Introduction to Synthetic Biology

Topic 1 | Topic 2 | Topic 3 | Topic 4 | Topic 5

Foundations for Synthetic Biology

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Bioengineering Department
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Introduction to Synthetic Biology

Topic 1 | Topic 2 | Topic 3 | Topic 4 | Topic 5

Standard for Physical DNA Composition

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Topic 1 | Topic 2 | Topic 3 | Topic 4 | Topic 5

Standards for Functional Composition

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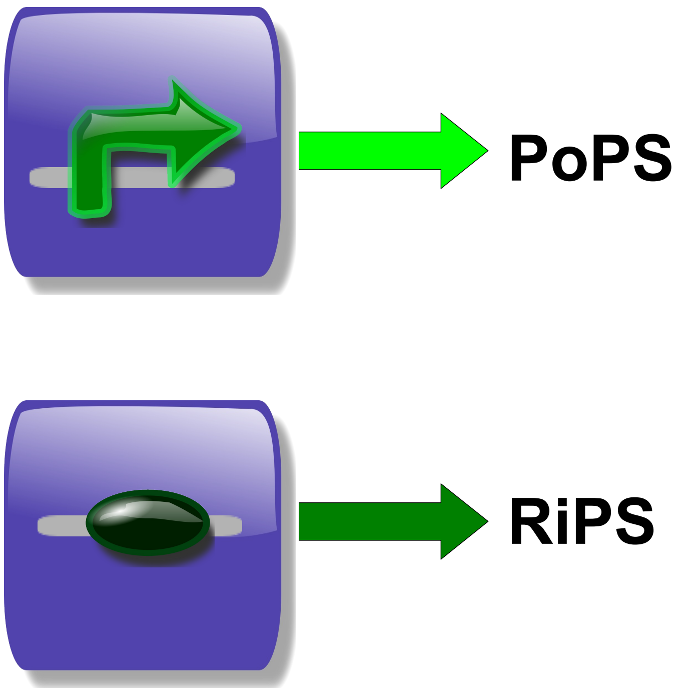
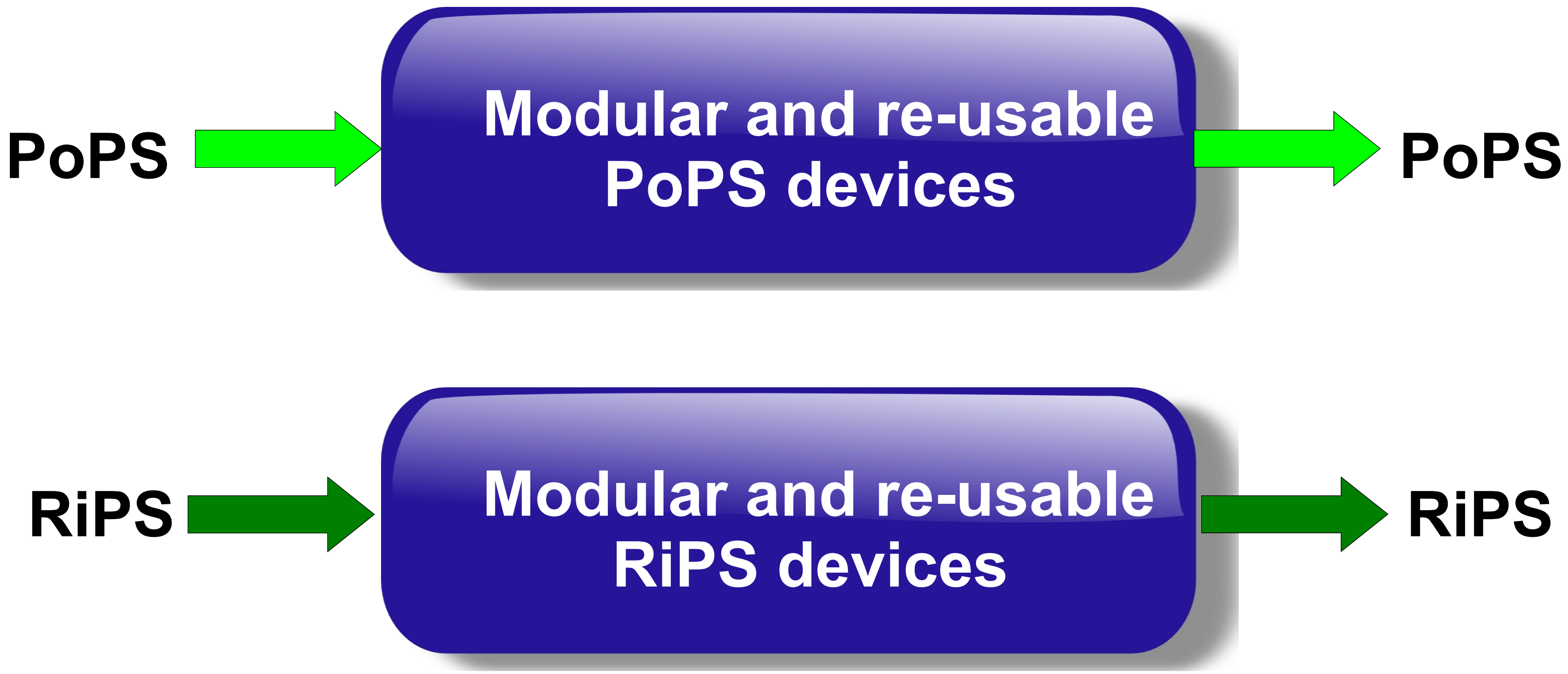
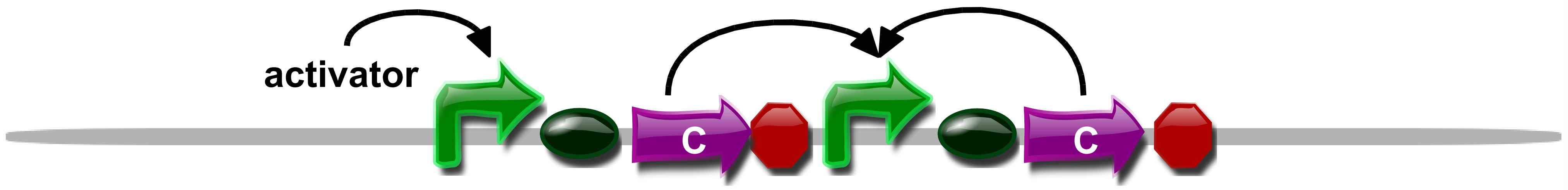
PoPS / RiPS Generators



PoPS / RiPS Devices



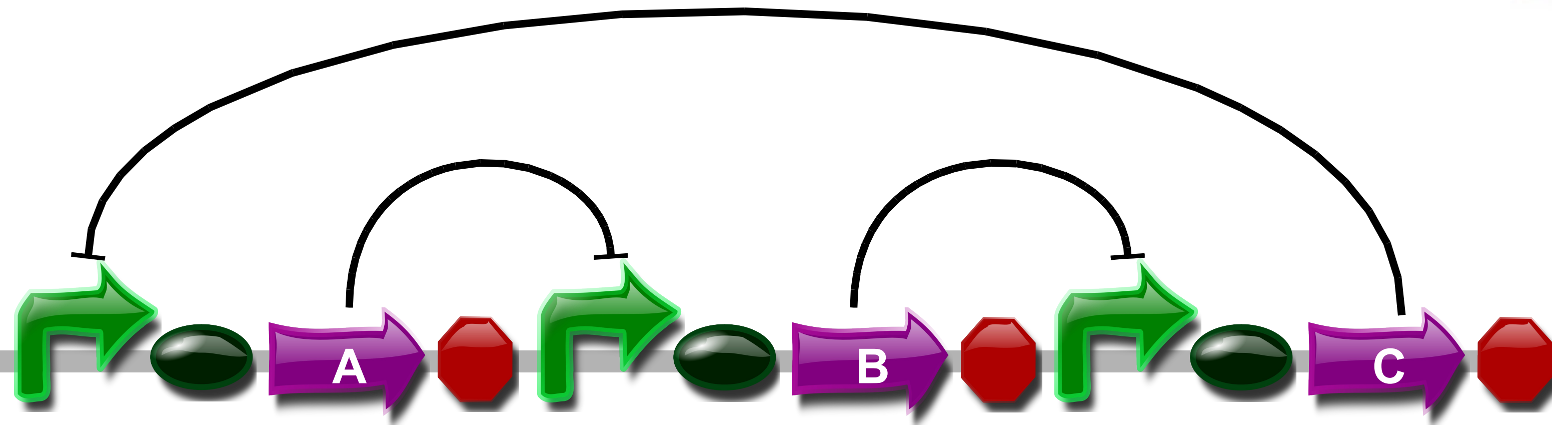
Transcriptional Device



Question 1:
What does this system do ?

Question 2:
Extract a modular and re-usable device from this circuit.

Transcriptional Device

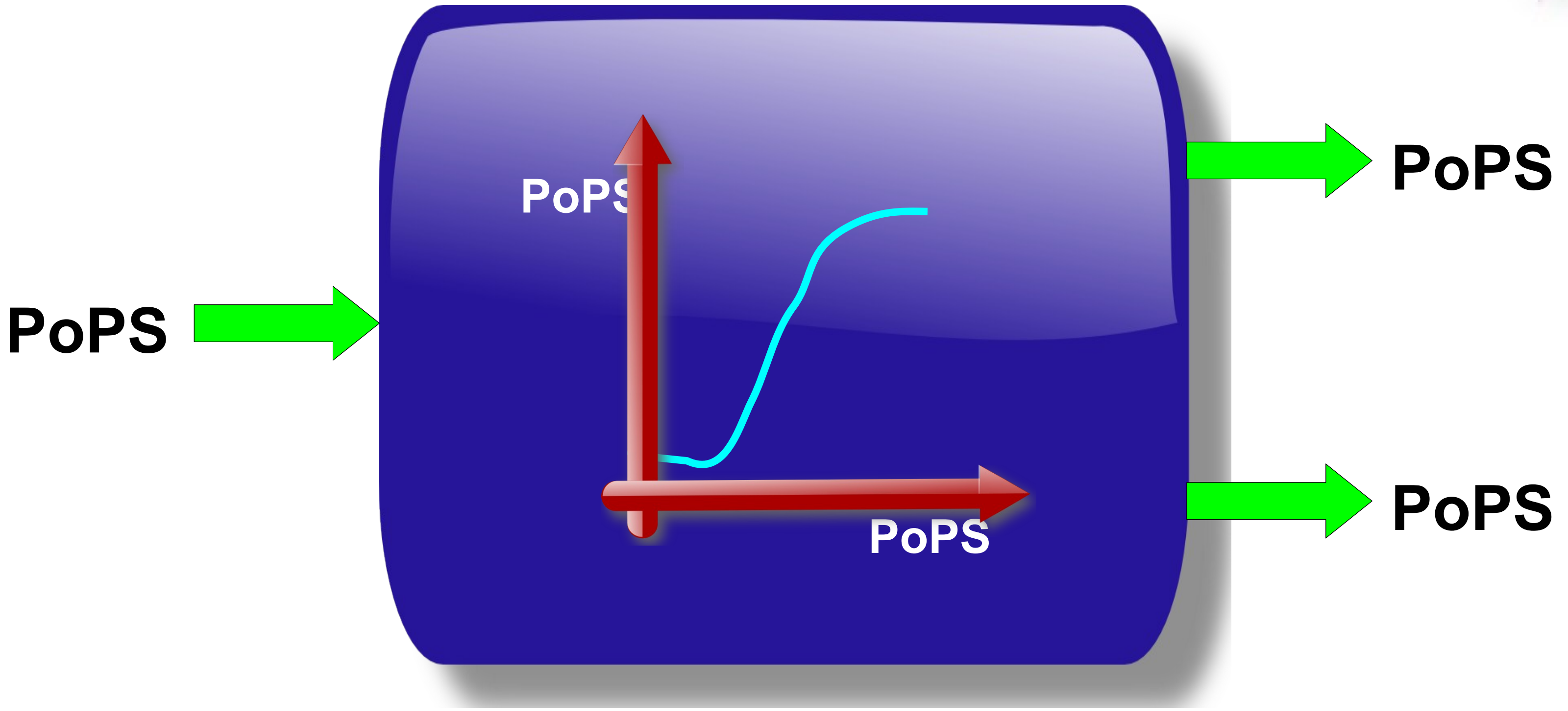


Question 1:
What does this system do ?

Question 2:
Extract 3 modular and re-usable devices from this circuit.

Question 3:
Update the design so this system can be easily plugged into a larger system

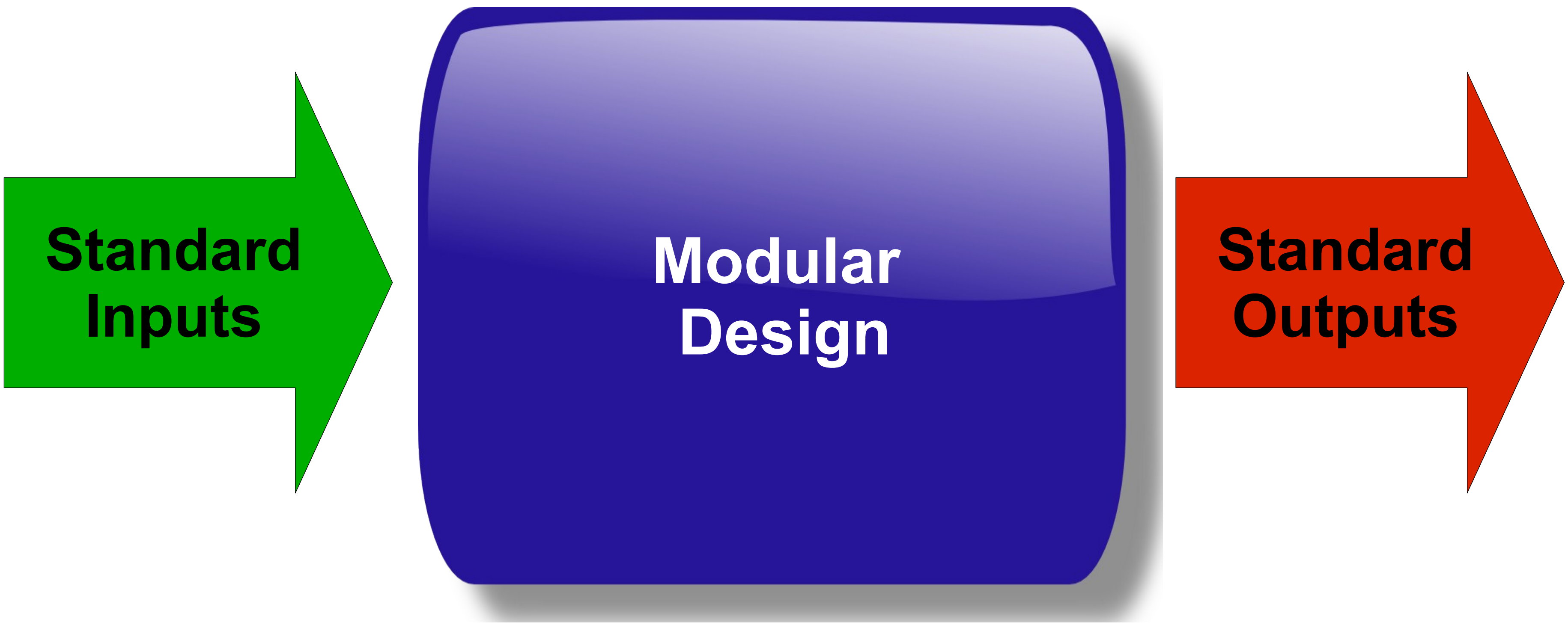
Transcriptional Device



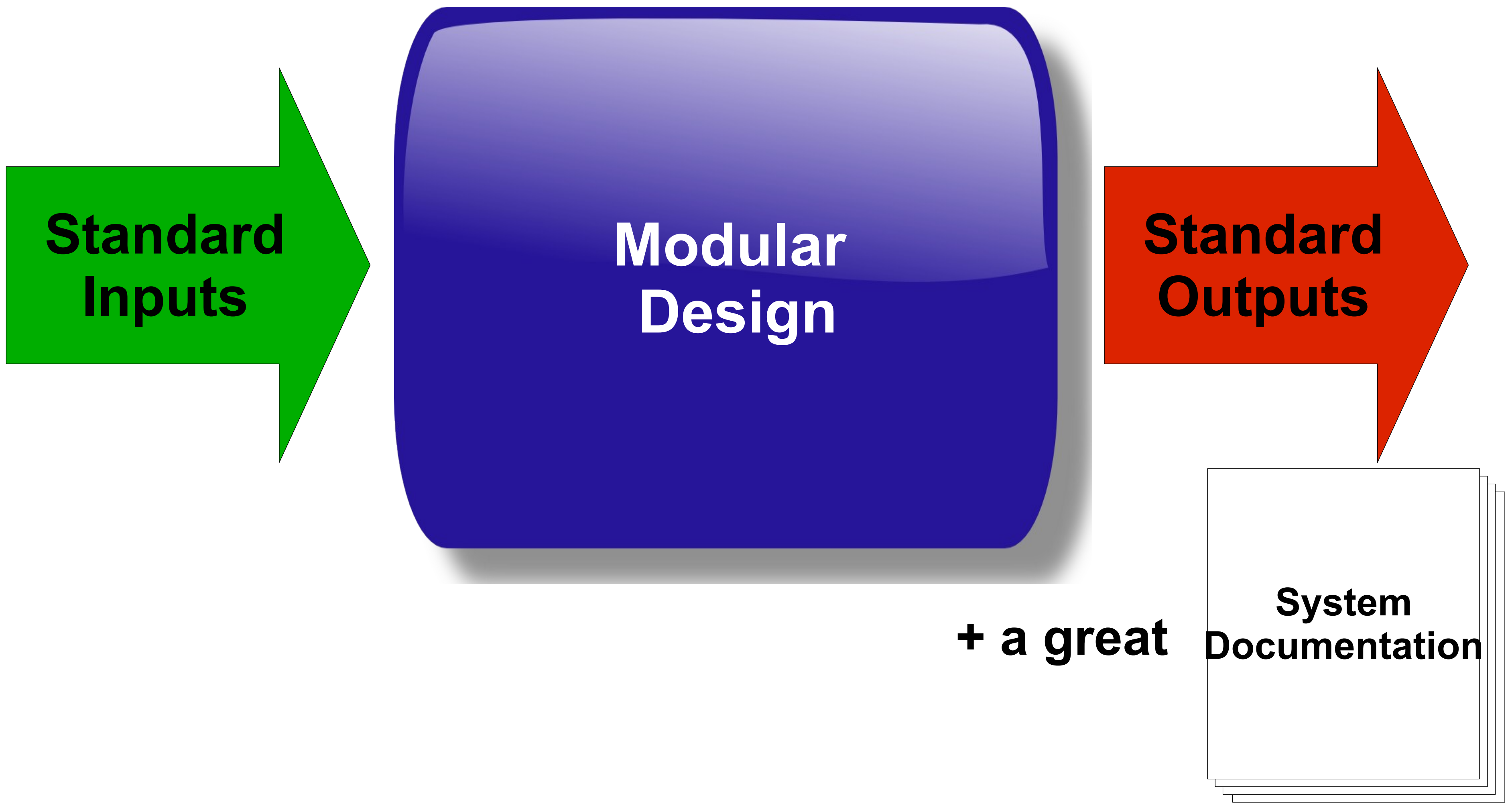
Question:

How to implement this device ?

To Promote Re-usability



To Promote Re-usability



Introduction to Synthetic Biology

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Characterising Standard Biological Parts

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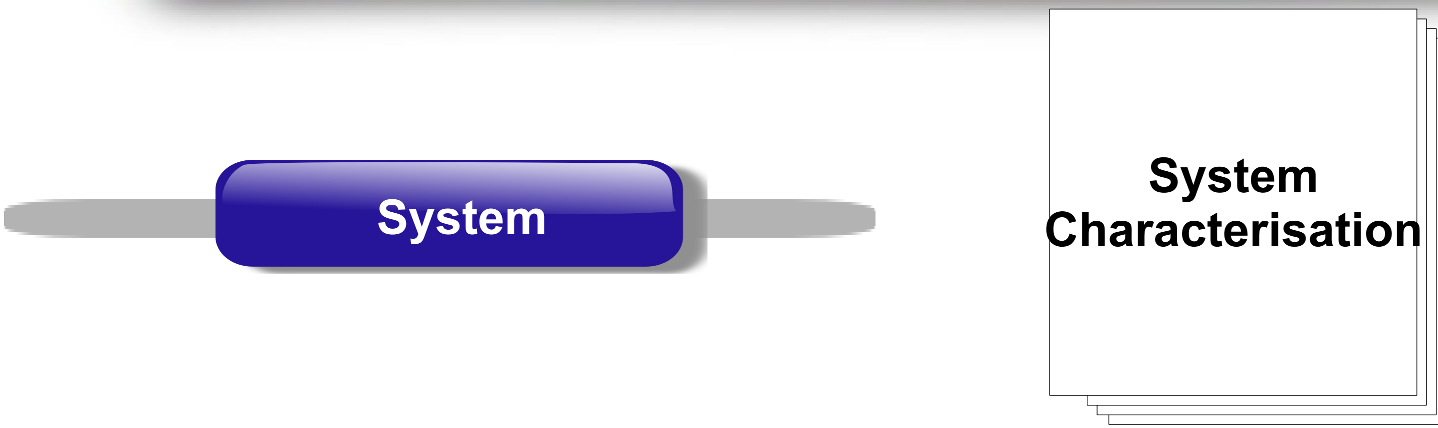
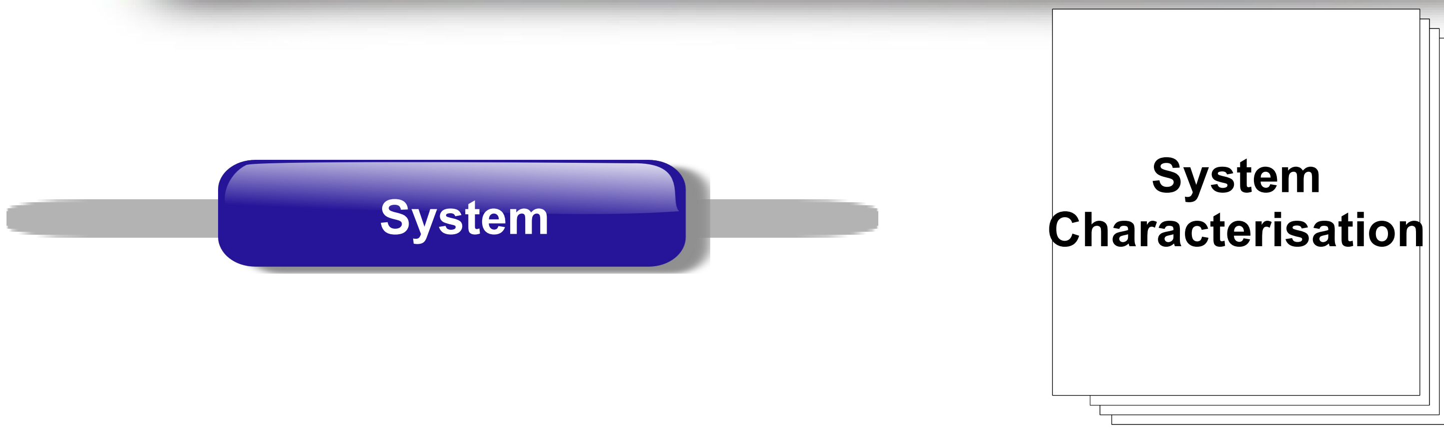
Imperial College London

What is a good Part / Device / System characterisation ?



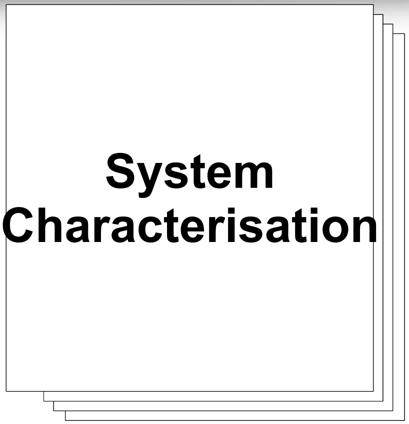
What is a good Part / Device / System characterisation ?



A good Device Characterisation is:

the minimum amount of information someone needs to reuse the part without any prior knowledge

What type of info do we expect ?



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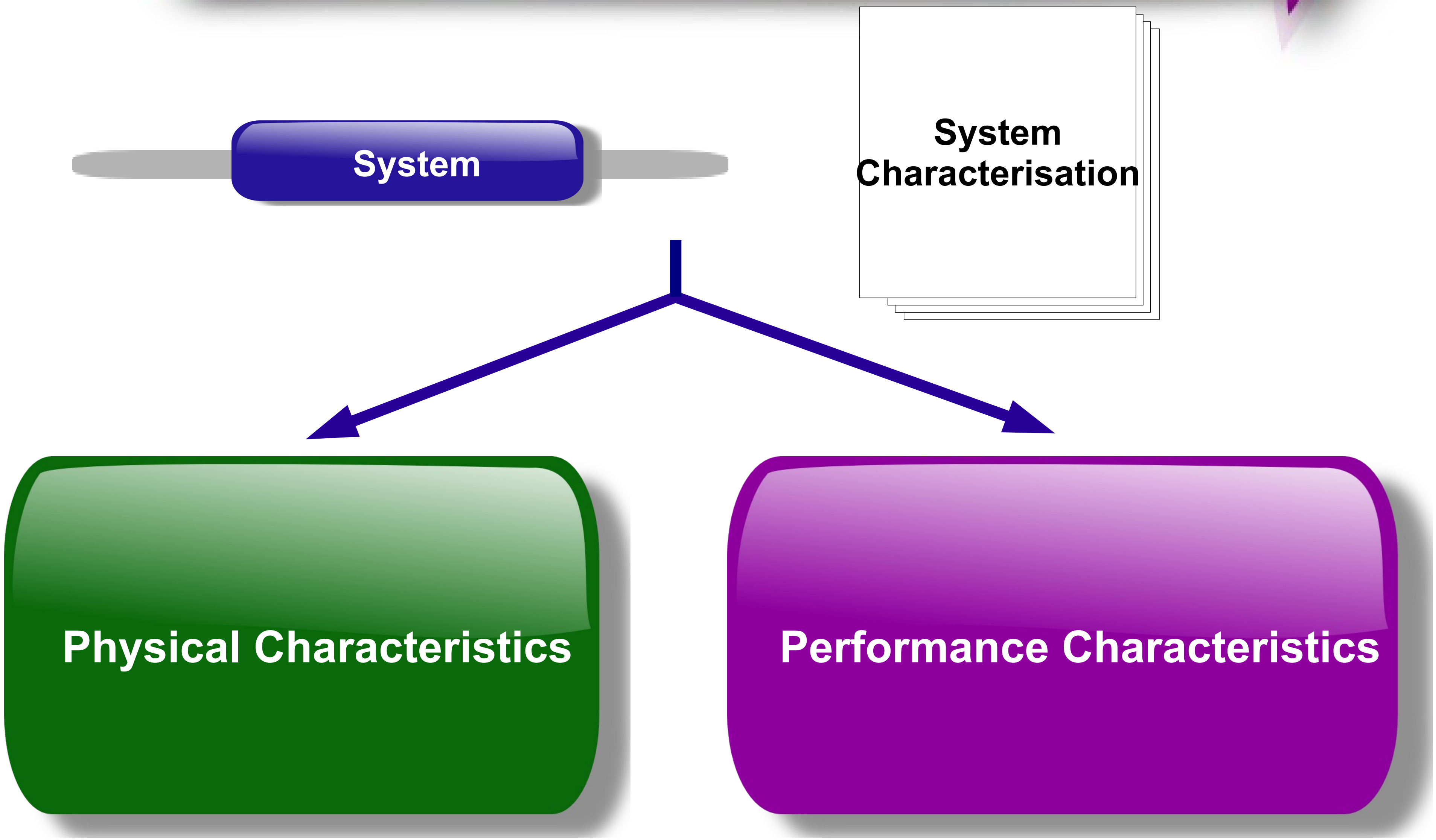


What type of info do we expect ?

System

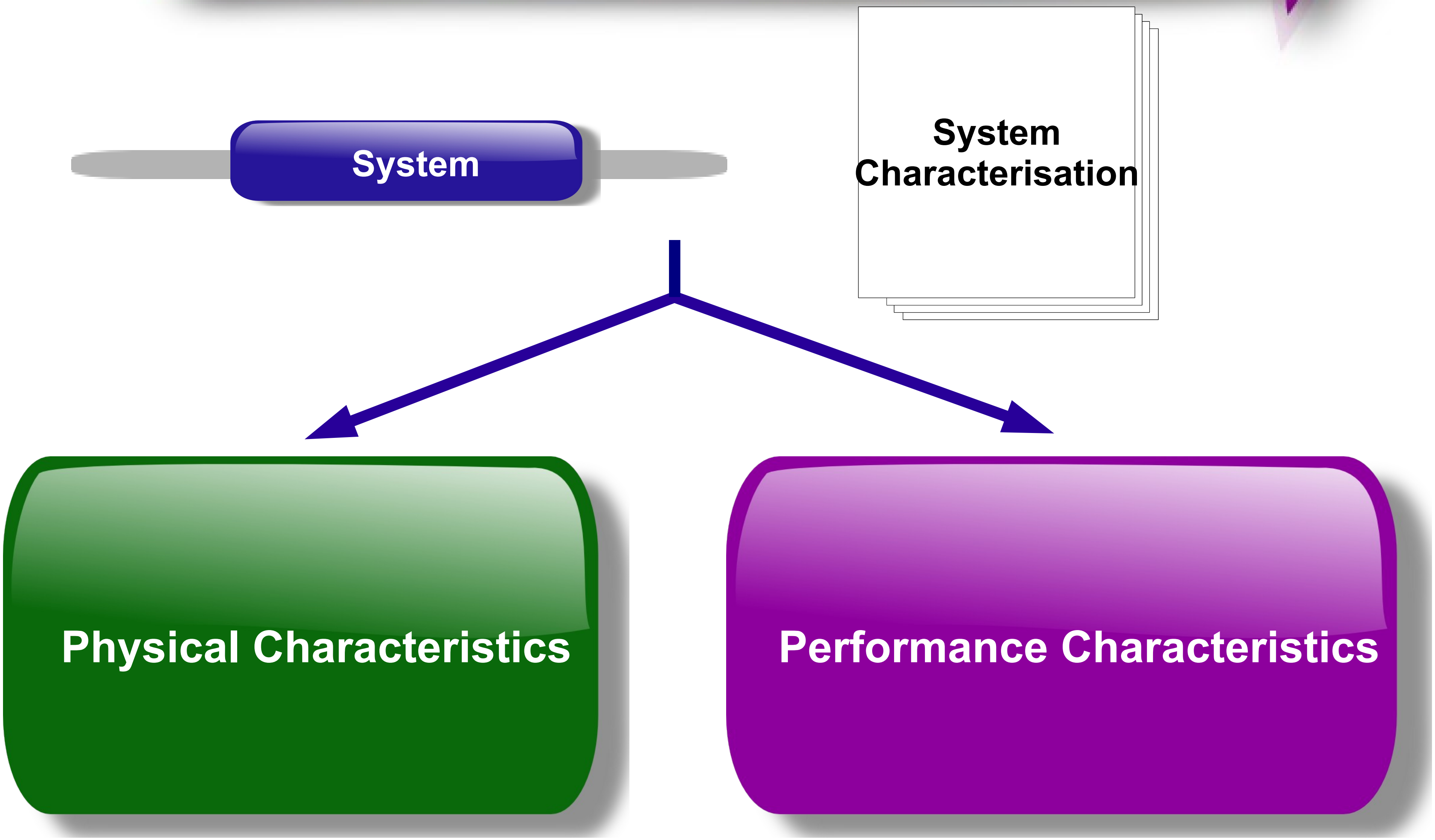
System Characterisation

Physical Characteristics

- Background info
- Sequencing certificate
- Sequence analysis
- ...

Performance Characteristics

What type of info do we expect ?



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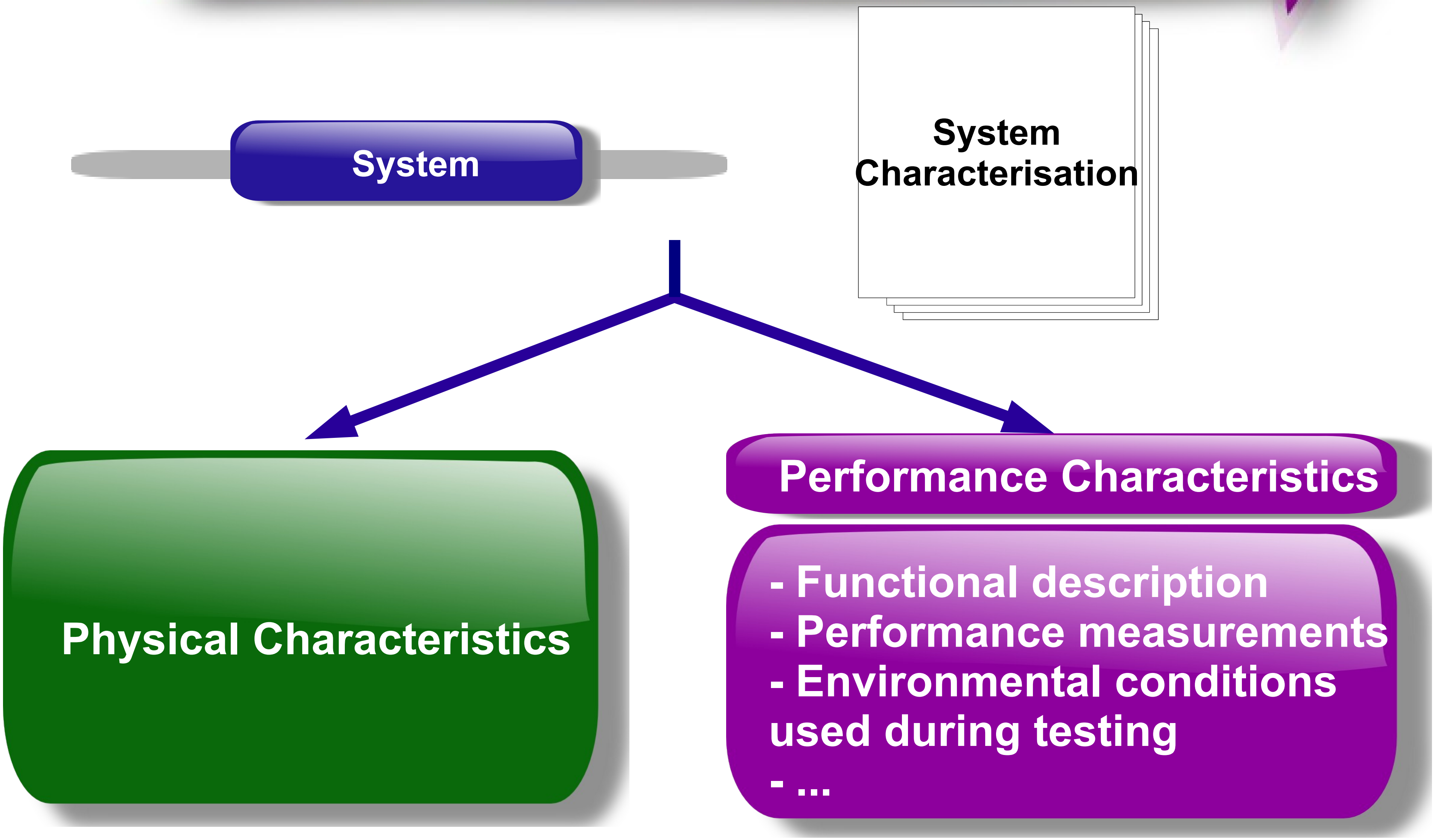
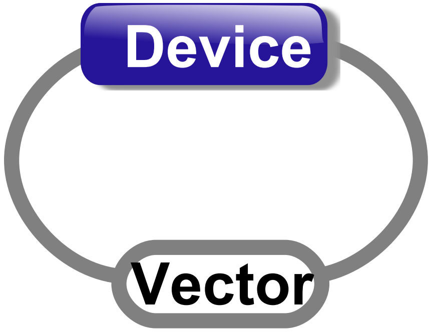


Functional Properties Dependencies

Device

Functional Properties Dependencies



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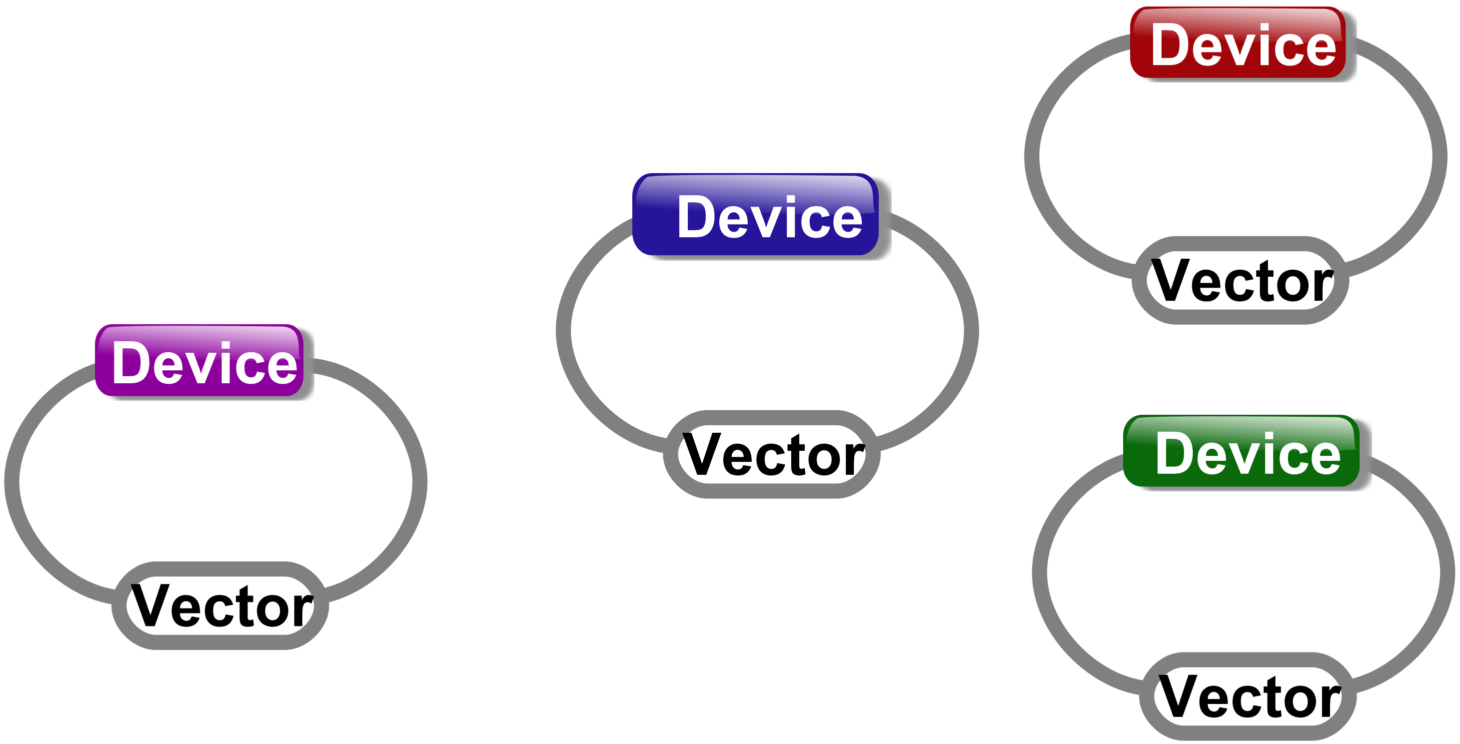

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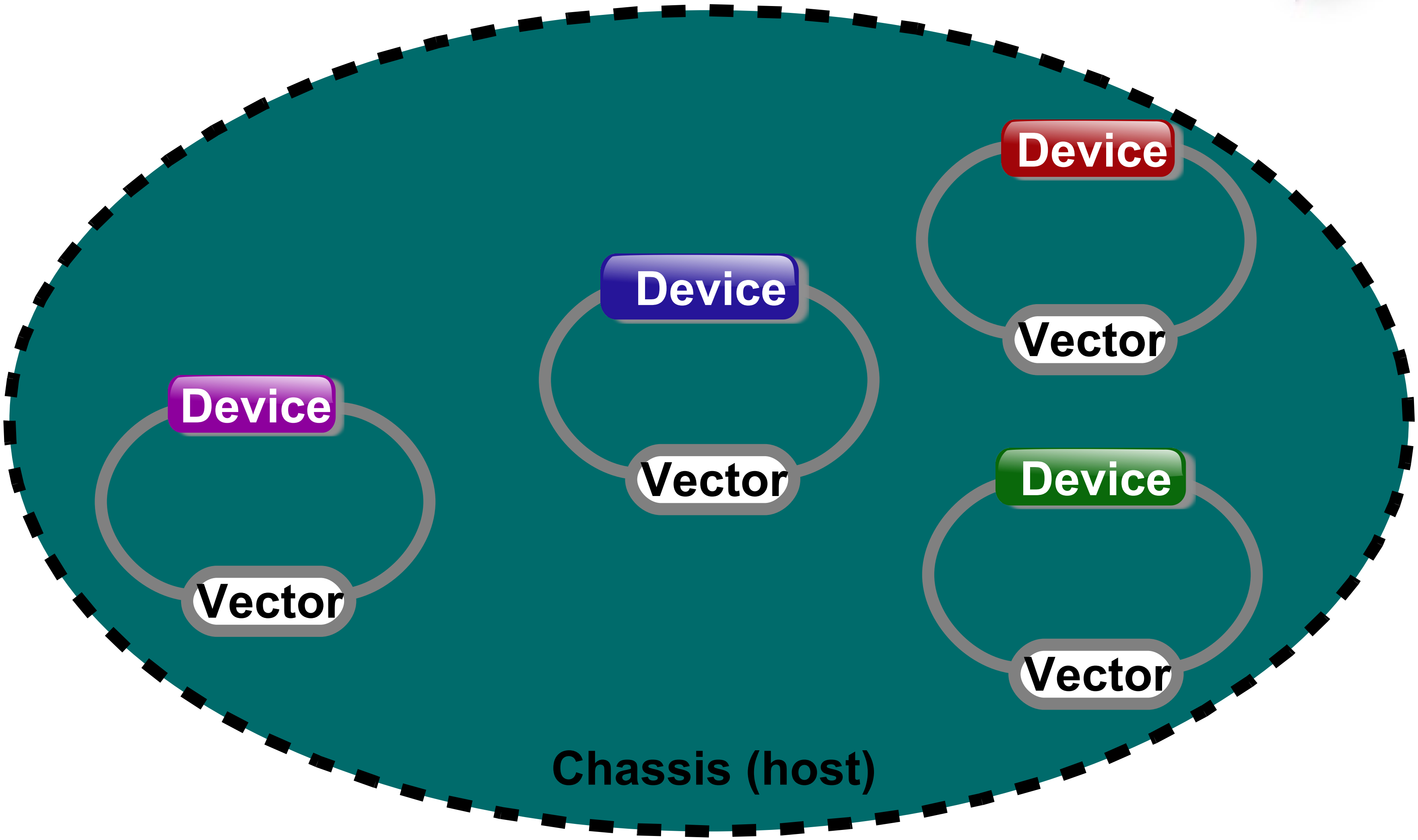

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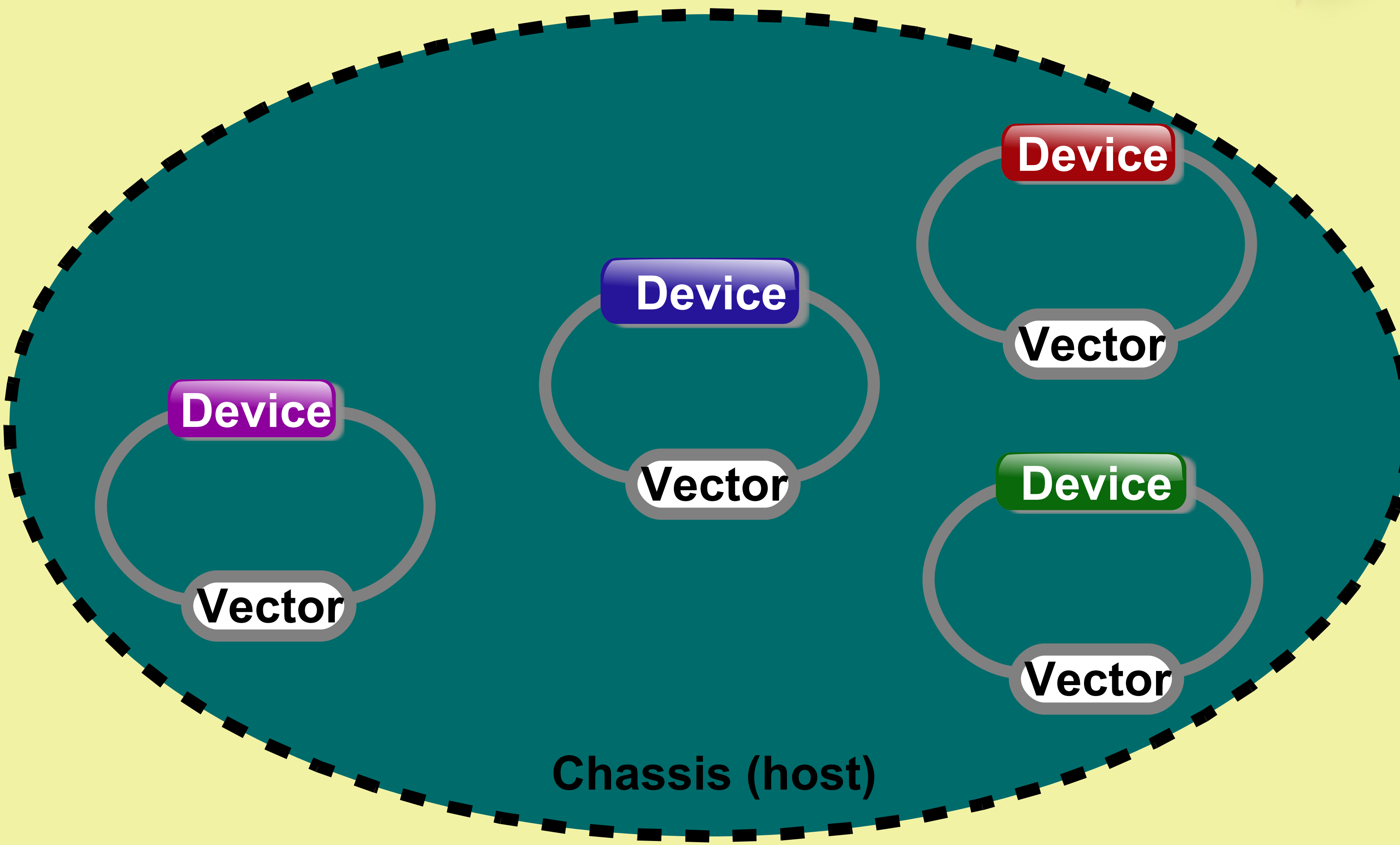


Environmental Conditions

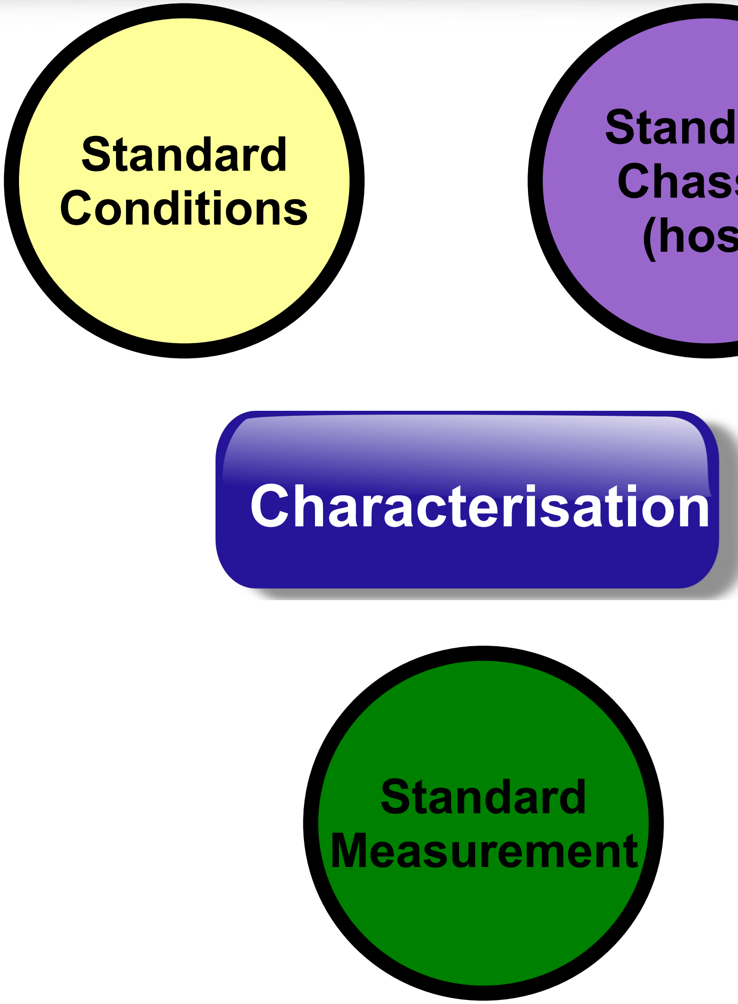
Functional Properties Dependencies

Too high level of complexity

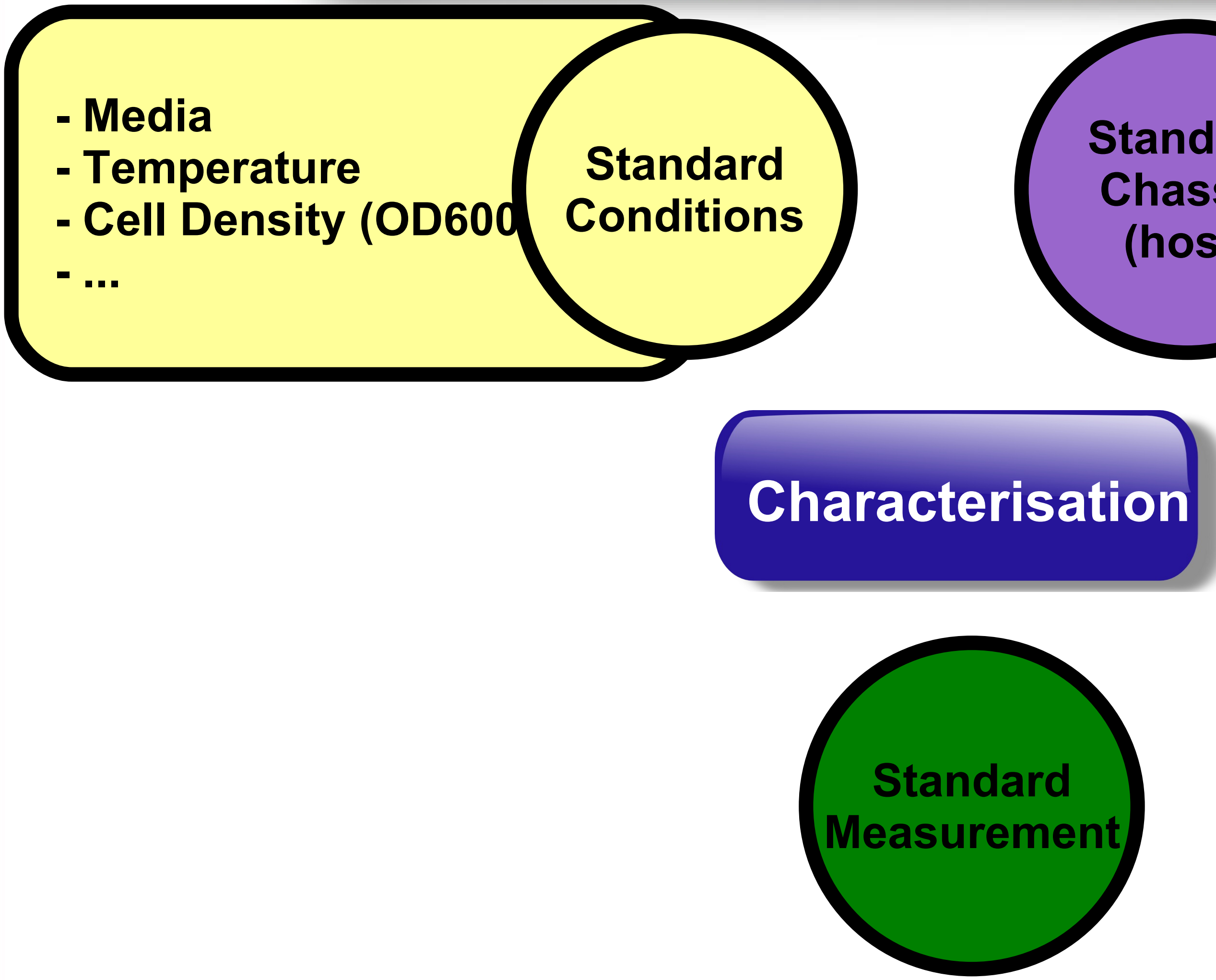
To obtain a reliable and reproducible characterisation we need to standardise the way we are probing the system

Environmental Con

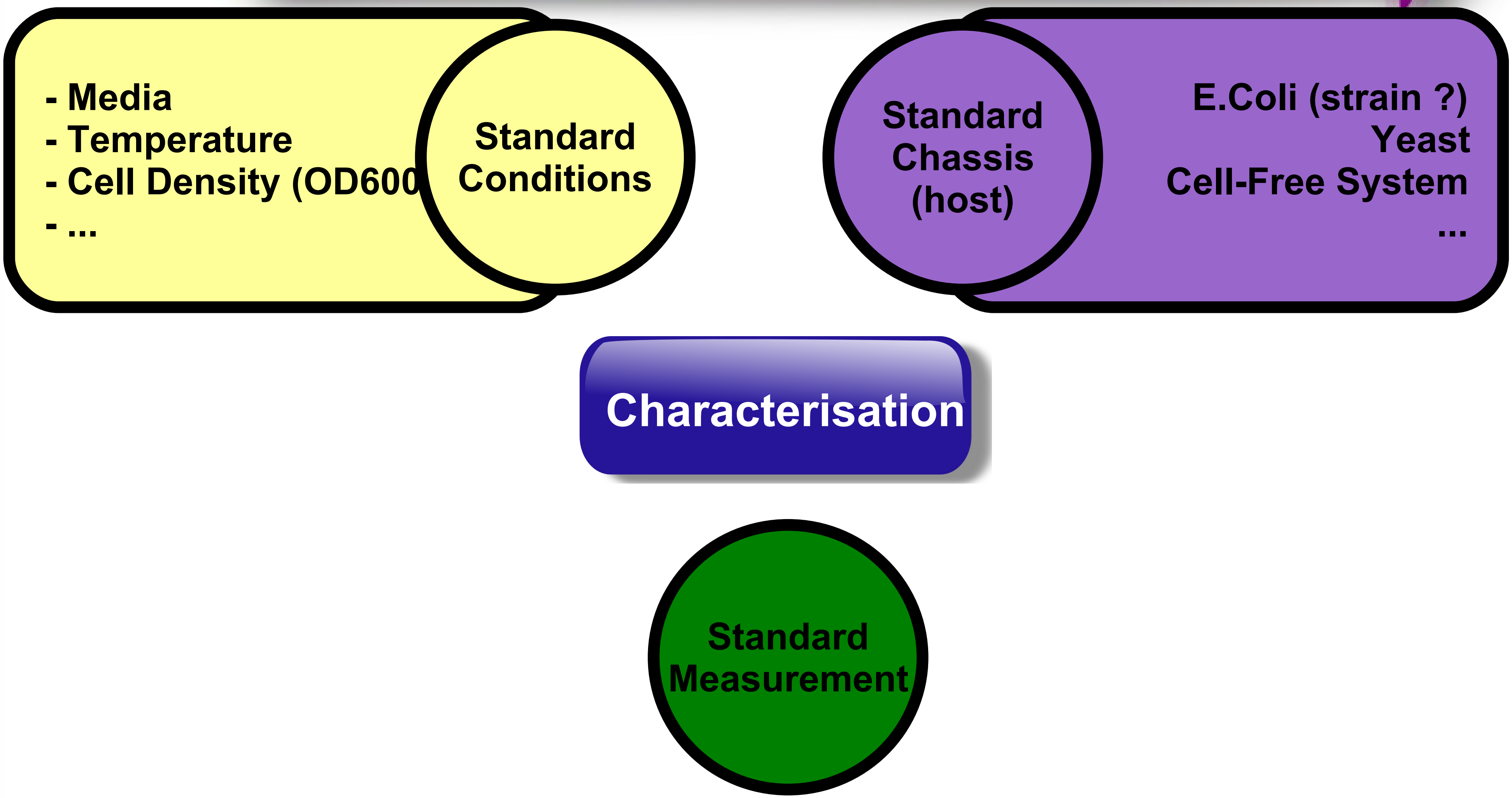
Characterisation and Standards



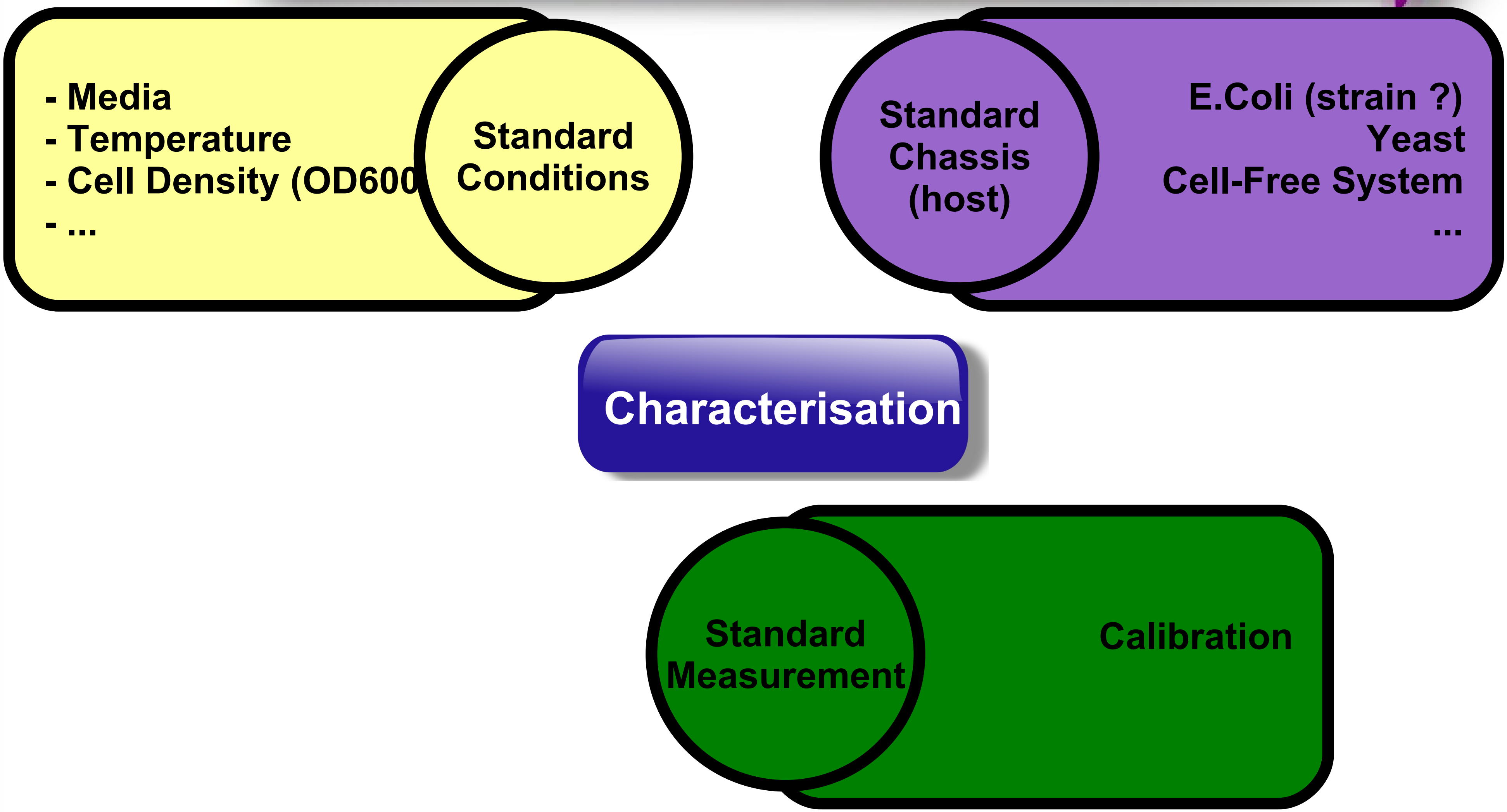
Characterisation and Standards



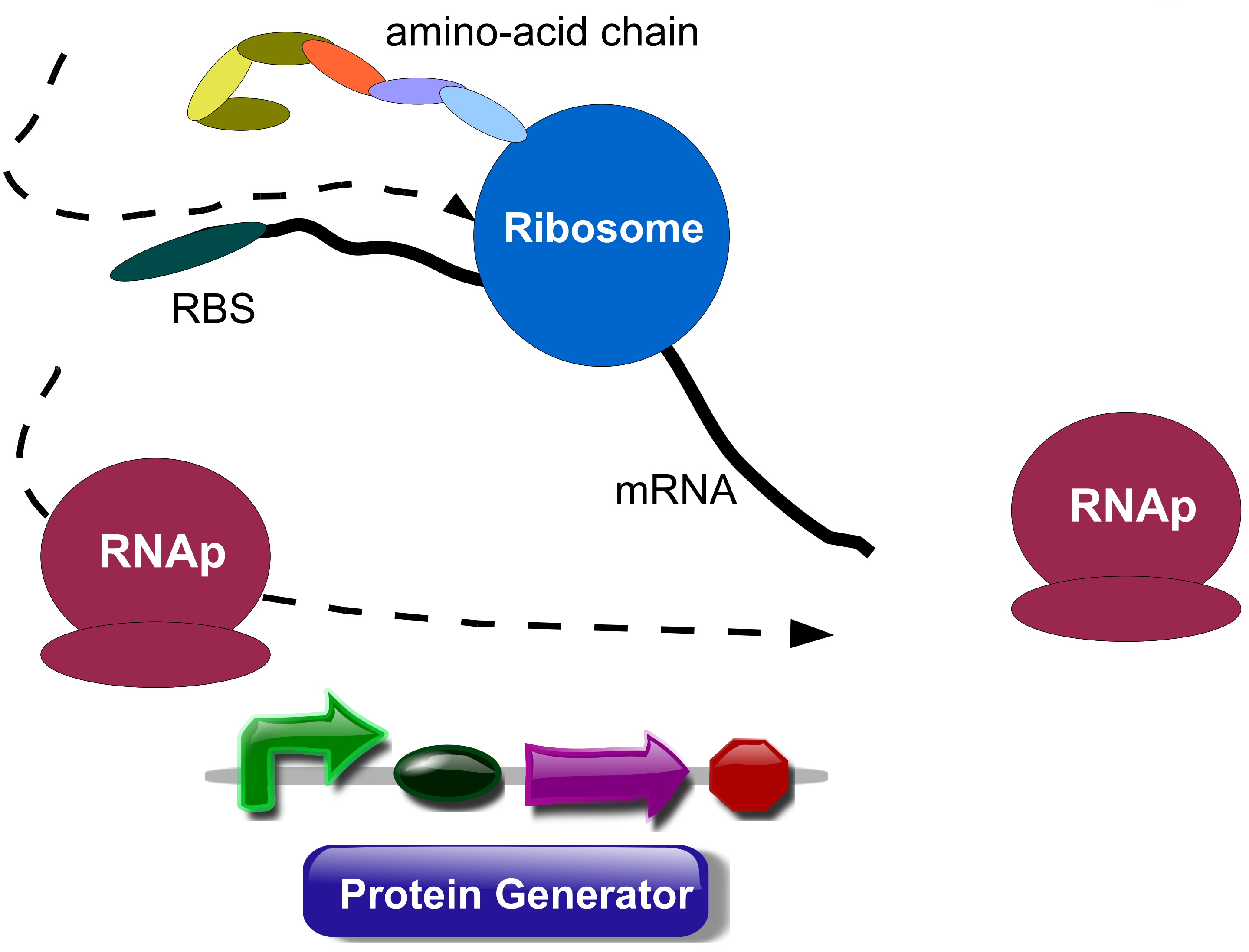
Characterisation and Standards



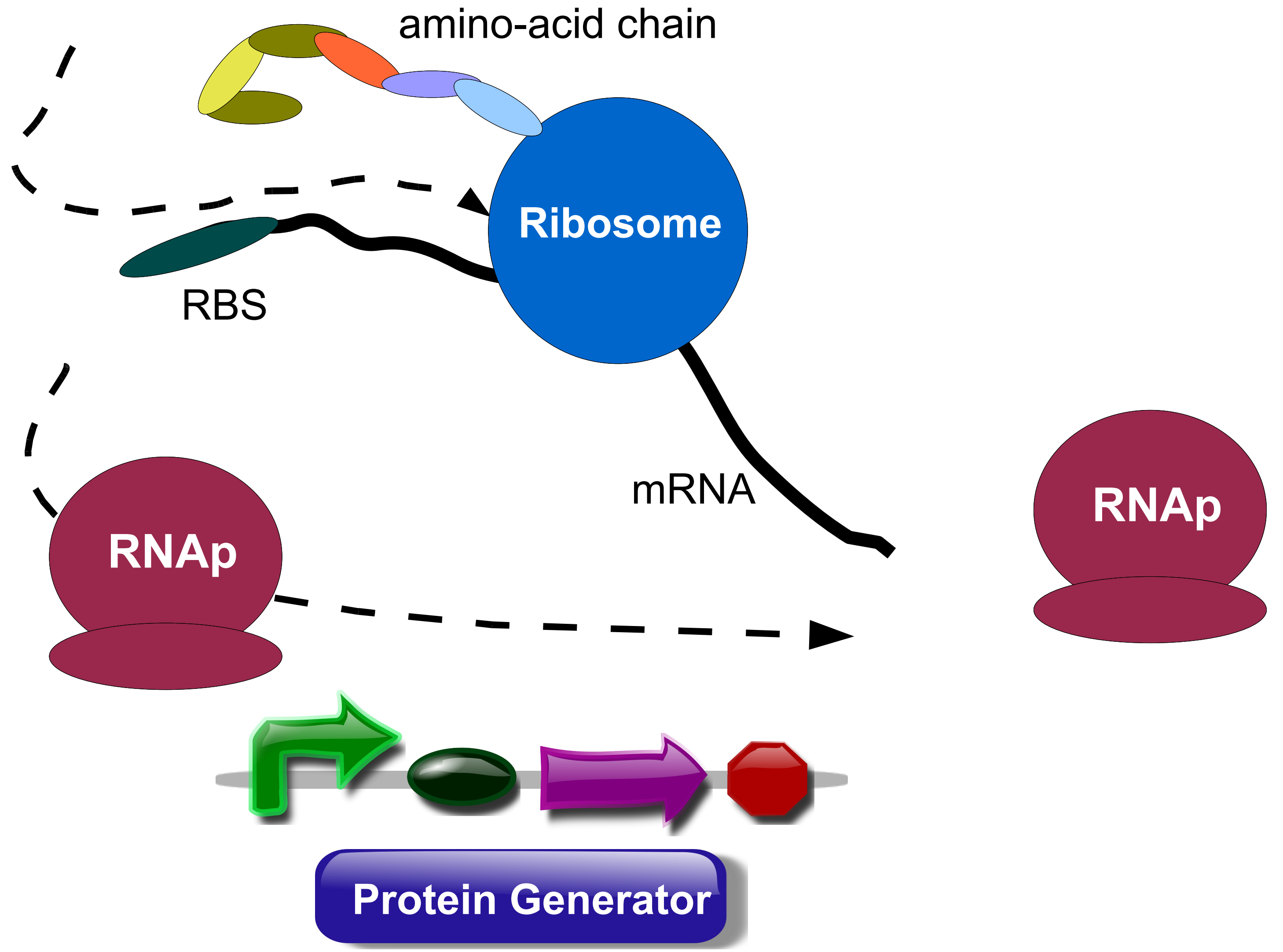
Characterisation and Standards



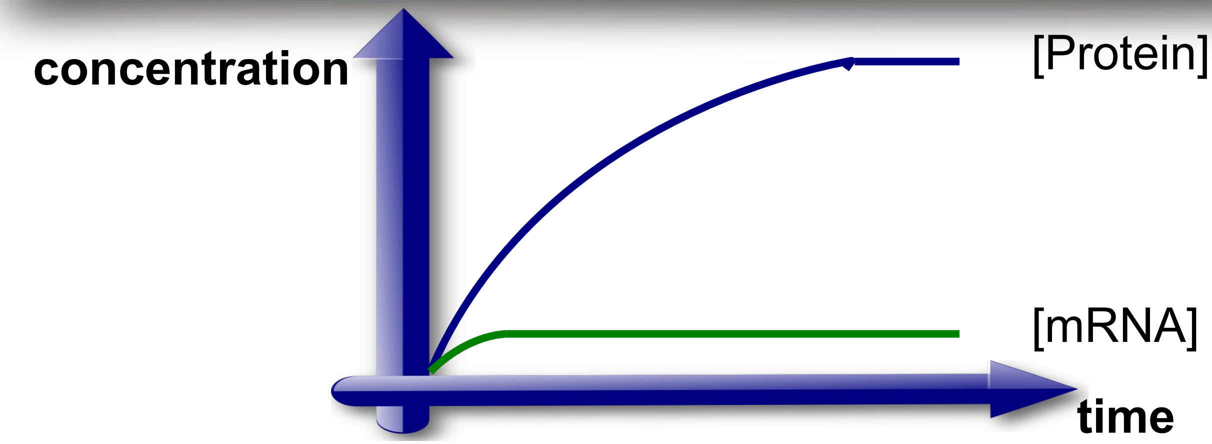
How do you measure PoPS ?



How do you measure RiPS ?

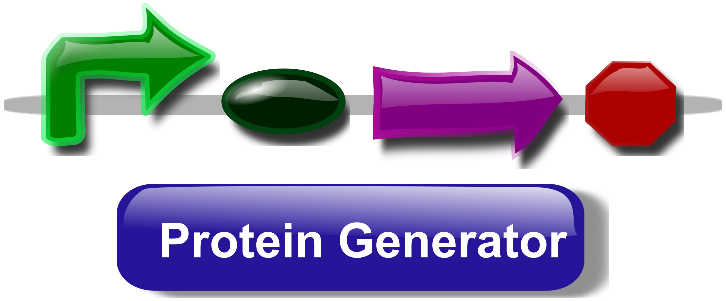


Indirect Measurement

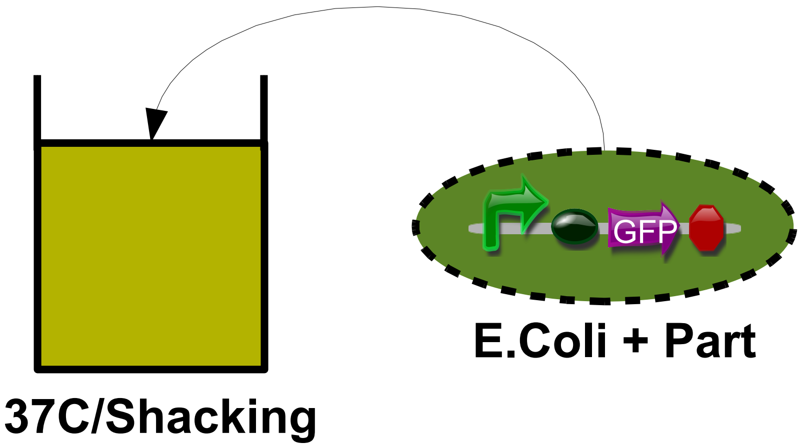


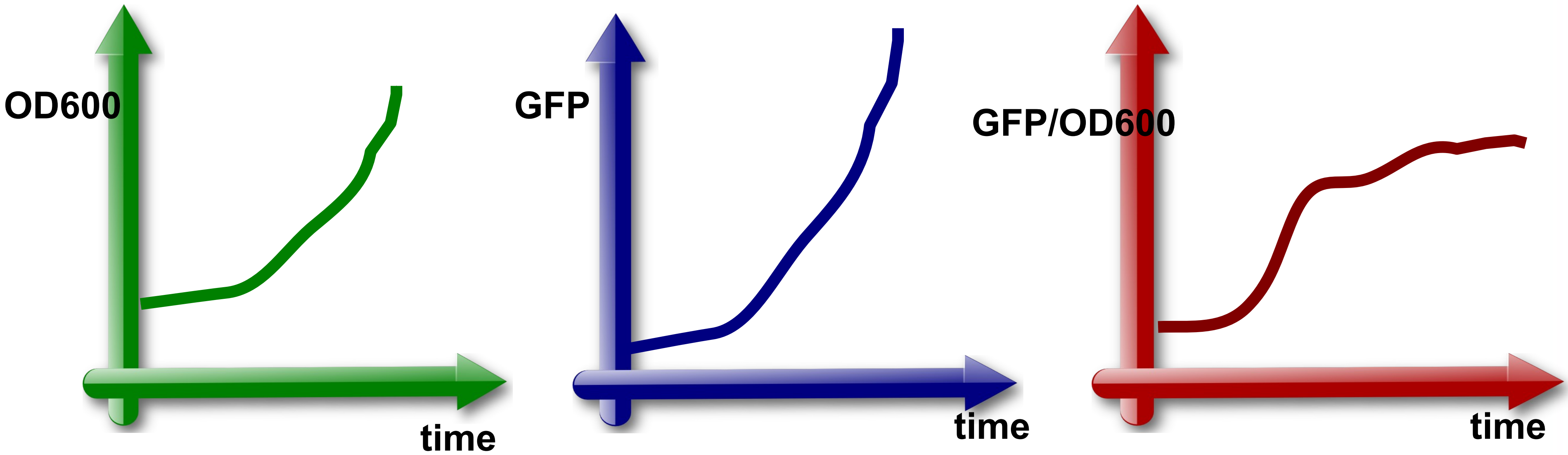
$$\frac{d[mRNA]}{dt} = k1 - d1[mRNA]$$

$$\frac{d[Protein]}{dt} = k2.[mRNA] - d2[Protein]$$

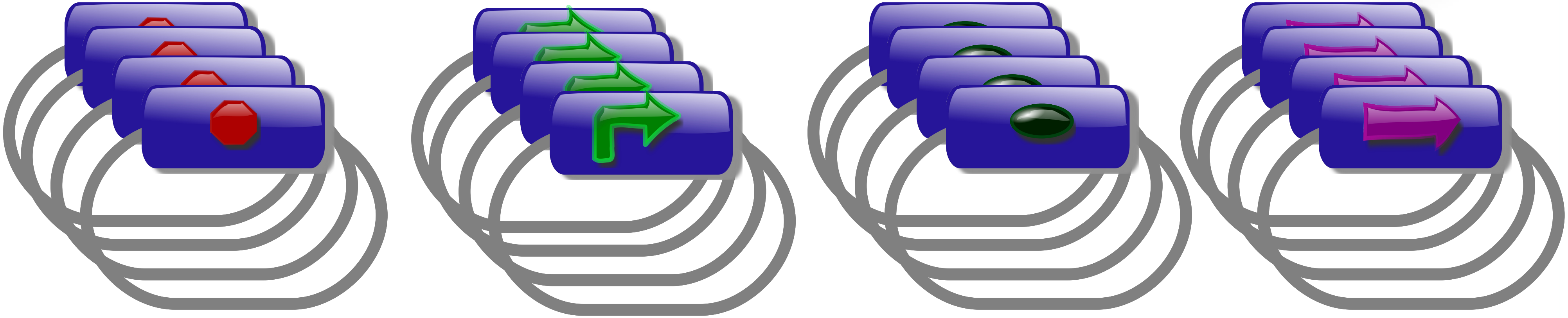


GFP Measurement



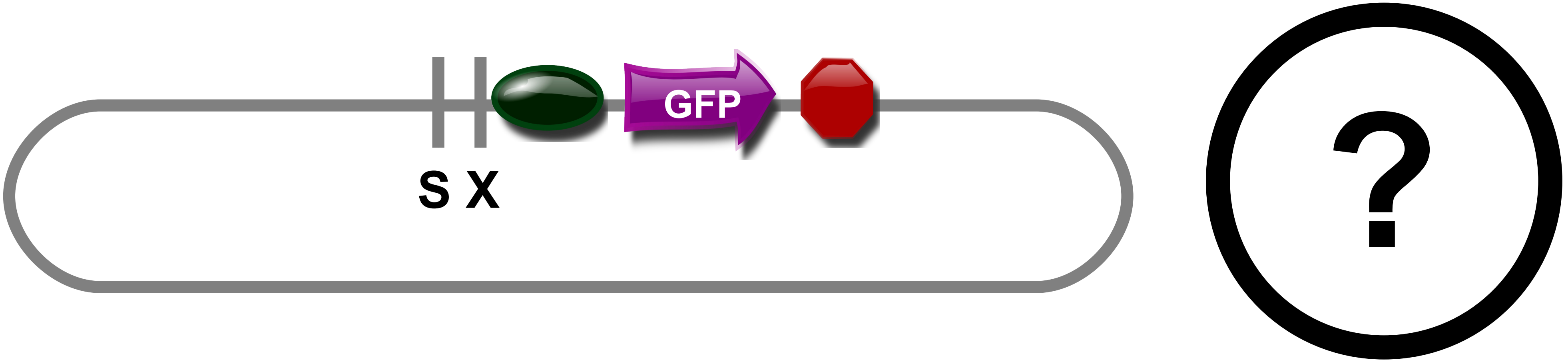


Measurement Kit

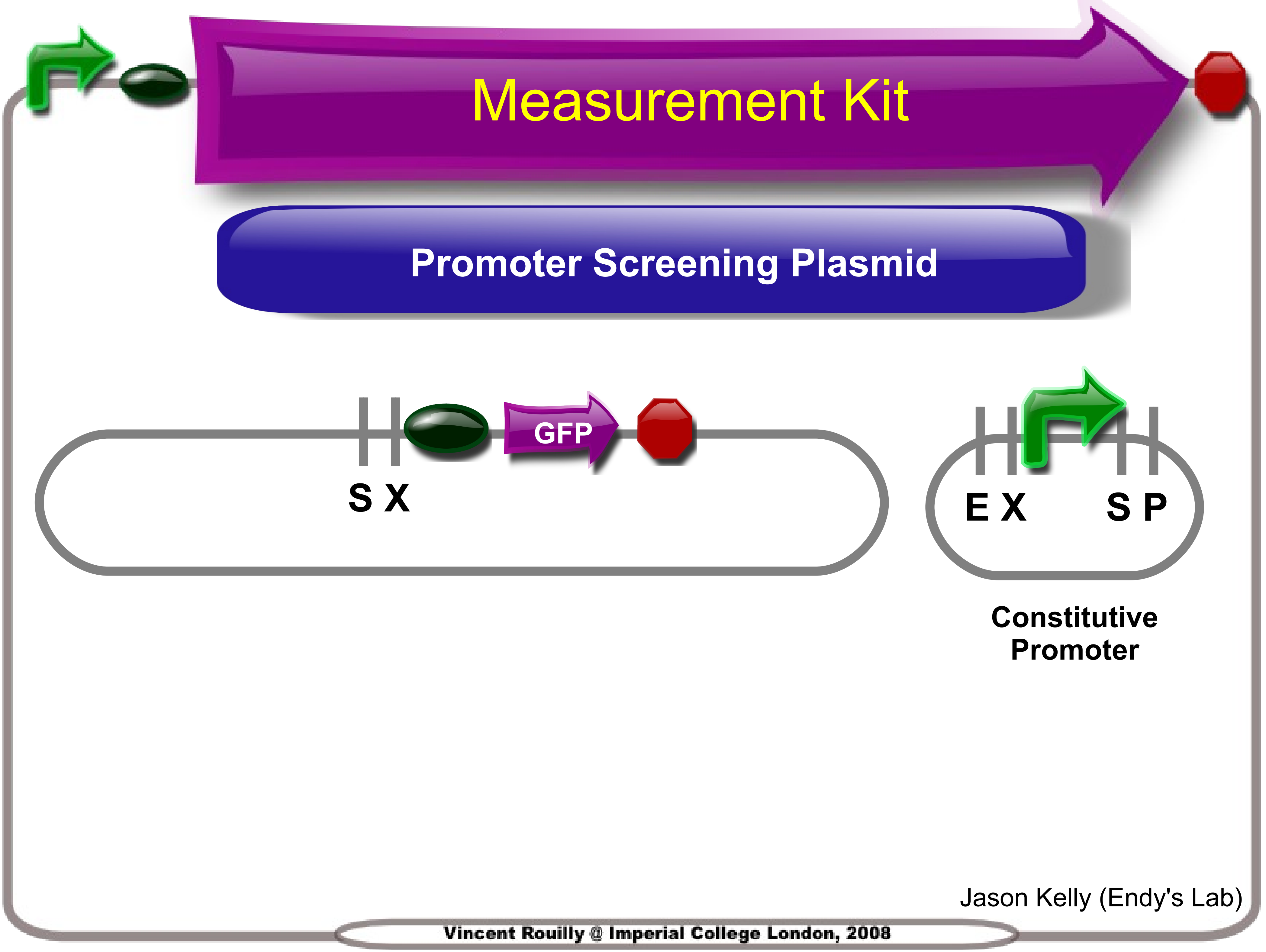


Back to Basic Parts

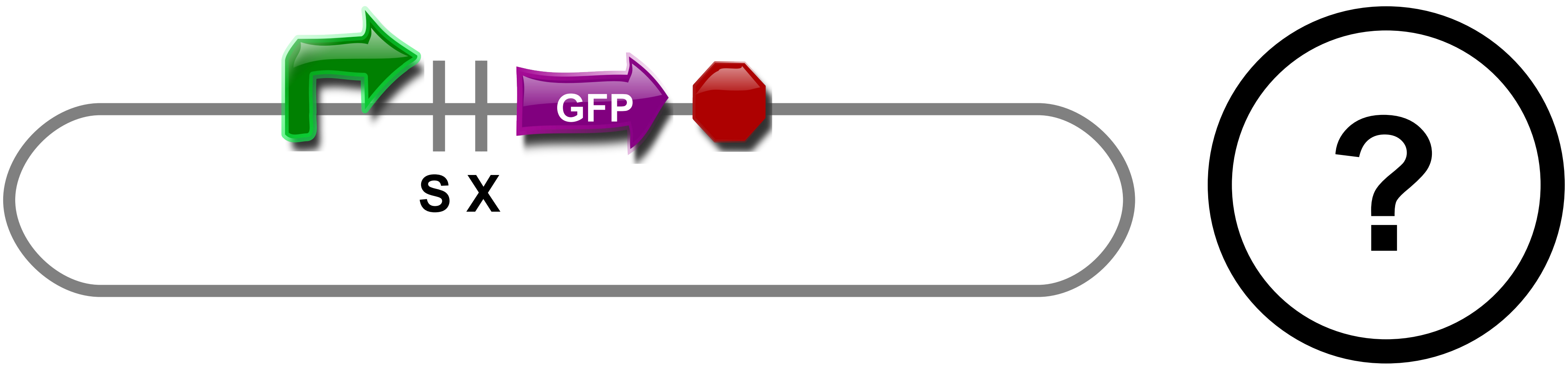
Measurement Kit



Jason Kelly (Endy's Lab)


Measurement Kit
Promoter Screening Plasmid
S X
GFP
E X
S P
Constitutive Promoter
Jason Kelly (Endy's Lab)
Vincent Rouilly @ Imperial College London, 2008

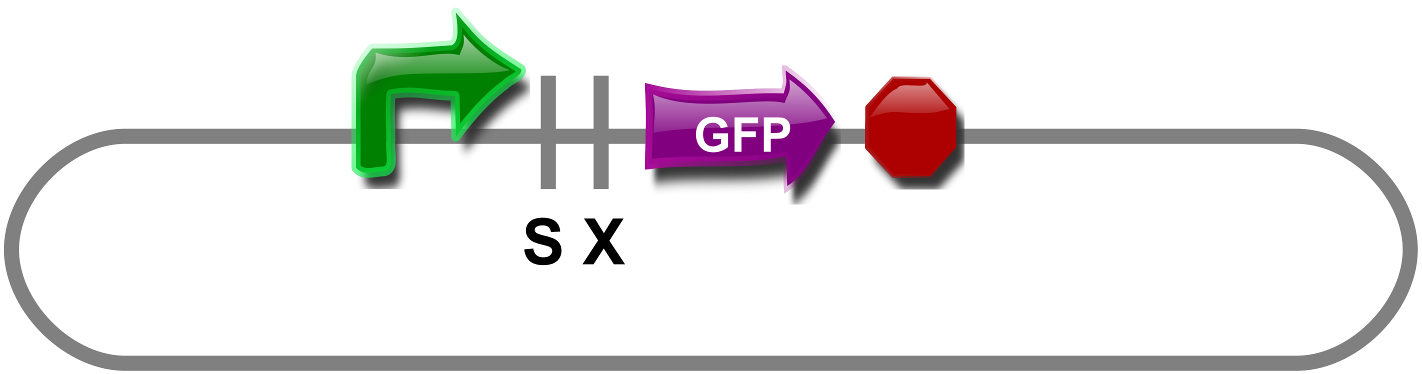
Measurement Kit

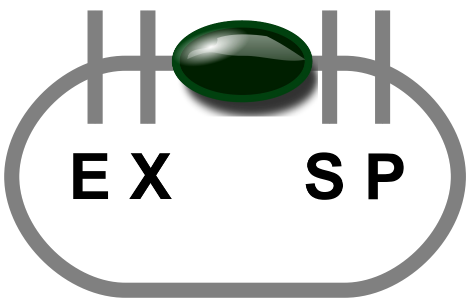


Jason Kelly (Endy's Lab)

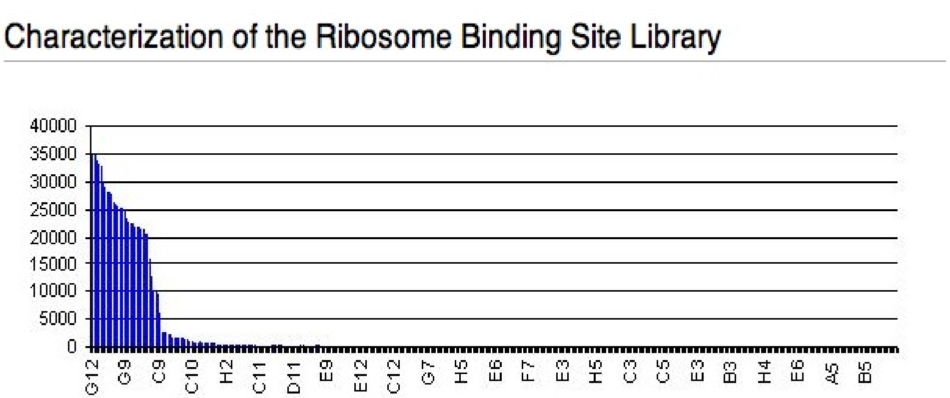
Measurement Kit

RBS Screening Plasmid





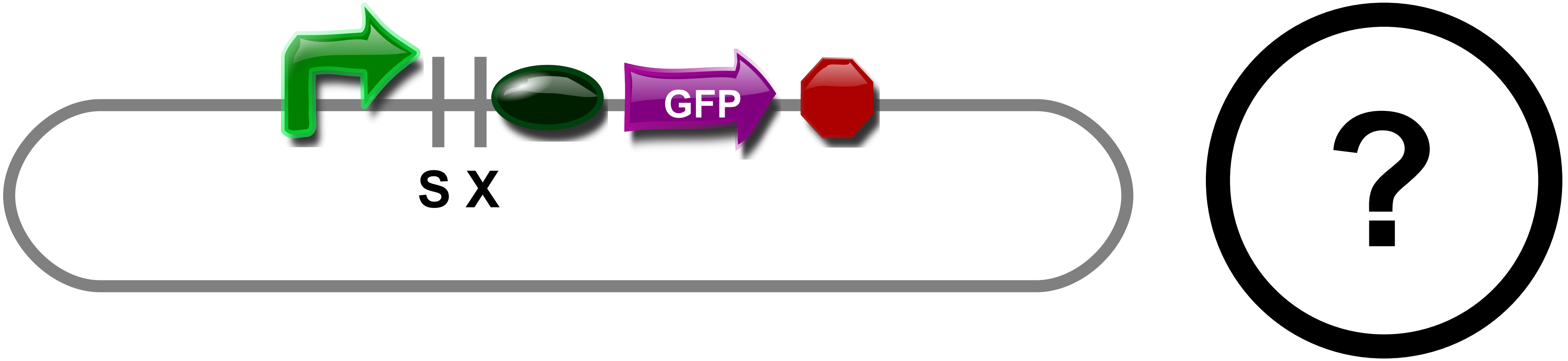
Ribosome Binding Site



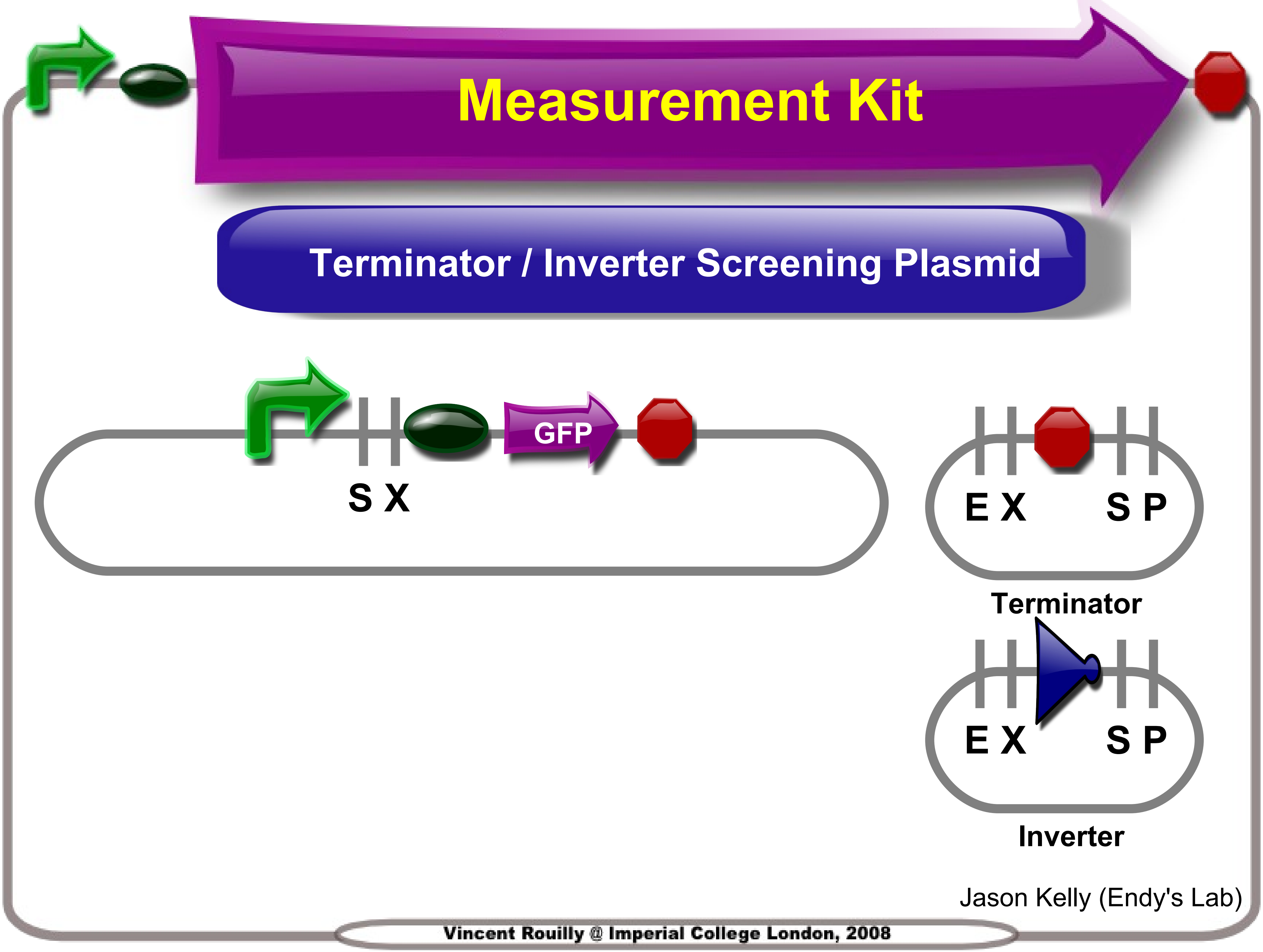
JC Anderson (MIT Registry)

Jason Kelly (Endy's Lab)

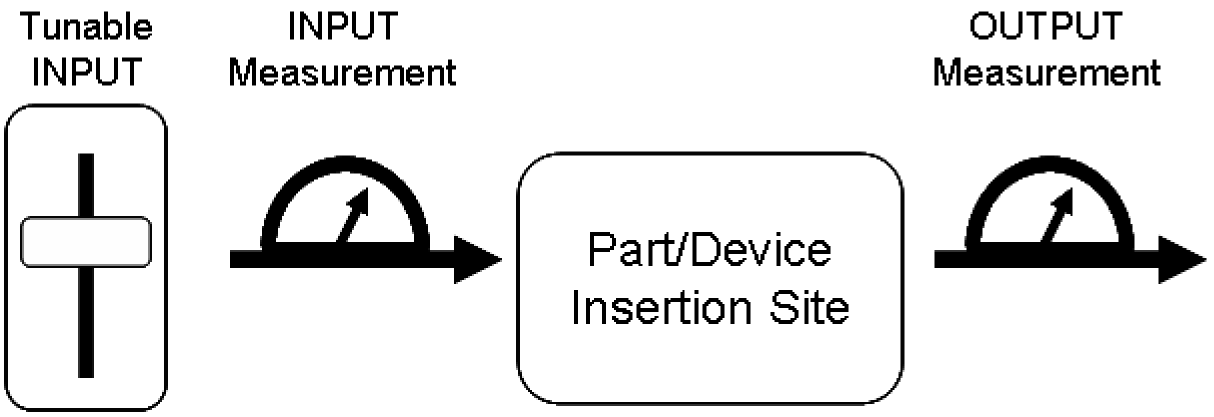
Measurement Kit



Jason Kelly (Endy's Lab)

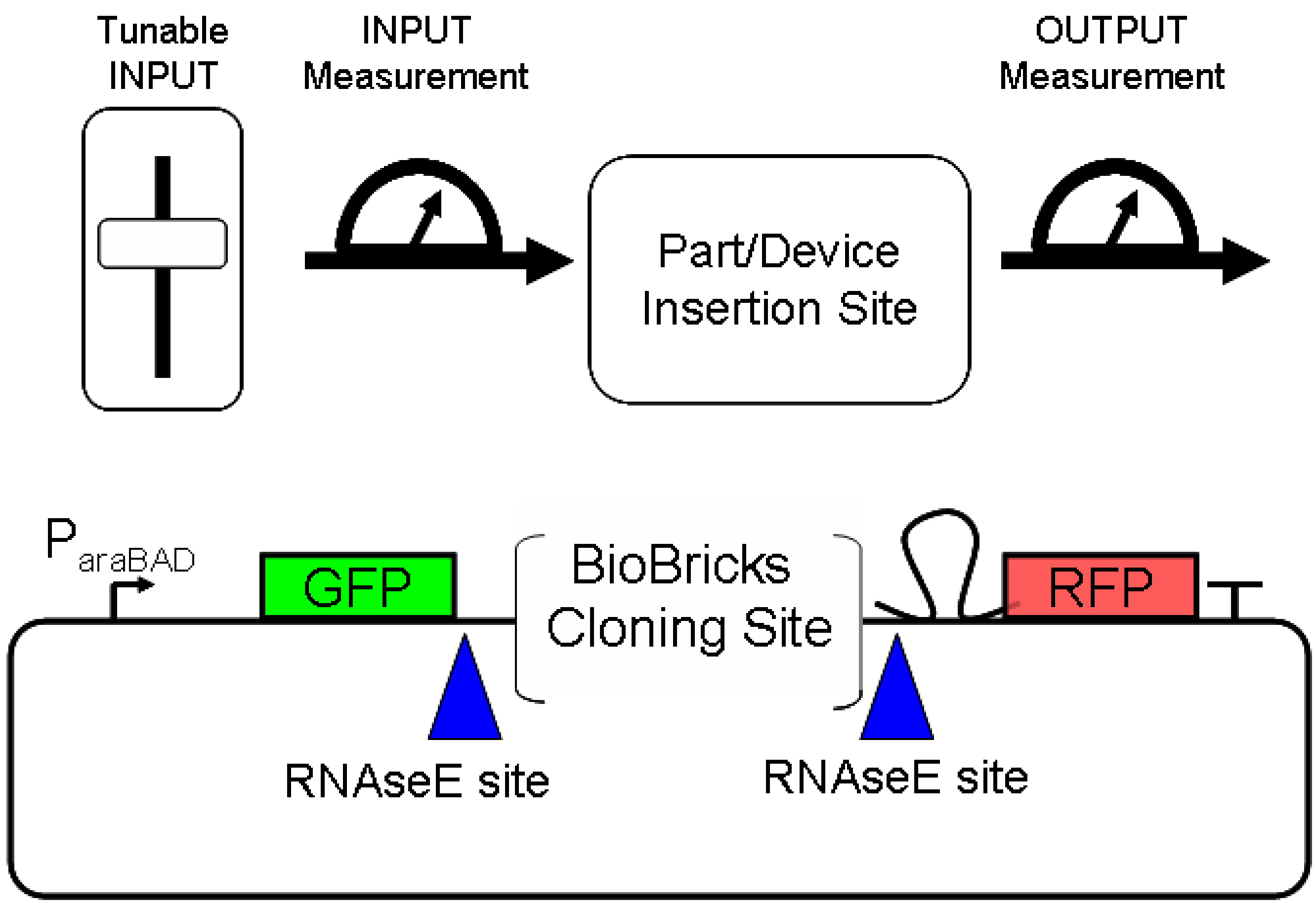

Measurement Kit
Terminator / Inverter Screening Plasmid
S X
GFP
E X
S P
Terminator
E X
S P
Inverter
Jason Kelly (Endy's Lab)
Vincent Rouilly @ Imperial College London, 2008

PoPS based Screening Plasmid



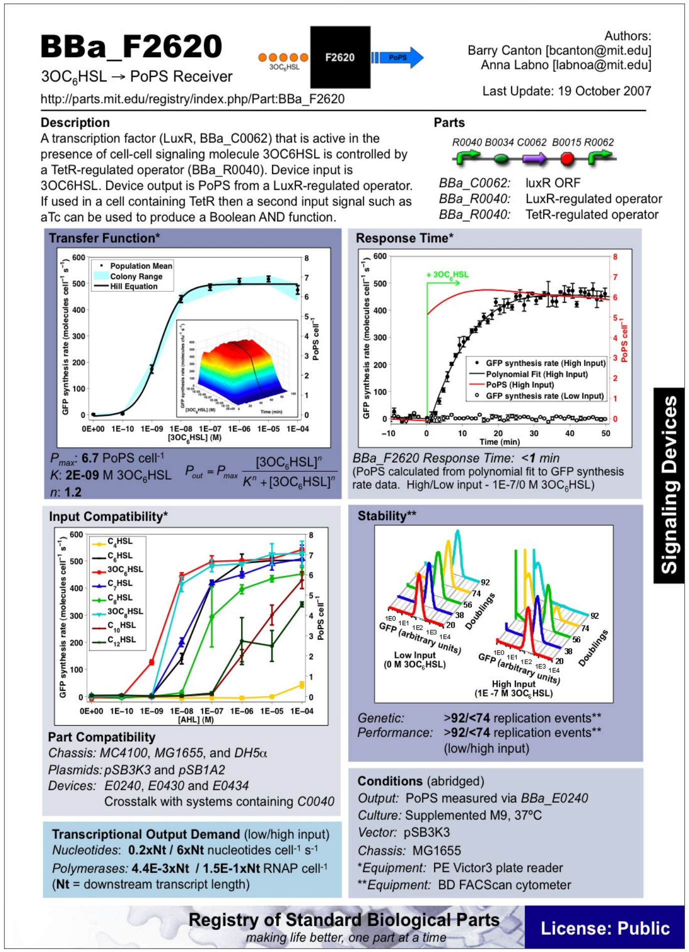
Jason Kelly (Endy's Lab)

PoPS based Screening Plasmid



Jason Kelly (Endy's Lab)

F2620 / T9002



BBa_F2620

3OC$_6$HSL → PoPS Receiver

http://parts.mit.edu/registry/index.php/Part:BBa_F2620

Authors:
Barry Canton [bcanton@mit.edu]
Anna Labno [labnoa@mit.edu]

Last Update: 19 October 2007

Description

A transcription factor (LuxR, BBa_C0062) that is active in the presence of cell-cell signaling molecule 3OC6HSL is controlled by a TetR-regulated operator (BBa_R0040). Device input is 3OC6HSL. Device output is PoPS from a LuxR-regulated operator. If used in a cell containing TetR then a second input signal such as aTc can be used to produce a Boolean AND function.

Parts

R0040 B0034 C0062 B0015 R0062

BBa_C0062: luxR ORF
BBa_R0040: LuxR-regulated operator
BBa_R0040: TetR-regulated operator

Transfer Function*

P_{max}: **6.7** PoPS cell^{-1}
K: **2E-09** M 3OC$_6$HSL
n: **1.2**

$$P_{out} = P_{max}\frac{[3OC_6HSL]^n}{K^n + [3OC_6HSL]^n}$$

Response Time*

BBa_F2620 Response Time: **<1** *min*
(PoPS calculated from polynomial fit to GFP synthesis rate data. High/Low input - 1E-7/0 M 3OC$_6$HSL)

Signaling Devices

Input Compatibility*

Stability**

Genetic: **>92/<74** replication events**
Performance: **>92/<74** replication events**
(low/high input)

Part Compatibility

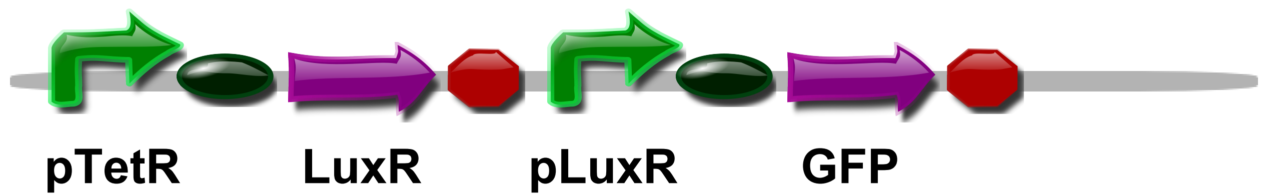
Chassis: MC4100, MG1655, and *DH5α*
Plasmids: pSB3K3 and pSB1A2
Devices: E0240, E0430 and E0434
Crosstalk with systems containing C0040

Transcriptional Output Demand (low/high input)
Nucleotides: **0.2xNt / 6xNt** nucleotides cell^{-1} s^{-1}
Polymerases: **4.4E-3xNt / 1.5E-1xNt** RNAP cell^{-1}
(**Nt** = downstream transcript length)

Conditions (abridged)
Output: PoPS measured via *BBa_E0240*
Culture: Supplemented M9, 37ºC
Vector: pSB3K3
Chassis: MG1655
**Equipment:* PE Victor3 plate reader
***Equipment:* BD FACScan cytometer

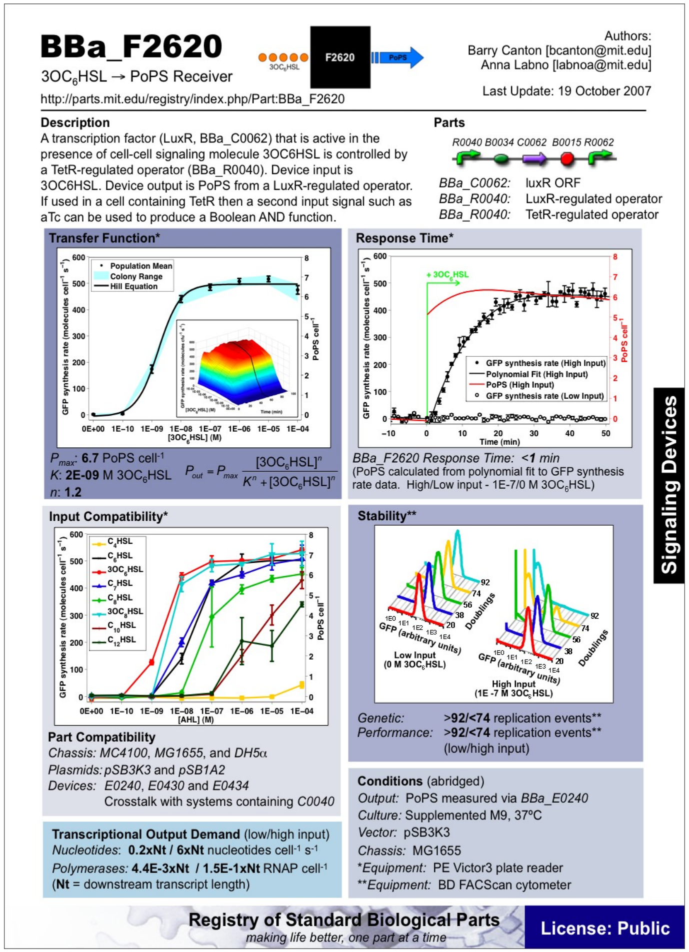
Registry of Standard Biological Parts
making life better, one part at a time

License: Public



Barry Canton (Endy's Lab)

F2620 / T9002


BBa_F2620

3OC$_6$HSL → PoPS Receiver

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Signaling Devices

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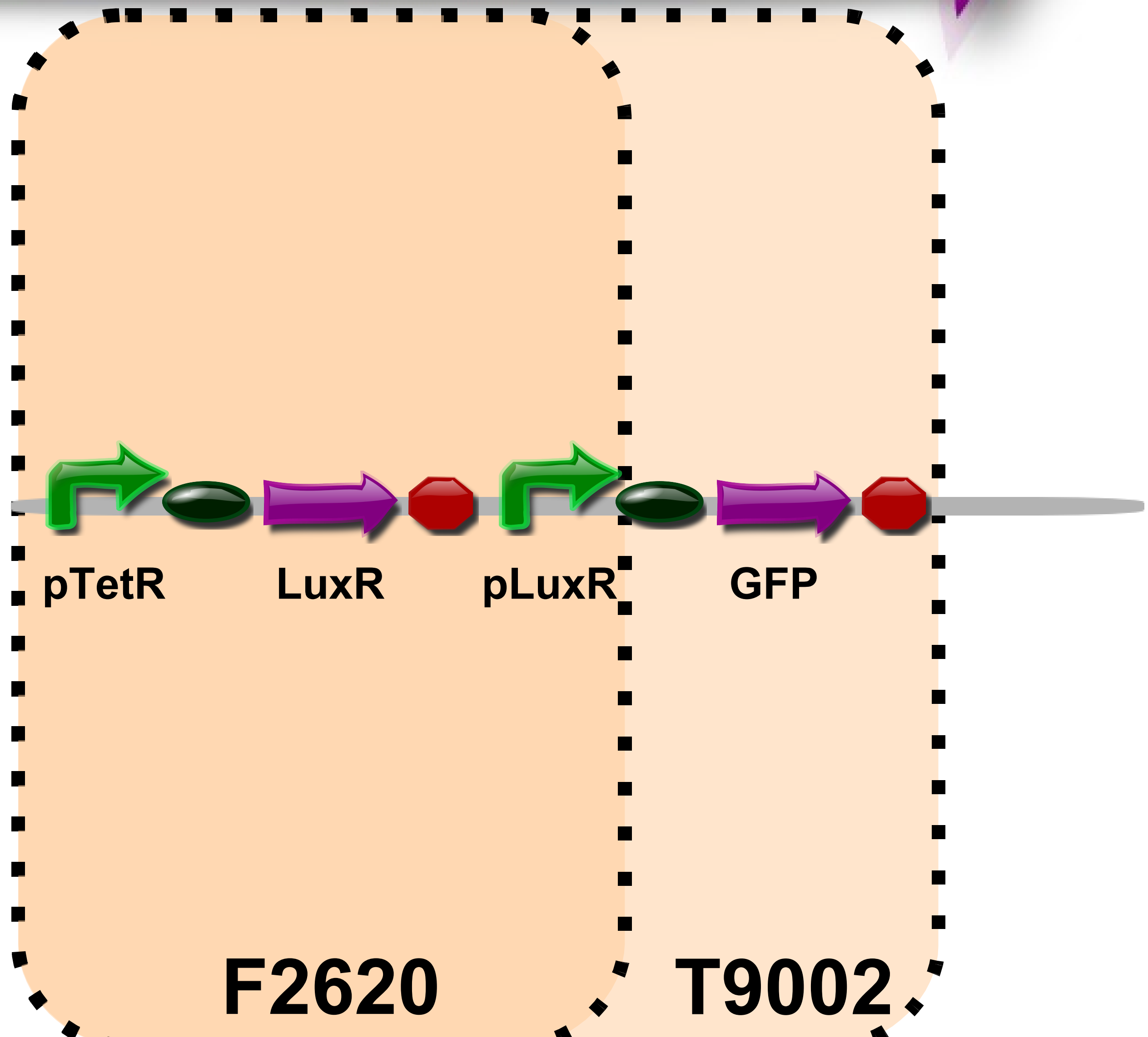

Barry Canton (Endy's Lab)

F2620: Description

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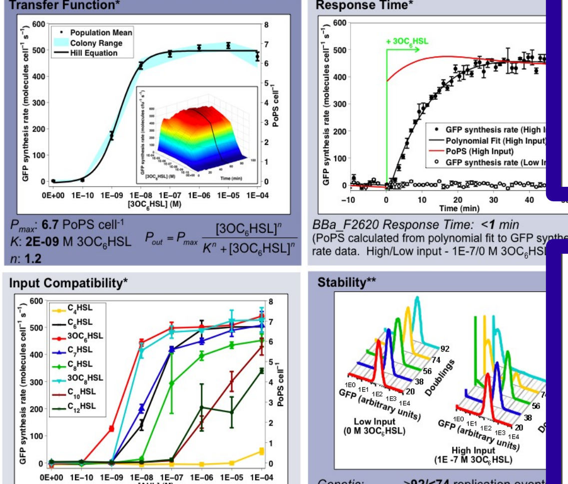

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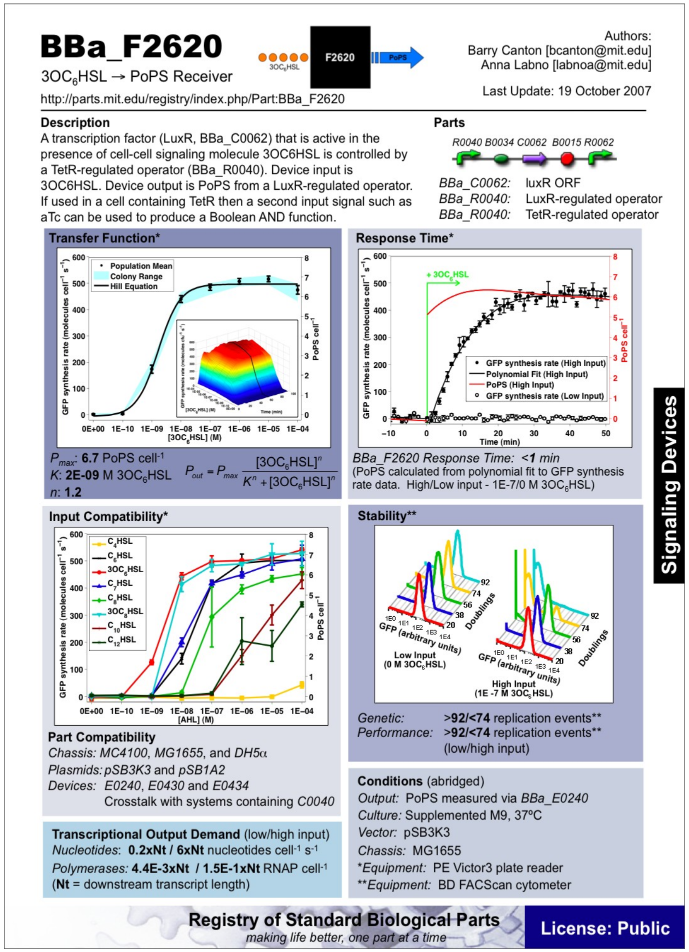
F2620: Transfer Function



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Chassis: MG1655
**Equipment:* PE Victor3 plate reader
***Equipment:* BD FACScan cytometer

Signaling Devices

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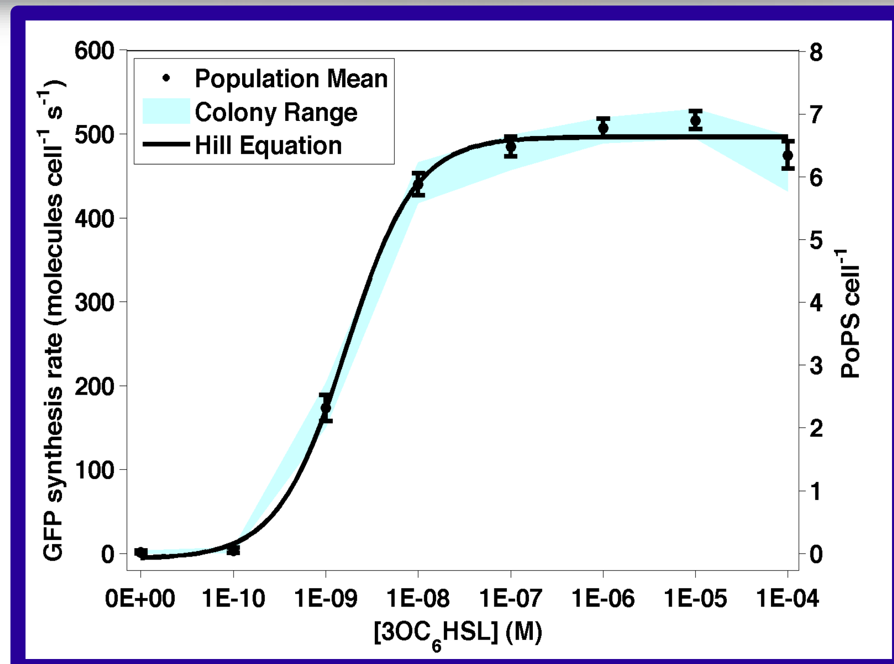

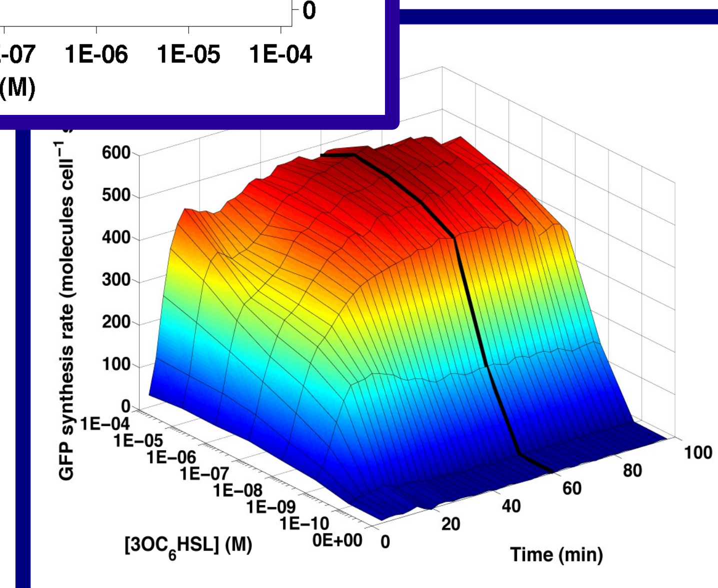

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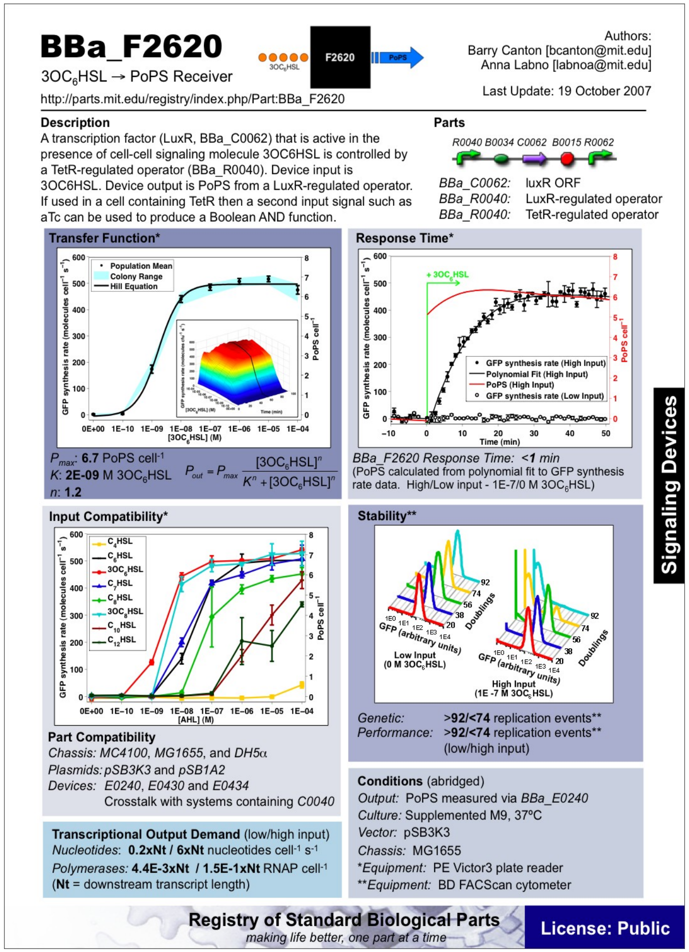
F2620: Specificity



BBa_F2620

3OC$_6$HSL → PoPS Receiver

http://parts.mit.edu/registry/index.php/Part:BBa_F2620

Authors:
Barry Canton [bcanton@mit.edu]
Anna Labno [labnoa@mit.edu]

Last Update: 19 October 2007

Description

A transcription factor (LuxR, BBa_C0062) that is active in the presence of cell-cell signaling molecule 3OC6HSL is controlled by a TetR-regulated operator (BBa_R0040). Device input is 3OC6HSL. Device output is PoPS from a LuxR-regulated operator. If used in a cell containing TetR then a second input signal such as aTc can be used to produce a Boolean AND function.

Parts

R0040 B0034 C0062 B0015 R0062

BBa_C0062: luxR ORF
BBa_R0040: LuxR-regulated operator
BBa_R0040: TetR-regulated operator

Transfer Function*

P_{max}: **6.7** PoPS cell^{-1}
K: **2E-09** M 3OC$_6$HSL
n: **1.2**

$$P_{out} = P_{max} \frac{[3OC_6HSL]^n}{K^n + [3OC_6HSL]^n}$$

Response Time*

BBa_F2620 Response Time: <*1 min*
(PoPS calculated from polynomial fit to GFP synthesis rate data. High/Low input - 1E-7/0 M 3OC$_6$HSL)

Input Compatibility*

Stability**

Genetic: >92/<74 replication events**
Performance: >92/<74 replication events**
(low/high input)

Part Compatibility

Chassis: MC4100, MG1655, and DH5α
Plasmids: pSB3K3 and pSB1A2
Devices: E0240, E0430 and E0434
Crosstalk with systems containing C0040

Conditions (abridged)

Output: PoPS measured via BBa_E0240
Culture: Supplemented M9, 37°C
Vector: pSB3K3
Chassis: MG1655
**Equipment:* PE Victor3 plate reader
***Equipment:* BD FACScan cytometer

Transcriptional Output Demand (low/high input)

Nucleotides: **0.2xNt / 6xNt** nucleotides cell^{-1} s^{-1}
Polymerases: **4.4E-3xNt / 1.5E-1xNt** RNAP cell^{-1}
(**Nt** = downstream transcript length)

Signaling Devices

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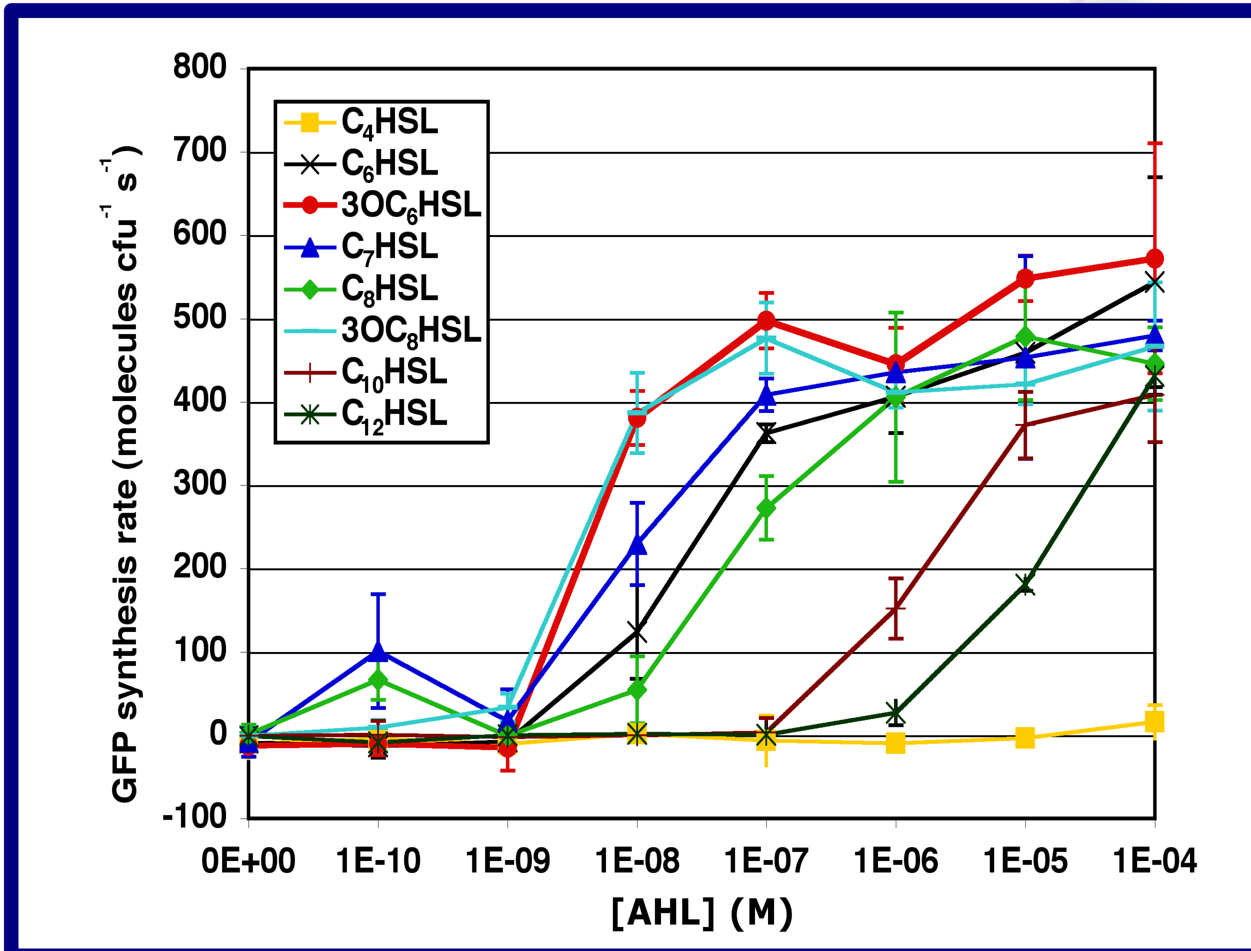

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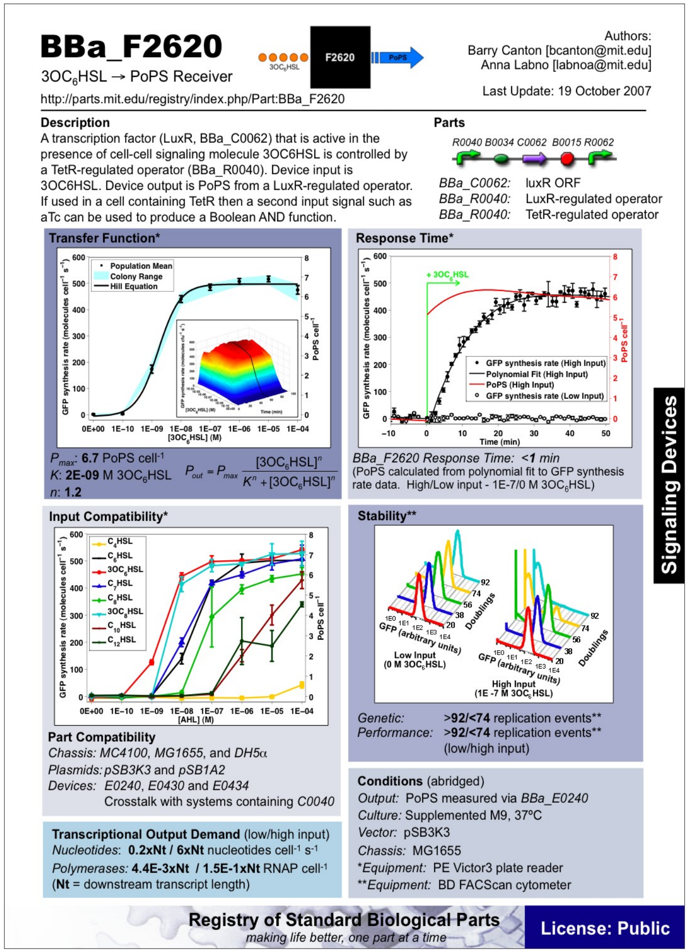
F2620



BBa_F2620

$3OC_6HSL$ → PoPS Receiver

http://parts.mit.edu/registry/index.php/Part:BBa_F2620

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Response Time*

*BBa_F2620 Response Time: <**1** min*
(PoPS calculated from polynomial fit to GFP synthesis rate data. High/Low input - 1E-7/0 M $3OC_6HSL$)

Input Compatibility*

Part Compatibility
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Plasmids: pSB3K3 and *pSB1A2*
Devices: E0240, E0430 and *E0434*
Crosstalk with systems containing *C0040*

Stability**

Genetic: >**92/<74** replication events**
Performance: >**92/<74** replication events**
(low/high input)

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Nucleotides: **0.2xNt / 6xNt** nucleotides cell^{-1} s^{-1}
Polymerases: **4.4E-3xNt / 1.5E-1xNt** RNAP cell^{-1}
(**Nt** = downstream transcript length)

Conditions (abridged)
Output: PoPS measured via *BBa_E0240*
Culture: Supplemented M9, 37ºC
Vector: pSB3K3
Chassis: MG1655
**Equipment:* PE Victor3 plate reader
***Equipment:* BD FACScan cytometer

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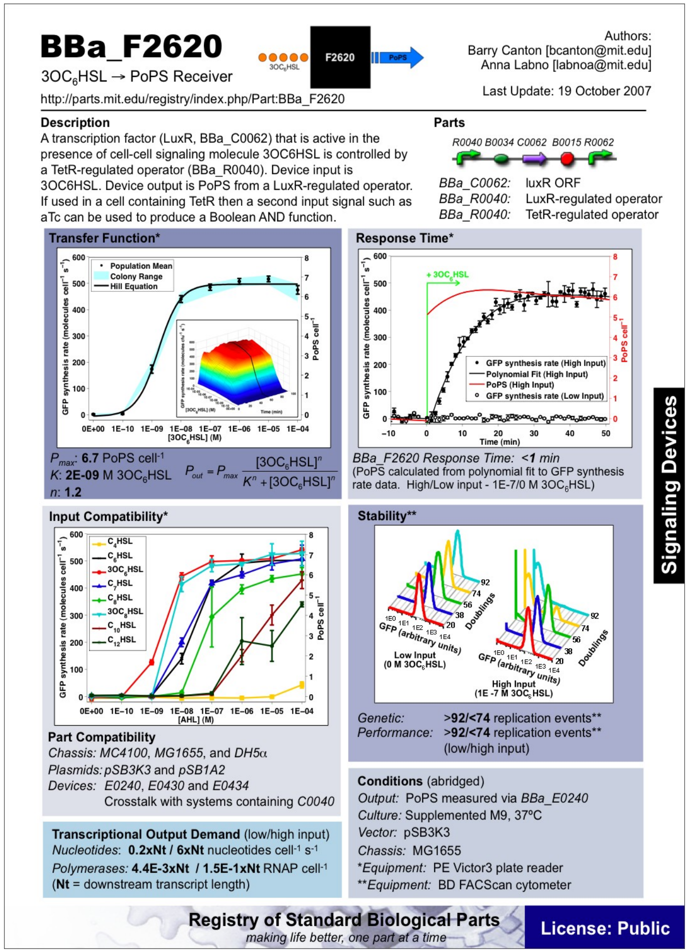
F2620: Stability



BBa_F2620

3OC$_6$HSL → PoPS Receiver

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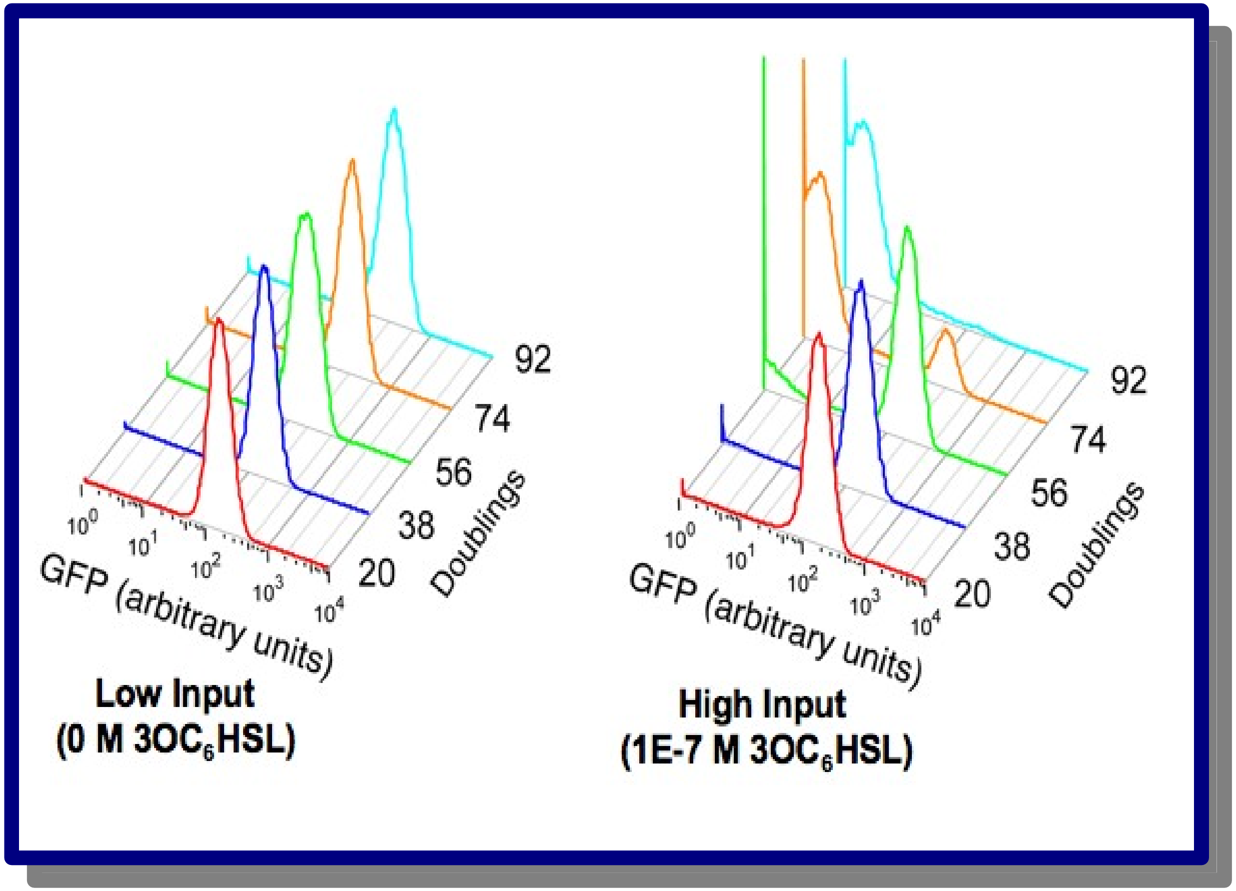

Barry Canton (Endy's Lab)

References

> Drew Endy Talks
> OpenWetWare Folks
> iGEM Competition
> BioBricks Foundation

Measurement