

Extraction of genomic DNA extraction with zirconium beads © J. de Lorgeril 2017 (in prep.)

Genomic DNA extraction (Kit Genomic DNA from tissue, Macherey-Nagel, ref. 740952.250)

- a. Materials
 - i. 1 tray for liquid nitrogen
 - ii. Zirconium beads (0.1 mm diameter, ZIRCONIA/SILICA. Cat. No. 11079101z BioSpec Products)
 - iii. 2 plastic tube racks
 - iv. 2 Beakers (1L)
 - v. 1 Micro spoon and spatula
 - vi. Absorbent paper
 - vii. Micropipettes + filter sterile tips (200 and 1000 µl)
 - viii. 2.0 ml Screw Cap Microtubes (starlab E1420-2341)
 - ix. 1.5 ml Microtubes (DNAse/RNase free)
 - x. 2 Laboratory water bath (56 and 70°C)
 - xi. Mixer Mill MM 400 + 2 racks
 - xii. Centrifuge at room temperature
 - xiii. Vortex
 - xiv. EtOH 100% et 70%
 - xv. DNAaway (ATTENTION: not on metals and joints)
 - xvi. SDS 0.1% (or ALCONOX detergent)
- b. Before extraction
 - i. Clean the laboratory bench and the micropipettes with EtOH 70% then DNAaway then EtOH 70%
 - ii. Clean the laboratory water baths , the racks, the Micro spoon and spatula, the plastic trays and the centrifuge
 - iii. Turn on the water baths to 56 and 70°C
 - iv. Number the tubes for each sample
 1. 1 Screw Cap Microtube 2 ml
 2. 2 Eppendorff Microtubes 1.5 ml
 3. 1 Collection tube from extraction kit (1 column and 2 collection tubes)
 - v. Have 100% ethanol at room temperature (if stored at -20 ° C)
 - vi. Prepare 2 beakers with diluted bleach and MilliQ water
 - vii. Check that the 2 solutions to be completed from the Kit are ready
 1. Wash Buffer B5: 200mL EtOH 100%
 2. Proteinase K (PK): 3.35mL Proteinase Buffer (Store 1mL aliquots at -20 ° C)
- c. For the extraction
 - i. **Lysis of samples**
 1. Preparation of Lysis Buffer
 - a. Add the zirconium beads with the top of a 200 µL filter tip into a 2 mL screw microtube
 - b. Add 180 µl of Lysis Buffer
 - c. Add 25 µl of PK in the last moment (10 minutes max before adding the sample to the tube)
 2. **Tissue preparation**
 - a. Place the samples in a tray containing liquid nitrogen
 - i. The samples have to be just out of -80°C

- ii. Micro spoon and spatula
 - b. For each sample
 - i. Transfer the sample with the weighing spoon into the corresponding 2 mL tube which contains the lysis buffer (rack and tube on the bench at RT)
 - ii. Vigorously homogenize the sample with the vortex
 - iii. Clean the weighing scoop in the bleach beaker, then in MilliQ beaker and then wipe with absorbent paper.
 - iv. Return the weighing spoon to liquid nitrogen and wait until it is at the right temperature to process the next sample
- 3. Mixer Mill MM 400**
- a. Place the tubes in the racks (RT) and then in the mixer mill MM 400 (always put both racks, even if only one contains the samples).
 - b. Shake at a frequency of 30s-1 for 12 min
 - c. Incubate tubes for 1h30 at 56 °C in water bath (vortex every 30 min during incubation)
- 4. ARN digestion and DNA precipitation**
- a. Add 20 µl of RNase A (10mg/mL) 5 min to RT
 - b. Vortex
 - c. Incubation 10 min at 70°C
 - i. Preheat the BE buffer at 70 °C for the last step of the protocol
 - ii. Take advantage to clean the centrifuge
 - d. Vortex the samples
 - e. Centrifugation 5 min 11000 g to RT (25°C)
 - f. Transfer the supernatant (420 µl) into a 1.5mL microtube
 - g. Addition of 210 µl EtOH (100%) to the 420 µl
 - h. Vortex
- 5. Washing and eluting DNA**
- a. Load the mixture into a column
 - b. Centrifugation 1 min 11000 g at RT
 - c. Place the column in a new collection tube and discard the old one
 - d. Add 500 µl of Wash Buffer BW
 - e. Centrifugation 1 min 11000 g at RT
 - f. Discard the eluate in a 15 mL Falcon tube (trash container), wipe the tube on an absorbent paper to remove the drops
 - g. Add 600 µl of Washing Buffer B5
 - h. Centrifugation 1 min 11000 g at RT
 - i. Discard the eluate in a 15 mL Falcon tube (trash container)
 - j. Centrifugation 1 min 11000 g at RT
 - k. Place the column in a 1.5 ml microtube
 - l. Add 100 µl of elution buffer BE(preheated at 70°C)
 - m. Incubate 1 min at RT (closed column)
 - n. Centrifugation 1 min 11000 g at RT
 - o. Discard the column and close the tube
 - p. Store at -80°C