# iGEM Measurement committee webinar series

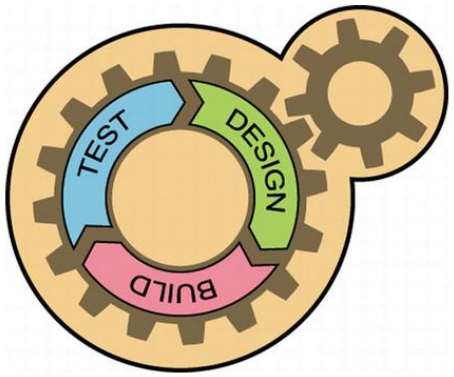
## Existing part collections and measurement

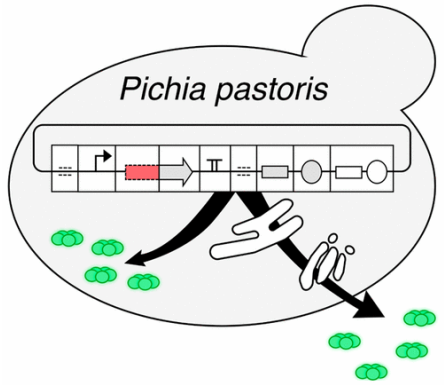
René Inckemann 19.06.2020

# Overview

- Pre-existing part collections outside from iGEM
- Pre-existing iGEM part collection
- Importance of measurements
- Part documentation

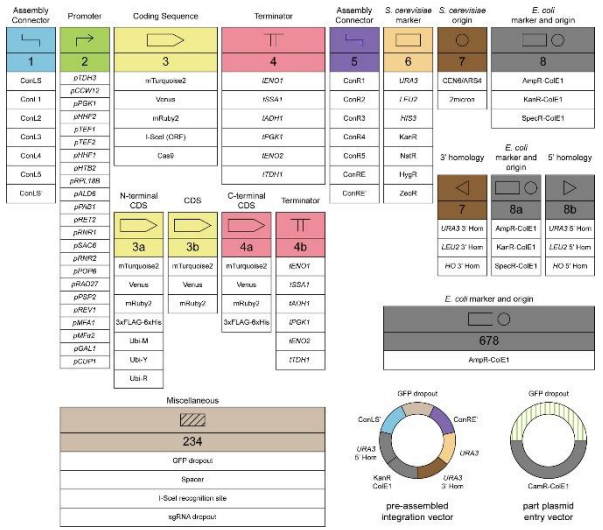
# Pre-existing part collections outside from iGEM

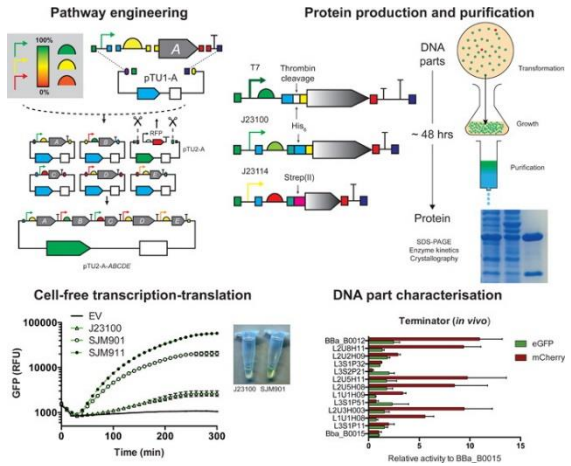


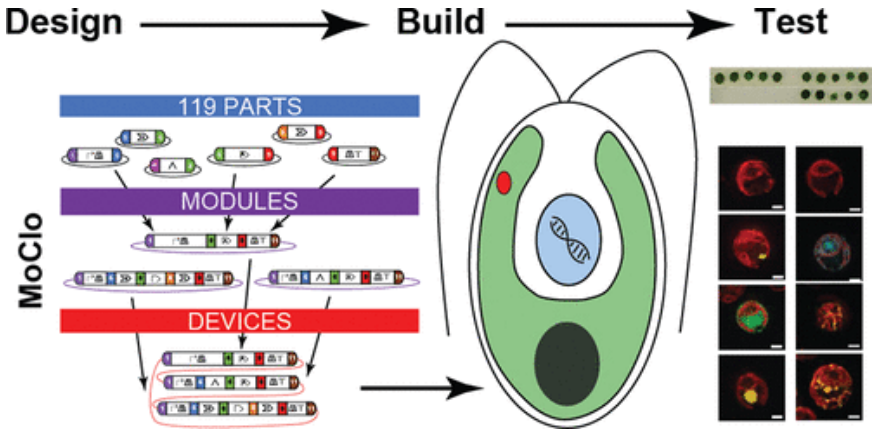




OpenPlant

sharing tools for a sustainable future



# Pre-existing iGEM part collections

## Anderson promoter collection

| Identifier | Sequence[a] | Measured Strength[b] |
|---|---|---|
| BBa_J23119 | ttgacagctagctcagtcctaggtataatgctagc | n/a |
| BBa_J23100 | ttgacggctagctcagtcctaggtacagtgctagc | 1 |
| BBa_J23101 | tttacagctagctcagtcctaggtattatgctagc | 0.70 |
| BBa_J23102 | ttgacagctagctcagtcctaggtactgtgctagc | 0.86 |
| BBa_J23103 | ctgatagctagctcagtcctagggattatgctagc | 0.01 |
| BBa_J23104 | ttgacagctagctcagtcctaggtattgtgctagc | 0.72 |
| BBa_J23105 | tttacggctagctcagtcctaggtactatgctagc | 0.24 |
| BBa_J23106 | tttacggctagctcagtcctaggtatagtgctagc | 0.47 |
| BBa_J23107 | tttacggctagctcagcctaggtattatgctagc | 0.36 |
| BBa_J23108 | ctgacagctagctcagtcctaggtataatgctagc | 0.51 |
| BBa_J23109 | tttacagctagctcagtcctagggactgtgctagc | 0.04 |
| BBa_J23110 | tttacggctagctcagtcctaggtacaatgctagc | 0.33 |
| BBa_J23111 | ttgacggctagctcagtcctaggtatagtgctagc | 0.58 |
| BBa_J23112 | ctgatagctagctcagtcctagggattatgctagc | 0.00 |
| BBa_J23113 | ctgatggctagctcagtcctagggattatgctagc | 0.01 |
| BBa_J23114 | tttatggctagctcagtcctaggtacaatgctagc | 0.10 |
| BBa_J23115 | tttatagctagctcagccttggtacaatgctagc | 0.15 |
| BBa_J23116 | ttgacagctagctcagtcctagggactatgctagc | 0.16 |
| BBa_J23117 | ttgacagctagctcagtcctagggattgtgctagc | 0.06 |
| BBa_J23118 | ttgacggctagctcagtcctaggtattgtgctagc | 0.56 |

# Pre-existing iGEM part collections

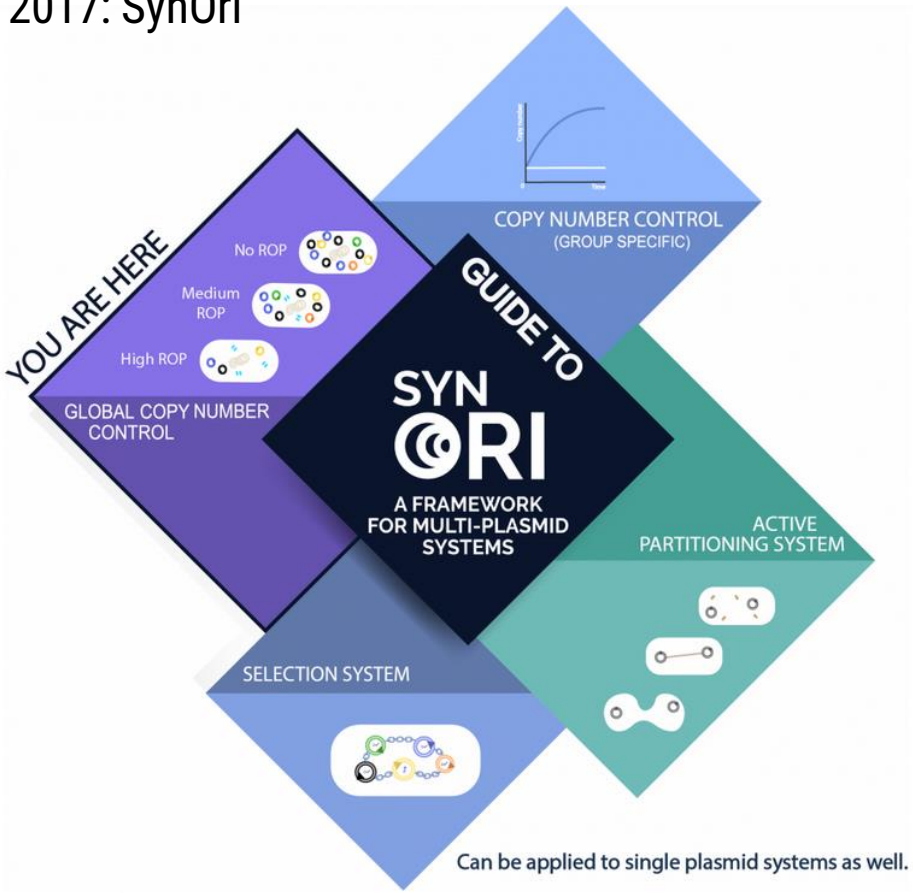
## Reporter collection

More...

| Name | Protein | Description | Tag | Direction | Fluorescent Color | Emission | Excitation | Length | Status |
|---|---|---|---|---|---|---|---|---|---|
| BBa_E0030 | EYFP | enhanced yellow fluorescent protein derived from A. victoria GFP | None | Forward | Yellow | 527 | 514 | 723 | In stock |
| BBa_E0020 | ECFP | engineered cyan fluorescent protein derived from A. victoria GFP | None | Forward | Cyan | 476 | 439 | 723 | In stock |
| BBa_E1010 | mRFP1 | **highly** engineered mutant of red fluorescent protein from Discosoma striata (coral) | None | Forward | Red | 607 | 584 | 706 | In stock |
| BBa_E2050 | mOrange | derivative of mRFP1, yeast-optimized | None | | Orange | 562 | 548 | 769 | In stock |
| BBa_E0040 | GFPmut3b | green fluorescent protein derived from jellyfish Aequeora victoria wild-type GFP (SwissProt: P42212 | None | Forward | Green | 511 | 501 | 720 | In stock |
| BBa_J52021 | | dnTraf6-linker-GFP | | | Green | | | 1446 | In stock |
| BBa_J52026 | | dnMyD88-linker-GFP | | | Green | | | 1155 | In stock |
| BBa_I715022 | | Amino Portion of RFP | | | Red | | | 462 | In stock |
| BBa_I715023 | | Carboxyl portion of RFP | | | Red | | | 220 | In stock |
| BBa_I712028 | | CherryNLS - synthetic construct monomeric red fluorescent protein with nuclear localization sequence | | Forward | Red | | | 733 | In stock |
| BBa_K125500 | | GFP fusion brick | | Forward | Green | | | 718 | In stock |
| BBa_K165005 | | Venus YFP, yeast optimized for fusion | | Forward | Yellow | | | 744 | In stock |
| BBa_K082003 | GFP | GFP(+LVA) | | | Green | | | 756 | In stock |
| BBa_K156009 | | OFP (orange fluorescent protein) | | Forward | Orange | | | 864 | In stock |
| BBa_K156010 | | SBFP2 (strongly enhanced blue fluorescent protein) | | | Blue | | | 720 | In stock |

# Pre-existing iGEM part collections

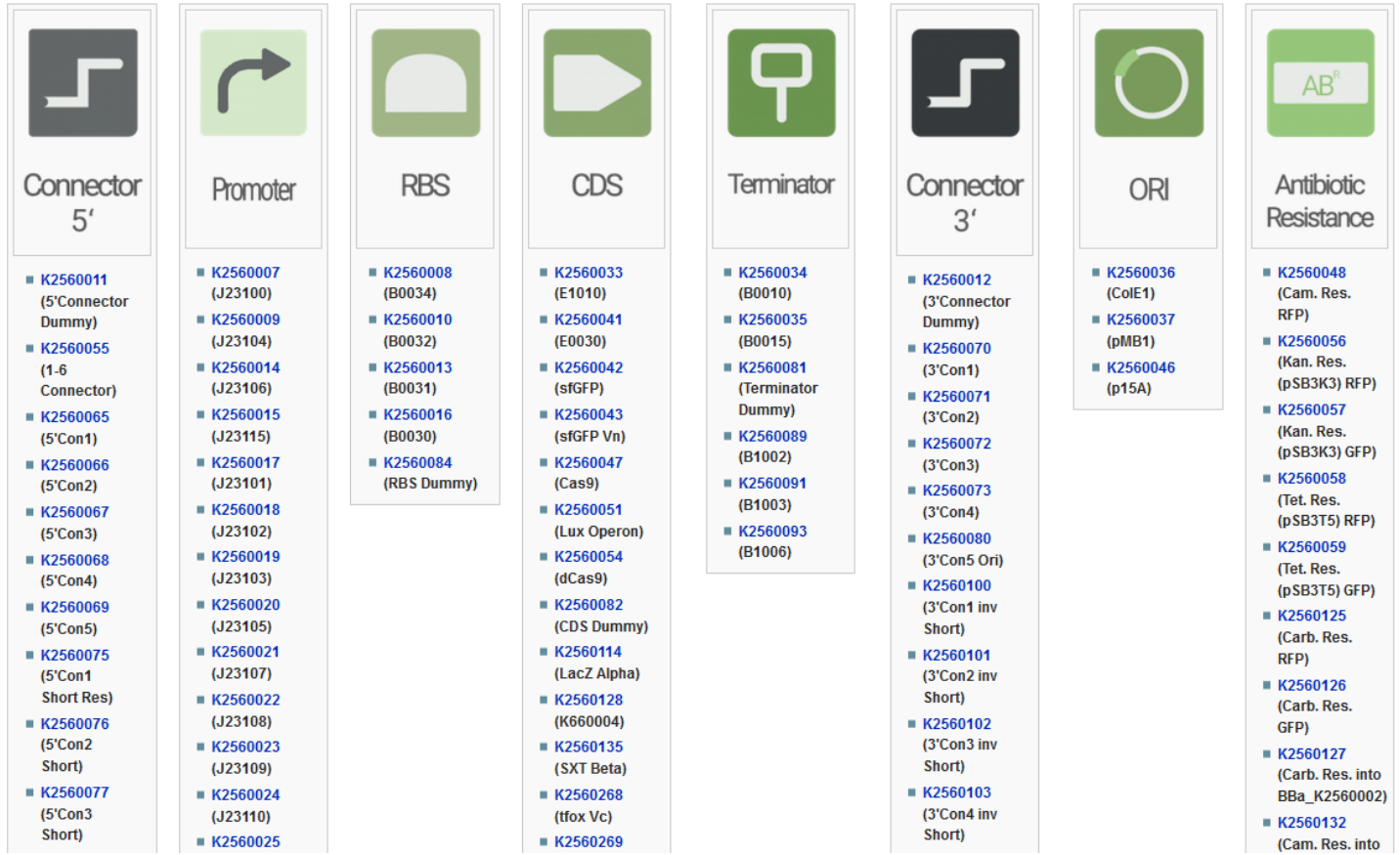
iGEM Vilnius 2017: SynOri



# Pre-existing iGEM part collections

## iGEM Marburg 2018: Vibrigens (Basic set of standard MoClo parts)

**Parts of the Marburg Toolbox** [edit]



### Connector 5'

- K2560011 (5'Connector Dummy)
- K2560055 (1-6 Connector)
- K2560065 (5'Con1)
- K2560066 (5'Con2)
- K2560067 (5'Con3)
- K2560068 (5'Con4)
- K2560069 (5'Con5)
- K2560075 (5'Con1 Short Res)
- K2560076 (5'Con2 Short)
- K2560077 (5'Con3 Short)

### Promoter

- K2560007 (J23100)
- K2560009 (J23104)
- K2560014 (J23106)
- K2560015 (J23115)
- K2560017 (J23101)
- K2560018 (J23102)
- K2560019 (J23103)
- K2560020 (J23105)
- K2560021 (J23107)
- K2560022 (J23108)
- K2560023 (J23109)
- K2560024 (J23110)
- K2560025

### RBS

- K2560008 (B0034)
- K2560010 (B0032)
- K2560013 (B0031)
- K2560016 (B0030)
- K2560084 (RBS Dummy)

### CDS

- K2560033 (E1010)
- K2560041 (E0030)
- K2560042 (sfGFP)
- K2560043 (sfGFP Vn)
- K2560047 (Cas9)
- K2560051 (Lux Operon)
- K2560054 (dCas9)
- K2560082 (CDS Dummy)
- K2560114 (LacZ Alpha)
- K2560128 (K660004)
- K2560135 (SXT Beta)
- K2560268 (tfox Vc)
- K2560269

### Terminator

- K2560034 (B0010)
- K2560035 (B0015)
- K2560081 (Terminator Dummy)
- K2560089 (B1002)
- K2560091 (B1003)
- K2560093 (B1006)

### Connector 3'

- K2560012 (3'Connector Dummy)
- K2560070 (3'Con1)
- K2560071 (3'Con2)
- K2560072 (3'Con3)
- K2560073 (3'Con4)
- K2560080 (3'Con5 Ori)
- K2560100 (3'Con1 inv Short)
- K2560101 (3'Con2 inv Short)
- K2560102 (3'Con3 inv Short)
- K2560103 (3'Con4 inv Short)

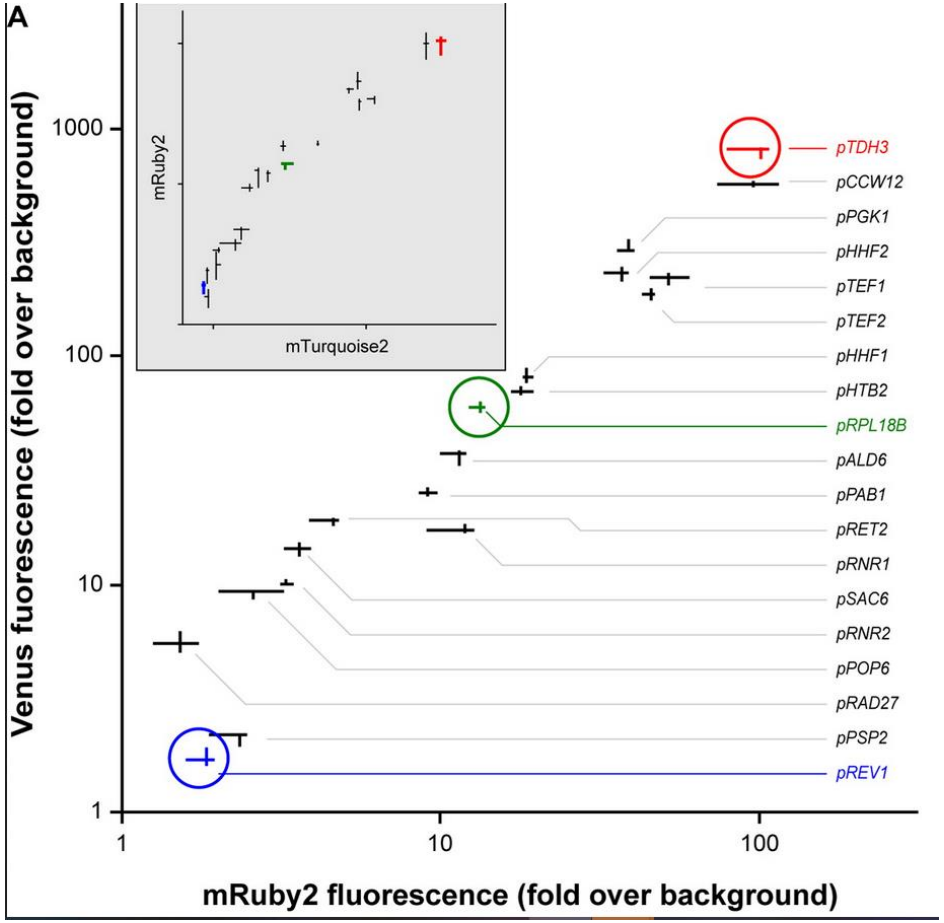
### ORI

- K2560036 (ColE1)
- K2560037 (pMB1)
- K2560046 (p15A)

### Antibiotic Resistance

- K2560048 (Cam. Res. RFP)
- K2560056 (Kan. Res. (pSB3K3) RFP)
- K2560057 (Kan. Res. (pSB3K3) GFP)
- K2560058 (Tet. Res. (pSB3T5) RFP)
- K2560059 (Tet. Res. (pSB3T5) GFP)
- K2560125 (Carb. Res. RFP)
- K2560126 (Carb. Res. GFP)
- K2560127 (Carb. Res. into BBa_K2560002)
- K2560132 (Cam. Res. into

# Importance of measurements



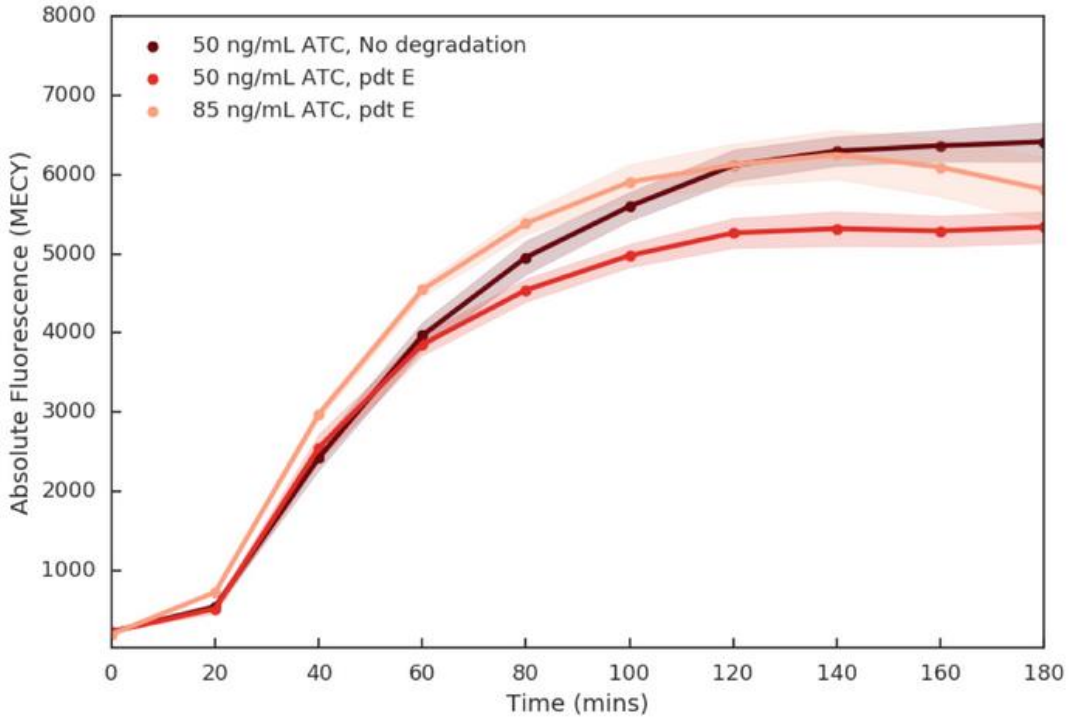

Lee *et al* 2015

Characterized parts are much more valuable for future projects and iGEM teams, in order to facilitate more complex genetic designs

<u>Example</u>:
Promoter, RBS, terminator collections for different chassis

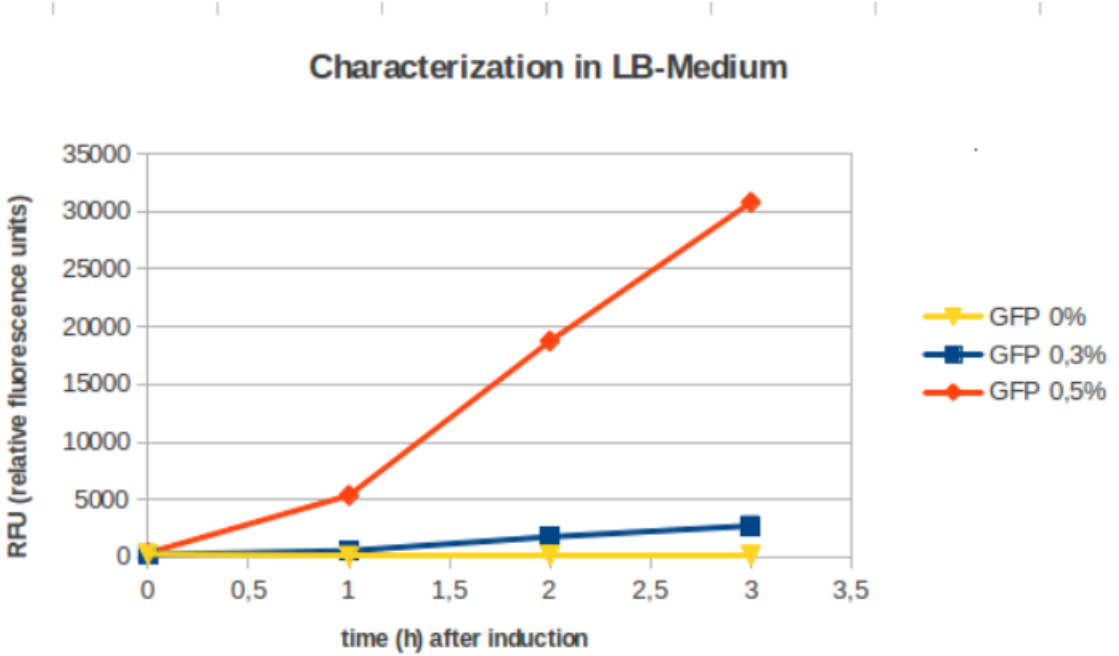
# Importance of measurements

absolute units




Example: iGEM William Mary 2017

relative units



# Part documentation

### pLac0-1 mf-Lon

This is an IPTG-inducible mf-Lon construct containing the pLlac 0-1 promoter. It was a cornerstone in William and Mary 2017's efforts to produce a modular method to alter gene expression speed, enabling them to test a wide variety of protease concentrations with ease. All of their primary characterization was done using this part, and the construct should prove to be useful to anyone in the future who wants to test a variety of mf-Lon concentrations without having to undergo a large number of cloning steps. This part can also be used to produce fully functional circuit motifs, as was demonstrated with W&M's mScarlet IFFL cricuit, and as such could contribute to proof of concept or final implementation of other projects.

### Usage and Biology [edit]

This composite part is a combination circuit with the LacI repressor under the constitutive promoter J23105 and mf-Lon under the control of the PLlac 0-1 promoter. William and Mary 2017 modified the mf-Lon gene via codon-optimization for iGEM use and added a double terminator. The mf-Lon protease specifically targets different protein degradation tags with varying affinities corresponding to varying degradation rates. This IPTG-inducible mf-Lon construct was used in tandem with aTc-inducible pdt reporter constructs by William and Mary 2017 to obtain gene expression speed measurements.


### Characterization [edit]

W&M 2017 characterized this mf-Lon containing composite part in combination with aTc-inducible pdt reporter constructs as well as with copper sulfate inducible pdt reporter constructs. The graphs below show this speed data along with the data from the other tags in their series.

### mScarlet Experiments [edit]

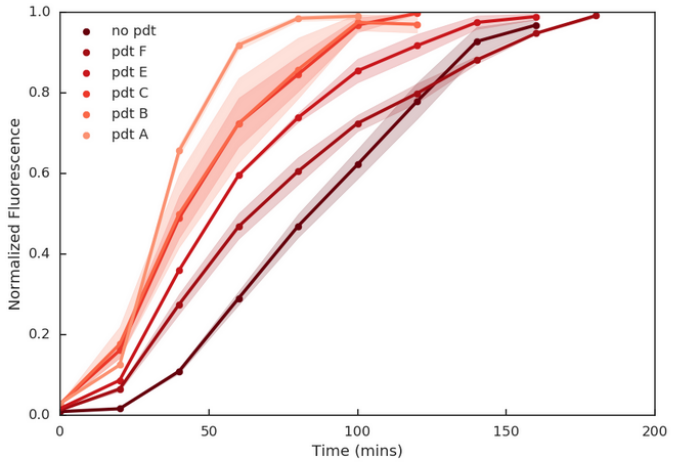
BBa_K2333427-BBa_K2333433

- Create an entry for all of your parts in the registry with a description
- You should have confirmed your parts by sequencing
- You should have all your data for a specific part on the correct **registry page (not just on the wiki)**

# Part documentation

**mScarlet Experiments** [edit]

BBa_K2333427-BBa_K2333433




Graph 1: Time course measurements were performed according to standard protocol, and fluorescence was normalized to steady state based upon when fluorescence no longer increased. Data is shown for each construct until steady state is reached (this means at least two consecutive subsequent data points do not increase fluorescence). As the no-pdt condition had not reached steady state when time course was ended, it was normalized to the final collected data point, which is likely close to the true steady state. Geometric mean of 10,000 cells each of three biological replicates. Shaded region represents one geometric standard deviation above and below the mean.

- Create an entry for all of your parts in the registry with a description
- You should have confirmed your parts by sequencing
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# practical part

# iGEM Measurement committee webinar series

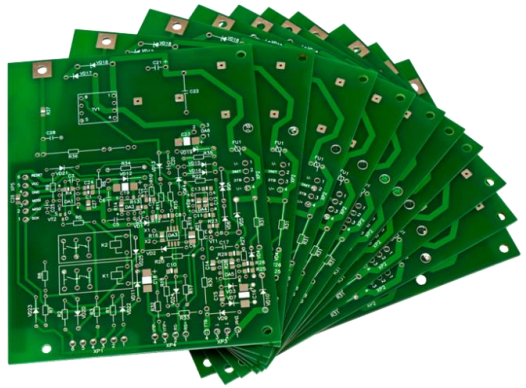
## The importance of Parts

René Inckemann 19.06.2020

# Overview

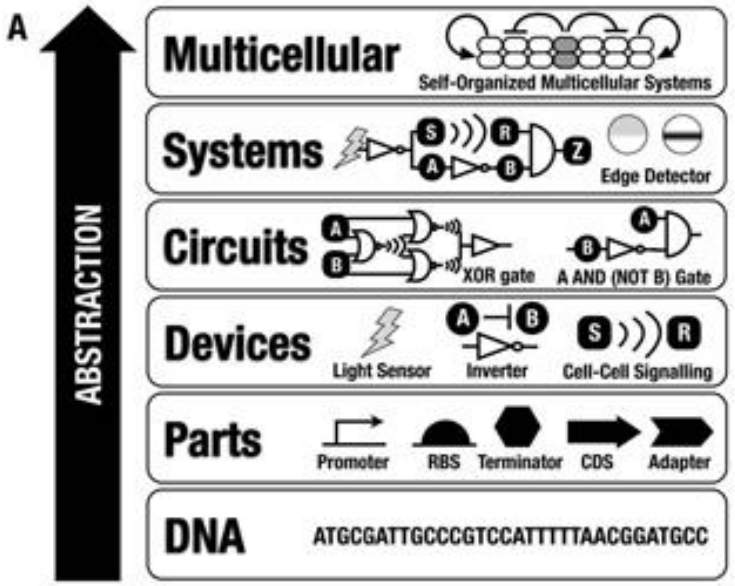
- Parts in engineering/Parts in sythetic biology
- Why is standardization important for synbio?
- The invention of the first part standard for Synbio: Biobrick assembly + Registry of parts
- The “new“ standard: Phytobricks and TypeIIS

# Parts in common engineering



- Building airplanes or complex circuit boards without standard parts impossible
- Well defined parts, with perfectly known properties and characteristics
- Parts can be reused for other engineering efforts
- Parts are tested in different setups and conditions

# Parts in synthetic biology




Federici *et al* 2013

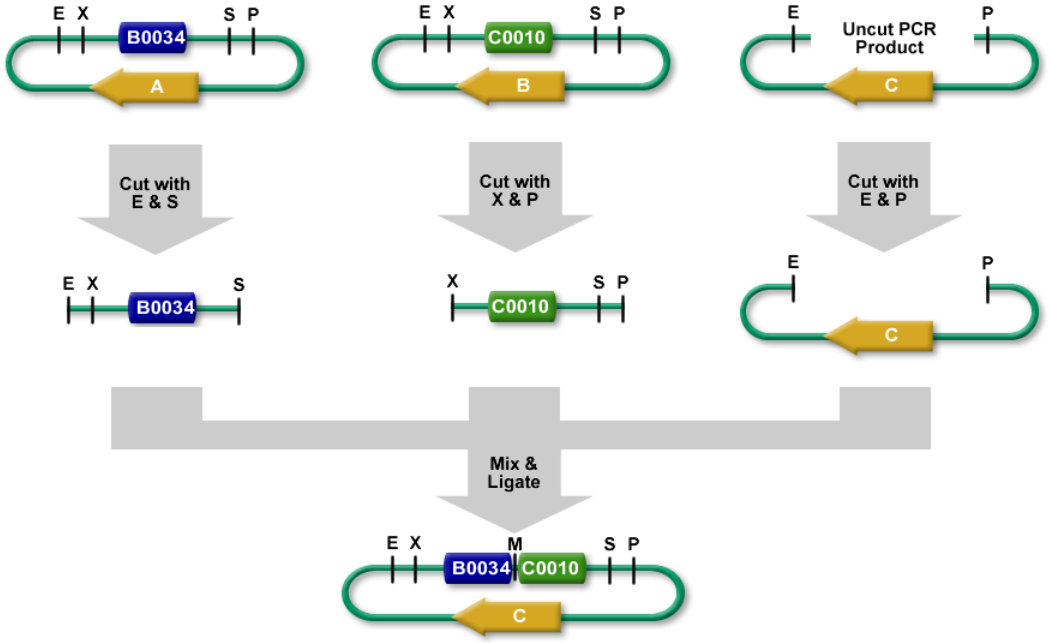
- Using engineering principles like standardization and abstraction for engineering biology
- DNA on the lowest level
- Parts could be definded by function, such as: promoters, RBS, CDS, terminator, UTR, tags

# Why is standardization important for iGEM and Synbio?

- Parts can be exchanged with other labs/iGEM teams
- New teams can use already existing parts and combine them with their own parts
- Parts can be further characterized and tested in different setups and condtions to define properties and characteristics

→ parts get better via collective efforts

# The invention of the first part standard for Synbio: Biobrick assembly

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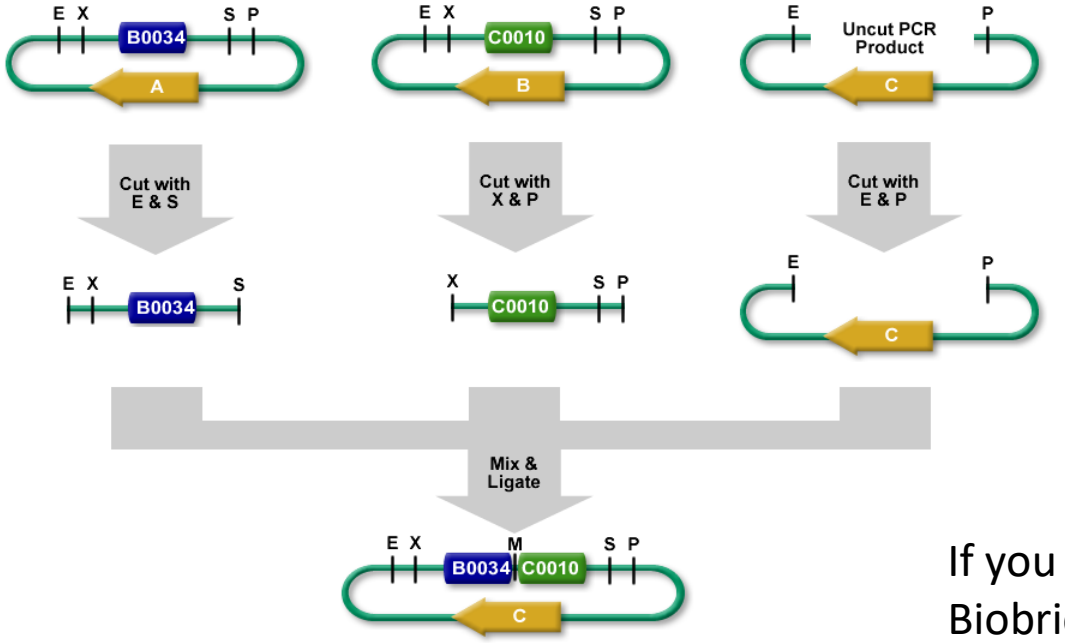


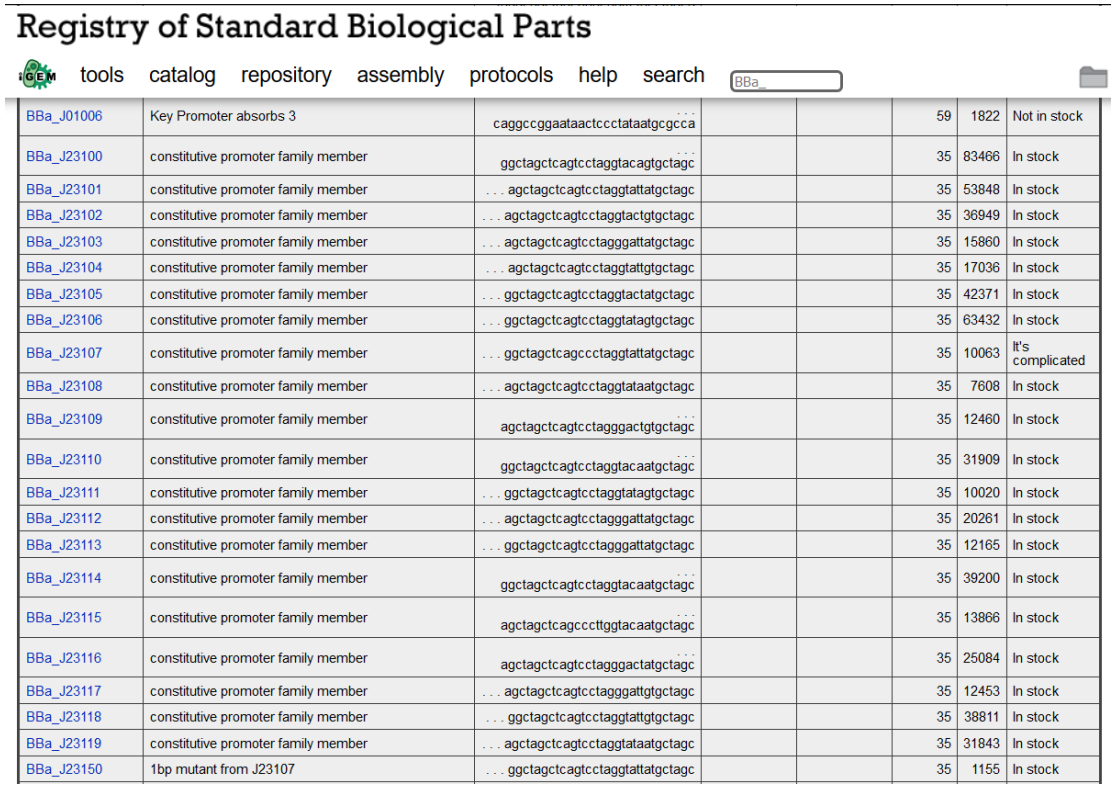


If you want to learn more about Biobrick assembly /3A assembly go to the following links:

https://www.youtube.com/watch?v=F1mXoy1-Vr0

https://www.youtube.com/watch?v=Zrtdwvn4G6s

# Registry of parts



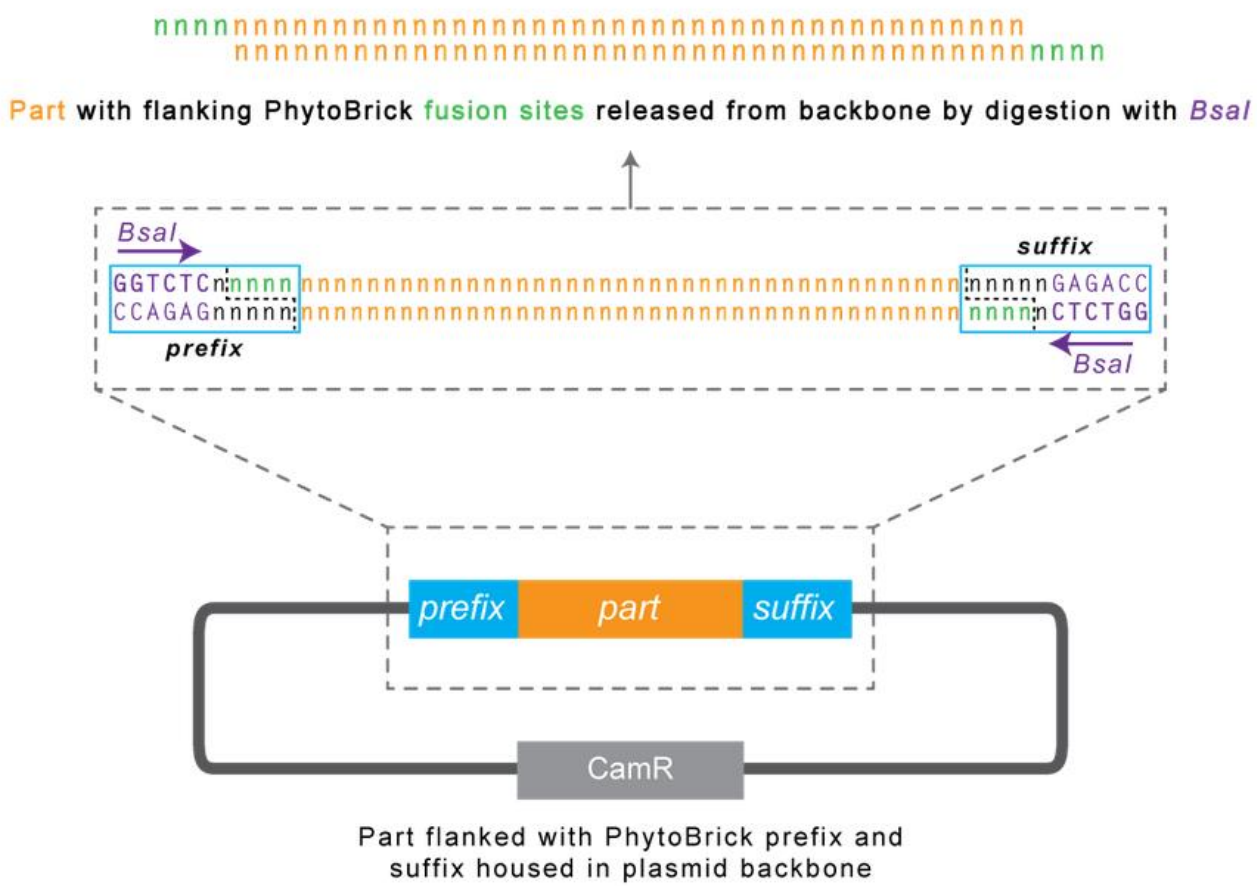
Registry of Standard Biological Parts

tools catalog repository assembly protocols help search BBa_

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| BBa_J01006 | Key Promoter absorbs 3 | caggccggaataactccctataatgcgcca | | | 59 | 1822 | Not in stock |
| BBa_J23100 | constitutive promoter family member | ggctagctcagtcctaggtacagtgctagc | | | 35 | 83466 | In stock |
| BBa_J23101 | constitutive promoter family member | agctagctcagtcctaggtattatgctagc | | | 35 | 53848 | In stock |
| BBa_J23102 | constitutive promoter family member | agctagctcagtcctaggtactgtgctagc | | | 35 | 36949 | In stock |
| BBa_J23103 | constitutive promoter family member | agctagctcagtcctagggattatgctagc | | | 35 | 15860 | In stock |
| BBa_J23104 | constitutive promoter family member | agctagctcagtcctaggtattgtgctagc | | | 35 | 17036 | In stock |
| BBa_J23105 | constitutive promoter family member | ggctagctcagtcctaggtactatgctagc | | | 35 | 42371 | In stock |
| BBa_J23106 | constitutive promoter family member | ggctagctcagtcctaggtatagtgctagc | | | 35 | 63432 | In stock |
| BBa_J23107 | constitutive promoter family member | ggctagctcagccctaggtattatgctagc | | | 35 | 10063 | It's complicated |
| BBa_J23108 | constitutive promoter family member | agctagctcagtcctaggtataatgctagc | | | 35 | 7608 | In stock |
| BBa_J23109 | constitutive promoter family member | agctagctcagtcctagggactgtgctagc | | | 35 | 12460 | In stock |
| BBa_J23110 | constitutive promoter family member | ggctagctcagtcctaggtacaatgctagc | | | 35 | 31909 | In stock |
| BBa_J23111 | constitutive promoter family member | ggctagctcagtcctaggtatagtgctagc | | | 35 | 10020 | In stock |
| BBa_J23112 | constitutive promoter family member | agctagctcagtcctagggattatgctagc | | | 35 | 20261 | In stock |
| BBa_J23113 | constitutive promoter family member | ggctagctcagtcctagggattatgctagc | | | 35 | 12165 | In stock |
| BBa_J23114 | constitutive promoter family member | ggctagctcagtcctaggtacaatgctagc | | | 35 | 39200 | In stock |
| BBa_J23115 | constitutive promoter family member | agctagctcagcccttggtacaatgctagc | | | 35 | 13866 | In stock |
| BBa_J23116 | constitutive promoter family member | agctagctcagtcctagggactatgctagc | | | 35 | 25084 | In stock |
| BBa_J23117 | constitutive promoter family member | agctagctcagtcctagggattgtgctagc | | | 35 | 12453 | In stock |
| BBa_J23118 | constitutive promoter family member | ggctagctcagtcctaggtattgtgctagc | | | 35 | 38811 | In stock |
| BBa_J23119 | constitutive promoter family member | agctagctcagtcctaggtataatgctagc | | | 35 | 31843 | In stock |
| BBa_J23150 | 1bp mutant from J23107 | ggctagctcagtcctaggtattatgctagc | | | 35 | 1155 | In stock |

- Library of parts created by iGEM teams in all the years
- Every iGEM team sends biobricks and fills in data and description
- All parts are standardized and can be easily combined

# The „new“ standard: Phytobricks and TypeIIS




Part flanked with PhytoBrick prefix and suffix housed in plasmid backbone

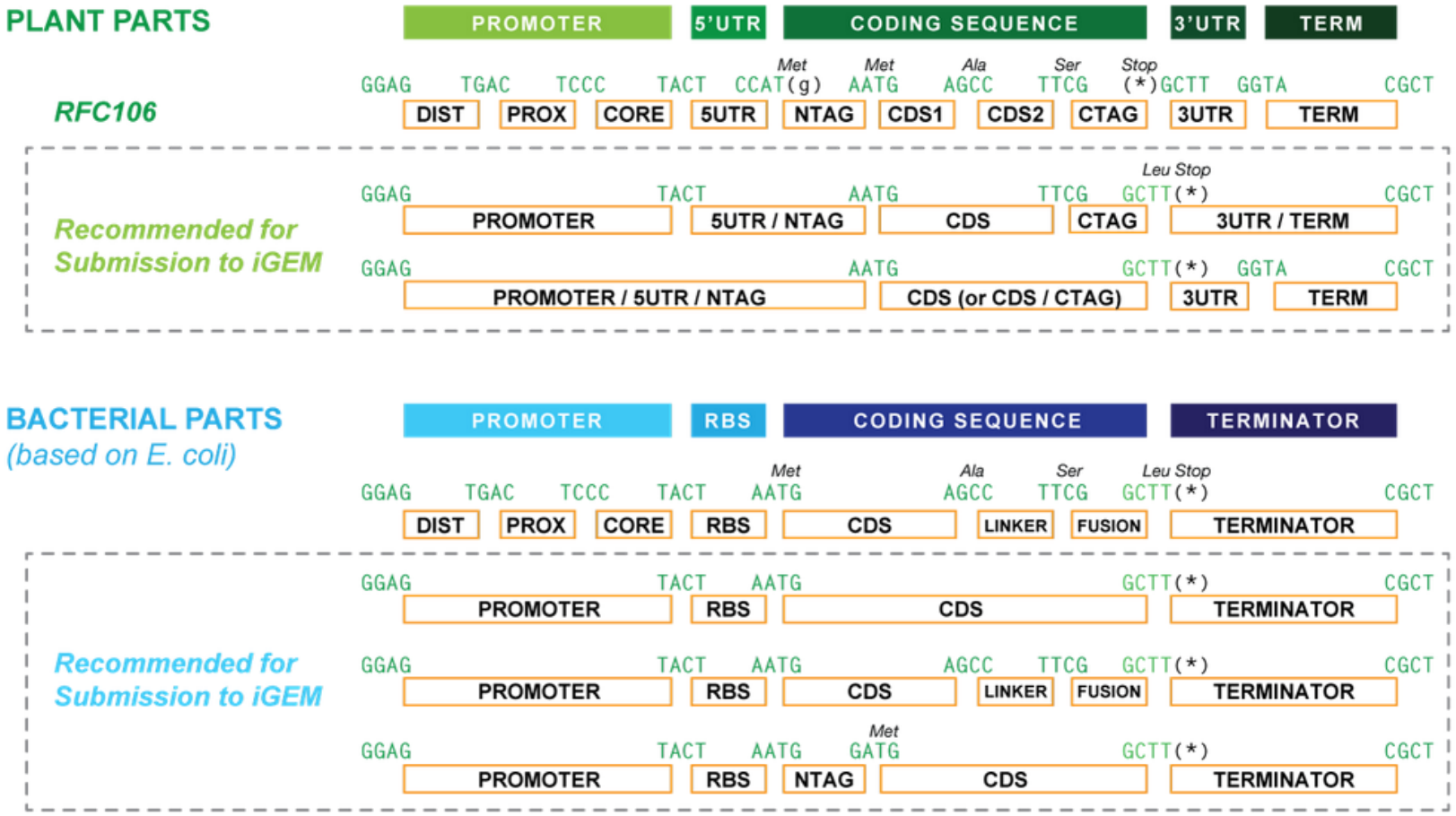
# The „new“ standard: Phytobricks and TypeIIS

| Enzyme | Type | Sequence (Forward) | Sequence (Reverse) |
|---|---|---|---|
| BsaI | Illegal | 5'...GGTCTC >>...3'<br>3'...CCAGAG >>...5' | 5'...<< GAGACC...3'<br>3'...<< CTCTGG...5' |
| SapI | Illegal | 5'...GCTCTTC >>...3'<br>3'...CGAGAAG >>...5' | 5'...<< GAAGAGC...3'<br>3'...<< CTTCTCG...5' |

| Fusion Site 5' | Transcriptional Unit (TU) | Fusion Site 3' |
|---|---|---|
| ATG | TU 1 | GCA |
| GCA | TU 2 | TAC |
| TAC | TU 3 | CAG |
| CAG | TU 4 | GGT |

| Level 2 Assembly | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ATG | TU 1 | GCA | TU 2 | TAC | TU 3 | CAG | TU 4 | GGT |
| ATG | Multi-transcriptional Unit | | | | | | | GGT |

# The „new“ standard: Phytobricks and TypeIIS




Much more information next week in the Golden Gate and Modular Cloning webinar!

# The „new“ standard: Phytobricks and TypeIIS

practical part

# Basic Techniques in Molecular Biology

Tania Pozzo Ph.D.

June 23, 2020

# Polymerase Chain Reaction (PCR)

- PCR is the fundamental technique used to make copies of gene sequences.
- PCR products are commonly referred to as amplicons.
- Metabolic engineering requires DNA manipulation using PCR.
- Understanding how PCR works is essential for developing new DNA based technologies.

https://youtu.be/QwT-Tj89VLo

# Restriction Digestions

## Digestion of DNA with Restriction Enzymes

- Polymers of dsDNA are cleaved using restriction endonucleases that recognize specific nucleotide sequences (see video).

- Circular Plasmids (Vectors) used for gene cloning are cleaved by restriction enzymes in order to insert PCR products such as protein coding sequences into the plasmid.

https://youtu.be/4CsLLcveIB0

# DNA Ligation with DNA Ligase

- Amplicons from PCR are inserted into open vectors to form recombinant plasmids.
- DNA ligase forms phosphodiester linkages between the ends of the amplicons and the open ends of the plasmid (see video).
- A common DNA ligase used for gene cloning is T4 DNA Ligase.

https://youtu.be/xMBC3q_CbTA

# Bacterial transformation

- Bacterial cells are “transformed” by taking recombinant plasmids into the cell.
- Bacterial transformation occurs via chemical transformation or electroporation (see video).
- Transformed bacterial cells are cultured on selective medium corresponding to selection marker gene expression (e.g. antibiotic resistance).

https://youtu.be/7Ul9RVYG5CM


Thanks for listening!

# Upcoming webinars

- Week 3 - Tuesday  June 30th - 7am EDT
  - Modeling circuits with ODEs and experimental data
- Week 3 - Tuesday June 30th - 10am EDT
  - DNA assembly techniques