

Extraction of large DNA fragments with the FastGene® Gel/ PCR Extraction Kit

Cat. No. FG-91202, FG-91302

Introduction

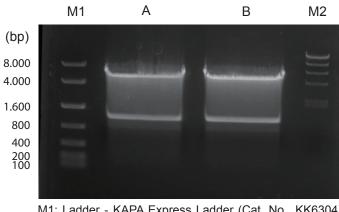
It is a well-known problem that the recovery of DNA fragments larger than 1 kb proves to be difficult and leads to the loss of large amounts of DNA. The FastGene® Gel/PCR Extraction Kit was used for the isolation of two DNA bands resulted from a restriction digest.

Methods

A 6.9 kb large plasmid was digested with a restriction enzyme. The restriction digest was analysed by agarose gel electrophoresis at 100 V for 20 min. The 0.7 % agarose gel was produced using 1 x TAE buffer. The target fragments were excised out of the gel and transferred in a 1.5 ml tube. The fragments were purified with the FastGene[®] Gel/PCR Extraction Kit. For the DNA elution 20 μ l of GP3 elution buffer was used. 100 ng of each purified DNA fragment were electrophoresed again at 100 V for 20 min.

Results

The plasmid contains two restriction sides for the used restriction enzyme, so that the digest results in two fragments with the size 5.4 kb and 1.5 kb.



M1: Ladder - KAPA Express Ladder (Cat. No.. KK6304) A: Sample

B: Sample

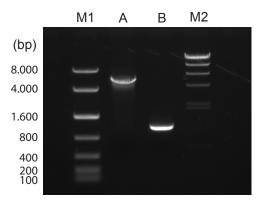
M2: Ladder - Lambda/HindIII (commercially available)

Electrophoretic conditions: 0.7 % agarose gel, 100 V, 20 min 1 x TAE

The gel electrophoresis of 100 ng of both obtained DNA fragments shows that independent of the DNA fragment size, a good recovery could be achieved.

Conclusions

- 1. Excellent purification independent of DNA size
- 2. Downstream application can be performed without difficulties



M1: KAPA Express Ladder (Cat. No.. KK6304)

A: With the FastGene[®] Gel/PCR Extraction Kit purified 5.4 kb DNA fragment

B: With the FastGene[®] Gel/PCR Extraction Kit purified 1.5 kb DNA fragment

M2: Lamda/HindIII (commercially available)

Electrophoretic conditions: 0.7 % agarose gel, 100 V, 20 min 1 x TAE

The table shows that 30% of the 5.4 kb and 60% of the 1.5 kb large fragment could be recovered by the FastGene[®] Gel/PCR Extraction Kit. A recovery rate of 30% for large plasmids is very high.

| DNA | DNA conc. | Vol. | DNA yield | Recovery rate |
|----------|-----------|------|-----------|---------------|
| Fragment | (ng/µl) | (μl) | (ng) | (%) |
| 5.4 kb | 50.0 | 20 | 1000 | 30 |
| 1.5 kb | 33.0 | 20 | 660 | 60 |

Concluding, the customer highlighted the fast preparation, easy handling, high recovery rate for large fragments and the unproblematic performance of downstream applications.

Genetics NIPPON Genetics EUROPE

www.nippongenetics.eu
info@nippongenetics.eu