Engineered promoters enable constant gene expression at any copy number in bacteria

Group 4

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Table of Contents

Introduction

The internal environment of growing cells is variable and dynamic, making it difficult to introduce reliable parts, such as promoters, for genetic engineering.

Authors applied control-theoretic ideas to design promoters that maintained constant levels of expression at any copy number.

Theory predicts that independence to copy number can be achieved by using an incoherent feedforward loop (iFFL) if the negative regulation is perfectly non-cooperative.

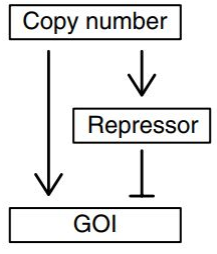
They engineered iFFLs into Escherichia coli promoters using transcription-activator-like effectors (TALEs).

Main aim

Genetic engineering projects often require balancing of gene expression; however, achieving and maintaining this balance is difficult.

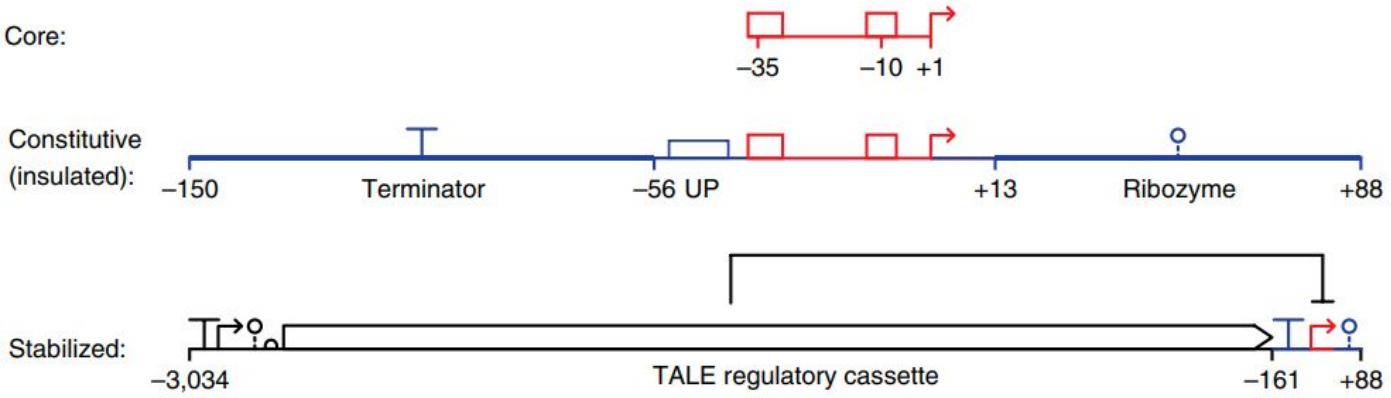
For example, in metabolic engineering the expression of enzymes may need to be regulated to optimize flux to the product, variable expression could result in diversion of carbon to other pathways or in the accumulation of toxic intermediates. → Important to stabilize gene expression

→ Main aim to stabilize the expression of gene is to interest by this incoherent feedforward loop (iFFL) meaning that the inputs both negatively and positively control the output



Methods

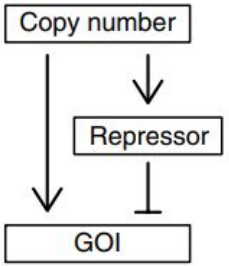
Methods



Insulated constitutive promoter regulates a repressor protein, which represses the GOI promoter

→ increasing copy-number = more repression

→ “stabilized” promoters



Theory behind the method

Under steady-state conditions transcriptional repression follows Hill's equation

$$f(X^*) = \frac{\beta}{1+\left(\frac{X^*}{K}\right)^n}$$

If the dynamic range of the repression is high, the repression concentratio (X*) will go so much over the dissociation constant, that we can rewrite it just as b/(X*)^n OR in our case:

$$G \propto \frac{c}{R^n}$$

G= gene expression, c= copy number, R=repression concentration

And because in our case the repression is under an unregulated promoter, it will be transcribed in proportion with the copy number → c = R

Which means we can rewrite the equation like this:

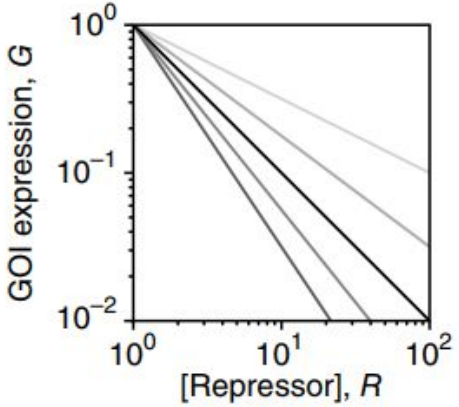
$$G \propto c^{1-n}$$

And n=1 when repression is non-cooperative → c^0 =1

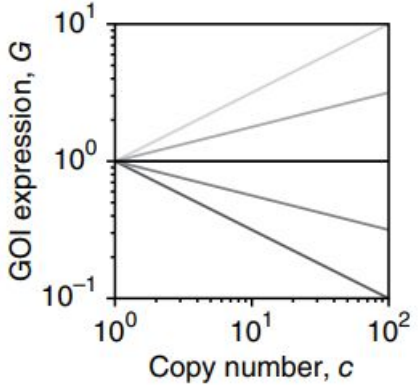
→ G ∝ 1 → gene expression is independent from copy number

So what this means:

We go from this:

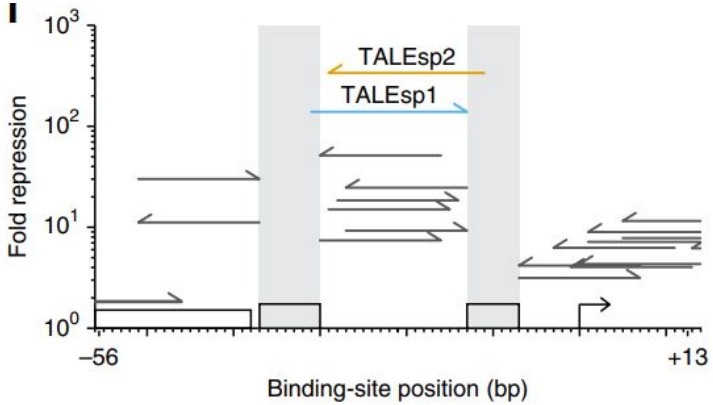


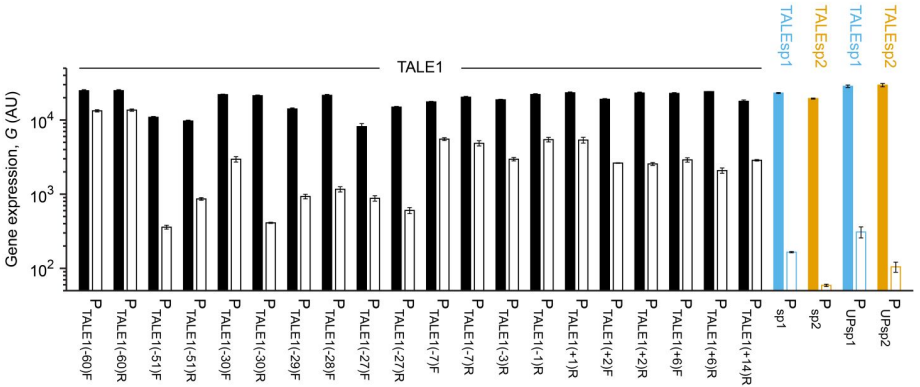
To this:

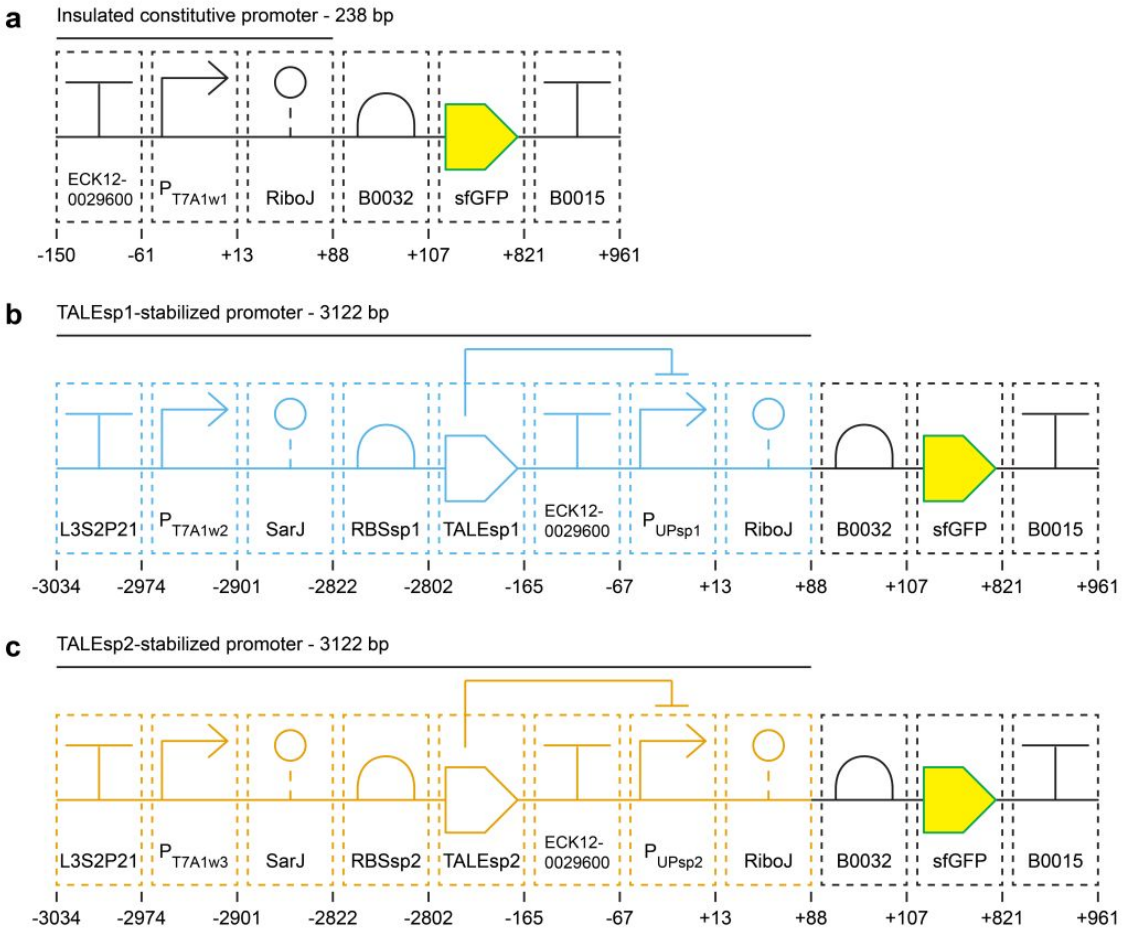


TALEs

- TALE proteins for stabilized promoter
  - Shown great repression activity in *E. coli*
- TALEs bind as monomers to the operator site
  - -> screened various sites for the operator
  - TALEsp1 and TALEsp2
  - 90- and 230-fold repression






a
Insulated constitutive promoter - 238 bp
ECK12-0029600
P T7A1w1
RiboJ
B0032
sfGFP
B0015
-150
-61
+13
+88
+107
+821
+961
b
TALEsp1-stabilized promoter - 3122 bp
L3S2P21
P T7A1w2
SarJ
RBSsp1
TALEsp1
ECK12-0029600
P UPsp1
RiboJ
B0032
sfGFP
B0015
-3034
-2974
-2901
-2822
-2802
-165
-67
+13
+88
+107
+821
+961
c
TALEsp2-stabilized promoter - 3122 bp
L3S2P21
P T7A1w3
SarJ
RBSsp2
TALEsp2
ECK12-0029600
P UPsp2
RiboJ
B0032
sfGFP
B0015
-3034
-2974
-2901
-2822
-2802
-165
-67
+13
+88
+107
+821
+961

Results

Stabilization of expression with synthetic promoter system

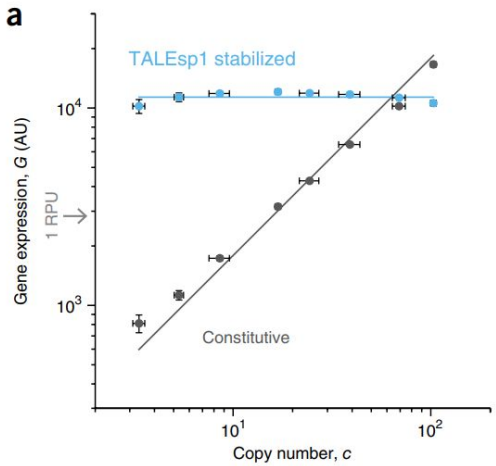
1. Against varying copy numbers

2. Against genomic locations

3. Against external environment

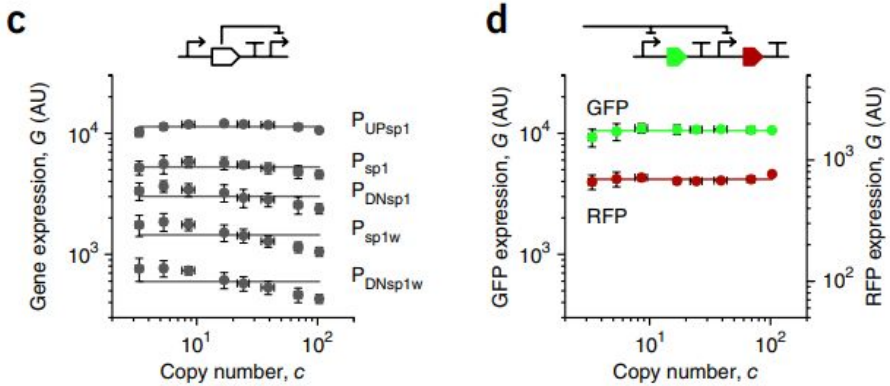
Independent expression of copy number

Basic idea



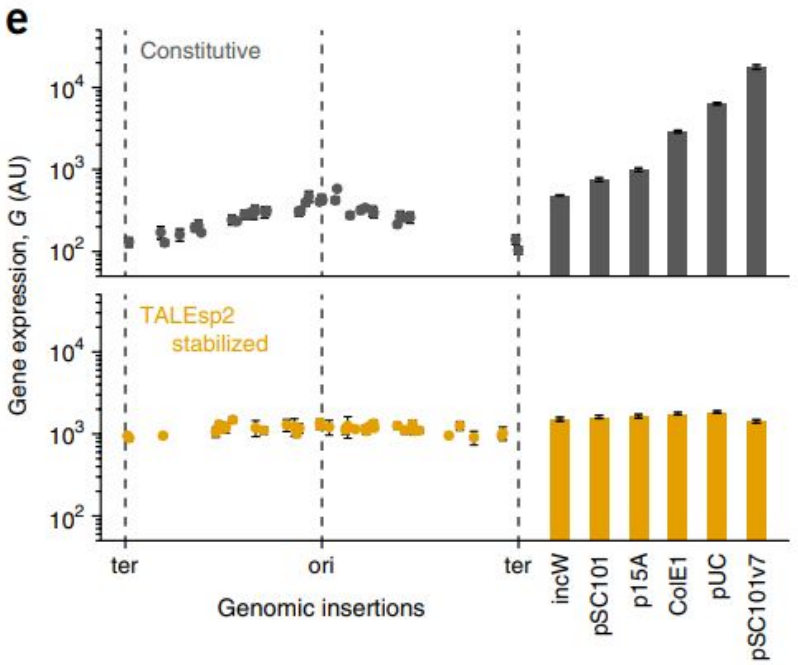
→ **Synthetic promoter TALEsp1 maintains expression**

Variety of promoters (pSC101 variants) achieve **variety of stabilized expression levels**



→ **One TALE with varying synthetic promoters allow the stabilization of multiple genes**

Independent expression of location



TALEsp2 promoter with higher TALE expression to buffer against rapid cell divisions; **stronger repression**

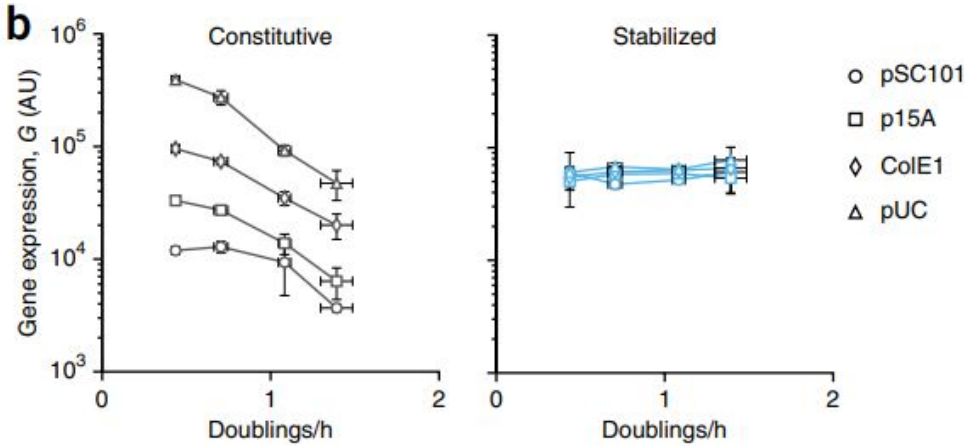
→ **Maximal expression near ORI**

→ **Similar expression in all genomic locations**

→ **Similar expression from the genome and plasmids**

Independent expression of (some) changes in the environment

External environment can affect plasmid copy numbers and break metabolic pathways

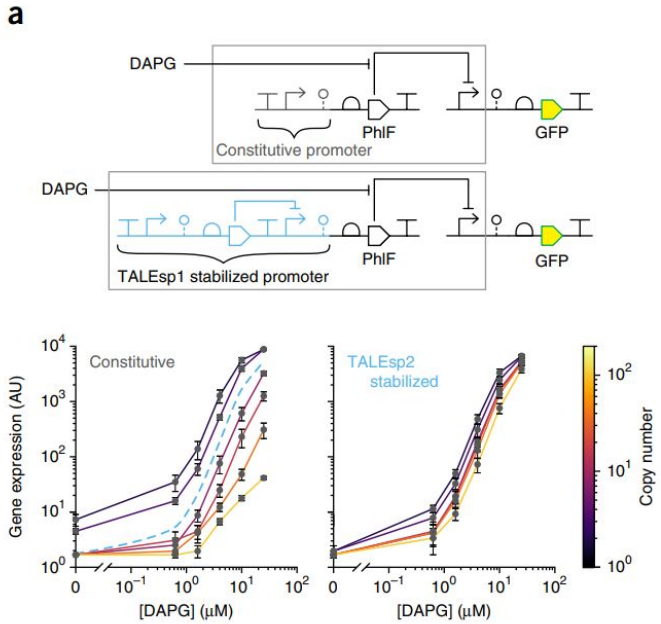


Four medium variants varying in the carbon source led to **differences in growth rate** and **90-fold spread in expression levels**

→ Stabilized promoter was able to balance out most of the differences

Performance of the synthetic promoter

Stabilized **synthetic regulation** against copy-number differences



2,4-diacetylphloroglucinol (DAPG) sensor based on PhlF repressor:

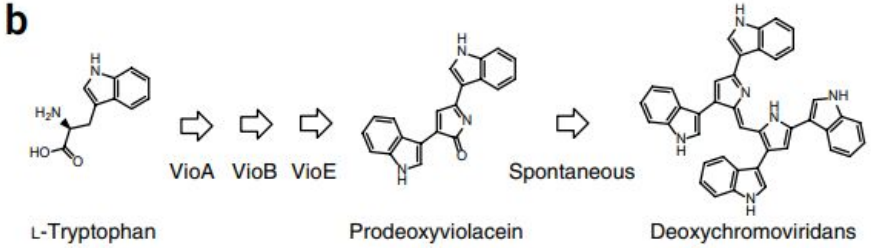
High concentration of DAPG inhibits the repression of GFP

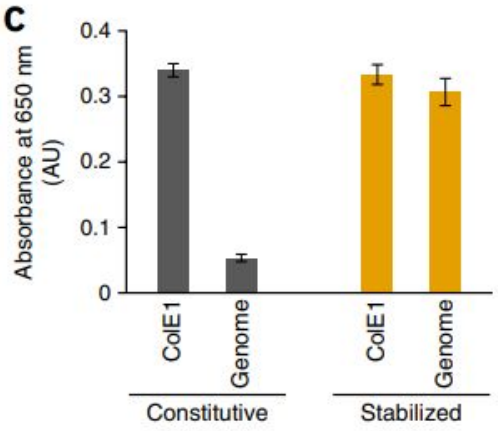
→ **High copy-number** backbones resulted in **low GFP expression** and **low copy-number** backbones **high GFP expression**

→ Stabilized PhF expression resulted to more the GFP expression to collapse in a single curve

Performance of the synthetic promoter

Optimizing a three-gene **metabolic pathway** for the production of deoxchromoviridans





Three-gene operon was optimized with RBSs and was inserted in the genome

→ Production collapsed after inserting the pathway in the genome

→ Production was preserved in the genome without further -retuning

Future Prospects

Why is this important?

The stabilized promoters are able to reduce the copy-number dependence of synthetic regulation and thus can be applied to maintain a constant response function irrespective of the genetic location of the regulatory protein

→ genetic stability of cells growth & effective product synthesis.

Project question: “Could we design a promoter that produces the same protein concentration no matter where it is placed?”

Current result: “Class of stabilised promoter was designed that maintained the same level of gene expression irrespective of the plasmid backbone or its location in the genome”

Why is this important?

- Helps to achieve balancing of gene expression in genetic engineering projects.

    For example, in metabolic engineering the expression of enzymes need to be regulated to optimize flux to the product.

- The construction of intracellular molecular machines requires the correct ratios of components (provided by gene expression stability).

Open Questions