BIOE.44

Synthetic Biology Lab

6 April 2010 Lecture / Discussion notes

http://openwetware.org/wiki/Stanford/BIOE44

Today's take aways:

1. Why technical standards matter? (cont.)

2. What the heck is standardized idempotent assembly? (i.e., BioBrick Assembly Standard #10)

3. Features of BB_AS#10

4. Problems with BB_AS#10

from last time

1973

Construction of biologically functional bacterial plasmids in vitro

Cohen et al., PNAS, 1973

MATERIALS AND METHODS

E. coli strain W1485 containing the RSF1010 plasmid, which carries resistance to streptomycin and sulfonamide, was obtained from S. Falkow. Other bacterial strains and R factors and procedures for DNA isolation, electron microscopy, and transformation of *E. coli* by plasmid DNA have been described (1, 7, 8). Purification and use of the *Eco*RI restriction endonuclease have been described (5). Plasmid heteroduplex studies were performed as previously described (9, 10). *E. coli* DNA ligase was a gift from P. Modrich and R. L. Lehman and was used as described (11). The detailed procedures for gel electrophoresis of DNA will be described elsewhere (Helling, Goodman, and Boyer, in preparation); in brief, duplex DNA was subjected to electrophoresis in a tube-type apparatus (Hoefer Scientific Instrument) (0.6 × 15-cm gel) at about 20° in 0.7% agarose at 22.5 V with 40 mM Tris-acetate buffer (pH 8.05) containing 20 mM sodium acetate, 2 mM EDTA, and 18 mM sodium chloride. The gels were then soaked in ethidium bromide (5 μg/ml) and the DNA was visualized by fluorescence under long wavelength ultraviolet light ("black light"). The molecular weight of each fragment in the range of 1 to 200 × 10^6 was determined from its

1985

Cloning and expression of the human erthropoietin gene

Lin et al., PNAS, 1985

Assembly of Expression Vector for the Epo Gene. For direct expression of the genomic Epo gene, the 4.8-kilobase (kb) *Bst*EII–*Bam*HI fragment of λHE1 (see *Results*), which contains the entire Epo gene, was used. After converting the *Bst*EII site into a *Bam*HI site with a synthetic linker, the fragment was inserted into the unique *Bam*HI site of the expression vector pDSVL (unpublished data), which contains a dihydrofolate reductase (DHFR) minigene from pMg1 (24). The resulting plasmid pDSVL-gHuEPO (Fig. 1*A*) was then used to transfect Chinese hamster ovary (CHO) DHFR$^-$ cells (25) by the calcium phosphate microprecipitate method (26). The transformants were selected by growth in medium lacking hypoxanthine and thymidine. The culture medium used was Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin, streptomycin, and glutamine (25).

2006

Production of the antimalarial drug precursor artemisinic acid in engineered yeast

Ro et al., Nature, 2006

2005-11-12577

Plasmid construction. To create plasmid pRS425ADS for expression of *ADS* with the *GAL1* promoter, *ADS* was PCR amplified from pADS[7] using primer pair 9 and 10. (Supplementary Table I). Using these primers the nucleotide sequence 5'-AAAACA-3' was cloned immediately upstream of the start codon of *ADS*. This consensus sequence was used for efficient translation[8,9] of *ADS* and the other galactose-inducible genes used in this study. The amplified product was cleaved with *Spe*I and *Hin*dIII and cloned into *Spe*I and *Hin*dIII digested pRS425GAL1[10].

For integration of an expression cassette for *tHMGR*, plasmid pδ-HMGR was constructed. First *Sac*II restriction sites were introduced into pRS426GAL1[10] at the 5' end of the *GAL1* promoter and 3' end of the *CYC1* terminator. To achieve this, the promoter-multiple cloning site-terminator cassette of pRS426GAL1 was PCR amplified using primer pair 11 and 12. The amplified product was cloned directly into *Pvu*II-

Genetic engineering remains expert driven artwork

from last time

ON A SYSTEM OF

SCREW THREADS AND NUTS.

By WILLIAM SELLERS.

[Read before the FRANKLIN INSTITUTE, April 21, 1864.]

The importance of a uniform system of screw threads and nuts is so generally acknowledged by the engineering profession, that it needs no argument to set forth its advantages; and in offering any plan for their acceptance, it remains only to demonstrate its practicability and its superiority over any of the numerous special proportions now used by the different manufacturers. In this country no organized attempt has as yet been made to establish any system, each manufacturer having adopted whatever his judgment may have dictated as the best, or as most convenient for himself; but the importance of the works now in progress, and the extent to which manufacturing has attained, admonish us that so radical a defect should be allowed to exist no longer. The importance of this subject was long ago recognised in England, and the engineers of that country, by mutual agreement, adopted the proportions now in universal use there. Our standard of length being the same as theirs, it would seem desirable that the system which they have adopted should also be employed by us, unless grave objections can be urged against it and a better one substituted. In examining the details of their system, the first in importance appears to be the pitch or the distance from centre to centre of the threads upon each diameter of screw, which is as follows, viz:

— FIG. 1 —

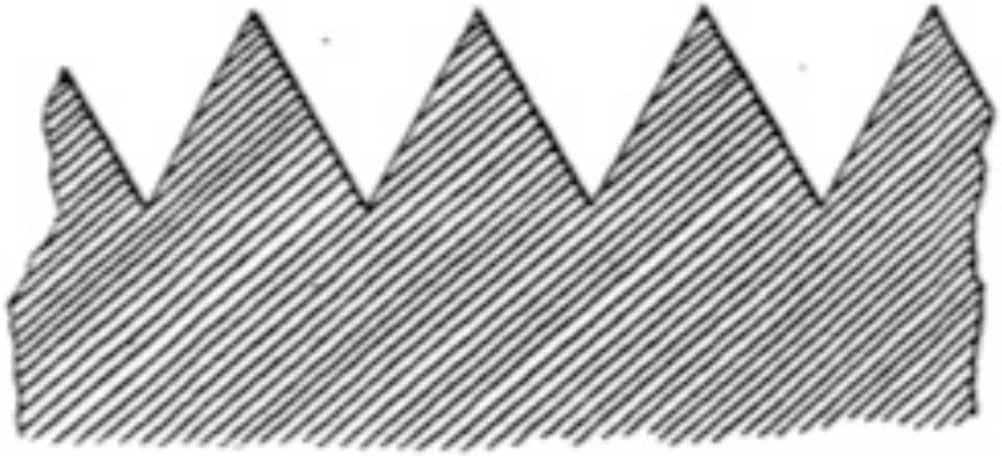
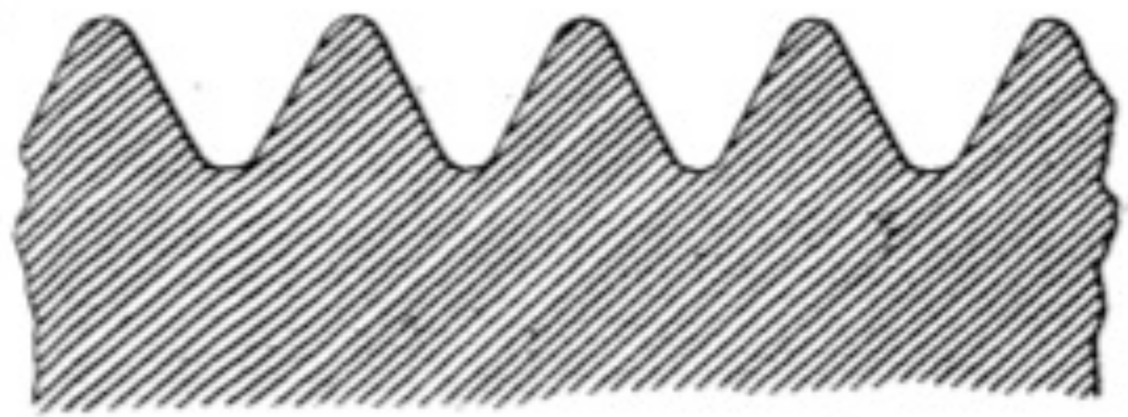
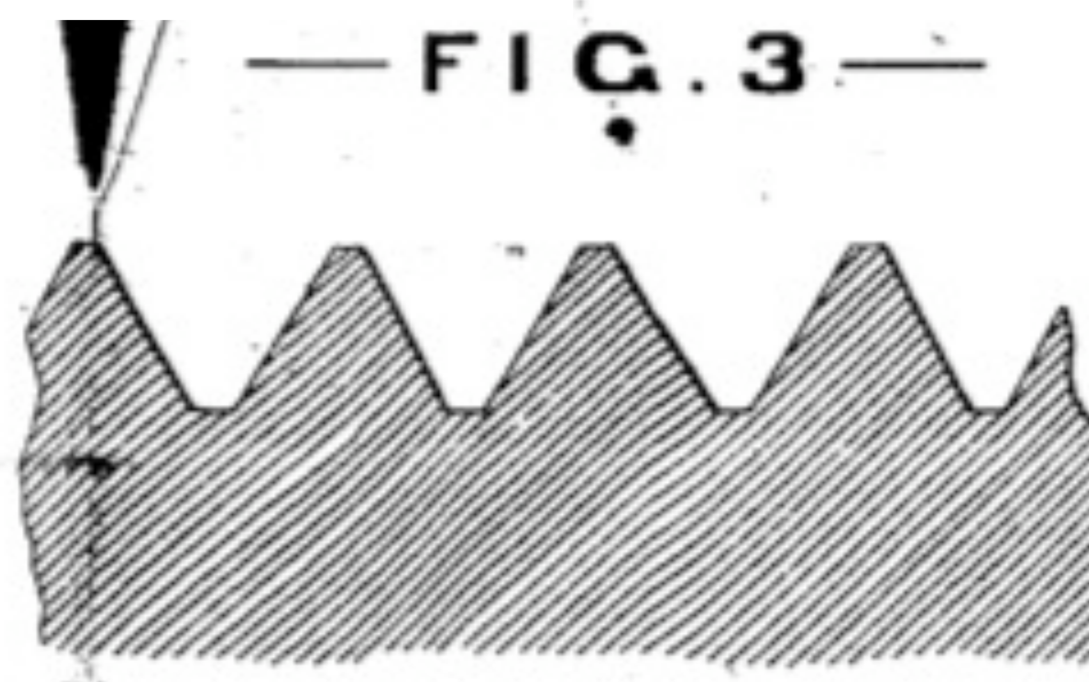


FIG. 2 —



— FIG. 3 —



from last time

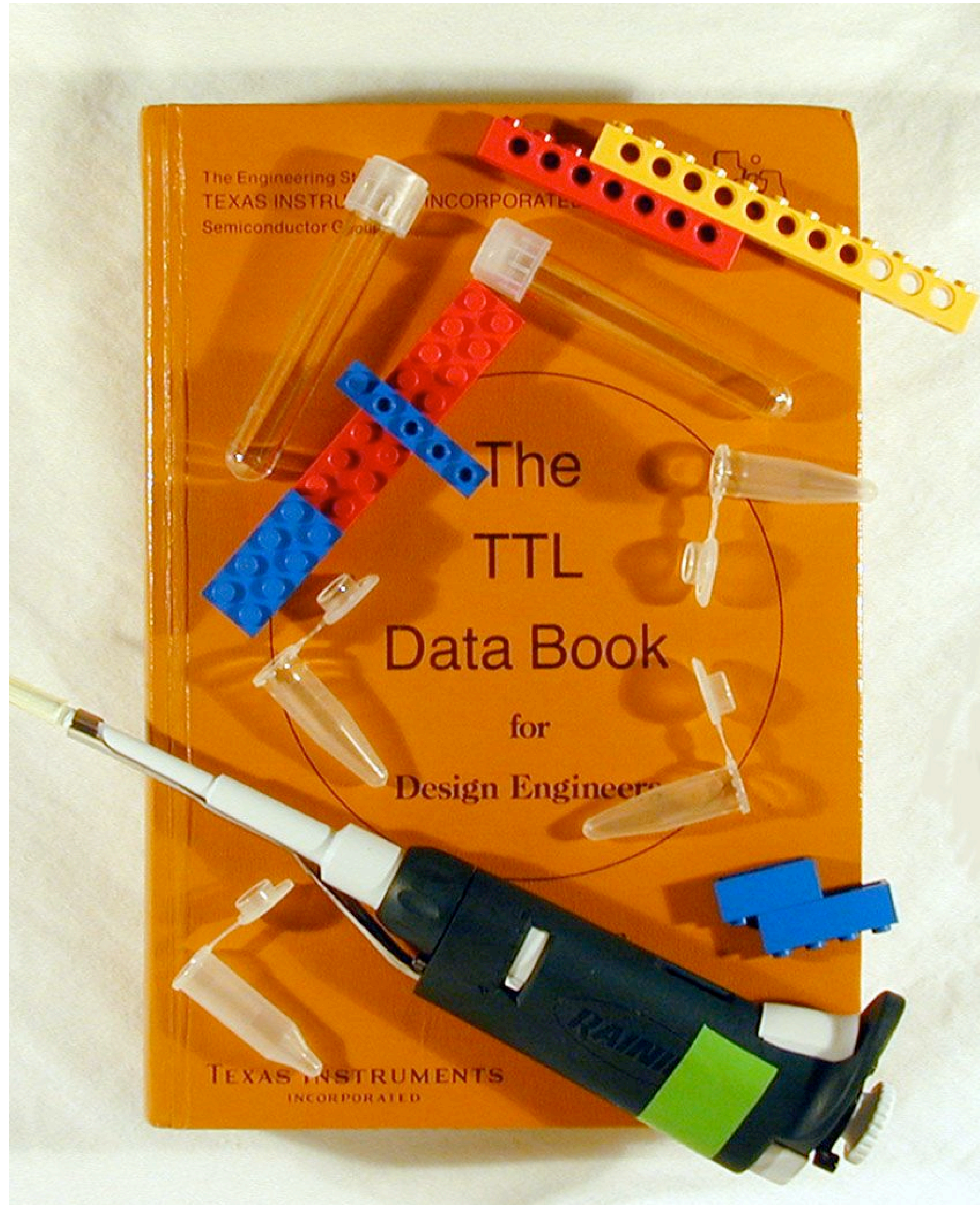
Why std. screw threads matter greatly

http://www.paloaltohardware.com/

Topic #2

Idempotent Vector Design for Standard Assembly of Biobricks

Tom Knight
MIT Artificial Intelligence Laboratory



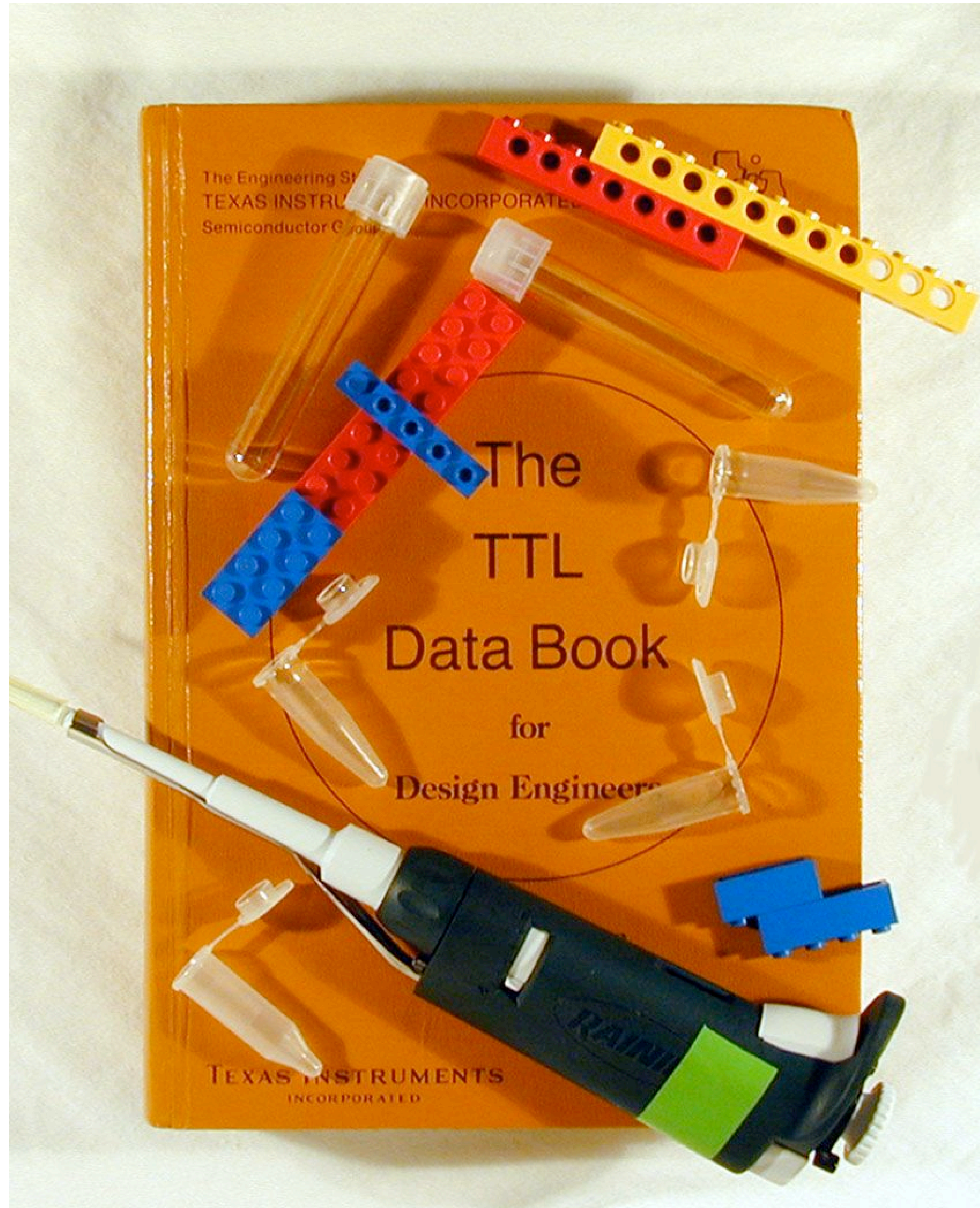
http://dspace.mit.edu/handle/1721.1/21168

Topic #2

Idempotent Vector Design for Standard Assembly of Biobricks

Tom Knight
MIT Artificial Intelligence Laboratory

"The lack of standardization in assembly techniques for DNA sequences forces each DNA assembly reaction to be both an experimental tool for addressing the current research topic, and an experiment in and of itself."



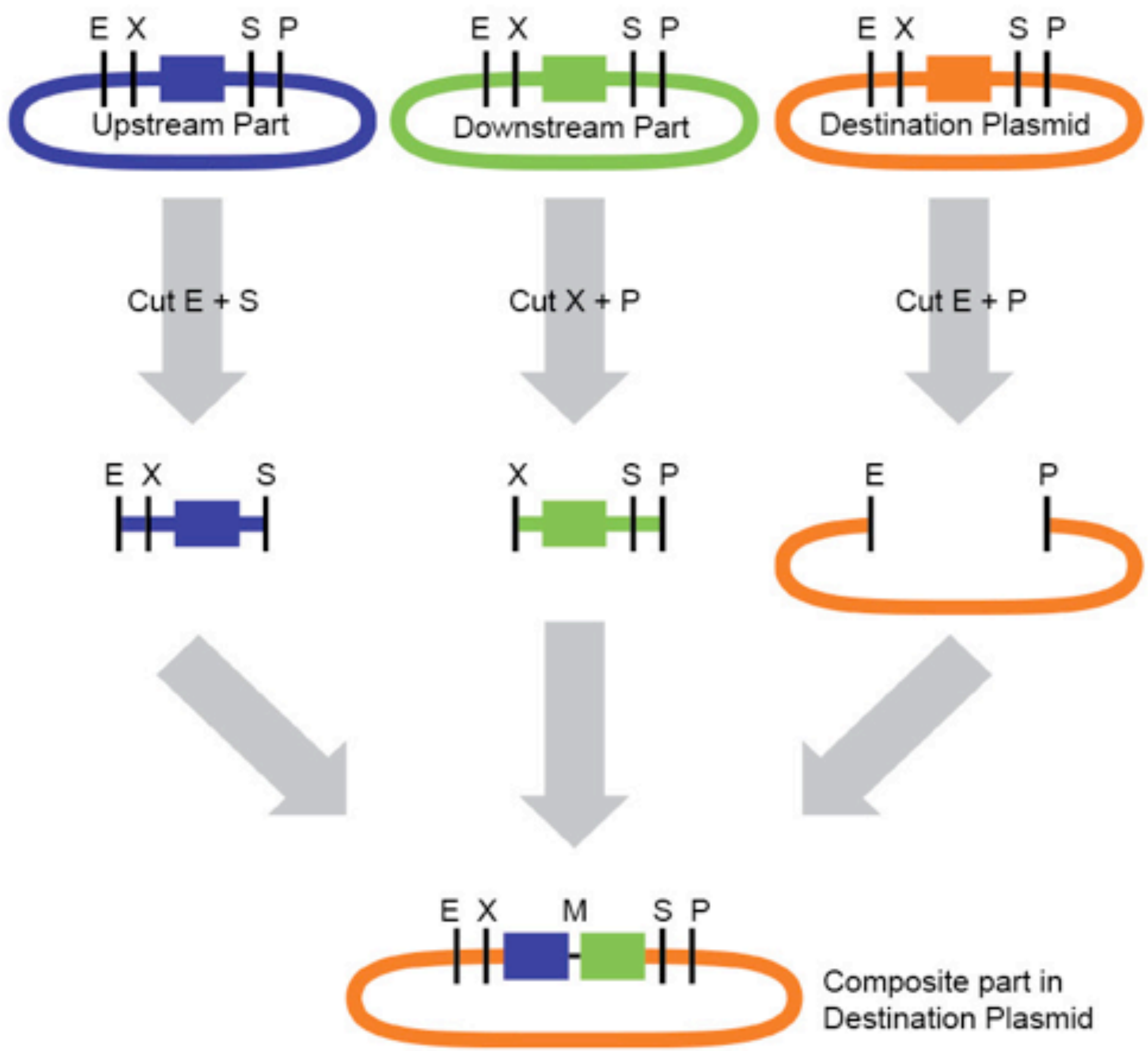
http://dspace.mit.edu/handle/1721.1/21168

BioBrick™ Assembly Manual

ginkgobioworks
NEW ENGLAND BioLabs Inc.

This manual describes the major steps of BioBrick assembly using BioBrick Assembly Standard 10. The input to the protocol is DNA for the two parts to be assembled and a destination plasmid. The manual includes protocols for the digestion of the three input DNA molecules and the ligation of the digested DNA to form a circularized plasmid containing the composite part. The product of the ligation reaction can be used to transform competent cells with the composite part. To read more about the BioBrick system and browse the BioBrick collection, visit the Registry of Standard Biological Parts at http://partsregistry.org.

E=EcoRI-HF™
X=XbaI
S=SpeI
P=PstI
M=Mixed site



BioBrick assembly overview

1 Start with two BioBrick parts and a BioBrick destination plasmid. The destination plasmid contains a toxic gene, *ccdB*, in the BioBrick cloning site and a different antibiotic resistance marker to the upstream and downstream parts.

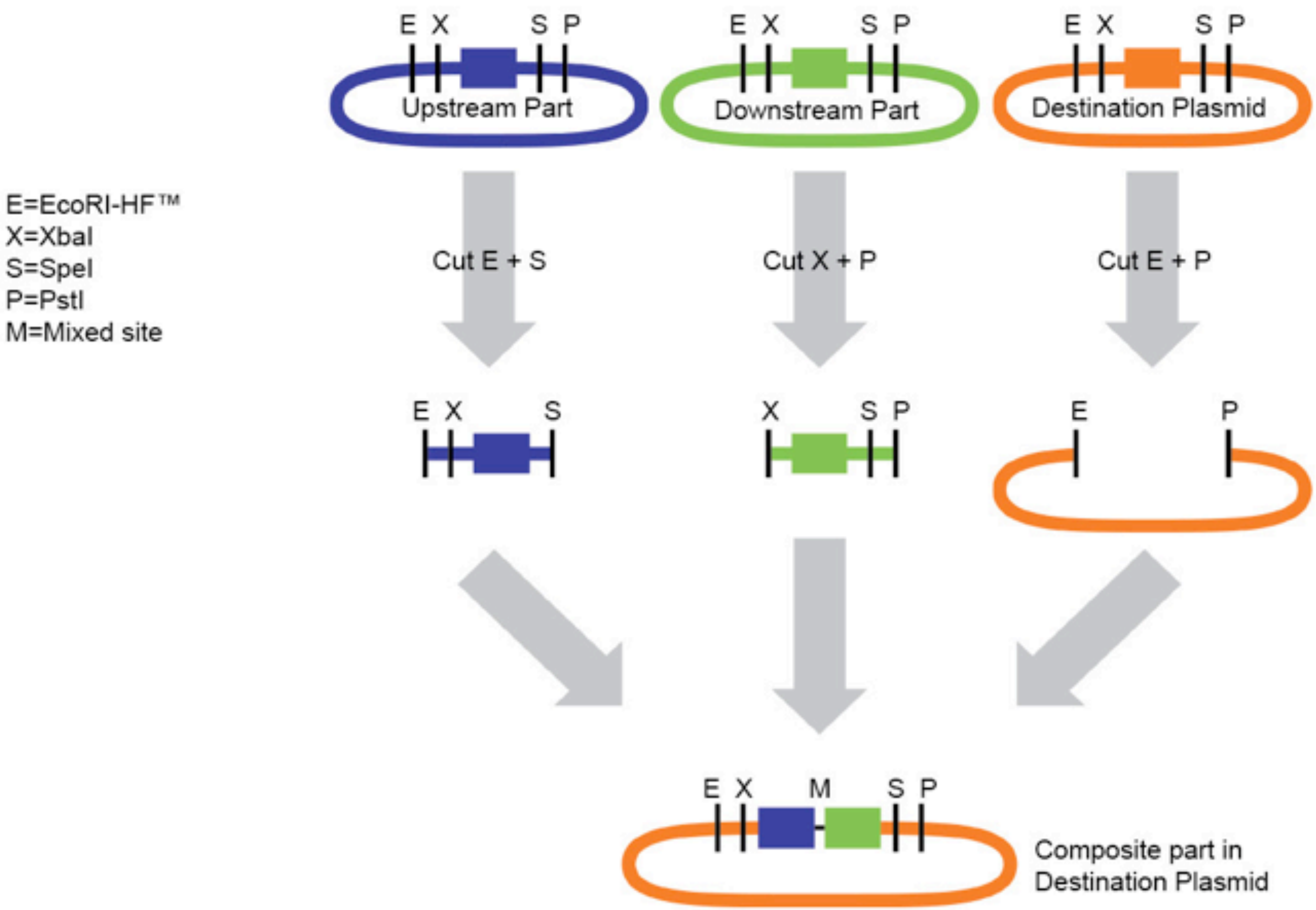
2 Digest each of the parts with the appropriate restriction enzymes.

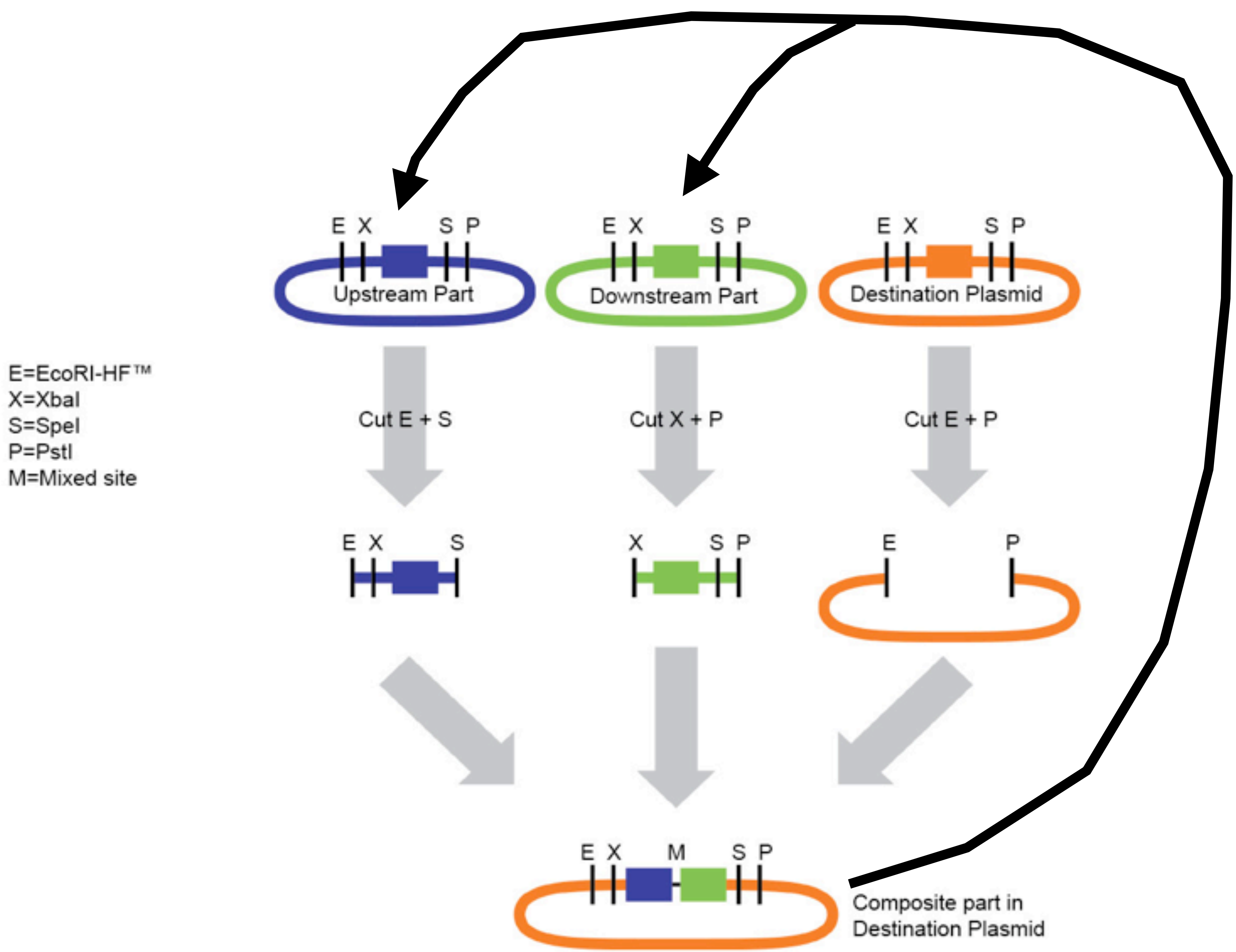
3 Mix the digests together and perform a ligation step. One of the ligation products formed will be the correctly assembled composite part in the destination plasmid. You can use the ligation mix to transform competent cells with the new composite part.

The BioBrick™ Assembly Kit from NEB and Ginkgo BioWorks has been designed for use with this manual. Download this manual from http//ginkgobioworks.com/support

Version 1.0

http://ginkgobioworks.com/support/BioBrick_Assembly_Manual.jpg


E X S P
Upstream Part
E X S P
Downstream Part
E X S P
Destination Plasmid
E=EcoRI-HF™
X=XbaI
S=SpeI
P=PstI
M=Mixed site
Cut E + S
Cut X + P
Cut E + P
E X S
X S P
E P
E X M S P
Composite part in
Destination Plasmid



E=EcoRI-HF™
X=XbaI
S=SpeI
P=PstI
M=Mixed site
E X S P
Upstream Part
E X S P
Downstream Part
E X S P
Destination Plasmid
Cut E + S
Cut X + P
Cut E + P
E X S
X S P
E P
E X M S P
Composite part in
Destination Plasmid

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

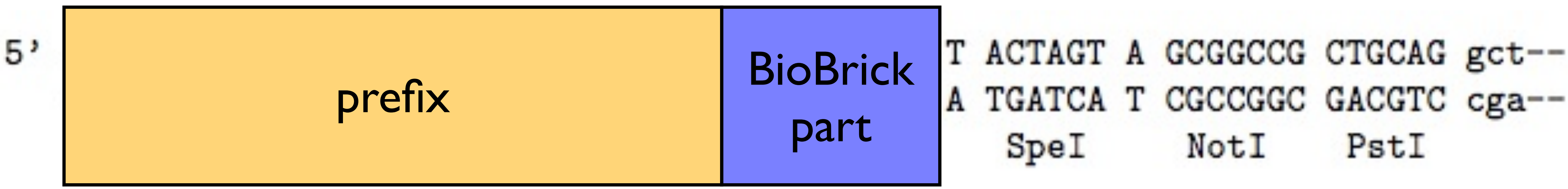
adapted from http://dspace.mit.edu/handle/1721.1/21168

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

adapted from http://dspace.mit.edu/handle/1721.1/21168

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

adapted from http://dspace.mit.edu/handle/1721.1/21168



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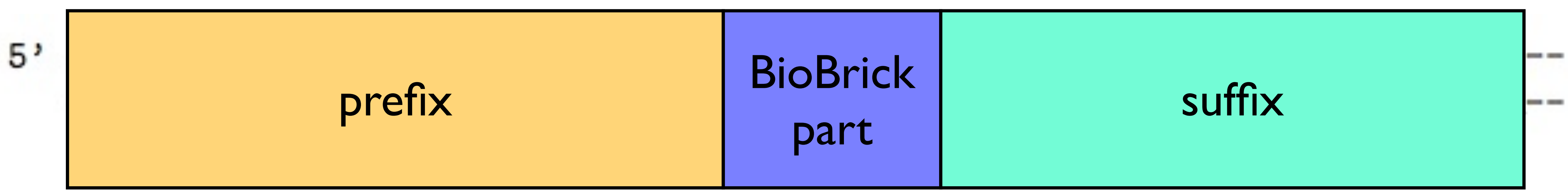


adapted from http://dspace.mit.edu/handle/1721.1/21168

BB assembly standard 10 supports 3 operations

1. *Prefixing reaction (put one part upstream of another)*

2. *Suffixing reaction (put one part downstream of another)*

3. *Three-way assembly (put any two parts together, in either order)*

Prefixing

```
5' --gca G                   *CTAGA G---- 3'               ACTAGT A GCGGCCG CTGC
3' --cgt CTTAA*                   T C---- 5'               TGATCA T CGCCGGC GACG
       EcoRI                   XbaI                         SpeI     NotI    Pst
```

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

```
5' --gca GAATTC GCGGCCGC T TCTAGA G       A    *CTAGA      T ACTAGT A GCGGCCG CTGCAG gct--
   --cgt CTTAAG CGCCGGCG A ACATCT C       TGATC*    T      A TGATCA T CGCCGGC GACGTC cga--
         EcoRI   NotI       XbaI             Mixed            SpeI     NotI    PstI
```

Prefixing

```
5' --gca G                *CTAGA G--  Your favorite part #1  ACTAGT A GCGGCCG CTGC
3' --cgt CTTAA*                T C--                         TGATCA T CGCCGGC GACG
         EcoRI              XbaI                              SpeI     NotI     Pst
```

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

```
5' --gca GAATTC GCGGCCGC T TCTAGA G      A     *CTAGA       T ACTAGT A GCGGCCG CTGCAG gct--
   --cgt CTTAAG CGCCGGCG A ACATCT C      TGATC*     T       A TGATCA T CGCCGGC GACGTC cga--
         EcoRI   NotI      XbaI               Mixed            SpeI     NotI     PstI
```

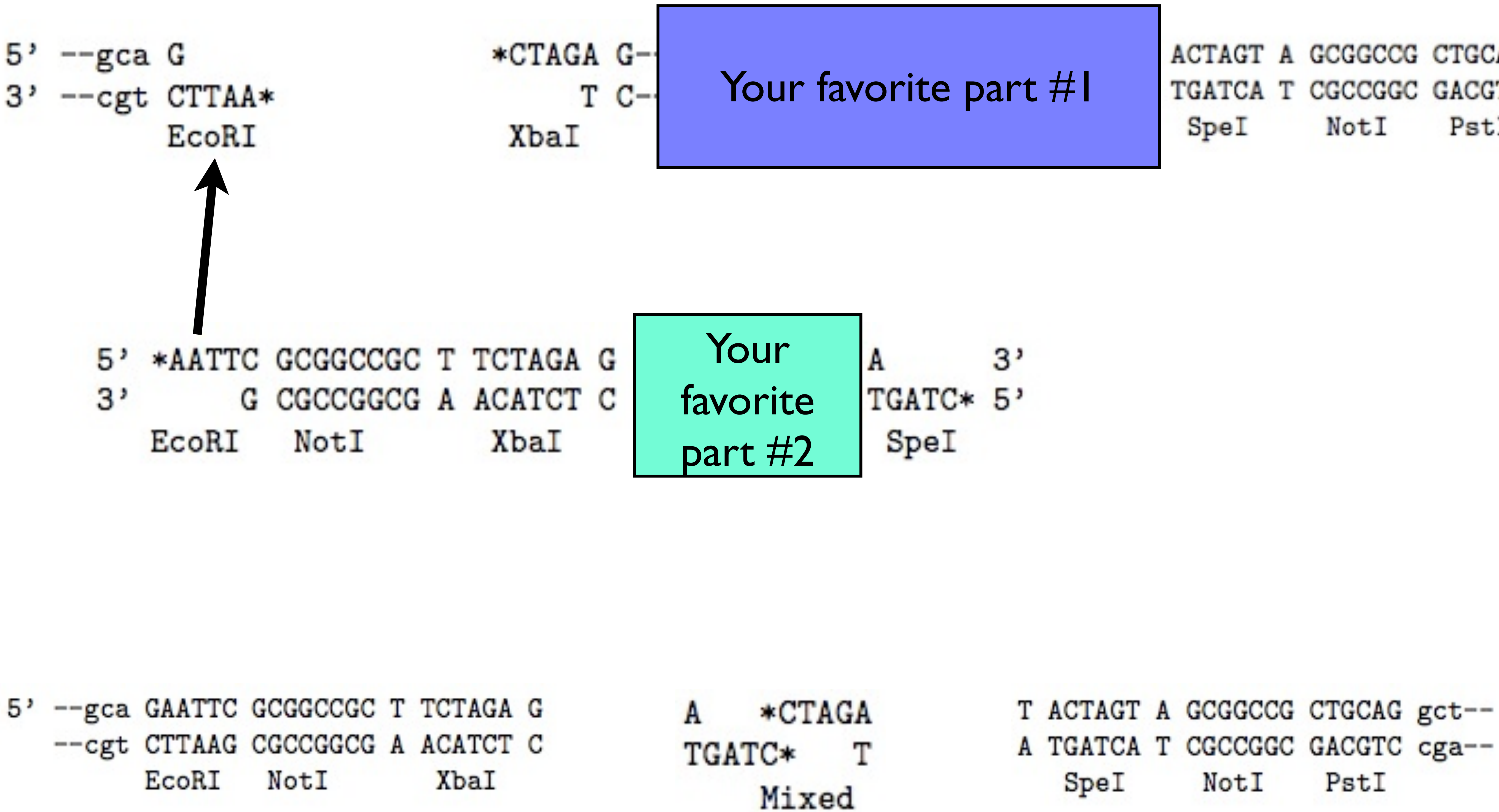
Prefixing

```
5' --gca G                *CTAGA G--   Your favorite part #1   ACTAGT A GCGGCCG CTGCA
3' --cgt CTTAA*                T C--                           TGATCA T CGCCGGC GACGT
         EcoRI              XbaI                                SpeI     NotI    PstI
```

```
5' *AATTC GCGGCCGC T TCTAGA G   Your            A       3'
3'      G CGCCGGCG A ACATCT C   favorite        TGATC*  5'
   EcoRI   NotI        XbaI     part #2          SpeI
```

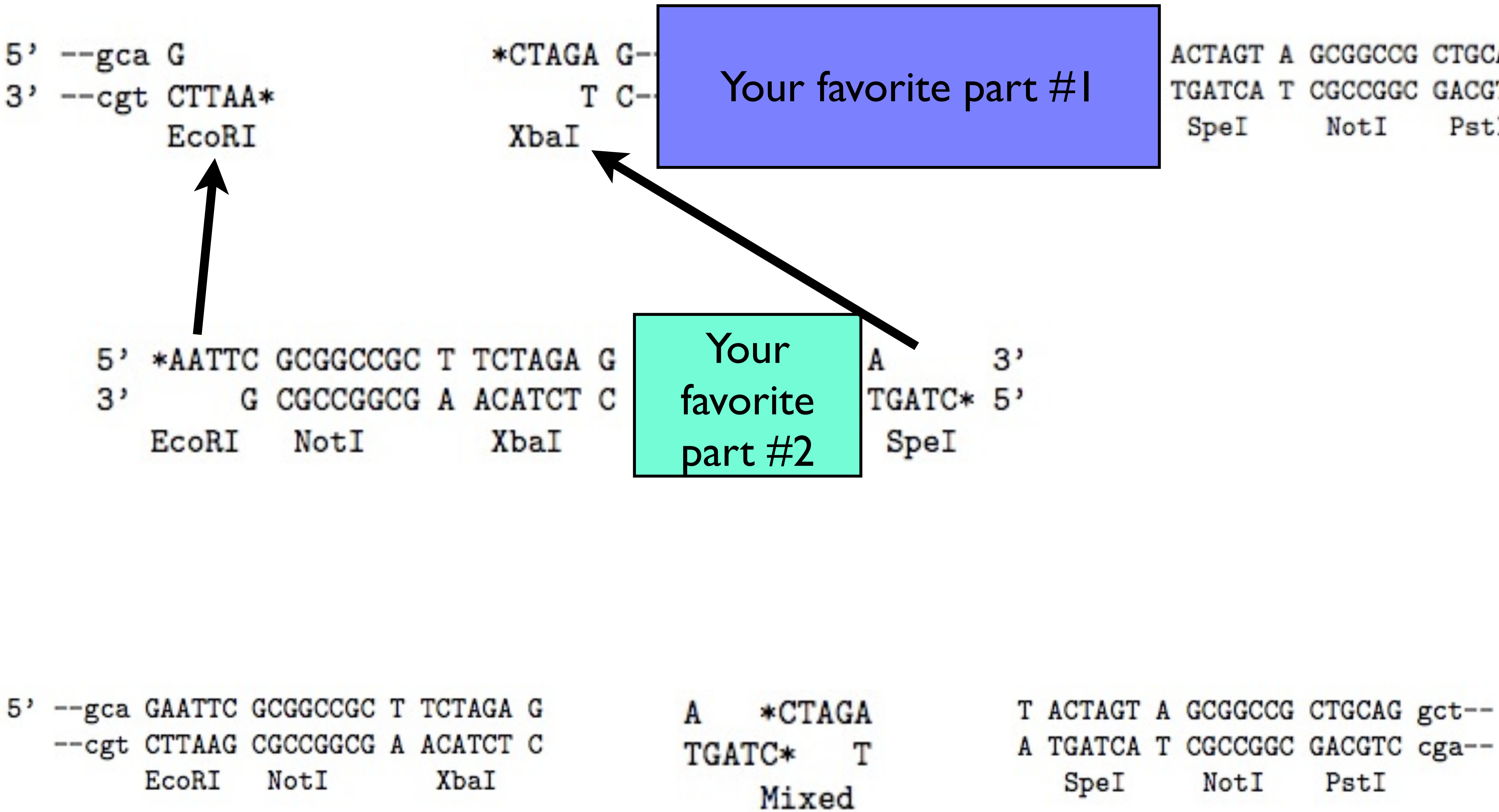
```
5' --gca GAATTC GCGGCCGC T TCTAGA G      A     *CTAGA      T ACTAGT A GCGGCCG CTGCAG gct--
   --cgt CTTAAG CGCCGGCG A ACATCT C      TGATC*     T      A TGATCA T CGCCGGC GACGTC cga--
         EcoRI   NotI        XbaI            Mixed            SpeI     NotI    PstI
```

Prefixing



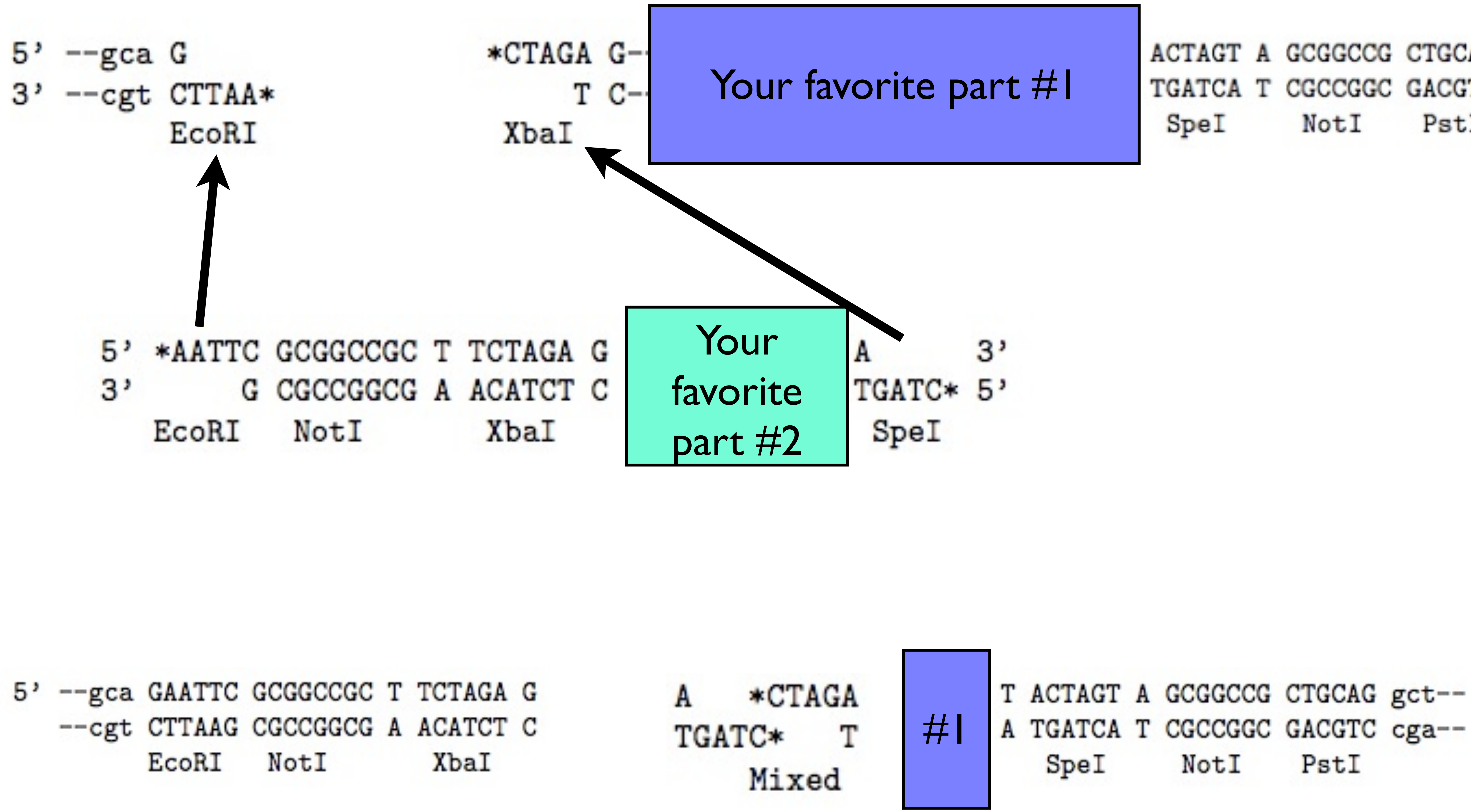
5' --gca G
3' --cgt CTTAA*
EcoRI
*CTAGA G--
T C--
XbaI
Your favorite part #1
ACTAGT A GCGGCCG CTGC
TGATCA T CGCCGGC GACG
SpeI NotI Pst
5' *AATTC GCGGCCGC T TCTAGA G
3' G CGCCGGCG A ACATCT C
EcoRI NotI XbaI
Your favorite part #2
A 3'
TGATC* 5'
SpeI
5' --gca GAATTC GCGGCCGC T TCTAGA G
--cgt CTTAAG CGCCGGCG A ACATCT C
EcoRI NotI XbaI
A *CTAGA
TGATC* T
Mixed
T ACTAGT A GCGGCCG CTGCAG gct--
A TGATCA T CGCCGGC GACGTC cga--
SpeI NotI PstI

Prefixing

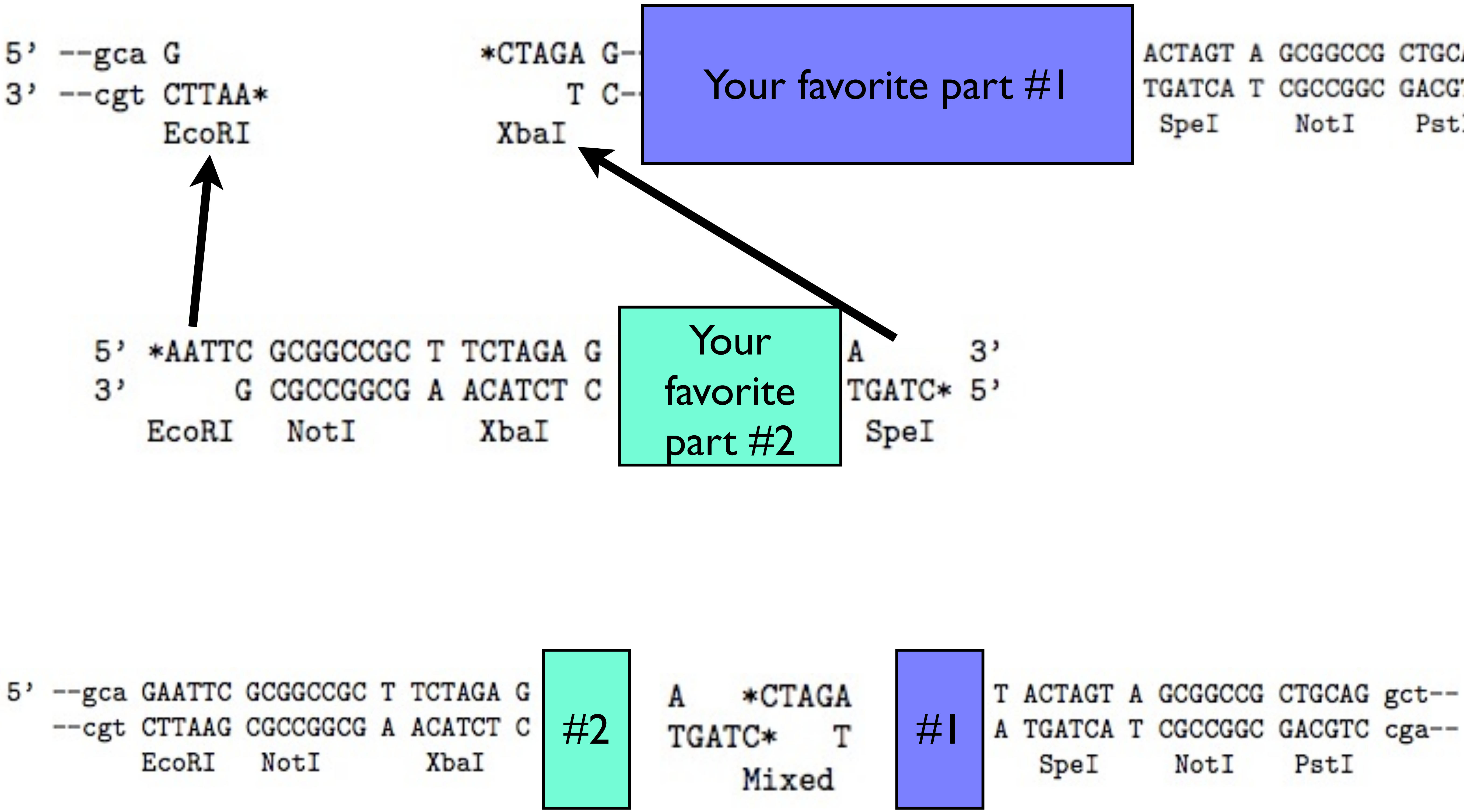


5' --gca G
3' --cgt CTTAA*
EcoRI
*CTAGA G--
T C--
XbaI
Your favorite part #1
ACTAGT A GCGGCCG CTGC
TGATCA T CGCCGGC GACG
SpeI NotI Pst
5' *AATTC GCGGCCGC T TCTAGA G
3' G CGCCGGCG A ACATCT C
EcoRI NotI XbaI
Your favorite part #2
A 3'
TGATC* 5'
SpeI
5' --gca GAATTC GCGGCCGC T TCTAGA G
--cgt CTTAAG CGCCGGCG A ACATCT C
EcoRI NotI XbaI
A *CTAGA
TGATC* T
Mixed
T ACTAGT A GCGGCCG CTGCAG gct--
A TGATCA T CGCCGGC GACGTC cga--
SpeI NotI PstI

Prefixing


5' --gca G
3' --cgt CTTAA*
EcoRI
*CTAGA G--
T C--
XbaI
Your favorite part #1
ACTAGT A GCGGCCG CTGC
TGATCA T CGCCGGC GACG
SpeI NotI Pst
5' *AATTC GCGGCCGC T TCTAGA G
3' G CGCCGGCG A ACATCT C
EcoRI NotI XbaI
Your favorite part #2
A 3'
TGATC* 5'
SpeI
5' --gca GAATTC GCGGCCGC T TCTAGA G
--cgt CTTAAG CGCCGGCG A ACATCT C
EcoRI NotI XbaI
A *CTAGA
TGATC* T
Mixed
#1
T ACTAGT A GCGGCCG CTGCAG gct--
A TGATCA T CGCCGGC GACGTC cga--
SpeI NotI PstI

Prefixing



5' --gca G
3' --cgt CTTAA*
EcoRI
*CTAGA G--
T C--
XbaI
Your favorite part #1
ACTAGT A GCGGCCG CTGC
TGATCA T CGCCGGC GACG
SpeI NotI Pst
5' *AATTC GCGGCCGC T TCTAGA G
3' G CGCCGGCG A ACATCT C
EcoRI NotI XbaI
Your favorite part #2
A 3'
TGATC* 5'
SpeI
5' --gca GAATTC GCGGCCGC T TCTAGA G
--cgt CTTAAG CGCCGGCG A ACATCT C
EcoRI NotI XbaI
#2
A *CTAGA
TGATC* T
Mixed
#1
T ACTAGT A GCGGCCG CTGCAG gct--
A TGATCA T CGCCGGC GACGTC cga--
SpeI NotI PstI

0.8 Considerations in the selection of the restriction enzymes

The choice of restriction enzymes was a significant issue in the design of the pSB103 vector and assembly plan. We wanted restriction enzymes which were easy to use and reliable, which functioned in compatible buffer systems and at compatible temperatures, which could be heat killed, provided complete digestion, with few required bases outside of their recognition site, and exhibited low star activity. In addition, we wanted four base overhangs to enhance ligation efficiency.

The sequence of the recognition site was also an issue. Avoiding the accidental creation of ATG start codons at awkward places in combined sequences was one goal. Another challenge was the avoidance of methylation sensitive sequences. Choice of enzymes which ignore DNA methylation was one approach, but other requirements forced the choice of some enzymes which were methylation sensitive. Then, avoiding the accidental creation of DNA methylation sites in common cloning strains, such as DH5α, was a goal. The EcoBI and EcoKI methylases are still active in these, and most other laboratory cloning strains, potentially methylating sites which we must be able to cut. By careful choice of flanking bases, we eliminated the possible creation of EcoBI and EcoKI methylation sites at the critical sequences we required for our assembly technique to reliably function.

(remember debates between 55 and 60 degree screwthreads?)

adapted from http://dspace.mit.edu/handle/1721.1/21168

3. Features:

1. *Works*
2. *Idempotent*
3. *Used widely*
4. *Geometric assemly*

4. Bugs

1. *Doesn't support re-work*

2. *Protein fusions???? (no)*

3. *Physical composition only (will it function?)*

4. *Geometric < Parallel*

-WILL ALLEN DROMGOOLE

An old man, going a lone highway,
Came at the evening cold and gray,
To a chasm, vast and deep and wide,
Through which was flowing a sullen tide.
The old man crossed in the twilight dim-
That sullen stream had no fears for him;
But he turned, when he reached the other side,
And built a bridge to span the tide.

-WILL ALLEN DROMGOOLE

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Came at the evening cold and gray,
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Through which was flowing a sullen tide.
The old man crossed in the twilight dim-
That sullen stream had no fears for him;
But he turned, when he reached the other side,
And built a bridge to span the tide.

"Old man," said a fellow pilgrim near,
"You are wasting strength in building here.
Your journey will end with the ending day;
You never again must pass this way.
You have crossed the chasm, deep and wide,
Why build you the bridge at the eventide?"

-WILL ALLEN DROMGOOLE

An old man, going a lone highway,
Came at the evening cold and gray,
To a chasm, vast and deep and wide,
Through which was flowing a sullen tide.
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But he turned, when he reached the other side,
And built a bridge to span the tide.

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"You are wasting strength in building here.
Your journey will end with the ending day;
You never again must pass this way.
You have crossed the chasm, deep and wide,
Why build you the bridge at the eventide?"

The builder lifted his old gray head.
"Good friend, in the path I have come," he said,
"There followeth after me today
A youth whose feet must pass this way.
This chasm that has been naught to me
To that fair-haired youth may a pitfall be.
He, too, must cross in the twilight dim;
Good friend, I am building the bridge for him."

-WILL ALLEN DROMGOOLE

An old man, going a lone highway,
Came at the evening cold and gray,
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Good friend, I am building the bridge for him."

-WILL ALLEN DROMGOOLE

Good engineers solve problems.

Great engineers also solve tomorrow's problems.