PURIFYING GENOMIC DNA FROM CULTURED CELLS AND HUMAN OR ANIMAL TISSUE

1 **Sample preparation**

1.1 **Human or animal tissue**

Cut up 25mg tissue and transfer to 1.5ml microcentrifuge tube (proceed to step 2).

1.2 **Cultured cells**

Resuspend up to 10^7 cells in 200µl Lysis Buffer GL.
Add 25µl Proteinase K solution and 200µl Lysis Buffer G3.
Incubate at 70°C for 10-15 min (proceed to step 4).

2 **Pre-lysis**

Add 180µl Lysis Buffer GL and 25µl Proteinase K solution.
Completely cover sample with solution and vortex.
Incubate at 56°C for 1–3 hours (until completely lysed), shake or vortex occasionally.

3 **Lyse sample**

Vortex sample briefly and add 200µl Lysis Buffer G3.
Vortex vigorously and incubate at 70°C for 10 min.

4 **Adjust DNA binding conditions**

Vortex briefly and add 210µl ethanol (96-100%) to sample.
Vortex vigorously.

5 **Bind DNA**

Place ISOLATE II Genomic DNA Spin Column (green) in a 2ml Collection Tube.
Load sample to column and centrifuge 1 min at 11,000 x g.
Discard flow-through and reuse Collection Tube.

6 **Wash silica membrane**

- Add 500µl Wash Buffer GW1.
  Centrifuge 1 min at 11,000 x g.
  Discard flow-through and reuse Collection Tube.
- Add 600µl Wash Buffer GW2.
  Centrifuge 1 min at 11,000 x g.
  Discard flow-through and reuse Collection Tube.

7 **Dry silica membrane**

Centrifuge 1 min at 11,000 x g, to remove residual ethanol.
Place ISOLATE II Genomic DNA Spin Column in a 1.5ml microcentrifuge tube (not supplied).

8 **Elute DNA**

Add 100µl preheated Elution Buffer G (70°C) onto center of silica membrane.
Incubate at room temperature for 1 min.
Centrifuge 1 min at 11,000 x g.