



Sanger Sequencing

- Deliver your samples in 0,2ml 8-well reaction tubes sealed with strip caps (Applied Biosystems part# N801-0580 and N801-0535)



- All tubes must be numbered on the side, not on the lids.**
- We need 6ul template and primer mix for the sequencing reaction. However we ask you to deliver 15 µl template and primer mix to be able to repeat the sequencing reaction when unexpected technical failures occur. It is your responsibility to deliver 15 µl of template and primer mix. At all times when you deliver less than 15 µl total volume, the LGTC cannot be held responsible for failed reactions.

The table below is the amount of template and primer needed for 15 µl template and primer mix.

Template amount	PCR product	Template quantity
	100 - 200 bp	3 - 7 ng
	200 - 500 bp	7 - 12 ng
	500 - 1000 bp	12 - 25 ng
	1000 - 2000 bp	25 - 45 ng
	>2000 bp	45 - 80 ng
	<i>Double stranded</i>	500 - 1500 ng
	<i>Single stranded</i>	250 - 500 ng
	<i>Cosmid, BAC</i>	2 - 4 µg
Primer	10 pmol	
H₂O	xx µl	
Total volume	15 µl	

- There are also several formulas to calculate the amount of template needed:

- PCR fragments: length of fragment / 50 = amount of template in ng.
- Plasmids: 25 * length (plasmid + insert) in kb = ng total input.

There is not one general rule about the amount of template and primer in the sequencing. It is a trial and error process to get the concentration right for your own specific template.

- Purify PCR products before sequencing. We have good results with the QIAGEN QIAquick PCR Purification Kit or the QIAGEN Nucleotrap PCR Purification Kit. This is necessary to get rid of primers, primer dimers, enzyme, dNTPs, and MgCl₂ left over from the PCR reaction.
- We advise to use the NanoDrop for an accurate measurement of the DNA concentration but correct for the small fragment size when measuring PCR fragments. You can also use the Caliper LabChip-90 (when you have more than 48 samples). We recommend starting with the minimal DNA concentration. This usually gives good results from the start, although in some cases further optimization might be needed.
- The quality of oligonucleotides (used for sequencing or any other cDNA synthesis application) is absolutely critical. Aliquot your sequencing primer(s) into small amounts for one or two uses only, since oligonucleotides, particularly longer ones, deteriorate rapidly on freeze/thawing.
- Enter your samples in the LGTC LIMS-system.
- Print out the Order Report for the LGTC. To print out your submission, go to 'View submissions', click on your submission ID, and print out the web page.
- Place samples in a rack in the LGTC-freezer (room R5-23), the racks are provided by the LGTC, and can be found on top of the LGTC freezer. Number all the wells clearly on the side, that we know their correct order. Please do not wrap your samples with tape, parafilm or otherwise. Use a single piece of painters tape to label your samples with the submission ID provided by the LGTC LIMS. Complete the Order Report with an authorization signature and leave the Order Report in the Sequence delivery tray in room R5-23 (on top of the freezer, top shelf). See figure 1 for Order report with a submission ID and authorization signature.
- You will receive a notification from our server for collection of your results. You can also check that yourself by logging into LGTC LIMS. When after 5 days the Status still is "Submitted" or "Checked", please contact us.

Figure 1: Order report with submission ID and authorization signature.

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Einthovenweg 20 2333 ZC Leiden Tel : +31 (0) 71 526 9500 Fax : +31 (0) 71 526 8285																										
Email : Info@LGTC.nl http://www.LGTC.nl/		Submission ID: 1015376 																								
Order report Ordered by:																										
Address: LGTC user LUMC Department Albinusdreef 2, 2333 ZA Leiden Tel. 071-526xxxx Email: mail@lumc.nl	Invoice address: LGTC user LUMC Department Albinusdreef 2, 2333 ZA Leiden Tel. 071-526xxxx Email: mail@lumc.nl																									
Tuesday February 17th, 2009																										
Client KostenPlaats/order number : XXXX																										
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