**eDNA metabarcoding reveals a rich but threatened and declining elasmobranch community in West Africa's largest marine protected area, the Banc d'Arguin**

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**Online Resource 4** eDNA library preparation

| **Two step PCR** | | |
|---|---|---|
| | **1st step PCR** | **2nd step PCR** |
| **Characteristics** | With Illumina-compatible overhangs needed for the second round of indexing PCR | Dual-indices and Illumina-compatible adapter sequences added to the amplicons created during the first round PCR |
| **Reagents** | Total volume: 20 µL<br>10 µL Qiagen Multiplex PCR Kit mastermix (Qiagen)<br>1 µL of each primer (5 µM of MiFish-E primer)<br>4 µL of sterile distilled water 4.0 µL eDNA template (10 ng/µL). | Total volume: 17 µL<br>7 µl of 2X Kapa HiFi Hot Start<br>0.7µl of each of two indexes (P7 and P5, 10 µM)<br>2.8 µl of autoclaved water<br>2.8 µl of cleaned PCR products |
| **Conditions** | Initial denaturation: 15 min at 95°C<br>9 cycles of denaturation at 95ºC for 5 sec, annealing at 54°C for 10 sec with 0.5°C decrease per cycle, extension at 72°C for 10 sec | Initial denaturation: 3min at 95°C<br>10 cycles of 30s denaturation at 95°C, a 30s annealing phase at 55°C and a 30s extension phase at 72°C |

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| | 31 cycles of denaturation at 95°C for 5 sec, annealing at 50°C for 10 sec, extension at 72°C for 10 sec, and a final extension phase of 5 min at 72°C | Final extension phase of 5min at 72°C. |
| Three PCR replicates per eDNA extraction sample were performed to avoid missing rare sequences and to check for consistency. PCR blanks were used as controls and sequenced as described for the samples. The quality of purified PCR products was assessed by electrophoresis using 2% agarose gel | | |