# ISOLATING DNA FROM ADHERENT TISSUE CULTURE CELLS

WITH DAVID DANKORT

**Reagents Required**

1xPBS

Lysis Buffer: 100mM Tris-HCl [pH 8.5]
5mM EDTA
0.2% SDS
200mM NaCl
add proteinase K to 100ug/ml

Sodium Acetate (3M, pH 5.2)

Phenol/Chloroform/Isoamyl alcohol (25:24:1)

2.0ml **PLG Light** Eppendorf tubes

100% EtOH (-20°C)

70% EtOH

TE pH 8

1. Trypsinize cells from plate, inactivate with serum, and pellet cells by centrifugation (1 000 rpm, 5-10 min). Aspirate media, resuspend cells in 5 ml 1xPBS, pellet cells by centrifugation.

2. Aspirate PBS, resuspend cells in 500ul Lysis Buffer, and transfer to a 1.5ml Eppendorf tube. Incubate at 55°C for 4-16 hours.

3. Prepare PGL Light Eppendorf tubes by brief centrifugation (30sec @ 14 000rpm).

4. Add 400-500ul Phenol-chloroform-isoamyl and vortex 30-60 seconds. Transfer to a 2.0ml PGL tube and mix by inversion (see manufacturer's protocol). Centrifuge at 14 000 rpm for 5 minutes.

5. Remove aqueous (upper) phase and transfer to a new tube. Add 400ul chloroform, mix and centrifuge at 14 000 rpm.

6. Transfer aqueous (upper) phase to a new tube. Add 1/10$^{th}$ volume of 3M Sodium Acetate and 3 volumes cold 100% Ethanol (-20°C), mix by inversion, and centrifuge at 14 000 rpm for 10 minutes.

7. Wash the DNA pellet twice with 70%EtOH and dry for 10 minutes at room temperature. Resuspend DNA pellet in 100-200ul TE and incubate at 55°C for 3-4 hours. Store DNA at 4°C.