FAQ LACEseq

How much RNA input is needed?

At least 5 ng of purified Ribosome Protected Fragments (RPFs) is needed, quantified using miRNA QuBit assay.

Usually 5 ng of RPFs are obtained with 1 RiboLace pull-down, corresponding to a numbers of Hela cells ranging from 100.000 to 1.000.000. The efficiency of RPFs recovery depends on the cell protein synthesis rate.

If you are using sucrose cushioning or sucrose gradient fractionation you should start from at least 5.000.000 Hela cells.

Can LACEseq be used for microRNA sequencing?

No, because miRNAs are known to have a 3' OH end. LACEseq is able to capture and sequence only RNA fragment with 3' phosphate end.

How much linker should be used in the first ligation reaction?

From unpublished data, it is known that only about 50% of the fragments between 25 and 35 nt are actual nuclease generated fragments, i.e RPFs with a 3' phosphate end. We recommend to use 1:2 molar between linker and fragments

For example:

RPF (ng)	RPF (pmol)	Linker (pmol)	Molar ratio (linker:RPF)
10 ng	1 pmol	0.5 pmol	1:2
20 ng	2 pmol	1 pmol	1:2

Does your linker already have ILLUMINA adapter sequence (SP1, SP2, P5, P7)?

No. We add specific ILLUMINA sequence during the 2-steps of PCR.

Do I have all of the kits necessary to do ribosome profiling if I have RiboLace Module 1, PAGE Extraction Kit, LACEseq and iUDI plate LACEseq?

Yes. You only need basic lab instruments listed in the protocols and a few additional common reagents.

What are the compatible ILLUMINA indexing primers?

TruSeq Compatible indexing primer or Illumina TruSeq HT (currently known as TruSeq CD).

What kind of index (i5, i7) are included in iUDI plate for LACEseq?

IDT for Illumina – 8 nt.

Can I use LACEseq also for RPFs generated with other ribosomal profiling protocols apart from RiboLace?

Yes, as long as you don't dephosphorylate the samples prior to library prep.

Do I have to perform T4 PNK dephosphorylation prior to LACEseq library preparation?

No

What sequencing platform is suitable for LACEseq?

We've tested on Novaseq6000, HiSeq2500, NextSeq550 and MiSeq so far.

Can you recommend a data analysis pipeline for Riboseq data?

Yes, we have our own pipeline. The code is available here.

Regarding the positive control reaction mentioned for the first time the kit is used, should it be used from LACEseq step 1 until the final purification of the library? Or even starting at the previous RPFs PAGE extraction?

Yes, Positive CTRL can be used till the end of the protocol (it's a way to check that the kit is working properly and can be also useful as a size marker for library gel purification). The Positive CTRL is already purified and you can start directly with the PK reaction.

In LACEseq step 2 (ligation), the note under the table mentions the ratio between RPFs and Linker_MC, for which it says that the RPFs are quantified by miRNA Qubit. My question is: do we have to quantify again after step 1's purification or do we calculate the ratio based on the quantification performed after the PAGE extraction (before step 1)?

No, you may use the initial RPFs quantification to calculate the ratio here.

After PCR 1, the sample is eluted in 40uL after purification with AMPURE XP beads, only 24uL are used for PCR2. Why do we elute in 40uL if only 24uL are needed? Will we have too much product? Do we keep the rest in case it's needed?

Yes, you can keep the remaining part for an extra PCR 2 (if something goes wrong or you need to increase the library concentration).

In case I need to wait a day or 2 for the NGS sequencing (depending on the availability of the NGS facility, etc), should I keep the library at -80ºC during that step 9 or can I finish the gel extraction and then freeze the final purified library. In the second option, should it be frozen at -80 or -20ºC?

You can finish the gel extraction and then store the libraries eluted in TR buffer at -20°C until you are ready with the sequencing run.

For the iUDI primers, I understood that samples can be pooled in groups of 4. But what if we have less samples?

Please use the ImmaginaExampleImmagina_SampleSheet file to input the primer pairs of your experiment. It is recommended to ask your NGS Facility and verify the sample sheet with them before starting the sequencing run.

I don't know how to verify the color balance as suggested. And can we pool more than 4 samples? What is the maximum? And what combination should be used again? (for example, would it be possible to pool together 8 samples using primers 1-8? again, for now will not have that many at the same time, but I want to know if it's possible in case we have more in the future).

The maximum depends on your sequencing depth, but you can pool the indexes down a column (Do not pool libraries across a row).