Protocol to Extract DNA From Paraffin Blocks

Day 1

1. Cut 10-20X (30um) sections of formalin fixed paraffin samples into eppendorf tubes.
2. Add 1ml Xylene and incubate at RT for 15min.
3. Spin down for 5 min at 13000 rpm and discard supernatant.
4. Add 1ml of 100% ethanol and incubate at RT for 15 min.
5. Spin down for 5 min at 13000 rpm and discard supernatant.
6. Add 500ul of proteinase K buffer (50mM Tris pH 8, 1mM EDTA, 0.5% Tween 20).
7. Incubate overnight at 55°C shaker.

Day 2-5

1. Add 20ul proteinase K (stock solution 20mg/ml in water, store at -20°C) Final Conc.=0.4mg/ml
2. Incubate overnight at 55°C shaker.
   (Solution will become clear. You may increase proteinase K conc. up to 1 mg/ml)

Day 6

1. Add 500ul Phenol Chloroform into tube and wait for 5 min at RT.
2. Spin down at 5 min with 13000 rpm.
3. Get off the upper layer. (we need the upper layer)
4. Mix it with 500ul of PCI in eppendorf tubes.
   (Phenol-chloroform-isoamylalcohol extraction 1:1 v/v)
5. Shake gently and incubate for 10 min at RT.
6. Spin down at 5 min with 13000 rpm.
7. Collect the supernatant into new eppendorf tubes.
8. Add 300ul of 7.5M ammonium acetate, 1ml cold 100% ethanol and 5ul glycogen(stock
   (stock = 20ug/ml)
9. Shake gently and incubate at -20°C for 2 hours or overnight.
10. Spin down for 30min at 13000 rpm and discard the supernatant.
11. Air dry pellet and dissolve in 25ul of water or TE buffer.

Proteinase K buffer

Tris pH=8		0.5ml
0.5M EDTA		40ul
10% Tween		1ml
dd H2O		28.5ml
Total		20ml

Proteinase K dissolve solution

Tris HCL pH=7.5	0.1ml
CaCl	0.2ml
Glycerol	10ml
Water	10ml