ISOLATION OF TOTAL DNA FROM MAMMALIAN CELLS
(DNA-fragmentation assay)

Materials:

0.5 M EDTA 8.0
1 M Tris 8.0
4 M NaCl
10% Triton X-100
2 mg/ml Proteinase K (PK)
isopropanol
RNase A (10mg/ml stock)
Lysis buffer: 20 mM EDTA
10 mM Tris 8.0
200 mM NaCl
0.2% Triton X-100
100 μg/ml PK

Procedure:

1. Resuspend cells in 0.5 ml lysis buffer.
2. Incubate 1.5h in 37°C incubator.
3. Centrifuge 14,000rpm/RT/5 min.
4. Transfer supernatant into new tube.
5. Add equal volume of isopropanol and 25μl 4M NaCl (100 mM final concentration).
6. Incubate tubes overnight at -20°C.
7. Centrifuge 14,000rpm/RT/20-25 min.
8. Dissolve DNA pellet in 30-50μl ddH_20, add 1-2 μl RNase A.
9. Incubate 1h/37°C.
10. Measure concentration of DNA and run 0.7μg DNA/lane on 1% agarose gel.

Submitted by: Ola Germaniuk