Basic gDNA preparation for Genotyping

Based on "Hot shot" protocol (https://pubmed.ncbi.nlm.nih.gov/10907076/).

Material required:

- Clean 50 mM NaOH prepared from solid NaOH dissolved in sterile Milli Q water (or ddH_2O).
- Autoclaved 100 mM Tris-HCL pH 8.0 prepared with sterile Milli Q water (or ddH_2O).
- Sterile Milli Q water (or ddH_2O)

Protocol for ear punch bipsy:

- Harvest mouse biopsy and transfer to 1.5 mL clean tube.
- Process the biopsies on the same day or store them at -20˚C to process them later.
- Localize the biopsy in each tube and ensure it is at the bottom of the tube. A 1 minute centrifugation at 13,000 rpm will normally get the tissue at the bottom.
- Add 150 µL of 50 mM NaOH to each tubes. (bigger biopsies require larger volume, for example 300 µL for a tail clip)
- Flick each tubes and make sure the piece of tissue is submerged in the liquid.
- Incubate the tubes at 95˚C for 60 minutes.
- Centrifuge the tubes at room temperature (RT) for 1 minutes at 13,000 rpm and let them cool down to +/- RT (could be done on the bench, in fridge or on ice).
- When temperature is low enough to open the tubes safely, add an equal volume of 100 mM Tris pH 8.0 (150 µL).
- Mix well by inverting (when doing several tubes, using a 2nd rack on top of the tubes will allow to mix all tubes at once). It is not recommended to vortex extensively at this stage but it is important to achieve a complete mixing.
- Pellet down the solid fragments remaining by centrifugation at RT for 5-10 minutes 13,000 RPM.
- Immediately after, transfer 1 volume (50-100 µL) of supernatant to a new labeled tube containing 4 volumes (200-400 µL) of sterile Milli Q water (or ddH_2O). (dilution 1/5).
- Store the stock gDNA at -20˚C for later use and store diluted gDNA at 4˚C for short-medium term storage (usually diluted gDNA works well in PCR even after 2 weeks at 4˚C).
- Use 5 µL of diluted gDNA to setup a genotyping PCR reaction of 15 µL.

Clean gDNA Extraction (from Lungs or Liver, Michel V. Levesque)

Protocol adapted from MacDougald lab
(https://macdougald.lab.medicine.umich.edu/lab-protocols/protocolsmethods/gitschier-protocol-for-genomic-dna-isolation-from-mice)

- Digestion with Proteinase K in 1X Modified Gitschier Buffer (1x MGB) (recipe below)
- Add ~50-100 µL of Digest buffer with Port. K and squish tissue with small Pestel. Complete volume to 500µL of digestion buffer, mix quickly (shaking tubes or quick vortex). Depending on the tissue source and size, mechanical force might not be required at this stage.
- Incubate O/N @ 55˚C (12-18 hours). (An alternative could be using the heat block shaker on low speed to promote complete digestion of the tissue).
- After digestion (next morning), quick spin and check for digestion efficacy. If incomplete, quick vortex and incubate for an extra 1-2 hours.
- Quick Spin, keep on ICE. Tubes should only be open once back to ~25˚C or lower to avoid any spill and potential cross contaminations.
- Add 500µL of Phenol/CHCl3/Isoamyl-Alcohol. (careful pipetting is required to avoid cross contamination of stock solution and samples)
- Mix well by shaking for 30-60 seconds. When processing multiple samples, the use of a second rack on top of the tubes allows to shake all at once. Avoid vortex since it promotes gDNA shearing.
- Incubate 5 min @ RT.
- Centrifuge for 15 min @ RT, >13K RPM (max speed table top centrifuge).
- Transfer aqueous phase to new tube and procced to CHCl3 extraction.
- Mix well by shaking for 30-60 seconds (see above for details) and incubate 5 min @ RT.
- Centrifuge for 15 min @ RT, >13K RPM (max speed table top centrifuge). (a 2nd CHCl3 extraction might help removing the white material that can remain with the aqueous phase).
- Transfer aqueous phase to new tube and procced to Isopropanol precipitation.
- Add 10% V. of 5M Na-Acetate pH5.2 (50µL) and mix well (same has organic extractions).
- Add 110% V of Isopropanol (550µL), incubate 30 minutes to O/N @-20˚C.
- Centrifuge for 20 min @ RT, >13K RPM (max speed table top centrifuge).
- Wash with 70% EtOH (500-900 µL). Ideally the pellets should come off the wall of the tube. Centrifuge for 5-10 min @ RT, >13K RPM. Remove 70% EtOH and repeat wash.
- Make sure to remove all remaining 70% EtOH by quick spin followed with small pipetting (P10 or P20). Air dry the pellets at RT for a couple of minutes (5-10 minutes). Avoid over drying pellets to favor good and easy gDNA resuspension.
- Resuspend gDNA pellets in 5 mM Tris pH 7.5-8 (clean as described in the Basic protocol). The volume will vary depending on the tissue input and the recovery efficacy. Start with a smaller volume and adjust for the next time.
- To facilitate resuspension, add the Tris and let the pellets re-hydrate for 30-60 minutes @ RT before performing up/down pipetting to complete the resuspension process. Alternatively, DNA can be incubated at 50-55˚C to improve solubilization of the gDNA.
- Measure gDNA concentration by spectrophotometry. Dilute stock gDNA to ~500ng/µL.
- Prepare sub-dilution for PCR use. (10 to 50 ng/15µL PCR reaction should work well).

10x Modified Gitschier Buffer (10x MGB)

- 223 mL Tris (1.5 M, pH 8.8)= 670 mM Tris
- 10.97 g Ammonium Sulfate =166 mM Ammonium Sulfate
- 3 g Magnesium Chloride= 65 mM Magnesium Chloride
- Fill to 500 mL with Milli-Q H_20, mix well and autoclave

1x MGB (for 10 mL) (could be prepared and stored -20˚C or prepared fresh)

- 8.85 mL ddH_2O
- 1 mL 10x MGB
- 100 µL 2-mercaptoethanol
- 50 µL Triton X-100 (final 0.5%)
- Vortex vigorously to make it soluble Triton X-100.

- Alternatively, prepare a 10% stock of Triton X-100 in clean H2O and use 500µL/10mL.

Proteinase K mastermix

- 10 parts 1x MGB
- 1 part 20 mg/mL Proteinase K (we have 10mg/mL)
- 800µL buffer + 100µL Prot.K (The amount of Prot. K could be doubled if poor digestion is achieved). For example: 4 mL of 1X MGB + 500 µL of Prot. K 10 mg/mL.