DEEP PROTEOME COVERAGE THROUGH RIBOSOME PROFILING AND MS INTEGRATION.

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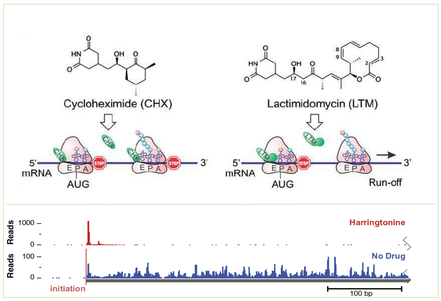
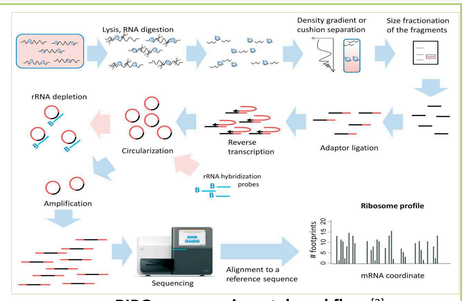
INTRODUCTION

Recent studies point to an underestimation of the complexity of the (eukaryotic) proteome [1]. With evidence pointing to the existence non-annotated translation products from alternative translation initiation resulting in the creation of N-terminal extended or truncated proteoforms, internal out-of-frame translation sequence variations products amongst others [1,2]. Typically, such alternative translation products cannot be identified from canonical protein databases, hence the need for a tailored search space.

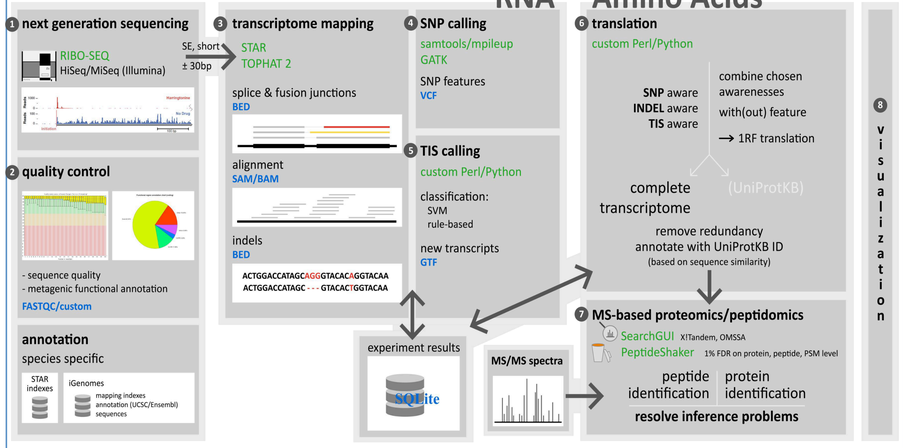




RIBO-seq experimental workflow [3]

The recently developed ribosome profiling (RIBO-seq) strategy provides a means to monitor protein synthesis at single to sub-codon level [1,3]. RIBO-seq derived data gives a **more representative expression state** and accounts for non-canonical information.. Without taking this information into account, MS-based proteomic studies may fail to detect novel protein forms. We developed a pipeline [2] that takes advantage of RIBO-seq information to generate an optimized search space

MATERIALS & METHODS (PROTEOFORMER Pipeline[1])



WORKFLOW OVERVIEW

1. Ribosome profiling (**RIBO-seq**) performed on HiSeq, Illumina sequencing platform

2. **Quality control** based on existing tools as FastQC and custom metagenic functional assessment.

3. **Transcriptome mapping** based on STAR [4] and/or TopHat2 [5] local aligners.

4. Single nucleotide polymorphism **(SNP) calling** based on samTools-mpileup.

5. Translation initiation site **(TIS) calling** based on trained Support Vector Machine (SVM) [2] or rule-based algorithm [1].

6. **Translation product assembly**, taken into account the SNP, TIS, INDEL awareness. Construct complete proteome, optionally combine with Canonical.

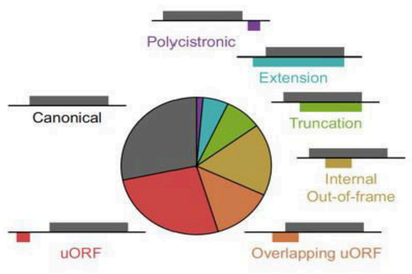
7. **MS-based proteomics/peptidomics** using SearchGui [6] and PeptideShaker [7] tools.

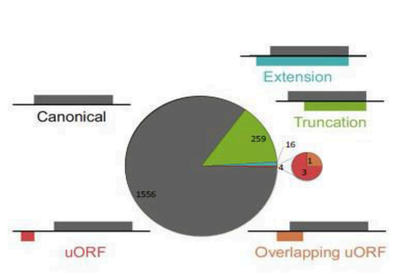
8. **Genome-centric visualization** of all generated information tracks (Ensembl, UCSC, IGV genome browsers).

RESULTS

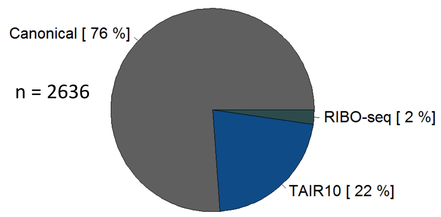
MS based proteomic and RIBO-seq experiments on two Mouse and Arabidopsis thaliana:

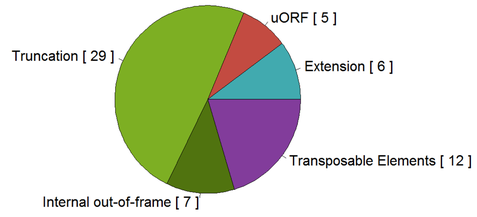
Mouse:



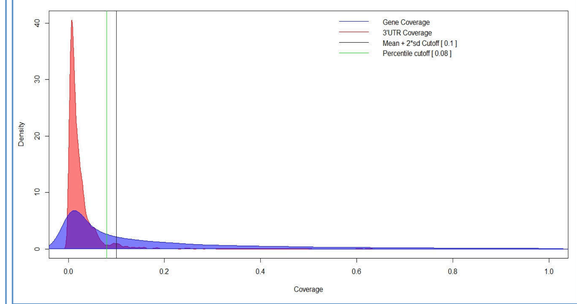


Arabidopsis Thaliana

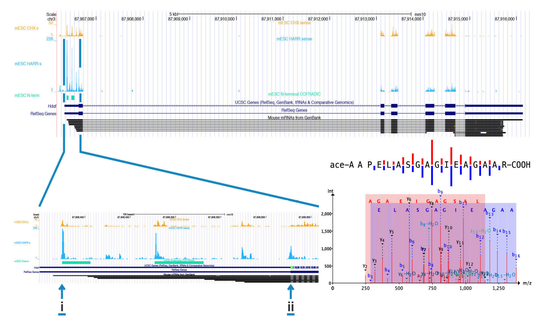


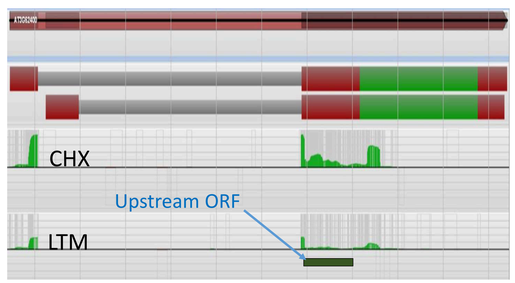


RPF coverage to determine transcripts being translated. Translation cutoff determined by 95th percentile of coverage values on untranslated regions



Examples:





LEFT: Depiction of the mouse HDGF gene's 5'-extension predicted by RIBO-seq and identified using N-terminal proteomics experiment.

RIGHT: uORF with TIS called by RIBO-seq and a peptide [AGHKVAHATLKGPSVVKE] at the TIS identified by N-terminomics for gene AT3G62400 of Arabidopsis Thaliana TAIR10 genome.

CONCLUSION & FUTURE WORK

Deep proteome coverage based on **ribosome profiling aids mass spectrometry-based protein and peptide discovery** and provides evidence of **alternative translation products** and **near-cognate translation initiation events** [1].

Future work will mainly focus on :

➔ Investigating read density and coverage to identify genes undergoing translation.

➔ Implementing SVM in TIS calling algorithm

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