PURIFYING GENOMIC DNA FROM CULTURED CELLS AND HUMAN OR ANIMAL TISSUE

1 SAMPLE PREPARATION

1.1 HUMAN OR ANIMAL TISSUE

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

1.2 CULTURED CELLS

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

2 PRE-LYSIS

Add 180 µL Lysis Buffer GL and 25 µL 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 200 µL Lysis Buffer G3.
Vortex vigorously and incubate at 70˚C for 10 min.

4 ADJUST DNA BINDING CONDITIONS

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

5 BIND DNA

Place ISOLATE II Genomic DNA Spin Column (green) in a 2 mL 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 500 µL Wash Buffer GW1.
  Centrifuge 1 min at 11,000 x g.
  Discard flow-through and reuse Collection Tube.
- Add 600 µL 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 1.5 mL microcentrifuge tube (not supplied).

8 ELUTE DNA

Add 100 µL 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.