A Simplified Protocol for Genomic DNA (gDNA) Preparation from Mammalian Cell Lines

(Zongyue Zeng @ 09/2019; commented by TCH)

Background: There are a number of different procedures for the preparation of genomic DNA. They all start with some form of cell lysis, followed by deproteinization and recovery of DNA. The main differences between various approaches lie in the extent of deproteinization and in molecular weight of the DNA produced. The isolation procedure described here is relatively brief and relies on the powerful proteolytic activity of proteinase K combined with the denaturing ability of the ionic detergent SDS. Use of proteinase K for DNA purification was described by Gross-Bellard et al. (1972) and Enrietto et al., (1983). EDTA is included in the digestion buffer to inhibit DNases.

Critical Parameters: To minimize the activity of endogenous nucleases, it is essential to rapidly isolate, mince and freeze tissue. Tissue culture cells should be cooled and washed quickly. As soon as the tissue is frozen or the tissue culture cells are added to the lysis buffer, DNA is protected from the action of nucleases throughout this protocol. It is important that the tissue be well dispersed and not left in large lumps to permit rapid and efficient access to proteinase K and SDS.

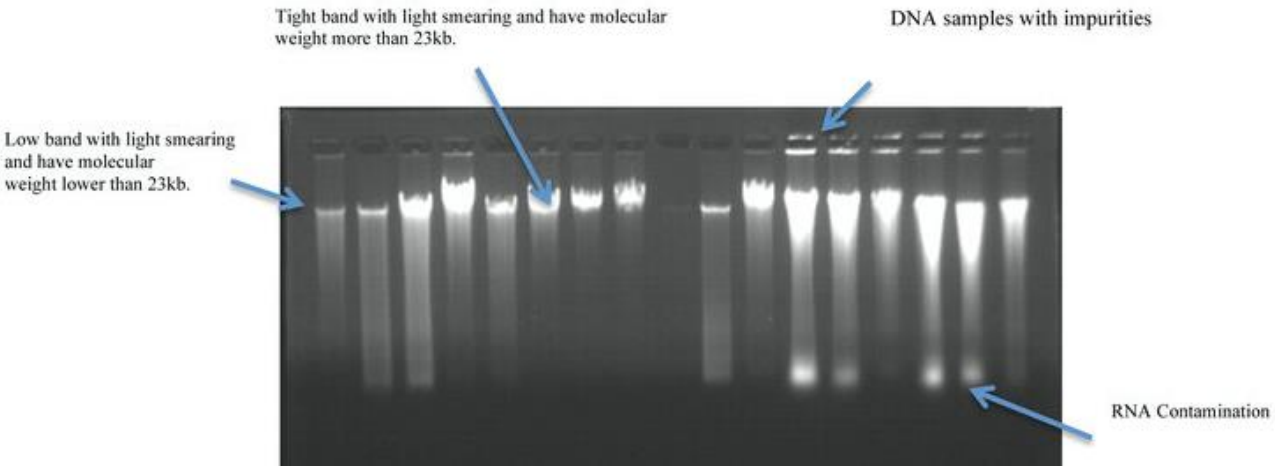
The absence of both cellular proteins and proteinase K in the final DNA solution is important for the susceptibility of the genomic DNA to the restriction enzyme action; therefor, care should be exercised in deproteination**.** To remove protein completely it may be necessary to repeat the proteinase K digestion or PC-8 extraction. In general, highly pure DNA has an A260/A280 ration >1.8, while 50% protein/50% DNA mixtures have ratios of 1.5.

1) Grow your favorite cells to 80-90% confluence in a 100mm-dish (for the next-gen sequencing analysis, one 100mm dish is more than sufficient);
2) Remove/aspirate culture medium completely, and wash the cells with 5ml PBS once;
3) Add 2ml **Extraction Buffer (GDEB)** (see below; containing RNase A 20µg/ml), and lyse the cells completely (Note: the lysate should be clear. Otherwise, you may start with too many cells, and more GDEB would be needed);
4) Collect the cell lysate, mix well, and divide into two **2.0ml Eppendorf tubes**: 1.0ml/tube (kept @-80°C immediately **as a backup**) and 1.0ml/tube;
5) To the **1.0ml cell lysate**, add **5µl** of **Proteinase K** (stocks = 20mg/ml);
6) Gently mix well, and incubate the lysed cell mix @ 50°C for **3h to overnight** with occasional gentle agitations (Note: the solution can be very viscous);
7) Cool the solution to room temperature;
8) Add **800µl** PC-8; mix the two phases slowly by turning the tube end over end for ~5 min, and spin at room temp for 10 min (top speed, benchtop microfuge);
9) Recover the (top) aqueous phase, and transfer it to a new 2-ml tube;
10) Repeat (600µl) PC-8 extraction as in **Step #8**; Recover the (top) aqueous phase (~900µl), and transfer it to **a new** 2-ml tube;
11) Precipitate the gDNA: ~900µl gDNA solution + 100µl 7.5M $(NH4)_2OAC$ + 1,000µl isopropanol; Precipitate gDNA at top speed for 5min (benchtop microfuge) (Note: you do not need to add glycogen although it won't hurt if you add);
12) Wash gDNA pellet with 800µl of 70% ethanol twice;
13) Aspirate liquid completely, and dissolve gDNA in **50µl ddH_2O** by mixing gently @ RT (Note: your gDNA concentrations should be in the range of **0.1~0.3µg/µl**).
14) Use 2~3µl to run 0.8% agarose gel (**see next page for quality check**) (**Note:** You will need to run through the **Mag-Bind Beads** to remove RNA completely for **DNA-Seq library preparation**).

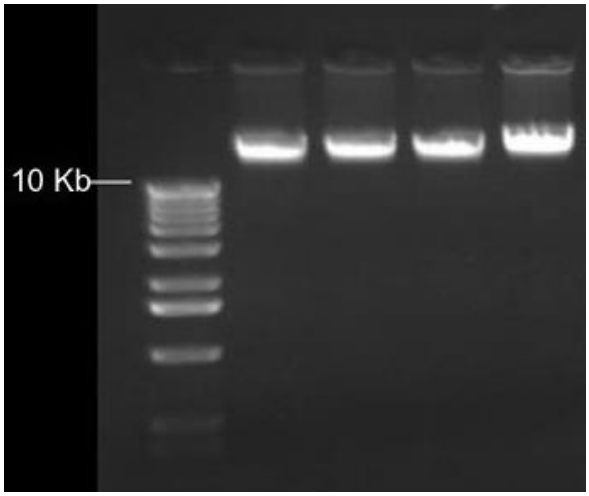
Genomic DNA Extraction Buffer (GDEB)

100mM	NaCl
10mM	Tris-HCl, pH 8.0
25mM	EDTA, pH 8.0
0.5%	SDS
20µg/ml	RNase A (Pancreatic)

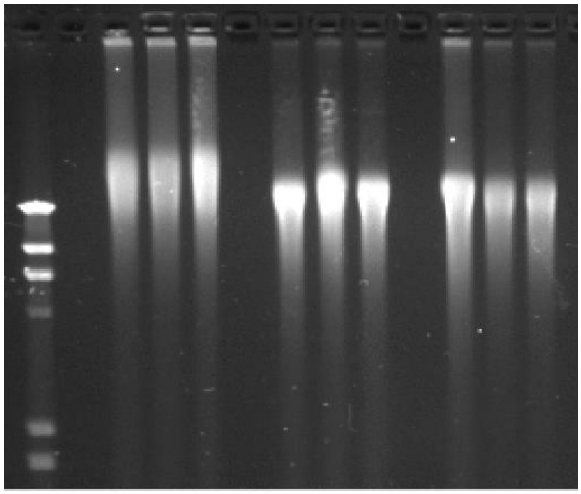
Typical Results of gDNA Preps



Best Quality gDNA Preps



Acceptable Quality gDNA Preps



Poor Quality gDNA Preps