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 T1
         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. Add 200 µl of Lysis Buffer B3
d. Vortex vigorously
e. 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
f. Vortex briefly
g. Centrifugation 5 min 11000 g to RT (25°C)
h. Transfer the supernatant (420 µl) into a 1.5mL microtube (safe at -80°C if interruption is necessary)
i. Addition of 210 µl EtOH (100%) to the 420 µl
j. Vortex vigorously

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