**Appendix 1.**

**Förster et al “Targeted re-sequencing of coding DNA sequences for SNP discovery in non-model species”**

***Library building and capture details***

Oligonucleotides used:

|  |  |
| --- | --- |
| **Oligo** | **Sequence** |
| IS4 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTT |
| P7-indexing-primer\* | CAAGCAGAAGACGGCATACGAGATxxxxxxxxGTGACTGGAGTTCAGACGTGT |
| IS5 | AATGATACGGCGACCACCGA |
| IS6 | CAAGCAGAAGACGGCATACGA |
| BO1.P5.F  | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-Pho |
| BO2.P5.R  | AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT-Pho |
| BO3.P7.part1.F | AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-Pho |
| BO4.P7.part1.R | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-Pho |
| BO5.P7.part2.F  | ATCTCGTATGCCGTCTTCTGCTTG-Pho |
| BO6.P7.part2.R  | CAAGCAGAAGACGGCATACGAGAT-Pho |

Following Meyer & Kircher 2010

\* 8bp long index marked by x’s

*Library building*

DNA extracts were sheared using a Covaris M220 (Covaris Inc., Woburn, Massachusetts, USA) in a volume of 50µL using microTUBEs (screw cap). In total, we used between 1.2µg and 1.5µg of DNA per sample. DNA was sheared to ~350bp using manufacturer’s instructions (temperature: 18 to 22°C; treatment: 65sec, Peak Incident Power: 50, Duty Factor: 20%, Cycles/Burst: 200). Sheared DNA was visualized on the Agilent TapeStation (Agilent Technologies, Santa Clara, CA, USA) using D1000 ScreenTapes.

Libraries were then built following Fortes & Paijmans (2015). This adaptation of the protocol by Meyer & Kircher (2010) omits some steps in order to reduce loss of template and to reduce costs. Specifically, there is no clean-up step between (i) blunt-end repair and adapter ligation, and (ii) adapter fill-in and indexing PCR. The clean-up between adapter ligation and adapter fill-in was carried out using the Qiagen (Qiagen, Hilden, Germany) MinElute kit (20µL elution in 2 x 10µL EB).

Indexing PCRs were carried out with 10µL template and 30µL reaction mix (final concentration): Herculase buffer (1x), BSA (0.1mg/mL), dNTPs (0.25mM), IS4 primer (0.75µM), P7-indexing primer (0.75µM), and Herculase (0.05 U/µL). Samples were amplified with the following programme: 94°C for 2mins, then 5 to 8 cycles of 94°C for 30 sec, 60°C for 45 sec, 72°C for 45 sec, followed by 72°C for 3min. Amplified products were assessed on an Agilent TapeStation using D1000 ScreenTapes.

*Hybridization capture*

Enrichment was carried out as detailed in Li et al. (2013) with the following exceptions.

We carried out clean-up steps using Qiagen MinElute columns (rather than SPRI beads).

We included more blocking-oligos in the **Library Master Mix:**

|  |  |
| --- | --- |
| **Reagent** | **Volume (µL) per sample** |
| Block #1 | 2.5 |
| BO1.P5.F (200µM) | 0.08 |
| BO2.P5.R (200µM) | 0.08 |
| BO3.P7.part1.F (200µM) | 0.08 |
| BO4.P7.part1.R (200µM) | 0.08 |
| BO5.P7.part2.F (200µM) | 0.08 |
| BO6.P7.part2.R (200µM) | 0.08 |

We used an 8-fold dilution of RNA baits (0.6µL per sample), rather than the 10-fold dilution used by Li et al. (2013).

We performed post-capture amplification “off beads” – that is, an aliquot of beads (with attached baits and library) was used as template for PCRs. Post-capture amplification was carried out with 10µL template and 30µL reaction mix (final concentration): Herculase buffer (1x), BSA (0.1mg/mL), dNTPs (0.25mM), IS5 primer (0.75µM), IS6 primer (0.75µM), and Herculase (0.05 U/µL). Samples were amplified with the following programme: 94°C for 2mins, then 10 to 18 cycles at 94°C for 30 sec, 60°C for 45 sec, 72°C for 45 sec, followed by 72°C for 3min. Amplified products were assessed on an Agilent TapeStation using D1000 ScreenTapes.