

TubeSeq Service

Sample Submission & Ordering Guide.



Sample Preparation & Submission.

- Templates should be provided in a volume of **15 µl for 1–4 reactions resp. 20 µl for 5–8 reactions** with either of the concentrations given in below table.
- If you send us **premixed samples**, add **2 µl of primer** with a concentration of 10 pmol/µl (10 µM).
- The **total volume of the premixed sample must be 17 µl**.
- Use **1.5 ml safe-lock tubes** for your samples and primers.
- Do not tape or wrap tubes with parafilm. Safe-lock tubes offer perfect sealing and evaporation protection.
- **Label your sample tubes** with our TubeSeq Labels or Tube Labels.
- Use our Tube Labels for your enclosed primers.



Sample concentration & volume

Sample type	Product length	Sample conc.	Vol. 1–4 rct.	Vol. 5–8 rct.
Plasmid DNA	<30 kbp	50–100 ng/µl	15 µl	20µl
BAC/PAC/Cosmid DNA	30–200 kbp	200–1000 ng/µl	15 µl	20µl
Purified PCR Products	150–300 bp	1 ng/µl	15 µl	20µl
	300–1000 bp	5 ng/µl	15 µl	20µl
	1000–3000 bp	10 ng/µl	15 µl	20µl
Unpurified PCR Products	150–300 bp	4 ng/µl	15 µl	20µl
	300–1000 bp	10 ng/µl	15 µl	20µl
	1000–3000 bp	20 ng/µl	15 µl	20µl

Quantify your template concentration via agarose gel or a photometer to ensure accurate results.

Sequencing Primers.

Optimum primer conditions

- The optimum primer length is between 16-25 bases.
- Primer melting temperature (T_m) should be 50-62 °C.
- The GC content of the primer should be 35-60 %.
- Ideally one G or C should be located at the 3' primer end.
- The number of 3' Gs or Cs should not exceed 2 Gs or Cs.
- If possible, avoid >3 identical bases in a row in the sequence.
- Primers must not contain phosphorylation or fluorescent dyes.

Use our free **Sequencing Primer Design Tool** to design the optimum sequencing primers.

SEQUENCING PRIMER DESIGN

Assay Design

Primer Name *

Target Sequence: *

>CATH_RAT
GTACGACGGAGTGGTTATAAGATGGGAAATCGGATACAGATGAAATTGTGGATCAGGTGCAAAA
GTCGGCAGATATCGTTGAAGTCATAGGTGATTATGTTCAATTAAAGAAGCAAGGCCGAACTAC
TTTGGACTCTGTCCTTTTCATGGAGAAAGCACACCTTCGTTTCCGTATCGCCCGACAAACAGA
TTTTTCATTGCTTTGGCTGCGGAGCGGGCGGCAATGTTTCTCTTTTAAAGGCAGATGGAAGG
CTATTCTTTGCCGAGTCGGTTTCTCACCTTGCTGACAAATACCAAATTGATTTTCAGATGAT

Length: 700 bases

Delete Input

Design Parameters

Sequencing Direction: ☒ Forward ☐ Reverse

Target Region: From: To:

Number of Primers (output)

* mandatory

Design Primers

Primer concentration & volume

- Exactly **10 pmol/μl** primer concentration is required per sequencing reaction.
- Each primer must have a **total volume of 15 μl** (double distilled water or 5mM Tris-HCl).
- **5 μl of primer volume** is required for every additional sequencing reaction.
- Concentration of **primers with wobble bases** must be calculated according to the following formula:

$$n^X \times \text{Conc}_{\text{Primer}}$$

n = number of bases within a wobble according to IUPC code; X = number of wobbles within the primer sequence. E.g. 1 V (AGC) = 31 x 10 pmol/μl; 2 V (AGC) (AGC) = 32 x 10 pmol/μl

Shipping Options.

Use our **metal sample box** or **tube bags** for secure shipping of your samples.



Drop it in your nearest **DropBox** for **free pick-up**.



Alternatively use our **Sample Bag (padded envelope)**, which you may have ordered online already.



If you have a postal service in your institute or company, **address stickers** with our sequencing lab address are available online, free of charge.



Online Ordering.

Go to the **TubeSeq Service** order page on eurofinsgenomics.eu and select either single input or file-upload as entry format.

The screenshot shows the 'TubeSeq Service' 'INTRO & INFO' section. It has two tabs: 'Select your entry format' (active) and 'Define your sequencing reactions'. Under 'ENTRY FORMATS', there are four radio buttons: 'Single Input' (selected), 'File-Upload', 'Reuse stored plate samples', and 'Reuse stored tube samples'. To the right, there is a 'Number of Samples' dropdown menu set to '2'. Below this, a note states: 'Please select the number of samples you want to order and press "Next". You can always add more or leave lines empty on the next page.'

On the next step you can:

- Select your **sample type** and **additional service**.
- Select "premixed" when the DNA already contains the primers.
- **Add primers** which you want to **enclose** or which should be **synthesised** by us.
- When you selected single input as entry format, specify the **additional reactions per sample** by clicking on the "plus icon"; **up to 8 reactions** per sample are possible.
- If you are uploading a sample sheet, the additional reactions with barcodes and primers are prepopulated.
- **Enter the first barcode you stucked on your tube**; the "auto-fill" icon are filling the next barcode fields sequentially.

The screenshot shows the 'Define your sequencing reactions' section. It has two tabs: 'Select your entry format' and 'Define your sequencing reactions' (active). On the left, there are two sections: 'SEND A PRIMER' and 'ORDER A PRIMER'. The 'SEND A PRIMER' section has fields for 'Primer Name' (up to 16 characters), 'Tube Label (optional)' (10-digit code), and 'Conc' (10 pmol/ul), with an 'Add primer' button. The 'ORDER A PRIMER' section has fields for 'Primer Name' (EP_rev), 'Sequence' (atgtgacactgactgactg), 'TubeSeq Label (optional)' (Aud0001722), and a checkbox for 'Please store for 1 year', with an 'Add primer' button. The main area is titled 'SAMPLE TYPES & REACTION SETUP'. It has a 'Define Your Samples' section with 'Sample type' (Plasmid selected), 'Additional Service' (Please select), and 'Sample specifications' (Premixed). Below is the 'Define Your Reactions' table with columns: No., Barcode, Sample Name, Sequencing Primer, and Options. The table contains 4 rows of reactions. At the bottom right, there is an 'Add 1 more samples' link and an 'Add to Cart' button.

No.	Barcode	Sample Name	Sequencing Primer	Options
1	Aud0001730	demo1	M13 uni (-21)	+ [icon] [icon] [icon]
2	Aud0001731		M13 rev (-29)	[icon] [icon] [icon]
3	Aud0001732	demo2	SP 6	+ [icon] [icon] [icon]
4	Aud0001736		EP_rev	[icon] [icon] [icon]

Save your tube sequencing reactions in your cart.

Select your **DropBox location** during checkout or in advance under "**My Dropboxes**".

DROPBOX LOCATION

Your nearest DropBox for free shipping

DropBoxes within 5 km around your location

Karte

Satellit

Eurofins Genomics GmbH

X

Anzinger Str.

Gewerbegebiet Nord

Aldi Süd

Dr. Collin

Autohaus Ebersberg GmbH & Co.

TUV SÜD

Service-Center Ebersberg

Google

Kartendaten © 2017 GeoBasis-DE/BKG (©2008), Google

Nutzungsbedingungen

Fehler bei Google Maps melden

Distance	Address	Pickup Time
<input checked="" type="radio"/> 0.35 km Overnight unselect	Eurofins Genomics GmbH Sequencing lab Anzingerstrasse 7a Ebersberg Bayern Germany 85560	Mon - Fri 19:00

In case you do not have a DropBox location nearby you, make use of our alternative shipping options.

Select your preferred **analysis options**, result **file types** and **email notifications** for each order during checkout or any time under "**My Preferences**".

SEQUENCING PREFERENCES

Advanced Analysis Options (not for Mix2Seq)

☒ IUPAC bases for heterozygous analysis
 ☐ Sequence should contain "N" for unclear bases

Quality value for sequence clipping:
 ☒ Q20
 ☐ Q30
 ☐ Q40

Sequencing Result Info

☒ Send email to ursuladoer@eurofins.com as soon as my results are available
 ☐ Include FASTA sequences directly in this email (plates excluded)

The result email should also go to:
 ☐

Desired File Types

☒ *.ab1 trace files - Original ABI trace files including all available raw data information
 ☒ *.scf files - Trace files with base calls and quality values
 ☒ *.pdf quality report - Trace file with quality scores [Example]
 ☐ *.phd.1 files - Text file which contains base call and quality Information [Example]
 ☒ *.seq files - Text file with FASTA sequences [Example]

TubeSeq Label Setting

☐ My TubeSeq Labels (prepaid barcodes) can non-restrictively be used by others.

Sequencing Lab Notifications

☒ Inform me per email when my sample material arrives.
 ☒ Inform me per email when my sample material has not arrived after 10 days.
 ☒ Inform me per email when my TubeSeq Labels run below: