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Phenol/Chloroform Genomic DNA extraction from Tissue Culture cells V.1 [↗](#)

In 1 collection

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1 *Works for me* [dx.doi.org/10.17504/protocols.io.7ejhcn](https://doi.org/10.17504/protocols.io.7ejhcn)



ABSTRACT

Old-School Phenol/Chloroform Genomic HMW DNA Preparation

In order to mitigate damage/shearing of genomic DNA we have avoided kits etc. that employ beads or a matrix that your DNA must associate with or sieve through (blend :o)). We have not gone the whole hog at this point and used nuclei preps, dialysis or plug extractions etc. as we have found that material produced from a simple and rapid phenol/chloroform prep is more than adequate and high yielding. We have done some limited salting out experiments as a substitute for the phenol/chloroform approach but have some remaining questions around size and stability in the fridge for extended periods that need resolving. We will be revisiting this. The jumping off point for us was using methods detailed in "Molecular Cloning: A laboratory Manual" by Sambrooke and Russell. If you are at a large institution there will probably be copies around on people's shelves or in the library collecting dust. It's time to dust those off, they have been patiently waiting for their day in the sun again :o)). Chapter 6 is a good place to start. This approach produces DNA that is more than large enough for any nanopore sequencing currently.

EXTERNAL LINK

<https://www.longreadclub.org/mountain-protocol/>

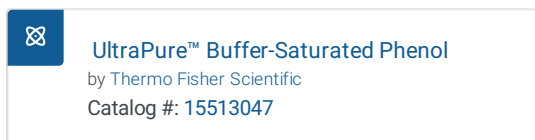
MATERIALS TEXT

Lysis Buffer

- 10 mM Tris-Cl (pH 8.0)
- 25 mM EDTA (pH 8.0)
- 100 mM NaCl
- 0.5 % (w/v) SDS
- 20 µg/ml RNase A (added fresh)

Additional Materials



- Proteinase K
- Buffer-Saturated Phenol



- Chloroform
- NaCl
- Ethanol
- Buffer EB (10mM Tris-Cl pH 8.0)


SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

- 1 Remove tissue culture media from two T25 flasks of cells ($\sim 1 \times 10^7$ cells / per flask, or 60 – 80 % confluency)
- 2 Lyse cells by adding  **5 ml** of **Lysis buffer** per flask and gentle rocking back and forth for \sim  **00:01:00** .
- 3 Pour the viscous sample into a 50 ml tube after gently scrapping the surface of the two T25's with a cell scraper.




Incubate for  **01:00:00** at  **37 °C** .

- 5 Add **proteinase K** (20mg/ml) to a final concentration of  **100 Mass Percent** .
- 6 Mix by gentle inversion of the tube several times.



Incubate the lysate at  **56 °C** for  **03:00:00** . (occasionally mix by inversion).

- 8 Allow to cool to  **Room temperature** before adding an equal volume of **buffer-saturated phenol**.

- 9 Gently mix by inversion for a few minutes until an emulsion is formed.



Separate the two phases by centrifugation at  **5000 x g**  **5000 x g** for  **00:30:00** –  **01:00:00** at  **Room temperature** .

- 11 Use a *wide-bore pipette* to transfer the upper viscous aqueous phase to a fresh tube being careful not to disturb any material at the interface.



Further Phenol extraction steps may be required if there is a large quantity of material at the interface i.e. when performing extractions from tissue samples.

- 12 Add an equal volume of **Chloroform** and mix by gentle inversion for a minute or so.



Separate the two phases by centrifugation at  **5000 x g** for  **00:30:00** at  **Room temperature** .

- 14 Use a *wide-bore pipette* to transfer the upper viscous aqueous phase to a fresh 50 ml tube being careful not to disturb the interface.

- 15 Add **NaCl** to increase concentration by  **0.3 Molarity (M)** from a  **5 Molarity (M)** stock.

- 16 Mix by gentle inversion.
- 17 Add 2 volumes of **ethanol**.
- 18 Mix by inversion a couple of times and then gentle swirling.



You should see a stringy mass of DNA condense together and this is hooked out using a glass capillary with a hook made at the end.

- 19

Wash the DNA condensate by dipping into a series of three 1 ml tubes containing **70 % ethanol** and holding there for ~ **00:00:20** .

- 20 Allow the DNA on the glass capillary to air-dry for ~ **00:01:00** (hold upside down and allow any excess liquid to rundown and dab away with a tissue avoiding the DNA).
- 21 Place the DNA on the end of the glass capillary into a tube containing **100 µl** – **200 µl** **Buffer EB** (**10 Molarity (m)** Tris-Cl pH 8.0).
- 22 Let sit at **Room temperature** for ~ **00:30:00** .
- 23 Gently dislodge the DNA from the glass capillary.



You should be left with a viscous solution with no “gel” mass on the glass capillary.

- 24 Place the DNA solution in the fridge and leave for a few days to fully hydrate before use. We generally see a yield of 200 – 400 µg of genomic DNA. High viscosity is a good indication of HMW DNA being present (using a pipette draw a small volume up and then pull out from the surface of the DNA solution. If you see a continuous strand connecting the tip and solution you are good).



It can take a while for HMW DNA solutions to “homogenise” when diluted due to viscosity and this often makes reliable quantification hard. We will often prepare 1/5 or other appropriate dilutions a day ahead of intended processing to help with this issue.



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