Fraunhofer IME

Department Functional and Applied Genomics

DNA Sequencing

The 48-capillary 3730 DNA Analyzer is the gold standard in medium to high throughput genetic analysis with applications ranging from standard Sanger DNA sequencing to DNA fragment analysis such as microsatellites, AFLP, SNP analysis as well as mutation detection. For those applications, contact Dr. Jost Muth (0241 6085-12050).

With capillary electrophoresis (CE) good quality sequences usually reach a length of up to 800 clear bases under optimal conditions, while the first 50 bases of your template are not readable due to the limitations of CE. Sequencing primers should be chosen accordingly to cover the whole area of interest especially when sequencing long and short DNA fragments. Routine DNA sequencing is performed on Tuesdays and Thursdays with prices per samples of 4,50€ for less than 24 samples and 3,00€ for more than 24 samples, if handed in on a PCR plate. Sequencing results are given in standard .abi and .seq format and can be obtained from a password secured ftp server.

Sample Handling for DNA Sequencing

Template

The quality of the sequencing results is directly proportional to the quality and quantity of the template. Template DNA should be purified thoroughly and the carryover of contaminants (e.g. ethanol), as well as the use of elution buffers containing EDTA avoided. The strain of ***E. coli*** used as host can have a significant impact on the quality of template DNA for plasmids, cosmids, and BACs. Strains optimized for protein expression should be avoided. For the optimal concentration of template DNA, refer to table 1 below.

Primers

Primers used for sequencing should bind specifically to the template at a single site and not form secondary structures or hybridize to form dimers or oligomers. When sequencing PCR templates, using nested primers for sequencing can improve the quality of the sequencing result.

Table 1 Optimal concentration of template DNA.

Plasmid	Quantity (≥100 ng/µL)	PCR Product	Quantity
“standard” plasmid	400-600 ng (140-160 ng/kb)	100 - 200 bp	6-15 ng
“gateway” plasmid	600-900 ng (210-240 ng/kb)	200 - 500 bp	15-30 ng
siRNA plasmid	600-900 ng (210-240 ng/kb)	500 - 1000 bp	25-50 ng
Cosmid, λDNA	2-5 µg	1000 - 2000 bp	40-100 ng
BAC, YAC, PAC	2-5 µg	> 2000 bp	80-200 ng

Sample Preparation

Sequencing samples should comprise the template DNA diluted in **10 mM Tris buffer** (pH 8.0, **no EDTA**) in a volume of 14 µL and 2 µL sequencing primer (10 µM, standard plasmid and PCR product) for a final volume of 16 µL. For other plasmids primer concentration can be adapted (2-5 µL, 10 µM).
Samples should be handed in in **0.5 mL reactions tubes** (< 24 samples) or in sealed standard **96-well PCR plates** (> 24 samples). On PCR plates, samples are arranged horizontally (sample 1 in well A1, sample 2 in well A2, etc.). Samples should be given a unique 'Sample Initial' comprising customers initials (up to 4 letters) and **consecutive** numbers, which can start at any number without gaps in between samples. For reaction tubes, sample initial should be written on **every cap**, while PCR plates are **labelled** with name and date.

Sequencing Order Form

With every sample delivery a Sequencing Order Form should be filled in, which provides all necessary information for successful high quality sequencing.

Sample Initial

Sample initial should comprise customers initials (up to 4 letters) and consecutive numbering without special characters. The sample initial (first column on the Order Sheet) is not the sample name ('Template' column) and should be kept straightforward to simplify the processing of samples and to ensure a correct assignment of sequences to templates.

Template (14 µL)

Cells should be filled with the names of templates to be sequenced to allow for assignment of sample initials to templates.

Primer (2 µL, 10 µM)

This column should contain the name of the used primers.

Product length

The expected product length of each template should be provided, especially when handing in large amounts of samples.

Customer Information

Customer information has to be filled in digitally and completely to ensure communication and billing.

Sample Delivery

Samples can either be dropped off on site or sent by mail.

For on-site drop-off, samples should be placed in a plastic bag, labeled with name and date, along with the completely filled in Sequencing Order Form. Samples may be dropped off at the front desk between 8:00 a.m. and 4:15 p.m. or outside of these hours in the mailbox in front of the entrance. On sequencing days (Tuesday and Thursday), samples can be dropped off until 10:30 a.m. for same-day sequencing. Samples handed in later may not be analyzed until the next sequencing day.

To send samples by mail, sample tubes should be chained together with string, PCR plates thoroughly sealed with appropriate sealing film, placed in a padded envelope along with the Sequencing Order Form, and mailed to this address:

Contact

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