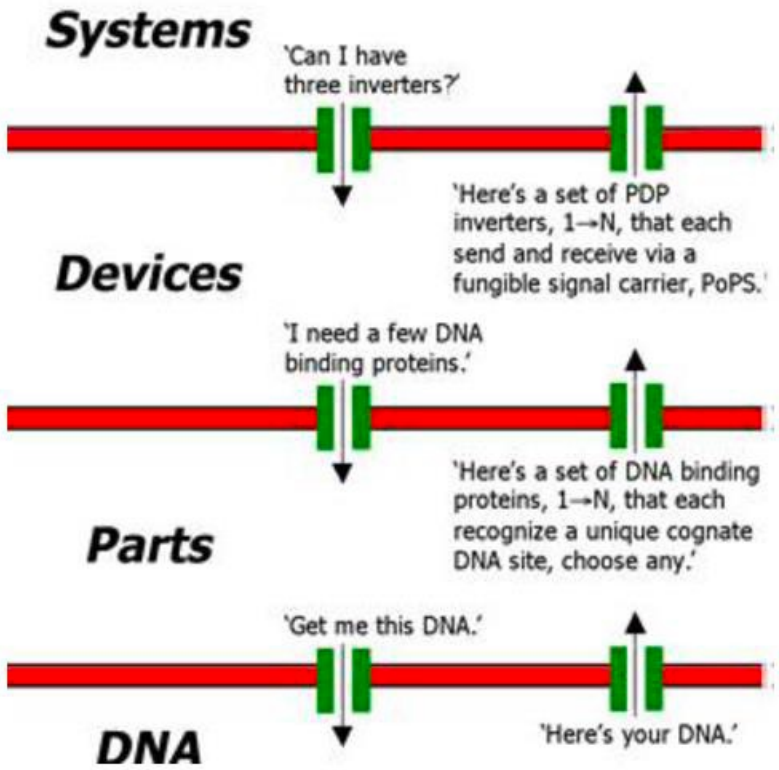
Abstraction hierarchy

Engineers in all disciplines take advantage of abstraction hierarchies to design and build complicated systems. For instance, software engineers write in high level programming languages like C++ or Java which are designed to be easy for humans to read and write. These programs are then translated into lower level sets of instructions that are more easily translatable to bit strings that are machine interpretable and implementable. Thus, the people who write C++ programs do not need to know how to translate their programs to machine code and the people who work on instruction sets do not need to envision all possible programs that the software engineer might write.

http://syntheticbiology.org/Abstraction_hierarchy.html



Layer	Definition
System	multiple devices hooked together to realize a goal
Device	multiple parts with a higher level function
Part	a finite sequence of nucleotides with a specific function
DNA	sequence of nucleotides

http://parts.igem.org/Abstraction_Hierarchy

Assemblage de « parts »: Wet Lab vs CA0

- « Wet Lab » = paillasse, laboratoire
- CAO = conception assistée par ordinateur

TP: approche comparative

Wet lab: Techniques de clonage

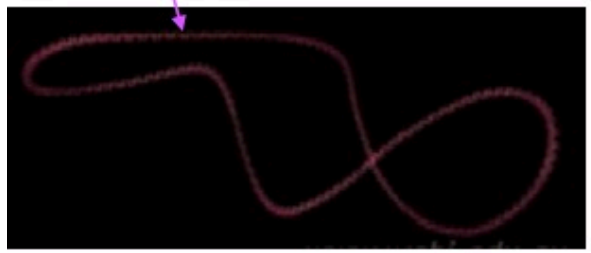
1

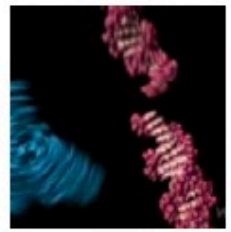
- Traditional Cloning Standard Biobrick™
- PCR Cloning Method
- Recombinational Cloning
- Ligation Independent Cloning (LIC)

Utilise les plasmides comme vecteurs d'amplification de l'ADN à l'intérieur de bactéries

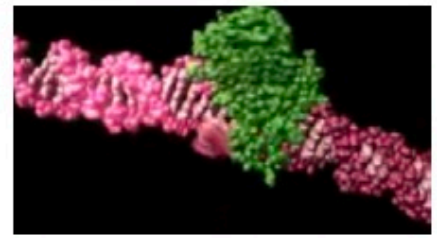
plasmide

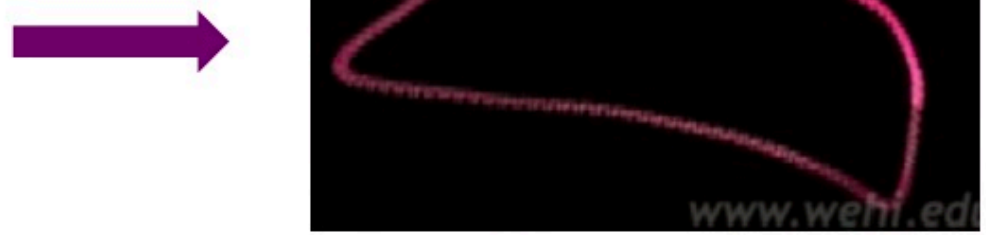
Traditional Cloning





Bleu: enzyme de restriction
Vert: ligase

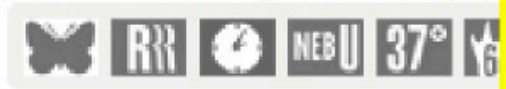




Standard Biobrick™

Basé sur la compatibilité SpeI/XbaI pour la ligation

EcoRI has High Fidelity (



5′...G▼AATTC...3′
3′...CTTAA▲G...5′

SpeI has High Fidelity (HF)

5′...A▼CTAGT...3′
3′...TGATC▲A...5′

XbaI has RE-Mix Master

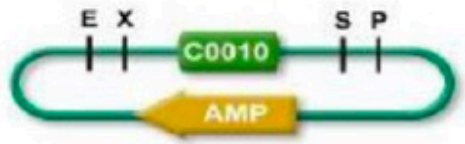
5′...T▼CTAGA...3′
3′...AGATC▲T...5′

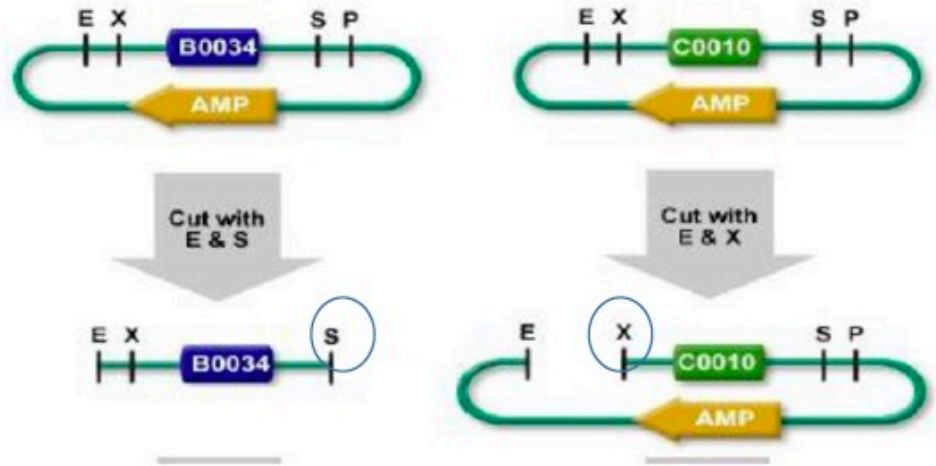
PstI has a High Fidelity (HF

5′...CTGCA▼G...3′
3′...G▲ACGTC...5′

Objectif:

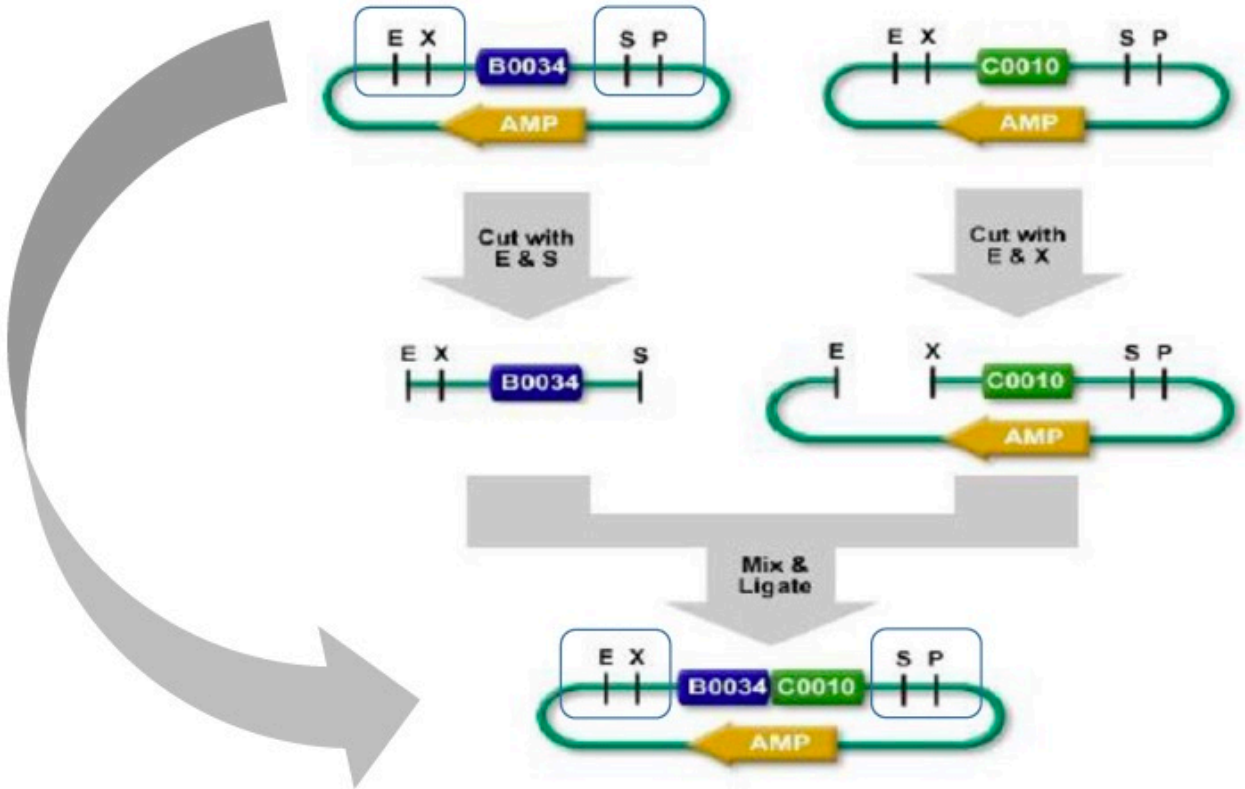

E X
B0034
S P
AMP


E X
C0010
S P
AMP



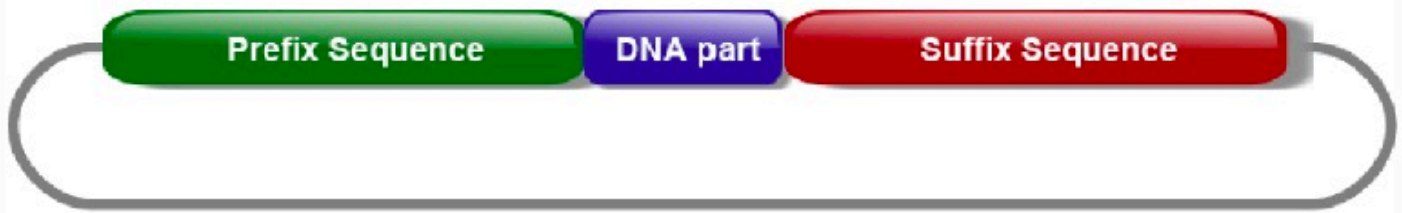
compatibilité SpeI/XbaI

Standard Biobrick™



Standard Assembly

Towards a BioBrick Standard



Standard part fabrication

Use this approach for promoters, ribosome binding sites, terminators and most other BioBricks parts.

Prefix

```
5' GTTTCTT C GAATTC GCGGCCGC  T  TCTAGA  G   [part] 3'
3' CAAAGAA G CTTAAG CGCCGGCG  A  AGATCT  C   [part] 5'
   (1)    (2)(3)    (4)      (5) (6)    (7)  (8)
```

1. Extra bases designed to both
    1. permit cutting of the PCR product with EcoRI by providing extra "spacer" bases. See notes on cutting near the ends of linear DNA fragments.
    2. promote addition of an A base on the opposite strand by Taq polymerase for high efficiency TA cloning if desired. See notes on TOPO TA cloning.
2. Random extra spacer base
3. EcoRI recognition site
4. NotI recognition site
5. Extra base to prevent inadvertent creation of EcoBI or EcoKI methylation sites which could inhibit efficient digestion by the BioBricks enzymes.
6. XbaI recognition site
7. Extra G base to prevent inadvertent creation of either
    1. a GATC site (which can undergo methylation in some strains thereby inhibiting digestion by the BioBricks enzymes.)
    2. an ATG start codon
8. Approximately 20 bp of sequence that matches the 5' end of the part you wish to construct.

The majority of *E.coli* strains used for propagation of plasmid DNA contain two site-specific DNA methyltransferases – **Dam** and **Dcm.**

The methylase encoded by the *dam* gene methylates the N6-position of an adenine residue within the **GATC** sequence.

The methylase encoded by the *dcm* gene methylates the C5-position of the internal cytosine residue within the **CCWGG** sequence.
To cleave with a restriction enzyme which is sensitive to the Dam or Dcm methylation, DNA should be purified from *dam-*, *dcm- E.coli* strains respectively.

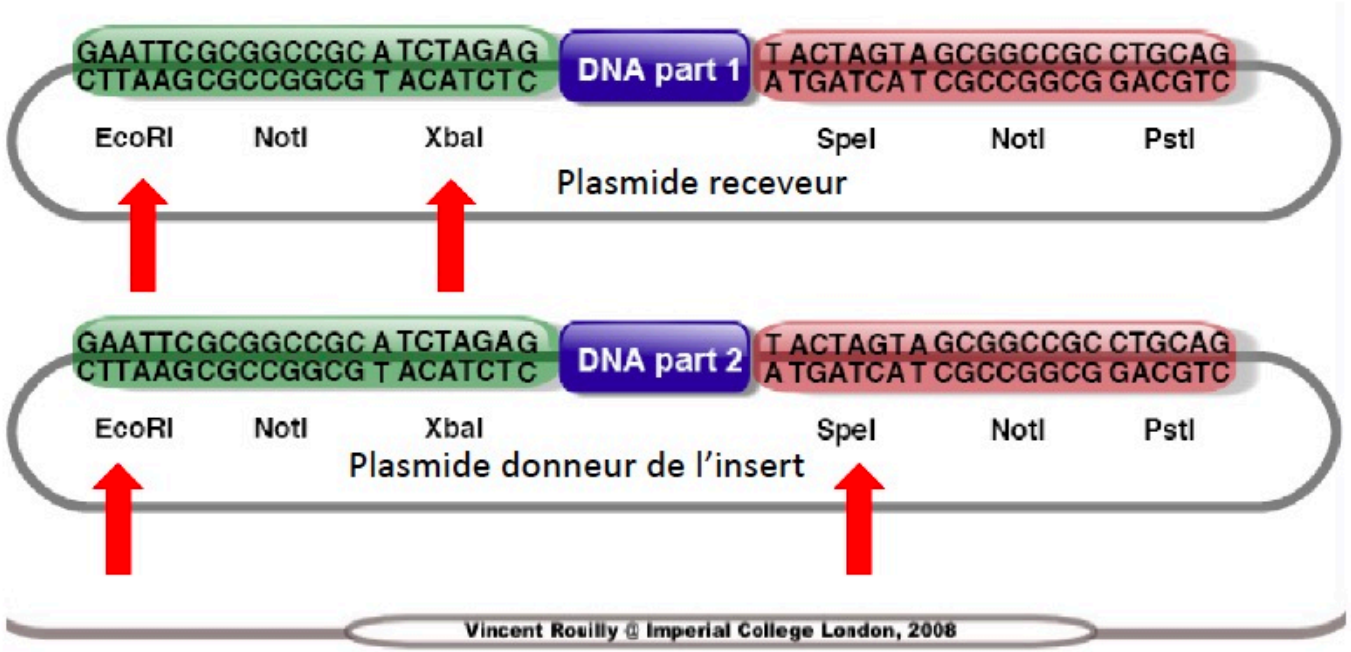
In addition to Dam and Dcm methylases, laboratory strains of *E.coli* K12 and B may contain **EcoKI** or **EcoBI** enzymes, respectively, encoded by a type I R-M system. These methyltransferases modify adenine residues within their respective recognition sequences: AAC$(N)_6$GTGC for EcoKI and TGA$(N)_8$TGCT for EcoBI

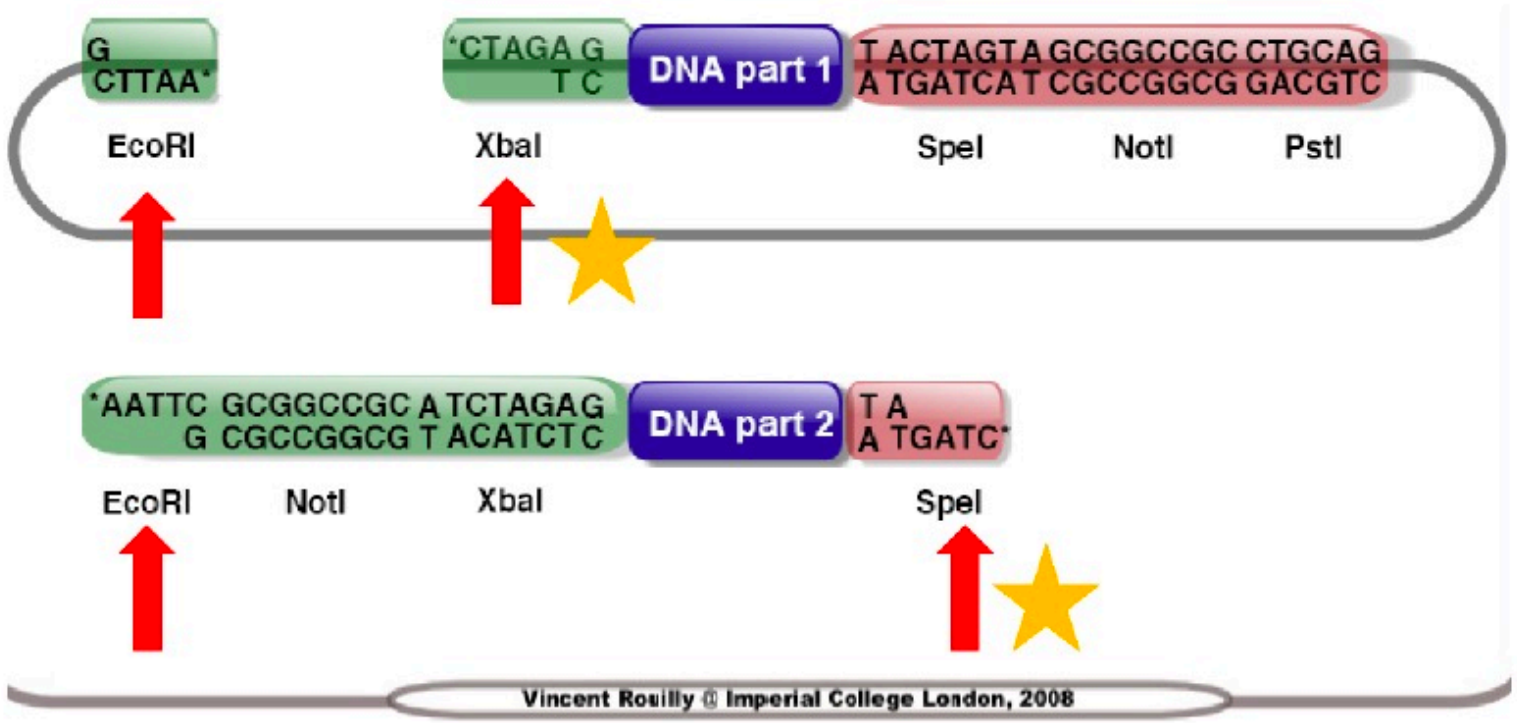
Suffix

```
5' [part] T ACTAGT  A  GCGGCCG CTGCAG G AAGAAAC   3'
3' [part] A TGATCA  T  CGCCGGC GACGTC C TTCTTTG   5'
   (1)      (2)    (3) (4)     (5)    (6)  (7)
```

- The above sequence assumes that your part is on the forward strand running in the 5' to 3' direction. To construct a PCR primer, you will need to use the bottom strand in the reverse direction.
  1. Approximately 20 bp of sequence that matches the 3' end of the part you wish to construct.
  2. SpeI recognition site
  3. Extra base to prevent inadvertent creation of EcoBI or EcoKI methylation sites which could inhibit efficient digestion by the BioBricks enzymes.
  4. NotI recognition site
  5. PstI recognition site
  6. Random extra spacer base
  7. Extra bases designed to both
     1. permit cutting of the PCR product with PstI by providing extra "spacer" bases. See notes on cutting near the ends of linear DNA fragments.
     2. promote addition of an A base on the opposite strand by Taq polymerase for high efficiency TA cloning if desired. See notes on TOPO TA cloning.

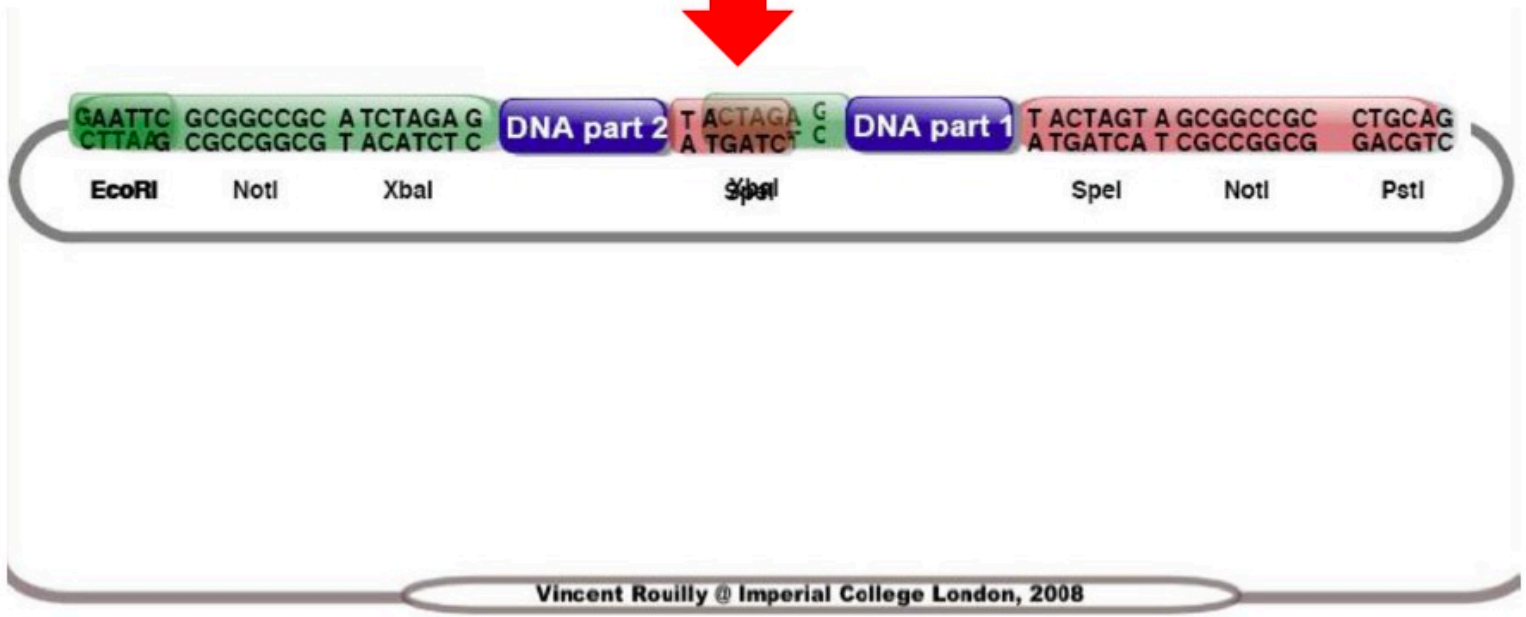
Clonage de la part 2 en amont de la part 1



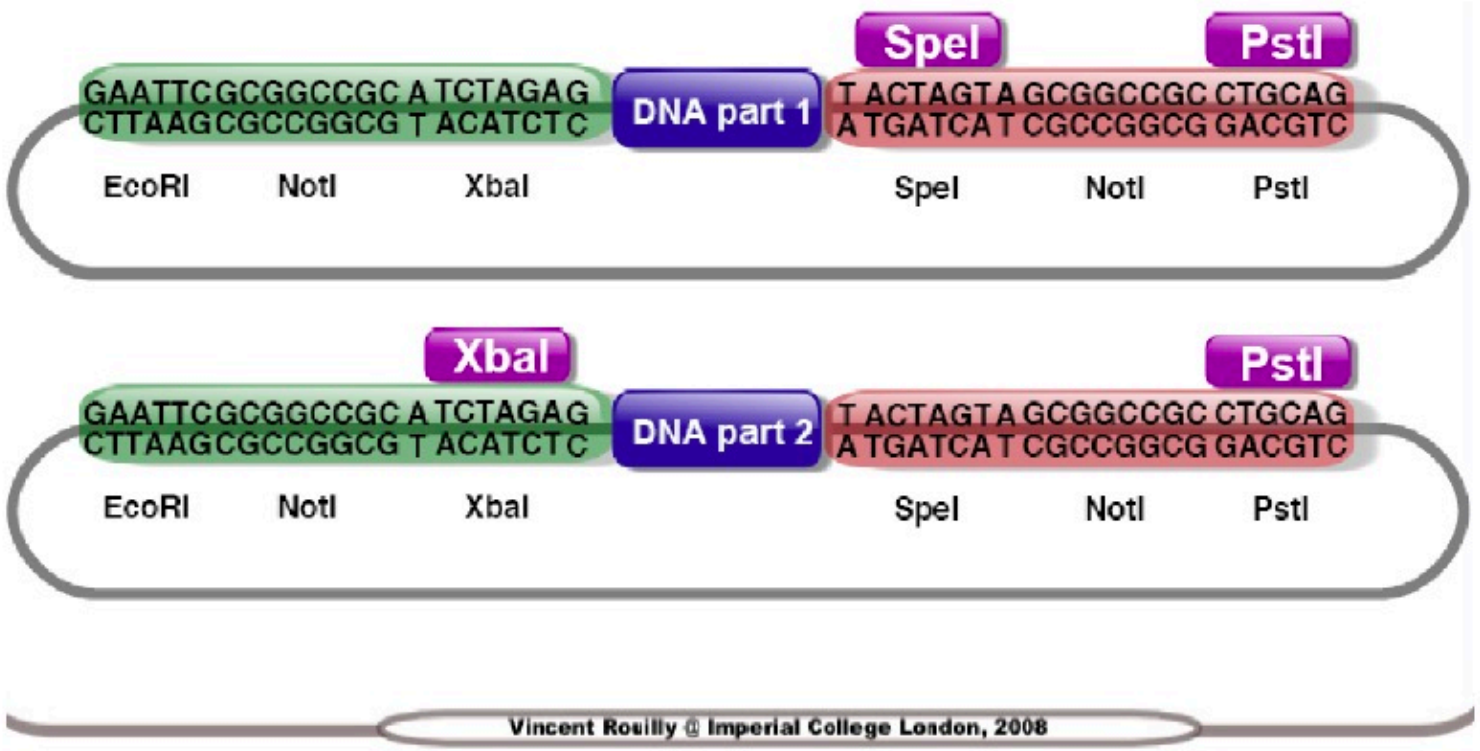

G
CTTAA*
*CTAGA G
T C
DNA part 1
T ACTAGT A GCGGCCGC CTGCAG
A TGATCA T CGCCGGCG GACGTC
EcoRI
XbaI
SpeI
NotI
PstI
*AATTC GCGGCCGC A TCTAGAG
G CGCCGGCG T ACATCT C
DNA part 2
T A
A TGATC*
EcoRI
NotI
XbaI
SpeI
Vincent Rouilly @ Imperial College London, 2008

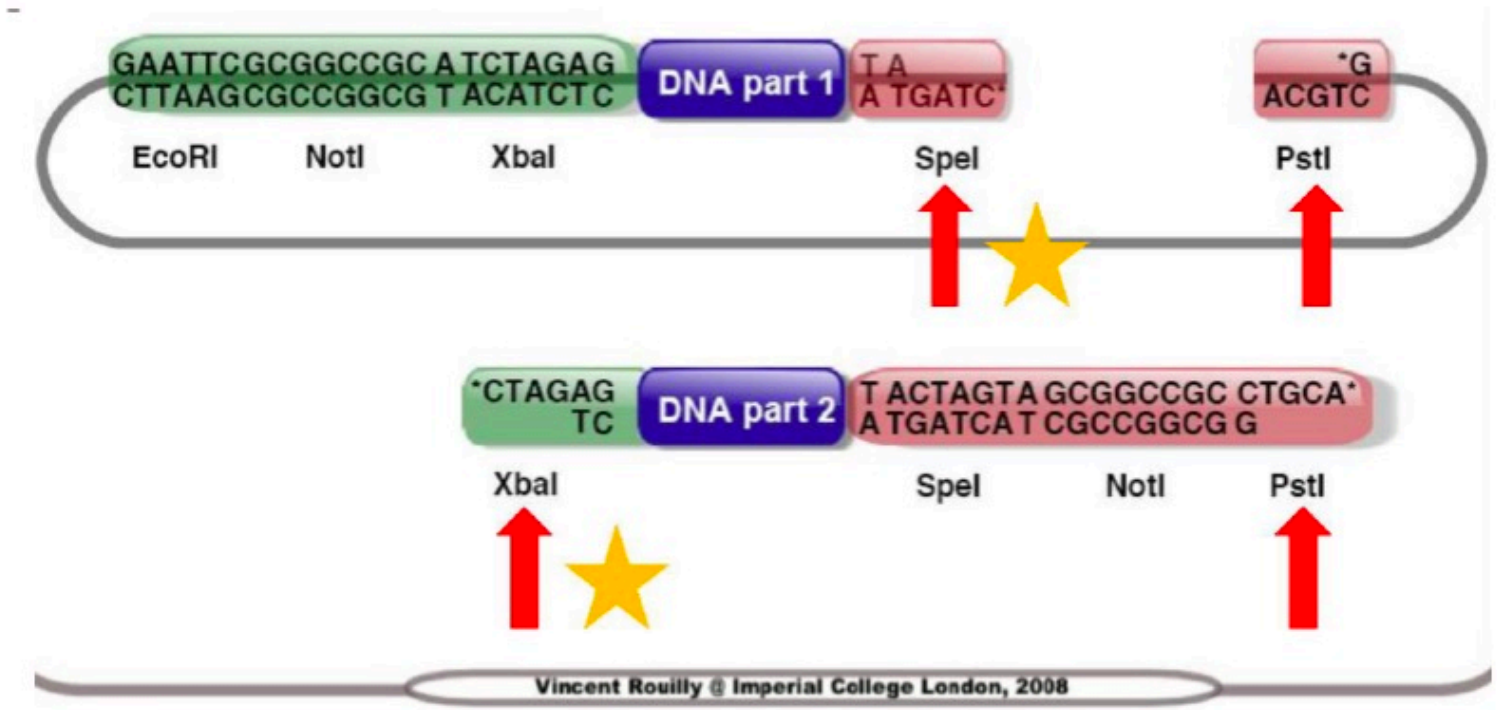
Clonage de la part 2 en amont de la part 1

Cicatrice non clivable



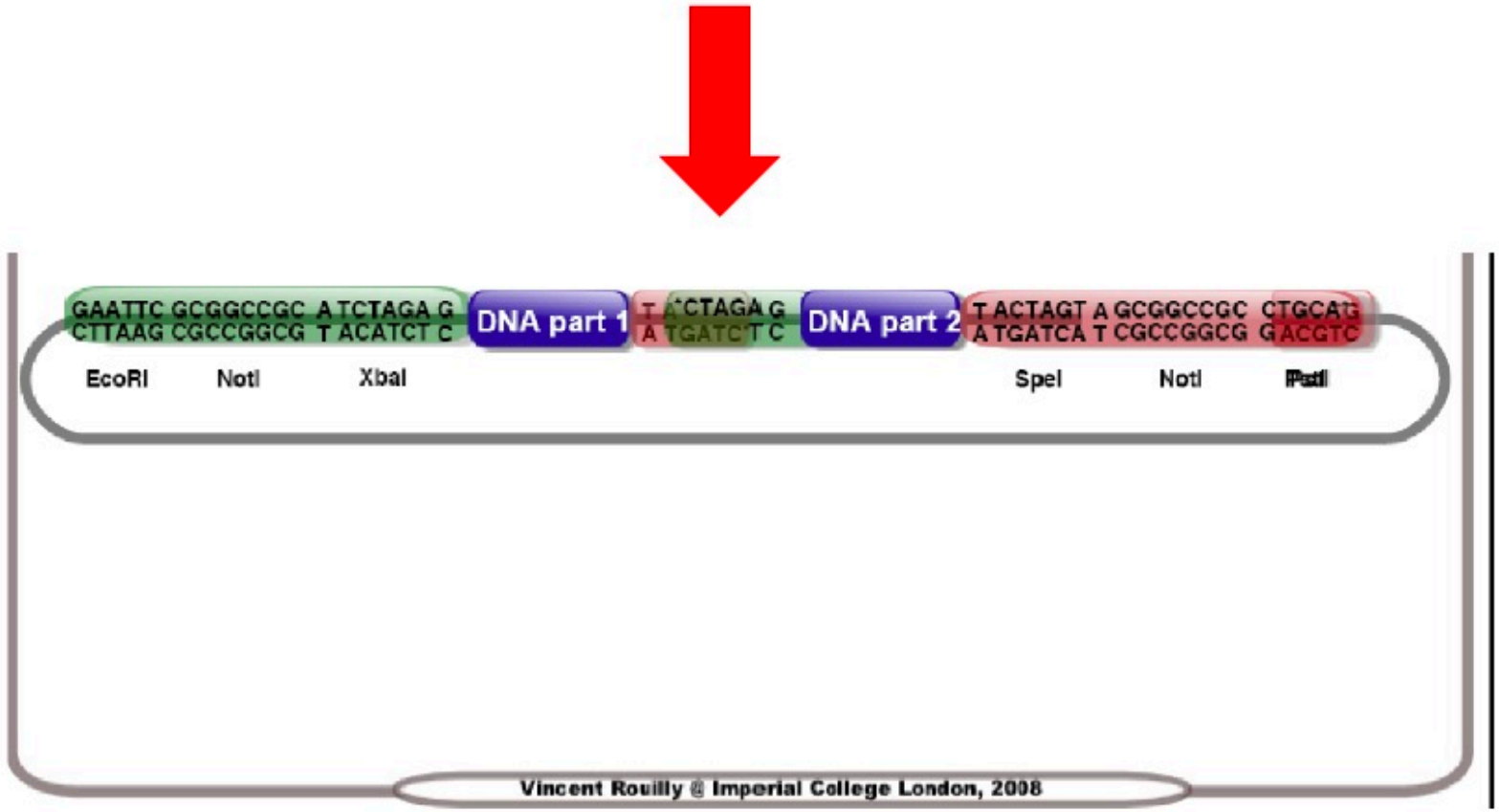
Clonage de la part 1 en amont de la part 2




GAATTCGCGGCCGC A TCTAGAG
CTTAAGCGCCGGCG T ACATCT C
DNA part 1
T A
A TGATC*
*G
ACGTC
EcoRI
NotI
XbaI
SpeI
PstI
*CTAGAG
TC
DNA part 2
T ACTAGT A GCGGCCGC CTGCA*
A TGATCA T CGCCGGCG G
XbaI
SpeI
NotI
PstI
Vincent Rouilly @ Imperial College London, 2008

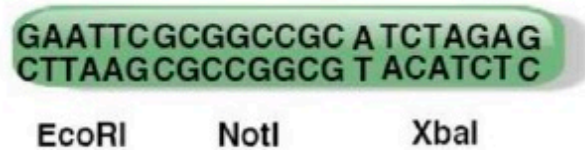
Clonage de la part 1 en amont de la part 2

Cicatrice non clivable



BioBricks Standard Summary

Upstream Sequence



Downstream Sequence

Restriction sites clean-up

Vincent Rouilly @ Imperial College London, 2008

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

Standard?
Plusieurs standards!

"BioBrick assembly standard 10" [modifier | modifier le code]

Cette nouvelle norme, développé par Tom Knigh est la plus largement utilisée parmi les standard d'assemblage. Elle implique l'utilisation d' enzymes de restriction. Chaque Bio-brique est une séquence d'ADN qui est portée par un plasmide circulaire, lequel est utilisé comme vecteur[12]. Ce vecteur agit comme un système de transport pour déplacer la bio-brique. La première approche allant dans ce sens a été l'introduction de séquences standard, des séquences "préfixe" et "suffixe" respectivement aux extrémités 5' et 3' d'une séquence d'ADN.[13] Ces séquences standard encodent des sites spécifiques à l'enzyme de restriction. Le préfixe code pour les sites *EcoRI* (E) et *XbaI* (X), tandis que le suffixe code les sites *SpeI* (S) et *PstI* (P). Ces préfixe et suffixe ne sont pas considérés comme faisant partie de la bio-brique[3]. Pour faciliter le processus d'assemblage, les bio-briques elles-mêmes ne doivent pas contenir l'un de ces sites de restriction. Lors de l'assemblage de deux briques différentes, l'un des plasmides est digéré avec *EcoRI* et *SpeI*. Le plasmide portant l'autre bio-brique est digéré avec *EcoRI* et *XbaI*. Cela laisse deux plasmides avec 4 paires de base (pb) au niveau des extrémités 5' et 3'. Les sites *EcoRI* vont le lier car ils sont complémentaires les uns des autres. Les sites *XbaI* et *SpeI* seront également liés car la "digestion" laissera des extrémités compatibles. Alors les deux briques de l'ADN se retrouvent dans un même plasmide. La ligature donne un zone "cicatricielle" de 8 paires de base entre les deux bio-briques. Comme le site de cette cicatrice est un hybride des sites *XbaI* et *SpeI*, il n'est pas reconnu par l'enzyme de restriction[13]. Les séquences préfixe et suffixe demeurent inchangées par ce processus de digestion et de "collage", ce qui permet de nouvelles étapes de montage avec plus de bio-briques.

Cette méthode d'assemblage est un processus idempotent : de multiples applications ne change pas le produit final, et maintiennent le préfixe et le suffixe. Cependant si l'assemblage de bio-briques standard permet la formation de modules fonctionnels, le standard 10 a des limites. Le site cicatriciel à 8 paires de bases (bp) ne permet pas la création d'une vraie protéine de fusion[12]. Le site-cicatrice cause un décalage ribosomique (aussi dénommé décalage du cadre de lecture) qui empêche la lecture en continu de codons, qui est nécessaire à la formation d'une protéine de fusion.

Tom Knight a ensuite (en 2008) développé un protocole (BB-2 assembly standard) pour répondre à ce problème, en utilisant d'autres enzymes pour la digestion de la première partie ; ce sont presque les mêmes mais avec des préfixes et suffixes modifiés[14].

Standard "BglBricks assembly" [modifier | modifier le code]

Le "BglBrick assembly standard" est une autre norme, proposée par J. Christopher Anderson, John E. Dueber, Mariana Leguia, Gabriel C. Wu, Jonathan C. Goler, Adam P. Arkin, and Jay D. Keasling en septembre 2009. Il s'agit d'une méthode permettant de faire de multiples fusion de domaines protéiques de briques biologiques sans modifier le cadre de lecture ni introduire de codons stop. Cette méthode crée une cicatrice GGATCT pouvant ensuite être assemblée par une autre méthode[14].

Standard Argent (Biofusion) [modifier | modifier le code]

Le Pam Silver lab a créé cet autre standard pour surmonter le problème de la formation d'une protéine de fusion. Ce standard d'assemblage est également connu sous le nom de Biofusion. Il s'agit d'une amélioration du "'BioBrick assembly standard 10"' ; il implique la suppression d'un nucléotide du site *XbaI* et *SpeI*, ce qui réduit la cicatrice de 2 nucléotides, permettant de maintenant former une séquence cicatricielle de 6 paires de base (bp) . Cette dernière permet au cadre de lecture d'être maintenu. La séquence cicatricielle code pour l'acide aminé thréonine (ACT) et l'arginine (AGA)[15]. Cette légère amélioration permet la formation d'une protéine de fusion dans le cadre. Cependant, l'arginine est un acide aminé chargé, ce qui est un désavantage pour les technique d'assemblage Biofusion : ces propriétés de l'arginine induisent une déstabilisation de la protéine selon le principe "N-end rule".

Prefixe, suffixe: bornes de la Biobrick

Wet lab: Techniques de clonage

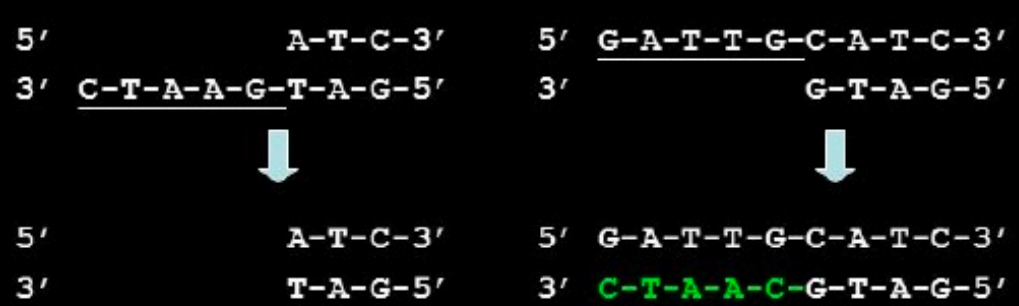
- Traditional Cloning
- PCR Cloning Method
- Recombinational Cloning

2 - Ligation Independent Cloning (LIC)

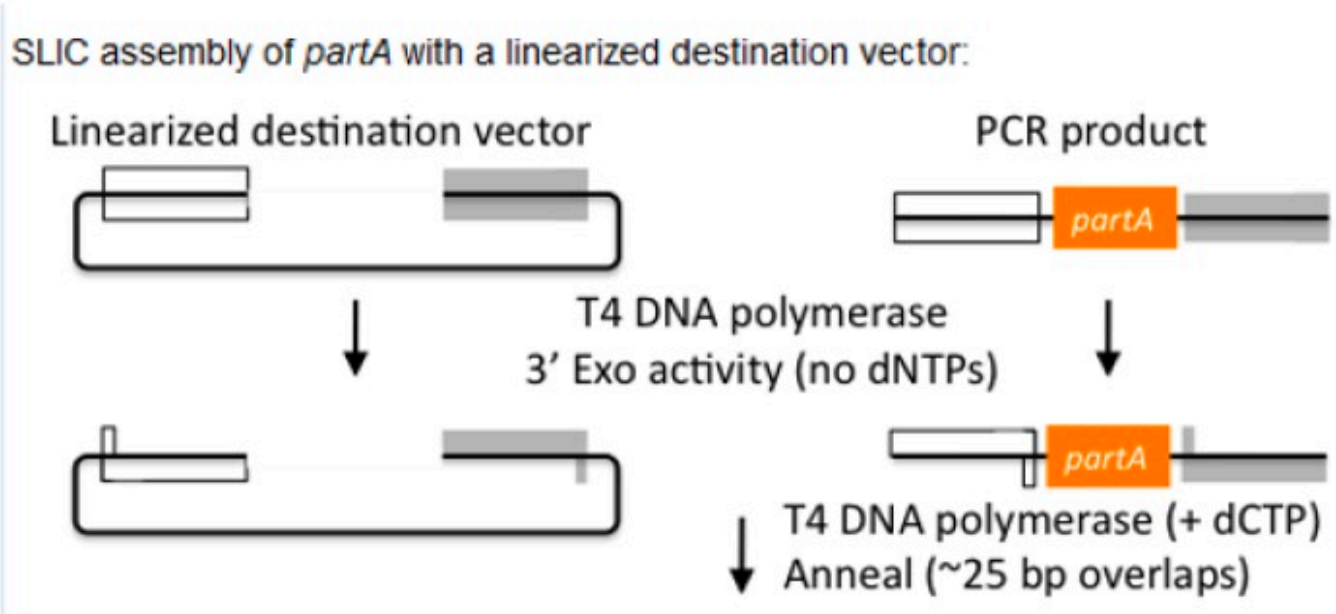
TP

Clonage SLIC

- T4 DNA polymerase
  – strong 3' to 5' exonuclease activity but deficient in 5'to3' exo activity
  - use to form blunt ends by either – removal of 3' overhangs or fill-in 5' overhang.



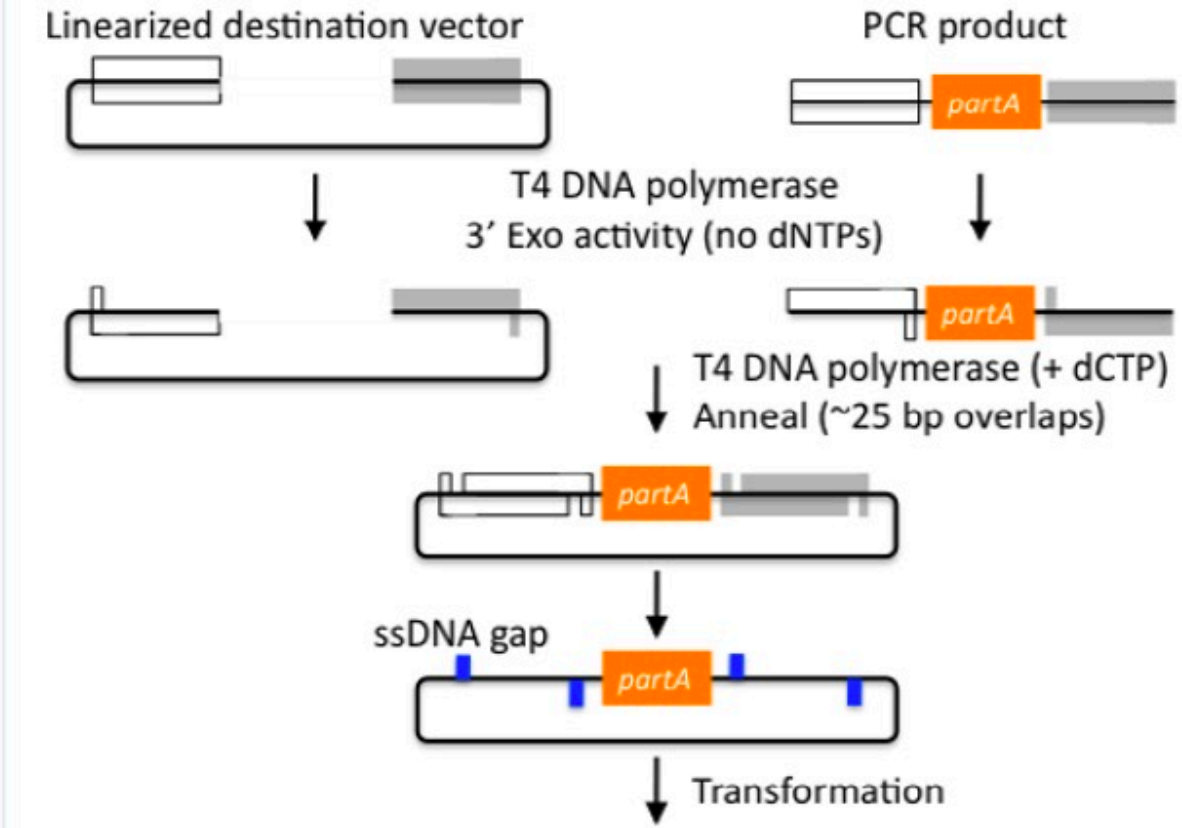
Clonage SLIC



SLIC, or sequence and ligase independent cloning (Li 2007), as its name implies, does not utilize restriction enzymes or ligase (see also a variation of the SLIC protocol for use with j5). A DNA sequence fragment to be cloned into a destination vector is PCR amplified with oligos whose 5' termini contain about 25 bp of sequence homology to the ends of the destination vector, linearized either by restriction digest or PCR amplification. Sequence homology regions are depicted in white and grey in the figure below.

Clonage SLIC

SLIC assembly of *partA* with a linearized destination vector:



The linearized destination vector and the PCR product containing *partA* are separately treated with T4 DNA polymerase in the absence of dNTPs. In the absence of dNTPs, T4 DNA polymerase has 3' exonuclease activity, which begins to chew-back the linearized destination vector and the PCR product from 3' to 5'. Once the termini of the linearized destination vector and the PCR product have sufficient complementary single-stranded 5' overhangs exposed, dCTP is added to arrest the chew-back reaction. With the addition of dCTP, the T4 DNA polymerase changes activity from 3' exonuclease to polymerase, but stalls because not all dNTPs are present, retaining most, if not the entirety, of each chewed-back overhang. Alternatives to the 3' chew-back with T4 DNA polymerase in the absence of dNTPs include the use of mixed or incomplete PCR products (so this does not apply to the linearized vector backbone if it is derived from a restriction enzyme digest), which can also result in the desired 5' overhangs, as described in the original SLIC publication (Li 2007).The chewed-back linearized destination vector and PCR product are mixed together, and annealed to each other. Since there is no ligase in the reaction, this results in a plasmid with four single stranded gaps or nicks. Once transformed into competent *E. coli*, the gaps are repaired. Note that SLIC assembly is standardized, in that it always uses the same reaction components and conditions, scar-less, since there is no sequence in the resulting assembly that is not user-designed, and sequence-independent, as the method is not (at least to a large extent, but see below) sensitive to the sequences of either the destination vector or the part to be incorporated.

CAO

Ecriture directe ->
Synthèse de gènes :
Ni Préfixe ni Suffixe