NIPPON Genetics EUROPE

Customers product feedback

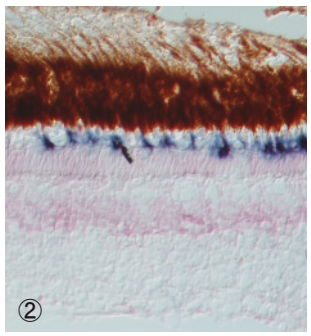
Product name: **FastGene® Gel/PCR Extraction Kit** (FG-91302)
FastGene® Plasmid Mini Kit (FG-90502)
FastGene® Gel Cutter (FG-830)

Manufacture name: **NIPPON Genetics EUROPE GmbH**

Application: **Sample Preparation for in situ Hybridization of targeted tissue specific genes in medeka fish**

Data kindly provided by Keita Sato, Okayama University - Graduate School of Medicine Dentistry and Pharmaceutical Sciences, Japan.

Background



This application was carried out in order to visualize cell groups in which various genes are activated involved in photoreception in the nerve tissue (retina / brain) of medaka, using the expression of messenger RNA as an indicator.

Figure ①: Medaka fish
Figure ②: Retina tissue of the medeka fish after in situ hybridization

Method

The in situ hybridization of tissue-specific genes of the medaka fish starts with the extraction of RNA from medaka tissue followed by the amplification of the target gene. After that a plasmid is required to synthesize the RNA which allows then the in situ hybridization of the gene of interest.

cDNA synthesis

1. RNA extraction (e.g. medaka brain, eyeball)
2. Reverse transcription of the extracted RNA and cDNA synthesis

PCR

3. Amplification of the target gene using specific primers.
   PCR amplification with TaKaRa PrimeSTAR®
   max. amplification size (500 - 2500 bp)

Electrophoresis

4. Electrophoresis of the PCR product
5. Gel cutting（Figure 1）
6. 20 µL elution buffer required
   Recovered DNA concentration: about 5 µg/mL (not quantified here)

FastGene® Gel Cutter

Advantages
- Easy band extraction from agarose gels
- Very fast gel cutting
- Reduce damage of the DNA
- Saving time, because fragments from the same gels have basically the same weight
- Reduce damage of the filter on the illuminator surface

FastGene® Gel/PCR Extraction Kit

Characteristic
- Both centrifugal and section can be used
- It can be used for both purification of PCR products and extraction of DNA from agarose
- 5 “gel band cutters” for cutting out gel sections are included

Transformation

7. Ligation of the PCR product to the pBluescript II KS plasmid
8. Transformation into E. coli DH5α and Transfer to X-gal, ampicillin-coated LB plate
9. Pick a white colony to make a replica plate
10. Confirm the lenght of the insert by colony PCR
    Primer: T7, T3
    Polymerase: TaKaRa Emerald amp max or Genedire X OnePC

Sequencing

11. The PCR product was diluted to about 1/50 and sequenced by T7 primer (BigDye Termiantor v1.1)
    * PCR product was not purified
12. After precipitation of the reaction product by ethanol, it was dissolved in HiDi formadine. Sequence was analysed (Fig.2) (Using ABI 3130)
13. Liquid culture (shaking overnight at 37°C) was performed from clones for which the sequence of the inserts was confirmed. Clones were used from the replica plate (s.step 9)

Plasmid preparation for RNA synthesis

14. Using Plasmid Mini Kit for plasmid purification
    * Type of medium: 2x YT medium (ampicilin added)
    * Amount used per prep: 1 mL
    * Strains used: DH5α
    * Plasmid size: 2961 bp
    * Insert size: 500 - 2500 bp
    * Amount of elution buffer used: 50 µL
    * Plasmid DNA concentration: Approx. 250 µg/mL
15. Using plasmid diluted 1/100 as a template
    PCR amplification with TaKaRa Prime STAR® MAX
    * Primer: T7, T3 primer
    Confirm single band by electrophoresing the PCR product

RNA synthesis

16. PCR product was purified with Gel/PCR Extraction Kit
    RNA was sythesied from this template by T7, T3 pol respectively

in situ hybridization

17. in situ hybridization

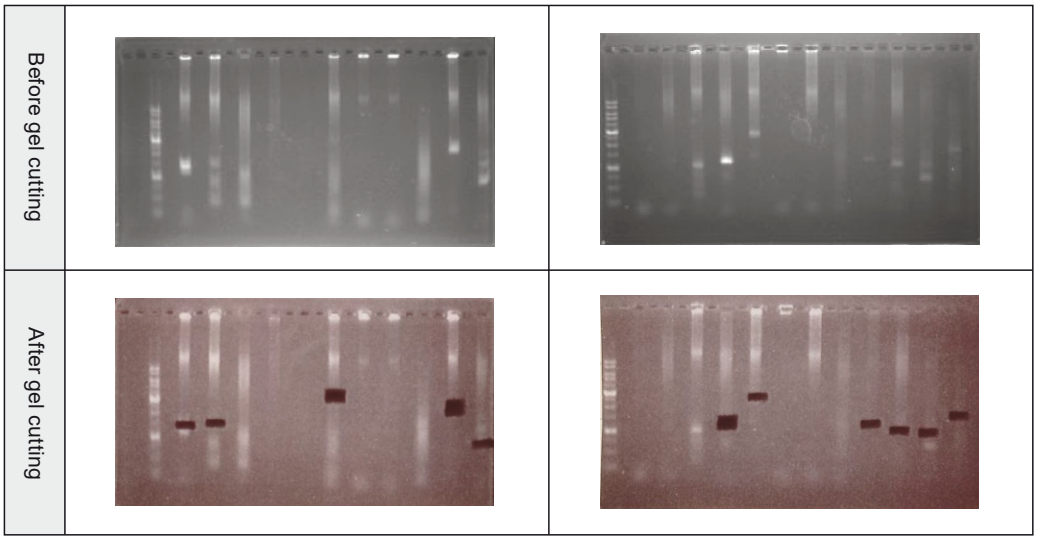
FastGene™
FastGene® Plasmid Mini Kit

Characteristic
- Stable yield can be optained!
- LB medium tablet free of charge!
- Both centrifugal and suction can be used
- Fast protocol can be used if you want to purify more easily and quickly

FastGene™
FastGene® Gel/PCR Extraction Kit

Result

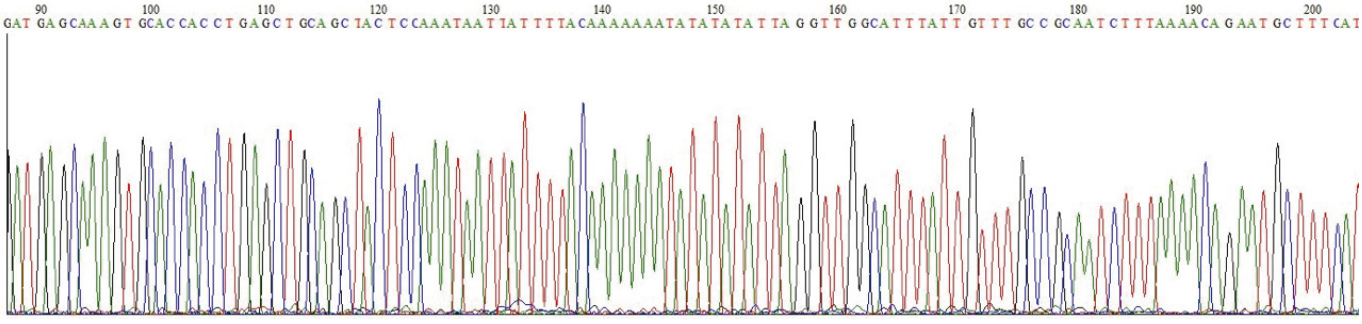
Figure 1. Cutting of bands using the gel cutter



FastGene® Agarose Gel Band Cutter

Illuminator: UV
Nucleic acid staining reagent:
Ethidium bromide (after dyeing)

Figure 2. Sequence result of the fragment cut out in Figure 1



<Customer's comments>
I am using FastGene' s Extraction Kit and Plasmid mini kit because of the quick protocol and the low cost.
Also we used razor blades so far, when extracing gels, but it became very easy to work after using gel cutter.
Although we used the gel cutter repeatedly, no contamination was detected so far.

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