

## Recommendations for Additional Services to accompany Service à la Carte

### Quality control of incoming DNA

For successful sequencing results, it is important to state the DNA concentration correctly. If you prefer not to carry out the concentration measurement yourself, we will be happy to do it for you: simply order "Quality control of incoming DNA". This additional service is performed using an agarose gel to check:

- DNA concentration
- Possible DNA degradation
- Contamination with RNA or chromosomal DNA
- Remaining primers or additional bands (PCR products)

You will be informed immediately by our sequencing lab if the quality control check reveals the wrong concentration or any potential risks for the sequencing reaction.

### Concentration adjustment of DNA (by dilution)

For each type of sample, the DNA needs to be in a specific concentration range. If the concentration of your DNA is higher than required, and you do not want to dilute it yourself, please order this additional service.

### Concentration adjustment of DNA (by desalting)

This additional service actually includes two services: increasing the DNA concentration and desalting the DNA.

If the concentration of your DNA is not high enough for the sequencing reaction, we can increase it, if the necessary amount is available in a higher volume.

At the same time, we desalt the DNA to avoid high salt concentrations which are the main reason for bad sequencing results. For this reason, we recommend this additional service in the following cases, even if your DNA concentration is sufficient:

- If you precipitate your DNA e.g. after a Maxi Preparation
- In the case of a column purification where the DNA binds to a column with high salt concentration
- If you eluted PCR bands from an agarose gel

Finally, we perform a quality control check on an agarose gel. You will be informed immediately by our sequencing lab if this shows any potential risks for the sequencing reaction.

### Sequencing by using special chemistry

For sequences with structural problems, e.g. hairpin structures which mostly occur in shRNA constructs or for sequences with G/T repeats, the sequencing reaction has to be carried out using a special chemistry. Therefore, we recommend ordering this additional service for those kinds of samples.

For G/C-rich samples, the standard sequencing chemistry may be sufficient, but it is also possible that the sequencing reaction stops. In that case we will ask you for approval to repeat the sequencing reaction with the special chemistry. To be on the safe side, we recommend always ordering the special chemistry for G/C-rich samples at the beginning.

### **Special cycle sequencing conditions**

The annealing temperature of our sequencing reactions is usually 50°C. If the melting temperature of your sequencing primer is below 50°C, we need to modify our cycle sequencing conditions; this is especially relevant to polyA/T primers. Furthermore, primers with a melting temperature higher than 65°C could be a reason for bad sequencing results. In these cases, please order the additional service “Sequencing with special cycle sequencing conditions” and state the melting temperature of your primer.

### **DNA preparation**

It is possible to send either a stab or glycerol culture with the selected E.coli clone, an overnight culture, or a streak on an agar plate by ordering the DNA preparation either as Mini or Midi Scale or as Large Construct Prep. The DNA preparation service includes:

- DNA quality control
- Concentration adjustment by dilution
- Concentration adjustment by desalting, if necessary

### **PCR product standard purification**

PCR products have to be purified from remaining primers because they may be the reason for mixed sequences or sequences with a high background. We recommend using a commercially available PCR purification kit with a minimum of two washing steps to remove interfering salts.

If you do not want to purify your PCR products yourself, we can offer you PCR product standard purification as an additional service.

### **PCR product gel purification**

If your PCR product shows more than one band and the PCR primers should be the sequencing primers too, then we recommend choosing the PCR product clean up using an agarose gel purification.

Please indicate in the comment field of your sequencing order if you eluted your PCR product from an agarose gel, as this could cause difficulties in the sequencing reactions. Using internal (nested) primers could be an alternative to avoid an agarose gel elution. Or you can simply order the PCR product gel purification as additional service at Eurofins MWG Operon, which also includes a DNA desalting step. In that case please include a gel picture and identify the desired fragment.

### **PCR amplification from genomic DNA**

With our Service à la Carte, it is possible to send in genomic DNA. To perform the sequencing, we first carry out a PCR amplification and then sequence the PCR product, rather than sequencing the genomic DNA directly.

If you require this service, simply define your template type as genomic DNA and the corresponding PCR primers for the amplification in your sequencing order. The additional service “PCR amplification from genomic DNA” will be performed automatically.