FUTURE FORESTS RESEARCH

DIVERSIFIED SPECIES TECHNICAL NOTE

Number: DSTN-016
Date: June 2010

DNA Fingerprinting of *Eucalyptus nitens*

Summary

DNA fingerprinting of the *Eucalyptus nitens* breeding population is an integral part of the FFR breeding program for this species. All parents who have contributed to progeny trials are to be fingerprinted to reconstruct parentage of the current generation of trees. Use of this technology will also allow control of inbreeding in future generations, as all selections will have their parents determined. This will allow the amount of representation of any given genotype selected to be restricted so that inbreeding does not build up in the breeding population. This note details fingerprinting carried out during the 2009/10 programme year. An overview is given for the clones selected for fingerprinting, the DNA extraction process and the markers used for DNA fingerprinting.

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Fingerprinting of *Eucalyptus nitens*

In the current *E. nitens* breeding plan, the use of DNA fingerprinting is a vital component, and all parents contributing to the program will be fingerprinted before 2011 to allow parental reconstruction. This parental reconstruction will be used when the next generation of plus trees are selected in the progeny trials to minimise relatedness.

2010

In 2010 a budget was allocated to fingerprint a further 51 trees. Fifty-one individuals had leaves collected from the Waiouru clonal archive, including three individuals that had been previously genotyped. All these genotypes are from the 898 series. In the table below, number refers to Clone Number from the Waiouru clonal archive.

Table 1: List of 898 series E. nitens collected for fingerprinting from the Waiouru clonal archive

1	5	8	10	12	14	21	22	23
25	26	28	33	37	41	47	48	49
51	53	54	56	57	60	64	66	68
72	74	77	73	80	84	86	87	88
89	90	91	94	95	97	98	104	108
109	110	113	114	115	118			

DNA Extraction and PCR Amplification

Genomic DNA was extracted from leaf material for all samples, using the reliable and widely used genomic DNA isolation method similar to that described by Stacey & Isaac (1990). Typically this is described as a CTAB method because the principal component of the buffer is a DNA complexing detergent called CTAB (cetyltrimethyltetraammonium bromide). A full description of the technique can be found in Scion output 38651- *Parental reconstruction in breeding programmes: An efficient approach for breeding, deployment and orchard management for Eucalyptus nitens.* Isolated DNA was quantified fluorometrically and the average concentration was found to be 72ng/µL. DNA was diluted to a working concentration of 20ng/µL and the 16 SSRs developed at Scion were amplified using fluorescently labelled primers.

A trial set of 15 known genotypes is currently being optimised at the Waikato DNA sequencing facility. The ABI PRISM® 3130 Genetic Analyzer from Applied Biosytems separates the different alleles electrophoretically and the individual fluorescent tags are detected using laser excitation. Once the 15 known samples have had their genotypes correctly reconstructed, the remaining 47 unknown samples will be analysed at the Waikato DNA sequencing facility and the records will be added to the file for future use.

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Table 2: *E. nitens* 16-plex SSR marker kit

Marker Name	Dye[1]	Actual Size (base pairs)[2]
Es054	FAM	102-118
FRMSA3	FAM	165-199
Es211	VIC	090-103
EMBRA10	VIC	116-148
Eg98	VIC	175-192
Eg65	VIC	244-279
FRMSA4	VIC	308-320
En6	NED	088-107
Es140	NED	117-151
Eg99	NED	184-202
Eg126	NED	344-384
FRMSA2	PET	109-121
EMBRA39	PET	128-152
EMBRA63	PET	182-230
EMBRA64	PET	256-266
Eg61	PET	315-373

1 Fluorescent dye attached to marker-specific DNA primers. Allows multiple markers of similar size to be analysed simulataneously

2 Size range of all alleles belonging to a single marker locus

References

Stacey & Isaac (1990). Chapter 3 Isolation and Purification of DNA from Plants *The Nucleic Acids Protocols Handbook* pg14-16

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