biodonostia
health research institute

Sanger sequencing sample requirements

The quality and concentration of the sample and the quality of the sequence are directly related. Aspects to bear in mind include:

- **Purification:** The purification of the sample is a key procedure in obtaining good sequences. It is important to avoid an excess of salts. Commercial kits are usually recommended, which take into account certain purification issues that may result in poor quality sequencing data. As for PCR products, it is essential to prevent primer dimers from forming, as they will make it difficult to view the 100-110 primers on which the reading is based. Also, the conditions for the sequencing reaction can only be satisfactory if a single mould is used. If more than one band appears in the PCR and this cannot be prevented, we recommended cutting the band of interest from the agarose gel and then purifying said band. We will request that, insofar as possible, you provide a picture of the agarose gel on which the PCR product has been smeared; if this is not possible, we will ask you to provide a statement indicating that the PCR in question was made in one single band.

- **Concentration:** The concentration in the mould is crucial for the sequencing reaction. Therefore it is requested that the sample meet some minimum standards and be quantified. The minimum amount we accept per request is 15-20 µl.

DNA type	Minimum concentration
Plasmid	75-150 ng/µl
Product PCR (pPCR)	20 ng /µl
pPCR higher 1000 pb	Please check with person in charge

- **Primers:** The primers must be submitted in separate tubes with a concentration of 3-5 µM and labelled accordingly. The minimum amount of primers per sequencing reaction is 4 µl. Should you request fewer than three sequences, the minimum amount of primers to be received will be 10 µl. The composition of the primer has an influence on the quality of the sequencing. Normally all primers that work well for PCR will also work well on the sequencing reaction. As a rule of thumb, primers must have a Tm between 55 and 65 ºC, be at least 18 pb in size and prevent the formation of secondary structures, mainly in the outer 3'; it is therefore very important to avoid any repetitions of 4 or more Gs or Cs as far as possible. If the primer you request has a different Tm, the person in charge of the sequencing service must be notified. The purification of the primer is also critical for the sequencing; if the primer is not properly purified or is even slightly degraded, it will cause shadow peaks and a lot of background noise. To minimize the likelihood of such issues, commercial primers are normally purified

using DHPLC or other methods, and they must be correctly maintained (few freezing/thawing cycles).

Results delivery

The usual period for delivering the results is 3 to 5 working days for PCR products and plasmids. For other services, please check. Both the chromatogram and the sequence (a text file) will be delivered via email.

The chromatogram file can be viewed with most programs to display sequences. Some examples might be:

- Chromas
- Bioedit
- Sequence Scanner
- Staden Package
- Peak Scanner

Check prices for large-volume samples or specific applications.

Genomics Plataform
Pº Dr. Beguiristain, s/n
20014 Donostia-San Sebastian
T +34 943 00 61 27 / 61 07 · F +34 943 00 62 50
plataforma.genomica@biodonostia.org
www.biodonostia.org