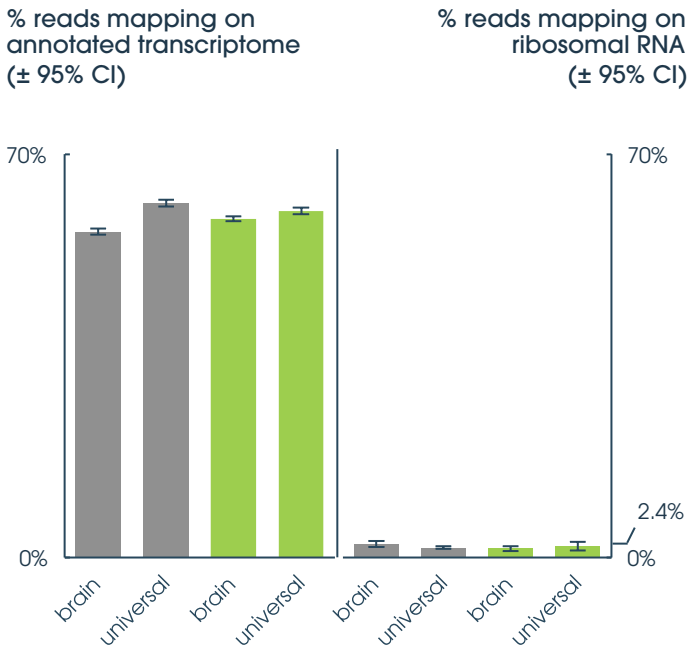
Biogazelle Tech Note 201401B

TruSeq total RNA sequencing performance assessment

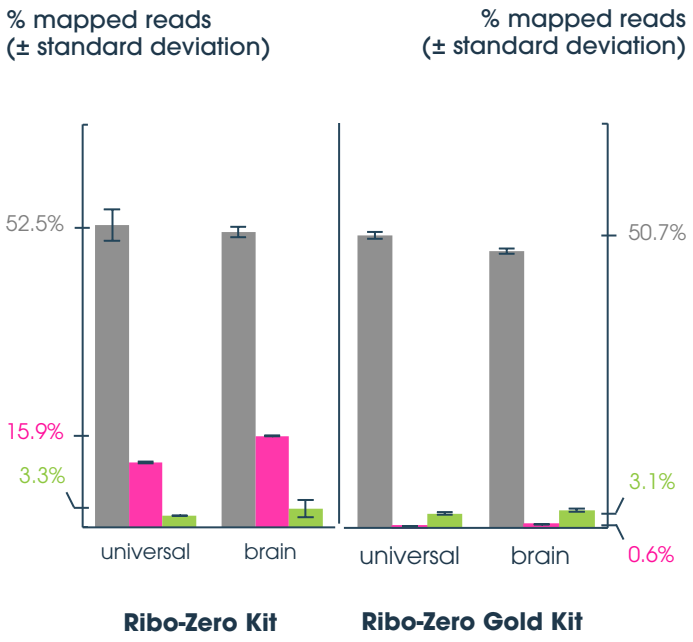


library prep & RNA sequencing performed at
- Illumina
- Biogazelle

Conclusions
- excellent reproducibility
- high transcriptome coverage (mRNA + lncRNA)
- low rRNA contamination
- perfect correspondence between providers

Data represent the percentage of reads mapping on the annotated transcriptome (left panel) or the ribosomal RNA (45S rRNA, right panel) of 4 (or 3 for Illumina universal RNA) independent replicate experiments. Illumina data represent the BaseSpace Public Data (NextSeq 500: RNA-Seq (8plex)).

Libraries for RNA-sequencing were prepared from 100 ng RNA using the Illumina TruSeq Stranded Total RNA (w/Ribo-Zero) sample prep kit, involving depletion of ribosomal RNA, fragmentation, adapter ligation, reverse transcription and PCR amplification. Libraries were quantified by Qubit (Life Technologies) prior to sequencing on a NextSeq 500 sequencer (Illumina). Sequencing reads were analysed using the Sailfish algorithm, taking into account annotated coding (mRNA) and non-coding (lncRNA) transcripts (Ensembl 76). Sailfish enables rapid alignment-free quantification of transcript isoform abundance.



% reads mapping on
- transcriptome (mRNA + lncRNA)
- mit-rRNA
- 45S rRNA

Conclusions

- strong depletion of mit-rRNA transcripts w/Ribo-Zero Gold
- excellent reproducibility
- consistent high transcriptome (mRNA + lncRNA) coverage and low rRNA contamination

Data represent the percentage of reads mapping on the annotated transcriptome (gray bars), the mitochondrial ribosomal RNA (mit-rRNA, pink bars) or the cytoplasmic ribosomal RNA (45S rRNA, green bars) of two independent replicate experiments.

100 ng total RNA was depleted of rRNA using the Illumina **Ribo-Zero Kit** (depletion of 45S rRNA) or the Illumina **Ribo-Zero Gold Kit** (depletion of 45S and mit-rRNA). Subsequently, libraries for RNA-sequencing were prepared using the Illumina TruSeq Stranded Total RNA sample prep kit involving fragmentation, adapter ligation, reverse transcription and PCR amplification. Libraries were quantified by Qubit (Life Technologies) prior to sequencing on a NextSeq 500 sequencer (Illumina). Sequencing reads were analysed using the Sailfish algorithm, taking into account annotated coding (mRNA) and non-coding (lncRNA) transcripts (Ensembl 76). Sailfish enables rapid alignment-free quantification of transcript isoform abundance.