Preparation of High Molecular Weight DNA from Cells

NOTES: *When pipetting genomic DNA samples, only use pipette tips from which the tips have been snipped off. This will prevent shearing the DNA and keep the average size in excess of 80-100 kb. Never vortex!*

1. Aspirate the media off the plate of cells (30mm, 60mm, or 100 mm). Trypsinize the cells and transfer to a 15 ml tube. Pellet the cells by spinning @ low speed (7000 rpm) for 5 minutes. Carefully aspirate off the media, leaving behind the cell pellet.

2. Resuspend and wash the cells in 5 ml of PBS. Spin @ 7000 rpm for 5 minutes. Carefully aspirate off the PBS.

3. Add 1 ml of Cell Lysis Buffer and 20 µl of freshly prepared 40 mg/ml Proteinase K to each tube.

4. Rock the tubes gently overnight @ 55°-60°C. Alternatively, wash the 30mm or 60mm plate of cells twice with PBS and add 1 ml of Cell Lysis Buffer directly to the plate. Leave 15-20 minutes to lyse and gently heave the lysate into a 2.2 ml Eppendorf tube, add the Proteinase K and rock the tubes overnight @ 55°-60°C.

5. Add 500µl of phenol/CHISAM mix and rotate @ 5-10 rpm for 15 minutes, or rock back and forth by hand (DO NOT VORTEX). Spin the tubes in the microcentrifuge @ maximum speed for 10 minutes. Transfer the aqueous phase (top layer) to clean Eppendorf tubes using pipette tips from which the tips have been removed.

6. Add an equal volume of isopropanol to the aqueous phase. Invert the tube a few times to precipitate the DNA.

7. Spool the DNA onto a flame-sealed micropipette, or recover DNA with a genomic pipet tip, or spin down the DNA 1 min on high in microfuge.

8. Rinse once in 80% EtOH and let the genomic DNA air dry.

9. Add 100-300µl of TE and dissolve overnight @ room temp.

Cell Lysis Buffer:		for 500 ml:	
100 mM	NaCl	10 ml	5.0 M NaCl
50 mM	Tris pH 7.5	25 ml	1.0 M Tris pH 7.5
10 mM	EDTA pH 8.0	10 ml	0.5 M EDTA pH 8.0
0.5%	SDS	25 ml	10% SDS
		430 ml	sterile water