

iGEM TU/e 2018
Biomedical Engineering

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Sequencing at BaseClear

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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 Baseclear for sequencing

1.1 Materials

- Nuclease free water
- 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.
- Verify how large the plasmid is. BaseClear requires certain amounts of DNA for sequencing to work. For each sample, 0.75 ug plasmid DNA is required.
- Add the plasmid DNA first up till the required amount of DNA (to a maximum of 6 µl).
- Add 2.5 µl of 10 µM primer necessary for sequencing. Make sure you use the correct primer. Only use one primer per sample. Dependent on the length of the construct, multiple samples with a single primer are needed for a certain sample.
- If necessary, add nuclease free water up to a total volume of 20 µl.
- Number the PCR tubes. The PCR tubes can be put in a BaseClear box which can be sent out to BaseClear.
- Send the box to BaseClear for sequencing.

2 BaseClear preparations

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

Primer: 25 pmol, i.e. 2.5 µl of a 10 µMolar 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 µl 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.