1. Genomic DNA Extraction from Blood of Animal

QuickGene

QuickGene DNA whole blood kit S (DB-S)
QuickGene SP kit DNA whole blood (SP-DB)

DA-a-1

Genomic DNA Extraction from Bone Marrow Fluid

Protocol

1.5 ml micro tube

← EDB*1 : 30 µl

← Diluted bone marrow fluid*2 : 200 µl

(bone marrow fluid 100 µl + PBS 100 µl)

← LDB : 250 µl

Pipetting 5 times (or mixing by inversion 5 times)

Vortex (maximum speed) : 15 sec

Flash spin down

↓

Incubation at 56°C : 2 min

↓ ← >99% ethanol : 250 µl

Vortex (maximum speed) : 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)

*1 Leave EDB for 30 min at room temperature after adding nuclease-free water and mixing, and use it after perfect dissolution.

*2 Dilute bone marrow fluid double with PBS in advance. Add 100 µl of PBS to 100 µl of bone marrow fluid, and add after mixing well.

Results

No Data

Common protocol is usable for the following

No Data

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).

KURABO

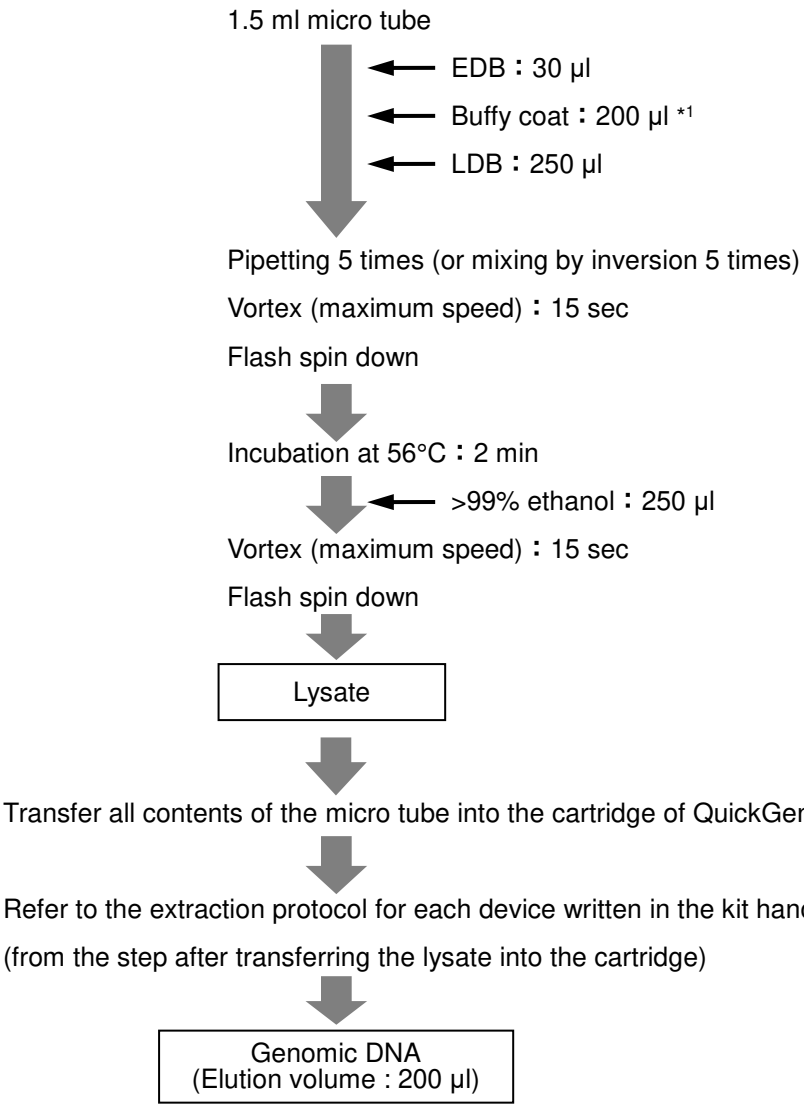
QuickGene

QuickGene DNA whole blood kit S (DB-S)
QuickGene SP kit DNA whole blood (SP-DB)

DA-a-2

Genomic DNA Extraction from Buffy Coat

Protocol



*1 Cell number of 3×106 were suspended by PBS 200 µl.

Results

No Data

Common protocol is usable for the following

No Data

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The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

QuickGene

QuickGene DNA whole blood kit S (DB-S)
QuickGene SP kit DNA whole blood (SP-DB)

DA-a-3

Genomic DNA Extraction from Whole Blood of Aves

Protocol

1.5 ml micro tube

← EDB*1 : 30 µl

← Blood diluted with PBS : 200 µl

(Aves blood 2 µl + PBS 198 µl)

← LDB : 250 µl

Pipetting 5 times (or mixing by inversion 5 times)

Vortex (maximum speed) : 15 sec

Flash spin down

↓

Incubation at 56°C : 2 min

↓ ← >99% ethanol : 250 µl

Vortex (maximum speed) : 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)]

*1 Leave EDB for 30 min at room temperature after adding nuclease-free water and mixing, and use it after perfect dissolution.

Results

No Data

Common protocol is usable for the following

No Data

Depending on sample and storage conditions, nucleic acid may not be extractable.
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KURABO

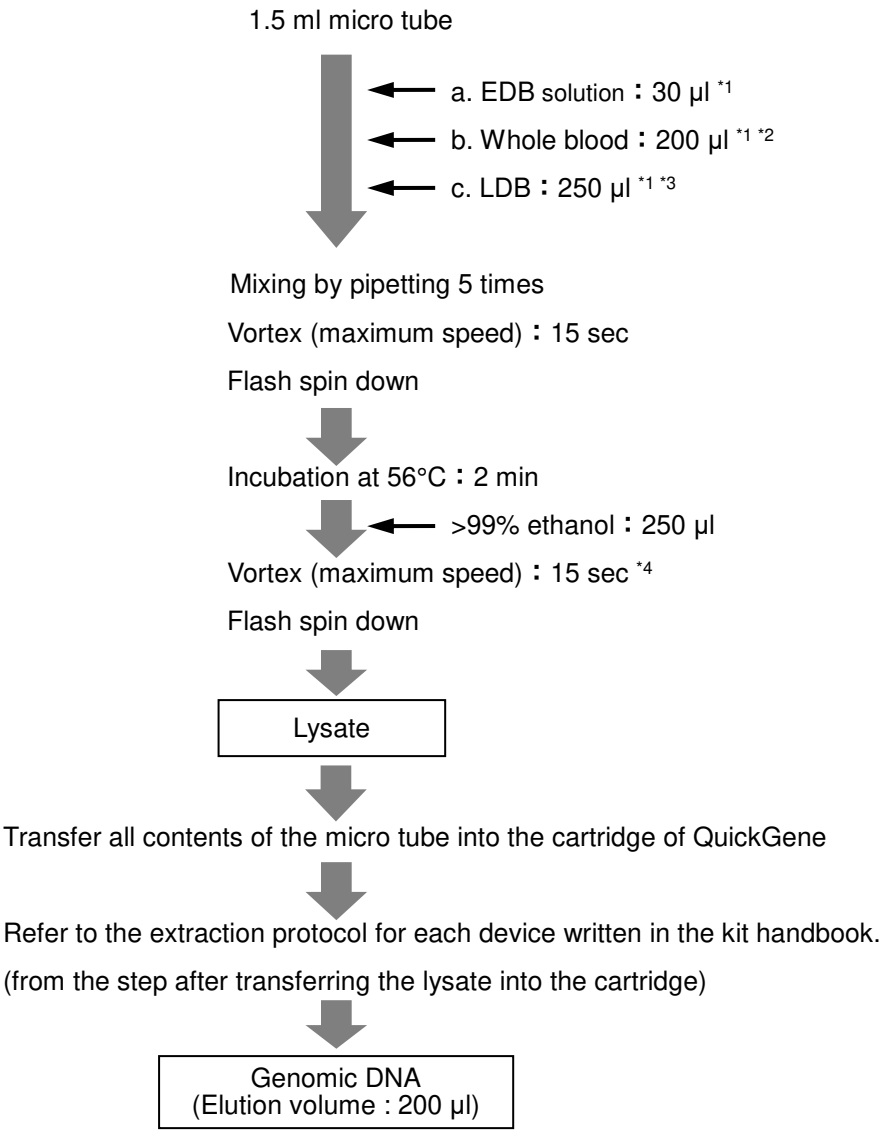
QuickGene

QuickGene DNA whole blood kit S (DB-S)
QuickGene SP kit DNA whole blood (SP-DB)

DA-a-4

Genomic DNA Extraction from Whole Blood of Human

Protocol



*1 Must follow the steps a, b and c.

*2 Recommended to use the whole blood collected in EDTA-2Na or EDTA-2K

*3 Proceed the step C immediately after adding whole blood

*4 Mix completely by vortexing at the maximum speed. If the mixing is not enough by vortexing, use the tapping, pipetting or inverting.

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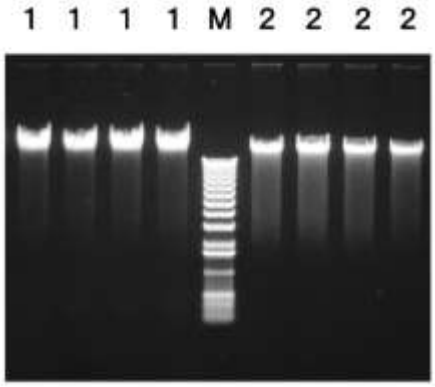
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Results

Electropherogram



M : 1k bp ladder

1 : QuickGene

2 : A company (spin method)

The yield of genomic DNA (Sample: 200µl of human whole blood)

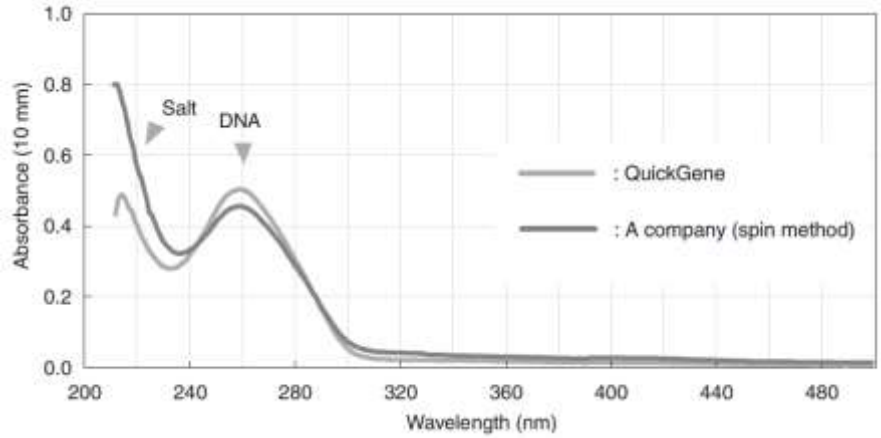
(µg)	Average	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
QuickGene	5.9	7.2	5.3	5.9	5.5	5.5
A company (spin method)	4.5	6.3	4.4	5.2	3.2	3.6

Protein contamination：A260/280

	Average	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
QuickGene	1.94	1.91	1.94	1.96	1.91	1.96
A company (spin method)	1.84	1.86	1.82	1.80	1.87	1.86

Chaotropic salt contamination：A260/230

	Average	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
QuickGene	1.61	1.76	1.69	1.43	1.76	1.42
A company (spin method)	1.12	1.21	0.89	1.07	1.24	1.21



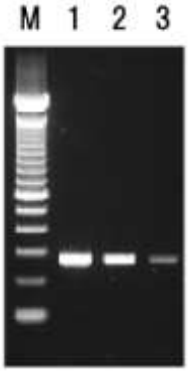
Hemoglobin contamination : A400

	Average	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
QuickGene	0.036	0.023	0.032	0.070	0.031	0.025
A company (spin method)	0.054	0.076	0.040	0.085	0.026	0.043

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).

Other

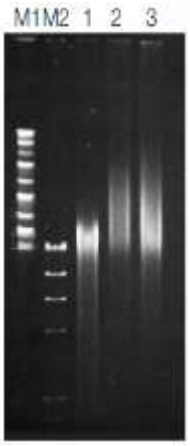
・ PCR



Serial dilution of isolated genomic DNA was used for PCR template to amplify p53 exon6 gene.
PCR amplification was performed successfully by using 0.1ng/µl genomic DNA.

M : 100 bp ladder
1 : Genomic DNA 10ng/µl
2 : Genomic DNA 1ng/µl
3 : Genomic DNA 0.1ng/µl

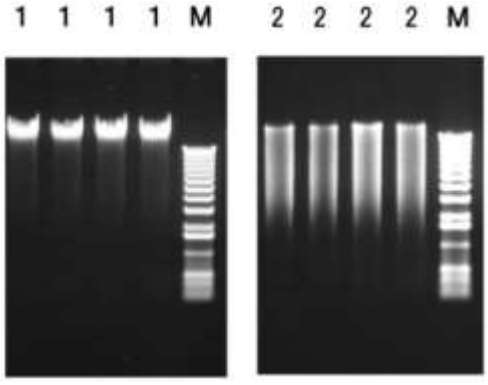
・ Pulsed-field electrophoresis



The use of QuickGene-810 (automatic nucleic-acid isolation system) and QuickGene DNA whole blood kit S enables the isolation of long genomic DNA same as manual method using phenol / chloroform.

M1 : MidRange PFG Marker II
M2 : *Hin*d III digest
1 : Comparison method using spin column (<~70kb)
2 : Using QuickGene isolation system and reagents (<~140kb)
3 : Manual method using phenol / chloroform (<~140kb)

・ Restriction Enzyme Digestion



The eluted genomic DNA sample had been digested with *Eco*R I.
The success of enzyme digestion is shown by the comparison of lane1 and 2.

M : 1k bp ladder
1 : Before digestion
2 : After digestion using *Eco*R I

- ## Next Generation Sequencing (Exsome sequence analysis)

Genome DNA extracted from whole blood by QuickGene was evaluated for Next Generation Sequencing Analysis, and confirmed it is suitable for NGS.

1	Non-redundant reads (de-duplicated by Picard tools)	Number of non-redundant reads	**132,541,212**
2	Non-redundant unique reads (uniquely mapped to human genome)	Number of unique reads mapped in human genome	**116,879,297**
3 (2÷1)	% Non-redundant unique reads (out of non-redundant reads)	Ratio of non-redundant unique reads to non-redundant reads (% for non-redundant reads)	**88.2%**
4	Target regions (bp)	Number of bases in target regions	**62,085,286**
5	Number of target genotypes (more than 10X)	Bases covered more than 10x coverage	**56,460,863**
6 (5÷4)	% Coverage of target region (more than 10X)	Percent bases covered more than 10x coverage	**90.9%**
7	Mean depth of target regions (X)	Average coverage of target regions	**115.8**

The quality of DNA library from QuickGene sample was sufficient for NGS with minimal sequence bios and was reliable enough with high depth sequencing across target region.

Common protocol is usable for the following

Canine Whole Blood

QuickGene

QuickGene DNA whole blood kit S (DB-S)
QuickGene SP kit DNA whole blood (SP-DB)

DA-a-5

Genomic DNA Extraction from Whole Blood of Canine

Protocol

1.5 ml micro tube

↓ ← a. EDB solution : 30 µl *1
← b. Whole blood : 200 µl *1
← c. LDB : 250 µl *1

Mixing by pipetting 5 times

Vortex (maximum speed) : 15 sec

Flash spin down

↓

Incubation at 56°C : 2 min

↓ ← >99% ethanol : 250 µl

Vortex (maximum speed) : 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)]

*1 a to c exactly.
Do not add LDB directly after addition of EDB.

Results

The yield of genomic DNA / Protein contamination : A260/280 / Chaotropic salt contamination : A260/230

amount of whole blood	Yield(µg)	A260/280	A260/230
200 µl	2.52	1.68	0.61

Common protocol is usable for the following

Human Whole Blood

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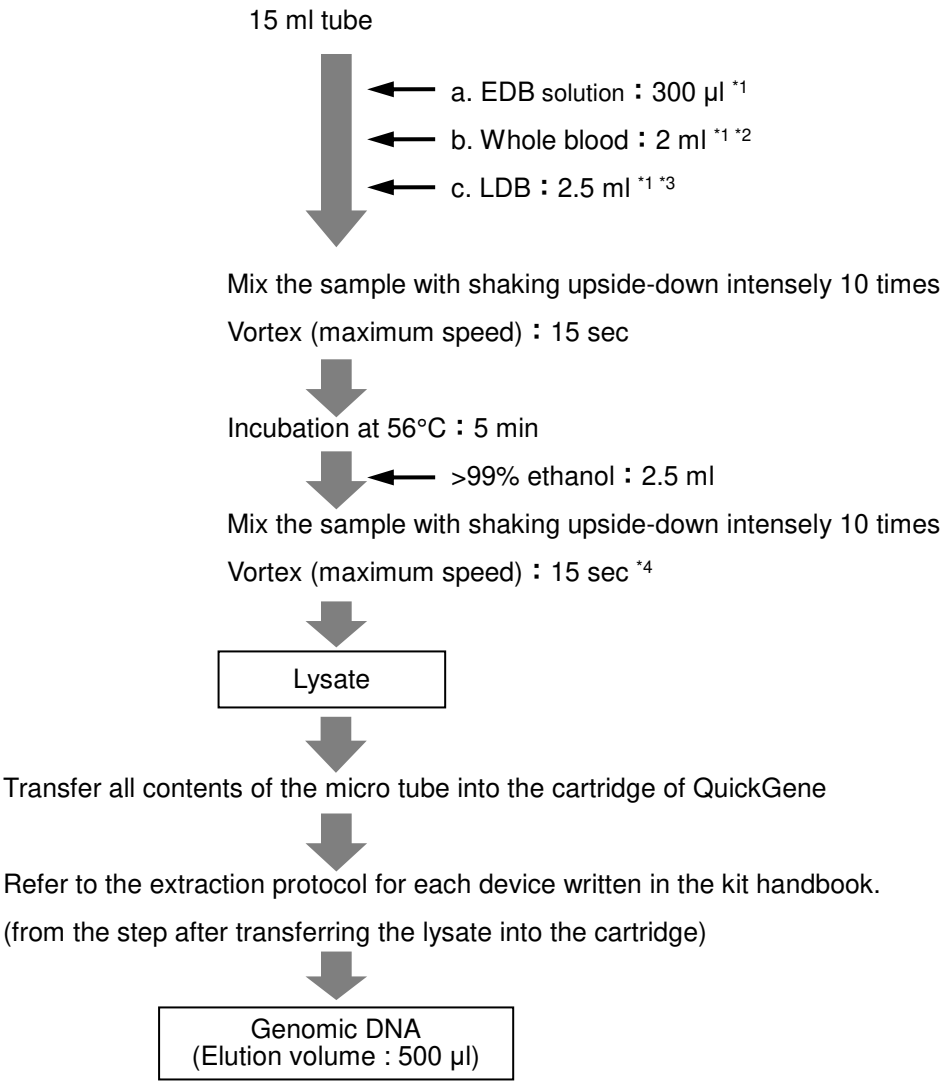
KURABO

DA-a-6

Large-scale Genomic DNA Extraction from Whole Blood of Human

Protocol



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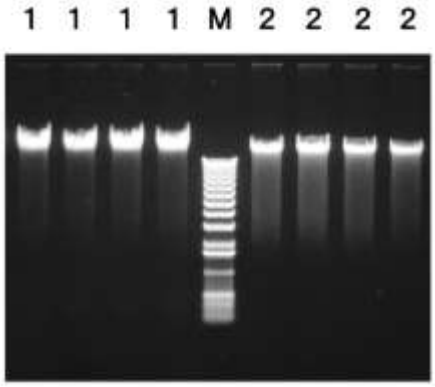

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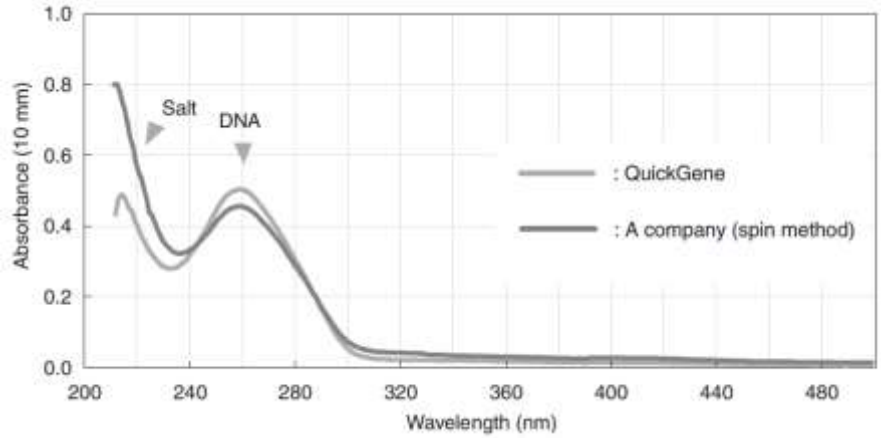

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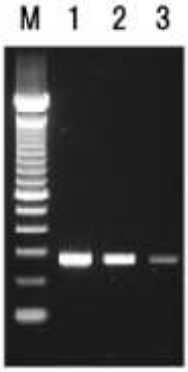

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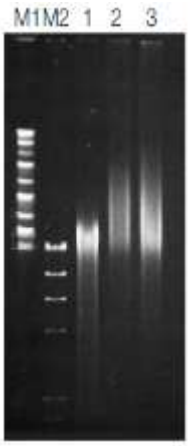

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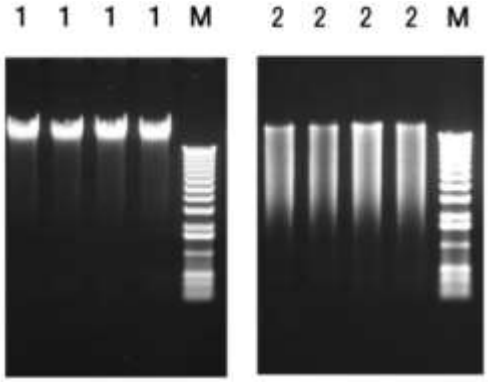

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