

RNase III as a Tool to Manipulate Transcript Stability: Identifying *in vivo* Targets in *Escherichia coli*

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Project Goals:

For this project we will use RNA-sequencing techniques to study mRNA stability in *Escherichia coli* and *Synechococcus* sp. PCC7002, a sequenced cyanobacteria with promising industrial traits. We will use this method to study how various RNA processing enzymes affect decay of mRNA from a global perspective. We will use the knowledge gained from our studies of mRNA turnover to develop design rules for (de)stabilizing transcripts and apply these rules to ongoing metabolic engineering projects in PCC 7002 for use in photosynthetic biorefineries.

Our specific objectives are:

- (1) Quantify decay rates for each nucleotide in the *E. coli* and PCC 7002 transcriptome with RNA sequencing
- (2) Design and test strategies for (de)stabilizing transcripts in PCC 7002
- (3) Apply design rules to improving biofuel production in PCC 7002

Messenger RNA (mRNA) is a labile intermediate that affects protein expression levels. For metabolic engineering purposes we would ideally be able to predict and precisely control protein expression based on a given DNA sequence. Unfortunately there is insufficient foundational knowledge about RNA stability to be able to predict the half-life of a given transcript. This arises due to the numerous and complex ribonucleases (RNases) that facilitate mRNA degradation. We are particularly interested in RNase III, a ribonuclease that cleaves long double-stranded regions of RNA, because of its potential use to alter transcript stability and process structure RNAs (e.g. CRISPR guide RNAs). RNase III recognizes mRNA secondary structure and not a conserved nucleotide sequence, but it is still more amenable to engineering than other RNases that have less selectivity. Using RNA-seq we have identified novel targets of RNase III in *Escherichia coli*, and we have shown that RNase III regulates protein expression of important metabolic enzymes, processes read-through transcripts, and is involved in the turnover of mRNA of leader peptides. With our greatly expanded list of RNase III sites and sequences, we will test if these sites can be placed next to genes of interest to alter their stability and subsequent protein expression.

CRISPRi is a trans-acting regulator strategy that provides novel opportunities to study essential genes and regulate metabolic pathways. We have adapted the clustered regularly interspersed palindromic repeats (CRISPR) system from *Streptococcus pyogenes* to repress genes *in trans* in the cyanobacterium *Synechococcus* sp. strain PCC 7002 (hereafter PCC 7002) and achieved conditional and titratable repression of heterologous YFP, downregulation of phycobilisomes, creation of a conditional auxotroph, and demonstrated a novel strategy for increasing central carbon flux by conditionally downregulating a key node in nitrogen assimilation. We will describe our system and its future application to studying mRNA stability in PCC 7002.

References

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