Methodology for DNA Extraction from Dry Museum samples

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Checklist for Day 1:

- Appropriate volume of digestion buffer - use the DNA extraction calculator to dilute the buffer according to the number of samples. Make this fresh or only add the proteinase K immediately before incubation (make sure to take that into account once calculating the volume of TRIS)
- Incubator set at 55ºC
- Sharpie
- 1.5 ml Tubes, or plates (2 per sample) > always label tube and lid! or plate
- Pipettes
- Tips
- Notebook to log the information on all samples
- Pencil

Methods for day 1:

- Prepare digestion buffer
    - Weight all solid reagents, calculated with the DNA extraction calculator, except proteinase K in an appropriate falcon tube (15ml-50ml) and add Tris buffer up to the total volume of buffer. Warm in an incubator or water bath set at 37ºC to solubilize the SDS (which can also be done if the buffer is stored on a refrigerator, provided that no proteinase K was added to it).
    - Add proteinase K right before proceeding to incubation with samples.
- Place samples into tubes/plate well
- Add 90ul of digestion buffer per sample. *This volume can change depending on the sample size. It needs to be enough to submerge the sample. Just pay attention to the relative volumes neede on day 2 of the extraction.
- Spin down (slowly) for 15''
- Incubate > 18 hrs (3pm-10am) on slow shaker (25 rpm for mosquitos, 15 rpm for specimens that will be put back in the collection, such as beetles and hemipterans) at 55ºC

Checklist for Day 2:

- Notes from day 1
- Pipettes
- Tips (2-20ul, 200ul, 200-1000ul > make sure to have at least 3 tips of each per sample)
- Notebook to log the information on all samples
- Pencil
- Isopropanol 100%
- 3M Sodium Acetate pH 5.2
- Glycoblue Ambion
- Ice cold 85% EtOH - overnight in the -20ºC or >30' in the -80ºC

Methods for day 2:

- Vortex and spin down samples
- Transfer supernatant to a new tube/plate
- Add 54 ul of Isopropanol 100% + 9 ul of 3M Sodium Acetate pH 5.2 + 1ul of Glycoblue Ambion to each sample. (Total volumes needed calculated on DNA extraction sheet – keep in mind that these volumes are dependent on the volume of digestion buffer used).
    - Isopropanol: 0.6* volume of digestion buffer
    - 3M Sodium Acetate pH 5.2: 0.1*volume of digestion buffer
    - Glycoblue Ambion: 0.01* volume of digestion buffer
- Vortex
- Centrifuge at maximum speed for 25 minutes (Nucleic acids will pellet within the tube) * A good trick is to always position the tube facing the same way, so the pellet is always on the same side and, even if it is "invisible" it minimizes the chance of loss.
- Remove Excess liquid to leave only the pellet in the Eppendorf tube
- Add 1.5 mL ice-cold 85% EtOH
- Centrifuged at maximum speed for 3 minutes
- Remove Excess liquid to leave only the pellet in the Eppendorf tube
- Add 1.5 mL ice-cold 85% EtOH
- Centrifuge at maximum speed for 3 minutes

- Remove Excess liquid to leave only the pellet in the Eppendorf tube
- Allow pellet to air dry at 65ºC (leave tubes/plate open in the incubator)
- Resuspend pellet in 100 ul of PCR grade H2O
- Allow DNA to come to solution overnight at 4ºC before quantifying.