Modified protocol for DNA isolation from tissue using Promega Wizard Genomic DNA Purification Kit

Materials:

- Promega Wizard Genomic DNA Purification Kit (order no. A1120 or A1125)
- Isopropanol (2-propanol)
- 75 % EtOH

Procedure:

- Tissue: cut 10-50 sections of 20 µM from a fresh frozen tissue block using a cryostate or for cells: collect between $1*10^4$ to $5*10^6$ tissue culture cells. (Or follow the homogenization with morter, see below).
- Add 600 µl Nuclei Lysis Solution to the frozen tissue or cell pellet and vortex.
- Add 17,5 µl proteinase K (20 mg/ml) and vortex (when using 10 mg/ml: add 35 µl!
- Incubate over night at 55 degrees C (w/shaking, cover with alu foil just to keep temp uniform).
- Next day : Turn on a heating block at 37 degrees and 65 degrees.
- Add 3 µl RNase solution and mix gently. Incubate 15-30 min on 37 degrees.
- Add 200 µl Protein Precipitation Solution and vortex for 20 seconds. Put the sample on ice for 5 minutes.
- Centrifuge for 4 minutes at 13 000 rpm at room temperature. The precipitated proteins will form a pellet.
- Pipet the supernatant to a clean tube and spin again at 13 000 rpm for 4 minutes. Pipet the supernatant into a clean tube and add 600 µl Isopropanol.
- Mix gently until the DNA becomes visible (by inverting the tube approx 10 times).
- Centrifuge for 2 minutes at 13 000 rpm. The DNA will now form a white pellet. Gently remove the supernatant. (Sometimes it can be difficult to see the pellet)
- Add 600 µl Ethanol and gently mix to wash the DNA. Spin for 1 minute at 13 000 rpm.
- Remove the Ethanol and let the DNA dry for 10-15 minutes.
- Add 60 µl SigmaH2O (the protocol says 100 µl DNA Rehydration Solution, but we use Sigma H2O, and if pellet very small use a bit less than 60 µl…) and put the tube at 65 degrees for 15 minutes. The tube can be put on gentle rocking/rotating overnight in room temp for the pellet to dissolve completely (or in 4 degrees).
- Store DNA at 2-8 degrees C.

From: http://www.qiagen.com/literature/qiagennews/weeklyarticle/dec03/e48/default.aspx

- Freeze sample in liquid nitrogen immediately after harvesting. Do not allow the sample to thaw at any time during disruption.
- Precool the mortar to –20°C and keep on dry ice.
- Pour liquid nitrogen into the mortar and precool the pestle by placing the grinding end in the liquid nitrogen.
- Place frozen tissue in the mortar and grind until a fine, whitish powder is formed. Add liquid nitrogen as necessary during the grinding.
- Transfer the powder using a precooled spatula to precooled containers of the appropriate size.
- Allow the liquid nitrogen to evaporate. To prevent the sample from thawing after the liquid nitrogen has evaporated, containers should be cooled by placing on dry ice or in liquid nitrogen.
- Add lysis buffer as quickly as possible and continue with the purification procedure.