

The use of DNA profiling for Plant Breeder's Rights registration

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Plant Breeders Rights

- Varietal descriptors that provide protection for the breeder, nursery and producer
- Generally rely on a series of observed traits
- Varieties can come from breeding programs
- Or from sports or mutants, which may appear very different, but be genetically “the same”
- System accepted widely, but has frequent issues with confused identity

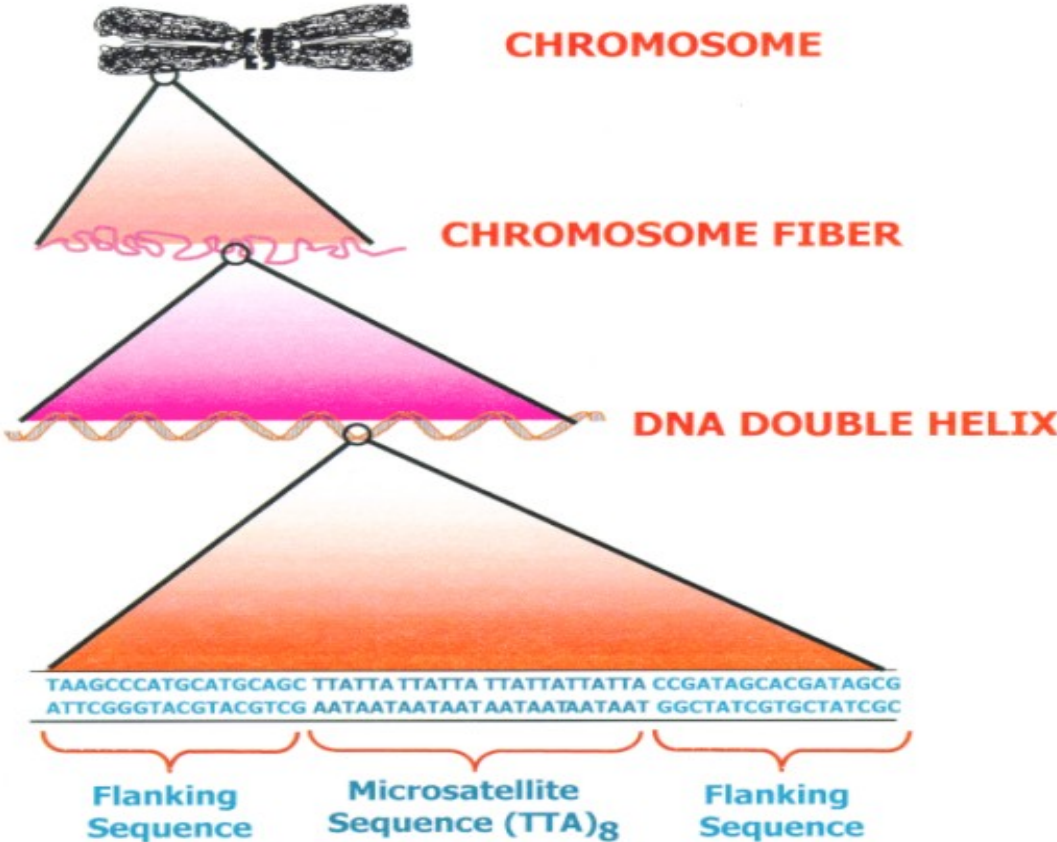
DNA Profiling techniques

- All depend on natural variation in DNA sequences in plants and animals
- Used widely for genetics and forensics
- Application in PBR is the same
- Potentially can use RFLPs, AFLPs, Microsatellites or SNPs
- Microsatellites the most widely used technology

SSRs : Microsatellites

- Short (2-6 base) repeats in genomic DNA sequence
- Found throughout genomes
- Caused by replication errors
- Highly polymorphic
- Usually genetically neutral
- PCR based mapping is portable between cultivars and closely related species

Microsatellites



PCR Analysis of Microsatellites

Allele 1

TAAGCAGCTGGTGACGTAGCTGTGTGTGTGTGTGTGTGTGACACATCGATGCACGTACGACTAGC
TAAGCAGCTGGTGACGTAGCTGTGTGTGTGTGTGTGTGTGACACATCGATGCACGTACGACTAGC



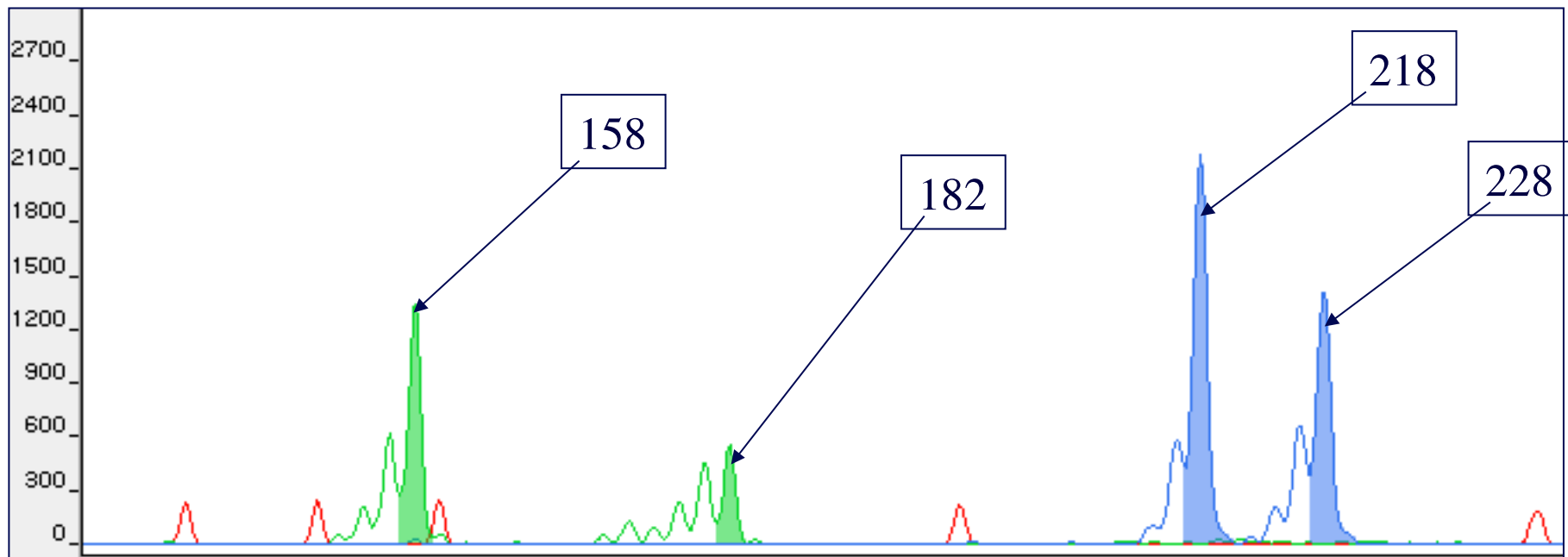
Allele 2

TAAGCAGCTGGTGACGTAGCTGTGTGTGTGTACACATCGATGCACGTACGACTAGC
TAAGCAGCTGGTGACGTAGCTGTGTGTGTGTACACATCGATGCACGTACGACTAGC



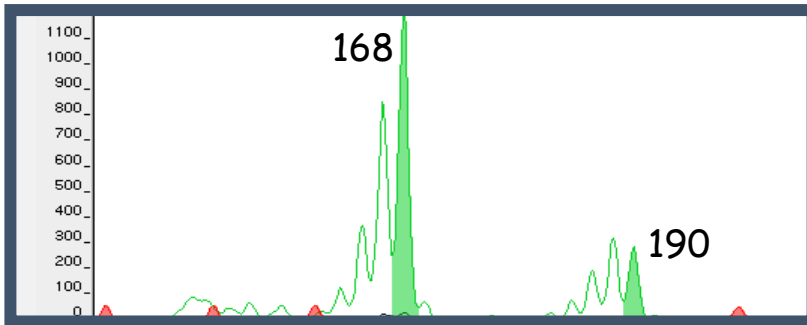
Microsatellite Analysis

- PCR with fluorescent primers
- Analysis by capillary electrophoresis
- Detection and scoring of alleles



Detection of alleles in Bon Rouge x Packhams mapping population

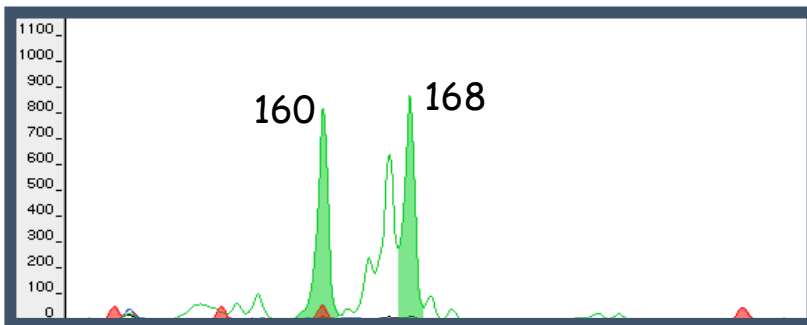
Bon Rouge



2

8

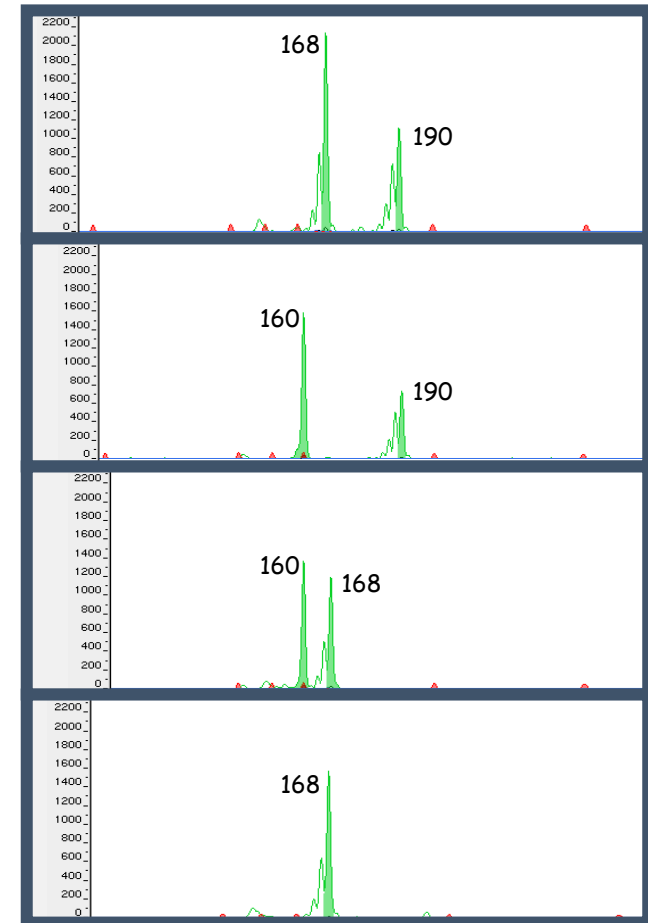
Packhams Triumph



9

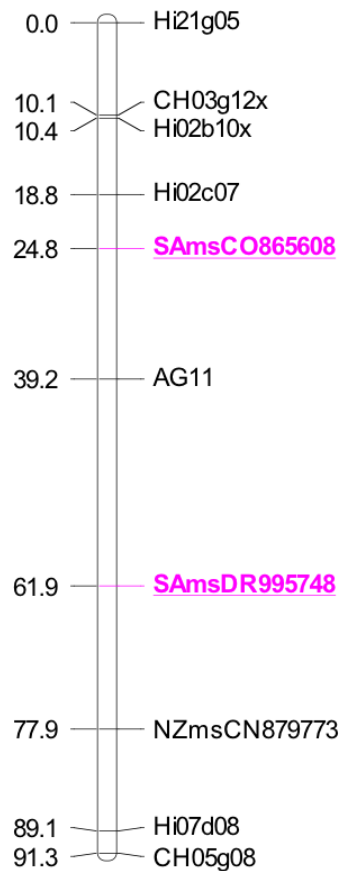
10

BR x PT progeny

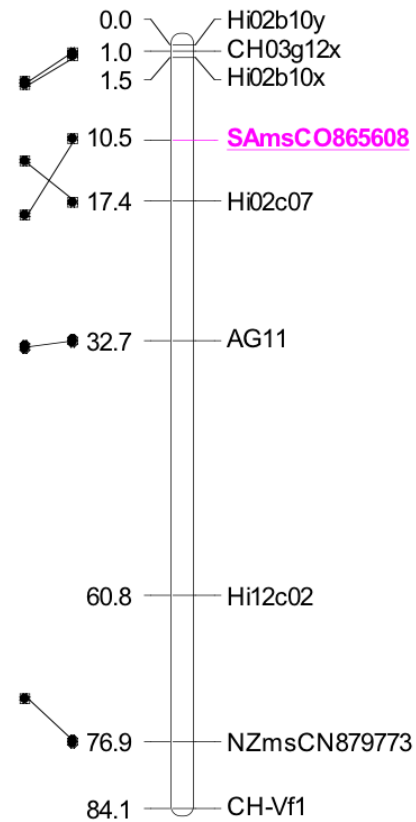


Apple Genetic Map: Chr 1 – 2 (of 17)

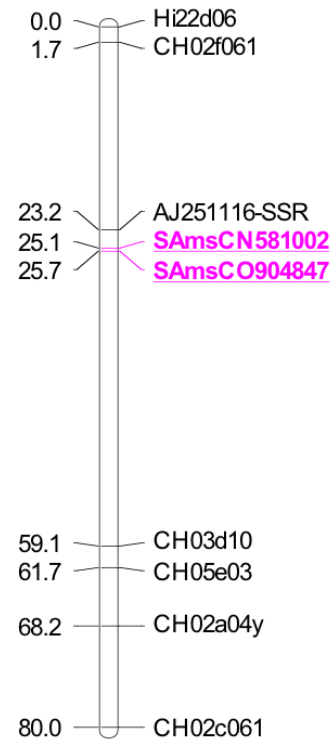
AnnaLG1



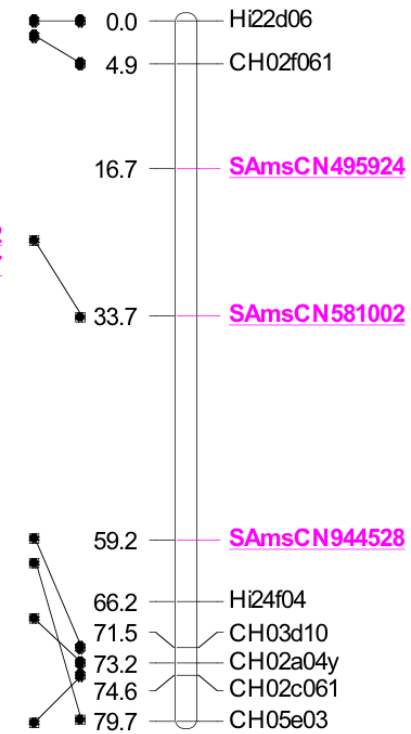
GDLG1



AnnaLG2



GDLG2

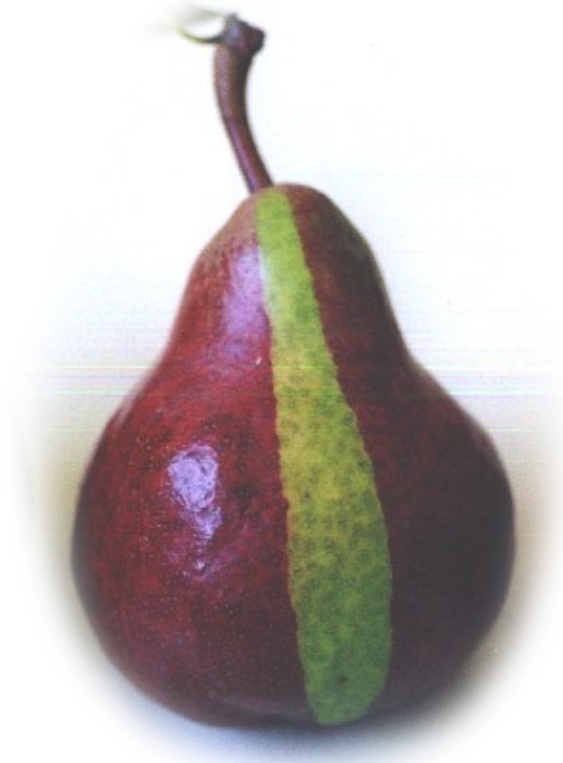


Selection of markers for cultivar typing

- Need to cover the whole genetic map
- Need 10-15 markers for apple, 8-12 for peach
- Need to be highly polymorphic on many different varieties
- Not all loci will be polymorphic in all varieties
- Varieties from the same parents will be more similar, but easily distinguished
- Tri and Tetra markers are better
- Need standard allele data for known varieties.
- Should have allele ladders for standardisation

What can we **not** differentiate?

- All clones look the same to microsatellite technology
- We cannot determine the difference between, for example, Bon Rouge and Bon Chretien, or clones of Royal Gala
- So far we have no technology to do this at the level of DNA markers.
- But you can still get PBR for such clonal selections



Why would you want this technology?

- To protect your PBR
- To ensure you have the variety you thought you had in your orchard
- To be sure you are buying or selling the right variety
- To be sure you are not use the rootstock as a scion!

Where has DNA fingerprinting been used for PBR?

- In South Africa: for Eucalyptus only
- We do not have pome and stone fruit protected by DNA markers
- But they can be used for testing for PBR



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