High-quality and Highly Scalable Cell DNA Extraction using GenEx™ Cell, the Solution-type Genomic DNA Extraction Kit

Experimental Conditions

Materials Required

- GenEx™ Cell Sx (221-101)
- 200 U of lyticase or 20 U of zymolase (for yeast cell lysis)
- 30 mg/ml of lysozyme or 300 µl/ml of lysostaphin (for gram-positive cell lysis)
- Ice (for incubation or maintaining the normal state of the enzyme solution and Proteinase K solution)
- Microcentrifuge tube
- Microcentrifuge (≤15,000 x g)
- Vortex mixer
- Heating block
- 70% ethanol
- Isopropanol (≥99.5%, C_3H_8O, CAS No. 67-63-0)
- Pipette & sterile pipette tips
- Suitable protector (e.g., lab coat, disposable gloves, goggles, etc.)

Sample Information

- 5 x 10^6 of K562 (human erythroleukemia cell line)
- 2 x 10^9 of DH5α (gram-negative bacteria)
- 2 x 10^9 of *Lactobacillus* (gram-positive bacteria)
- 5 x 10^7 of yeast

Protocol

Enzymatic pre-treatment of lysozyme or lysostaphin is required for gram-positive and yeast cell DNA extraction.
Gram-positive bacteria : manual handbook of Exgene™ Cell SV mini (Step 1~2 of Protocol L, page 38) was observed.
Yeast : manual handbook of Exgene™ Cell SV mini (Step 1~4 of Protocol M, page 40) was observed.
For more details, **please refer to handbook of Exgene™ Cell SV mini and GenEx™ Cell Sx.**

Sample Preparation and protocol

K562
Manual handbook of Protocol E (page 24~25) was observed.
DH5α
Manual handbook of Protocol G (page 28~29) was observed.
Lactobacillus
Following enzymatic pre-treatment, manual handbook was observed starting with Step 3 of Protocol G (page 28).
Total yeast
Following enzymatic pre-treatment, manual handbook was observed starting with Step 2 of Protocol G (page 28).

Result

Kit	GenEx™ Cell Sx			
	Yield (ng/µl)	$A_{260/280}$	$A_{260/230}$	CV (%)
K562	15.62	1.98	2.15	1.10
	15.51	2.01	2.21	
	15.29	1.98	2.18	
DH5α	14.92	1.98	2.19	0.85
	15.11	1.99	2.20	
	14.86	2.01	1.99	
Lactobacillus	12.26	1.98	2.19	3.78
	12.68	2.02	2.18	
	13.21	1.98	2.20	
Yeast	6.11	2.01	2.11	2.77
	6.35	1.98	2.19	
	6.02	2.03	2.20	

Table 1. Result of yield, purity and CV (coefficient of variation) of DNA extracted from 4 different samples using GenEx™ Cell Sx.
The DNA were extracted from four different samples using GenEx™ Cell. All eluates were analyzed with a absorbance using NanoDrop™ 2000. The absorbance was performed in triplicated. The yield and CV values were calculated based on the measured absorbance values.

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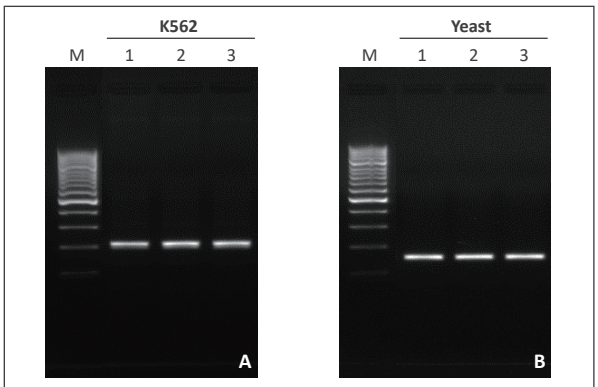
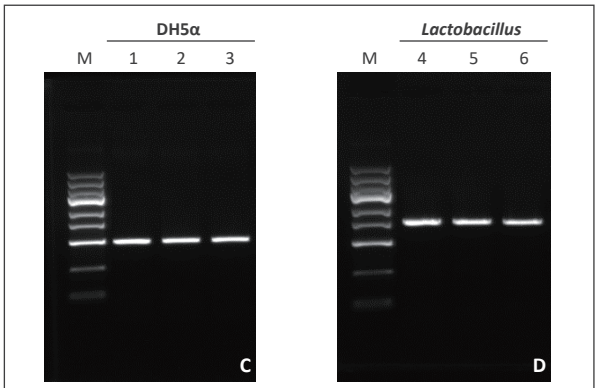


2.0% agarose gel, 5 µl loading, 150 V, 30 min
DNA ladder size : 100 bp
PCR product size : K562 - 205 bp / Yeast - 150 bp



1.2% agarose gel, 5 µl loading, 150 V, 30 min
DNA ladder size : 250 bp
PCR product size : DH5α - 500 bp / *Lactobacillus* - 763 bp

Figure 1. PCR amplification of DNA extracted from four different samples using GenEx™ Cell Sx.
PCR reaction was performed in triplicate with extracted DNA from four different samples using GenEx™ Cell Sx. Eluted PCR products were analyzed with gel-electrophoresis using ethidium bromide staining.

- **PCR primer**
  A : K562 cells : human GAPDH primer
  B : Total yeast : Scer primer
  C : DH5α : bacteria universal primer
  D : *Lactobacillus* : uvrC primer

- **DNA ladder**
  Lane M : DNA ladder
  A, B : GENESTA™ 100 bp DNA ladder (GA-010)
  C, D : GENESTA™ 250 bp DNA ladder (GA-025)

- **PCR instrument and kit**
  MultiGene™ Optimax Thermal Cycler (TC9610, Supplier : L)
  2X Taq PCR Master Mix (TAQ-OV-500R, Supplier : M)