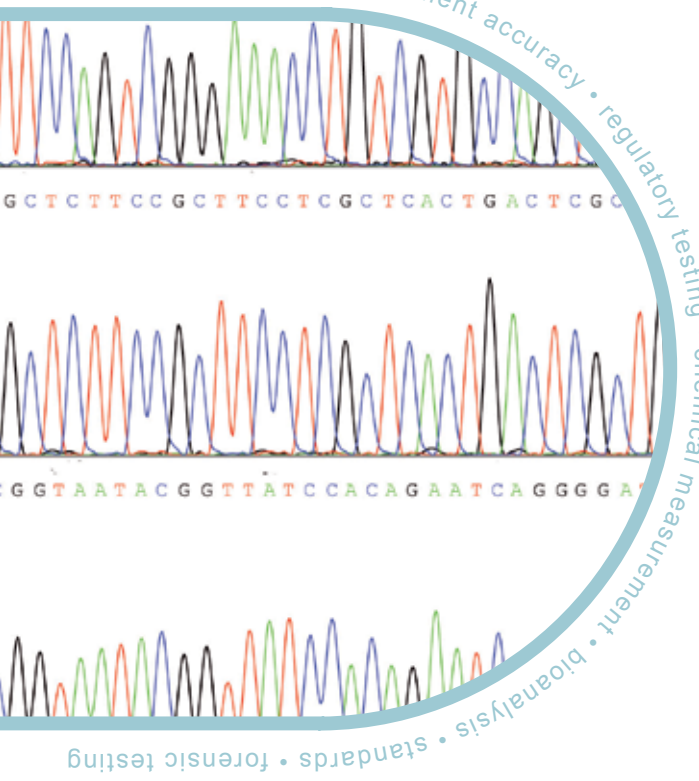
Single sample sequencing



“Ready2 run with flexible solutions and premium quality”

Please choose between our three services.

Ready2 Run

- Sequencing of **purified** PCR products or plasmid DNA
- **Pre-pipetted** primer by customers
- Fixed volume & adjusted DNA concentration (see sample specification)
- Volume: **10 µL DNA + 4 µL primer** (see sample and primer specification)
- **No repetition** of failed runs.

Flexi Run

- Sequencing of **purified** PCR products or plasmid DNA
- Submitted primer in separated tube
- > 100 universal primers available or shipment of customer primers
- **Up to 15 runs** per sample, Volume: min.15 µL (1 run) + 4 µL for each additional run of template DNA
- Adjusted DNA concentration (see sample specification)
- Storage of templates, submitted primers, and primers synthesised by LGC **for 3 months**
- **Repetition** of failed runs.

Additional service

- Primer synthesis

Premium Run

- **Quality check and DNA quantification** via agarose gel
- Complex / difficult templates / large constructs (e.g Cosmid, BACs)
- Technical support by our experienced scientific team
- > 100 universal primers available
- Storage of templates, submitted primers and primers synthesised by LGC **for 3 months**
- **Repetition** of failed runs.

Additional services

- Primer design and synthesis
- PCR clean-up / plasmid preparation

For further information please contact us on
Tel: +49 (0)30 5304 2230 or sequencing@lgcgenomics.com

Template DNA requirements

The main factors influencing read length are the DNA concentration and the quality of the template.

- Template DNA must be **free** of EtOH, EDTA, RNA, salts, genomic DNA and proteins
- Please use ultrapure water for elution
- Plasmid DNA should be present in covalently closed circular form.
- PCR products need to appear as a single band in an agarose gel and have to be purified from reaction buffer, primers and nucleotides.
- LGC recommends silica membrane-based spin column kits for template purification.

Custom primer requirements

- Length of 18 - 25 bases
- No wobble bases
- GC-content of at least 40%
- The annealing temperature must be T_m-3 and should be at least 52°C
- To calculate T_m please use T_m=4x(G+C)+2x(A+T)
- 3'- end should be G or C
- No self-hybridisation (primer dimer, loops) or binding to several sites on the template
- **For Ready2 Run:** Primer concentration **5 µM ≙ 5 pmol / µL**
- **For Flexi Run:** We recommend to ship stock solutions (100 µM) dissolved in ultrapure water. Please specify the concentration on the tube.
- Deprotected
- Without modifications (fluorophore or others)
- Free of salts and other contaminants.

Plasmid preparation (Premium Run only)

- Pour 200 - 250 µL of 1.5 % LB-medium agar, together with the appropriate antibiotic, in a tube. Avoid overfilling and bubbles in the agar.
- Inoculate single colonies using a sterile toothpick; stab the toothpick into the agar an incubate at 37°C overnight.
- We do not recommend the transfer of small amounts of a liquid bacterial culture to the tube filled with agar.

Sample specification

Please use **1.5 mL tubes** (no screw cap) for sample submission. Affix the appropriate label to each tube. Send sufficient volume of template DNA at the concentrations detailed below.

Please note: **All sample tubes must be clearly barcode labelled.** We cannot otherwise guarantee reliable sample tracking during sample processing.

Template DNA	Concentration	Volume Ready2 Run	Volume Flexi
Plasmids*	100 ng/ µL	DNA + Primer = 14 µL	min. 15 µL DNA
PCR products*			
200 - 500 bp	10 ng/ µL		
500 - 1,000 bp	20 ng/ µL		
1,000 - 2,000 bp	40 ng/ µL		

* The sequencing service for Ready2 Run and Flexi Run is optimised for plasmids up to **10 kb** and PCR fragments up to approximately **2 kb** with normal nucleotide composition.

Sequencing of PCR fragments smaller than **150 bp** is not recommended due to base calling limitations of the current technology.

For an accurate determination of the DNA concentration of your plasmid please use the LGC plasmid DNA standard which is supplied free of charge.

Sequencing results

All sequencing data are available **for three months** from the password-protected download area of the sequencing online ordering system.

We provide the chromatograms (.ab1 and .scf files) and the text sequence (.txt file) extracted by the LGC software.

Additionally, the secure download area provides **free programmes for sequence data visualisation**.

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Tel: +49 (0)30 5304 2230 or sequencing@lgcgenomics.com

Single sample sequencing service includes

FREE
- repeats for failed sequences (**Flexi / Premium Run**)
- use of our Pick-up boxes at various locations
- universal primers (**Flexi / Premium Run**)
- LGC plasmid DNA standard (simply load and compare 2 µL (100 ng) of plasmid DNA standard to your plasmid preparation)
- sample bags for secure sample shipment

- **Automated and standardised** ABI 3730 XL sequencing run with a read length up to 1,100 nt (PHRED20 quality)
- **Overnight** turnaround if samples are delivered before 10 am
- **Stored** customer-specific primers are selectable during online ordering for 3 months
- **Return** of aliquots of synthesised primers on request.

Expert advice and customer support on
Tel: +49 (0)30 5304 2230 from 8 am to 6 pm
Monday to Friday

Online ordering system

To place your order please visit our webpage and log onto our online ordering system at https://shop.lgcgenomics.com

- Register as a new user
- Choose your sequencing service, order labels, manage your data and shipment order
- Please prepare your samples according to the given requirements and send your samples in a padded envelope to us.

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Ostendstr. 25 / TGS Haus 8
12459 Berlin
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