

Sanger Sequencing sample requirements

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 (T_m) = 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 numbers (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 (*Plasmid - Ready To Go* or *PCR - Ready To Go*) 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.
- You do not need to print labels, just put your tubes in a bag with your name and the order number on it.
- Please be advised that 'ready to go' samples cannot be repeated if the reaction doesn't work or the results are not satisfactory.

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

'The good old way': primer and template separately

Please bring your samples in 1.5 ml Eppendorf tubes.

Create a NEW order (*Plasmid* or *PCR*) in [Bookit](#) (do not copy old reservations). For those who are sending their orders from Neve Sha'an an campus, make sure to specify the order number on the envelope and to address it to the sanger sequencing unit.

Template type	Template concentration	Template volume	Primer volume and concentration
Plasmid	100 ng/μl (min 60 ng/μl; max 200 ng/μl)	10 μl	5 μl of 5 pmol/μl (5μM)
PCR product (purified*)	30 ng/μl (min 20 ng/μl; max 50 ng/μl)		
BAC (**)	500 ng/μl		

* 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.

** For BAC sequencing please contact us in advance.

For more information regarding Sanger sequencing, please contact Liza (gliza@technion.ac.il) or Doron (fdoron@technion.ac.il).