Optimized Loopable Translation as a Platform for the Synthesis of Repetitive Proteins

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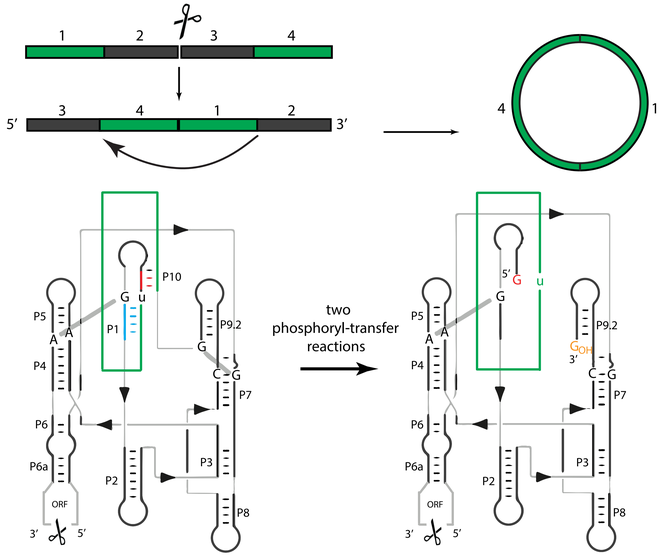
Hijacking the Natural Mechanism of Self-Splicing Intron

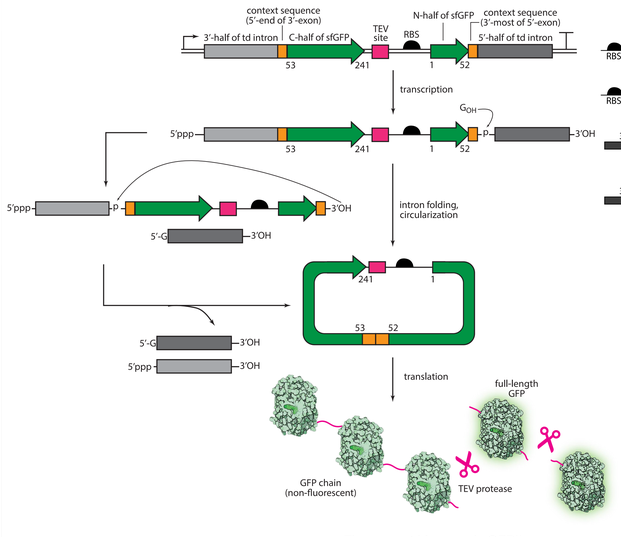
The expression of long proteins with repetitive amino acid sequences often presents a challenge in recombinant systems. We report a genetic construct that circularizes mRNA *in vivo* by rearranging the topology of a group I self-splicing td intron, thereby enabling "loopable" translation. Using a fluorescence-based assay to probe the translational efficiency of circularized mRNAs, we identify several conditions (30°C; 20 mM Mg^{2+}) that optimize protein expression. Our data suggested that translation of circularized mRNAs could be limited primarily by the rate of ribosomal initiation; therefore, using a modified error-prone PCR method, we generated a library that concentrated mutations into the initiation region of circularized mRNA and discovered mutants with markedly higher expression levels. Combining our rational improvements with those discovered through directed evolution, we report a loopable translator that achieves protein expression levels within 1.5-fold of the levels of standard vectorial translation.

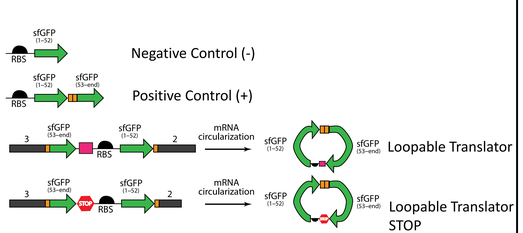
Natural Self-Splicing Group I Intron

Group I Intron Based Self-Circularizing Construct

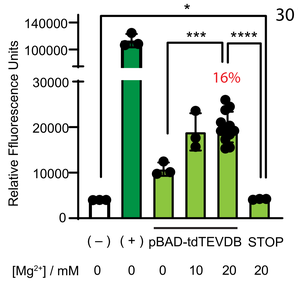


Loopable Translator Generated Fluorescence Signal

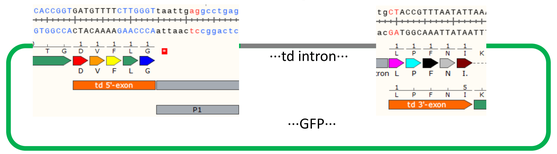




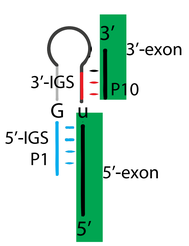
Fluorescence Signal of Loopable Translator

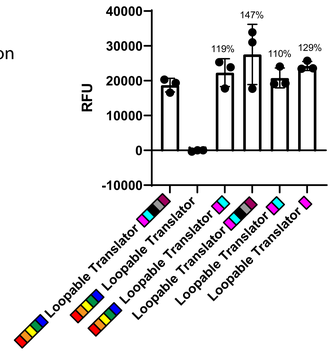


Context Requirement



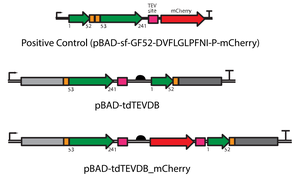
Fluorescence Assay on Context Requirement

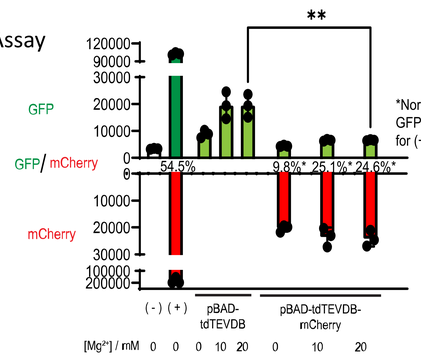




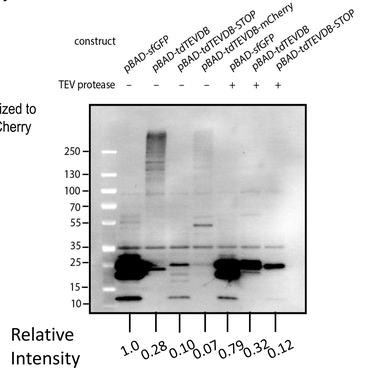
Verification of mRNA Looping: Fluorescence Assay and Western Blot

Constructs for Fluorescence Assay & Western Blot



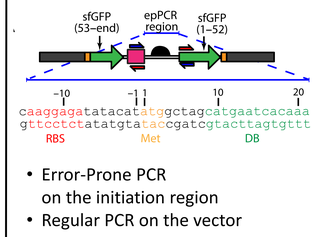


*Normalized to GFP/mCherry for (+)

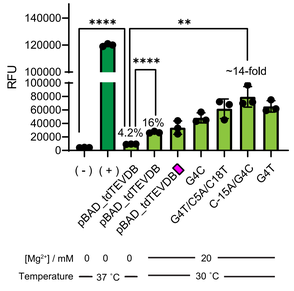


Improvement by Directed Evolution

Fluorescence Assay on Screened Constructs



- Error-Prone PCR on the initiation region
- Regular PCR on the vector



Screened 720 constructs

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Perriman, R. & Ares, M. Circular mRNA can direct translation of extremely long repeating-sequence proteins in vivo. *RNA* **4**, 1047–1054 (1998).
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