

**SECOND GENERATION
REFERENCE PROMOTERS TO
MEASURE TRANSCRIPTION
IN VIVO**

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BioFAB

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WHO I AM

- Daniel Bibl
- Undergraduate at Johns Hopkins University, Baltimore
- 10 week internship at BIOFAB

WHO I AM WORKING WITH

- BIOFAB Team:

- Vivek Mutalik

- Lance Martin

- Gaymon Bennett

- Jenhan Tao

- Cesar Rodriguez

- Joao Guimaraes

- Arthur Yu

CURRENT STATE OF THE REFERENCE PROMOTER

- Kelly et. al. : Using J23101 as a reference reduces variation of measured promoter activity across several measurement approaches by ~50%

CURRENT STATE OF THE REFERENCE PROMOTER

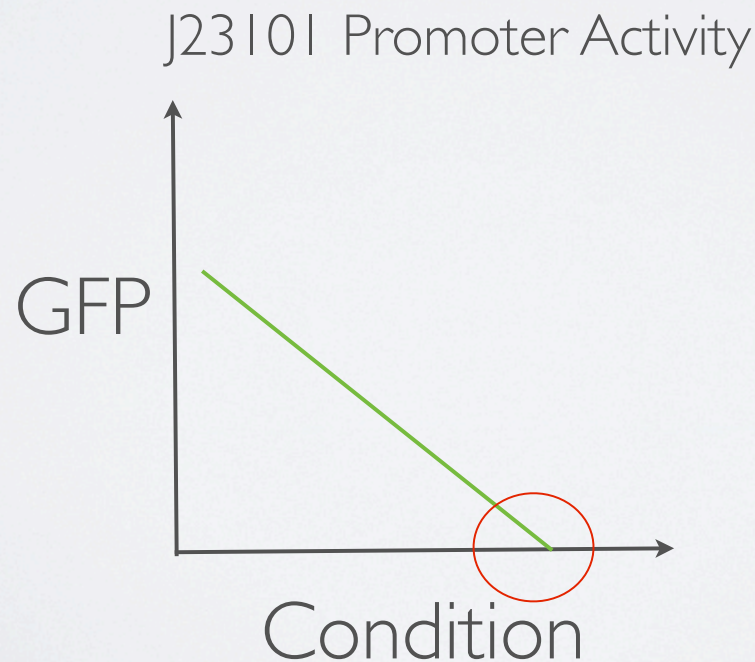
- Bottom line: Kelly et. al. showed that using reference promoters greatly reduces the effect of the measurement approach and media conditions on measurement of promoter activity.

CAN WE DO BETTER?

- >50% reduction of variation?
- Can we better tailor RPs to suit the needs of industry?

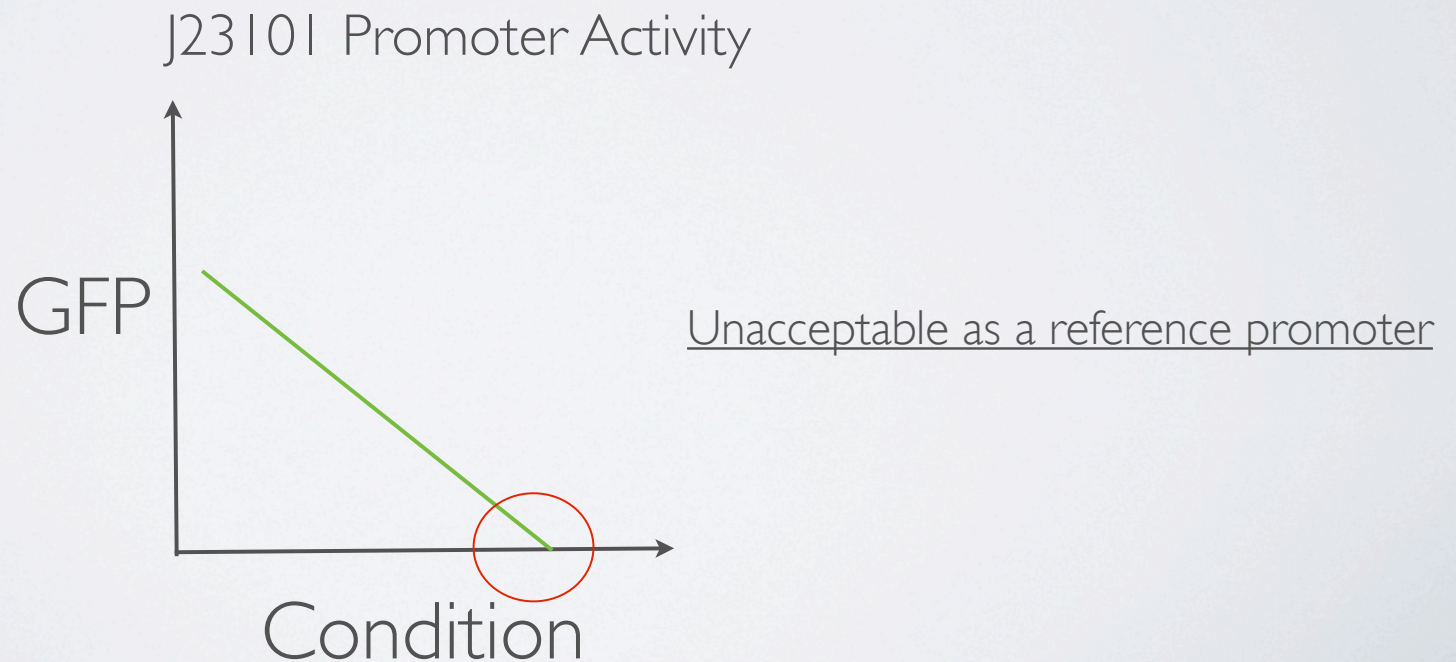
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CAN WE DO BETTER?

- **Goal:** Develop a set of reference promoters that have a dynamic range within the set of conditions representative of what is commonly used by people that make money by engineering DNA

Ist Gen.

**Proof of
Concept**



3rd+ Gen.



**Master Reference
Promoter**

1st Gen.

**Proof of
Concept**

J23101



3rd+ Gen.

**Master Reference
Promoter**

1 promoter for all conditions



1st Gen.

**Proof of
Concept**

J23101



2nd Gen.

Set of RPs

1 promoter for each condition



3rd+ Gen.

**Master Reference
Promoter**

1 promoter for all conditions

WORK DONE SO FAR

Gather information from industry contacts



Set of consensus operating conditions



Set of promoters known to be sensitive to these conditions

INDUSTRY CONTACTS

- Using feedback from the following industry contacts, developed the context in which metabolic engineers operate
 - Amyris Biotechnologies
 - DSM
 - Genencor
 - Joint BioEnergy Institute

CONDITIONS

- Consensus operating conditions known to affect metabolic pathways the most:
 - pH
 - Temperature
 - Dissolved Oxygen Levels
 - Carbon source
 - Growth phase

WORK DONE SO FAR - PROMOTERS

- 8 inducible promoters sensitive to key operating conditions
- 10 constitutive promoters insensitive to environmental conditions
- All promoters are well characterized and studied

PROMOTERS

- Your feedback needed!

PH RESPONSIVE PROMOTERS

- **Needs:** a promoter that has pH dependent activity within the common metabolic engineering operating regime of E. Coli: pH 4.0 - 7.0
- **Promoters:**
 - **asr** - acid shock response promoter. Known to be responsive to pH between 4.0 and 5.0.
 - **cadA** - Gene encoding for lysine decarboxylase. Regulatory region has variable degree of fully reversible induction between pH 7.0 and 5.5

OXYGEN RESPONSIVE PROMOTERS

- **Needs:** a promoter responsive to varying levels of dissolved oxygen.
- **Promoters:** The *nar* promoter had the best induction characteristics to be used as an oxygen-dependent inducible promoter, maximal induction of which occurred at low DO level
 - **nar P1** - weak induction by anaerobic conditions
 - **nar P2** - very strong induction by anaerobic conditions
 - Both created using site directed mutagenesis of wild type *nar* promoter yielding two promoters of varying oxygen-dependent activity.

TEMPERATURE RESPONSIVE PROMOTERS

- **Needs:** a promoter that has temperature dependent activity within the common operating regime of 30C - 42C.
- **dnaK P2** - well studied, medium strength heat shock promoter.
- **groEL** - highly expressed heat shock promoter within the groE regulon.

GROWTH PHASE RESPONSIVE PROMOTERS

- **Needs:** a promoter that is sensitive to various growth rates and states.
- **rrnB PI** - Well characterized growth state promoter. Induced in exponential phase. Reduced activity at low growth rate.
- **PoxB** - Sigma S promoter induced in stationary phase.

PROMOTERS INSENSITIVE TO OPERATING CONDITIONS

- 10 constitutive promoters

- J23101

- J23111

- J23119

- J23115

- Ptrc

- Dpsp

- RpsJ

- U46D46

- U66D56

- U66D66

PROMOTERS INSENSITIVE TO OPERATING CONDITIONS

- 10 constitutive promoters
 - J23101
 - J23111
 - J23119
 - J23115
 - Ptrc
 - Dpsp
 - RpsJ
 - U46D46
 - U66D56
 - U66D66
- Chosen to represent low, medium and high levels of constitutive activity

CONDITIONS TO BE TESTED

Condition	Range
pH	4.0 - 7.0
Temperature	30C - 42C
Oxygen	0-100% saturation
Growth Rate	Exponential phase, Stationary phase
Carbon Source	Glucose, Glycerol, Rich Media, Minimal media

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- All promoters to be tested in all conditions

QUESTIONS AND OPPORTUNITIES

- Are these the correct promoters (i.e. are there better)?
- Are these the correct conditions to focus on (i.e. are there other more important conditions)?
- Off-site verification of finished set of reference promoters (i.e. do they work; do they reduce variation of measurement)?

REFERENCE OBJECTS FOR OTHER PARTS?

- Quick and easy characterization and sharing of information of a part is key to the ongoing effort of standardizing biological parts
- Reference promoters have been shown to reduce the complexity of measuring and sharing information of promoter activity

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- Quick and easy characterization and sharing of a part is key to the ongoing effort of standardizing biological parts
- Reference promoters have been shown to reduce the complexity of measuring and sharing information of promoter activity
- **Can the idea of a reference object be applied to other parts?**