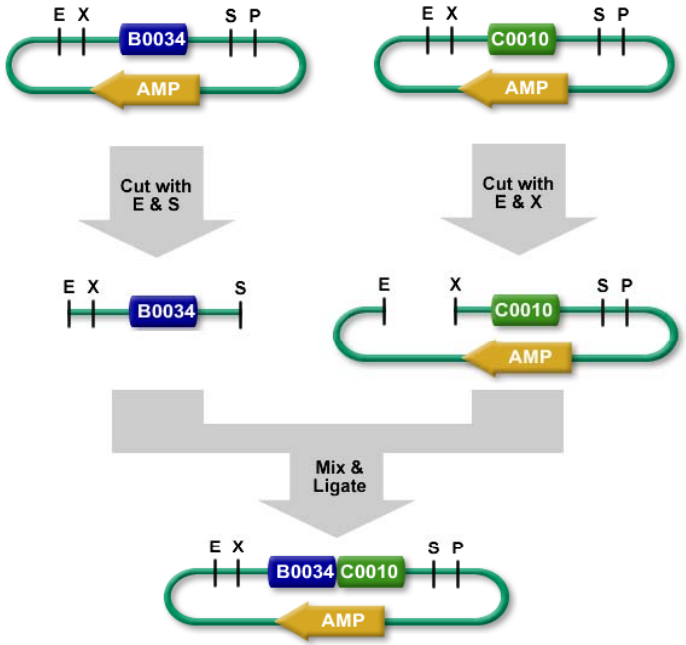
iGEM 2008 Lab Meeting

2008.05.22

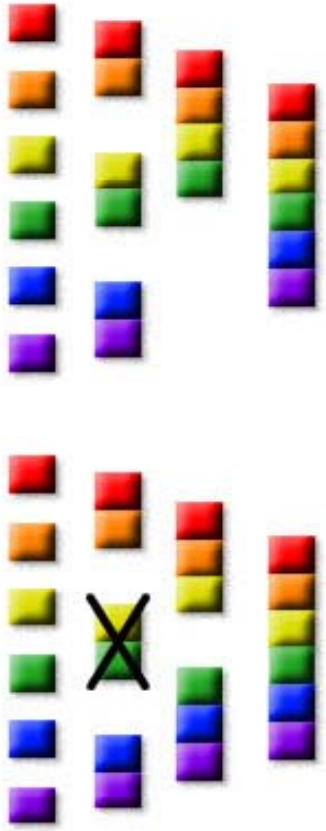
Assembly Process

Standard Assembly



"Idempotent Vector Design for Standard Assembly of Biobricks"
http://dspace.mit.edu/handle/1721.1/21168

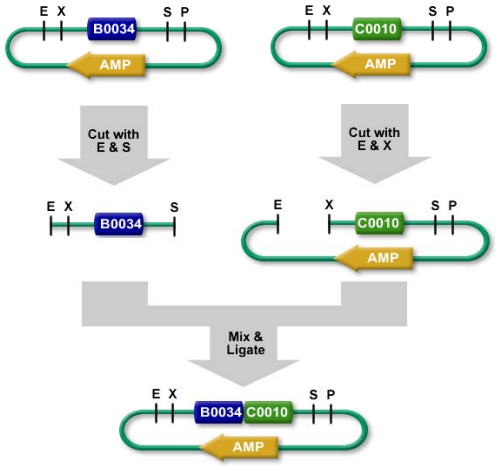
Rolling Assembly

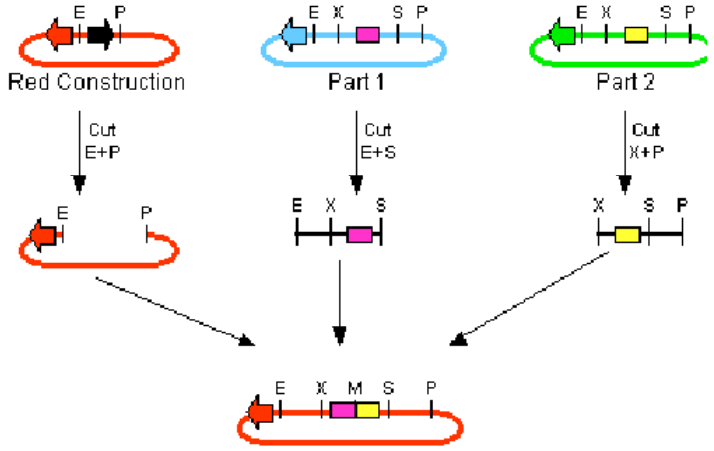


1. parallel process
2. anticipate failure

Assembly Methods

Standard Assembly (Knight Lab)



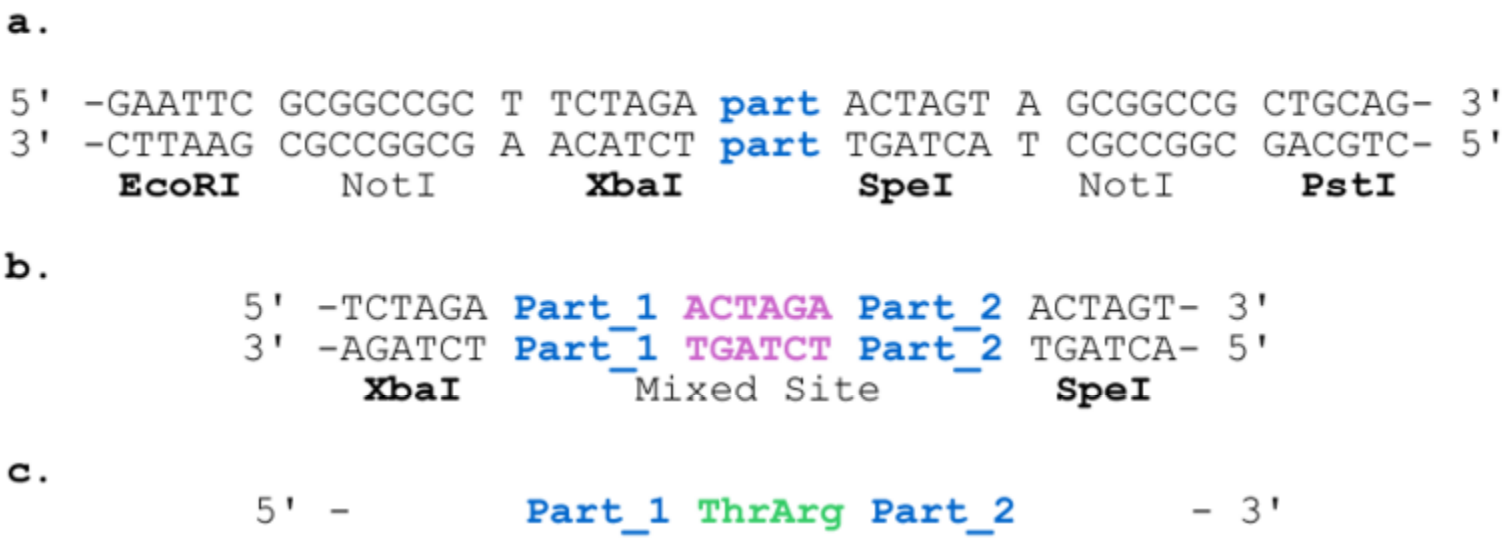


Three Antibiotic (3A) Assembly

http://openwetware.org/wiki/Synthetic_Biology:BioBricks/3A_assembly

Fusion Bricks (Silver Lab)

- Developed by Pam Silver, Ira Philips & the lab at Harvard
- Scar after fusing BioBrick
  creates a frameshift when translating to a protein
- FusionBricks allow reading frame to be maintained



http://2008.igem.org/Image:FusionBricks_image.png
http://dspace.mit.edu/handle/1721.1/32535

BioBrickExtreme... (Anderson Lab, Berkley. Incompatible “standard”)

Prefixing

- **The Standard BioBrick Restriction Enzyme Sites:**

```
5' --gca GAATTC GCGGCCGC T TCTAGA G --Insert-- T ACTAGT A GCGGCCG CTGCAG gct--- 3'
3' --cgt CTTAAG CGCCGGCG A AGATCT C --Insert-- A TGATCA T CGCCGGC GACGTC cga--- 5'
        EcoRI  NotI       XbaI                  SpeI     NotI    PstI
```

- **Front Vector (FV) [EcoRI/XbaI]**

```
5' --gca G                *CTAGA G --Insert-- T ACTAGT A GCGGCCG CTGCAG gct--- 3'
3' --cgt CTTAA*                T C --Insert-- A TGATCA T CGCCGGC GACGTC cga--- 5'
        EcoRI             XbaI                  SpeI     NotI    PstI
```

- **Front Insert (FI) [EcoRI/SpeI]**

```
5'       *AATTC GCGGCCGC T TCTAGA G --Insert-- T A                            3'
3'            G CGCCGGCG A ACATCT C --Insert-- A TGATC*                       5'
        EcoRI  NotI       XbaI                  SpeI
```

- **Front Ligation (FV + FI)**

```
5' --gca GAATTC GCGGCCGC T TCTAGA G --Insert-- T ACTAGA G --Insert-- T ACTAGT A GCGGCCG CTGCAG gct--- 3'
3' --cgt CTTAAG CGCCGGCG A ACATCT C --Insert-- A TGATCT C --Insert-- A TGATCA T CGCCGGC GACGTC cga--- 5'
        EcoRI  NotI       XbaI                  N/A                    SpeI     NotI    PstI
```

Postfixing

- **The Standard BioBrick Restriction Enzyme Sites:**

```
5' --gca GAATTC GCGGCCGC T TCTAGA G --Insert-- T ACTAGT A GCGGCCG CTGCAG gct--- 3'
3' --cgt CTTAAG CGCCGGCG A AGATCT C --Insert-- A TGATCA T CGCCGGC GACGTC cga--- 5'
         EcoRI  NotI       XbaI                 SpeI     NotI    PstI
```

- **Back Vector (BV) [SpeI/PstI]**

```
5' --gca GAATTC GCGGCCGC T TCTAGA G --Insert-- T A                   *G gct--- 3'
3' --cgt CTTAAG CGCCGGCG A ACATCT C --Insert-- A TGATC*             ACGTC cga--- 5'
         EcoRI  NotI       XbaI                 SpeI              PstI
```

- **Back Insert (BI) [XbaI/PstI]**

```
5'                       *CTAGA G --insert-- T ACTAGT A GCGGCCG CTGCA         3'
3'                            T C --insert-- A TGATCA T CGCCGGC G*            5'
                         XbaI                 SpeI     NotI    PstI
```

- **Back Ligation (BV + BI)**

```
5′ --gca GAATTC GCGGCCGC T TCTAGA G --Insert-- T ACTAGA G --insert-- T ACTAGT A GCGGCCG CTGCAG gct--- 3'
3' --cgt CTTAAG CGCCGGCG A ACATCT C --Insert-- A TGATCT C --insert-- A TGATCA T CGCCGGC GACGTC cga--- 5'
         EcoRI  NotI       XbaI                 N/A                   SpeI     NotI    PstI
```

BioBrick Parts

Part Number	Binding Efficiency	Sequence
BBa_B0030	0.6	att aaa gag gag aaa
BBa_B0031	0.07	tca cac agg aaa cc
BBa_B0032	0.3	tca cac agg aaa g

RBS

A ribosome binding site (RBS) is a segment of the 5' (upstream) part of an mRNA molecule that binds to the ribosome to position the message correctly for the initiation of translation.

Other Parts

BBa_B0033 efficiency 0.01

BBa_B0034 efficiency 1.0

e.g. Shine Delgarno Seq.

- lies ~7 nucleotide upstream of the AUG
- mRNA containes consensus seq AGGAGG
- 16S RNA contains the complementary CCUCCU

BioBrick Part

RNA

novel structures such as stem loops or riboregulators

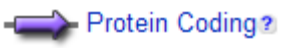
DNA

Act as DNA itself

e.g. spacers, restriction sites, recombinational enhancers.

Recombination Enhancer (RE) is a DNA sequence contains Fis binding sites. Fis bound to RE facilitates Hin-mediated DNA inversion. See BBa_J31001 for details on Hin invertase.

BioBrick Part

Proteins  Protein Coding ?

protein coding regions

Enzymes

Enymes catalyze biochemical reactions.

 Enzymes

Repressors and Activators

These are proteins that bind to control sequences in DNA and either down- or up-regulate the expression of nearby genes. (Some of them do both depending on conditions.)

 Repressors and Activators

Reporter Coding Sequences

These are the basic parts for fluorescent proteins and other reporters. See the reporter *devices* that use these basic parts.

Reporter Coding Regions

Uncategorized Coding Sequences

Some of the proteins do not fall into these categories or have not yet been categorized.

 Uncategorized Protein Coding

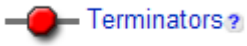
All Protein-Coding Regions

The old table of unsorted Protein Coding Regions plus the table of Reporter Coding Regions (also proteins). Click on the ? for general introductory information about coding regions.

 Protein Coding ?

BioBrick Part

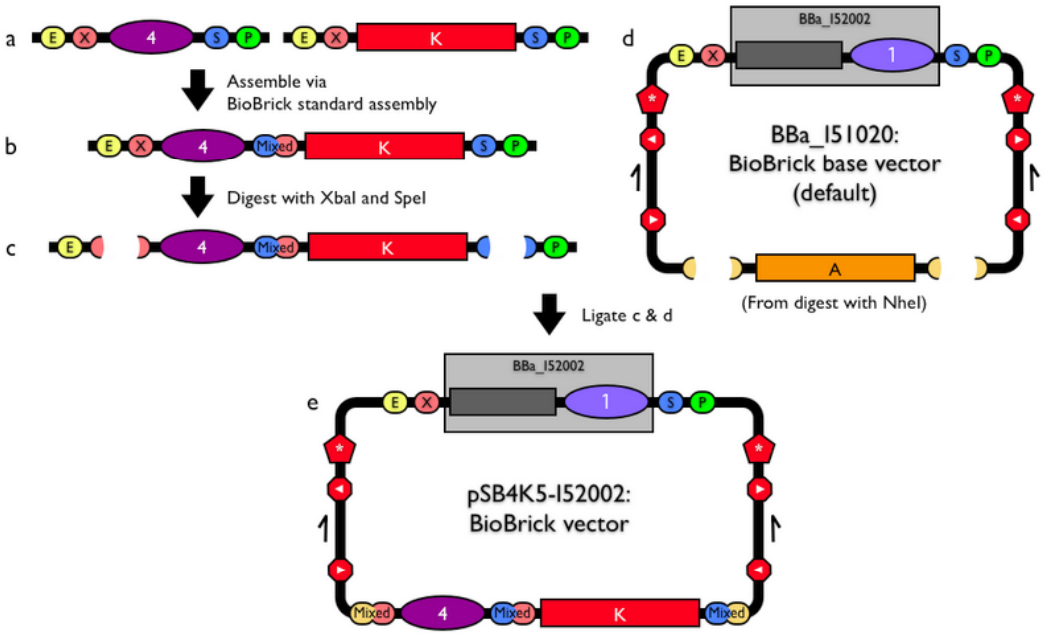
Terminators



transcriptional terminators
forward/reverse

Plasmid Construction

- http://parts.mit.edu/registry/index.php/Help:Plasmids/Construction



Part Number	Function	Notation
BBa_G00000	BioBrick cloning site prefix	
BBa_G00001	BioBrick cloning site suffix	
BBa_P1016	*ccdB* positive selection marker	
BBa_I50022	minimal pUC-derived high copy replication origin	
BBa_B0042	translational stop sequence	
BBa_B0053 & BBa_B0054	forward transcriptional terminator	
BBa_B0055 & BBa_B0062	reverse transcriptional terminator	
BBa_G00100	forward sequencing primer annealing site (VF2)	
BBa_G00102	reverse sequencing primer annealing site (VR)	
BBa_B0045	NheI restriction site	
BBa_P1006	ampicillin resistance marker (reverse orientation)	
BBa_P1002	ampicillin resistance marker	
BBa_P1003	kanamycin resistance marker	
BBa_P1004	chloramphenicol resistance marker	
BBa_P1005	tetracycline resistance marker	
BBa_I50042	pSC101 replication origin	
BBa_I50032	p15A replication origin	

Recognition Site:
5′...GCTAGC...3′
3′...CGATCG...5′

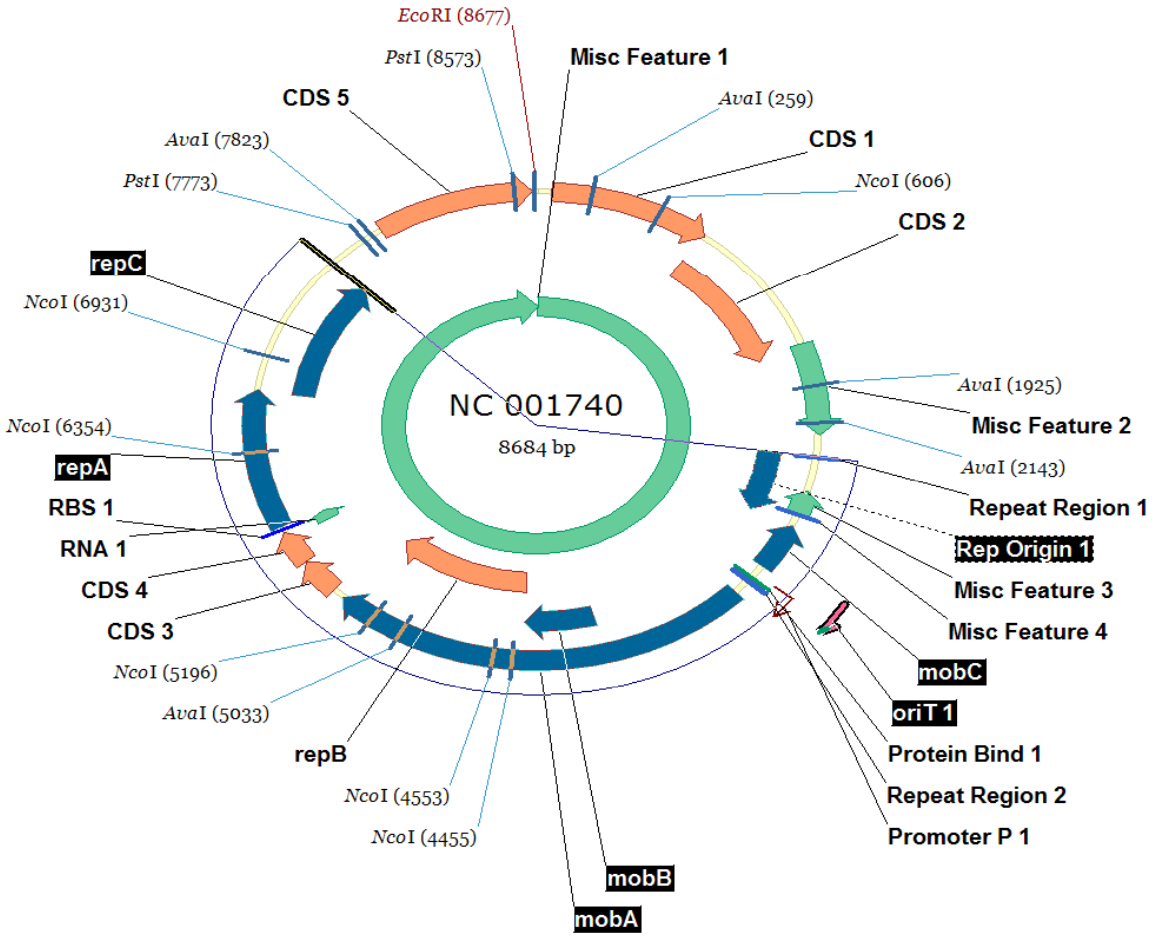
Recognition Site:
5′...GAATTC...3′
3′...CTTAAG...5′

Recognition Site:
5′...TCTAGA...3′
3′...AGATCT...5′

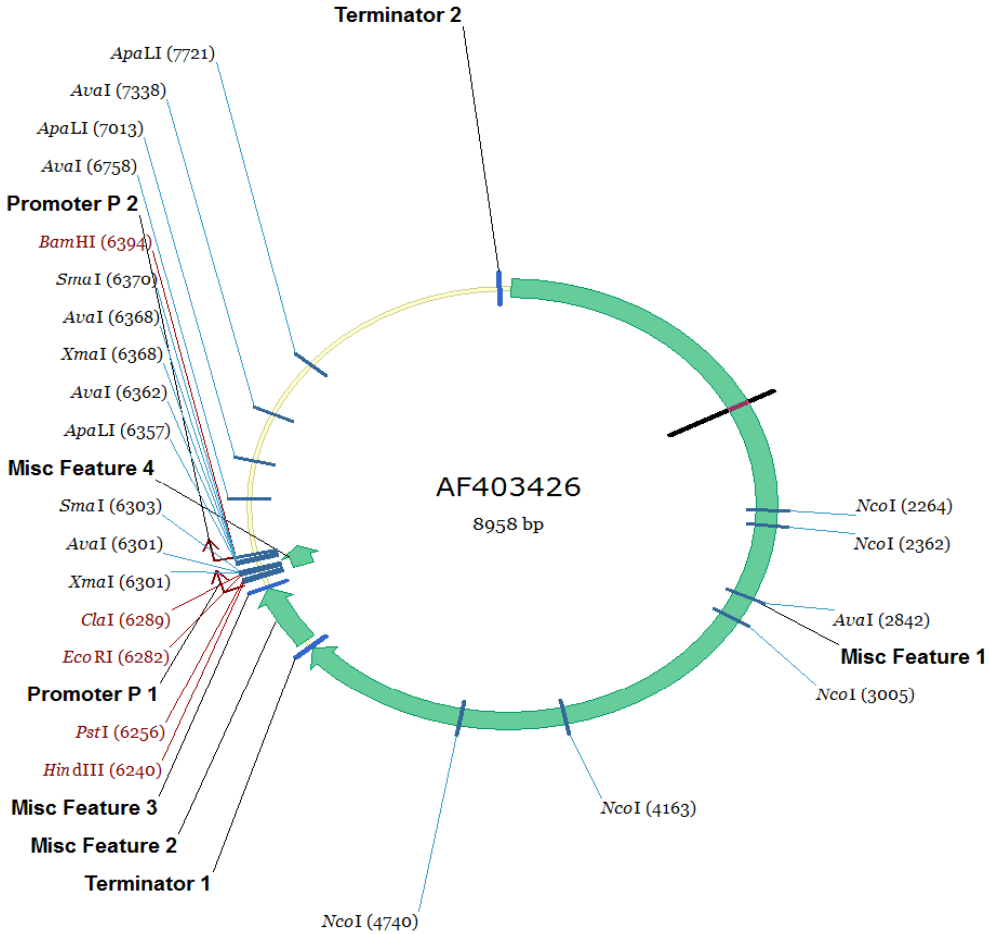
Recognition Site:
5′...ACTAGT...3′
3′...TGATCA...5′

Recognition Site:
5′...CTGCAG...3′
3′...GACGTC...5′

RSF1010


EcoRI (8677)
PstI (8573)
Misc Feature 1
AvaI (259)
CDS 5
CDS 1
AvaI (7823)
NcoI (606)
PstI (7773)
CDS 2
repC
NcoI (6931)
AvaI (1925)
Misc Feature 2
NC 001740
8684 bp
NcoI (6354)
AvaI (2143)
repA
Repeat Region 1
RBS 1
Rep Origin 1
RNA 1
Misc Feature 3
CDS 4
Misc Feature 4
CDS 3
mobC
NcoI (5196)
oriT 1
AvaI (5033)
Protein Bind 1
repB
Repeat Region 2
NcoI (4553)
Promoter P 1
NcoI (4455)
mobB
mobA

pRL1383a


Terminator 2
ApaLI (7721)
AvaI (7338)
ApaLI (7013)
AvaI (6758)
Promoter P 2
BamHI (6394)
SmaI (6370)
AvaI (6368)
XmaI (6368)
AvaI (6362)
ApaLI (6357)
Misc Feature 4
SmaI (6303)
AvaI (6301)
XmaI (6301)
ClaI (6289)
EcoRI (6282)
Promoter P 1
PstI (6256)
HindIII (6240)
Misc Feature 3
Misc Feature 2
Terminator 1
AF403426
8958 bp
NcoI (2264)
NcoI (2362)
AvaI (2842)
Misc Feature 1
NcoI (3005)
NcoI (4163)
NcoI (4740)