190	190
sn (or3)	areka	177	183	321	321	181	195	165	167	231	248	136	136	192	207
sn1	areka	177	191			195	195	165	167	231	248	136	136	192	207
sn2	areka	177	191	321	321	195	195	165	167	231	248	136	136	192	207
sn3	areka	177	191	321	321	181	195	165	167	231	248			192	207
sn4	gedeo	177	177	321	321	195	195	165	169	248	248	124	124	190	190
sn5	sidamo	177	183	321	321	195	195	165	169	248	248	124	124	190	190
sn6	areka	177	191	321	321	195	195	165	167	231	248	136	136	192	207
sn7	gedeo	177	183	321	321	195	195	165	169	248	248	124	124	190	190
sn8	areka	183	183			193	203	167	167					186	207
sn9	areka	177	191	321	321	195	195	165	167	231	248	136	136	192	207
sn10	welaiyta	177	191	321	321	179	193	167	171	227	231	124	138	188	203
sn11	gedeo	177	191	321	321	181	195	165	169	248	248	124	124	190	190
sn12	areka	177	191	321	321	181	195	165	167	231	248	136	136	192	207
sn13	areka	177	179	321	321	181	195	167	167	221	248	136	136	190	190
sn14	gedeo	177	183	321	321	181	195	165	169	248	248	124	124	190	190

sn15	**areka**	**177**	**191**	**321**	**321**	**181**	**195**	**165**	**167**	**231**	**248**	**136**	**136**	**192**	**207**
sn16	**areka**	**177**	**191**	**321**	**321**	**181**	**195**	**165**	**167**	**231**	**248**	**124**	**136**	**190**	**207**
sn17	**areka**	**177**	**183**	**321**	**321**	**195**	**195**	**165**	**169**	**248**	**248**	**124**	**124**	**190**	**190**
sn18	**areka**	**177**	**191**	**321**	**321**	**195**	**195**			**231**	**248**			**192**	**207**
sn19	**gamugofa**	**183**	**183**	**321**	**321**	**193**	**203**	**167**	**167**	**248**	**248**	**124**	**136**	**186**	**207**
sn20	**gamugofa**	**177**	**191**	**321**	**321**	**195**	**195**	**165**	**167**	**231**	**248**	**136**	**136**	**192**	**207**
sn21	**areka**	**183**	**183**	**321**	**321**	**193**	**203**	**167**	**167**	**248**	**248**	**124**	**136**	**186**	**207**
sn22	**areka**	**177**	**183**			**189**	**203**					**136**	**136**	**184**	**184**
sn23	**areka**					**195**	**195**	**179**	**179**	**221**	**234**			**184**	**190**
sn24	**gedeo**	**177**	**183**	**321**	**321**	**181**	**195**	**165**	**169**	**248**	**248**	**124**	**124**	**188**	**188**
sn25	**areka**	**183**	**183**	**321**	**321**	**193**	**203**	**167**	**167**	**248**	**248**	**124**	**124**	**186**	**207**

Appendix 7. Microsattelite presence/absence data used to infer taxonomic relationships among the taxa using NTYSYS

catagory	DabyOR	DabyOR	DabyOR	DabyOR	DabySH	DabySN	DabySN	DabySN	DabySN	DabySN	DprhSN6	DcayOR	DcayOR	Dcaysh	Dcaysh	DcaySh	DcaySh	DcaySh	DcaySH	DcaySh	DcaySH	DcaySH	DcaySh	DcaySH	DcaySH	DcaySH	DcaySh
178	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
184	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
186	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
188	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
190	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	1	1	0	1	0	0	1	1	1
192	0	0	1	1	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	1	1	0	0	0
194	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
196	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
198	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
201	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
203	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
205	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
207	0	0	0	0	0	1	0	1	1	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0
211	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
213	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
115	0	0	-9	0	-9	0	-9	0	0	0	0	0	0	0	0	-9	0	-9	0	0	-9	0	0	-9	0	0	-9
122	0	0	-9	0	-9	0	-9	0	0	0	0	0	0	0	0	-9	0	-9	0	0	-9	0	0	-9	0	0	-9
127	0	0	-9	0	-9	0	-9	0	0	0	0	0	0	0	0	-9	0	-9	1	0	-9	0	0	-9	0	0	-9
129	0	0	-9	0	-9	1	-9	0	0	0	0	0	0	1	0	-9	1	-9	1	1	-9	1	1	-9	1	0	-9
130	0	0	-9	0	-9	0	-9	1	1	1	1	0	0	0	0	-9	0	-9	0	0	-9	0	0	-9	0	1	-9
132	1	1	-9	1	-9	0	-9	0	0	0	0	1	1	0	1	-9	0	-9	0	0	-9	0	1	-9	0	0	-9
215	0	0	0	0	0	0	-9	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
221	0	0	0	0	0	0	-9	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
227	0	0	0	0	0	0	-9	0	0	0	0	-9	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
229	0	0	0	0	0	0	-9	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
231	0	0	0	1	0	1	-9	0	0	0	1	-9	1	0	1	1	1	0	0	1	0	0	0	0	1	0	0
234	0	0	0	0	0	0	-9	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
236	0	0	0	0	0	0	-9	0	0	0	0	-9	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0
238	0	0	0	0	0	0	-9	0	0	0	0	-9	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
248	1	1	1	1	1	1	-9	1	1	1	1	-9	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
118	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0
120	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	-9	0	0	0	0	0	0
124	1	1	0	0	-9	0	0	1	1	1	0	1	0	1	0	0	1	0	0	1	-9	0	1	0	1	0	0
127	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0
129	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0
132	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	-9	0	0	0	0	0	0
134	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	1	0	0	0	0	0
136	0	0	0	0	-9	1	1	1	1	1	1	0	0	0	0	1	1	0	0	1	-9	0	1	1	1	1	1
138	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0
140	1	1	1	1	-9	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	-9	0	0	0	0	1	0
142	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	1	0	0	0
177	1	1	1	0	1	1	1	0	0	0	1	0	0	1	0	1	1	1	-9	1	-9	1	1	0	1	1	1
179	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	-9	0	0	0	0	0	0
181	1	1	1	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	-9	0	-9	0	0	1	0	0	0
183	0	0	0	0	0	0	1	1	1	1	1	0	0	1	0	1	0	1	-9	0	-9	0	0	0	0	0	0
191	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	-9	1	1	1	0	1	1
193	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	-9	0	0	0	0	0	0
195	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	-9	0	0	0	0	0	0
302	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	-9	0	-9	0	0	-9	0	0	0	0	0	0
308	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	-9	0	-9	0	0	-9	0	0	0	0	0	0
317	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	-9	0	-9	1	0	-9	0	0	0	0	0	0

catagory	DabOR	DabOR	DabOR	DabyOR	DabySH	DabySN	DabySN	DabySN	DabySN	DabySN	DprhSN6	DcayOR	DcayOR	DcaySh	DcaySh	DcaySh	DcaySh	DcaySh	DcaySH	DcaySh	DcaySH	DcaySH	DcaySh	DcaySH	DcaySH	DcaySH	DcaySh
319	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	-9	0	-9	0	0	-9	0	0	0	0	0	0
320	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	-9	0	-9	0	0	-9	0	0	0	0	0	0
321	1	1	1	1	0	1	-9	1	1	1	1	1	1	1	1	-9	1	-9	0	1	-9	1	1	1	1	1	1
328	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	-9	0	-9	0	0	-9	0	0	0	0	0	0
329	0	0	0	0	1	0	-9	0	0	0	0	0	0	0	0	-9	0	-9	0	0	-9	0	0	0	0	0	0
331	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	-9	0	-9	0	0	-9	0	0	0	0	0	0
173	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
175	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
179	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
181	0	0	0	1	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1
187	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0
189	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
191	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
193	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
195	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1
197	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
199	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
203	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
205	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
210	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
336	-9	-9	-9	0	-9	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	-9	0	0	-9	0	0	0
339	-9	-9	-9	0	-9	0	-9	0	0	0	0	0	0	0	0	0	1	-9	-9	1	-9	1	0	-9	1	1	1
346	-9	-9	-9	0	-9	0	-9	0	0	0	0	0	0	1	0	1	1	-9	-9	1	-9	0	1	-9	1	0	0
348	-9	-9	-9	0	-9	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	-9	0	0	-9	0	0	0
352	-9	-9	-9	0	-9	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	-9	0	0	-9	0	0	0
356	-9	-9	-9	0	-9	1	-9	1	1	1	1	0	0	0	0	0	0	-9	-9	0	-9	1	1	-9	0	1	1
365	-9	-9	-9	0	-9	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	-9	0	0	-9	0	0	0
360	-9	-9	-9	0	-9	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	-9	0	0	-9	0	0	0
367	-9	-9	-9	1	-9	0	-9	0	0	0	0	1	1	0	1	0	0	-9	-9	0	-9	0	0	-9	0	0	0
370	-9	-9	-9	0	-9	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	-9	0	0	-9	0	0	0
376	-9	-9	-9	0	-9	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	-9	0	0	-9	0	0	0
380	-9	-9	-9	0	-9	0	-9	0	0	0	0	0	0	1	0	1	0	-9	-9	0	-9	0	0	-9	0	0	0
390	-9	-9	-9	0	-9	0	-9	1	0	1	0	0	0	0	0	0	0	-9	-9	0	-9	0	0	-9	0	0	0
161	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0
165	1	1	1	1	0	1	-9	0	0	0	1	1	1	1	1	0	0	0	-9	0	0	0	0	0	0	0	0
167	1	1	1	0	1	1	-9	1	1	1	1	0	0	0	0	0	1	1	-9	1	1	1	1	1	1	1	1
169	0	0	0	0	0	0	-9	0	0	0	0	0	0	1	0	1	0	1	-9	0	0	0	1	0	0	0	0
171	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0
175	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	1	0	0	0
179	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0

catagory	DcaySH	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DabyOR	DprhOR	DprhOR	DprhOR	DprhOr	DprhSH	DprhSh	DprhSH	DprhSH	DprhSH	DabySh	DcayOR
178	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0
184	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	1	1	0	0	1	0	0
186	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	1	0	0	0
188	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	-9	0	0	0	1	0	0	0	0	0	0	0
190	0	0	0	0	1	1	0	1	0	0	1	0	0	1	1	0	1	1	0	0	-9	1	0	0	0	0	0	1	0	0	0	1
192	1	1	1	1	0	0	1	0	1	0	0	0	1	0	0	1	0	0	1	0	-9	0	0	0	0	1	0	0	0	0	0	0
194	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	1	0
196	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	1	0	0	0	0	0	0	0	0
198	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	1	0	0	0	0	0	0	0	0	0
201	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	1	0	1	0	0	0	0	0
203	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	-9	0	0	0	0	0	0	0	0	1	0	0
205	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0
207	0	1	1	1	0	0	1	0	1	0	0	0	1	0	0	1	1	0	1	0	-9	0	0	0	0	0	0	0	0	0	0	0
211	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	1	0	0	0	0
213	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	1
115	-9	0	-9	0	-9	0	0	0	-9	0	0	-9	-9	0	0	-9	0	-9	-9	0	-9	0	-9	-9	0	0	-9	-9	-9	0	-9	0
122	-9	0	-9	0	-9	0	0	0	-9	0	0	-9	-9	0	0	-9	0	-9	-9	0	-9	0	-9	-9	0	0	-9	-9	-9	0	-9	0
127	-9	0	-9	0	-9	0	0	0	-9	1	1	-9	-9	0	0	-9	0	-9	-9	0	-9	0	-9	-9	0	0	-9	-9	-9	1	-9	0
129	-9	1	-9	0	-9	1	1	1	-9	1	0	-9	-9	1	1	-9	0	-9	-9	1	-9	1	-9	-9	0	1	-9	-9	-9	0	-9	0
130	-9	0	-9	1	-9	0	0	0	-9	0	1	-9	-9	0	0	-9	1	-9	-9	0	-9	0	-9	-9	1	0	-9	-9	-9	0	-9	0
132	-9	0	-9	0	-9	0	0	0	-9	0	0	-9	-9	0	0	-9	0	-9	-9	0	-9	0	-9	-9	0	0	-9	-9	-9	1	-9	1
215	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	-9	-9	0	0	0	0
221	0	0	0	0	0	0	0	0	0	0	0	-9	0	1	0	0	0	0	0	0	0	0	-9	-9	0	0	-9	-9	0	0	0	0
227	1	0	0	0	0	0	0	0	0	1	0	-9	0	0	0	0	0	0	0	0	1	0	-9	-9	0	1	-9	-9	0	0	1	0
229	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	-9	-9	1	0	0	0
231	0	1	1	1	0	0	1	0	1	1	0	-9	1	0	0	1	1	0	1	0	0	0	-9	-9	1	0	-9	-9	0	0	0	1
234	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	-9	-9	0	1	0	0
236	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	-9	-9	0	0	0	0
238	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	-9	-9	0	0	0	0
248	1	1	1	1	1	1	1	1	1	0	1	-9	1	1	1	10	1	1	1	1	1	1	-9	-9	0	1	-9	-9	1	1	0	1
118	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	-9	0	0	0	-9	0
120	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	-9	1	0	0	-9	0
124	1	0	0	-9	1	1	0	1	0	0	1	1	0	0	1	0	1	1	-9	0	1	1	0	0	0	0	-9	0	1	1	-9	0
127	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	-9	0	0	0	-9	0
129	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	-9	0	0	0	-9	0
132	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	1	0	-9	0	0	0	-9	0
134	0	1	0	-9	0	0	1	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	-9	0	0	0	-9	0
136	0	0	1	-9	0	0	0	0	1	1	0	1	1	1	0	1	1	0	-9	1	0	0	0	1	0	1	-9	0	0	0	-9	0
138	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	1	0	0	0	1	0	-9	0	0	1	-9	0
140	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	1	0	0	0	0	0	0	-9	0	0	0	-9	1
142	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	1	0	0	1	-9	0	0	0	-9	0
177	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	-9	1	1	1	0	-9	-9	0	1	0	0
179	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	-9	0	0	0	0	-9	-9	0	1	0	0
181	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	-9	0	0	0	1	-9	-9	0	0	0	1
183	0	0	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	1	0	1	0	-9	1	0	0	0	-9	-9	1	0	1	0
191	1	1	1	1	0	0	1	0	1	1	1	1	1	0	0	1	1	0	1	0	0	-9	0	1	0	1	-9	-9	0	0	0	0
193	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	-9	-9	0	0	1	0
195	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	-9	-9	1	0	0	0
302	-9	-9	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	-9	0	-9	0	0	0

catagory	DcaySH	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DcaySN	DabyOR	DprhOR	DprhOR	DprhOR	DprhOr	DprhSH	DprhSh	DprhSh	DprhSh	DprhSH	DabySh	DcayOR
308	-9	-9	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	-9	0	-9	0	0	0
317	-9	-9	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	-9	0	-9	0	0	0
319	-9	-9	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	-9	0	-9	1	0	0
320	-9	-9	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	-9	0	-9	0	0	0
321	-9	-9	1	1	1	1	1	1	1	1	1	-9	1	1	1	1	1	1	1	0	1	1	-9	-9	1	1	-9	0	-9	1	1	1
328	-9	-9	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	1	0	0	-9	-9	0	0	-9	0	-9	0	0	0
329	-9	-9	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	-9	0	-9	0	0	0
331	-9	-9	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	-9	1	-9	0	0	0
173	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	0	-9	0	0	0	0
175	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	0	-9	0	0	0	0
179	0	0	0	0	0	0	0	0	0	1	1	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	0	-9	0	0	0	0
181	0	0	0	1	0	0	0	0	0	0	0	-9	1	1	1	1	1	0	0	0	0	1	-9	-9	0	0	0	-9	0	0	0	1
187	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	0	-9	0	0	0	0
189	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	1	0	-9	-9	0	0	0	-9	0	0	0	0
191	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	0	-9	0	0	0	0
193	0	0	0	0	0	0	0	0	0	1	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	0	-9	0	1	0	0
195	1	1	1	1	1	1	1	1	1	0	1	-9	1	1	1	1	1	1	1	1	0	1	-9	-9	1	1	1	-9	0	0	0	1
197	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	0	-9	0	0	0	0
199	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	1	0	0	-9	0	0	1	0
203	1	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	1	0	-9	-9	0	1	1	-9	1	1	0	0
205	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	0	-9	1	0	1	0
210	0	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	0	0	0	0	-9	-9	0	0	0	-9	0	0	0	0
336	-9	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	-9	-9	0	0	0	-9	-9	-9	0	-9	-9	0	-9	-9	0
339	-9	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	-9	-9	0	0	0	-9	-9	-9	0	-9	-9	0	-9	-9	0
346	-9	0	0	0	1	1	0	1	0	0	1	-9	0	0	1	0	0	-9	-9	1	0	1	-9	-9	-9	1	-9	-9	1	-9	-9	0
348	-9	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	-9	-9	0	0	0	-9	-9	-9	0	-9	-9	0	-9	-9	0
352	-9	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	-9	-9	1	0	0	-9	-9	-9	0	-9	-9	0	-9	-9	0
356	-9	1	1	1	0	0	1	0	1	0	0	-9	1	1	0	1	1	-9	-9	0	0	0	-9	-9	-9	1	-9	-9	1	-9	-9	0
365	-9	0	0	0	0	0	0	0	0	1	0	-9	0	0	0	0	0	-9	-9	0	0	0	-9	-9	-9	0	-9	-9	0	-9	-9	0
360	-9	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	-9	-9	0	1	0	-9	-9	-9	0	-9	-9	0	-9	-9	0
367	-9	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	-9	-9	0	1	0	-9	-9	-9	0	-9	-9	0	-9	-9	1
370	-9	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	-9	-9	0	0	0	-9	-9	-9	0	-9	-9	0	-9	-9	0
376	-9	0	0	0	0	0	0	0	0	1	0	-9	0	0	0	0	0	-9	-9	0	0	0	-9	-9	-9	0	-9	-9	0	-9	-9	0
380	-9	0	0	0	1	1	0	1	0	0	1	-9	0	1	1	0	0	-9	-9	0	0	1	-9	-9	-9	0	-9	-9	0	-9	-9	0
390	-9	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	-9	-9	0	0	0	-9	-9	-9	0	-9	-9	0	-9	-9	0
161	-9	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	-9	0	1	0	-9	-9	0	0	-9	-9	0	0	0	0
165	-9	1	1	1	1	1	1	1	1	0	1	-9	1	0	1	1	1	0	-9	0	0	1	-9	-9	0	0	-9	-9	0	0	0	1
167	-9	1	1	1	0	0	1	0	1	1	0	-9	1	1	0	1	1	1	-9	1	1	0	-9	-9	0	1	-9	-9	0	0	1	0
169	-9	0	0	0	1	1	0	1	0	0	1	-9	0	0	1	0	0	1	-9	0	0	1	-9	-9	0	0	-9	-9	1	1	0	0
171	-9	0	0	0	0	0	0	0	0	1	0	-9	0	0	0	0	0	0	-9	0	0	0	-9	-9	1	0	-9	-9	0	0	1	0
175	-9	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	-9	0	0	0	-9	-9	0	1	-9	-9	0	0	0	0
179	-9	0	0	0	0	0	0	0	0	0	0	-9	0	0	0	0	0	0	-9	0	0	0	-9	-9	0	0	-9	-9	0	0	0	0

Declaration

This thesis is my original work and has not been presented for a degree in any other University, and that all sources of material used for the thesis have been duly acknowledged.

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