DNA extraction Formol fixated animal tissue (Anouk Langerak – ARISE)

Materials

- Milli-Q water
- Boric acid - H_3BO_3 (Borsäure/Borat)
- Sodium hydroxide (10 M) NaOH
- Demi water
- SDS (85%) - Natriumlaurylsulfat (Sodium Lauryl Sulfate)
- Proteinase K

Buffer – 50 mM BNB 1% SDS pH11

For a 500 mL buffer

1. Weigh 1.55 g Boric acid and transfer to a 500 mL flask
2. Add 450 mL Milli-Q water and measure pH (probably around 4.5)
3. Add approximately 3.5 mL 10M NaOH in shifts to get pH 11. (Measure pH in between so pH will not exceed 11)
4. Add 1% SDS. End volume is 500 mL so 5mL. 5mL = 5000 mg. Stock SDS is 85% pure, so add 8.88 g SDS.
5. Fill up bottle to 500 mL end volume and check pH to be sure it is 11.
6. Buffer can be stored in the yellow chemical cabinets on the prelab.

Manual column extraction kit

1. Subsample specimens and add no more than 25 mg tissue in a clean 1.5 mL tube.
2. Rinse the samples twice with Milli-Q to remove traces of ethanol/formalin.
3. Add 980 ul of the BNB-SDS lysis buffer to each sample.
4. Add 50U ProtK to each sample.
5. Incubate samples for 24h at 60C.
6. Continue with suitable extraction kit after lysing step.

Extraction plate Kingfisher

1. Subsample specimens and add no more than 25 mg tissue in a deep well plate.
2. Rinse the samples twice with Milli-Q to remove traces of ethanol/formalin.
3. Add 225 ul of the BNB-SDS lysis buffer to each sample.
4. Add 12U ProtK to each sample.
5. Incubate samples for 24h at 60C.
6. Check pH (Must be ~ 6 (Same value as standard KingFisher lysis buffer))
7. Continue with MN Tissue KingFisher protocol.