

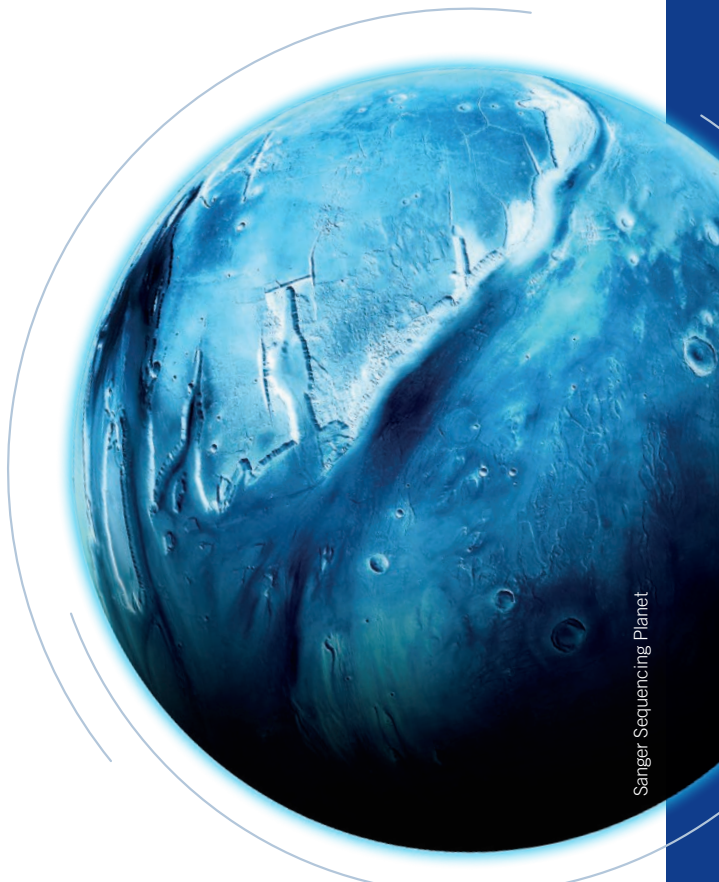
Online Ordering.



Go to the Direct Colony Sequencing order page on euofinsgenomics.eu and select the entry format.

Specify the material you want to send in on the second step. Select the barcode which is stuck on your plate in the first dropdown. Additional reactions can be paid with PlateSeq Coupons and redeemed in the 2nd to 4th dropdown.

On the last step you have the possibility to modify and finally check your plates. Confirm your sample plates by adding them to your cart.



Sanger Sequencing Planet

Direct Colony Sequencing Service

SAMPLE SUBMISSION & ORDERING GUIDE.

Sample Preparation & Submission.



- Order either our **PlateSeq Kit Colony** or the **ColonySeq Plate** first. (They contain the appropriate buffer)
- After receiving the plate, shortly centrifuge the received plate to avoid the liquid on the foil. Carefully remove the sealing foil from the plate.
- Grow your **E.coli** on agar plates long enough to have a colony diameter of at least 1 mm*.
- Use a toothpick to take as much of the **E.coli colony** as possible and inoculate into our plates. Swirl the toothpick for some seconds to transfer as many cells as possible into the wells.
- In case of **bacterial suspension** use a pipette to **add 5 µl** of the liquid culture in each well.
- Well H12 should be kept empty for internal quality control
- Seal your plates using **8-cap strips** to prevent material loss (8-cap strips are provided along with each PlateSeq Kit Colony).
- Plates can be sent / provided at **ambient temperature** to our sequencing lab.

Where to send samples

BY DROPBOX

There are many DropBoxes installed throughout Europe for free sample shipment.

BY POST:

Eurofins Genomics Sequencing GmbH
Gottfried-Hagen-Straße 20
51105 Köln

*We recommend to create a replica of your E.coli colonies right after growing. Use the same toothpick to inoculate LB media containing antibiotics to produce an overnight culture or streak out on a fresh agar plate.

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.

SEQUENCING PRIMER DESIGN

Assay Design
Primer Name * Demo

Target Sequence: *
ATACAGTCCATTCCGGAGCCCGCCAGAGTCTTCTGGAGACAAAATAAGTCAAGGCACATG
AGCTCTGAAGAAATTTACCACTCTTTGTTAATAAATACAAAGAAAGGTCAAGAGCACTGGA
TTATCTGCTTCTAGAGGCTTACGAAAGACTGATTAATGAATTCAGATTCGCTATGCTCTT
GATCTCTGGAGCTTATACCAAAATCTCTGTAAGAGAGGGGATTTAGTGGAGCCAAATGAAA
AAGCGGCTCTCTGATCAGAGCGAAGCGAAGCGGATATTTCCAGCCCTTCAGAAACCGTGT
CATGTTCCGATCCAGATCATCACAGGGGCTGTTGTTGCTTCTCAGACAGGGGCTCTTGG

Length: 700 bases

Design Parameters Open / Close
Sequencing Direction: Forward Reverse
Target Region: From: 1 To: 500
Number of Primers (output): 2

* mandatory

Results

No.	Main_Strand	Sequence	Begin	End	Order
1	Demo_1_forward	GTACGAGGAGTGTATAAGATGG	1	24	<input type="checkbox"/>
2	Demo_2_forward	AGTCGGTTTCTCACCTTGCTG	271	291	<input type="checkbox"/>

Use our free **Sequencing Primer Design Tool** to design the optimum sequencing 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 5 mM 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