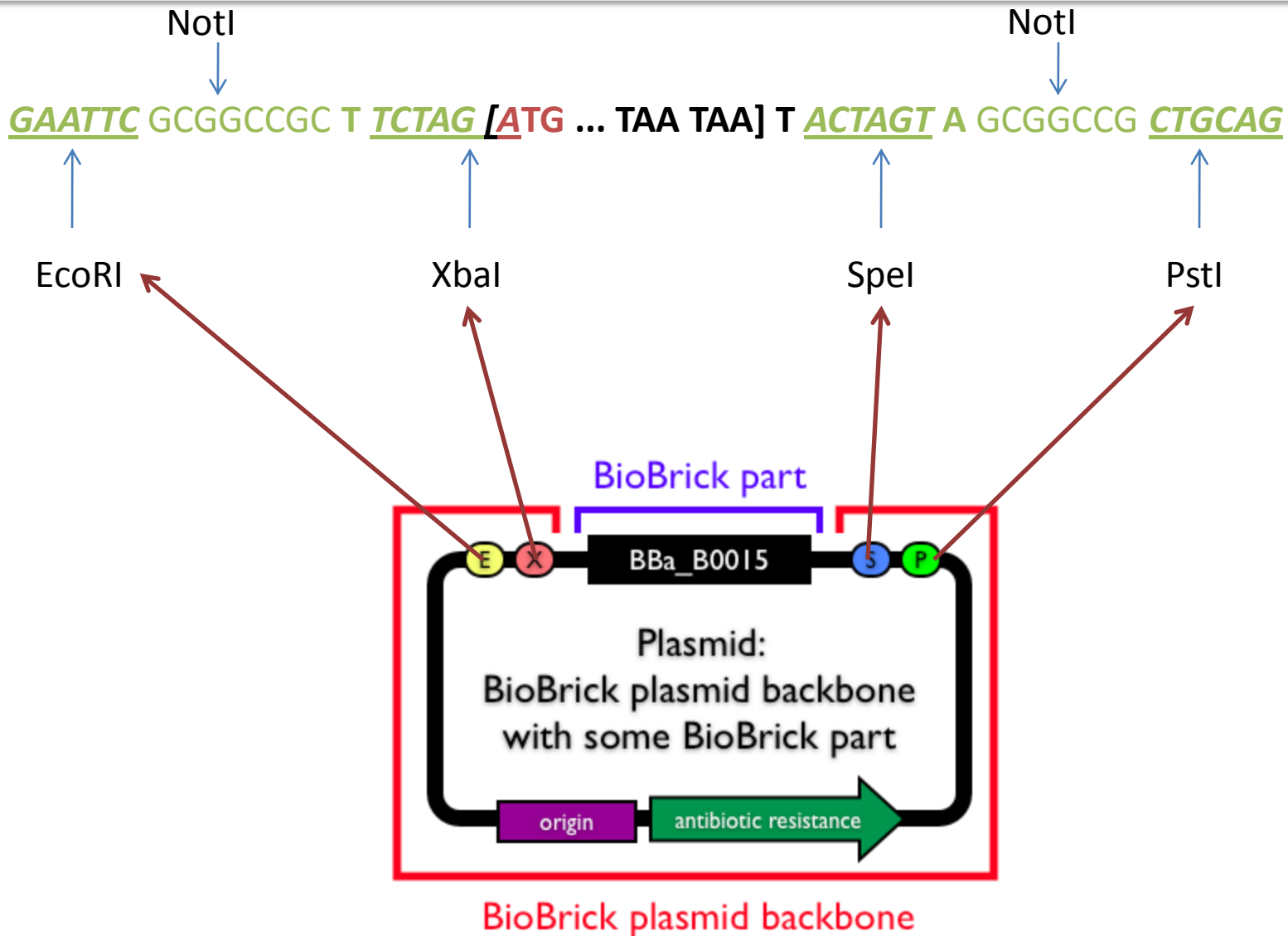


iGEM meeting

16 Jul 09

Protein Fusion – RFC10



Upon Fusion of 2 Protein Bricks

GAATTC GCGGCCGCT TCTAG [ATG ... TAA TAA] T ACTAGT A GCGGCCG CTGCAG

UPSTREAM PROTEIN PART
E + S

GAATTC GCGGCCGCT TCTAG [ATG ... TAA TAA] T ACTAGT A GCGGCCG CTGCAG

DOWNSTREAM PROTEIN PART
X + P

GAATTC GCGGCCGCT TCTAG [ATG ... TAA TAA] T ACTAG [ATG ... TAA TAA] T ACTAGT A GCGGCCG CTGCAG

Biobrick Scar

The biobrick scar problem

...TAC TAG...

Two stop codons found upstream of TAC (part of upstream protein gene)

TAG is a STOP CODON.

Cannot do in-line fusion and produce functional domains of proteins.

Different Type fusions (between proteins/non-proteins) will produce out of frame scars
e.g. ...**TACTAG AG** [ATG...TAA TAA]

Thus Still have Stop Codons and out of frame !

Suggested Solutions....I

- Different Assembly Standards
 - RFC23 ; Will allow in-frame fusions
 - » BUT Stop codons from upstream protein still present
 - » AND Will produce an Arginine residue in scar (non-favorable)
 - RFC25 ; Allows in-frame fusions
 - » BUT need to insert restriction enzyme sequence upstream of gene coding for Protein
 - » This will upset the RBS-ATG reading frame (no protein translation)
 - » Still a problem between any two consecutive proteins with stop codons in between

Suggested Solutions...II

- Usage of BioScaffold Parts (RFC15)
 - Parts that supposedly allow protein-fusions by removing protein heads/tails and scars.
 - True ?
 - http://partsregistry.org/Part:BBa_J70030:Design
 - http://partsregistry.org/Part:BBa_J70032
 - http://partsregistry.org/Part:BBa_J70012:Design

Some Good News

- Primers ordered for our first Biobricks
 - http://partsregistry.org/Part:BBa_K259000
 - http://partsregistry.org/Part:BBa_K259001
- Modelling Updates
 - <http://2009.igem.org/Team:BCCS-Bristol/Modeling/BSim-UML>