Prepare plate with 95 samples

- Create plate in Geneious/Biocode LIMS
- Enter sample IDs by scanning 2D barcodes
- Print plate layout with sample positions

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Make notes on plate layout sheet:

- 1st individuum will become morphological voucher
- 2nd individuum from same location for Biobank

Also note any other special requirements.

Print labels for morphological vouchers (in DiversityCollection)

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Photograph samples



Fill S-block with lysis buffer and ProteinaseK



Transfer tissue of each specimen into corresponding well

(make notes on printout)





Microscope & light source





Fresh gloves



S-block filled with ATL buffer and Proteinase K and silicon pad



2 forceps (1 soft and 1 sharp) and petri dish

tissues & ethanol for cleaning









Additional fresh vials for the voucher and empty box for Biobank subsamples



paper labels (DC) for vials with voucher and scissors





Garbage bin

Larger arthropods: remove up to 3 legs from same (right) side of body



Larger arthropods: remove up to 3 legs from same (right) side of body



1 leg in S-block for lysis (chopped up for a better inflow of Proteinase K)

Larger arthropods: remove up to 3 legs from same (right) side of body







Larger arthropods: remove up to 3 legs from same (right) side of body





Specimen with paper label in new vial as voucher (collection)



Examples of different sizes









ightarrow 1 leg for lysis, 1 leg for Biobank



ightarrow 2 legs for lysis, 1 leg for Biobank



ightarrow 3 legs for lysis, no leg for Biobank

 \rightarrow Whole body lysis

Do not take the pedipalps instead of legs!







 \rightarrow 1 leg for lysis, 1 leg for Biobank



 \rightarrow 2 legs for lysis, 2 legs for Biobank



 \rightarrow 4 legs for lysis, no leg for Biobank



 \rightarrow Whole body lysis

Tissue samples from larger animals



Piece of tissue (e.g. muscle) sent from collector

Other tissue sources: toe or quill (reptiles and birds) tip of tail (reptiles and rodents) fin (fish) Cover S-block with silicon pad and put into lysis overnight: gently shaking at 300rpm and 56°C, ~14-16 hours



Next day: recover small individuums that have been in lysis completely \rightarrow voucher vial with paper label

Fill 5 additional S-blocks and elution plate with buffers (pipetting by hand or using the "EpMotion 5075" robot)



Wash 1: 650µl Buffer AW1 Wash 2: 500µl Buffer AW1 Wash 3: 500µl Buffer AW2 Wash 4: 500µl Buffer AW2 Wash 5: 500µl H₂O Elution: 200µl Buffer AE



In a 50ml tube prepare magnetic bead solution:

- 22ml Buffer AL
- 22ml Isopropanol
- 3.2 ml MagAttract (vortex very well before using)

 \rightarrow Vortex solution, then add 450µl to each well

Load all blocks into BioSprint 96 machine and start according program.



Prepare PCR immediately after extraction, before storing the DNA

Using the Qiagen Multiplex Kit prepare a mix for 100 reactions:

- 440µl H2O
- 200µl Q-Solution
- 1000µl Multiplex-Mix
- 80µl forward-Primer: LCO1490-JJ (5´-CHACWAAYCATAAAGATATYGG-3')
- 80μl reverse-Primer: HCO2198-JJ (5´- AWACTTCVGGRTGVCCAAARAATCA-3')
- \rightarrow 1800µl Mix in total \rightarrow fill 18µl in each well of a PCR-plate

Then transfer 2µl DNA of each well from the elution plate into the corresponding well of the PCR-plate (using a multi-channel pipette)

Cover plate with slide and put directly into PCR machine



Transfer remaining DNA from elution plate to long term storage system (FluidX)

Used PCR program is a combination of a 'touchdown' and a 'step-up' routine:

```
hot start Taq activation:15 min at 95 °Cfirst cycle set (15 repeats):35 s denaturation at 94°C90 s annealing at 55°C (−1°C per cycle)90 s extension at 72°Csecond cycle set (25 repeats):35 s denaturation at 94°C90 s annealing at 40°C90 s extension at 72°Cfinal elongation10 min at 72 °Ccooling12°C (∞)
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Unpurified PCR products are sent for bidirectional Sanger sequencing to BGI (Hong Kong)

What we have in the end:



Vial with the specimen voucher \downarrow Temporary freezer until barcode has been validated \downarrow Permanent morphological collection What we have in the end:



Vial with the specimen voucher
↓
Temporary freezer until barcode has been validated
↓
Permanent morphological collection

Subsample (leg or whole animal) for Biobank



What we have in the end:



Vial with the specimen voucher
↓
Temporary freezer until barcode has been validated
↓
Permanent morphological collection

Subsample (leg or whole animal) for Biobank





DNA (in storage tubes) \rightarrow send to the different partners