Sequencing Sample preparation

Aim of the experiment

This protocol can be used for the subsequent preparation of samples for sequencing your DNA of interest.

The Sequencing preparation protocol given here follows the needs of the 2 big sequencing companies Eurofins and GATC:

Eurofins Sequencing

GATC Sequencing

For more detailed descriptions on how to prepare samples for other companies, visit the website of your local Sequencing company.

Materials

- DNA of interest (at least 500 ng in 7.5 µl of nuclease-free water)
- pair of appropriate primers flanking the sequence of interest (<2000 bp in between primers)
- nuclease-free H_2O (nf H_2O, Sigma Aldrich, Germany)
- Safe-lock tubes (i.e. Eppendorf)
- Sequencing labels

Procedure

1. Mix in a 1.5 ml safe-lock reaction tube:

Table 1: Sequencing sample

Volume (µl)	**Chemicals**
x	500 ng of DNA of interest
2.5	10 µMof appropriate primer
Fill up to 10	nf H_2O

2. Stick sequencing label (barcode) to tube and keep accompanying tag with barcode number in an ordered folder
3. Send out samples to sequencing company
4. Check your sequencing results on companies website (usually the next working day)

Possible follow up protocols

The following protocols are the next steps of a possible cloning cycle after a Sequencing run:

1. Transformation
2. Restriction digest
3. Mutagenesis-PCR (for point mutation)