TELL-Seq™ Demonstrated Protocol:

A Salting Out Method for High-Molecular-Weight DNA Extraction from Cultured Cells

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1. Introduction

This Demonstrated Protocol describes how to extract high-molecular-weight (HMW) genomic DNA (gDNA) from suspension cells (lymphocytes). It is expected to be effective similarly for the extraction of HMW gRNA from other cell types and culturing formats, including adherent cell culture.

The TELL-Seq Whole Genome Sequencing (WGS) Library Preparation kit uses Transposase Enzyme-Linked Long-read Sequencing (TELL-Seq™) technology to capture long-range information in large DNA segments. Using HMW gDNA as input material enables larger phase blocks and better structural variant (SV) calls after TELL-Seq sample processing.

This Demonstrated Protocol can produce HMW gDNA with an average size of over 100 kb typically which is an ideal DNA size for TELL-Seq library preparation. There are many variables that will affect extracted gDNA quality, including sample type, sample age, transportation methods, additives, storage method, etc. A quality check on the extracted DNA prior to TELL-Seq library preparation is strongly recommended.

2. Best Practices

Recommended best practices for maximizing cell quality and minimizing cell damage prior to gDNA extraction:

- Using sterile techniques
- Using nuclease-free reagents and consumables
- Minimizing pipetting steps
- Using wide-orifice pipette tips

Common best practices are recommended for minimizing gDNA sharing:

- Never using vortex to mix HMW gDNA and always mixing by pipetting with wide-orifice pipette tips; if wide-orifice tips are not available, use a sharp and clean razor blade to cut 4-5 mm off a standard tip top instead
- Pipetting slowly (either when mixing or transferring HMW DNA).
- For mixing, 4 second down strokes and 4 second up strokes using wide-orifice tips; slowly aspirate 80% of the HMW gDNA-containing solution and then dispense at the surface of the remaining solution 10 times
- Using standard pipette tips for transferring HMW DNA if accurate quantity is required

3. Consumables

Consumables	Supplier
0.5M EDTA pH 8.0	General lab supplier
1M Tris-HCl, pH 8.0	General lab supplier
10% SDS	General lab supplier
Ethanol 200 proof (absolute) for molecular biology	Sigma-Aldrich, # E7023
Low TE Buffer (10mM Tris-HCl pH8.0, 0.1mM EDTA)	General lab supplier
Proteinase K, 20 mg/ml	Qiagen, # 19133
Sodium Chloride Solution, 5M	General lab supplier
DNA LoBind Tubes, 2.0 ml	Eppendorf, # 022431048
Polypropylene centrifuge tubes, 15 ml	Thermo Fisher # 05-539-12

4. Step-by-step Protocol

4.1. Buffer Preparation

1. Prepare 3 ml of Lysis Buffer for each sample extraction:
   10 mM Tris-HCl, 400 mM NaCl, and 2 mM EDTA, pH 8.0
2. Prepare 0.5 ml of Proteinase K Solution for each sample extraction:
   1 mg/ml Proteinase K, 1% SDS, and 2 mM EDTA, pH 8.0

4.2. Cell Lysis

3. Collect 1.5 x 10^6 cells per extraction into a 15 ml centrifuge tube and centrifuge at 315 x g for 7 min (Beckman Coulter Allegra® X-14R)
4. Remove media without disturbing the cell pellet
5. Add 3 ml Lysis Buffer and resuspend cell pellet by gentle inversion (20 times)
6. Add 0.2 ml 10% SDS
7. Add 0.5 ml Proteinase K Solution and mix by gentle inversion (5 times)
8. Incubate overnight (12 – 18 hours) in a 37°C water bath

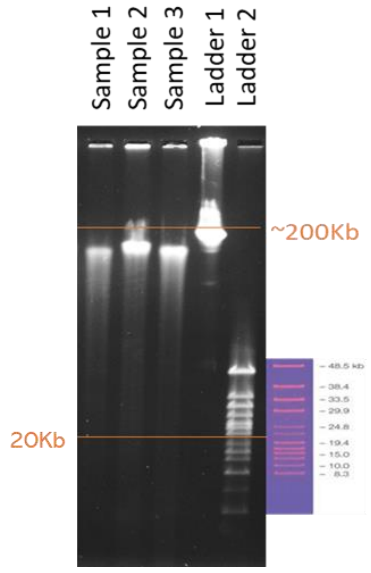
4.3. DNA Extraction

9. Add 1.2 ml 5M NaCl and mix by gentle inversion (5 times)
10. Centrifuge, 1,010 x g for 15 min at 4°C (Beckman Coulter Allegra® X-14R)

11. Using a wide-orifice pipette tip or a serological pipette, slowly transfer the supernatant containing the DNA to a new 15 ml tube and add 8 ml of 100% ethanol
12. Gently mix by inversion and a DNA precipitate should be clearly visibly
13. If possible, aliquot the DNA solution into multiple 2 ml Eppendorf DNA LoBind tubes (up to 6) to mitigate the risk of fully losing the sample in a later step. Mark the expected position of the pellet on the tube as it may be difficult to see in Step 15 and 16
14. Centrifuge, 6,250 x g for 5 min at 4°C (Eppendorf® Centrifuge 5425R)
15. Carefully remove the supernatant
16. Allow the pellets to air dry for 5 min (do not exceed for too long)
17. Using a wide-orifice pipette tip, add 150 µl Low TE Buffer to one tube and resuspend the DNA pellet with gentle pipette mixing, if necessary. Sequentially transfer the solution each to the remaining tubes to recombine all the DNA pellets into a single solution
18. Allow the combined solution to slowly homogenize at room temperature for at least 1 hour
19. Store the extracted HMW gDNA sample at 4°C for up to 2 weeks or at −20°C for up to 6 months

5. Results

The image shows pulsed-field gel electrophoresis analysis of three HMW DNA extractions (Sample 1-3) from a lymphocyte suspension culture and two ladder lanes.



6. Acknowledgement

This protocol was modified from Miller, S.A., Dykes, D.D., and Polesky, H.F. “A Simple Salting Out Procedure for Extracting DNA from Human Nucleated Cells” published in *Nucleic Acids Research*, *16*, 1215 (1988). PMID: 3344216

Revision History

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100034-USG	1.0	DCR-220001 Initial Release