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We understand that for some projects adhering to all of these requirements may not be possible. If this is the case, please discuss any and all deviations from these requirements with MOC before submission. Failure to adhere to these requirements or get approval from MOC to deviate from any of them may result in poor results that will not be corrected at MOC's expense.

Before submitting samples please review the Terms of Service and follow the guidelines below.

RNA for RNAtag-Seq

MOC offers a **standard** or **streamlined** version of the RNAtag-Seq-TS library construction protocol (Shishkin et al., *Nature Methods* 2015; Bhattacharyya et al. *Nature Medicine*, 2019). The **streamlined** version shortens turnaround time, but requires RNA extraction to be conducted in a way that excludes tRNAs. Our **standard** protocols for extraction from culture or mouse stool using the DirectZol kit does not exclude tRNAs can be found here. Using a decreased concentration of ethanol in the DirectZol protocol cited above (highlighted in red in protocol) will yield tRNA-excluded RNA, as will the standard Qiagen RNeasy protocol.

1. **Sample concentration and volumes**
    a. *Standard protocol*: 500ng total RNA in 15uL RNase free water (~33ng/uL) with 1uL of Superase-IN or other RNase inhibitor
    b. *Streamlined protocol*: 250ng tRNA-depleted RNA in 5uL RNase free water (~50ng/uL) with 0.5uL of Superase-IN or other RNase inhibitor
        i. Please select streamline protocol if you've used Direct-Zol extraction using 40% ethanol or if your kit excludes small RNAs (<100 bp).

2. **Sample quality**
    a. Please confirm the integrity of your RNA by electropherogram or electrophoresis before submitting samples. If you have any reservations if your samples meet the minimum quality requirements, please upload QC images (e.g. electropherograms of several representative samples) to your project's google drive directory and ask MOC to review it.
    b. QC can be conducted at MOC for a fee. If MOC will be performing QC, please provide 2uL aliquots of ~10% of your samples for spot-checking in a separate plate or strip tubes. This RNA doesn't need to be normalized and shouldn't contain an RNase inhibitor, but please provide concentrations.
3. **Pool size**
    a. 24-36 samples.
    b. Whenever possible please group samples in the same pool by row rather than by column.

    MOC can process pools/batches of 12-23 samples for a surcharge. Be aware that all members of a pool will undergo rRNA depletion together so significant differences in rRNA:mRNA ratios (such as large difference in phase of growth or exposure of some samples to transcriptional inhibitors) among samples in the same pool will likely translate to differences in total sequencing read counts per sample. If this is a concern for your project, please discuss a pooling strategy with MOC following submission of your Key File but before approving the project checklist.

4. **Sample Submission**
    a. Samples should be submitted in Axygen 96 well PCR Plates (47743-953) microplates at -80°C i.e. on dry ice sealed with Axygen PCR-AS-200 Foil Seal. Alternative plates and seals can be used but we have found the plates above to be the most reliable. Plates will be inspected for leaks that, if found, **will require samples to be resubmitted.**
    b. Group samples in the same pool **by row rather than by column.**
    c. Whenever possible, please provide two replicate aliquots in separate plates in the unlikely case that library construction needs to be repeated. In case of sample dropouts, we will only attempt to remake libraries if a backup plate is provided.
    d. Ensure **all** plates are labeled with a unique ID and that all sample information has been linked to the correct plate and well IDs in the **MOC Key file**.

5. **Reference file submission**

    MOC offers RNA-Seq analysis that includes alignment of reads to a single reference genome per sample, generation of tables containing reads per genomic features such as protein coding genes, non-coding RNAs, and intergenic regions for all samples, and

differential expression analyses using DESeq2 and edgeR. For these analyses, collaborators must upload genome sequence files (with .fna suffix, in fasta format) and annotation files (with .gff suffix, in gff format) and link samples to these references in the MOC Key file**.** Reference files can be obtained through RefSeq or GenBank FTP. Reference files should be uploaded in the Reference_Files subdirectory in the MOCP Google drive directory. Please make sure that:

1. The names of the files up to the suffix (.fna or .gff) are identical to each other and to the names entered in the "Bacterial_reference" field in the MOC Key file.
2. If using custom (i.e. non-RefSeq or GenBank) references, please ensure that each fasta header is unique and matches EXACTLY to its cognate replicon identifier (first field for every feature) in the .gff file

If using custom (i.e. non-RefSeq or GenBank) references, please email MOC after uploading the files to ensure they are compatible with our analysis pipeline. Not following these instructions will lead to (potentially significant) delays in data delivery and possibly additional project management fees. If any of this is unclear, please reach out to your project manager during submission to ensure that everything is read for analysis when sequencing is complete.

DNA for NEBNext WGS

1. **Sample concentration and volume**
    a. 30 uL of purified DNA at ≥2ng/ul in nuclease free water or 10mM Tris Cl [pH 8.5] Any EDTA present will inhibit initial reactions.

2. **Sample quality**
    a. Please confirm DNA integrity by electropherogram or electrophoresis before submission and upload any QC data collected to the project Google drive before sample submission.
    b. If the DNA is derived from stool please clean it using a 2x SPRI to remove inhibitors to downstream enzymatic reactions.

3. **Batch sizes**
    a. 24 samples/batch.

4. **Submission requirements**
    a. Samples should be submitted in Axygen 96 well PCR Plates (47743-953) sealed with Axygen PCR-AS-200 Foil Seal at -80°C. Alternative plates and seals can be used but we have found the plates above to be the most reliable. Plates will be

inspected for leaks that, if found, **will require samples to be resubmitted.**

b. Please plate samples by row, not column, leaving the last 3 wells (H10-12) empty on each plate.
c. When possible, please include 1-2 additional plates of replicated aliquots to expedite the repeating of library construction should that be necessary.
d. Ensure **all** plates are labeled with a unique ID and that all sample information has been linked to the correct plate and well IDs in the **MOC Key file**.

DNA for ITS

5. **Sample concentration and volume**
    a. 20 - 50 uL of DNA in nuclease free water.
    b. The minimum concentration required is 2 ng/ul, however the success of the protocol depends not only on the total amount submitted, but the proportion of that DNA that is fungal. We recommend you sending the highest concentration possible.

6. **Sample quality**
    a. Please confirm DNA integrity by electropherogram or electrophoresis before submission and upload any QC data collected to the project Google drive before sample submission.
    b. Please indicate the expected yield of fungal DNA (normal or low)
    c. Please clean DNA using a 2x SPRI and elute in nuclease free water to remove inhibitors to downstream enzymatic reactions.

7. **Batch sizes**
    a. 92 samples/batch
        i. Pricing is **per batch**

8. **Submission requirements**
    a. All samples must be in nuclease free water or 10mM Tris Cl [pH 8.5]. Any EDTA present will inhibit initial qPCR.
    b. Samples should be submitted in Axygen 96 well PCR Plates (47743-953) sealed with Axygen PCR-AS-200 Foil Seal at -80°C. Alternative plates and seals can be used but we have found the plates above to be the most reliable. Plates will be inspected for leaks that, if found, **will require samples to be resubmitted.**
    c. When possible, please include 1-2 additional plates of replicated aliquots to expedite the repeating of library construction should that be necessary.

d. Ensure **all** plates are labeled with a unique ID and that all sample information has been linked to the correct plate and well IDs in the **MOC Key file**.

General sample submission and shipping guidelines

Please follow this link for all shipping and delivery guidelines. Please note **MOC can only receive samples Tuesday-Friday excluding holidays**, so please ship between **Monday - Wednesday** to account for possible shipping delays.