Introduction to Synthetic Biology

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

Standard for Physical DNA Composition

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A Programming Language

ACAGGGTGACCTAAGACCTTATTGTCTAATTGAGAAGGCTTCGGAATTTGACCTAAG
ACCTTATTGTCTAATTGAGAAGGGCCGGTTGAACAGGGCTTCGGAATTTGACCTAAG
ACCTTATTGTCTAATTGAGATGACCTAAGACCTTATTGTCTAATTGAGAAGGAGGGCC
GGTTGAACAGGTGACCTAAGACCTTATTGTCTAATTGAGAAGGGCTTCGGAATTTGAC
CTAAGACCTTATTGTCTAATTGAGAAGGGCCGGTTGAACAGGGCTTCGGAATTTGACC
TAAGACCTTATTGTCTTGACCTAAGACCTTATTGTCTAATTGAGAAGGAATTGAGAAGG
GCCGGTTGAACAGGTGACCTAAGACCTTATTGTCTAATTGAGAAGGGCTTCGGAATTT
GACCTAAGACCTTATTGTCTAATTGAGAAGGGCCGGTTGAACAGGGCTTCGGAATTTG
ACTGACCTAAGACCTTATTGTCTAATTGAGAAGGCTAAGACCTTATTGTCTAATTGAGA
AGGGCCGGTTGAACAGGGCTTCGGAATTTGACCTAAGACCTTATTGTCTAATTGAGAAG
GGCCGGTTGAACAGGTGACCTAAGACCTTATTGTCTAATTGAGAAGGGCTTCGGAATTT
GACCTAAGACCTTATTGTCTAATTGAGAAGGGCCGGTTGAACAGGGTGACCTAAGAC
ACCTTATTGTCTAATTGAGAAGGGCCGGTTGAACAGGGCTTCGGAATTTGACCTAAG
ACCTTATTGTCTAATTGAGATGACCTAAGACCTTATTGTCTAATTGAGAAGGAGGGCC
GGTTGAACAGGTGACCTAAGACCTTATTGTCTAATTGAGAAGGGCTTCGGAATTTGAC
CTAAGACCTTATTGTCTAATTGAGAAGGGCCGGTTGAACAGGGCTTCGGAATTTGACC
TAAGACCTTATTGTCTTGACCTAAGACCTTATTGTCTAATTGAGAAGGAATTGAGAAGG
GCCGGTTGAACAGGTGACCTAAGACCTTATTGTCTAATTGAGAAGGGCTTCGGAATTT
GACCTAAGACCTTATTGTCTAATTGAGAAGGGCCGGTTG

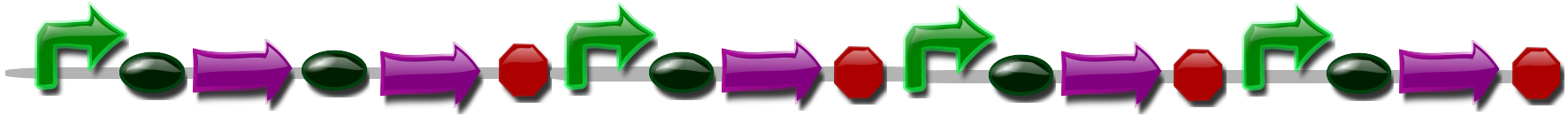
Sizes

	E.Coli	Yeast (S. cerevisae)	Mammalian (Human Fibroblast)
Genome Size	4.6 M bp 4500 genes	130 M bp 6600 genes	3000 M bp ~25000 genes
Reg. Binding Site	~10bp	~10bp	~10bp
Promoter	~100 bp	~1000 bp	~10000 to 100000 bp
Gene	~1000 bp	~1000 bp	~10000 to 1000000 bp

from “An Introduction to Systems Biology”, Uri Alon, 2004

Imaginary Project

Bird Flu Drug Production in E.Coli



5 Genes	4 Promoters	4 RBS	4 Terminators

1 Plasmid ~6000 bp

DNA Toolbox
(cut, copy, paste, read & write)

DNA Synthesis

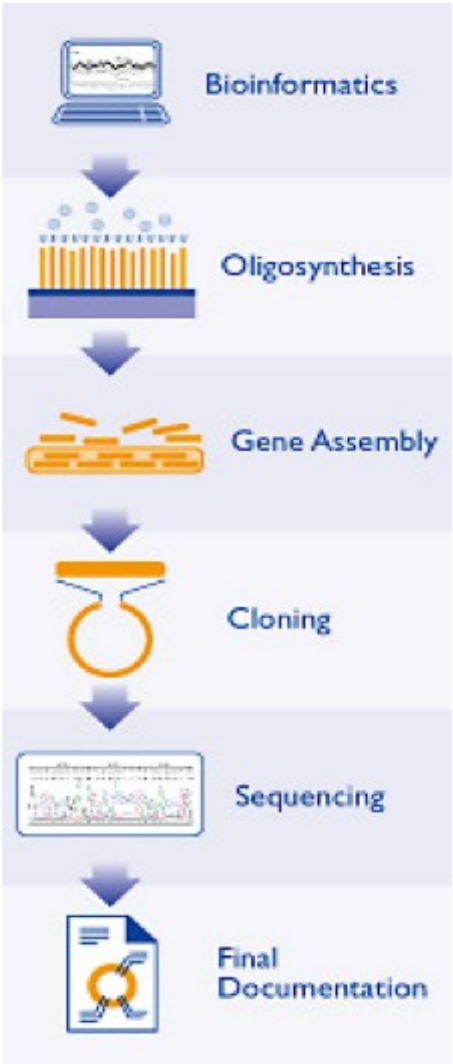
Restriction Enzymes

PCR

DNA Sequencing

DNA Ligase

DNA Synthesis

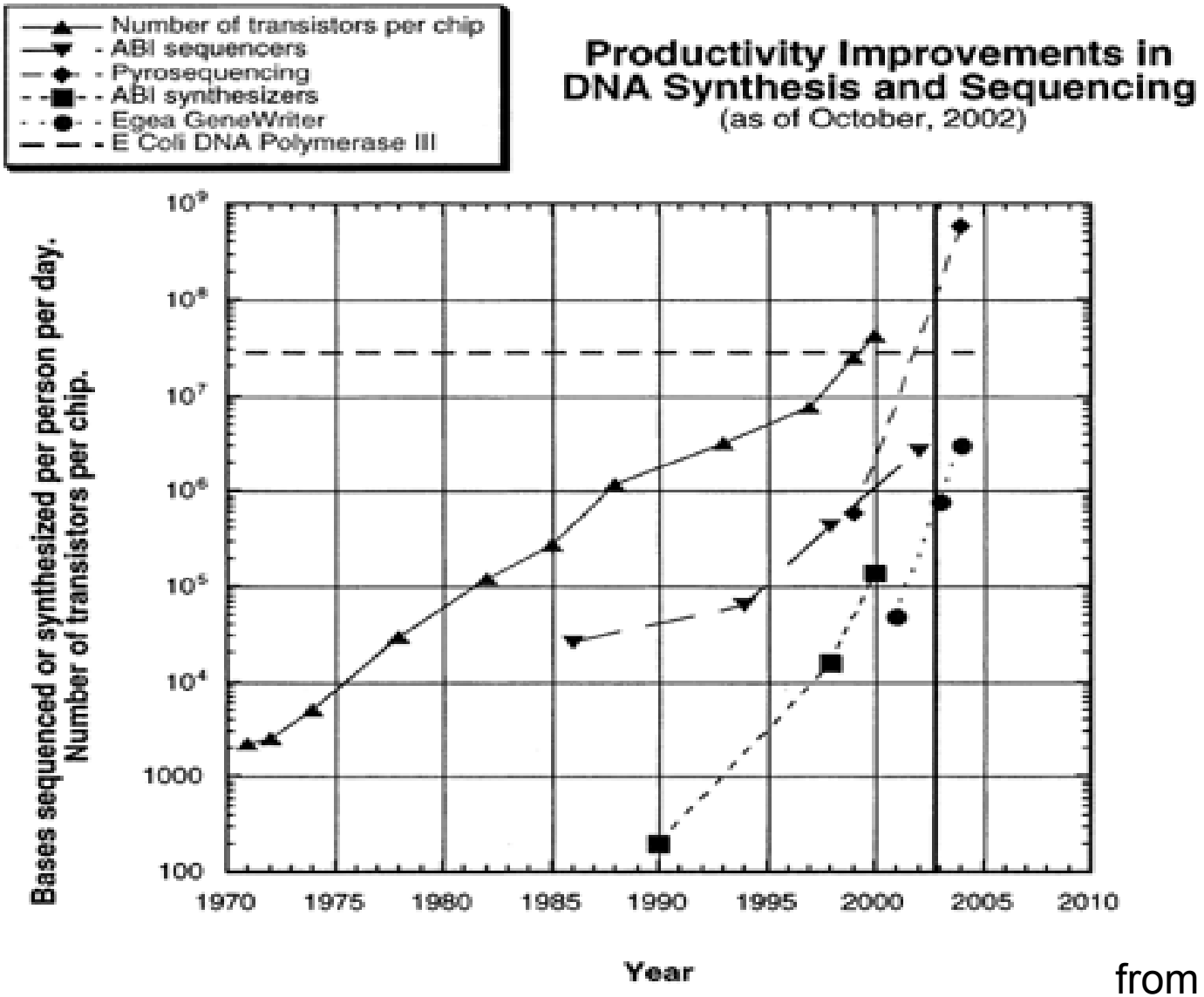


current price : from 0.80 euro/bp

production time: from 2 to 4 weeks

GENEART
THE GENE OF YOUR CHOICE

DNA Synthesis is the solution ...



from Carlson, 2003

DNA Synthesis

RESEARCH ARTICLES

Submitted on October 15, 2007
Accepted on January 11, 2008

Complete Chemical Synthesis, Assembly, and Cloning of a *Mycoplasma genitalium* Genome

Daniel G. Gibson [1], Gwynedd A. Benders [1], Cynthia Andrews-Pfannkoch [1], Evgeniya A. Denisova [1], Holly Baden-Tillson [1], Jayshree Zaveri [1], Timothy B. Stockwell [1], Anushka Brownley [1], David W. Thomas [1], Mikkel A. Algire [1], Chuck Merryman [1], Lei Young [1], Vladimir N. Noskov [1], John I. Glass [1], J. Craig Venter [1], Clyde A. Hutchison III[1], Hamilton O. Smith [1*]

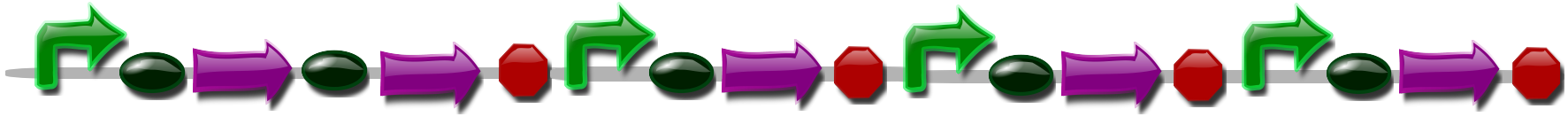
[1] The J. Craig Venter Institute, Rockville, MD 20850, USA.

[*] To whom correspondence should be addressed.
Hamilton O. Smith , E-mail: hsmith@jcvi.org

We have synthesized a 582,970 bp *Mycoplasma genitalium* genome. This synthetic genome, named *M. genitalium* JCVI-1.0, contains all the genes of wild-type *M. genitalium* G37 except MG408, which was disrupted by an antibiotic marker to block pathogenicity and to allow for selection. To identify the genome as synthetic, we inserted "watermarks" at intergenic sites known to tolerate transposon insertions.

Back to our Project

Bird Flu Drug Production in E.Coli



5 Genes | 4 Promoters | 4 RBS | 4 Terminators

1 Plasmid ~6000 bp

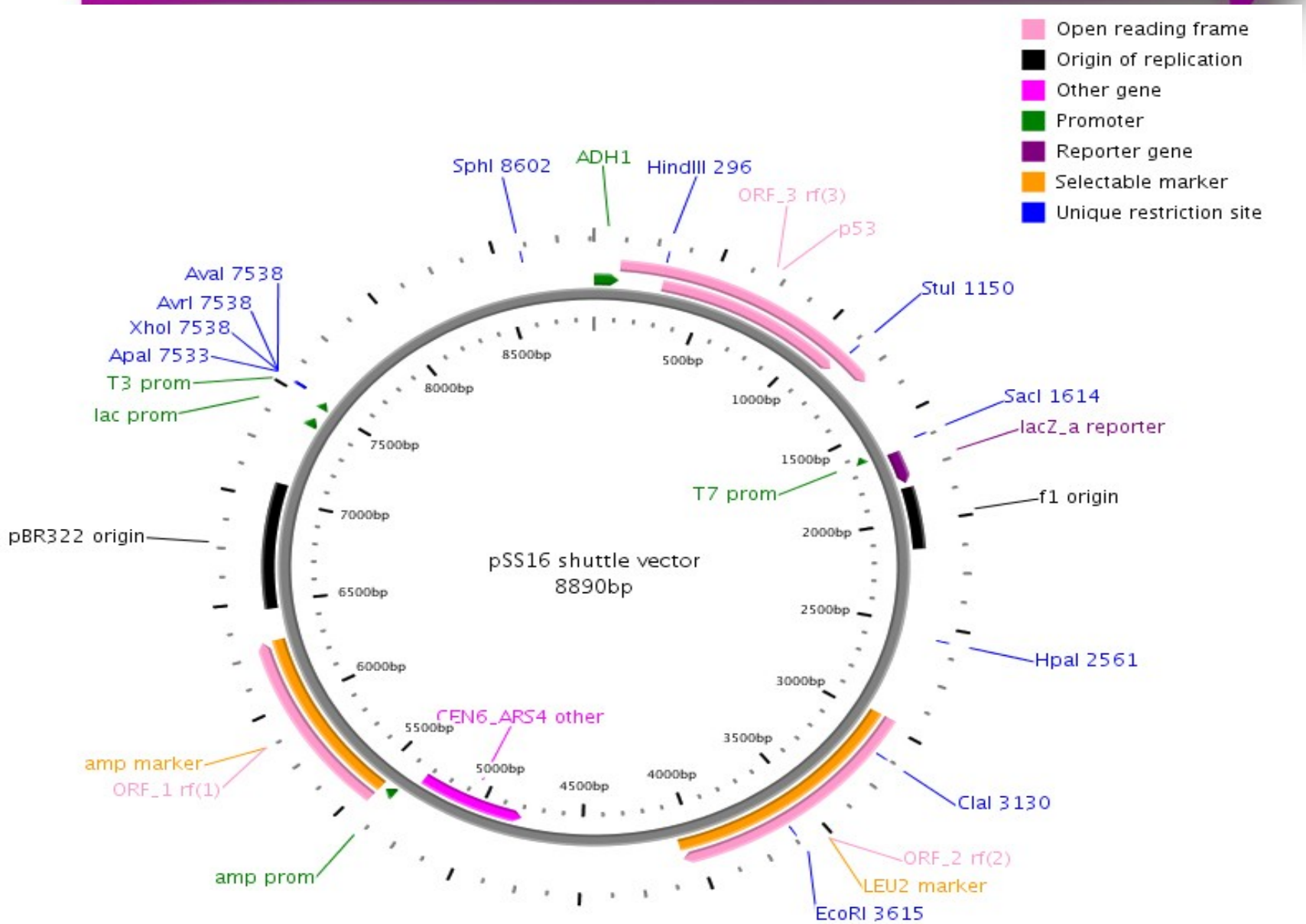
Maybe, we want to tune the level of expression for each gene

Maybe, we have different mutants for each gene

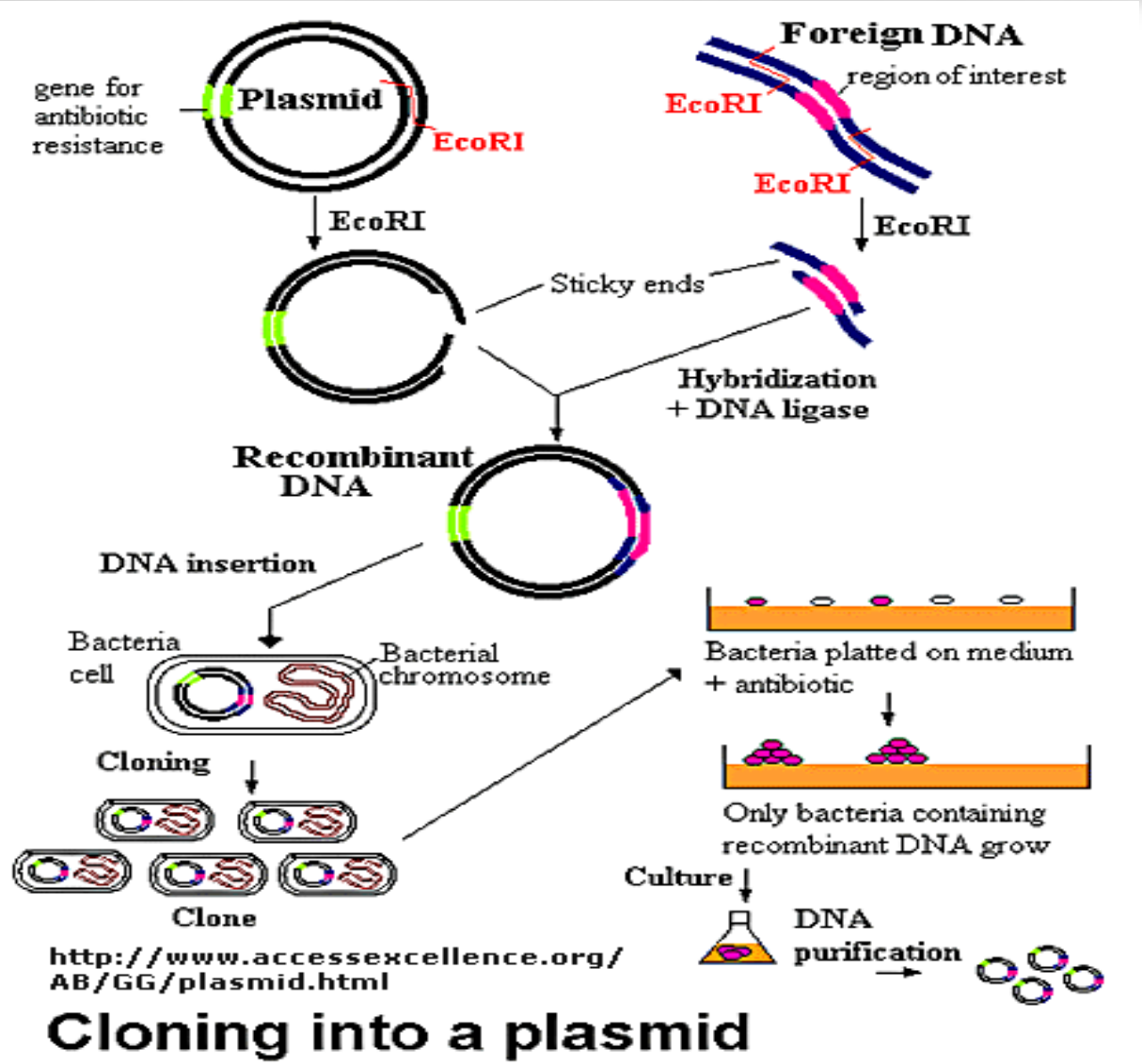
How many plasmids to synthesise ? £££££

Traditional cloning techniques

Traditional cloning techniques



Traditional cloning techniques



Cloning into a plasmid

Lack of Standardisation

"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."

"One of our goals is to replace this **ad hoc experimental design** with a set of **standard and reliable engineering mechanisms** to remove much of the tedium and surprise during assembly of genetic components into larger systems "

Tom Knight, MIT.

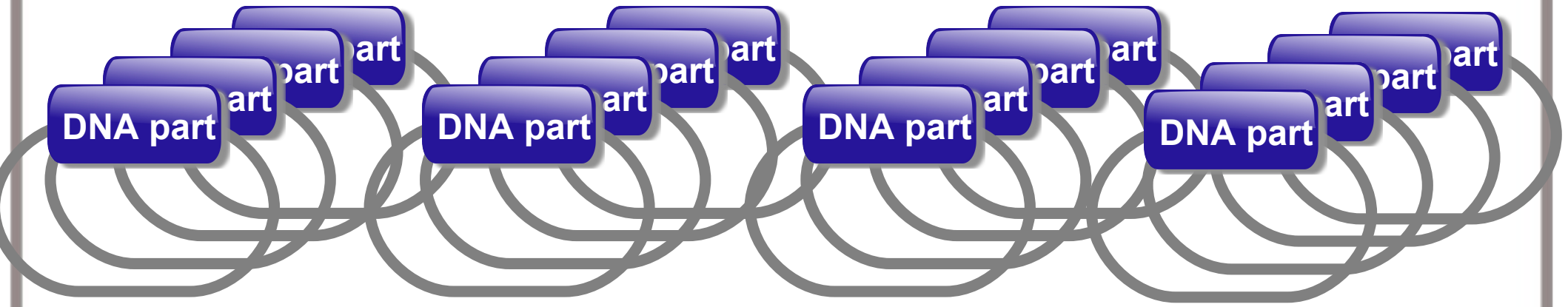
from "**Idempotent Vector Design for Standard Assembly of Biobricks** ", Tom Knight (MIT) 2003.

Wish List for DNA composition

Library of DNA Parts that can easily be plugged together

Efficient and Reliable process

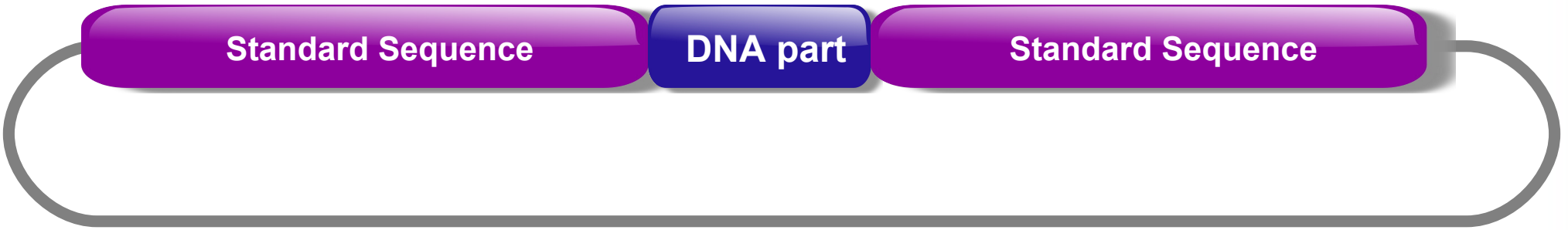
Support an Abstraction Hierarchy



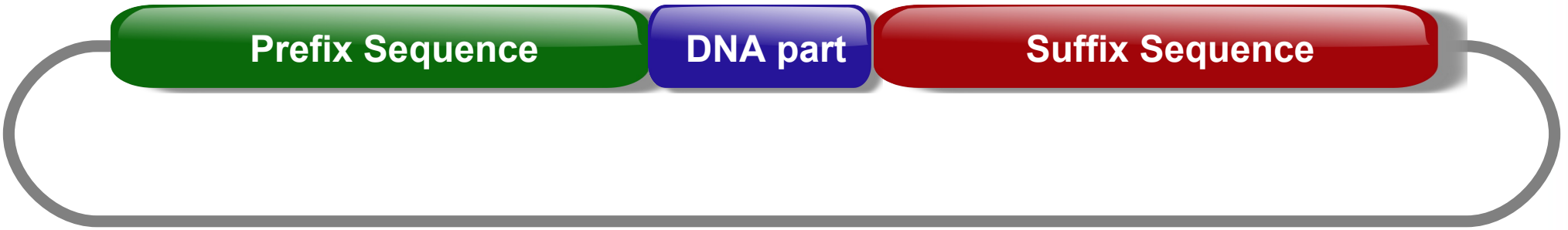
Towards a BioBrick Standard

DNA part

Towards a BioBrick Standard



Towards a BioBrick Standard



Towards a BioBrick Standard

BioBrick Standard (Tom Knight, MIT)

from "**Idempotent Vector Design for Standard Assembly of Biobricks** ", Tom Knight (MIT) 2003.

BioBrick: 4 Operations

Create Front-Insert

EcoRI

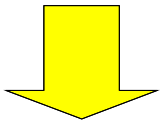
SpeI

GAATTC GCGGCCGC A TCTAGA G
CTTAAG CGCCGGCG T ACATCT C

DNA part

T ACTAGT A GCGGCCGC CTGCAG
A TGATCA T CGCCGGCG GACGTC

EcoRI NotI XbaI SpeI NotI PstI



*AATTC GCGGCCGC A TCTAGA G
G CGCCGGCG T ACATCT C

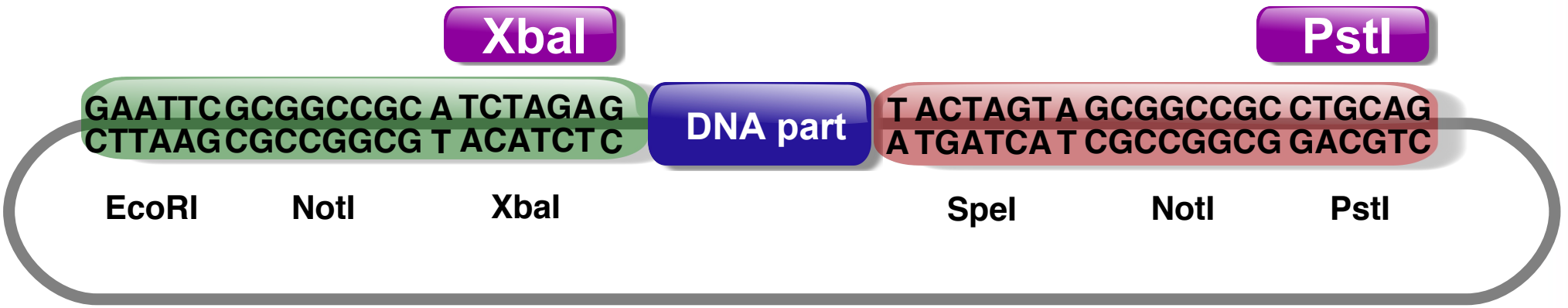
DNA part

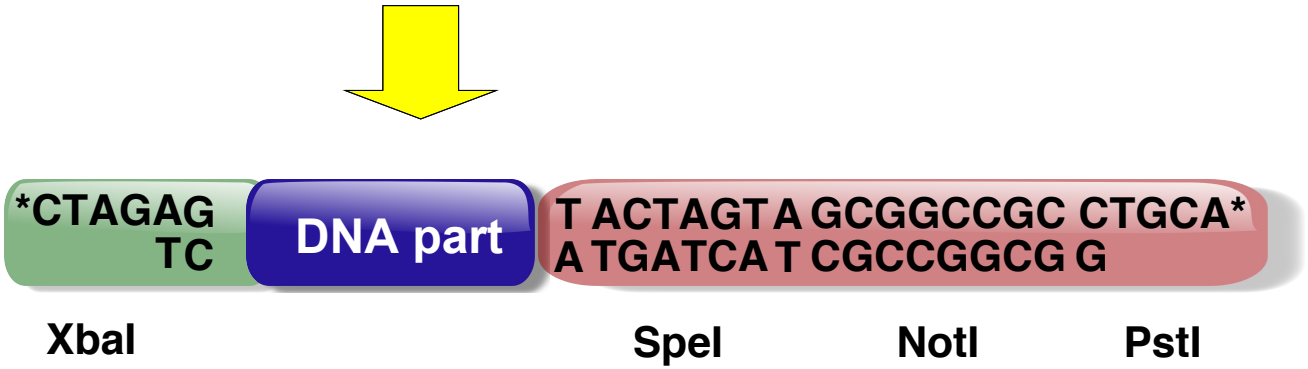
T A
A TGATC*

EcoRI NotI XbaI SpeI

BioBrick: 4 Operations

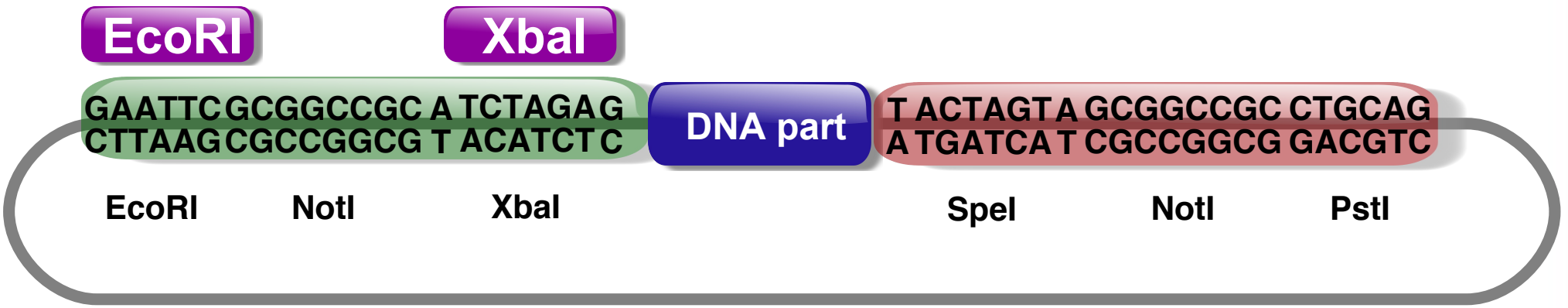
Create Back-Insert

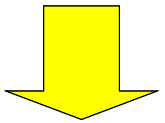




BioBrick: 4 Operations

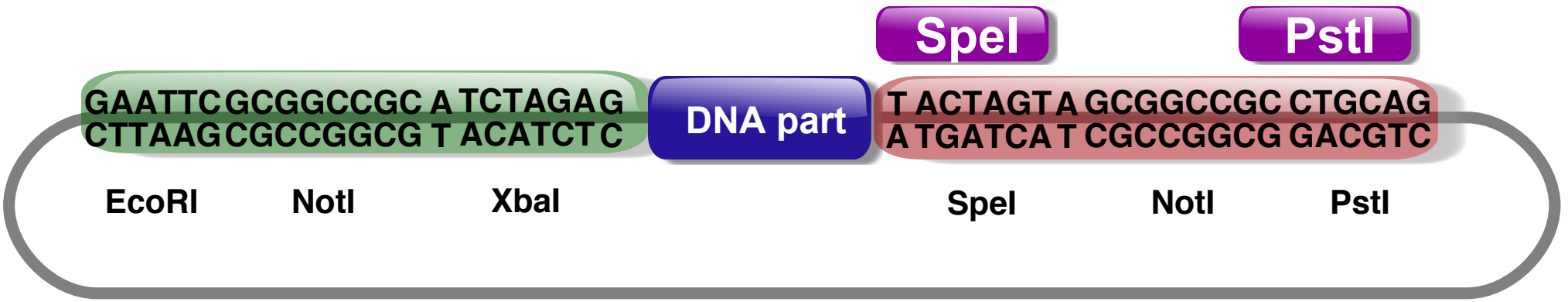
Create Front-Vector

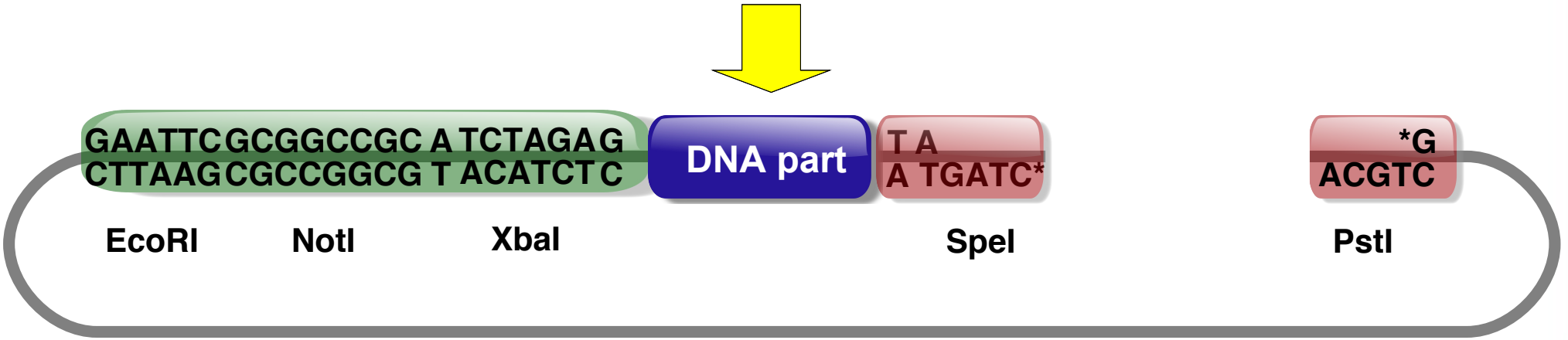




BioBrick: 4 Operations

Create Back-Vector





BioBricks: Composition Techniques

Prefixing Composition = Front Vector + Front Insert

GAATTC GCGGCCGC A TCTAGA G
CTTAAG CGCCGGCG T ACATCT C

DNA part 1

T ACTAGT A GCGGCCGC CTGCAG
A TGATCA T CGCCGGCG GACGTC

EcoRI NotI XbaI SpeI NotI PstI

GAATTC GCGGCCGC A TCTAGA G
CTTAAG CGCCGGCG T ACATCT C

DNA part 2

T ACTAGT A GCGGCCGC CTGCAG
A TGATCA T CGCCGGCG GACGTC

EcoRI NotI XbaI SpeI NotI PstI

BioBricks: Composition Techniques

Prefixing Composition = Front Vector + Front Insert

EcoRI XbaI

GAATTC GCGGCCGC A TCTAGA G
CTTAAG CGCCGGCG T ACATCT C

DNA part 1

T ACTAGT A GCGGCCGC CTGCAG
A TGATCA T CGCCGGCG GACGTC

EcoRI NotI XbaI SpeI NotI PstI

EcoRI SpeI

GAATTC GCGGCCGC A TCTAGA G
CTTAAG CGCCGGCG T ACATCT C

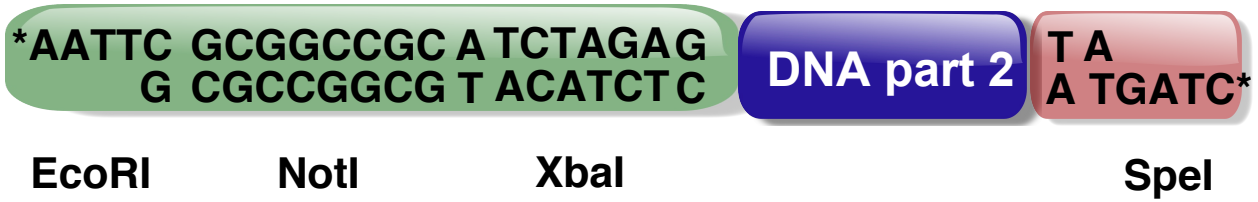
DNA part 2

T ACTAGT A GCGGCCGC CTGCAG
A TGATCA T CGCCGGCG GACGTC

EcoRI NotI XbaI SpeI NotI PstI

BioBricks: Composition Techniques

Prefixing Composition = Front Vector + Front Insert



BioBricks: Composition Techniques

Prefixing Composition = Front Vector + Front Insert

BioBricks: Composition Techniques

Postfixing Composition = Back Vector + Back Insert

GAATTC GCGGCCGC A TCTAGA G
CTTAAG CGCCGGCG T ACATCT C

DNA part 1

T ACTAGT A GCGGCCGC CTGCAG
A TGATCA T CGCCGGCG GACGTC

EcoRI NotI XbaI SpeI NotI PstI

GAATTC GCGGCCGC A TCTAGA G
CTTAAG CGCCGGCG T ACATCT C

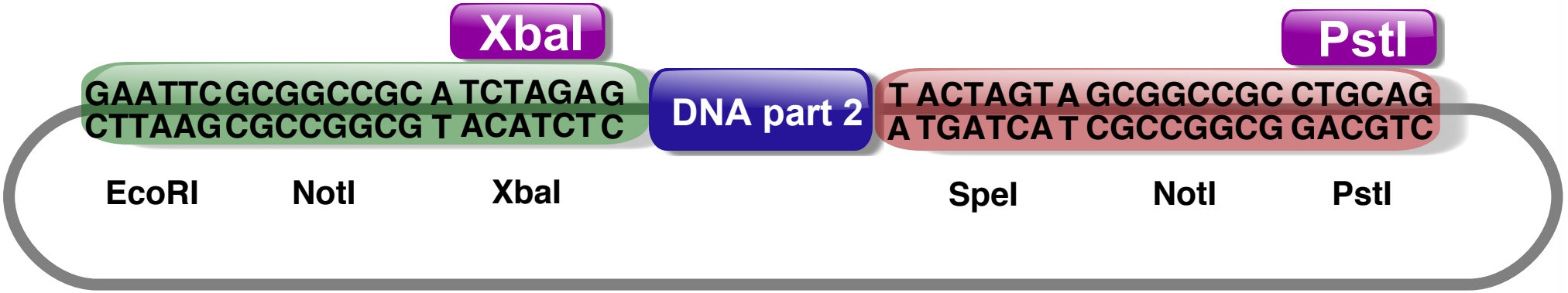
DNA part 2

T ACTAGT A GCGGCCGC CTGCAG
A TGATCA T CGCCGGCG GACGTC

EcoRI NotI XbaI SpeI NotI PstI

BioBricks: Composition Techniques

Postfixing Composition = Back Vector + Back Insert

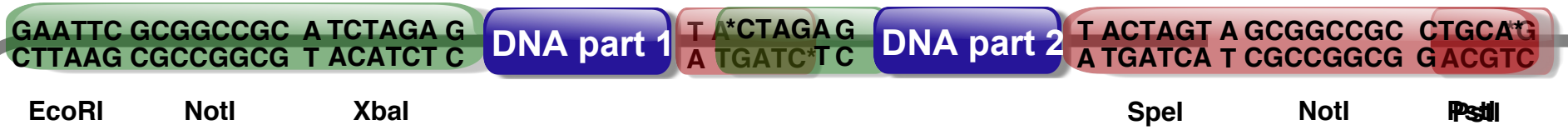


BioBricks: Composition Techniques

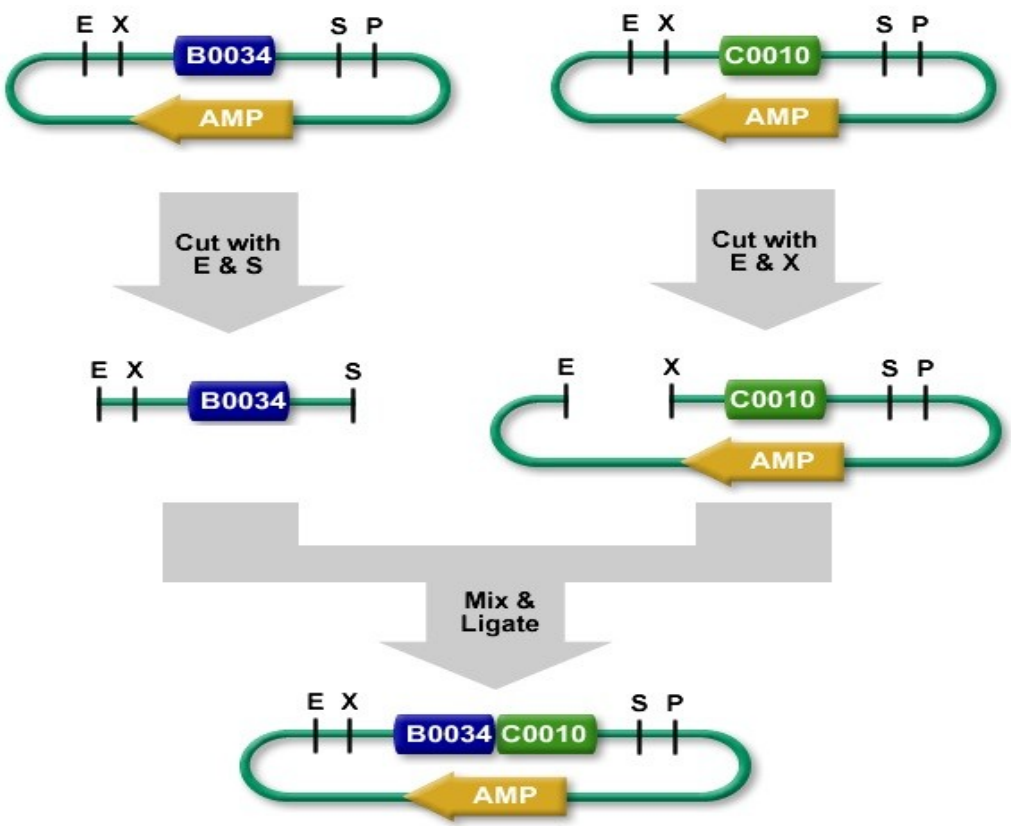
Postfixing Composition = Back Vector + Back Insert

BioBricks: Composition Techniques

Postfixing Composition = Back Vector + Back Insert

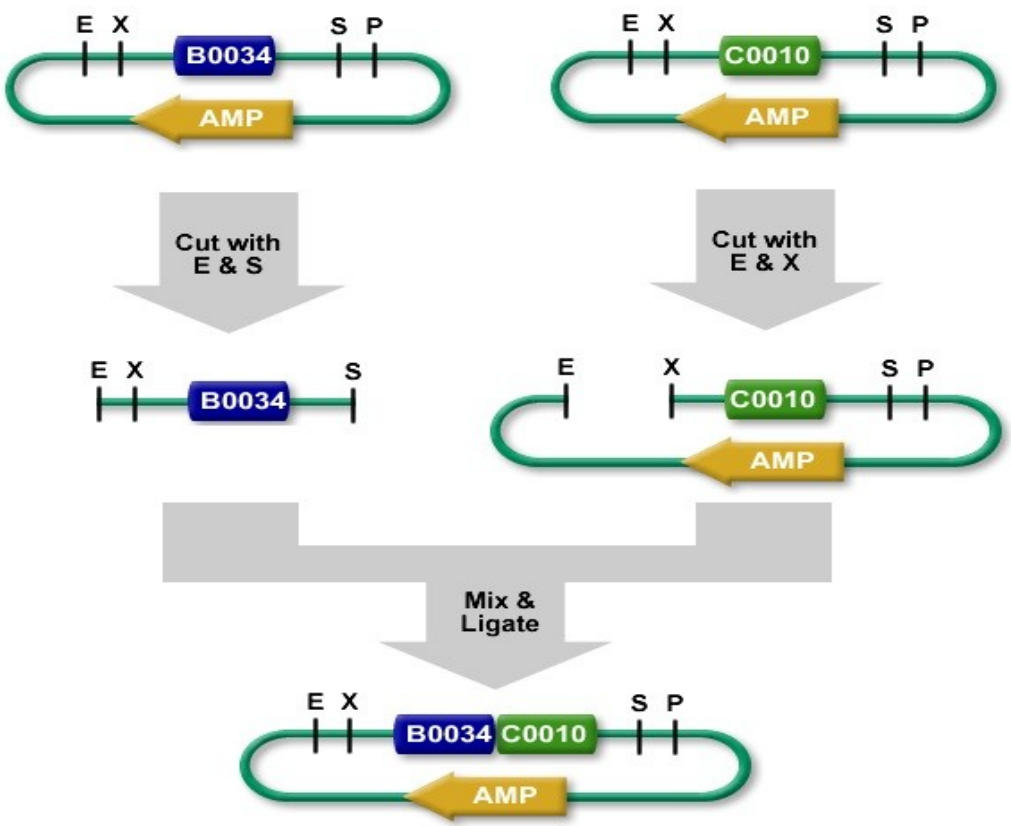


Idempotence and Abstraction Hierarchy

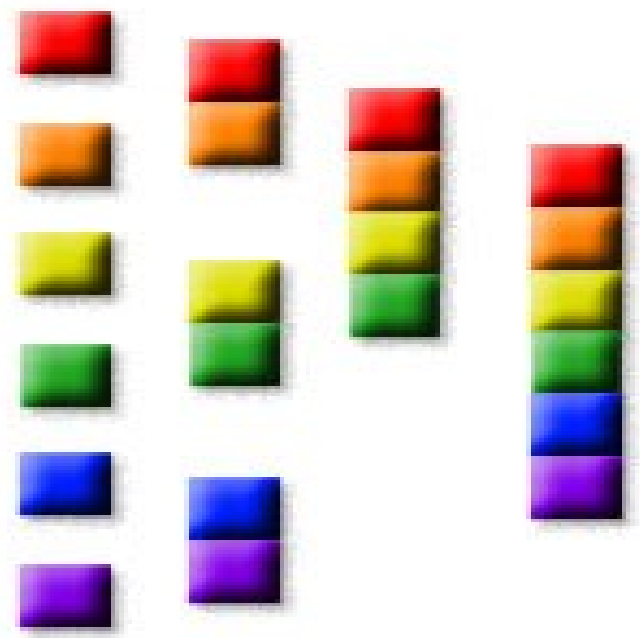


Standard Assembly

Idempotence and Abstraction Hierarchy



Standard Assembly



Rolling Assembly

BioBricks Standard Summary

Upstream Sequence

```
GAATTCGCGGCCGCATCTAGAG
CTTAAGCGCCGGCGTAGATCTC
```

EcoRI NotI XbaI

Downstream Sequence

```
T ACTAGTAGCGGCCGC CTGCAG
A TGATCATCGCCGGCG GACGTC
```

SpeI NotI PstI

Restriction sites clean-up

EcoRI XbaI SpeI PstI

Verification primers

Verification forward
5’ ttg tct cat gag cgg ata ca 3’

Verification reverse
5’ att acc gcc ttt gag tga gc

Behind the Standard: Technical considerations

Enzyme choice:

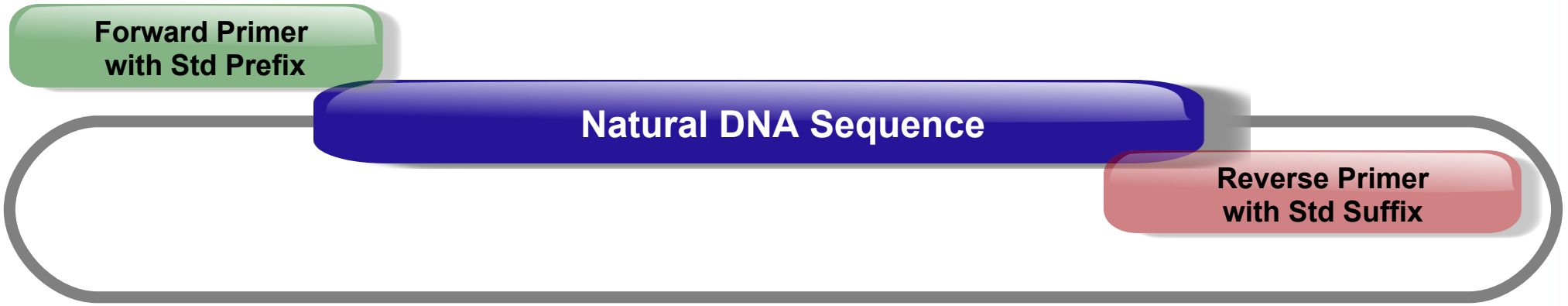
- Easy to use/reliable,
- Compatible buffers/temperature,
- Heat killed,
- Few required bases outside of their recognition sites,
- Low star activity,
- Four base overhangs to enhance ligation efficiency
- Avoiding accidental creation of ATG sequence,
- Avoid methylation sensitive sites

at the end ... as always ... concessions

How to make a BioBrick: using PCR

Natural DNA Sequence

How to make a BioBrick: using PCR



+ Restriction sites clean-up

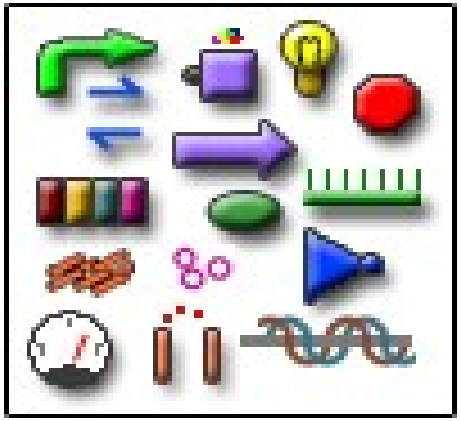
How to make a BioBrick: Direct Synthesis

DNA Sequence

How to make a BioBrick: Direct Synthesis

+ Restriction sites clean-up

Registry of standard biological parts



http://parts.mit.edu

“Competing / Alternative” standards

BioBrick++, Austin Che (MIT)

Pam Silver's Lab Biobrick Version

JC Anderson's Lab Biobrick Version

References

> Drew Endy Talks
> Austin Che Presentations
> OpenWetWare Folks
> iGEM Competition
> BioBricks Foundation

to be added

3 Antibiotics Assembly

Automated assembly

to be added

Study of the scare (frame shift)

detail of the protein fusion (Silver's version)

Specific BB version for Coding Sequence parts