

**Development of a Recombinant Bacteriophage
for the Concentration and Colorimetric
Detection of Escherichia coli O157:H7**

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Advantages of a phage-based detection system

- **Only live cells detected**
- **Reporter phenotype detection simple and inexpensive**
- **Phage can be used for capture and concentration**
 - **Irreversible binding**

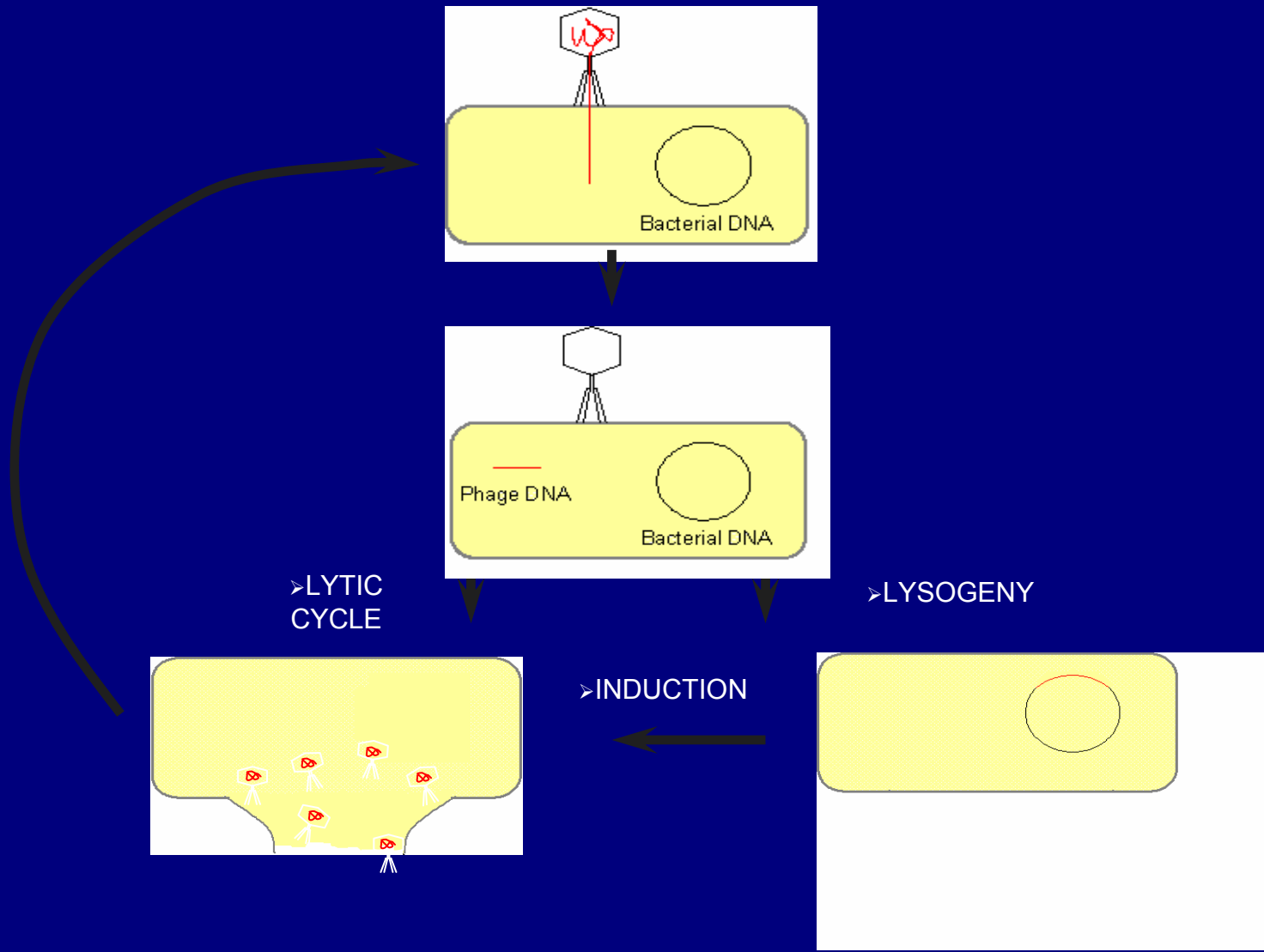
Important properties of Φ V10

- Available
- Specific for *E. coli* O157:H7
- Plaques on majority of O157:H7 strains
- Lysogenic
 - Easy to manipulate genetically

$\Phi V10$



Life Cycle of Tailed Phages



Key Scientific Hurdles

- **Construct *E. coli* O157:H7 detecting phage by:**
 - Replacement of targeted non-essential phage genes with reporter genes, resulting in a functional and packagable phage genome
- **Propagate reporter phage from non-O157 strain**
 - Introduce phage DNA into non-pathogenic strain by transformation, yielding a lysogen which can be induced to produce phage

Key Scientific Hurdles

- **Immobilize Phi V10 on plastic:**

- Attach the reporter phage to a plastic surface by a process which does not affect phage function

- **Develop a rapid method for phage purification:**

- Currently optimizing protocol utilizing scalable HPLC method

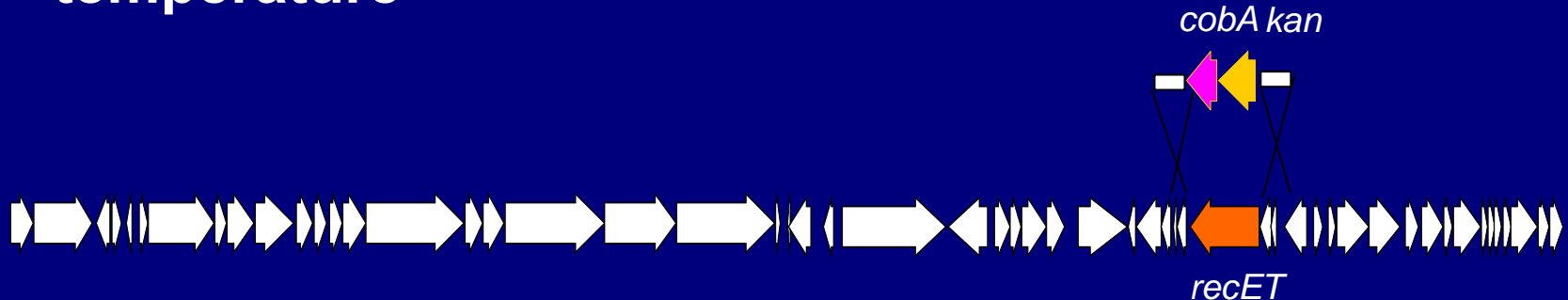
cobA reporter

- Uroporphyrinogen III methyltransferase from *Propionibacterium freudenreichii*
- High level expression of *cobA* leads to accumulation of a red fluorescent compound

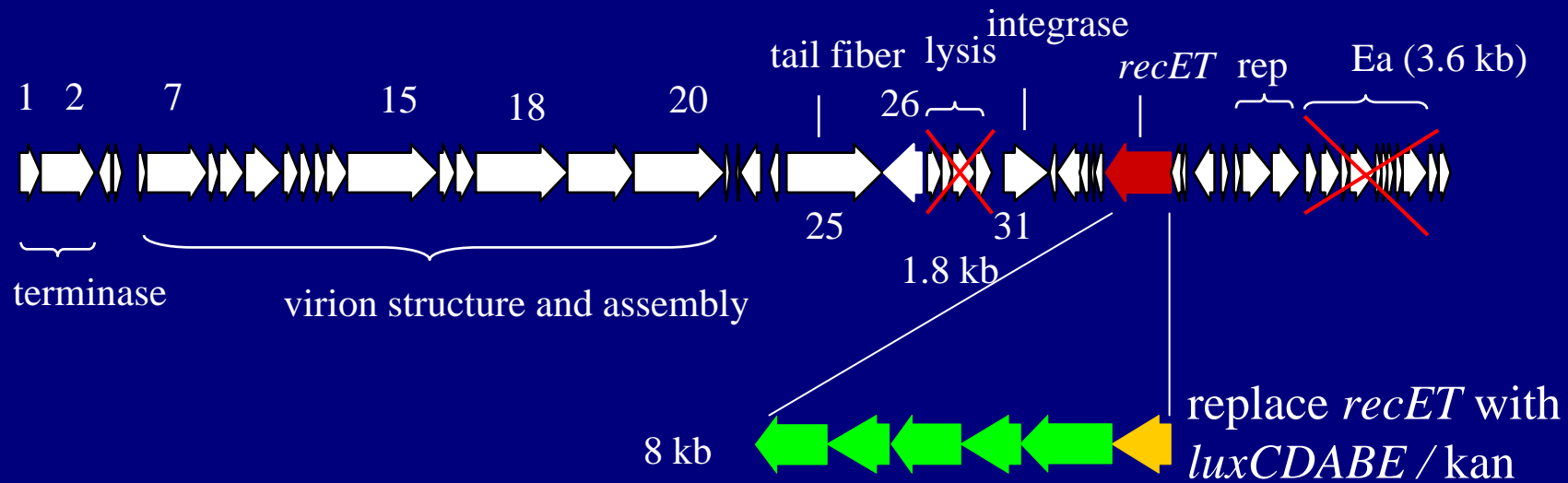


Replacement of *recET* with *kan-cobA*

- Transform Φ V10 lysogen with temperature-sensitive plasmid pKD46 carrying inducible λ recombination genes (Datsenko and Wanner. 2000. Proc. Natl. Acad. Sci. U. S. A. 97:6640-6645)
- Induce recombination genes and transform with PCR product (*kan-cobA* flanked by *recET*-flanking Φ V10 sequence)
- Select on kanamycin
- Eliminate pKD46 by incubation at non-permissive temperature



Future modifications



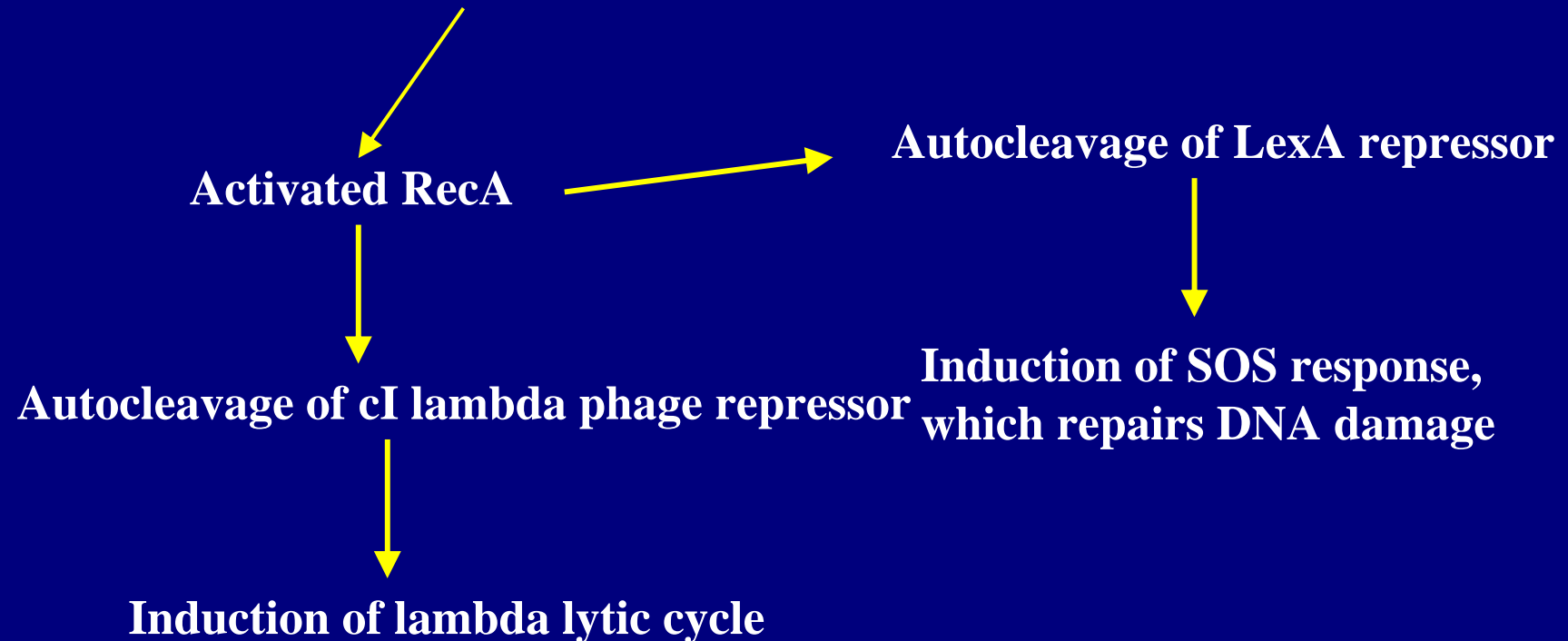
Φ V10 lysogen of *E. coli* Top10

- Top10 strain transformed with Φ V10 kan Δ recET DNA
- Clone isolated, insertion into normal attachment site confirmed by PCR and sequencing
- Supernatant from an *E. coli* O157:H7 strain lysogenic for Φ V10 grown to OD 1.0 normally contains 10^7 - 10^8 PFU /ml. Supernatant from Top10 lysogen contains no phage capable of infecting *E. coli* O157:H7
- Possible explanations:
 - Top10 is a *recA* strain
 - Induction may require O157:H7-specific factor
 - Restriction

Role of RecA in induction of lysogenic phages

In phage lambda, the major pathway of lytic induction is RecA-dependant. It occurs spontaneously at a low frequency, or in response to mutagenic agents at a high frequency

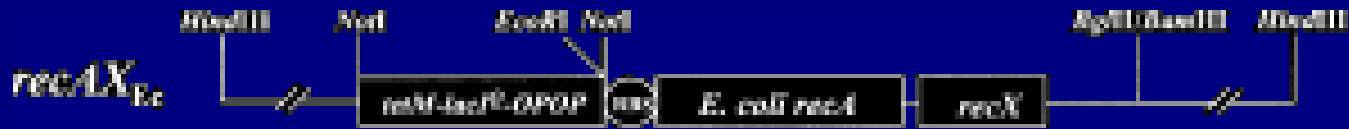
DNA damage (mitomycin C, UV light, etc)



Role of RecA in induction of lysogenic phages

- Induction of Φ V10 prophages in O157:H7, like lambda prophages, appears to be coupled to induction of the SOS response, although the coupling mechanism may work differently than in lambda.
 - Exposure to SOS-inducing mutagens (mitomycin C or UV light) increases the concentration of phage in the supernatant of a lysogenic culture
- Since induction of the SOS response is RecA-dependent, induction of Φ V10 prophages is likely to also be RecA-dependent

Restoration of RecA⁺ phenotype to Top10 Φ V10 lysogen



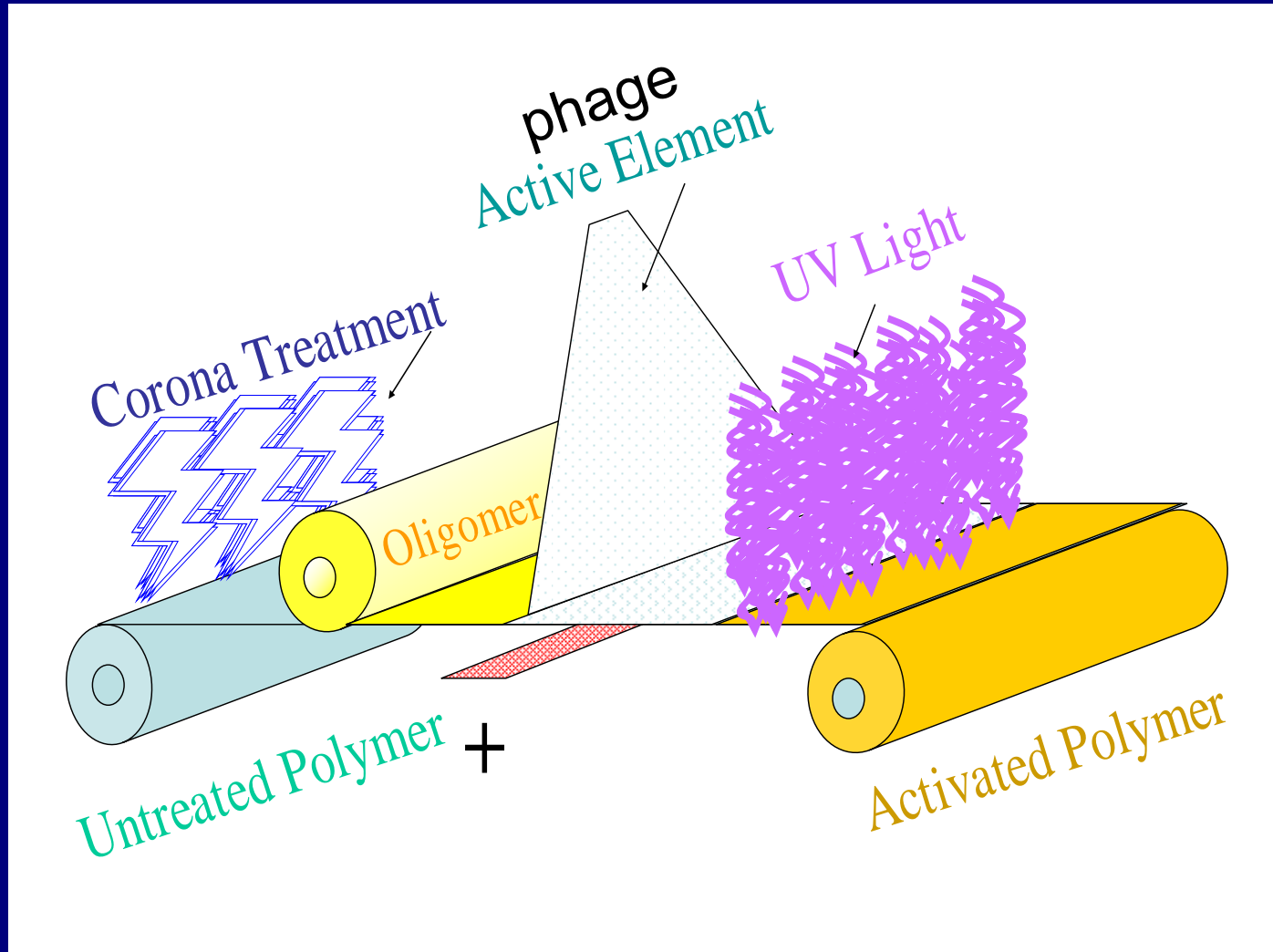
- Transformed *recAX_{Ec}* plasmid into Top10 Φ V10 lysogen
 - (Stohl et al, Microbiology (2002) 148:1821-1831)
- Induced *recA* and *recX* expression with IPTG
 - RecX is thought to be involved in regulation of *recA*
- Results: Phage were detected in the culture supernatant

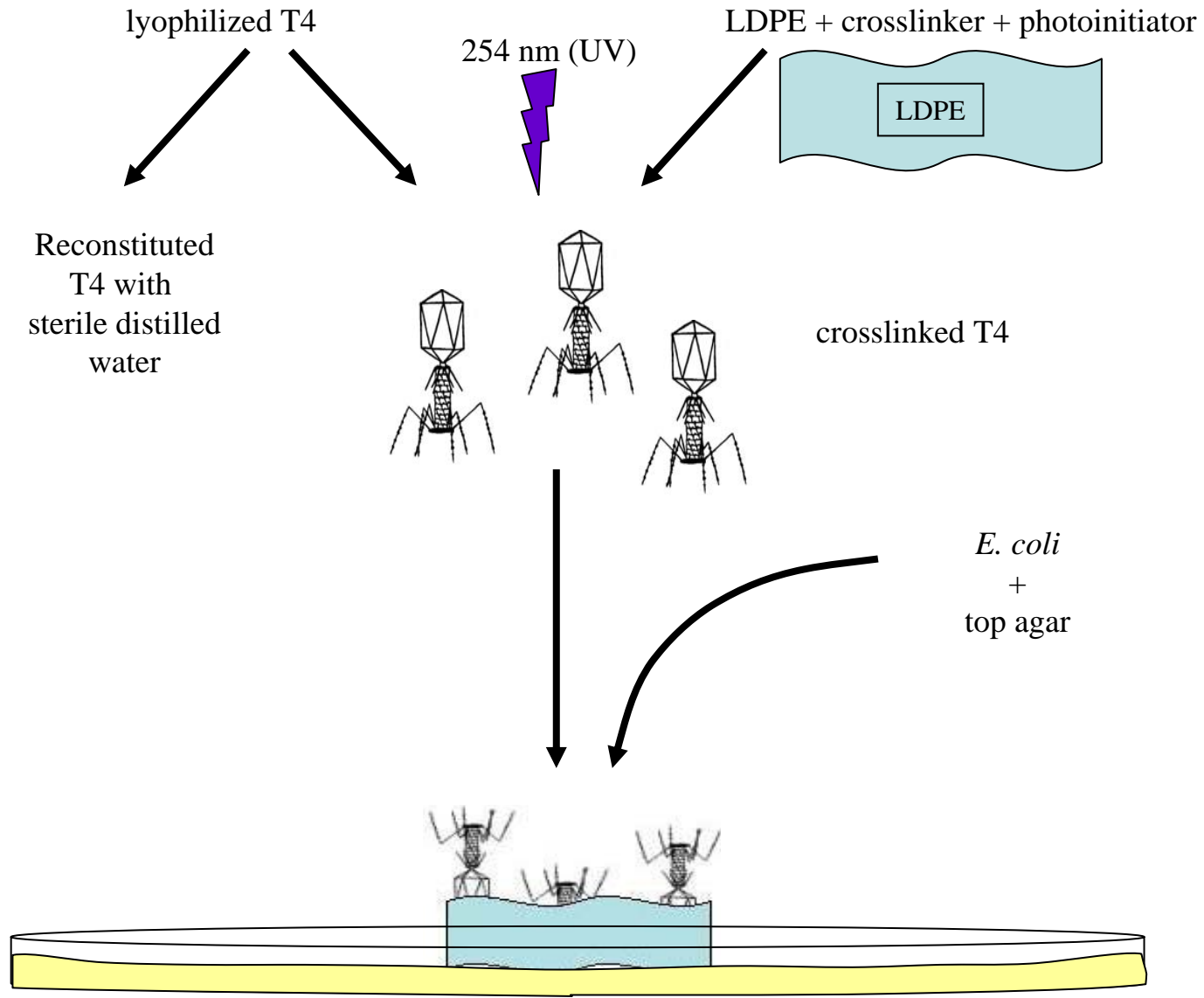
Spontaneous induction of Φ V10 prophages in different genetic backgrounds

Phage titers in supernatants of lysogenic cultures

Culture stage	O157:H7 lysogen	Top10 lysogen expressing RecA
OD 1.0	10^7- 10^8 CFU /ml	10 CFU /ml (only after UV exposure)
Overnight	ND	Estimated 10^4- 10^5 CFU /ml

Immobilization





Schematic representation of bacteriophage immobilization process.



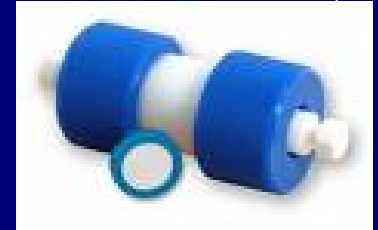
**Zone of *E. coli* Top 10 lysis
around T4 crosslinked LDPE**

**Negative control
LDPE with crosslinking treatment
and no T4 was also negative**

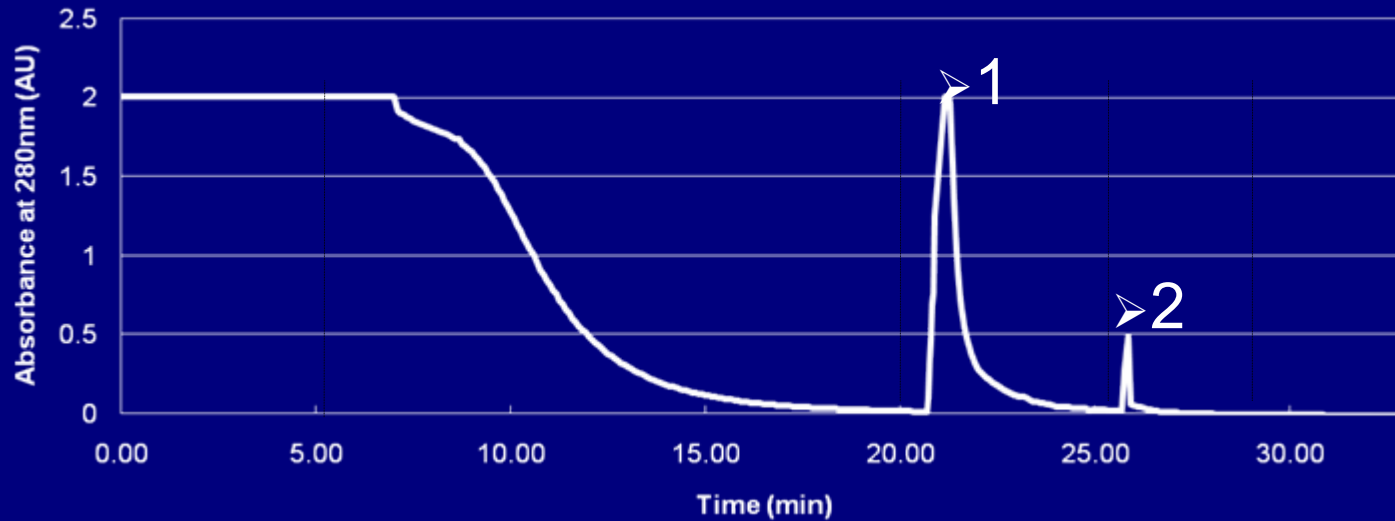
**Digital image showing the activity of immobilized phage on bacteria
(rough spots seen on the coupon show the immobilized phage)**

Phage Purification

- Smrekar et al. (2008) protocol
- Gradient column chromatography
- Stationary phase:
 - Anion exchange column (CIM-QA mololithic column)
- Mobile phase:
 - 125 mM Sodium Phosphate buffer
 - 1.5 M NaCl 125 mM Sodium Phosphate buffer
- Flow rate: 2ml/min
- Scalable



Phage Purification



TIME (min)	% BUFFER A	% BUFFER B
15	100	0
5	67	33
4	34	66
2	0	100



➤ 0.5 M NaCl

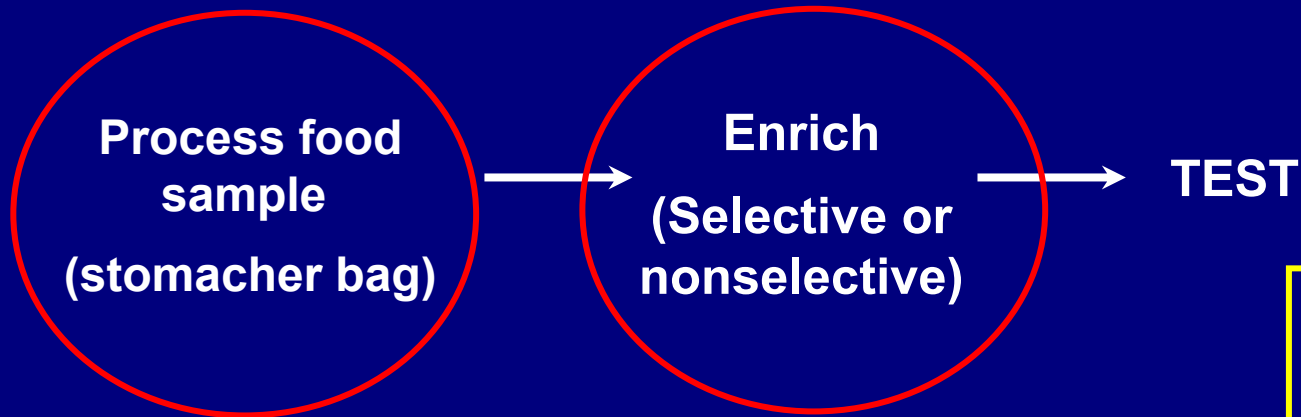


➤ 1.0 M NaCl



➤ 1.5 M NaCl

Food Testing Strategies



TEST

1. Standard plating culture techniques
2. Immunological testing (e.g. ELISA)
3. Real-time hygiene monitoring systems
4. Genetic testing (PCR)



*Test strip
format*

**BACTERIA ANIMATION
DR. BRUCE APPEGATE**

Summary/Future Work

- All of the individual components have been completed
- Currently evaluating sensitivity of recombinant *cobA* phage
- Working on large scale production procedure
- System can be tied into FTIR approach as a capture method to increase sensitivity and specificity due to ease of immobilization
- Due to our extensive knowledge of PhiV10 it may be possible to simply exchange regions of the genome to create specific phage for other pathogenic *E. coli* and *Salmonella*

Reporter Genes

- *lux* –large in size, require substrate addition
- *gfp* –lots of interference in the blue-green UV range
- *cobA* –bright red fluorescence