

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 below)
- Volume: 10 μ l DNA + 4 μ l primer (5 pmol/ μ l)
- No repetition of failed runs

Flexi Run

- Sequencing of purified PCR products or plasmid DNA
- **Up to 8 runs** per sample, Volume: 40 μ l of template DNA (minimum 15 μ l) & adjusted DNA concentration
- > 200 universal primers available
- Storage of templates, custom primers and synthesised primers by LGC Genomics (3 months)
- Repetition of failed sequences

Option: Primer synthesis

Premium Run

- Including PCR clean-up / plasmid preparation
- **Quality check and DNA quantification** via agarose gel
- Complex / difficult templates
- Large constructs (Cosmid, BACs)
- > 200 universal primers available
- Storage of templates, custom primers and synthesised primers by LGC Genomics (3 months)
- Repetition of failed sequences

Option: Primer design & synthesis in combination

Template DNA requirements

The quality of the template in addition to the required DNA concentration are the main factors influencing read length:

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

Custom primer requirements

- Length of 18 - 24 bases
- No wobble bases
- **The annealing temperature** (4 x number of G, C + 2 x number of A, T) **should be at least 52°C**
- GC-content of at least 40 - 50 %
- 3'- end should be G or C
- No self hybridisation (primer dimer, loops) or binding to several sites on the template
- Concentration of stock solution dissolved dd H₂O
20 µl of 100 µM solution
- Deprotected
- **Without modifications** (fluorophore or others)
- Free of salts and other contaminants

Plasmid

- 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 by stabbing it into the agar and 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

Label one 1.5 ml tube (no screw cap) per sequencing reaction using one label. Please send us the required template DNA at concentrations listed below.

Please note: Every template service must be labeled regardless of chosen service.

Template DNA	Concentration
Plasmids*	100 ng/ μ l
PCR products*	
200 - 500 bp	10 ng/ μ l
500 - 1,000 bp	20 ng/ μ l
1,000 - 2,000 bp	40 ng/ μ l

* The sequencing service for Read2 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 limitations of the current technology.

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

Sequencing results

The sequencing data are available from the password-protected download area of the LGC Genomics online order system.

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

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

Single Sample sequencing service includes

- **Automated and standardised** ABI 3730xl sequencing run with a read length up to 1,000 nt (PHRED20 quality)
- **Overnight** turnaround if samples are delivered before 10 a.m.
- **Free repeats** for failed sequences and multiple runs (Flexi / Premium Run)
- **Free** LGC Genomics plasmid DNA standard (simply load and compare 1 µl (80 ng) of plasmid DNA standard to your plasmid preparation)
- **Free** use of our Pick-up boxes at various location
- **Free** universal primers
- **Stored** customer specific primers are selectable during online ordering
- **Return** of aliquots of synthesised primers on request
- **Free** sample bags for secure sample shipment
- 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.

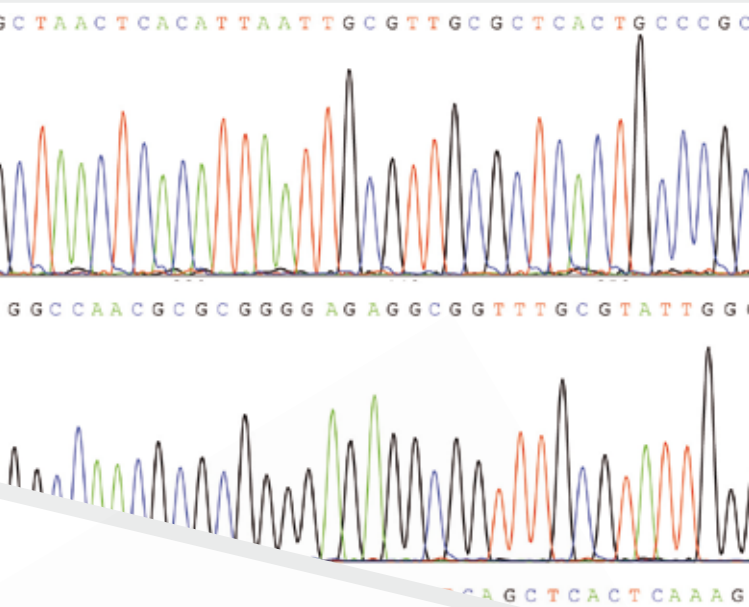
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