www.seqme.eu
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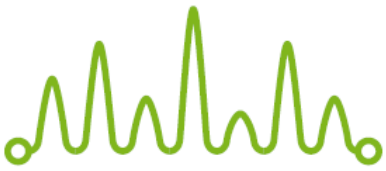
StandardSeq

Preparation of samples



Sample is a template mixed together with sequencing primer in a single test tube / well or template only if you wish to use some of our universal primers. Template is a PCR product or plasmid.

Number of samples ordered = number of tubes sent or number of plate wells used.

All templates must be purified (e.g., plasmids on cleanup columns or using other suitable method compatible with sequencing, PCR products enzymatically using Exo-SAP or otherwise), or at least precipitated with ethanol, and dissolved in water (HPLC-grade). We do not recommend buffers (TE, etc.) for dilution.

If you use your own primers, mix the following:

Template		Your primer	Total volume
PCR product < 500 bp	50 ng	Add 25 pmol of your primer (5 µl of 5 µM primer)	10 µl
PCR product 500 – 1000 bp	100 ng		
PCR product > 1000 bp	200 ng		
Plasmid	500 ng		

If you wish to use our universal primers, prepare the template as follows:

Template		Total volume
PCR product < 500 bp	50 ng	5 µl
PCR product 500 – 1000 bp	100 ng	
PCR product > 1000 bp	200 ng	
Plasmid	500 ng	

If you do not have the required amount of template available, you can reduce the total volume of sent samples while maintaining the concentration of template and primer. E.g.: If you have only 30 ng of PCR product of 400 bases, use 3 µl of this template and 3 µl of 5 µM primer solution. The total volume in this case will be 6 µl. Please note that in such cases we cannot guarantee the repetition of sequencing if the first sequencing reaction fails, whatever the reason.

The minimum total volume of template + primer you can send is 4 µl.

We recommend 8-tube strips with individual caps (eg Eppendorf 0030124359) or 96-well plates. Do not use parafilm. Please mark the tubes preferably with a number indicating their order in the order form.

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