This document provides generic guidelines which are applicable for most projects.
Other methods and protocols than those cited below are available.
Do not hesitate to email us in case of doubt or non-standard requests

# General information

## Starting amount

Whenever possible, we recommend you **provide twice the required amount** so that the procedure can be repeated in (rare) case of failure.

All samples must be **normalized to the same starting concentration**.

## Tube / Plate formatting

Tubes must be named with a clear code on the cap **and** the sidewall. We recommend numbering, to limit the risk of misunderstanding your writing. Avoid stickers since they usually do not resist freezing.

Tubes must be provided in a bag (can be found at the facility).

You may provide samples in MW96 plates. This may reduce processing time.
Plates must be filled **column-wise** (A1 is #01, C1 is #03 and A3 is #17).
Use a cold resistant foil and label the plate with date and name on side (not on the foil).

## Nucleic acid quantification

Quantification with Nanodrop is sensitive to non-nucleic acid contaminant.

We recommend using a fluorimetric based method (e.g. QubIT) for concentrations below 25 ng/uL. Nanodrop is unreliable for concentrations below 10 ng/uL.

## DNAse / RNAse treatment

While not always mandatory (see details below), it is good practice to perform:

- DNAse treatment on extracted RNA
- RNAse treatment on extracted DNA.

## Buffer

Samples must be free of organic contaminants (phenol, ethanol) and **free of EDTA**
Optimal buffer is 10mM Tris-HCl pH 7.5-8.0, Qiagen EB buffer.
Pure water (RNAse/DNAse free) can also be used, and is actually preferred for RNA.

# DNA sequencing

## Nextera DNA Flex (Illumina DNA Prep)

Least expensive and fastest protocol.

| Input amount | Volume |
|---|---|
| Minimum 1 ng gDNA<br>Optimal 100-500 ng<br>No normalization requires if > 200 ng | 32 uL |

DNA must not be too degraded (fragments >1kb, without much signal below 150 bp)
Amplicons must be > 150bp.

## DNA library prep

DNA must not be too degraded (fragments >1kb).

| Input amount | Volume |
|---|---|
| Standard: 100 pg - 1'000 ng<br>PCR free: > 100 ng | 12 uL |

PCR-Free libraries sequencing yield is difficult to control and cannot be guaranteed without running a small-scale sequencing run on our MiSeq. If possible, we recommend using the Nextera DNA Flex kit, which is only 5 PCR cycles if starting with > 100 ng DNA.

## Exome sequencing

We mostly use IDT or Twist panels.

| Input amount | Volume |
|---|---|
| 100 pg - 1'000 ng | 12 uL |

Input requirement (quality and quantity) can be flexible. Please enquire but the standard input recommendation is the one shown above.

The procedure works fine with FFPE samples, but details must be discussed ahead.

## SeqWell High Throughput DNA library prep

Samples must be provided "normalized" and arrayed in MW96 plates
As for most library types, the buffer should **NOT** contain EDTA

| Input amount | Volume |
|---|---|
| plexWell: 10 ng (tolerance ≈ 3-fold) | 6 uL |
| purePlex: 5 ng-50 ng | 5 uL |

seqWell are processed by batch of samples (full batches are billed):

- plexWell: batches of 48 samples
- purePlex: batches of 24 samples

# RNA sequencing

## Messenger RNA analysis

Messenger RNA is targeted through its polyA tail.
It is the cheapest RNAseq approach.
It is compatible with any organism with polyadenylated messenger RNA.

mRNA sequencing imposes an **RNA quality RQN/RIN of at least 8.0**.

TruSeq mRNA

The most robust protocol we propose.

| Input amount | Volume |
|---|---|
| 50 ng - 1'000 ng | 52 uL |

Illumina stranded mRNA

Nice alternative to TruSeq mRNA when less RNA is available.

| Input amount | Volume |
|---|---|
| 25 ng - 1'000 ng | 27 uL |

Takara SMART-Seq v4

Ultra-low input option, but more expensive.
This kit does not keep the RNA strand information.

| Input amount | Volume |
|---|---|
| 10 pg - 10 ng | 27 uL |

QC is generally difficult with such low input concentration.

## Total RNA analysis

No polyA selection is performed, but it generally requires **ribosomal RNA removal**.
A total RNAseq experiment is roughly twice the budget of an mRNA design (library preparation + sequencing).

It is compatible with lower quality partially compromised RNA
No definite RIN/RQN requirement, but DV200 must be > 50% (FFPE compatible).
It is highly recommended that all RNA samples have the same quality.

Ribosomal RNA depletion is not readily available for non-model organisms (see details). For Illumina kits, it is possible to use Qiagen FastSelect depletion modules to deplete total RNA from unwanted RNA (can be customized).

Dilution in H2O is preferred.
**DNAse treatment is MANDATORY**.

Truseq stranded RNA with Ribo-Zero depletion

Most robust protocol for total RNA input.

| Input amount | Volume |
|---|---|
| 100 ng - 1’000 ng | 12 uL |

Ribosomal depletion (capture) is officially validated for human, mouse, and rat.
We could also get decent depletion from some insect samples.

Illumina Stranded Total RNA Prep with Ribo-Zero Plus

Nice alternative to TruSeq Total RNA when less material is available or broader depletion is required.

| Input amount | Volume |
|---|---|
| 1 ng - 1’000 ng | 13 uL |

The Ribozero PLUS (RNAseH targeting) is validated for depletion of abundant transcripts from multiple species including: human cytoplasmic & mitochondria rRNA, mouse rRNA, rat rRNA, bacteria Gram +/- rRNA, human beta globin transcripts.

Takara SMART-Seq (or SMARTer) strand kit

Ultra-low input option.

| Input amount | Volume |
|---|---|
| 10 pg - 10 ng | 10 uL |

Ribosomal RNA depletion is best performing on human samples. Mouse is also working, though to a lesser extent.