Resource Summary Report

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ddRADSeq in a Field Setting

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Protocol Information

URL: https://dx.doi.org/10.17504/protocols.io.y2efybe

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Summary: 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: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.For the same reason, we also size selected larger fragments than can be sequenced on a MiSeq platform.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.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 usWe used gel-based size selection in the absence of methods such as Blue Pippin.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.

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