GeneCore DNA-Seq Checklist

- Read the requirements carefully
- Project discussed with GeneCore
- Samples registered with GeneCore
- Samples QCed as described in Section 2
- Samples normalized as described in Section 3
- What is the sample number?
- **Fewer than 24 Samples**:
    - Use **1.5 mL Eppendorf LoBind Tubes** (no other tube types) as per Section 1.1.1.
    - Label tubes on the lid.

- **24 Samples or More**:
    - Use **Full-Skirted Eppendorf TwinTec Plates** (no other plate types) as per Section 1.1.2
    - **Well H12 Empty** as per Section 1.1.2
    - Arrange samples in columns (Section 1.2)
    - Seal the plate securely with the recommended Seal (Section 1.2)
    - Clearly label the skirt of the plate (not on top or on the seal)
    - Provide an Excel file with plate positions and corresponding sample names

- Store samples in a sample box, clearly labeled with your group and user name.

General Requirements for:

NEBNext FS Ultra Express DNA

NEBNext Ultra Express DNA

NEBNext EM-Seq

Important:

Do not send your samples until they are registered in our system. To ensure safe arrival, wait for confirmation of registration.

Please read the following information carefully to ensure your samples are processed quickly and accurately.

Note: Any modification of these requirements is going to extend turn-around time.

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1. Do I Need Tubes or Plates?

1.1.1 Fewer than 24 Samples

- Use **1.5 mL Eppendorf Lo-Bind Tubes**. Avoid tubes from extraction kits, as their lids may break off, risking contamination.
- Label each tube on the lid in a simple, human-readable format (e.g., 1; 2; 3; 4 …) using a waterproof marker. Avoid using labels wrapped around the tube.

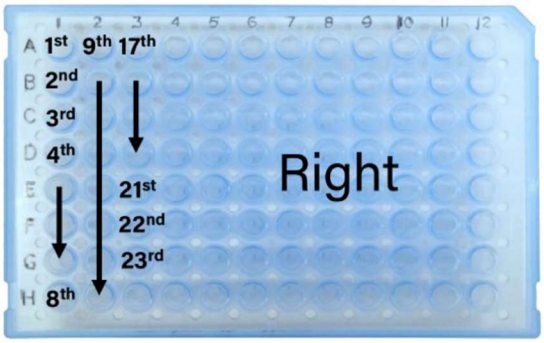
- **Important**: Ship samples in a 1.5 mL sample box, labeled with your group and user name. Avoid placing samples in a plastic bag.

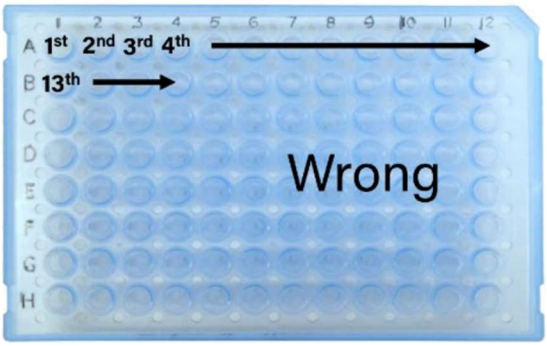
1.1.2 24 Samples or More

- Use a **Full-Skirted Eppendorf TwinTec Plate** (Order # 30128648). Please contact us if you need these plates.
- Avoid using other plate types to streamline sample processing.
  Alternative plates require extra steps, significantly increasing turnaround time.
- **<u>Leave Well H12 Empty, it is used for QC purposes and will not be processed</u>**

1.2 Sample Arrangement in a Plate

- Arrange samples in **columns** (not rows).





- Once samples are normalized and arranged, seal the plate with a heat-sealer or the aluminum seal (Beckman order # 538619). Contact us if you need these seals.
  This seal is stable at -80°C.
- Avoid transparent qPCR seals, which are going to slip off during shipping, risking contamination.
- Label the plate on the **skirt**, not on the top or seal.
- Provide an Excel file with plate positions and corresponding sample names.

Example:

Location (Well ID)	Sample Name
A1	Sample1
B1	Sample2
C1	Sample3
D1	Sample4
E1	Sample5
F1	Sample6
G1	Sample7
H1	Sample8
A2	Sample9
B2	Sample10
C2	Sample11
D2	Sample12
.	.

- **Important**: Place the plate in a box labeled with your group and user name for safe shipping. Avoid using a plastic bag.

2. Quality Scores

Please quantify your samples using a fluorometric, DNA specific method to reliably quantify your samples, like Qubit.

Avoid Nanodrop especially for very low (below 10 ng/µl) or very concentrated samples (above 200ng/µl). Due to the nature of the assay, it measures anything within the wavelength of DNA and is therefore not recommended to be used.

For DNA Samples, if possible, please provide a Gel-Image of your samples, to ensure the integrity and quality of your DNA.

Keep in mind that the Quality of your data is going to represent the quality of your sample. If you do have any questions regarding QC results do not hesitate to reach out to us.

If you need assistance with QC, please contact us before registering your samples.

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3. Sample Requirements

- Regardless of sample number, normalizing to a specific concentration significantly speeds up processing.
- **Do not use Nanodrop values** for normalization. Use **Qubit values**. See Section 2 for QC details.
- For any questions on QC results, feel free to contact us.

Sample Volumes for Sequencing Types:

Regardless of which library preparation type you choose, please normalize your samples to the following conditions:

- 20 ul of sample at a concentration of 25-100ng/ µl
    - If limited to, e.g., 30ng/µl for one of your samples, normalize the entire sample set to this amount.

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4. Frequently Asked Questions

- **What happens if a library prep fails?**
    - In case of dropouts, we offer a one-time repeat for the sample.

- **Can I use Nanodrop to normalize my samples?**
    - We recommend not to use Nanodrop to quantify your samples. Due to the nature of the assay, it measures anything in a specific wave length. That means it tends to be inaccurate in case of impurities, we therefore recommend using an assay which is specific to RNA, like Bioanalyzer or Qubit.

- **How much material should I sent?**
    - Sent as much as possible, within the range of the desired Method.

- **I have some samples in my large sample set that have a low concentration, how should I normalize? Should I dilute all the samples to the lowest sample?**
    - If you only have a few samples that are lower concentrated, we recommend not to dilute all of the samples to the lowest concentration. Consider extracting again to improve the yield for this sample or even excluding that sample. Diluting the majority of your sample set to the lowest sample increases the risk of multiple drop outs the lower you go.
    - If an entire subset is concentrated lower than the other samples, split the set and normalize one set as low input samples and the other as regular. This way we minimize the risk of dropouts and avoid unnecessary delays.
    - **Contact us to confirm your setup in advance, it will significantly reduce the turn-around time**