

## Sanger Sequencing

### DNA Requirements:

All samples should be dissolved in DDW

Nanodrop 260/280 = 1.8-2.0

If your DNA sample is GC-rich (>70%) you must indicate this in your order

### Primer Requirements:

All primers should be dissolved in DDW

18-22 bases with 50-60% GC content Melting  
point (Tm) = 50-60°C

### 'Ready to go' mode: primer and template together in the same tube

- Please bring your samples in 8-stripes or 96-well plate.
- The samples should be marked by num. (1, 2, 3,...);  
if you bring 96-well plate, please be advised that the numbering should be according to columns (e.g.: samples 1-8 should correspond to A1-H1 in the plate).
- Create a NEW order in bookit (do not copy old reservations) and under 'template name' enter the same numbers written on the tubes in the same

order as in the strip/plate. If you want to add a name to your samples, please enter it according to the following example: 1\_sample name.

- In the comment section of the bookit order please note 'ready to go' mode.
- You do not need to print labels just put your tubes in a bag with your name and the order number on it.
- Pay attention that samples in 'ready to go' mode will not be repeated in case it doesn't work or the results are not satisfactory.

Template type	Template concentration	Template volume	Primer volume and Primer concentration
Plasmid	50-100 ng/ $\mu$ l	8.5 $\mu$ l	1.5 $\mu$ l of 5 pmo/ $\mu$ l
PCR product column purification	30-50 ng/ $\mu$ l		
PCR product ExoSap purification	-		

*'The good old way': primer and template separately*

Please bring your samples in 1.5 ml Eppendorf tubes

DNA Preparation:

Required volume: 10  $\mu$ l per reaction Required concentration:

Plasmid- 100 ng/ $\mu$ l (min 60 ng/ $\mu$ l; max 200 ng/ $\mu$ l)

PCR product (purified\*) - 30 ng/ $\mu$ l (min 20 ng/ $\mu$ l; max 50 ng/ $\mu$ l)

BAC - 500 ng/ $\mu$ l

Primer Preparation:

Required volume: 5  $\mu$ l per reaction

Required concentration: 5 pmol/ $\mu$ l (5 $\mu$ M)

\* For PCR products there is an option to bring samples before purification and we will perform Exo/Sap purification for extra charge. In that case, please bring your samples in a numbered 0.2 PCR strip and add a comment to your order specifying a purification step is needed.