Sequencing Service
Fraunhofer-Institut für Molekularbiologie
und Angewandte Oekologie IME
Forckenbeckstr. 6 | 52074 Aachen

Fraunhofer IME

DNA Sequencing at IME

The 48-capillary 3730 DNA Analyzer is the gold Standard in medium to high throughput genetic analysis. This device is made for Sanger DNA sequencing and DNA fragment analysis applications such as microsatellites, AFLP, SNP analysis, mutation detection are as well possible. For those applications, contact Dr. Jost Muth (0241 6085 12050). Nevertheless, capillary electrophoresis (CE) has some limitations. Good quality sequence in the best conditions reaches ~650 to 800 clear bases. Thus, plan your experiment wisely if you want to cover the complete sequence of your genes. Better off with designing one or two primers more, than too few. Regarding primer design, you ought to consider a window of about 50 bases before the actual start of your sequence of interest, because CE is also not allowing to read directly from the first base of your oligonucleotide. This remark is particularly true if you´re sequencing short DNA fragments (~300 bases and less).

Template Quality:

The quality of the sequencing results is directly proportional to the quality of the template, i.e. clean DNA is absolutely critical. For plasmids, cosmids, and BACs, the strain of E. coli used as host can have a significant impact on the quality of the template DNA. Strains DH5α and TOP10 are the best, while XL-1 is okay. The JM101 series of strains are inconsistent at best, and strains optimized for protein expression should entirely be avoided and absolutely none DNA from TG1 cells.
Template DNA must be prepared with commercially available plasmid purification kits or any similar methods. PCR products must be cleaned up by PCR purification methods (column, membrane, or enzymatic). But in any case, DNA must be purified.

Template Quantity:

Ranges of template quantity to be given in total volume of 16 µL (DNA + 10 mM Tris pH 8.0 + primer):

Plasmid

concentration ≥100 ng/µL

"standard" plasmid	80-160 ng/kb
"gateway" plasmid	140-240 ng/kb
Cosmid, λDNA	2-5 µL of DNA [1µg/µL]
BAC, YAC, PAC	2-5 µL of DNA [1µg/µL]
siRNA plasmid	140-240 ng/kb

PCR product

100 - 200 bp	15 - 30 ng
200 - 500 bp	25 - 50 ng
500 - 1000 bp	40 - 100 ng
>2000 bp	80 - 200 ng

+

Primer

2 µL of 10 µM primer for standard plasmid & PCR product

2 - 5 µL of 10 µM primer for all other cases

Sample preparation:

Please hand in the required amount of DNA in 14 µL 10 mM Tris buffer (pH 8.0) and 2 µL Sequencing Primer = 16 µL (compare Table above for required amount of DNA). This amount is enough for three sequencing reactions. In case, the first attempt fails due to technical problems we will automatically re-sequence your samples for free. Strictly stick to 10 mM Tris buffer (pH 8.0). **Do not use TE buffer since it contains EDTA which will inhibit the sequencing reaction.**

Contact:
sequencing@ime.fraunhofer.de
Dr. Jost Muth +49 (0)241 6085 12050
Lena Freund +49 (0)241 6085 35045

If you have less than 24 samples, hand in the 16 µL in 0.5 mL reaction tubes. If you want to sequence more than 24 samples, please deliver them in a sealed 96-well PCR plate with the samples arranged horizontally (sample 1 in well A1, sample 2 in well A2…). This will reduce your sequencing price per sample and saves the Sequencing Team a lot of time.
Label your samples legibly with your initials (up to 4 letters) and **consecutive** numbers. You can start at any number but do not leave any gaps! The sample initial (first column on the Order Sheet) is not your sample name ('Template' column), so please keep the initials straightforward to simplify the processing of your samples and to ensure a correct assignment of sequences to your templates. For reaction tubes, write the sample initial on the cap, PCR plates must be labeled with your name and date.

Sample delivery:

For in-house IME samples: place your samples in the labelled drawer (Sequencing ABI) of the -20°C freezer (#1) in room A330 and place your printed Sequencing Order Sheet in the provided box outside the lab next to the door. On sequencing days (Tuesday and Thursday), samples can be submitted until **10:30 am** for same-day sequencing. Samples handed in later may not be sequenced until the next sequencing day.
For RWTH University customers: place your samples in a plastic bag, signed with name and date, in the designated -20°C freezer at RWTH University, Worringerweg 1, BIOVII 1st floor and the printed Sequencing Order Sheet in the provided box (D151). On sequencing days, samples can be submitted until **8:30 am** for same-day sequencing. Please make sure to notify us via mail (sequencing@ime.fraunhofer.de), we will collect your samples on sequencing days. Samples handed in later will not be sequenced until the next sequencing day.
For external customers: chain your samples together on a string and pack the chain with a copy of your Sequencing Order Sheet in a padded envelope and send by post to:

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Sequencing Order Sheet:

The samples you send us for sequencing must come along with the Sequencing Order Sheet. This form shows all the information we may need to get high quality sequences. Read carefully and fill out accordingly.
Sample Initial: your initials plus a number for each reaction. Use consecutive numbers if you order more than one reaction. Don't use symbols, blank spaces or special characters in the name as the Analyzer database doesn't recognize those. (Special characters are: ? ! " ° ^ / . \ ´ # ~ & $ §…).
Template (14 µL): fill these cells with the names of your templates to be sequenced. With this column you can assign your template with the corresponding sample initial.
Primer (2 µL, 10 pmol/µL): fill these cells with the name of the primers being used.
Tm Oligo [C°]: fill these cells with the optimal Tm of the oligo being used (especially when Tm is outside 52°C to 58°C range).
Product length: indicate the minimum expected product length.
PCR Product: mark if your sample is a PCR product.
Relevant Feature: please fill out this column with the letter code provided on the Sequencing Order Sheet. All the information added here are of relevant interest for successful sequencing. Please indicate also the GC content (in %) of the region to be sequenced (this is even more important if you have sequences with higher than 55% GC content).
Plasmid Backbone & Size: please indicate which vector backbone was used (including the length of the whole construct, plasmid and insert).

Do not forget to fill out the cells containing your name and contact information!

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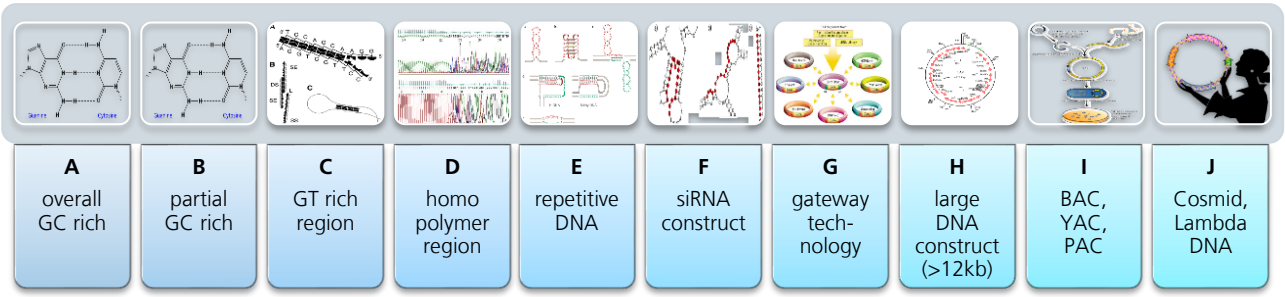

Further Information:

This is just a little 'list' of all troubles, oddities and other rarities encountered through the years.
Best sequences are obtained when DNA and primer are mixed together inside the same tube. Using an appropriate protocol for sequencing preparation is very important, thus the complete fill-in of your Sequencing Order Sheet is very helpful.
For plasmid purification using methods such as Miniprep columns (i.e Qiagen), loading more cells doesn't necessarily mean more DNA at the end. Miniprep kits are designed to be used with a 12-16 h culture (DH5α, XL-1blue, DH1, and C600) in LB medium. Hence, adjust your protocol when using other strains and/or media!
DNA quality will also suffer if columns are overloaded. Elution is pH dependent - if you're using water, be sure that the pH is around 7 to 8.5. Ethanol is a very strong inhibitor of the sequencing BigDYE™ chemistry. Even traces may impair the PCR reaction. Thus, incubation of your column for 5 min at 60°C before elution is helpful. Elution with EB buffer is fine, but please never use TE buffer. It contains EDTA which chelates Mg^{2+} which in turn is required for the sequencing reaction.
Show that your DNA is clean with a picture and an $OD_{260/280}$ rather than just assuming it.
siRNA constructs aren't plain constructs. Let them be clearly noticeable.
Avoid primers that can bind at multiple sites as it will result in multiple sequences. When designing your primers, verify that they aren't able to show secondary structure or hybridize to form dimers or oligomers. Use a primer that binds to your template.
Clean DNA in 10 mM Tris (pH 8.0) will not suffer when sent uncooled.

The six golden rules for sequencing at IME:

To run the sequencing service as effectively and easily as possible, we have come up to edit these basic rules which are the least things we must ask for to be able to provide you with quality sequences twice a week. Please, read them all carefully and be sure that you understand them to the full extent of their meaning.

Rule #1: Tuesdays & Thursdays are sequencing days.
Rule #2: Sample submitting is until 10:30 am (for in-house IME customers) and 8:30 for outside customers who bring samples to RWTH University (Worringerweg 1, BIOVII-1st floor).
Rule #3: No other reaction tube than 0.5 mL is accepted for less than 24 samples. For more than 24 samples use PCR plates.
Rule #4: No sequencing order sheet = No sequencing.
Rule #5: Illegible sheets will not be processed.
Rule #6: Whenever, due to client mistake or omission, extra work has been performed and/or extra costs have been caused, the sequencing service has the right to charge client for that extra costs.

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