Phase Lock Gel™ Light and Heavy facilitate most applications that require extraction with organic solvents

Phase Lock Gel Light can be used to improve the recovery of DNA fragments from Low Melting Point (LMP) Agarose with only minor changes to the standard protocol.

Phase Lock Gel Heavy may be used for the preparation of total RNA by homogenization in Guanidinium Isothiocyanate followed by organic extraction and for isolating genomic DNA from mouse tail.

Recovery of DNA from LMP agarose[1]

1. Resolve DNA fragments on a Low Melting Point (LMP) agarose gel in 1x Tris- Acetate- EDTA (TAE) buffer. Tris-Borate-EDTA (TBE) is not recommended as TBE gels are much more difficult to solubilize.
2. Stain gel with Ethidium Bromide, visualize with a longwave UV light, and carefully cut out the band(s) of interest with a sharp razor blade.
   → **Caution:** Wear gloves when handling Ethidium Bromide stained gels.
   → **Caution:** Wear safety glasses or a face shield when using a UV light.
3. Transfer slice to a pre-spun (12,000 x g for 20 to 30 seconds), pre-weighed PLG 2 ml Light tube and determine the weight of the slice.
4. Add a volume (in µl) of TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0) equivalent to 5x the weight (in mg) of the slice and melt the LMP agarose in the TE in a thermomixer set at 65°C for 5 to 10 minutes. If a thermomixer is not available a heat block can be used with intermittent mixing.
5. Mix well to ensure LMP agarose slice is fully dissolved, allow the dissolved sample to come to room temperature, and then add an equal volume of room temperature Tris- buffer saturated Phenol (pH 8.0) to the sample and mix until homogeneous.
   → **Do not vortex.**
6. Centrifuge at full speed (12,000 x g or greater in a microcentrifuge) for 2 minutes to separate the phases.
   → If the resulting aqueous phase still appears cloudy, the extraction should be repeated with room temperature Tris-buffer saturated Phenol (pH 8.0).
7. Recover aqueous phase to a fresh PLG 2 ml Light tube and extract with an equivalent volume of room temperature Phenol:Chloroform:Isoamyl Alcohol (PCI, 25:24:1).
   → **Do not vortex.**
8. Centrifuge as in step 6 above. Recover aqueous phase to a fresh PLG 2 ml Light tube, and extract with an equivalent volume of room temperature Chloroform:Isoamyl Alcohol (CI, 24:1).
9. Centrifuge as in step 6 above and recover the aqueous phase to a suitably sized microcentrifuge tube.
10. Add 0.25x volume of 10 M Ammonium Acetate and 2.5x volume of 100% Ethanol to the sample and mix well.
11. Incubate at room temperature for 20 minutes, pellet by centrifugation, wash pellet two to three times with cold 70% Ethanol, air-dry pellet, and resuspend in a suitable buffer.

Mouse tail genomic DNA isolation protocol[2]

→ **Note:** Genomic DNA is fragile. High molecular weight DNA is sheared easily by mechanical forces. Use suitable large-bore pipette tips or equipment when pipetting genomic DNA. Do not vortex solutions containing genomic DNA.

1. Place a 1 cm tail sample into a 1.5 ml microcentrifuge tube; this may be stored at -20°C. To minimize possible cross-contamination, do not mince the sample. Add 700 µl Lysis Buffer (50 mM Tris-HCl, pH 8.0, 100 mM EDTA, 100 mM NaCl, 1% SDS) to the sample.
2. Add 35 µl 10 mg/ml Proteinase K to the sample and mix briefly.
3. Incubate at 55 to 60°C overnight with mixing. This step should result in the complete solubilization of the tail fragment. In the case of incomplete digestion, more Proteinase K can be added and the samples incubated for several more hours.
4. Add 20 µl 10 mg/ml RNase A (DNase-free) to the sample. Mix briefly and incubate at 37°C for 1 to 2 hours.
5. Transfer entire solution to a pre-spun (1,500 x g for 1 to 2 minutes) PLG 2 ml Heavy tube.
6. Add 0.5 ml Phenol:Chloroform:Isoamyl Alcohol (PCI, 25:24:1) to the sample in the PLG 2 ml tube and mix well by repeated inversion.
   → **Do not vortex.**
7. Centrifuge at full speed (12,000 x g or greater) for 5 minutes in a microcentrifuge, then carefully transfer the resultant aqueous phase to a fresh pre-spun PLG 2 ml Heavy tube.
8. Add 0.5 ml Chloroform:Isoamyl Alcohol (CI, 24:1) to the sample in the PLG 2 ml tube and mix well by repeated inversion.
   → **Do not vortex.**
9. Centrifuge at full speed (12,000 x g or greater) for 5 minutes in a microcentrifuge, then carefully transfer resultant aqueous phase to a fresh microcentrifuge tube.
10. Fill sample-containing tube with 100% Isopropanol and mix thoroughly by repeated inversion. Do not vortex. A visible DNA precipitate should form.

    Proceed immediately to step 11.
11. Recover DNA precipitate by touching it to a heat-sealed glass micropipette tip or by lifting the DNA with a yellow pipette tip and partial suction from a pipettor. Transfer the DNA to a 1.5 ml microcentrifuge tube containing 70% Ethanol. If DNA is not stringy, pellet by a brief, low speed centrifugation.
12. Wash DNA with the 70% Ethanol, then wash twice with 95% Ethanol.
13. Allow DNA to partially dry and then either transfer the DNA to a microcentrifuge tube containing 400 µl TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0) or add 400 µl TE to the DNA in the tube. Do not vortex or re-pipet to resuspend DNA.
14. Re-solubilize the DNA overnight (e.g. by rotation at 30-60 rpm). Resolubilization may be facilitated by heating the sample at 50°C.

Total RNA isolation protocol[3,4,5]

1. Sample preparation:
   → To extract RNA from washed and pelleted cultured cells, add 200 µl 4 M Guanidinium Isothiocyanate Solution (4 M Guanidinium Isothiocyanate, 25 mM Sodium Citrate, pH 7.0, 0.1 M β-Mercaptoethanol) to 0.5 x 10^4 cells - 1 x 10^6 cells.

   → To directly extract RNA from cultured cells growing in monolayer, add 200 µl 4 M Guanidinium Isothiocyanate Solution directly to each well of a 6, 12 or 24 well plate. Add 100 µl of the 4 M Guanidinium Isothiocyanate Solution directly to each well of a 48 or 96 well plate.
2. Homogenize monolayer cells by pipetting the mixture up and down several times, taking care to "wash" cell material free from the culture dish, tube or well in the process. Homogenize washed, pelleted cells by pipetting the mixture up and down until the pellet is fully suspended. Use a small bore pipette tip to collect the cell homogenate.
3. Transfer all of the homogenate to a pre-spun (12,000 - 16,000 x g for 1 to 2 minutes) PLG 2 ml Heavy tube.
4. Add 20 µl (10 µl per sample for 48 or 96 well plates) 2.0 M Sodium Acetate, pH 4.0 to the sample, cap the PLG tube and mix briefly.
   → **Do not vortex.**
5. Add 200 µl (100 µl per sample for 48 or 96 well plates) water-saturated Phenol to the sample, cap the PLG tube, and mix thoroughly by repeated inversion.
   → **Do not vortex.**
6. Add 60 µl (30 µl per sample for 48 or 96 well plates) Chloroform:Isoamyl Alcohol (CI, 49:1) to the sample in the same PLG tube and mix thoroughly by repeated gentle inversion.
   → **Do not vortex.**
7. Incubate on ice for 10 minutes.
8. Centrifuge at 12,000 - 16,000 x g for 5 minutes in a microcentrifuge to separate the phases.
9. Add 200 µl (100 µl per sample from 48 or 96 well plates) Phenol:Chloroform:Isoamyl Alcohol (PCI, 50:49:1) to the aqueous phase in the same PLG tube. Mix thoroughly by repeated gentle inversion.
   → **Do not vortex.**
10. Centrifuge at 12,000 - 16,000 x g for 5 minutes to separate the phases.
11. Collect resultant aqueous phase to an RNase-free microcentrifuge tube, add an equal volume of 100% Isopropanol, and mix by repeated inversion.
12. Centrifuge at 12,000 - 16,000 x g for 20 minutes.
13. Discard resultant supernatant and wash pellet several times with 200 µl 70% Ethanol, centrifuging 2 to 3 minutes at 12,000 - 16,000 x g to re-pellet the sample.
    → **Note:** Samples may be stored in the 70% Ethanol wash at this stage at -70°C or colder for extended periods.
14. Discard final wash and dry pellet at room temperature.
15. Re-dissolve pellet in a suitable volume (5 to 10 µl) of RNase-free water. Store the RNA solution at -70°C.
    → **Note:** Absorbance determinations should be performed in RNase-free TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0).15

References

1. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) “Molecular Cloning, A Laboratory Manual”, 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. pp.6.30-6.31.

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3. Chirgwin, J.M. et al. (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochem. 18:5294-5299.

4. Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanatephenol-chloroform extraction. Anal. Biochem. 162:156-159.

5. Birnboim, H.C. (1988) Rapid extraction of high molecular weight RNA from cultured cells and granulocytes for Northern analysis. Nucl. Acids Res. 16:1487-1497.

MK-TN-0003 REV 01 PLG Applications 0918