Genefish : an alternate metagenomic approach for capturing targeted bacterial diversity in an engineered recipient *E. coli* strain

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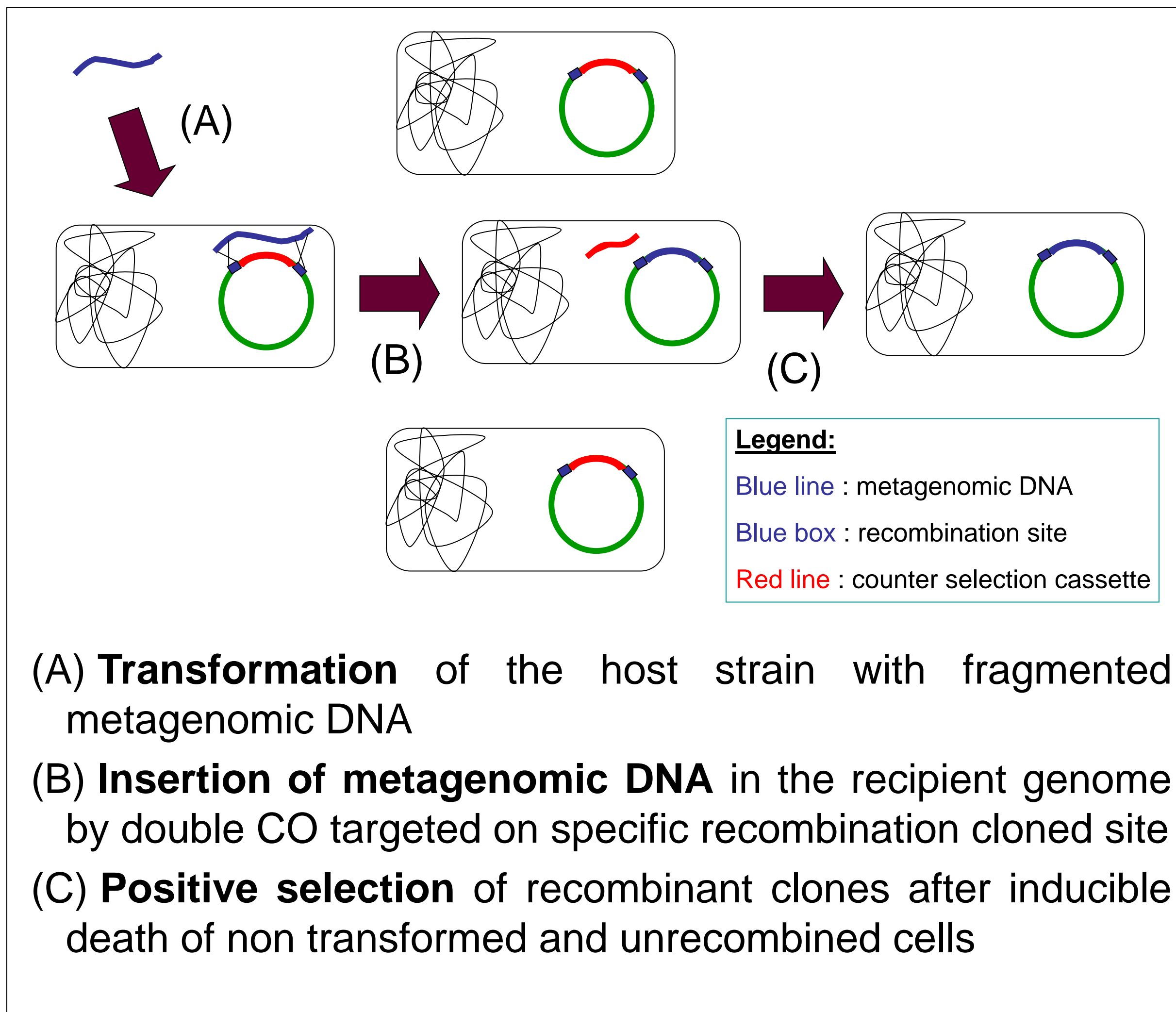[2] Soil and Environmental Microbiology, UMR1229, CMSE, INRA, 21065 Dijon cedex, France
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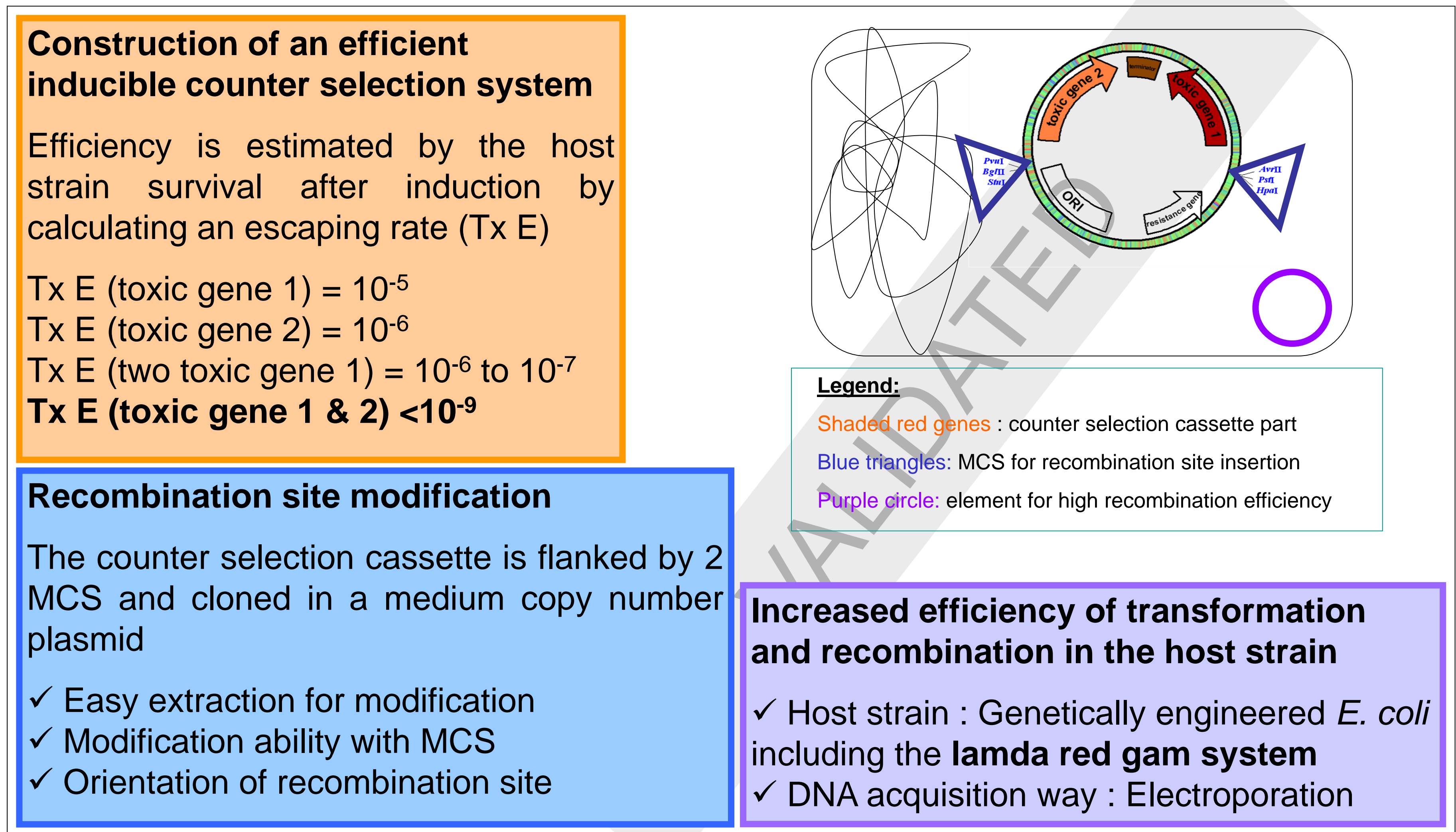
1-Introduction

Bacterial diversity in soil environment is so high that recovery of specific genes in soil extracted DNA requires construction and screening of metagenomic DNA libraries of several hundreds thousands of clones. The gene fishing approach we describe here can be considered as a simpler alternative to the traditional metagenomic technique to recover specific genes or DNA fragments in a metagenome.
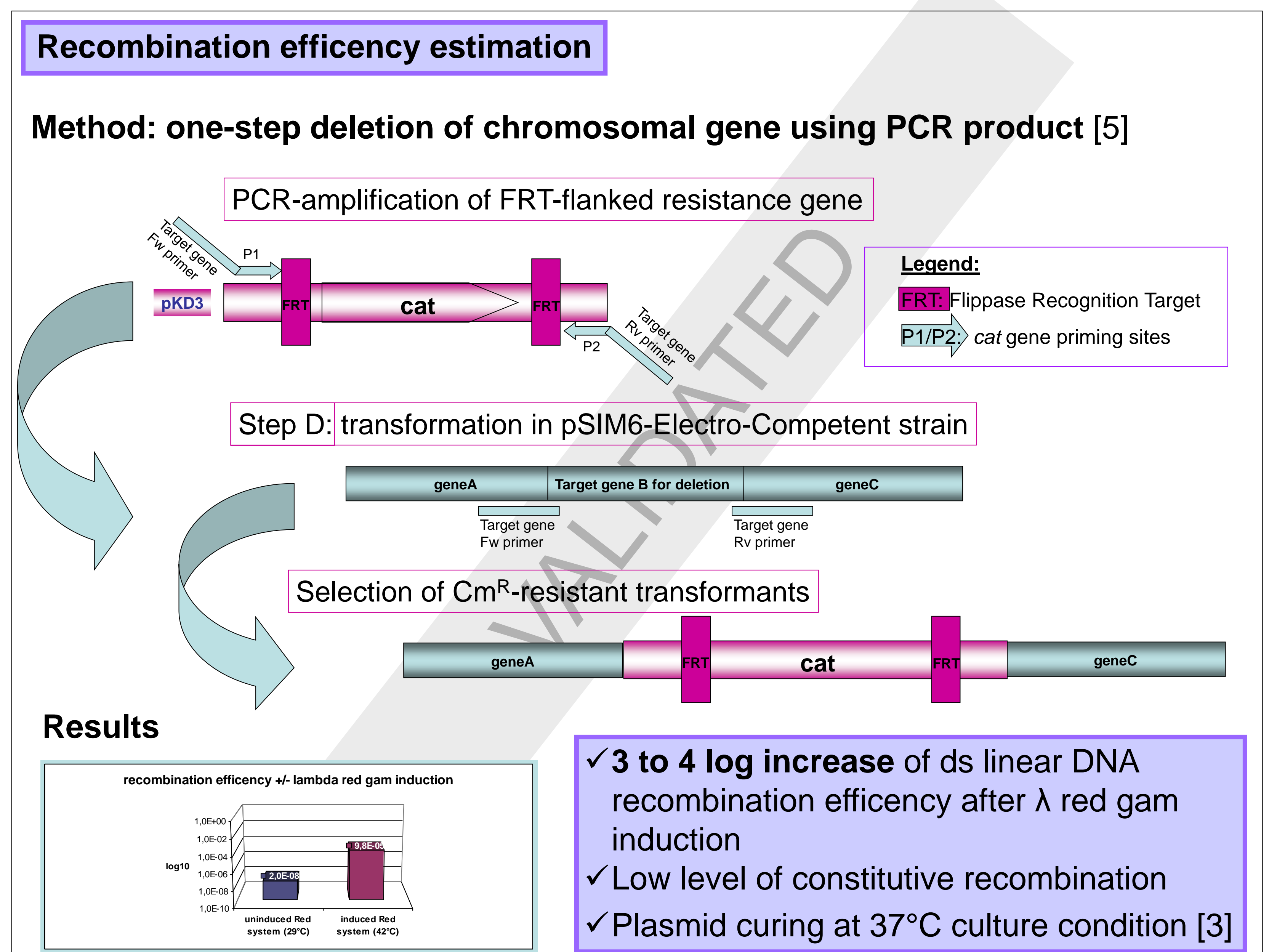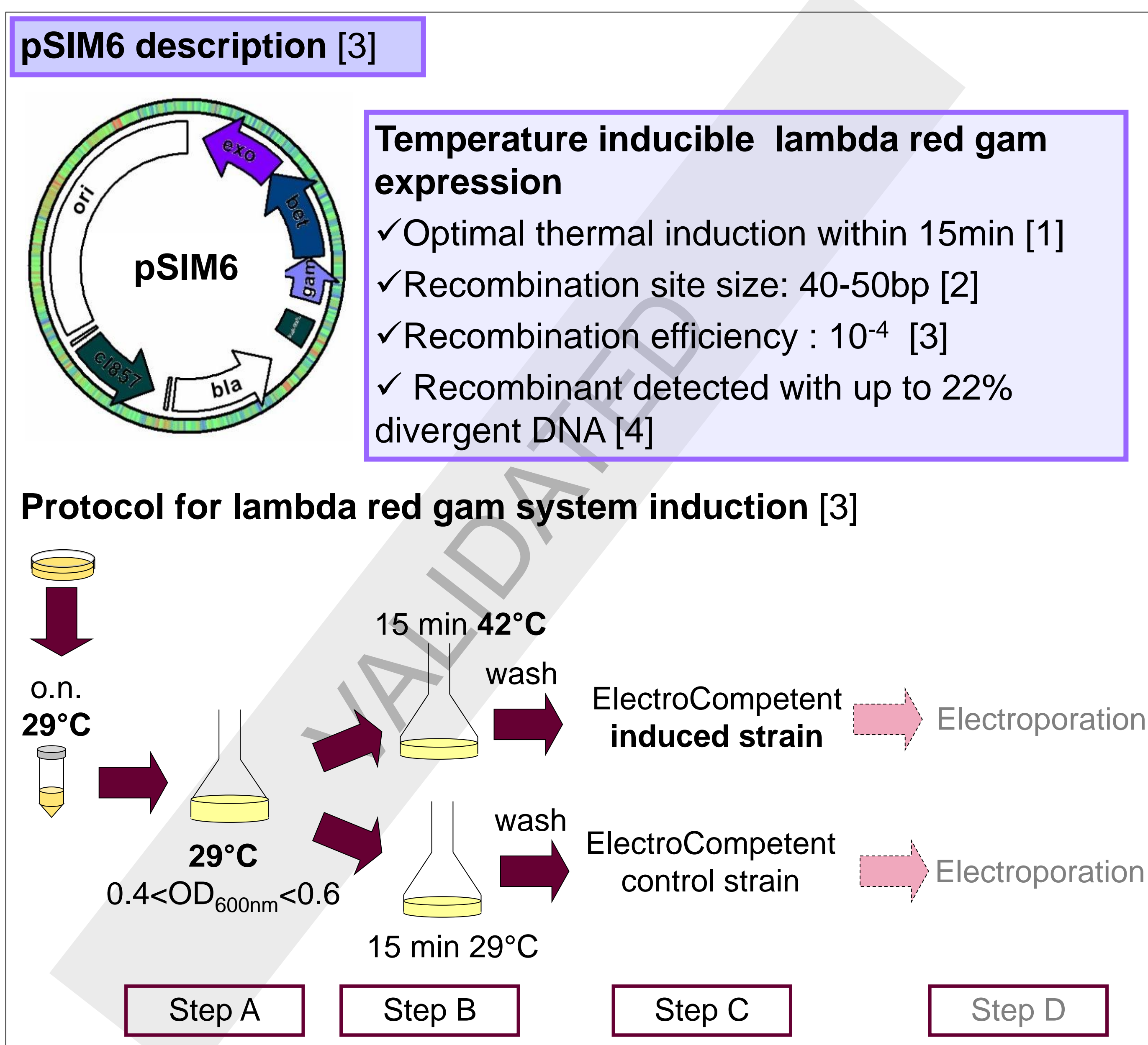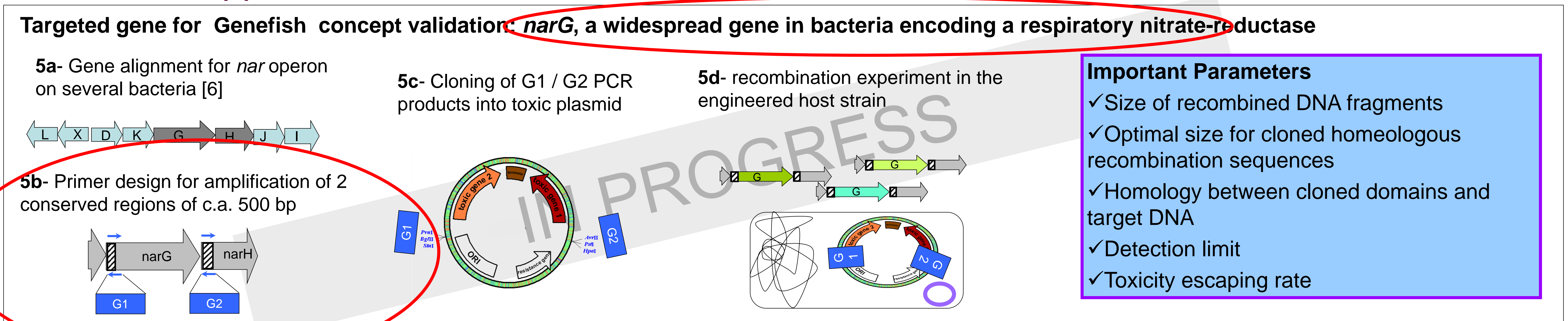
2-Genefish concept

Legend :
Blue line : metagenomic DNA
Blue box : recombination site
Red line : counter selection cassette

(A) **Transformation** of the host strain with fragmented metagenomic DNA

(B) **Insertion of metagenomic DNA** in the recipient genome by double CO targeted on specific recombination cloned site

(C) **Positive selection** of recombinant clones after inducible death of non transformed and unrecombined cells

3-Host strain construction

Construction of an efficient inducible counter selection system

Efficiency is estimated by the host strain survival after induction by calculating an escaping rate (Tx E)

Tx E (toxic gene 1) = 10^{-5}
Tx E (toxic gene 2) = 10^{-6}
Tx E (two toxic gene 1) = 10^{-6} to 10^{-7}
Tx E (toxic gene 1 & 2) < 10^{-9}

Recombination site modification

The counter selection cassette is flanked by 2 MCS and cloned in a medium copy number plasmid

✓ Easy extraction for modification
✓ Modification ability with MCS
✓ Orientation of recombination site

Legend:
Shaded red genes : counter selection cassette part
Blue triangles: MCS for recombination site insertion
Purple circle: element for high recombination efficiency

Increased efficiency of transformation and recombination in the host strain

✓ Host strain : Genetically engineered *E. coli* including the **lamda red gam system**
✓ DNA acquisition way : Electroporation

4-Lambda Red gam system evaluation for recombination

pSIM6 description [3]

Temperature inducible lambda red gam expression
✓ Optimal thermal induction within 15min [1]
✓ Recombination site size: 40-50bp [2]
✓ Recombination efficiency : 10^{-4} [3]
✓ Recombinant detected with up to 22% divergent DNA [4]

Protocol for lambda red gam system induction [3]

o.n. **29°C**

15 min **42°C** → wash → ElectroCompetent **induced strain** → Electroporation

29°C
$0.4 < OD_{600nm} < 0.6$

15 min 29°C → wash → ElectroCompetent control strain → Electroporation

| Step A | Step B | Step C | Step D |

Recombination efficency estimation

Method: one-step deletion of chromosomal gene using PCR product [5]

PCR-amplification of FRT-flanked resistance gene

Legend:
FRT : Flippase Recognition Target
P1/P2 : *cat* gene priming sites

Step D: transformation in pSIM6-Electro-Competent strain

Selection of Cm^R-resistant transformants

Results

recombination efficiency +/- lambda red gam induction

✓ **3 to 4 log increase** of ds linear DNA recombination efficency after λ red gam induction
✓ Low level of constitutive recombination
✓ Plasmid curing at 37°C culture condition [3]

5-Genefish applications

Targeted gene for Genefish concept validation: *narG*, a widespread gene in bacteria encoding a respiratory nitrate-reductase

5a- Gene alignment for *nar* operon on several bacteria [6]

5b- Primer design for amplification of 2 conserved regions of c.a. 500 bp

5c- Cloning of G1 / G2 PCR products into toxic plasmid

5d- recombination experiment in the engineered host strain

Important Parameters
✓ Size of recombined DNA fragments
✓ Optimal size for cloned homeologous recombination sequences
✓ Homology between cloned domains and target DNA
✓ Detection limit
✓ Toxicity escaping rate

6-Perspectives

Genefish efficiency evaluation with a wide range of DNA including PCR products, plasmid isolates, genomic DNA and soil metagenome

References:
[1] Yu, D., Ellis, H.M., Lee, E.C., Jenkins, N.A., Copeland, N.G. &Court,D.L. (2000). *PNAS* 97, 5979-5983
[2] Murphy, K. C. (1998). *J Bacteriol* 180, 2063-2071.
[3] Datta, S., Costantino, N. & Court, D.L. (2006). *Gene* 379, 109-115 .
[4] Martinsohn, J.T., Radman, M. & Petit, M.A. (2008). *Plos genetics* 4, 1-12.
[5] Datsenko, K.A. & Wanner, B.L. (2000). *PNAS* 97, 6640-6645.
[6] Philippot, L. (2002) *Biochimica et Biophysica Acta* 1577, 335-376.