

Measurement standards

RFC 19:

Measuring the activity of BioBrick promoters using an in vivo reference standard

RFC 41:

Units for Promoter Measurement in Mammalian Cells

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Standardization of assembly

Standardization of part properties



Physical composition

Functional composition

Standardization of part properties



Prediction of behaviour

Measurement standard

Standard reference object

Absolute promoter activity



PoPS:

Polymerases per second

=

Protein expression:

e.g. GFP synthesis rate



$$\frac{\gamma_M(a + \gamma_I)S_{cell}^{SS}}{\rho a n}$$

Relative promoter activity

RPU:

Relative promoter units

Promoter of interest

$$PoPS_{\varphi}^{SS}$$

=

$$\frac{PoPS_{\varphi}^{SS}}{PoPS_{J23101}^{SS}}$$

Standard reference promoter

Promoter of interest

$$P_o P S_{\phi}^{SS}$$

$$P_o P S_{J23101}^{SS}$$

Standard reference promoter

Protein expression: promoter of interest

$$S_{cell, \phi}^{SS}$$

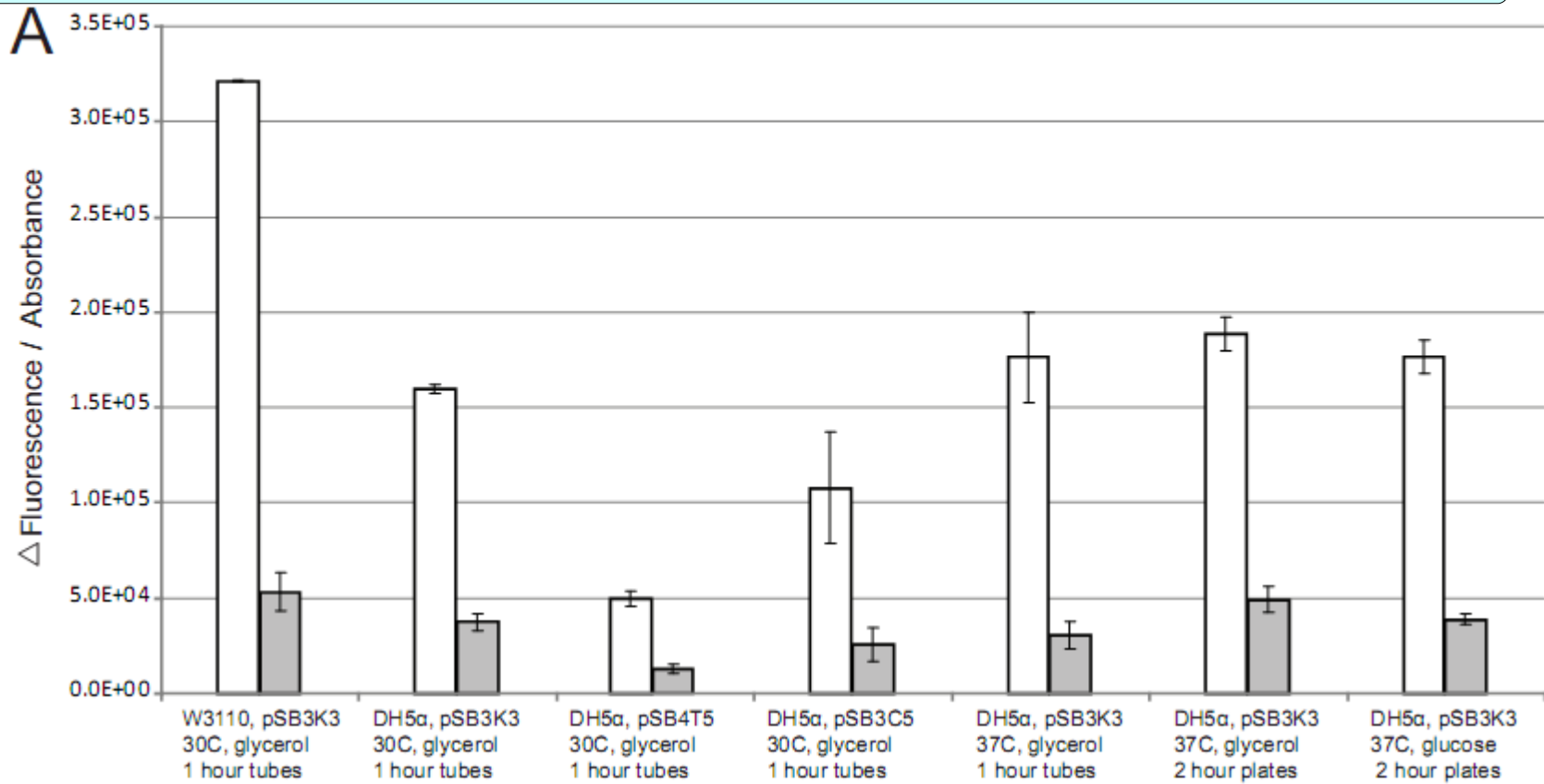
$$S_{cell, J23101}^{SS}$$

Protein expression: Standard reference promoter

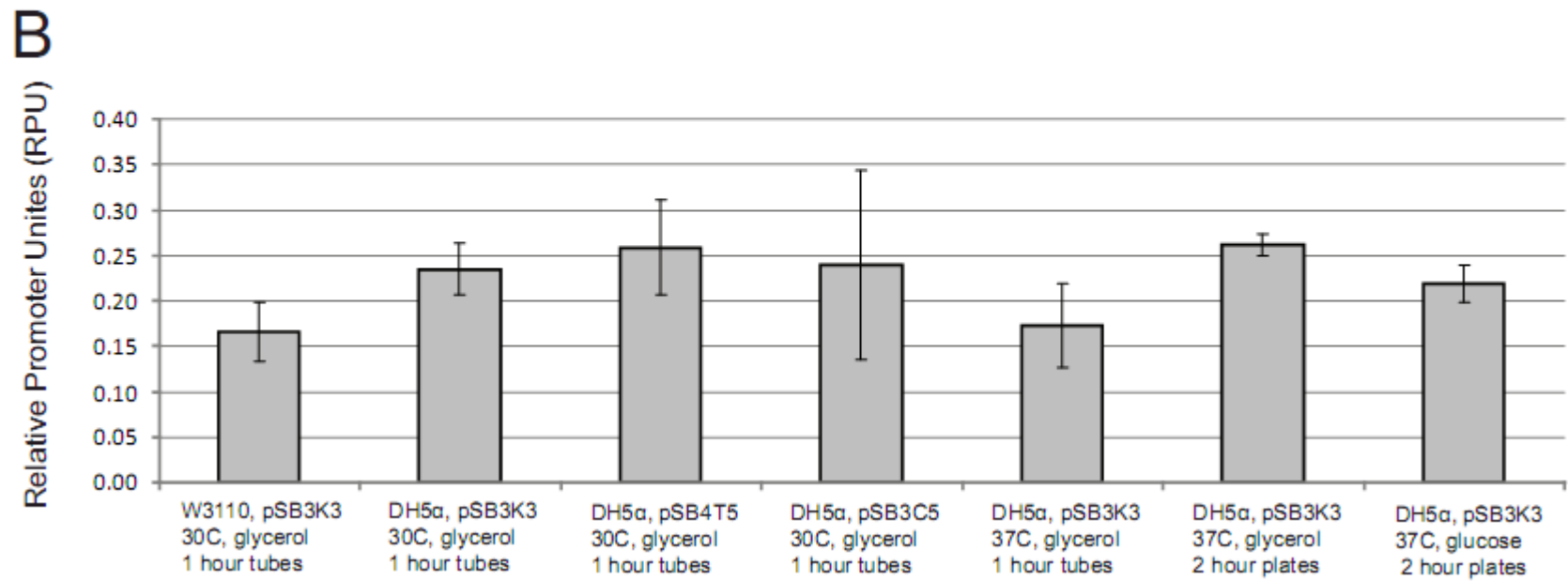
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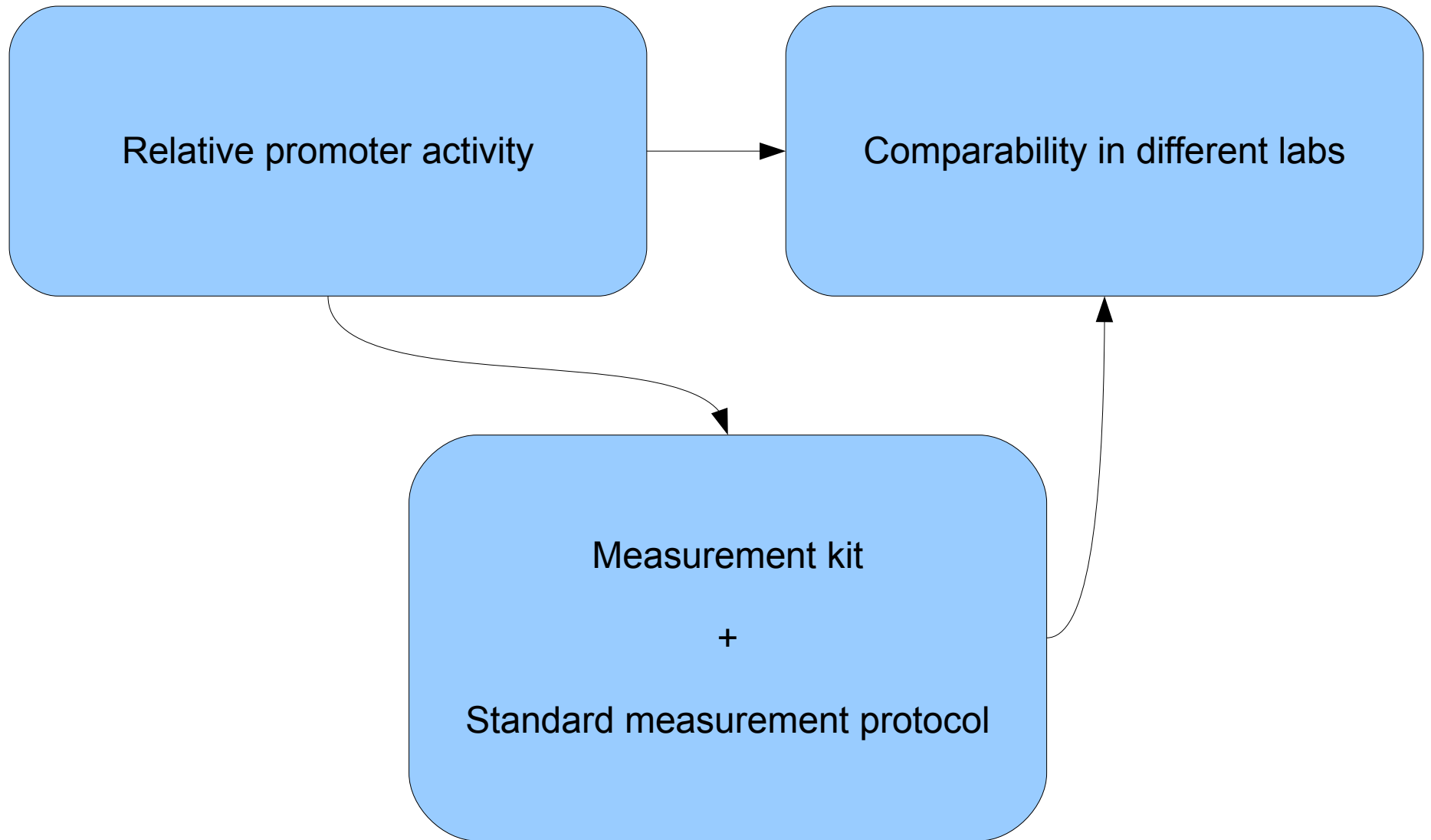
RFC19

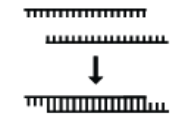
PoPS
-
Varies strongly



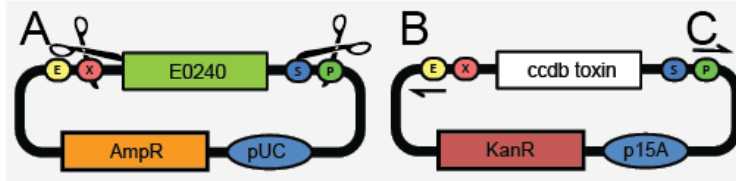
RPU
-
Very constant





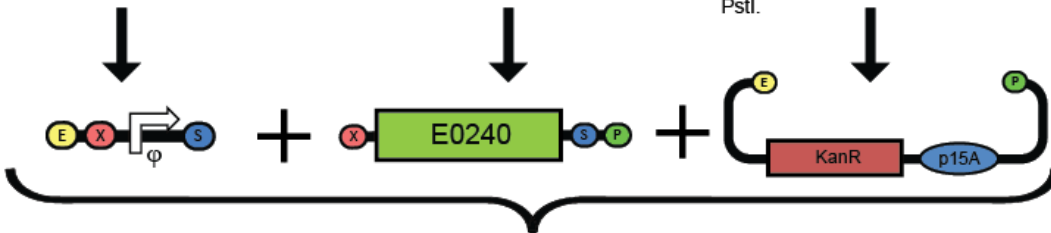


STEP 1: Prepare the test promoter ϕ by annealing synthesized oligos leaving EcoRI and SpeI sticky ends.

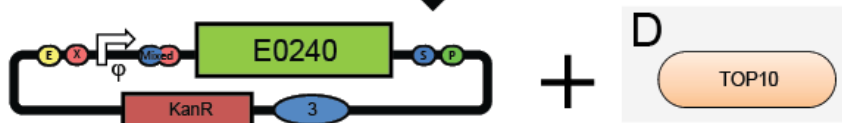


STEP 2: Prepare the GFP reporter device (BBa_E0240) by miniprep of pSB1A2-E0240 followed by restriction digest with XbaI and PstI.

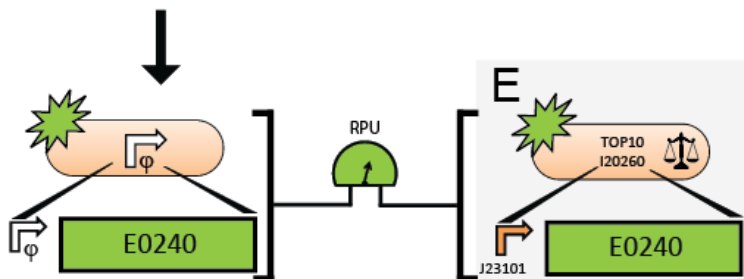
STEP 3: Prepare backbone plasmid (pSB3K3) by preparative PCR of pSB3K3-P1010 using provided primers followed by restriction digest with EcoRI and PstI.



STEP 4: Combine the test promoter ϕ , GFP reporter device, and backbone plasmid in a 3-way ligation to build the promoter test construct.

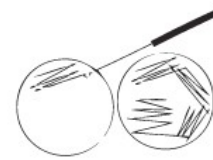


STEP 5: Transform the promoter test construct into TOP10 cells. Select for transformants on Kanamycin plates.



STEP 6: Measure the activity of the test promoter ϕ relative to the activity of the reference standard promoter (BBa_J23101). Report promoter ϕ activity in relative units of Relative Promoter Units (RPUs).

STEP1: Streak 3 plates



A: TOP10
B: BBa_I20260
C: Your promoter!

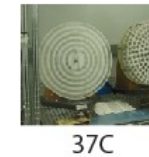
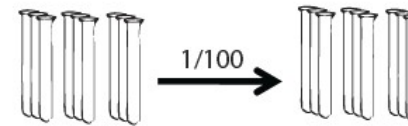


STEP 2: Pick 3 colonies from each plate to start overnight cultures in Supplemented M9 Media at 37 C (9 tubes)



16 hours

STEP 3: Dilute 1/100 into fresh, pre-warmed media incubate at 37C (9 tubes)



37C

STEP 4: After 3 hours measure GFP and OD



3 hours



STEP 5: After another half hour measure GFP and OD again



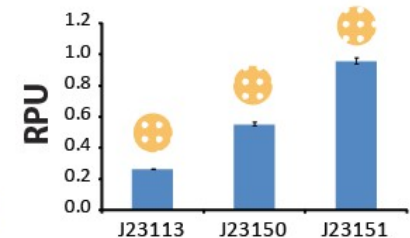
1/2 hour



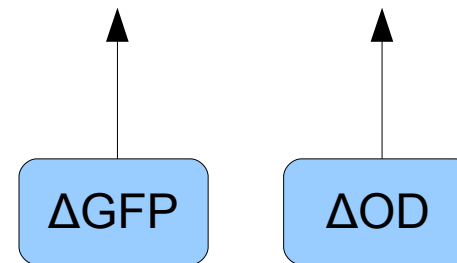
Practice Promoter Set

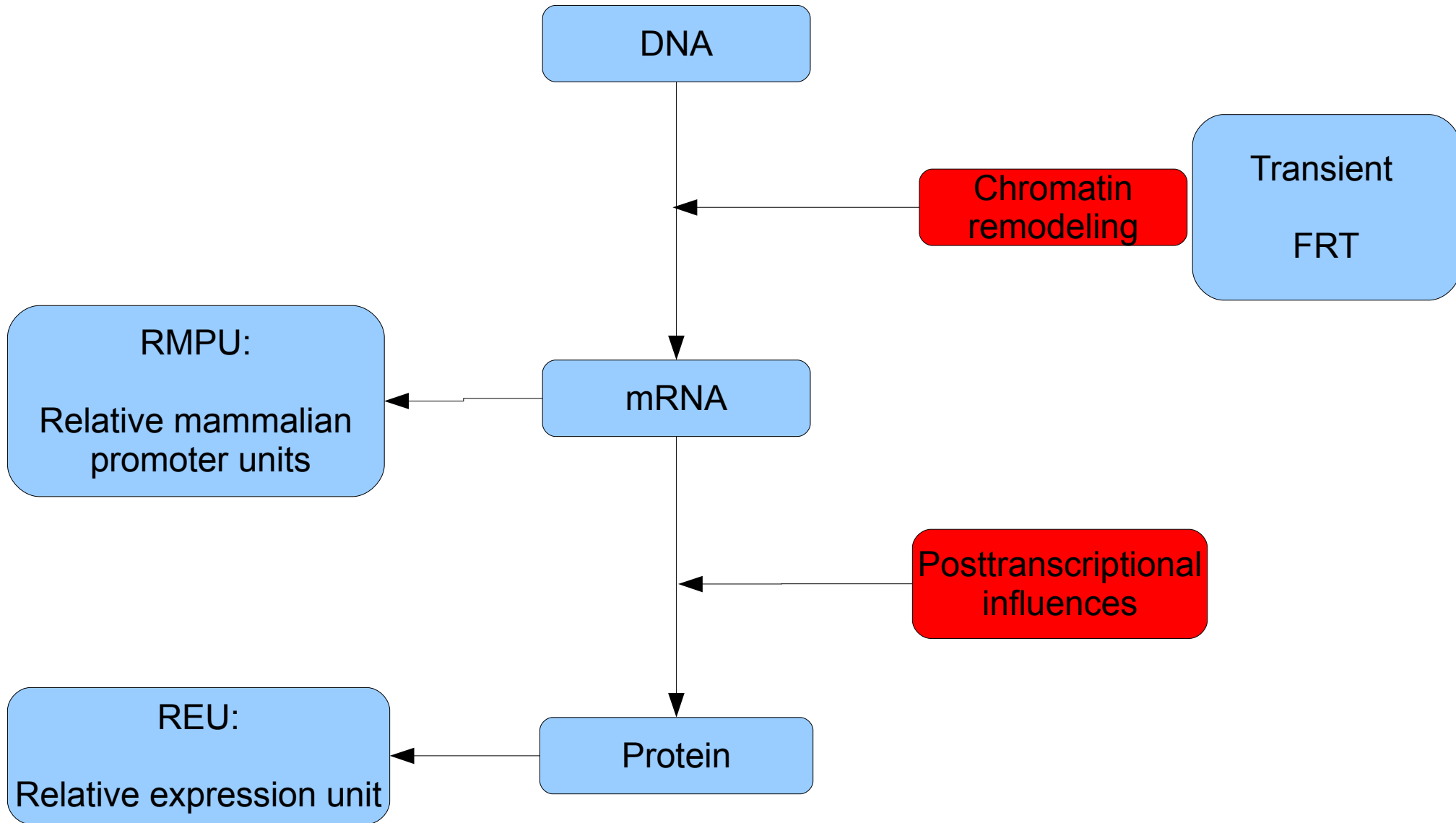
Transform the weak, medium, and strong test promoters into TOP10 and measure using the protocol above. Report your results at:

<http://partsregistry.org/measurement>



$$\text{Activity of Promoter } \varphi \text{ (RPU)} = \frac{[G]_{\text{cell},\varphi}}{[G]_{\text{cell},J23101}} * \frac{\mu_{\varphi}}{\mu_{J23101}} \quad (\text{SM.10})$$





Measurement compatible for mammalian cells

Polymerase

mRNA

RMPU:

Relative mammalian promoter units

=

$$\frac{PoPS_x}{PoPS_{JeT}} = \frac{M_x}{M_j}$$

qRT-PCR

Measurement compatible for mammalian cells

REU:
Relative expression unit

=

$$\frac{\text{Folded protein x}}{\text{Folded protein Jet}}$$

Not proportional to RMPU

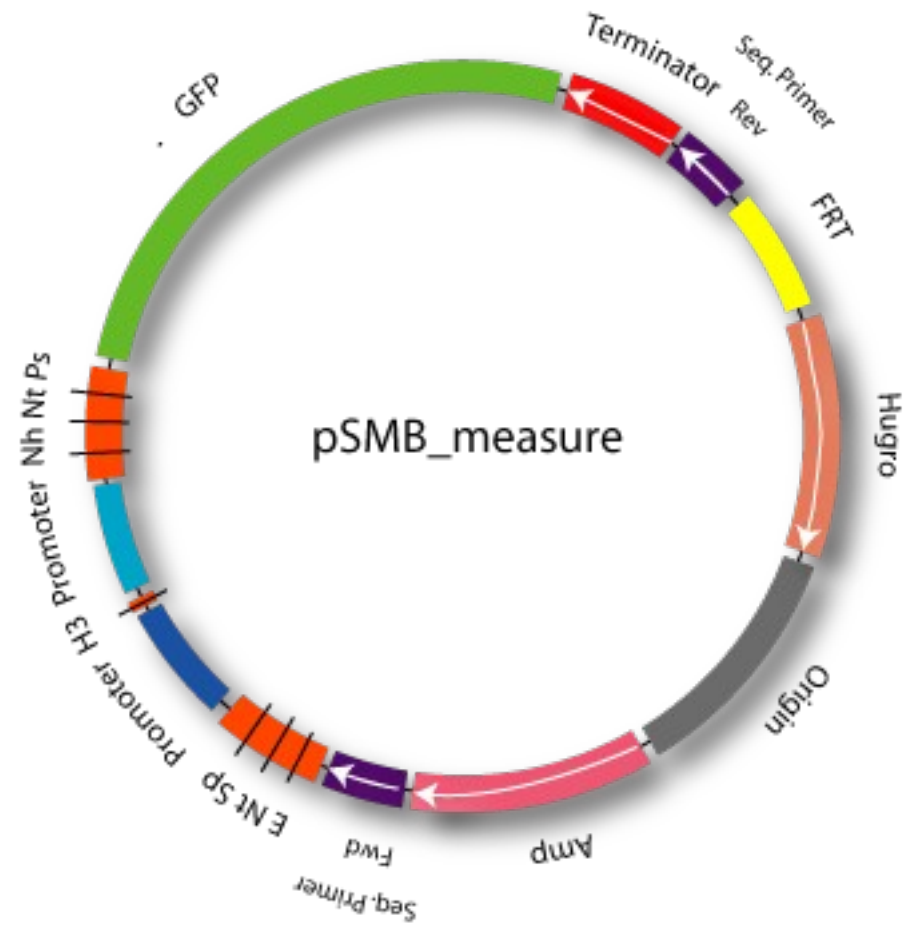
Flow cytometry
Microscopy and Image analysis
TECAN

Plasmid compatible for mammalian cells

Kit

pSMB_measure

pSMB_reference
(for measurement with
transient transfection)



Discussion

Transfection efficiency

Cell proliferation

Plasmid design

X-GFP / S-GFP

X-GFP + S-mCherry / S-GFP + S-mCherry

X-GFP-S-mCherry / S-GFP-S-mCherry

X-GFP-S-mCherry

Direct transfection eff. readout

No issues with transfection

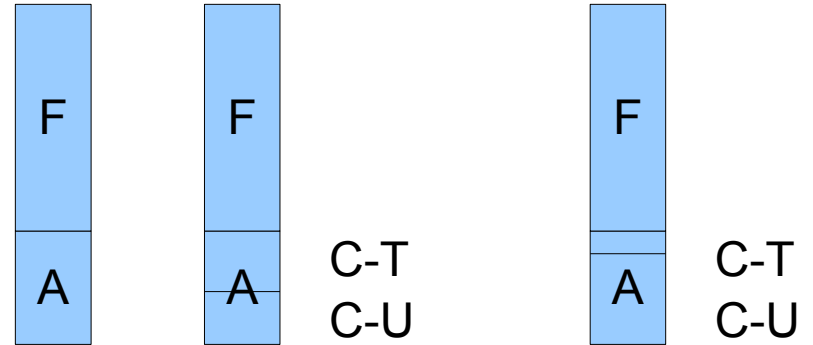
New unit !?

Measure / Ignore cells without plasmid

TECAN
qRT-PCR
Fluorometer

Flow Cytometry
Fl. Mi. + Im. an.

Measuring only cells with no plasmid



Significance

P-Value

Replicas

partsregistry.org/measurement

biobrick.org/standards