

BBF RFC 90 : The Measurement of rho-independent Transcription Terminator Efficiency.

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1. Purpose

The purpose of this RFC is to provide standard methodology for the measurement of the absolute strength of terminators in bacteria. Because we have characterized the performance of terminator in E.coli and used a simple equation model, it can be expressed in PoPS.

2. Relation to other BBF RFCs

This maybe doesn't relate to any other BBF RFC.

3. Copyright Notice

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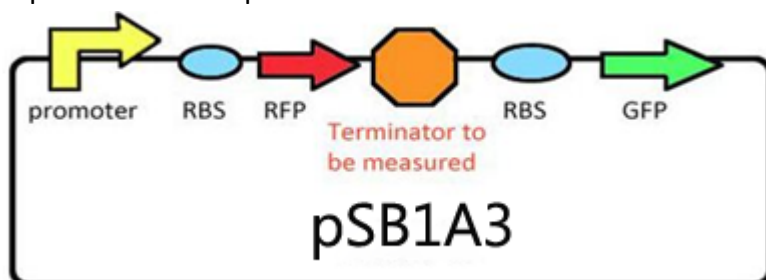
4. Standard terminator efficiency measurement kit for use in E.coli.

The terminator efficiency measurement kit MUST include promoter, ribosome binding sites, reporter gene and vector backbone. Standard transcription terminator efficiency measurement kit MUST comprise of promoter BBa_R0040, ribosome binding site BBa_B0030, reporter gene, RFP BBa_E1010, GFP BBa_E0040. It SHOULD comprise vector backbone pSB1A3, a high copy number plasmid carrying ampicillin resistance. This high copy number vector is chosen to facilitate growth of bacteria step in the experiment

5. Design and construction of characterization devices.

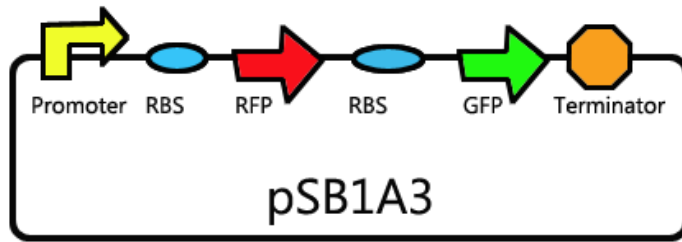
The fluorescence produced by the characterization devices are then measured using flow cytometry to calculate the termination efficiency of the terminators.

Experimental Group:



(Inputs are measured by RFP expression and outputs are measured by GFP expression.)

Control Group:



(calibrates RFP input to GFP output.)

6. Experimental procedure

6.1 Media:

Lysogeny Broth (LB) liquid and solid media.

6.2 Protocol:

- a. Plasmid pSB1A3 is chosen to be the vector that ligate GFP and RFP fragments. To protect the structural integrity of the constructed plasmid, we need to mutate a restriction enzyme cutting site named Pst I to Afl II using PCR. Proper primer are designed for this purpose.

PtoA-F:5'-CCACCTGACGTCTAAGAAAC-3'

PtoA-R:5'-ATGATCATCGCCGGCGAATTCAGGC-3'

- b. Check whether the mutation of restriction cutting site is successful.
- c. Transform the plasmid to competent cell DH5 α and incubate overnight.
- d. Pick up colonies, plasmid isolation, and digest to choose the positive one.
- e. Amplify GFP & RFP. Do an electrophoresis for verification the PCR is successful.
- f. Use specific restriction enzymes to digest plasmid mutant-psb1a3, GFP and RFP to get sticky ends and purify the DNA fragment after the Electrophoresis.

Digestion of plasmid mutant-pSB1A3 with Afl II and Not I;

Digestion of PCR product GFP with Not I and Spe I;

Digestion of PCR product RFP with Afl II and Spe I;

Put the tubes in 37°C environment for 4-8 hours.

- g. Ligation is needed to connect these 3 fragments together.
- h. Transform the plasmid to competent cell DH5 α and incubate overnight.
- i. Pick up colonies, plasmid isolation, and choose the positive one.
- j. Use restriction enzymes Xba I and Pst I to digest the constructed vector.
- k. Ligation is needed to terminators to be measured and the vector together.
- l. Transform the plasmid to competent cell and incubate overnight.
- m. Test the fluorescent using the method of flow cytometry.

7. Parts used to create the terminator characterization.

Left Part	Right Part	Description
BBa_E1010	BBa_E0040	RFP+BBa_K827086+GFP
BBa_E1010	BBa_E0040	RFP+BBa_K827087+GFP
BBa_E1010	BBa_E0040	RFP+BBa_K827088+GFP
BBa_E1010	BBa_E0040	RFP+BBa_K827089+GFP
BBa_E1010	BBa_E0040	RFP+BBa_K827090+GFP
BBa_E1010	BBa_E0040	RFP+BBa_K827091+GFP
BBa_E1010	BBa_E0040	RFP+BBa_K827092+GFP
BBa_E1010	BBa_E0040	RFP+BBa_K827093+GFP
BBa_E1010	BBa_E0040	RFP+BBa_K827094+GFP

8. Calculate terminator efficiency

$$TE=1-GE/GC$$

TE: Terminator efficiency

GE: Mean value of GFP from experimental group

GC: Mean value of GFP from control group

Under the ideal circumstances, the output of GFP and RFP should be almost the same and the value of TE should approach 0. If a strong terminator is placed into characterization experimental group plasmid, the only output should be RFP and the value of TE should approach 1.

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10. References

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- [2] Haiyao Huang "Design and Characterization of Artificial Transcriptional Terminators".
- [3] Lars Velten, Nao" Units for Promoter Measurement in Mammalian Cells"