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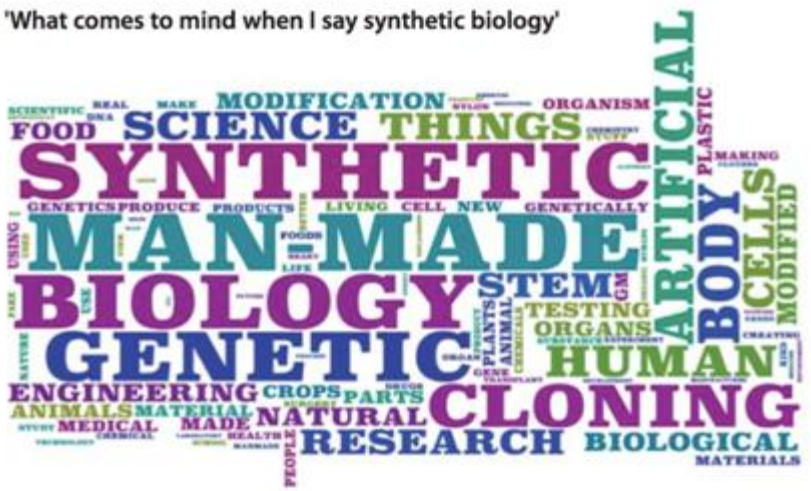
Encapsulation for Drug Delivery

21st July

iGEM 2009



Phenylketonuria - PKU

- Deficiency in PAH enzyme
- PAH enzyme catalyses Phenylalanine to Tyrosine
- The condition causes mental retardation
- 1/15000 in USA

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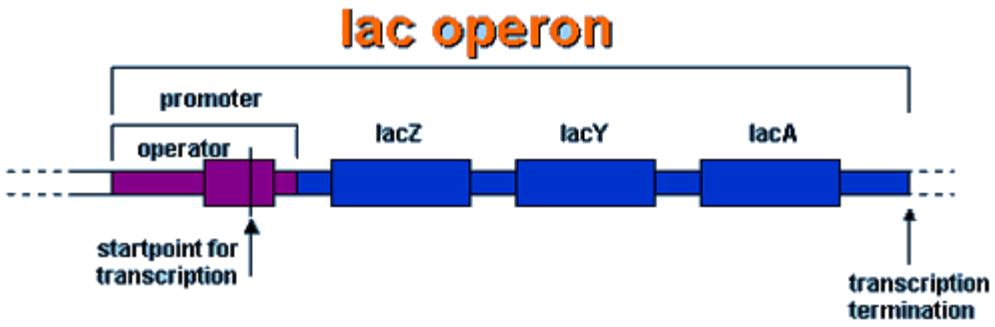
Application - Lactose Intolerance

- Inability to digest lactose due to deficiency of lactase
- Occurs in 25% of population (1994/NIDDK)
- E. *Coli* has three genes that code for lactase, lacZ, lacY, lacA
- When there is lactose, the repressor is removed and RNA polymerase binds to the promoter to begin translation and transcription



Calt

- e to
- c der to
- ly duction
- F
- B :

Registry Number	Plasmid	Part	Status
BBa_K137002	pSB1A2	LacY	Constructed
BBa_K137126	pSB1A2	Lysis Cassette	Constructed
BBa_S03970	pSB1A2	B0031 + LacY	Constructed
BBa_S03971	pSB1A2	B0033 + LacY	Constructed
BBa_S03973	pSB1AK3	B0034-LacZ + B0015	Constructed
BBa_S04107	pSB1AK3	B0031-LacY + B0015	Constructed
BBa_S04108	pSB1AK3	B0033-LacY + B0015	Constructed
BBa_S04109	J61002	J23113 + B0031-LacY-B0015	Constructed
BBa_S04122	J61002	J23106 + B0031-LacY-B0015	Unavailable
BBa_S04123	J61002	J23100 + B0031-LacY-B0015	Unavailable
BBa_S04110	J61002	J23113 + B0033-LacY-B0015	Constructed
BBa_S04111	J61002	J23106 + B0033-LacY-B0015	Constructed
BBa_S04112	J61002	J23100 + B0033-LacY-B0015	Constructed
BBa_S04041	pSB1A2	B0033 + LacY +H	Constructed
BBa_S04022	pSB1A2	B0033 + LacY +2H	Constructed
BBa_S04113	pSB1AK3	B0033-LacY +H + B0034-LacZ-B0015	Constructed
BBa_S04054	pSB1AK3	B0033-LacY +2H + B0034-LacZ-B0015	Constructed
BBa_S04055	J61002	Final synthetic LacYZ operon	Constructed
BBa_K137125	pSB1A2	LacI Repressed Promoter B4	Constructed
BBa_S04114	pSB2K3	Lysis + B0015	Constructed
BBa_S04105	pSB2K3	B0034-LacI + B0015-B4	Constructed
BBa_S04106	pSB2K3	J23100 + B0034-LacI-B0015-B4	Constructed
BBa_K137131	pSB2K3	J23100-B0034-LacI-B0015-B4 + Lysis-B0015	Constructed
BBa_K137132	pSB2K3	J23100-B0034-LacI-B0015-B4 + B0034-GFP-B0015	Constructed

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Application – Gaucher's Disease

- Genetic disease where lipid accumulates in cells and certain organs
- Type 1 occurs in 1 in 500 births in the Ashkenazi Jewish population. Occurrence depends on race
- Can be treated by enzyme replacement treatment with intravenous recombinant glucocerebrosidase
- Produced in yeast *Pichia pastoris* and Chinese hamster ovary cells (CHO cells)

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Application – Anti-cancer Anthocyanin

- Anthocyanins are red, purple, or blue pigments naturally occuring in plants
- Has anti-cancer properties, e.g. anti colon cancer
- Metabolic pathways in E. coli characterised
- Abstract: E. coli containing the recombinant plant pathway were able to take up either naringenin or eriodictyol and convert it to the corresponding glycosylated anthocyanin, pelargonidin 3-*O*-glucoside or cyanidin 3-*O*-glucoside

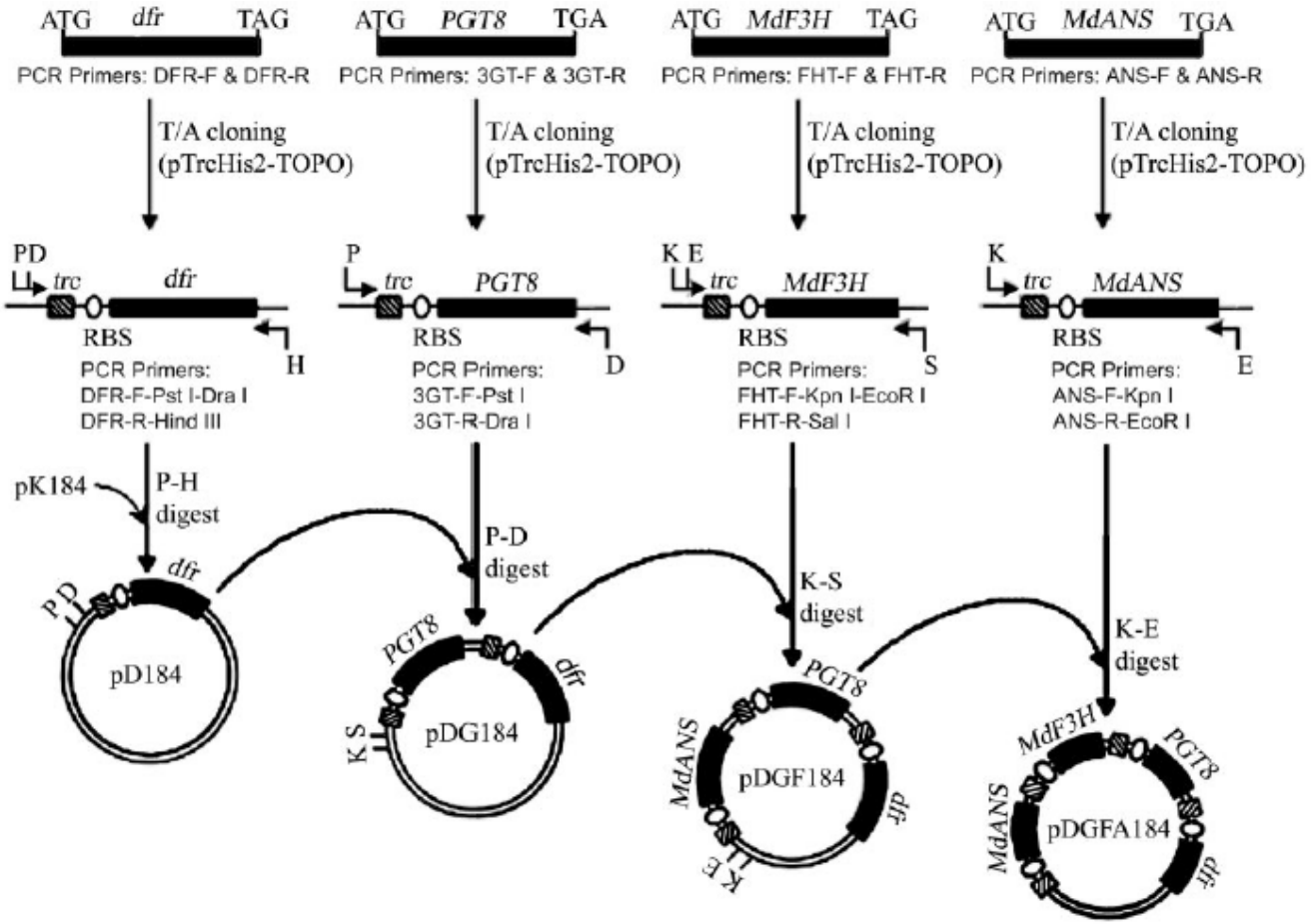

FIG. 2. Schematic representation of the strategy used for constructing vector pDGFA184. Abbreviations used for restriction enzymes: P, PstI; H, HindIII; D, DraI; S, SalI; K, KpnI; E, EcoRV. By performing a first round of PCR or RT-PCR, the *MdF3H*, *dfr*, *MdANS*, or *PGT8* gene was placed under the control of the *E. coli trc* promoter and an *E. coli* RBS derived from cloning vector pTrcHis2-TOPO. In a second round of PCR, each gene was amplified together with the *trc* promoter and RBS and placed sequentially into *E. coli* cloning vector pK184. The PCR and RT-PCR primer sequences used are presented in Table 1.

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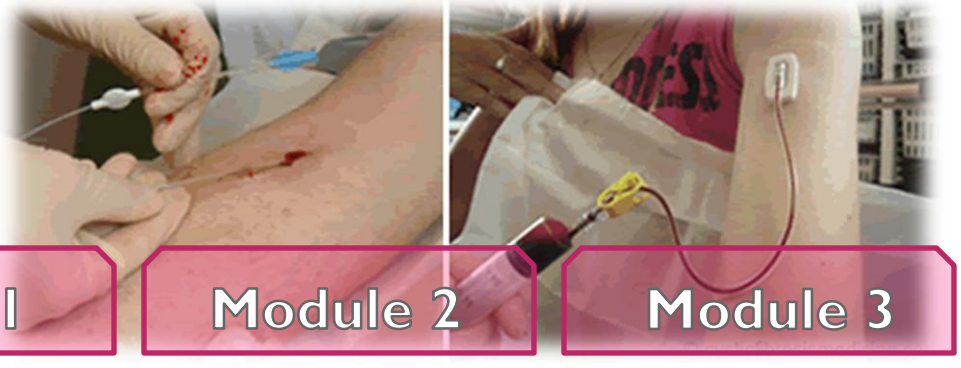
Application – Cystic Fibrosis

- Cannot be cured, hence management is necessary to prolong the lives of patients
- Occurs in 1 in 3300 Caucasians
- Home administered intravenous antibiotic therapy not very good e.g.
- Antibiotic Teicoplanin require reconstitution prior being used = troublesome
- Can be administered orally
- Biosynthetic gene cluster isolated for E. coli to produce Teicoplanin. 49 genes isolated. Too complicated!!!



Specifications of Encapsulation

- 1) Protection against low pH
- 2) Attachment in intestines
- 3) Efficient release of the bacteria within the gastrointestine
- 4) Use of materials that are inexpensive, stable, and of food grade
- 5) Inducibility

  and possibly
- 6) Protection against environmental stresses during drying, formulation, and storage

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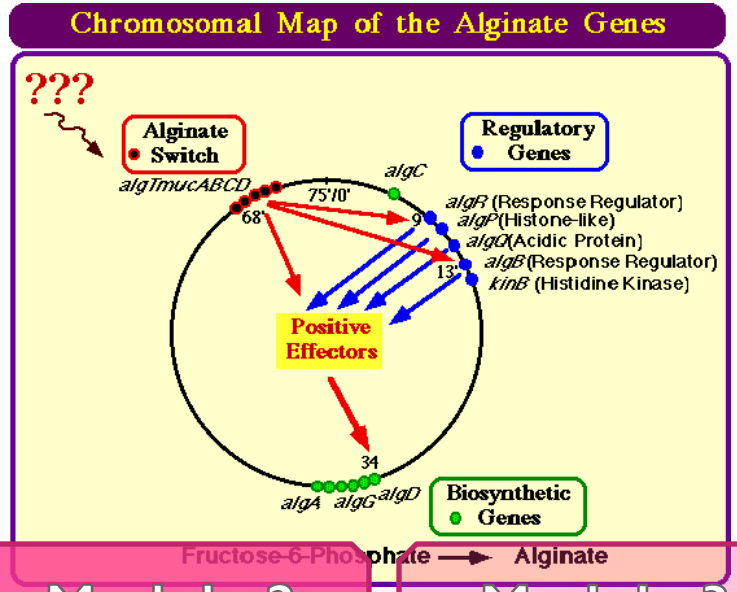
Exo-polysaccharides

Naturally secreting exo-polysaccharides

- Bacteria coat themselves – biofilm formation.

e.g. Pseudomonas aeruginosa biofilms encodes phosphomutase triggered naturally by glucose & osmotic levels.

- Function: defence
- 6 genes including algC required for alginate synthesis.



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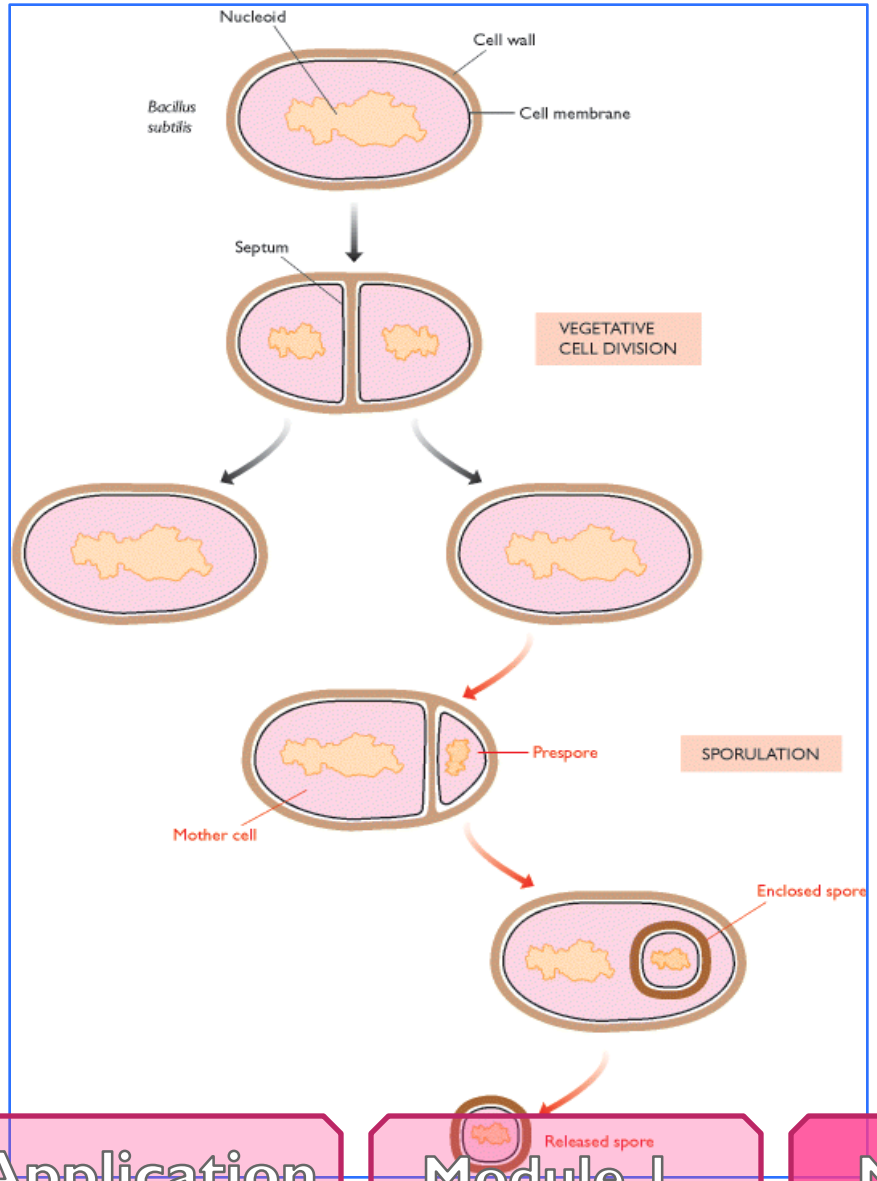
Sporulation Method



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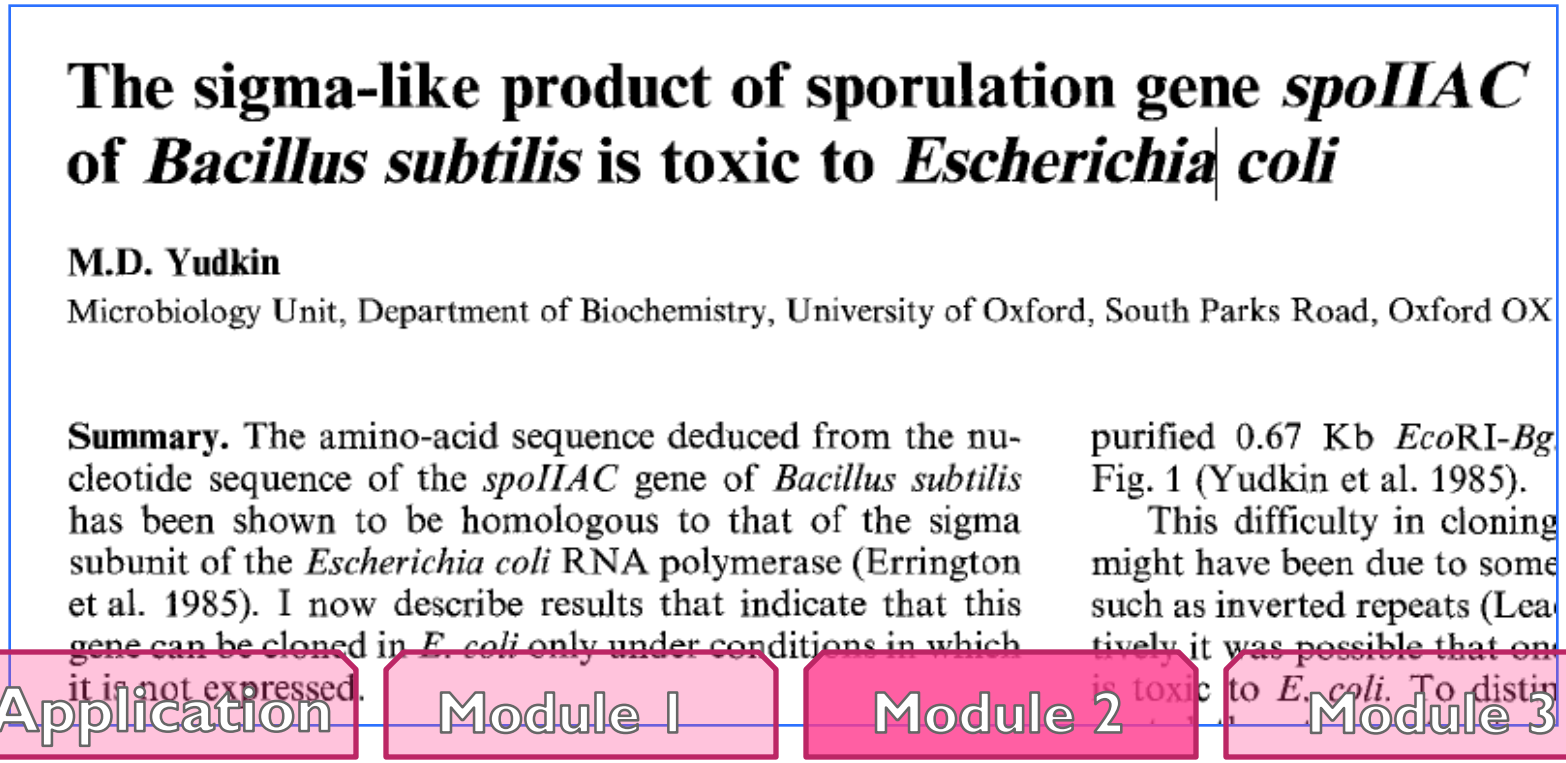
Sporualtion & E.coli

- Non-sporulating
- SpoIIAC gene homologous to E.coli sigma subunit of RNAP
- Can clone in E. coli **only** under conditions it is not expressed.



The sigma-like product of sporulation gene *spoIIAC* of *Bacillus subtilis* is toxic to *Escherichia coli*

M.D. Yudkin
Microbiology Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX

Summary. The amino-acid sequence deduced from the nucleotide sequence of the *spoIIAC* gene of *Bacillus subtilis* has been shown to be homologous to that of the sigma subunit of the *Escherichia coli* RNA polymerase (Errington et al. 1985). I now describe results that indicate that this gene can be cloned in *E. coli* only under conditions in which it is not expressed.

purified 0.67 Kb *Eco*RI-*Bg*
Fig. 1 (Yudkin et al. 1985).

This difficulty in cloning might have been due to some such as inverted repeats (Lea tively it was possible that on is toxic to *E. coli*. To distin

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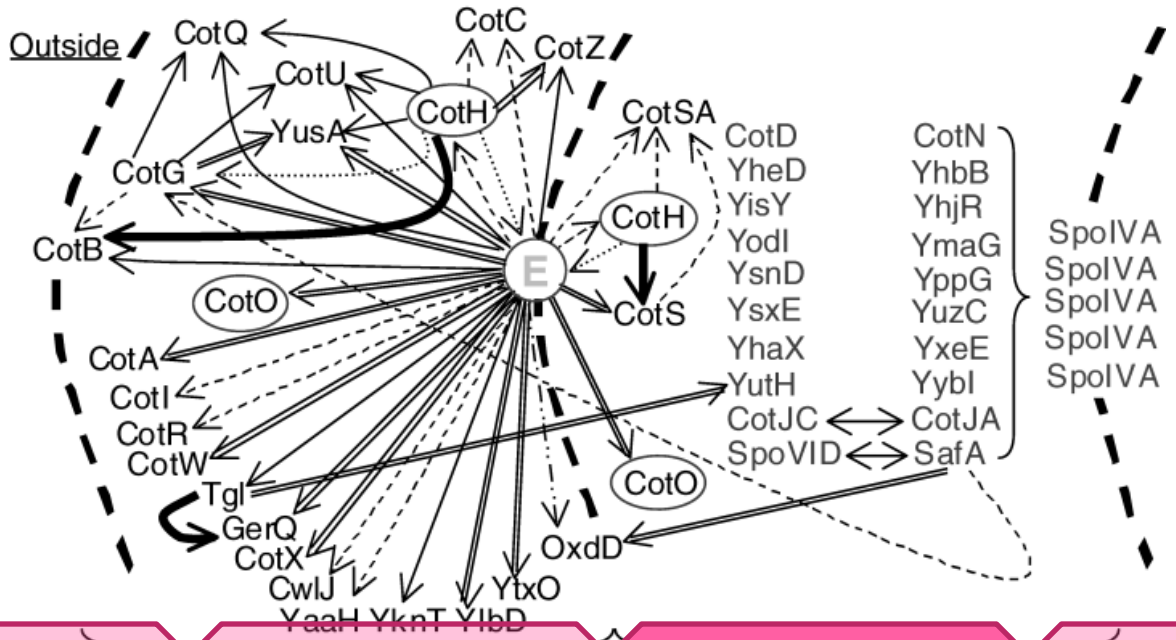
The Protein Coat

- Main chassis – Bacillus subtilis
  - easiest to manipulate & well characterised
- Number of transcription factors regulate each stage
- Each has a number of operons that it influences



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Control

DBTBS Release 5

Transcription Factors | Regulated Operons | Motif Conservation | Mail

Search | Promoters | Transcription Factors | Submit | Advanced Search | About

Transcription factor: Spo0A

Factor type	LuxR/UhpA
SWISS-PROT	P06534
SubtiList	BG10765
Consensus seq.	TGTCGAA
Comment	a key bi-functional regulator to control developmental transcription activities. Increases its affinity after phosphorylation (phosphorelay system). Spo0F is required for the phosphorylation. Two-domain st called 0A box and can be located downstream of the initiation site. Often, two adjacent boxes are found. These listed sites might be viewed in its complementary strand.
Link to	**Phylogenetic profile**, Weight matrix, Motif alignment & Similar conserved hexameric motifs

Operon	Regulated Gene	Sigma	Regulation	Absolute position	Location	Binding seq.(cis-element)	Ex
metS	metS	None	Negative	ND	ND	ND	Molle V.
rapA-phrA	rapA	SigA	Negative	ND	ND	ND	Mueller DB RG Molle V.
abrB	abrB	SigA	Negative	45172..45199	+7:+34	ATTTTGTCGAATAATGACGAAGAAAAAT	Perego N and Biot 129-134: Strauch Fujita M Molle V.
dltABCDE	dltA	SigX	Positive	3951092..3951112	+1:+21	ATGTGATTGTCGAAAAAACGG	Perego N
cdd-era	era	None	Positive	ND	ND	ND	Minkovs
kinA	kinA	SigH	Negative	1469285..1469305	+13:+33	ATCTGTATATGTCGAAACACG	Fujita M
kinC	kinC	SigA	Positive	1517584..1517613	-30:-1	ATTATTTGTCGAAGAATGGTACAATAAGTA	Kobayas
sinIR	sinI	SigA	Positive	2551503..2551530	-50:-26	ACCATTCGACATCATTCTCGTTTTTTTT	Shafikha
spo0A	spo0A	SigH	Positive	2518110..2518123	-18:-5	TTTGTCGAATGTAA	Strauch
spo0A	spo0A	SigA	Negative	2518187..2518221	+38:+72	AATTTCATTTTTAGTCGAAAAACAGAGAAAAACAT	Strauch
spo0A	spo0A	SigA	Negative	2518219..2518265	-3:+20	AAAAGAAGATTTTTCGACAAAATGA	Strauch
spo0F	spo0F	SigH	Positive	3809035..3809072	-84:-51	CAAAAGAGAAAATGTCAGAAAATGTCGTAAAGTAGAC	Strauch

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Spore Survival

- Spores offers unique resistance properties

- Can survive under extreme conditions eg. excessive temperature, desiccation, & exposure to solvents & other noxious chemicals.

- Spore - ideal vehicle for delivery of heterologous antigens to extreme environments such as the GI tract.

Oral administration of a *Bacillus subtilis* spore-based vaccine expressing *Clonorchis sinensis* tegumental protein 22.3 kDa confers protection against *Clonorchis sinensis*

Zhenwen Zhou[a], Huimin Xia[a], Xuchu Hu[b], Yan Huang[b], Yanwen Li[b], Li Li[b], Changling Ma[b], Xiaoxiang Chen[b], Fengyu Hu[b], Jin Xu[b], Fangli Lu[b], Zhongdao Wu[b] and Xinbing Yu[b],

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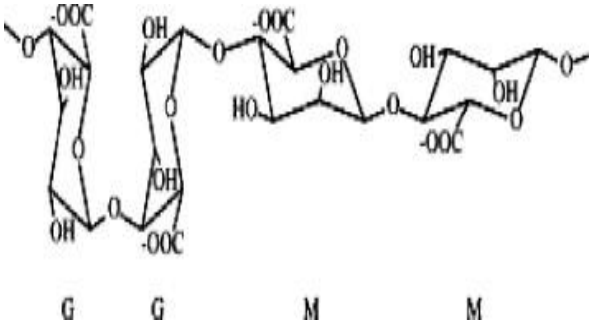
Germination

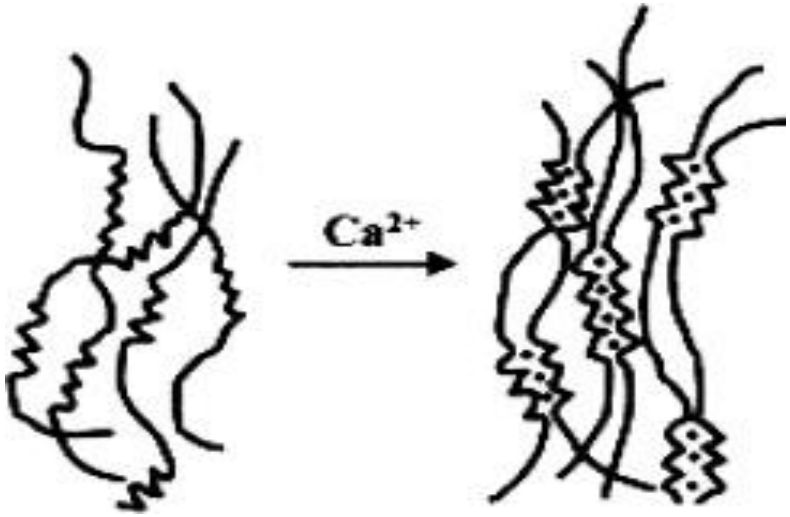
- Most common germinants
  - amino acids (L-alanine)
  - sugars
  - ribosides.

- Germinant penetrates the coat & cortex

- Interacts with a receptor complex located in the inner spore membrane

Alginate Encapsulation





form gels by reaction with divalent cations such as Ca^{2+}

Alginate encapsulation

- Alginate composed of β-D-mannuronic acid (M) and α-L-guluronic acid (G)
- Calcium ions are used to cross-link G-rich regions of the alginate chains
- Calcium Alginate (CaAlg) hydrogel beads are coated with crosslinkers to strengthen the bead surface and control permeability.

Benefits of using alginate

- Mild gelation conditions
- Biocompatibility
- Biodegradability
- Nontoxicity
- pH dependency

Method

- Bifidobacterial cells were centrifuged and added to alginate solution
- These were extruded to 0.1M calcium chloride through the end of a blunt needle using compressed air
- The cross-linking material were added
- The beads were gently stirred and hardened for an hour

1) Acid resistance

- Various materials can be used for coating:
- Polydextrose, soy fibre, skim milk, yeast extract, kappa- carageenen, chitosan and whey protein

- In one paper, skimmed milk exhibited highest resistance

Sphere diameter

- Spheres of diameter 1.5mm formed
- Corresponded to previous study that gel diameters of 1-3mm needed to protect bifidobacteria at gastric pH

Encapsulation in alginate-coated gelatin microspheres improves survival of the probiotic *Bifidobacterium adolescentis* 15703T during exposure to simulated gastro-intestinal conditions

N.T. Annan[a], A.D. Borza[a] and L. Truelstrup Hansen

Reasons for acid resistance

1) The carboxy charges of polymeric matrices neutralises acidity

- Moderate protection (22–26 %) afforded by native alginate beads seems related to D-mannuronate carboxylate groups to intercept proton access

2) Alginate is converted to alginic acid with release of calcium ions

2) Attachment

- The mechanism of sustained drug release is attributable to the fact that alginate is a mucoadhesive polymer
- Increased gastrointestinal residence time improves in drug bioavailability
- The ability of chitosan to modulate the intestinal tight junctions is an added virtue, which helps the encapsulated drugs in crossing the permeability barriers

3) Release

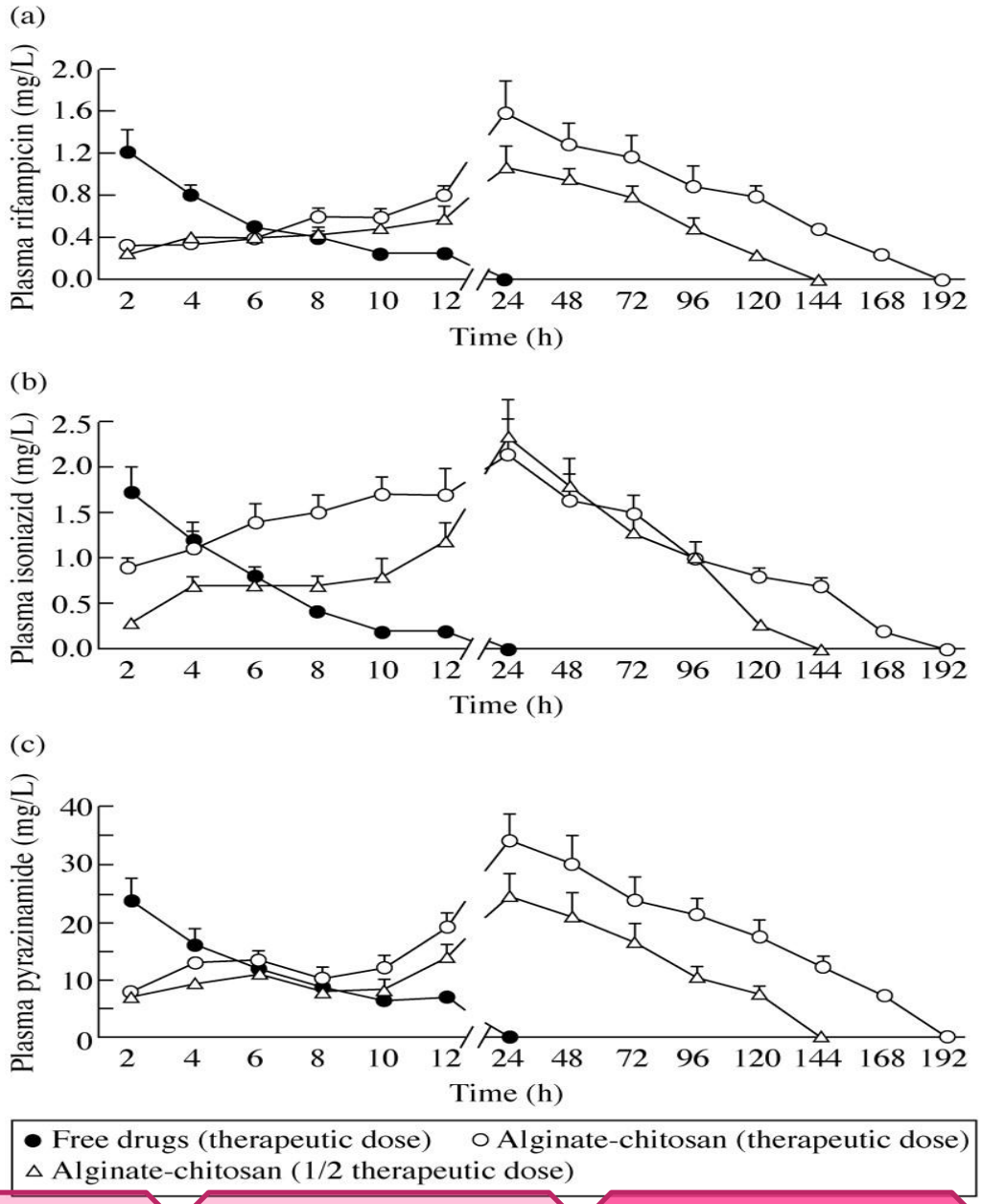
- Nominal release (less than 7% of the encapsulated drug) in the SGF throughout the 72 h study period.
- SIF in the initial 6 h:

Rifampicin (16%)

isoniazid (20.6%)

pyrazinamide (22.1%)

- Subsequently, there was a slow but sustained release of each drug, limited to less than 3% of the encapsulated drug



Chemotherapeutic application of Alginate-chitosan microspheres as anti-tubercular drug carriers
Rajesh Pandey and G. K. Khuller

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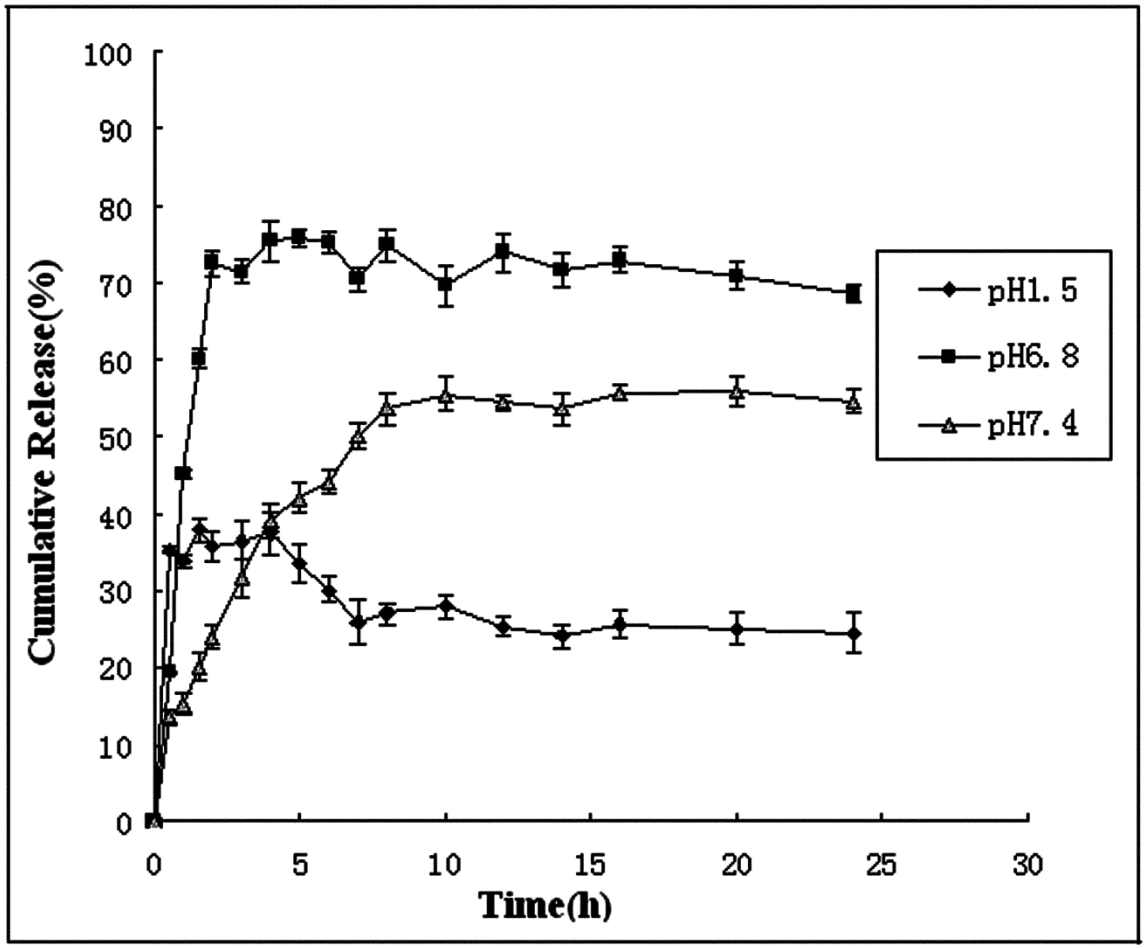

Chitosan–Alginate Nanoparticles as a Novel Drug Delivery System for Nifedipine

Pin Li1, YiNi Dai1, 2, Jun-Ping Zhang3, Ai-Qin Wang 3, Qing Wei1

- Drugs encapsulated in alginate–chitosan microspheres attained Cmax at 24 h as against 1 h in the case of orally administered parent drugs.
- In case of free drugs, the Cmax was achieved instantaneously
- The sustained release allows a reduction in dose/dosage frequency

Control of rate

- Polycationic macromolecules such as chitosan not only stabilize the alginate microspheres but also control the porosity of alginate to enhance the sustained release effect

Reasons for release

- A decrease in the pH leads to shrinkage in the alginate gel and a reduced permeability of the alginate–chitosan microspheres
- In a neutral/alkaline medium, the interpolymeric complex swells and disintegrates to release the drugs,
- Assisted by the sequestration of calcium ions by the phosphate present in the SIF

Induction?

- Calcium efflux in intact cells is coupled to the proton motive force via secondary calcium-proton exchange.
- CaxP, the first Ca^{2+} exporter reported in Pneumonia

- **Calcium efflux is essential for bacterial survival in the eukaryotic host**
- Jason W. Rosch, [1] Jack Sublett, [1] Geli Gao, [1] Yong-Dong Wang [2] and Elaine I. Tuomanen [1]*

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Quality Control - Encapsulation

- Optimum thickness of capsule → determined by conditions and time capsule will be subjected to, in the digestive track
- Uniformity of capsule
- Porosity and density of capsule
- Bacteria must be dead in the capsule
- Release of medication → ideally constant rate over a long time
- Shelf life of drug produced (since dead bacteria is inside capsule)

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Quality Control – Killing mechanisms

- Timer → control of timer, how long do we want the bacteria to live?

Timer

- Potential applications in Modules 1, 2, 3 & 5.
- Number of existing timers explored by previous iGEM teams (review in progress).

- Considerations for our timer:
  - Separate timer for each module vs. one timer that is 'continuous' between modules
  - Thresholds
  - Periodicity
  - Start/stop vs. oscillation vs. combination of both
  - Reset function
  - Pre-programmed function(s)

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Timer Specifications for Each Module

- **Module 1 – Compound:**

Induce production of compound at a specific time? - eg in response to something.

- **Module 2 - Encapsulation:**

Induce sporulation/encapsulation once desired threshold of compound X has been produced.

Module 3 - Killing Strategy:

Induce death after encapsulation.

Have a preset timer that induces death once the bacterium is encapsulated? (Chiba'08)

Induce death immediately - would a timer be required? Why not just use a promoter? We could set a threshold rather than using a timer mechanism.

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Programmed Plasmid Deletion

- As our bacteria are to be ingested, we wish to destroy all genetic material in the chassis.
- This is for a number of reasons:
  - To prevent any pathogenic/ antigenic response.
  - To prevent recombination with other bacteria
  - So live GM bacteria are not being eaten (acceptance).

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Criteria for Mechanism

- Should trigger after encapsulation
- Time taken for genetic material to be completely destroyed should be well characterised.
- Ideally would like a failsafe mechanism to ensure all material destroyed.
- Effectiveness of each method should be assessed, so the probability of any bacteria surviving can be calculated.

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Trigger for Cell Death

- Chassis and application dependant
- Number of Possibilities:
  - Hypoxic conditions from encapsulation
  - Timer mechanism for cell death
  - Environmental sensing mechanism
  - RNA thermometer *(BBa_K115020, TUDelft)*
  - External Factors

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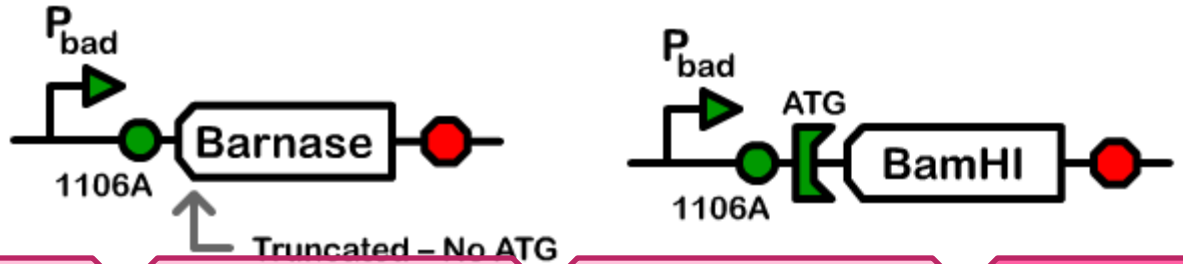
Berkeley '07 – Bactoblood

- Self destruct to remove genetic information whilst leaving cell membrane intact.
- Uses a plasmid with plasmid that can be translated to produce a toxin.
- Toxins are endonucleases/ RNAses



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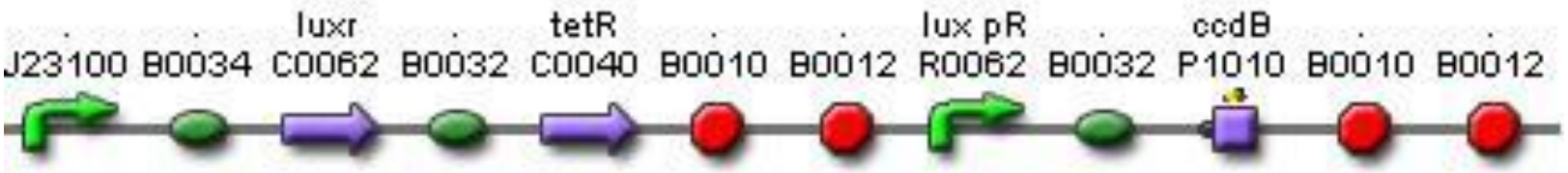
KU Leuven – Dr. Coli

- Programmable Self Destruct Mechanism.
- Uses CcdB as the toxic product, and expression is controlled by luxr gene.



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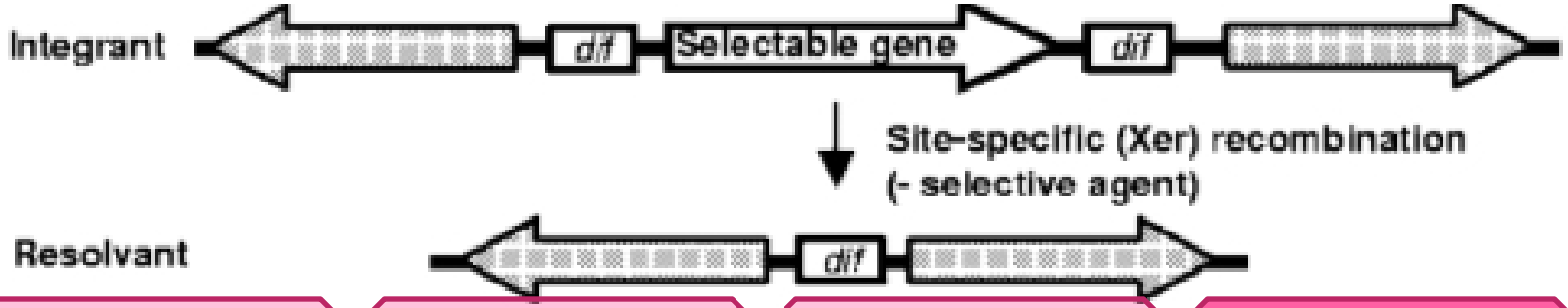
Our Method i)

- Using recombinase sequences to excise necessary parts of the genome.
- Thus removing possibility for viability of cell.
- E-coli: XerC and XerD
- B. Sub: CodV and RipX



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Our Method ii) BioBrick Schematic

- E. Coli: inducible promoter + restriction enzyme

- B. Subtilis: stress inducible promoter + restriction enzyme

σB inducible promoter

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Our Method

- Small Cutters – Would cut the genome in little pieces.

EcoRI

G A A T T C
C T T A A G

- Large Cutters – Would cut a specific gene

AsuHPI

G G T G A n n n n n n n n n
C C A C T n n n n n n n n n

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Modular Testing

- Self destruct signal given by transcription factor (Xylose or σB inducible promoter)
- Promoter must be non-leaky (could also use repressor in combination).
- Should destroy all genetic material rapidly