Regarding the Use of DNA Sequence Consignment Analysis

Open Facility Center

Protein and Genome analysis Laboratory

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1. Procedure for Utilization

You can utilize consignment analysis by following the steps ① to ⑤ below.

① Fill in the required information in the Consignment Analysis Request Form*

② Prepare the samples

③ Send the Consignment Analysis Request Form (Word file) by email

④ Submit the samples into the refrigerator

⑤ Receive the results by email at a later date

*For first-time users, a meeting with the faculty member in charge of the consignment analysis is required before preparing samples. Prior to this, please contact the Protein and Gene Analysis Ro om to arrange a meeting date and time (E-mail: k-tsuka@fujita-hu.ac.jp, extension 9928) . Please note that we cannot accept samples brought in without a prior meeting.

① Fill in the required information in the Consignment Analysis Request Form

Please download the "DNA Sequence Consignment Analysis Request Form" from the Open Facility Center's website.

URL:https://www.fujita-hu.ac.jp/~kyoriken/jyutaku/index.html

Fill in the required information on the request form. If there are any unclear sections, you may leave them blank. We will explain them during the initial meeting.

② Prepare the samples

Please prepare the template DNA* and sequencing primer.

***Purified** PCR products or **purified** plasmids can be requested.

Samples to be submitted

Dispense the template DNA, sequencing primer, and H_2O into 8-strip PCR tubes so that the total volume is 12 μL per tube. Seal with dome-shaped caps. Please write the sample numbers in Arabic numerals on the sides of the tubes. Use sticky notes or tape to indicate the requester's name and extension number. Submit the samples in this condition. Add 8 μL of BigDye Terminator v3.1 Cycle Sequencing kit to these samples to make a final volume of 20 μL, and proceed with the sequencing reaction. The person in charge will enter the "Client's Last Name + Sample Number" on the sample sheet. For details, see "**2. Sample Preparation**" and "**3. Regarding the Sequencing Work of Consignment Analysis**".

③ **Send the Consignment Analysis Request Form (Word file) by e-mail**

Please send a completed "DNA Sequence Consignment Analysis Request Form" Word file (PDF not acceptable) to the following address by e-mail.

E-mail: k-tsuka@fujita-hu.ac.jp

Protein and Gene Analysis Room Consignment Analysis Faculty Member in Charge

④ **Submit the prepared samples**

Please bring the adjusted sample to the following place by the "Sample Submission Date and Time" entered in the request form. The person in charge collects the sample and begins the analysis.

Submission location: Sample container (blue-lidded plastic container) inside the showcase-style refrigerator in Room 317, 3rd Floor, Building 1, ~~3rd Floor,~~ Central Research Center, at the university.

Sample collection time by the person in charge: Weekdays from 9 AM to 5 PM on open days.

(Sample collection is not available on weekends, public holidays, and when the faculty member in charge is absent, as these are closed days.)

*The request will be considered complete, and the submission date will be recorded, when both the request form (Word file) and the samples have been submitted.

⑤ **Receive the results by email at a later date**

Within three open days from the submission date (excluding weekends, public holidays, and days when the faculty member in charge is absent), we will send a file of the analysis results by e-mail to the e-mail address sent to us in ③*. There are two files of analysis results per sample: a waveform file (.ab1) and a text file (.seq). Specialized software is required to open the waveform file (.ab1) . You can view the waveform files in the Sequencing Analysis Software installed on the control PC of the capillary DNA sequencer. The files of the analysis results will be kept in our laboratory for approximately 30 days, but backup is not guaranteed. Please back up the data by yourself.

*Since the sequencer is used together with general users, there may be a delay in analysis depending on the reservation status. Consignment analysis may be temporarily interrupted due to sequencer congestion.

2. **Sample Preparation**

In DNA sequencing analysis, the quantity and purification of template DNA and the quality of primer have a significant impact on the results of the analysis. When using this Consignment Analysis Service, please consider the following items ① to ⑥ and prepare a sample.

① Check the required amount of Template DNA and primer

Prepare according to the amount of DNA shown in the table below. Too little or too much Template DNA and primer may interfere with the sequencing reaction. The amount of template DNA can be determined by 1) absorbance measurement using a spectrophotometer (such as the NanoDrop at the Central Research Center) or 2) agarose gel electrophoresis to estimate the amount of sample bands from the brightness of DNA size markers of known amounts.

Approximate amount of Template DNA and primer required (Reproduced from ABI protocol)

Template	Quantity
PCR product	
100–200 bp	1–3 ng
200–500 bp	3–10 ng
500–1000 bp	5–20 ng
1000–2000 bp	10–40 ng
>2000 bp	20–50 ng
Single-stranded	25–50 ng
Double-stranded	150–300 ng
Cosmid, BAC	0.5–1.0 μg
Bacterial genomic DNA	2–3 μg
Primer	3.2 pmol

*For plasmid DNA, please refer to Double-stranded.

② Check the purity of Template DNA

Measure the absorbance at 230 nm, 260 nm, and 280 nm using a spectrophotometer (such as NanoDrop) available at the Central Research Center to determine the purity of the DNA solution. 260 nm/280 nm = 1.8 or higher, 260 nm/230 nm = 2.0 or higher is recommended. If the requirements are not met, we recommend re-purification using columns or similar methods.

③ Use template DNA with as high a concentration as possible

Especially when using plasmid as the template DNA, using a large volume of DNA solution for sample preparation may lead to inhibition of the sequencing reaction due to contaminants present in the solution. Therefore, it is recommended to concentrate the template DNA as much as possible and to minimize the volume of DNA solution added during sample preparation. When performing DNA purification, the final concentration can be increased by reducing the volume of water dissolved in the final solution. If DNA is already dissolved, it may be possible to concentrate it by ethanol precipitation.

④ Use water as the solvent

When purifying DNA, please use water for the final dissolution of the DNA, rather than buffer solutions. Dissolving in water prevents the introduction of extra reagents into the sequencing reaction. Do not use solutions containing EDTA (TE, etc.) since EDTA significantly inhibits the sequence reaction.

⑤ Purify PCR products

When sequencing PCR products as templates, it is necessary to remove unreacted dNTPs and PCR primers from the sample. There are methods such as 1) cutting out the target band using agarose gel electrophoresis and
2) using commercially available enzymes (e.g., ExoSAP-IT) to degrade unreacted dNTPs and PCR primers. The method 1) is also effective for purifying the target band when multiple bands are observed in addition to the target band. The method 2) is effective when only the target band is amplified by PCR, and it can be processed quickly (20-30 minutes). However, if the target band contains multiple PCR products, cloning is necessary.

⑥ Use a new sequencing primer

Even when stored in the freezer for long periods, primers may degrade. Of course, if the primer is degraded, the sequencing reaction will not proceed. If primers have been stored for an extended period and degradation is suspected, it is recommended to use new ones.
Additionally, using primers that were used in PCR for sequencing reactions may sometimes result in poor sequencing reads. In such cases, it is recommended to design new sequencing primers.

3. **Regarding the Sequencing Work of Consignment Analysis**

① **Sequence Reaction Reagent**

Use BigDye Terminator v3.1 Cycle Sequencing kit (Applied biosystems) and dilute BigDye Terminator Ready Reaction mix to 1/8 in a 20 μℓ system.

Basically, pGEM3Z(+) and M13 primer are used as control samples.

*Reactions with other BigDye reagents are not performed.

② **Cycle conditions**

Sequence reactions are performed under the following cycle conditions.

Hold	96°C	1 min	
Cycle	96°C	10 sec	25 cycles
	50°C	5 sec	
	60°C	4 min	
Hold	4°C	Store	

③ **Sample purification after sequence reaction**

Sample purification is performed after sequencing reaction by ethanol precipitation method (ethanol/EDTA purification) . After purification, the sample is dried up (2 min at 50°C) and finally dissolved in 15 μℓ of Hi- Di Formamide (Applied biosystems). Heatshock the samples (at 95°C for 2 min) and perform on ice (at least 5 min) immediately before setting them on the sequencer.

④ **Capillary DNA sequencer**

Use one of the following sequencers (Applied biosystems, Inc.) .

- SeqStudio Genetic Analyzer (4 capillaries, 28 cm capillaries, POP1 polymer)
- 3500 Genetic Analyzer (8 capillaries, 50 cm capillaries, POP7 polymer)

⑤ Electrophoresis conditions

SeqStudio Genetic Analyzer (4 capillaries)

*In the case of a request for more than 8 samples, the fee for each 8 samples will be 3,500 yen.

Run module	Electrophoresis time	Number of bases decoded
Short #*	30 minutes	Max. approx. 300 bp
Medium #*	45 minutes	Max. approx. 500 bp
Long*	120 minutes	Max. approx. 700 bp

#It may be performed using the Long protocol.

*Depending on the usage status of the equipment, it may be performed using the 3500 Fast or Standard.

3500 Genetic Analyzer (8 capillaries)

Run module	Electrophoresis time	Number of bases decoded
Short**	30 minutes	Max. approx. 300 bp
Rapid**	40 minutes	Max. approx. 500 bp
Fast**	65 minutes	Max. approx. 700 bp
Standard	125 minutes	Max. approx. 850 bp

*It may be performed using the Standard protocol.

* Depending on the usage status of the equipment, it may be performed using the SeqStudio Long.

4. Consignment analysis fees and payment methods

① Consignment analysis usage fees

To use a device equipped with 4 capillaries or 8 capillaries, the fee per sample is 480 yen when requested in units of 4 samples. This fee includes a measurement reagent cost of 150 yen. Since the measurement reagent cost also applies to capillaries without samples during measurement, the fee per sample for less than 4 samples is as follows.

For 4 samples, the cost is 480 yen x 4 = 1920 yen, which is 480 yen per sample.

For 3 samples, the cost is 480 yen x 3 + 150 yen x 1 = 1590 yen, which is 530 yen per sample.

For 2 samples, the cost is 480 yen x 2 + 150 yen x 2 = 1260 yen, which is 630 yen per sample.

For 1 sample, the cost is 480 yen x 1 + 150 yen x 3 = 930 yen, which is 930 yen per sample.

(Example) Fee calculation for a request with 14 samples:

For 12 samples: 4 samples per unit (480 yen x 4) 1920 yen x 3 = 5,760 yen

For 2 samples: 480 yen x 2 + 150 yen x 2 = 1,260 yen

Total: 7,020 yen

*There is no discount based on the number of samples.

② Payment methods

Invoices for usage fees will be issued monthly. A billing request will be sent to your affiliated office from the Research Support Division of the Research Support Promotion Headquarters in early the following month after the reception date. Payment can be made using university research funds, public research grants, etc. Please direct any inquiries to the Research Support Division.

5. **Disclaimer**

This Consignment analysis does not guarantee the results. Our analysis lab assumes no responsibility if analysis results are not obtained due to the quality of the submitted samples or other factors. In that case, the usage fees will be charged.

6. **Reanalysis**

If this consignment analysis fails due to errors on the part of the analysis room or machine troubles, a reanalysis will be conducted free of charge. Please note that we cannot compensate for the costs of template DNA and primers in the event of a failure.

7. **Research Consultation**

If you have any questions about the sequencing results, please feel free to consult us. Additionally, we offer a research consultation service for technical issues such as sample preparation (including PCR and plasmid construction) , primer design, and difficulties in obtaining good sequencing results. Please do not hesitate to use this service.

Contact: Open Facility Center, Protein and Gene Analysis Room
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