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Working

ddRAD-Seq protocol

Version 2

PLOS One

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EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0216903

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Shepherd LD, Brownsey PJ, Stowe C, Newell C, Perrie LR (2019) Genetic and morphological identification of a recurrent *Dicksonia* tree fern hybrid in New Zealand. PLoS ONE 14(5): e0216903. doi: 10.1371/journal.pone.0216903

BEFORE STARTING

Quantify DNA and check its quality on an agarose gel. DNA should be of high molecular weight.

Purchase adaptors as described in:

Peterson BK, Weber JN, Kay EH, Fisher SH, Hoekstra HE. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS ONE. 2012; 7 (5): e37135.https://doi.org/10.1371/journal.pone.0037135

1 Digest 300ng of DNA with the enzymes Ava11 and Msp1 (New England Biolabs) in a total volume of 30 µl. Perform digestion in a PCR machine at 37˚C for 3 hours, followed by inactivation of the enzymes at 65˚C for 20 min. Confirm digestion is complete by running 4ul of the digest on a 2% agarose gel.

2 Ligate adaptors to the sticky ends of the digested DNA using T4 ligase (New England Biolabs) in 40 µl reactions by incubating for 2 hours at 23˚C in a PCR machine, then inactivate the enzyme at 65˚C for 20 min.

3 Perform a PCR on a subset of samples to test that the ligation was successful. PCR amplifications are performed in 12 µl reactions with 1× MyTaq reagent buffer (Bioline, Australia), 5 pmol of each primer (the oligo P2.2-Msp and the unique forward oligo used to make the adaptor used for that particular ligation). PCR thermocycling conditions are an initial denaturation of 2 min at 94˚C, followed by 30 cycles of 94˚C for 20 sec, 55˚C for 20 sec and 72˚C for 30 sec, then a final extension at 72˚C for 5 min. Load 5ul of PCR product on a 2% agarose gel next to unamplified ligated DNA to confirm amplification.

4 Pool ligations for each index. Purify each index pool with a Qiaquick PCR purification kit (Qiagen), following the manufacturer's instructions except elute in 35 µl of Buffer EB and leave on the column for 1 min prior to centrifugation. Leave the final elution in an open 1.7ml tubes on a heating block set at 40˚C for 20 min to ensure no EtOH is present.

5 A gel rig for running the pooled index should be prepared by cleaning the gel apparatus with 70% ethanol. Each pooled index will be loaded into a single well so adjacent wells may need to be taped together to create sufficiently wide wells. An empty well should be left between each index, to avoid cross-contamination and a suitable ladder included at both sides on the gel. Fresh buffer should be used. Pour a 2% agarose and load the samples, then run at 80V for 80 minutes.

6 Prepare 1.7ml tubes by labeling them with the index and the size range to be collected. It is recommended to remove several gel slices per index (e.g., 300-500 bp, 500-700 bp) and to store the spares at -20˚C as backups. Use a new razor blade for each index and try and minimize the time the gel is exposed to UV light during gel excision.

For the size range that will be processed, weigh the gel slice and subtract the weight of the tube. Process each index by putting it through a

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7 Qiaquick gel extraction kit (Qiagen), following manufacture's instructions except use 30 µl buffer EB for the final elution and leave the elution buffer on the column for 4 min prior to centrifugation.

8 Quantify each index with a Qubit dsDNA HS (high sensitivity) assay kit (Thermo Fisher Scientific), following manufacturer's instructions.

9 Perform 4-6 PCRs per index to add the Illumina sequences to the fragments. Perform PCRs in 20 µl reactions with 1x Phusion flash high fidelity PCR master mix (Thermo Scientific), 4 pmol of each primer (PCR1 and PCR-Index, with PCR-Index being a unique primer for each index), and 5 µl gel-extracted ligation.

10 Quantify one PCR of each index with a Qubit dsDNA HS (high sensitivity) assay kit (Thermo Fisher Scientific) to confirm that DNA concentration has increased with the PCR.

11 Combine the PCRs for each index and purify with a MinElute kit (Qiagen), eluting in 12 µl Buffer EB.

12 Quantify each index with a Qubit dsDNA HS (high sensitivity) assay kit (Thermo Fisher Scientific). Combine each index equimolar.

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