9. Gen	nomic DNA Extraction	from Cultured Cell	



Genomic DNA Extraction from Cultured HepG2 Cell of Human

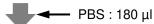
Protocol

 \leq 1 x 10⁶ cells in 1.5 ml micro tube

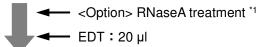


Remove the medium and wash with PBS

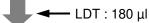
Remove the PBS completely



Tap the tube 5 times gently to suspend pelleted cells



Tap the tube 5 times gently to mix the solution



Mix thoroughly by vortexing for 15 sec *2

Flash spin down



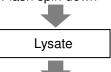
Incubate at 70°C, 10 min

Flash spin down



Mix thoroughly by vortexing for 15 sec *2

Flash spin down



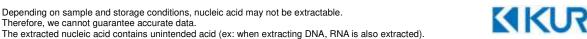
Transfer all contents of the micro tube into the cartridge of QuickGene



Refer to the extraction protocol for each device written in the kit handbook. (from the step after transferring the lysate into the cartridge)



- *1 RNaseA : 20 µl Tap the tube 5 times gently to mix the solution Flash spin down Set it down at room temperature for 2 min
- *2 Mix completely by vortexing at the maximum speed. If the mixing is not enough by vortexing, use the tapping, pipetting or inverting.







Results

The yield of genomic DNA / Protein contamination: A260/280

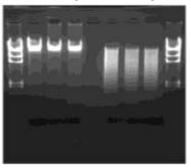
Number of HepG2 cells	Yield (μg)	A260/280
5 × 10 ⁵ cells	5.2	1.7

Other

Restriction Enzyme Digestion

AGE of *Hin* d III restriction enzyme digestion fragments of genomic DNA extracted from several cell lines using QuickGene isolation system and reagents

without digestion Hin d III digestion M 1 2 3 1 2 3 M



1 µg DNA / lane

Isolated genomic DNA with QuickGene-810 (automatic nucleicacid isolation system) and QuickGene DNA tissue kit S, had been digested with *Hin* d III successfully.

M: λ-Hin d III digest

1 : Genomic DNA from HepG2 cell line (0.5 x 10^6 cells)

2 : Genomic DNA from Huh6 cell line (0.5 x 106 cells)

3 : Genomic DNA derived from Huh6 cell line (0.5 x 106 cells)

Common protocol is usable for the following

Rat Cultured PC-12 Cell. Mouse Cultured ES Cells





Genomic DNA Extraction from Cultured HepG2 Cell of Human

Protocol

≤1 x 10⁶ cells in 1.5 ml micro tube

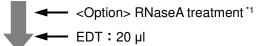


Remove the medium and wash with PBS

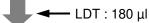
Remove the PBS completely



Tap the tube 5 times gently to suspend pelleted cells



Tap the tube 5 times gently to mix the solution



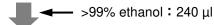
Mix thoroughly by vortexing for 15 sec *2

Flash spin down



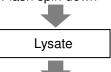
Incubate at 70°C, 10 min

Flash spin down



Mix thoroughly by vortexing for 15 sec *2

Flash spin down



Transfer all contents of the micro tube into the cartridge of QuickGene



Refer to the extraction protocol for each device written in the kit handbook. (from the step after transferring the lysate into the cartridge)



- *1 RNaseA: 20 µl
 Tap the tube 5 times
 gently to mix the solution
 Flash spin down
 Set it down at room
 temperature for 2 min
- *2 Mix completely by vortexing at the maximum speed.

 If the mixing is not enough by vortexing, use the tapping, pipetting or inverting.





Results

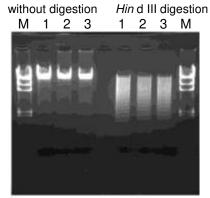
The yield of genomic DNA / Protein contamination: A260/280

Number of Huh6 cells	Yield (μg)	A260/280
Huh6	7.6	1.8
Derived from Huh6	6.6	1.7

Other

Restriction Enzyme Digestion

AGE of *Hin* d III restriction enzyme digestion fragments of genomic DNA extracted from several cell lines using QuickGene isolation system and reagents



1 μg DNA / lane

Isolated genomic DNA with QuickGene-810 (automatic nucleicacid isolation system) and QuickGene DNA tissue kit S, had been digested with Hind III successfully.

M: λ-Hin d III digest

1 : Genomic DNA from HepG2 cell line (0.5 x 10⁶cells)

2: Genomic DNA from Huh6 cell line (0.5 x 106 cells)

3: Genomic DNA derived from Huh6 cell line (0.5 x 106 cells)

Common protocol is usable for the following

Rat Cultured PC-12 Cell, Mouse Cultured ES Cells





Genomic DNA Extraction from Cultured ES Cell of Mouse

Protocol

 \leq 1 x 10⁶ cells in 1.5 ml micro tube



Remove the medium and wash with PBS

Remove the PBS completely

PBS : 180 μl

Tap the tube 5 times gently to suspend pelleted cells

■ EDT : 20 µl

Tap the tube 5 times gently to mix the solution

← LDT : 180 μl

Mix thoroughly by vortexing for 15 sec & Flash spin down



Incubation at 70°C: 10 min

Flash spin down

■ >99% ethanol : 240 μl

Mix thoroughly by vortexing for 15 sec & Flash spin down



Transfer all contents of the micro tube into the cartridge of QuickGene



Refer to the extraction protocol for each device written in the kit handbook. (from the step after transferring the lysate into the cartridge)



Results

The yield of genomic DNA

Number of ES cells	Yield (μg)	
1 × 10 ⁵ cells	about 1.0	

Common protocol is usable for the following

Human Cultured Cell Line, Rat Cultured PC-12 Cell

Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data. The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).





Genomic DNA Extraction from Cultured PC-2 Cell of Rat

Protocol

 \leq 1 x 10⁶ cells in 1.5 ml micro tube



Remove the medium and wash with PBS

Remove the PBS completely

← PBS : 180 μl

Tap the tube 5 times gently to suspend pelleted cells

← EDT : 20 µl

Tap the tube 5 times gently to mix the solution

← LDT : 180 μl

Mix thoroughly by vortexing for 15 sec & Flash spin down



Incubation at 70°C: 10 min

Flash spin down

Mix thoroughly by vortexing for 15 sec & Flash spin down



Lysate



Transfer all contents of the micro tube into the cartridge of QuickGene



Refer to the extraction protocol for each device written in the kit handbook. (from the step after transferring the lysate into the cartridge)



Genomic DNA (Elution volume : 200 µl)

Results

The yield of genomic DNA / Protein contamination: A260/280

Number of PC-12 cells	Yield (μg)	A260/280
1 × 10 ⁶ cells	about 15.0	1.45

Common protocol is usable for the following

Human Cultured Cell Line, Mouse Cultured ES Cells

Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.

