Promoter-driven expression of the enzyme Uptr

\[ \text{Uptr} \downarrow 4\text{tU} \]

Incorporation of 4tUMP into actively transcribed RNA

RNA isolation from Uptr-positive and Uptr-negative cells

Biotinylation of thio-groups

Biotin-Streptavidin affinity purification

Isolation of 4tU-labeled RNA containing: unprocessed RNA, mRNA, ncRNA, rRNA, tRNA

SMARTer mRNA amplification

Nextera library synthesis

Illumina sequencing

RNASeq data analysis: CutAdapt, GSNAP, HTSeq, DESeq2