High Molecular Weight genomic DNA from coral sperm

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Abstract

This protocol is based on that described by Blin and Stafford (1976).

Blin, N., Stafford, D., 1976. A general method for isolation of high molecular weight DNA from eukaryotes. Nucleic Acids Research. 3**,** 2303.

It uses RNAse and ProteinaseK follwed by phenol/Chlorofrom extraction. For the highest molecular weight, the DNA is purified by dialysis, avoiding alcohol precipitation.

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Protocol

Step 1.
1. Grind frozen sperm to a fine powder in liquid nitrogen using a pre-cooled mortar and pestle.

Step 2.
2. Add 10mls of lysis buffer (10mM Tris pH 8; 100mM EDTA; 0.5%SDS) per gram of starting material to the frozen powder and mix. Due to the sub-zero temperature of the mortar, the mixture quickly freezes.

Step 3.
3. Allow the mixture to thaw - mix gently with the pestle while thawing to ensure even dispersion of the tissue through the lysis buffer.

Step 4.
4. Place the thawed mixture in a 50 ml Falcon tube and add RNaseA (DNase-free) to 20 micrograms/ml

Step 5.
5. Incubation at 37°C for 1 hour with periodic gentle mixing.

Step 6.
6. Add Proteinase K to a concentration of 100 micrograms/ml and incubate at 50°C for 3 hours with periodic gentle mixing.

Step 7.
7. Add an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) and gently rotate end over end for 10 minutes at room temperature.

Step 8.

8. Centrifuge at 5000g for 15 minutes at room temperature.

Step 9.
9. Transfer the aqueous phase to a fresh 50ml falcon tube. The aqueous phase can be very viscous and difficult to handle- sacrifice yield for purity. If needed MaxTract High density (Qiagen) can be used to aid phase separation – this may require addition of extra chloroform.

Step 10.
10. Repeat the phenol/chloroform/isoamyl alcohol extraction twice and follow by an extraction using phenol/chloroform.

Step 11.
11. Precipitate the DNA by adding 1/9th volume 3M sodium acetate pH5.2 and 0.6 volumes of isopropanol. Mix gently until the DNA comes out of solution.

Step 12.
12. If the yield is high enough, remove the DNA using a sealed glass pasteur pipette, or a Gilson tip and place in a large volume of 70% ethanol. Mix gently for 5 minutes at room temperature. Repeat the 70% ethanol wash at least two times, either by transferring the DNA to fresh tubes of 70% ethanol, or by carefully removing the 70% ethanol and replacing it with fresh 70% ethanol.

Step 13.
13. Collect the DNA using a sealed glass pasteur pipette/Gilson tip and allow to air-dry.

Step 14.
14. Place the DNA in an appropriate volume of 1XTE (if EDTA is a problem use 0.1XTE), allowing it to detach from the pasteur pipette/Gilson tip. The yield should be >1mg per gram of starting material. Place at 4 degC. The DNA may take a long time (hours/days to dissolve properly).

Step 15.
15. If the yield is not high enough to remove the DNA from the isopropanol precipitation with a pasteur pipette/Gilson tip, use standard centrifugation to pellet the DNA. Wash at least two times with 70% ethanol.

Step 16.
16. Alternatively, for very high molecular weight DNA, dialyse the aqueous phase from the final phenol/chloroform extraction extensively against 50mM Tris pH 8; 10mM EDTA until the OD 260/280 is >1.8 (several changes of buffer over at least two days). If EDTA is a problem, change the dialysis solution to 0.1XTE. The dialysis option should only be considered if the expected yield is high.

ANNOTATIONS

Benjamin Schwessinger 31 Mar 2018

Hi David, How do you dialyse DNA?