Review of: "Deep sequencing of yeast and mouse tRNAs and tRNA fragments using OTTR"

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In the pre-print, authors have explored a latest technique of OTTR (ordered two template relay) to sequence the tRNA and tRNA halves from budding yeast and mouse tissues. Sequencing techniques involve cDNA synthesis while preparing the library. These techniques cannot capture tRNAs or their fragments efficiently due to secondary structures and modifications. Currently, in the context of tRNA derived fragments, sequencing data have revealed the presence of 5' tRFs and 5' tsRNAs as being abundant than the 3' tRFs or 3' tsRNAs (in humans, arabidopsis). The authors here, bring the contrary picture that classical technique such as Northern blot detects 3' fragments in the samples where technique such as Illumina true seq cannot capture the same. In order to get a real picture of tRNA (full length and tRNA fragments), they adopt to use a recently developed technique OTTR. Authors choose yeast (extensive in tRNA modifications) and mouse sperm (high small RNA content) samples. Their results suggest that mir vana purified OTTR libraries were more efficient in recovery of full length tRNAs than gel purified ones and the recovery is comparable to mim-tRNA seq and YAMAT seq. Also, they map mutation signatures to methylation modification. After overexpression of RNY1 in yeast, the sequencing by OTTR was compared with Neb and Fu methods and fragmented tRNA recovery (5' and 3') and coverage (from start to stop) was more in OTTR sequencing. For mouse, the RNA from sperm was treated with pnk or pnk/atp and OTTR sequencing had more recovery than other techniques. PNK treatment recovers RNAs cleaved by RNAse A/t1 which would not be possible to be included in ligation steps in usual seq techniques. To conclude, it is good work and the manuscript is constructed well to understand the methodology. It is a novel work and it will give a more authentic picture to t RNA researchers. The authors can refer to recent paper by Nagai et al (2021) (published in RNA).

1. Details of markers can be given in fig S1.
2. Figure 4d lacks y axis and x axis is not visible.
3. the ribosomal recovery can be elaborated from the results obtained.
4. A flow chart is required to describe the OTTR technique.
5. In figure 4a, is it fragmented RNA recovery or tsRNA recovery
6. Can the same experiments be done with yeast after overexpression of tRF or tsRNA generating enzyme.
7. The efficiency of OTTR technique in the context of tRFs and tsRNAs is lacking in the manuscript.