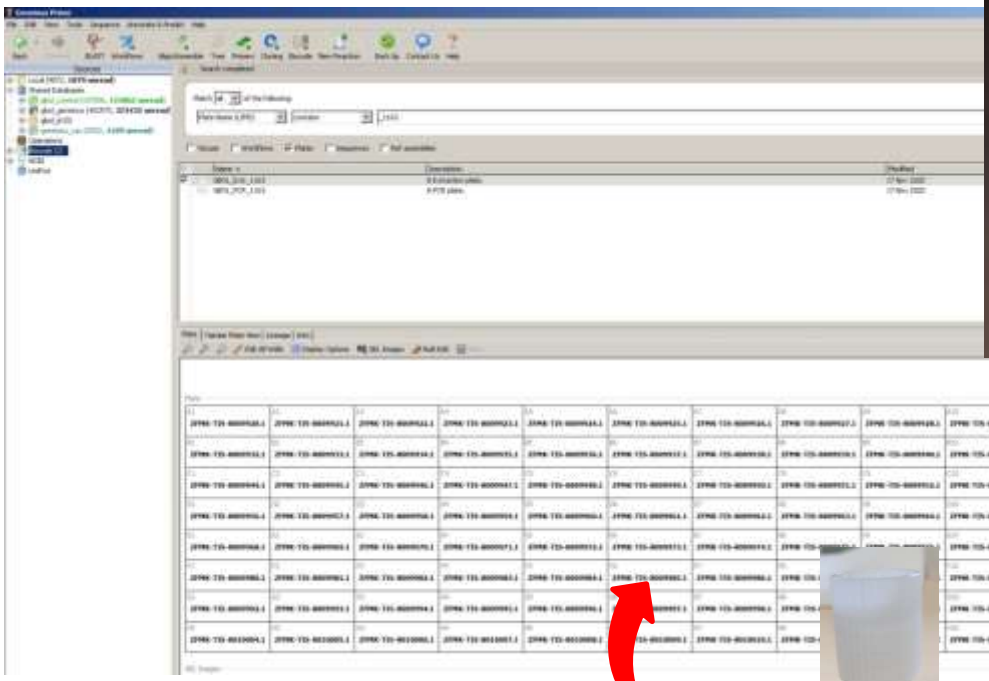
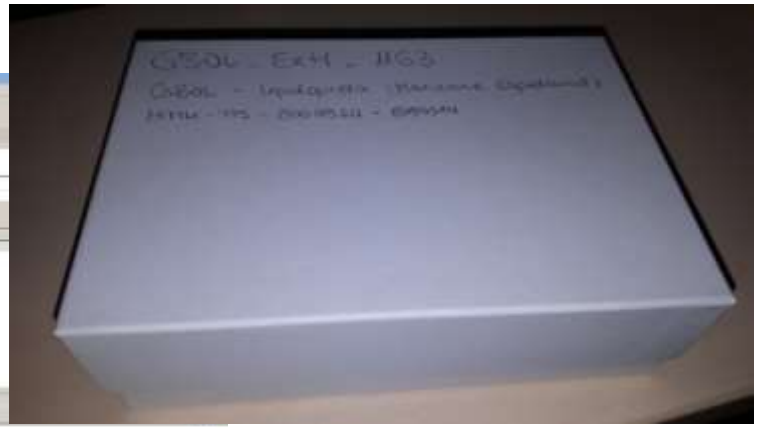
# Prepare plate with 95 samples

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





scan

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)

# Photograph samples

# Fill S-block with lysis buffer and ProteinaseK

→

180µl ATL buffer
20µl ProteinaseK

↓

Transfer tissue of each specimen into corresponding well

(make notes on printout)

# Lab space for subsampling

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Fresh gloves

# Lab space for subsampling

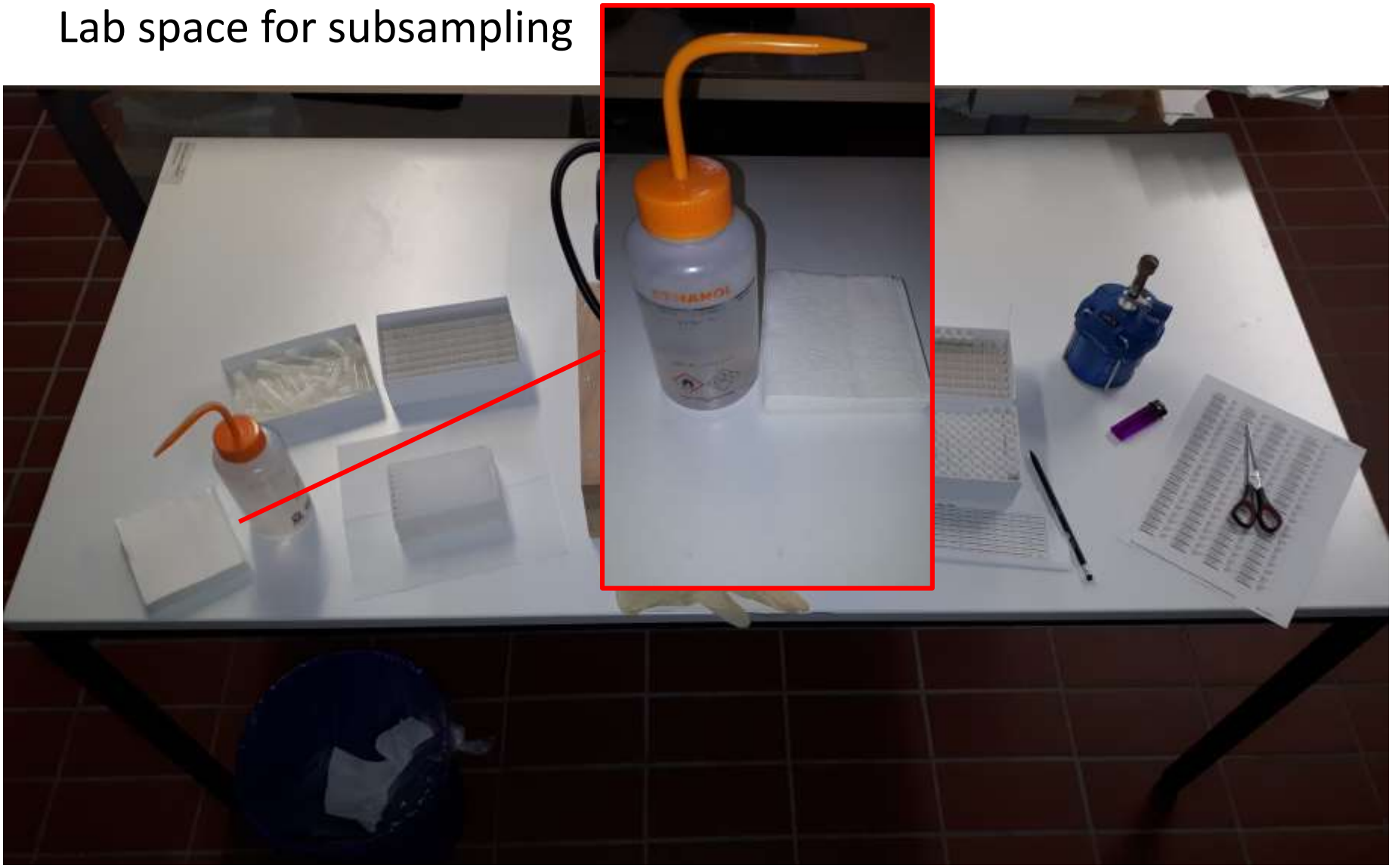
S-block filled with ATL buffer and Proteinase K and silicon pad

# Lab space for subsampling

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

tissues & ethanol for cleaning

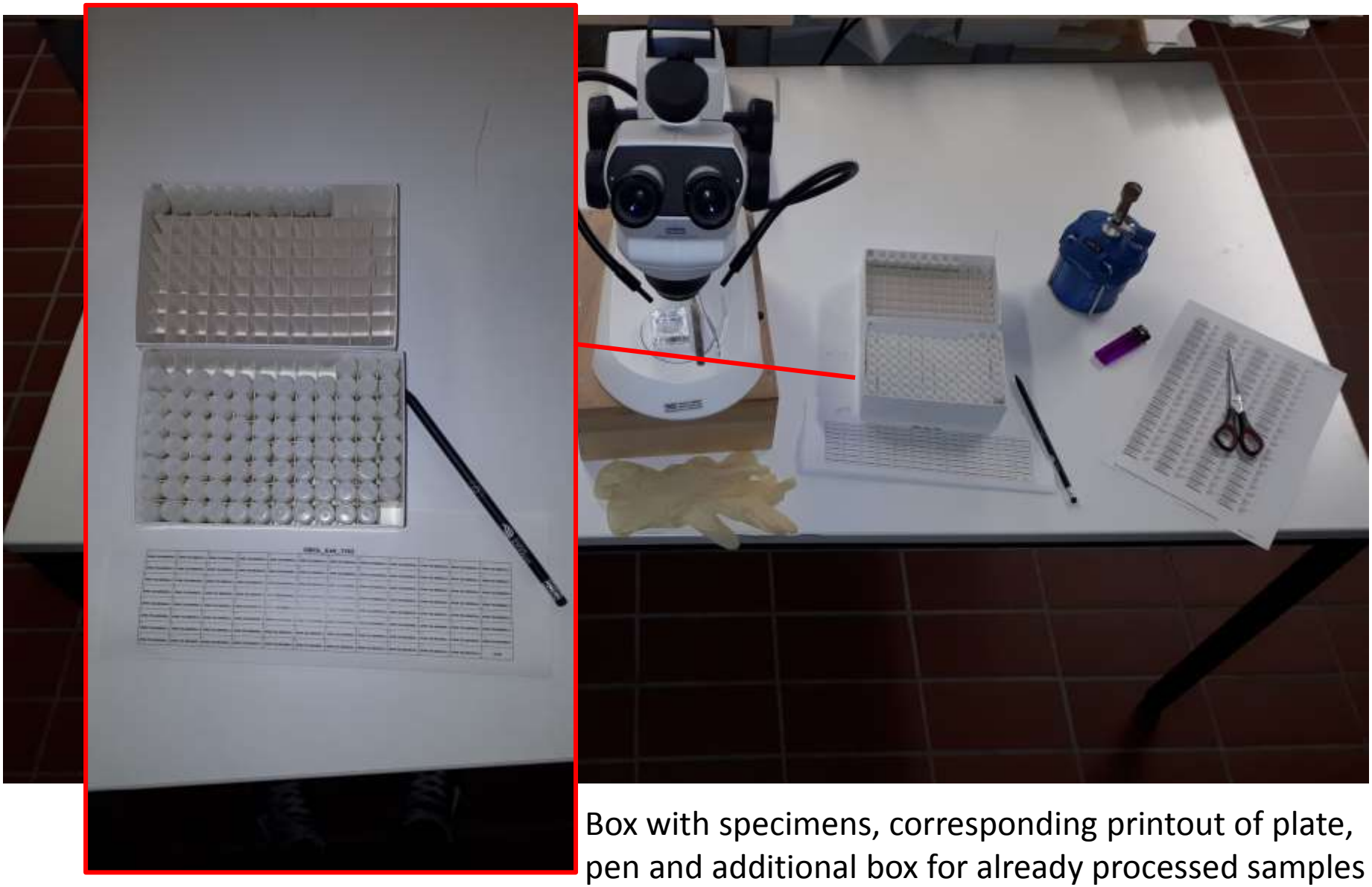
# Lab space for subsampling



Lab space for subsampling


lighter & burner

# Lab space for subsampling



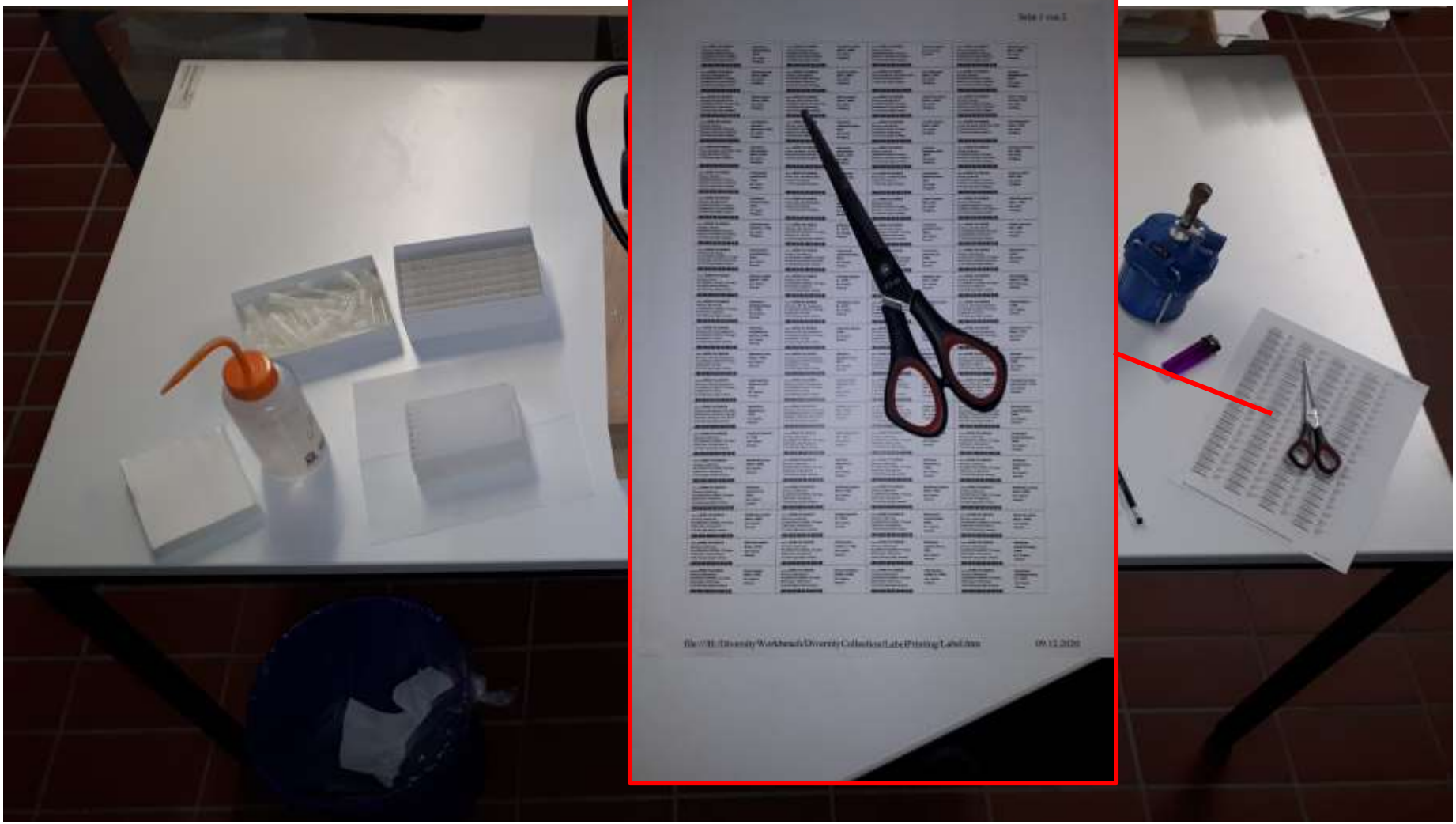
Box with specimens, corresponding printout of plate, pen and additional box for already processed samples

# Lab space for subsampling

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

paper labels (DC) for vials with voucher and scissors

# Lab space for subsampling



# Lab space for subsampling

Garbage bin

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

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

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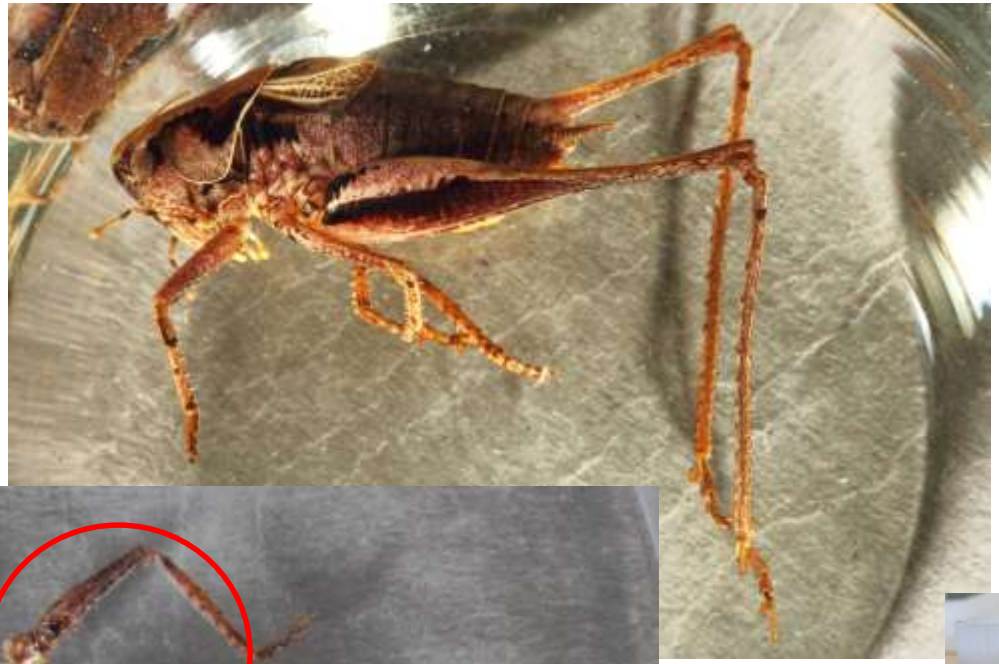

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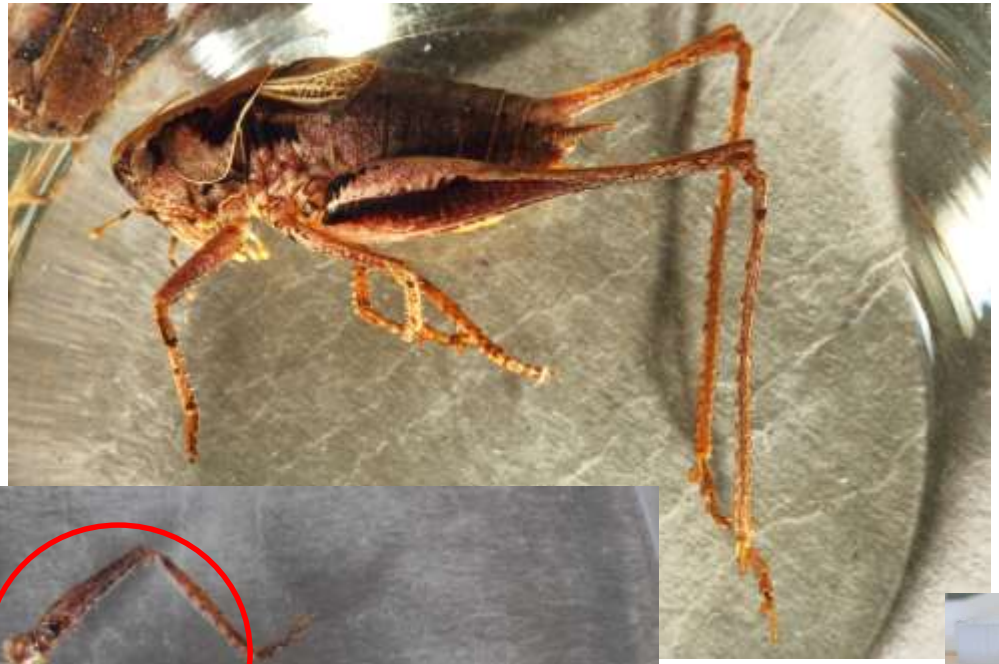
1 leg in labelled vial for Biobank

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

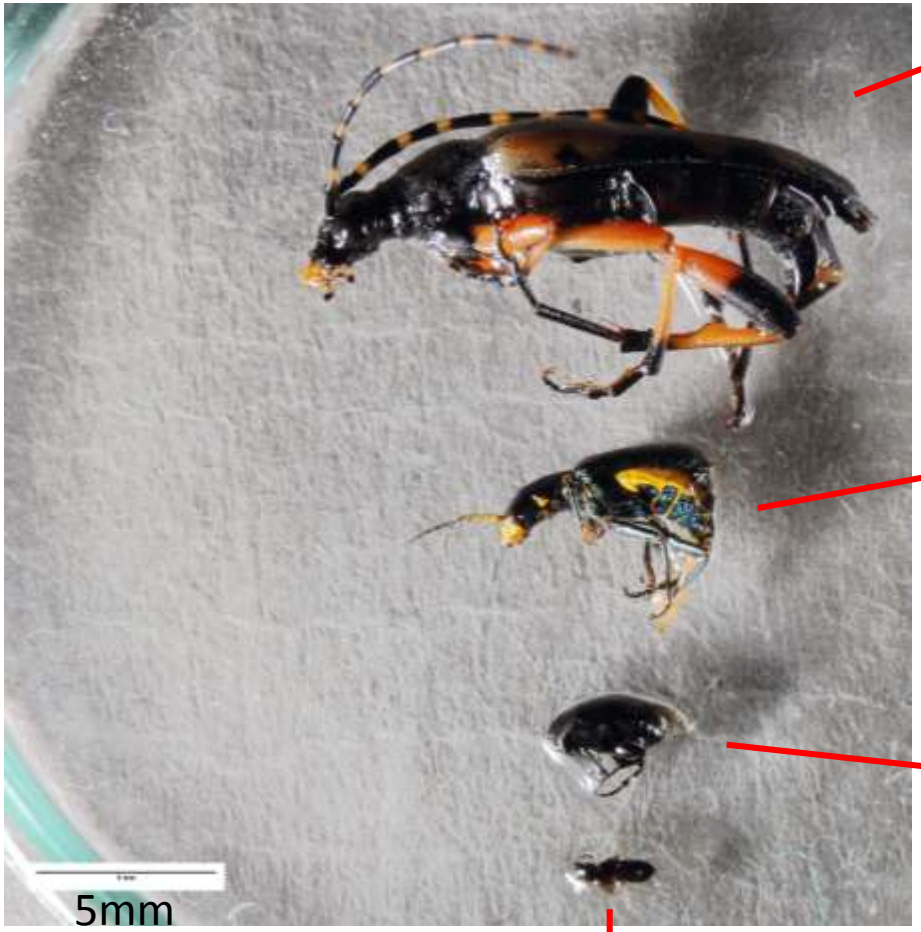


1 leg in S-block for lysis
(chopped up for a better inflow of Proteinase K)


1 leg in labelled vial for Biobank

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

# Examples of different sizes




→ 1 leg for lysis, 1 leg for Biobank

→ 2 legs for lysis, 1 leg for Biobank

→ 3 legs for lysis, no leg for Biobank

→ Whole body lysis

Do not take the pedipalps instead of legs!

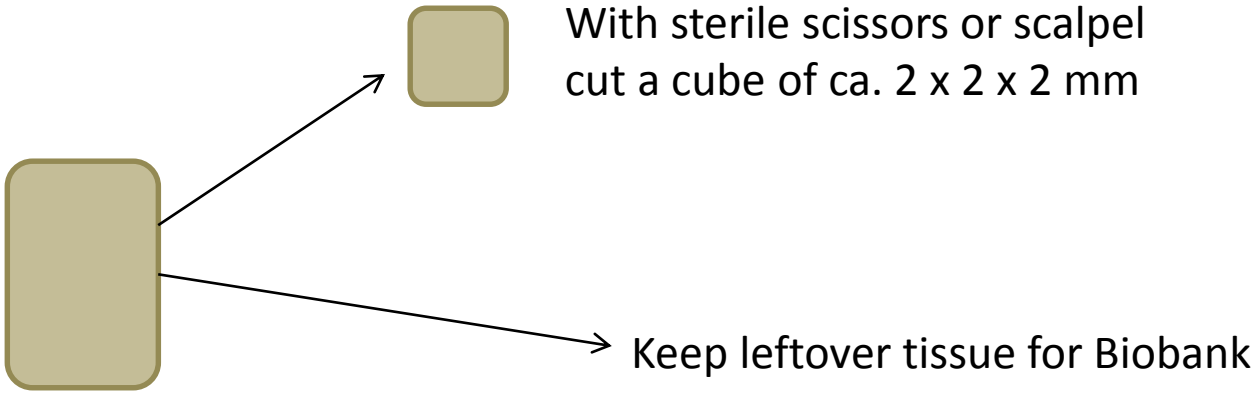

→ 1 leg for lysis, 1 leg for Biobank

→ 2 legs for lysis, 2 legs for Biobank

→ 4 legs for lysis, no leg for Biobank

→ Whole body lysis

# Tissue samples from larger animals

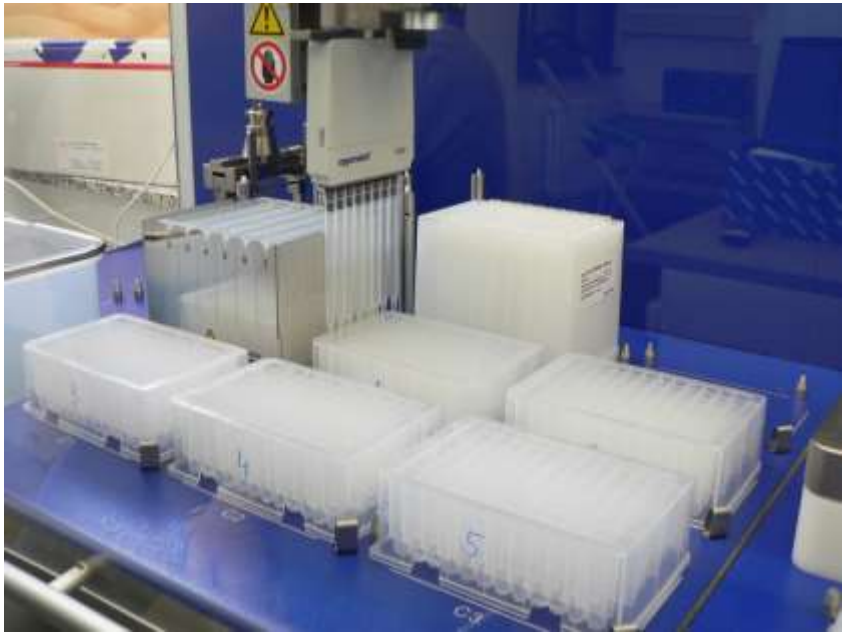



Other tissue sources: toe or quill (reptiles and birds)
tip of tail (reptiles and rodents)
fin (fish)

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 → voucher vial with paper label

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



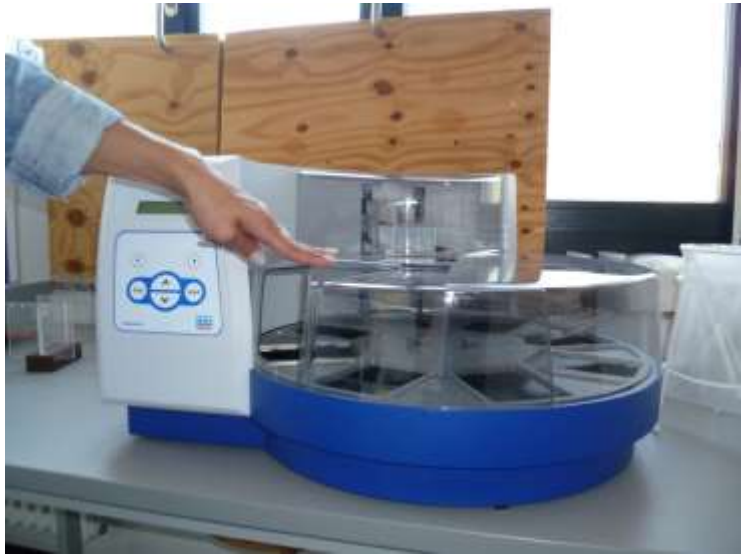
Wash 1: 650µl Buffer AW1
Wash 2: 500µl Buffer AW1
Wash 3: 500µl Buffer AW2
Wash 4: 500µl Buffer AW2
Wash 5: 500µl $H_2O$
Elution: 200µl Buffer AE

In a 50ml tube prepare magnetic bead solution:

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

→ 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')

→ 1800µl Mix in total → 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 °C |
| first cycle set (15 repeats): | 35 s denaturation at 94°C |
| | 90 s annealing at 55°C (−1°C per cycle) |
| | 90 s extension at 72°C |
| second cycle set (25 repeats): | 35 s denaturation at 94°C |
| | 90 s annealing at 40°C |
| | 90 s extension at 72°C |
| final elongation | 10 min at 72 °C |
| cooling | 12°C (∞) |

Unpurified PCR products are sent for bidirectional Sanger sequencing to BGI (Hong Kong)

# What we have in the end:

Vial with the specimen voucher

↓

Temporary freezer until barcode has been validated

↓

Permanent morphological collection

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Subsample (leg or whole animal) for Biobank

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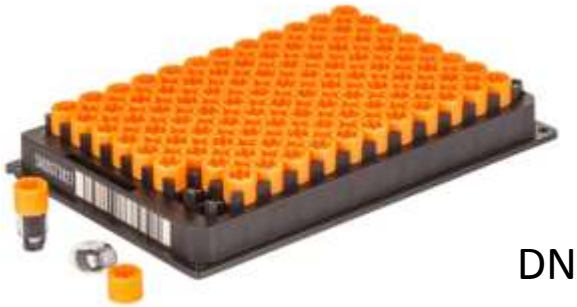

DNA (in storage tubes) → send to the different partners