

iGEM TU/e 2017
Biomedical Engineering

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Sequencing at StarSeq

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1 Preparation of samples

Estimated bench time: 2 minutes per sample

Estimated total time: 2 minutes per sample

Purpose: Sending the plasmids to StarSeq for sequencing

1.1 Materials

- Autoclaved H₂O
- Autoclaved PCR tubes with flat lids
- Falcon tube
- Pipettes and tips
- Plasmids which are to be sequenced
- Primers which are needed for sequencing

1.2 Setup & Protocol

- It is necessary to know the concentrations of the plasmids which are to be sequenced. We sequenced using the U-mix product StarSeq is offering. The sequencing method is StarSeq sequencing.
- Verify how large the plasmid is. StarSeq requires certain amounts of DNA for sequencing to work.
- Add the plasmid DNA first up till the required amount of DNA (to a maximum of 6 µl).
- Add 1 µl of 10 µM primer necessary for sequencing. Make sure you use the correct primer.
- If necessary, add H₂O up to a volume of 7 µl.
- Number the PCR tubes. The PCR tubes can be put in a small Falcon tube which can be sent out to StarSeq. Write the numbers of the PCR tubes on the Falcon tube.
- Send the Falcon tubes to StarSeq for sequencing.

2 StarSeq preparations

DNA: dissolved in water or TrisHCl (10mM), pH 7-8

Amounts:

PCR product: < 200 bp: 50 ng; 200 – 300 bp: 60 ng; 301 – 400 bp: 80 ng; 401 – 500 bp: 100 ng; 501 – 600 bp: 120 ng; 601 – 700 bp: 140 ng; 701 – 800 bp: 160 ng; 801 – 900 bp: 180 ng; 901 – 1 kb: 200 ng

Plasmid DNA: <3,5 kb: 350 ng; 3,5 – 4,5 kb: 400 ng; 4,6 – 5,5 kb: 450 ng; 5,6 – 6,5 kb: 500 ng; 6,6 – 7,5 kb: 550 ng; 7,6 – 10 kb: 600 ng; > 10 kb: 700 ng

Cosmid DNA, PACs, BACs: >1 µg

We recommend to measure the DNA concentration with fluorescent assay or on an agarose gel.

Primer: 10 pmol, i.e. 1 μ l of a 10 μ Molar primer solution

Melting temperature: 52 – 58°C

Optimal length: 18 – 25mer

Primer base composition: G or C should be at the 3' end, try to avoid more than 3 G's or C's at the end and 4 identical bases in a row.

Tube/Plate: 200 μ l PCR tubes with flat lids (e. g. Starlab, Art. Nr. I 1402-8100 or similar);

Close only, no Parafilm. Always fill plates from A1 to H1, A2 to H2, A3 to H3... Samples in

Plate: Its your choice (must be between 48 and 96 samples)!

Label: legible, with indelible black pen (e. g. Staedtler permanent lumocolor; Art. Nr. 318-9;

EAN 40 07817 304563 or similar). Note your abbreviation on the lids and number them

continuously. Please do not use special characters for the sample names like / [\ } & % , ; : . ä

ö ü ? ß () " ' # + * @ μ and blanks. Underscores and hyphen could be used.

Dispatch: Protect the tube with suitable firm boxes or bubble wrap and send them in stable tearproof padded envelope.