NIPPON Genetics EUROPE

Application

Purification of plasmids using FastGene® Plasmid Mini Kit

Product: FastGene® Plasmid Mini Kit（FG-90402）

Manufacturer: NIPPON Genetics EUROPE

The following data was kindly provided by Ken Honda, Department of Pharmacology, Yamaguchi University, Graduate School of Medicine, Japan

We tried transfection (luciferase activity) with a plasmid extracted with FastGene® Plasmid Mini Kit.

Experimental conditions

- Sample

  E. coli strain: TOP10

  Vector: pGL4 Vector 4.3kb (Insert size 0.5～2kb） * Insert: Promoter region

  Input amount: LB medium 2.5 mL

- Procedure

  1. Plasmid DNA-transformed TOP10 was cultured in LB medium
  2. *E.coli* was recovered from the culture (2.5 mL) and plasmid DNA was purified with FastGene® Plasmid Mini Kit (elution buffer 50 µL)
  3. DNA yield was measured with NanoDrop（Result ①）
  4. Plasmids were digested by restriction enzymes. Presumed fragment pattern was confirmed by electrophoresis（Result ②）
  5. After transfection, luciferase activity was measured（Result ③）

     Detection device: Perkin Elmer 2030 ARVO X series multi label reader

Result

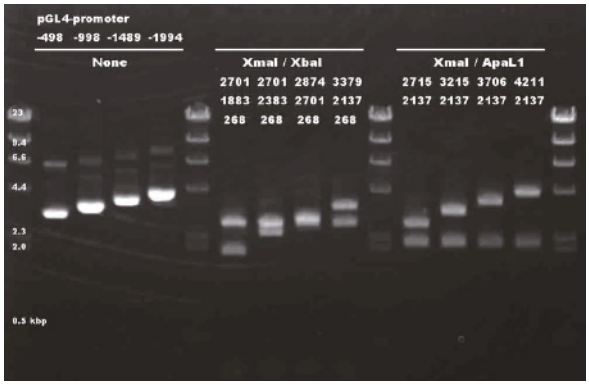
① DNA yield and purity

#	Elution buffer volume（µL）	yield（µg）	Nucleic AcidConc.Unit	A260	A280	A260/A280	A260/A230
pGL4-prom-498	50.0	16.3072	332.8 ng/µL	6.656	3.511	1.90	2.20
pGL4-prom-998	50.0	14.2541	290.9 ng/µL	5.818	3.088	1.88	2.17
pGL4-prom-1489	50.0	16.2092	330.8 ng/µL	6.617	3.655	1.81	1.97
pGL4-prom-1994	50.0	23.4465	478.5 ng/µL	9.571	5.063	1.89	2.24

② Electrophoresis

The purified plasmid was used for restriction enzyme check

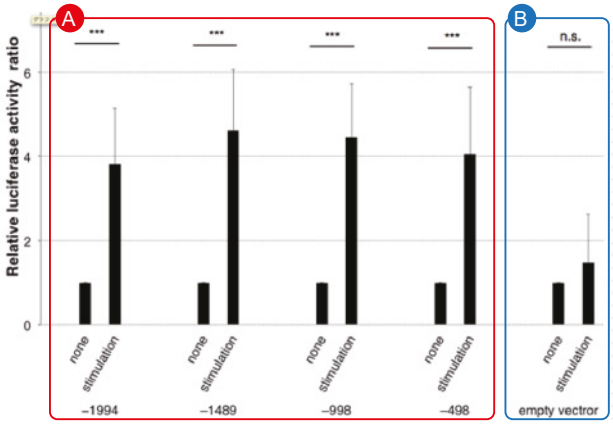
Conditions: 1 lane 10 µL (250 ng) used, TAE buffer,
0.7% TAE agarose, 100V・30 min



The numerical value is the assumed base lenth (bp)
* Bands below 500 bp are not detected in gel image.

③ Measurement of luciferase assay

A plasmid with the confirmed fragment was transfected into mouse myoblast C2C12 cells and a reporter gene assay was performed:
0.2 µg of DNA was transfected into 10^5 cells by Lipofectamine 2000.
Stimulation of cells after 24 h, the cell lysate was recovered and the luciferase assay was measured.



A A promoter-inserted vector

B Vector only (Without insert)

After stimulation, luciferase assay activity was meassured in all cells.

Customer comment

I think that purification degree and recovery rate is reproducible. The cost efficiency is also great.
Personally, I think it will be easier to use, if you can pull out the column from the collection tube a bit more smoothly, but handling is not complicated overall, and it was possible to handle multile samples in a short time without stress.

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