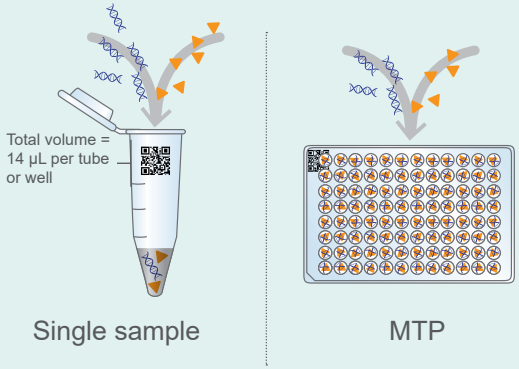
Sanger sequencing quick guide

Ready2 Run
1-shot reaction

- Turnaround time 24 hours*
- Template/primer preadjustments mandatory
- One run per sample/plate (10 µL sample + 4 µL primer (5 µM)

Purified template including primer
concentration preadjusted

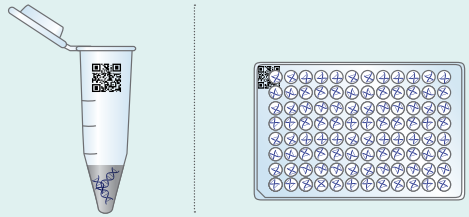


Flexi Run
n-reactions

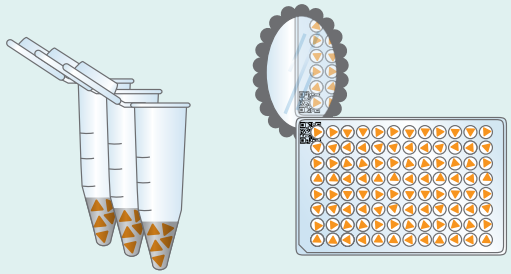
- Turnaround time 24 hours*
- Template preadjustments mandatory
- Several runs per sample/plate (in minimum 12 µL samples)
- Template/primer storage for three months

Additional service: Primer synthesis

Purified template
concentration preadjusted



Primers in tubes or mirror plate



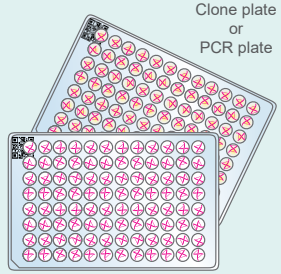
Premium Run
n-reactions

- Turnaround time ~24 hours* (except for clones)
- DNA quality check
- Protocol modifications possible
- Several runs per sample/plate
- Template/primer storage for three months

Additional services: Primer design and synthesis

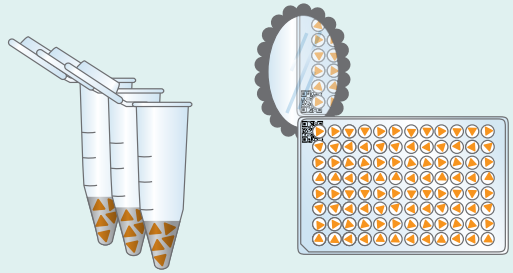
Unpurified or purified template

Unpurified template



Plasmid prep and PCR purification price included

Primers in tubes or mirror plate



* Results will be available 24 hours after recieving your sample.

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

Online ordering system

To place your order please visit our online shop and log in at **https://shop.lgcgenomics.com**

- Register as a new user.
- Choose your sequencing service, order labels (and shipping boxes for MTP sequencing), order free sample bags, manage your data and shipment.
- Please prepare your samples according to the given requirements and send your samples in a padded envelope (sample bag).

Universal barcode labels for all Sanger services

Template DNA requirements

Template conc. Ready2 Run and Flexi Run:

- Plasmid (up to 12 kb): 100 ng/µL
- PCR: 150-500 bp: 10 ng/µL
  500-1,000 bp: 20 ng/µL
  1,000-2,000 bp: 40 ng/µL

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

- 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 Biosearch Technologies recommends silica membrane-based spin column kits for template purification.

General information

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 Biosearch Technologies‘ plasmid DNA standard which is supplied free of charge.

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
- 20 pmol per reaction or stock solution (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.

For Single Sample Sequencing:

- Primer stocks should be submitted in 1.5 ml ‘safe-lock’ tubes and individually labelled with primer name and concentration.

For MTP sequencing (96-well plate):

- We accept a maximum of 6 different primers per plate - 1 primer per tube. Templates for given primers have to be arranged in blocks (i.e. lines or rows).
- Where more than 6 primers are required for a 96-well plate or a block arrangement of templates is not possible, Biosearch Technologies recommends a ‘mirror plate’ of primers .
- The mirror plate should comprise 15 µL primer solution per well (5 µM or 5 pmol/µL).
- Please use 8-strip caps to securely seal the primer mirror plate.

Free universal primers and free mass gel standard available.

For scientific advice and customer support, please contact us at:

telephone: **+49 (0)30 5304 22 30**

email: **sequencing@lgcgroup.com**

or visit our webpage: **www.biosearchtech.com**

Shipping specification

For Single Sample Sequencing:

- Use **1.5 mL tubes** preferably ‚safe lock‘ (no screw cap) for sample submission.
- Affix the barcode label to each tube. Make sure to use physically the same barcode label you choose when ordering. Send sufficient volume of template DNA - see preadjustments (page 1).
- **All sample tubes must be correctly barcode-labelled.**

For MTP Sequencing:

- Use semi-skirted or skirted plates only.
- For PCR products: v-shaped plates
  For clones: u-shape or flat bottom plates
- Please use 8-strip caps to seal the plates (preferably from the same supplier). Film or adhesive foils of plates is not sufficiently secure for shipping.
- Since an equal volume of sample will be used for all sequencing reactions, please ensure that the fragments do not vary in size by more than a factor of three (i.e. the largest fragment is not more than three times the size of the smallest). Plasmid DNA should be at a uniform concentration across the plate.

Via courier and post
LGC Genomics GmbH
Ostendstr. 25/TGS Haus 8
12459 Berlin
Germany

Alternative
Why not use the pickup box near you? Enquire at:
sequencing@lgcgroup.com

Sequencing results

Automated and standardised ABI 3730 XL sequencing run with a read length up to 1,100 bp (PHRED20 quality).

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 Biosearch Technologies software.

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

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