# Provisional Patent Draft: Synthetic Logic-Gated mRNA Expression System

# Title:

Synthetic mRNA Expression System for Tumor-Specific Logic-Gated Gene Activation

# Applicant:

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# Field of Invention:

This invention relates to synthetic biology, molecular therapeutics, and genetic engineering. More specifically, it concerns the design and function of a synthetic mRNA expression system that activates transcription and translation only under tumor-specific intracellular conditions.

# Background:

Modern cancer treatments struggle to distinguish between tumor and healthy cells. Single-marker therapies often fail due to expression overlap between malignant and normal tissues. A more robust approach is to require multiple tumor-specific signals to be present before a therapeutic payload is expressed. This invention achieves that via a logic-gated synthetic mRNA circuit.

# Summary of the Invention:

The invention provides a modular genetic construct that integrates three cancer-associated intracellular conditions as logical inputs:

1. 1. \*\*Elevated MYC activity\*\* (sensed by a synthetic MYC-responsive promoter)
2. 2. \*\*Elevated reactive oxygen species (ROS)\*\* (sensed by a riboswitch embedded in the 5' UTR)

3. \*\*Suppressed let-7 microRNA levels\*\* (sensed by four tandem let-7 response elements in the 3' UTR)

Only when all three conditions are met will the mRNA survive and be translated, producing the intended protein payload.

# Detailed Description:

* - \*\*Promoter:\*\*
* - Sequence: CACGTGCACGTGCACGTGCACGTGTATAAAAG
* - Composed of four E-box motifs (CACGTG) and a minimal TATA box core promoter.
* - Responds to elevated MYC activity, common in cancer cells.
* - \*\*5' UTR ROS Riboswitch:\*\*
* - Sequence: GGAAGAGGAGGAAGAGGAGGAACAGTACACGTAGCTGTACTCGGATGCTAC
* - Folds into a structure that blocks the ribosome binding site unless ROS levels are elevated.
* - \*\*Coding Sequence (Payload):\*\*
* - Example: Enhanced Green Fluorescent Protein (GFP) coding sequence.
* - Can be substituted with therapeutic genes such as Diphtheria toxin A (DTA), Granzyme B, or TRAIL.
* - \*\*3' UTR let-7 Response Elements:\*\*
* - Sequence (repeated 4x): AACTATACAACCTACTACCTCA
* - Matches the seed region of let-7a microRNA, promoting degradation in non-cancerous cells.
* - \*\*Polyadenylation Signal:\*\*
* - Sequence: AATAAAAGCATTTTTTTTTTTTTTTTTTTTTTTTTTT
* - Ensures proper mRNA termination and export.

# Claims:

1. 1. A nucleic acid construct comprising:

- (a) a MYC-responsive promoter;

- (b) a 5' untranslated region (UTR) containing a ROS-activated riboswitch;

- (c) a coding sequence encoding a detectable or therapeutic protein;

- (d) a 3' UTR containing multiple microRNA let-7 binding sites.

1. 2. The construct of claim 1, wherein the coding sequence encodes green fluorescent protein (GFP).

3. The construct of claim 1, wherein the coding sequence encodes a cytotoxic protein selected from the group consisting of diphtheria toxin A, TRAIL, or Granzyme B.

4. The construct of claim 1, wherein expression occurs only when MYC activity is elevated, ROS levels are elevated, and let-7 levels are low.

5. A method of selectively expressing a gene in tumor cells, comprising introducing the construct of claim 1 into a cell population and allowing expression only in cells exhibiting the specified input conditions.

# Appendix:

* - Full GenBank sequence export (attached)
* - mRNA folding analysis report (dot-bracket + MFE)
* - Benchling plasmid map screenshots