

Bioplatforms Australia Genomics for Australian Plants Initiative

Activity: Phylogenomics

Species: Various - <https://www.genomicsforaustralianplants.com/phylogenomics/>

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GAP Phylogenomics

The following protocols were used to generate target capture sequence data for Stages 1 and 2 of the Australian Angiosperm Tree of Life project.

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DNA extraction (for samples prepared by AGRF only)

Dried plant tissue (20–30 mg) was ground using a TissueLyser II (Qiagen) with tungsten carbide beads for simultaneous disruption and homogenization of the sample, per the manufacturer's instructions. Genomic DNA was extracted using the DNeasy® Plant mini kit (Qiagen) as per the manufacturer's instructions on a QIAcube Connect (Qiagen). DNA quantity and quality was assessed using 1% E-gel with Sybr Safe dye (Thermo Fisher) and concentrations assessed using Quantifluor dsDNA assay (Promega).

Library prep (for samples prepared by AGRF only)

DNA samples were fragmented enzymatically as part of the NEBNext Ultra II FS library preparation workflow. Libraries were prepared using the NEBNext Ultra II FS Library Prep Kit (New England Biolabs, Ipswich, MA, USA), following the manufacturer's instructions with inserts of approximately 350 bp.

Pooling and Hybridisation

Pooled libraries (12–16 plex) were enriched using the Angiosperms353 probe kit (Johnson et al. 2018) by hybridising at 65°C with the Arbor Biosciences MyBaits Expert Plant Angiosperms353 v1 bait set with V5 chemistry (Cat. # 308108.v5) [*Or Arbor Biosciences MyBaits custom OzBaits_NR set (Cat. # 300496R.V5) set with V5 chemistry and 64°C hybridization OR Arbor Biosciences MyBaits custom Salibaits set (Cat. # 300416.v5-ARB) with V5 chemistry and 62°C hybridization*] following the manufacturer's instructions.

Sequencing

Sequencing was performed on a NovaSeq 6000 (Illumina Inc., San Diego, USA) at the Australian Genome Research Facility (Melbourne, Australia) with v1.5 chemistry and 150bp paired-end reads.

Reference

Johnson MG, Pokorný L, Dodsworth S, Botigué LR, Cowan RS, Devault A, Eiserhardt WL, Epitawalage N, Forest F, Kim JT, Leebens-Mack JH, Leitch IJ, Maurin O, Soltis DE, Soltis PS, Wong GK, Baker WJ, Wickett NJ (2018), A universal probe set for targeted sequencing of 353 nuclear genes from any flowering plant designed using k-medoids clustering. *Systematic Biology* **68**, 594–606.