<Extraction genomic DNA from cultured cells>

1. Preparation

i) Cell culture in 6 cm dish

ii) Tail buffer (100mM NaCl, 50mM Tris-HCl (pH8.0), 100mM EDTA, 1%SDS)

iii) Proteinase K (20 mg/ml)

iv) 5M NaCl

v) Isopropanol & 70% Ethanol

2. Procedure (standard)

Cell culture in 6 cm dish

↓ Aspirate medium

↓ Add 0.5 ml of Tail buffer

↓ to 1.5 ml microtube

↓ Add 12.5 μl of Proteinase K (20 mg/ml)

↓ Overnight, 60℃

↓ Add 167 μl of 5M NaCl & vortex

↓ 15000 rpm, 5 min, 4℃

Sup (to new tube ~550μl)

↓ Add 550 μl of Isopropanol

↓ RT 10 min with rotating

↓ 15000 rpm, 10 min, 4℃

ppt (DNA)

↓ Add 1 ml of 70% Ethanol & vortex

↓ 15000 rpm, 5 min, 4℃

ppt (DNA)

↓ Air dry

↓ Dissolve in 100~150 μl of TE buffer (with shaking ~ O/N)

PCR using 1 μl as template