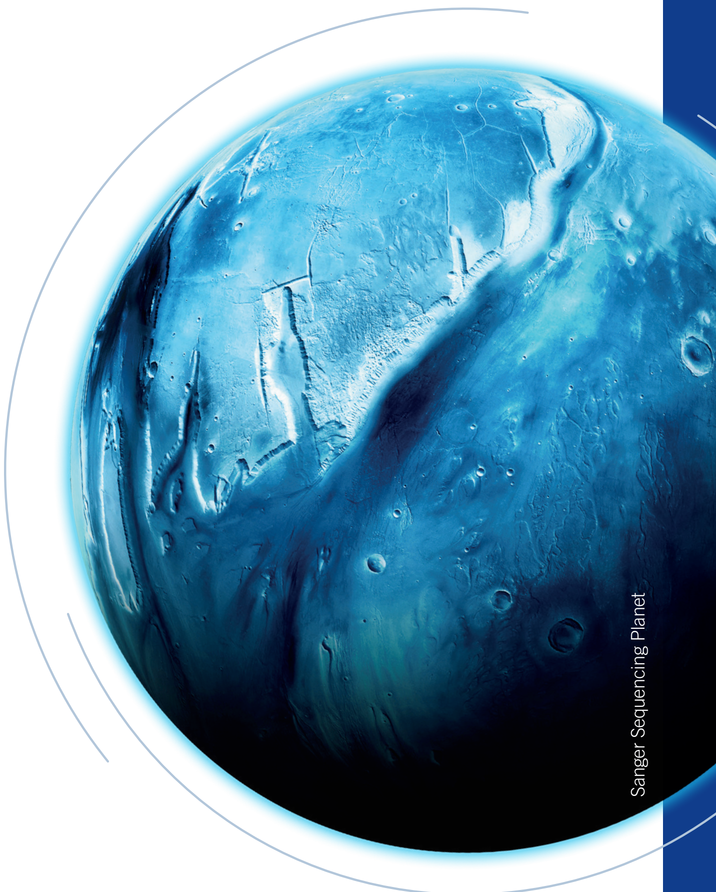
Online Ordering.

eurofins | Genomics

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

Specify the material you want to send in on the second step. Select the barcode which is sticked on your plate in the first dropdown. Additional reactions can be payed 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.



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 (Tm) 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.

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: $n^X \times Conc_{Primer}$

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