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Working

ddRADSeq in a Field Setting

Version 3

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ABSTRACT

This protocol begins with blood stored in Longmire's solution and has as its goal, the sequencing of genomic DNA from several individuals onto a single MinION sequencing run. It is based on a protocol outlined by Thrasher et al. (2018) for the same process on a large number of warbler samples, but it differs from this original protocol in a few key ways:

1. Since we ultimately were targeting sequencing by a MinION sequencer, the ultimate processing steps reflect library preparation for runs on this device, instead of Illumina sequencers.
2. For the same reason, we also size selected larger fragments than can be sequenced on a MiSeq platform.
3. This protocol is also meant to only analysed a few individuals (20 or less) since the MinION read coverage is lower than that of a MiSeq.
4. The entire protocol was carried out in a field laboratory in the southeastern Peruvian Amazon, and therefore many of the luxuries a institutional laboratory can afford were not present to us
5. We used gel-based size selection in the absence of methods such as Blue Pippin.
6. We stored blood on FTA cards and in Longmire's solution in our sampling program. We discovered that yields from FTA cards, regardless of including large numbers of hole punches per extraction, simply were too low for the sequencing goals of this project. Therefore, we include the protocol of a WGA amplification step for these low samples. Longmire's solution produced higher yields, but some times, multiple extractions might need to be pooled together with an SPRI bead cleanup to be fully effective.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Buffer AE	19077	Qiagen
Buffer AE	19077	Qiagen

P1 Adaptor Ligation

1 Resuspend P1 oligos to 100uM in 0.5X AE Buffer (Qiagen), and follow instructions provided by supplier to do so if needed.

2 In a PCR strip tube, mix equal volumes of each oligo together to get a final concentration of 50uM of adapter. E.g. **20 µl** of P1F_X and **20 µl** l of P1R_X 11.

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3 In a

MiniOne PCR System
PCR thermocycler
MiniOne M4000

heat the mixture at 80 °C for 00:01:00

4 Remove tube from the thermocylcer and place on a rack at 24 °C **or room temperature** .

5 Allow to cool to 24 °C **or room temperature** for at least 00:30:00 .

6 Further dilute the adapter to 5uM with 0.5X

Buffer AE
by Qiagen
Catalog #: 19077

in 1.5ml tube. E.g Add 40 µl of annealed adapter and 360 µl 0.5X AE.

7 Store adapters at 4 °C or in freezer (-20 °C **minimum**) for long term storage.

P1 1:20 Dilution

8 This can be done in a strip of PCR tubes to allow you to use the multichannel pipette to dispense the P1 adapters. (You will need 20 tubes if using all the P1 adapters)

9 Label tubes.

10 Add 19 µl of nuclease free water to each tube.

11 Add 1 µl of P1 adapter to corresponding one tube above.

12 Repeat for all P1 adapters being used.

13 Seal tubes, vortex briefly to mix, centrifuge.

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FTA Card DNA Extractions

14 2. Rinse each sample in ddH2O by vortexing three times for five seconds each

15 3. Using a steril tip, transfer each disk to a pre-labeled PCR tube.

16 4. Add 45 to 50 uL of ddH2O (should cover the disk)

17 5. Spin down tubes and make sure disk is submerged

18 6. Heat to 95°C for 30 minutes in a PCR machine (

19 7. Remove tube and pulse vortex or gently tap 60 times.

20 8. Centrifuge for 30 seconds

21 9. Transfer all liquid into a pre-labelled tube – this will be Elution A

22 10. Add 45 to 50 uL (Confirm that you did use 30 ul for each elution) to the disk that remains in the PCR tube and repeat steps 4 – 9 to get Elution B

23 11. Store at 4°C if using it soon, or store at -20°C otherwise

P2 Adaptor Ligation

24 5In a PCR tube mix equal volumes of each oligo together to get a final concentration of 50uM of adapter. E.g. **50 µl** of P2F_MspI and **50 µl** of P2R_MspI.

25 In a thermocylcer heat the mixture at **80 °C** for **00:01:00**

26 Remove tube from the thermocylcer and place on a rack at **24 °C or room temperature** .

27 Allow to cool to **24 °C or room temperature** for at least **00:30:00** .

28 Further dilute the adapter to 25uM with 0.5 X

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Buffer AE
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in 1.5ml tube. E.g Add 100 µl of annealed adapter and 100 µl 0.5X AE.

29 Store adapter at 4 °C or in freezer for long term storage.

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