Identification of bacterial virulence factors

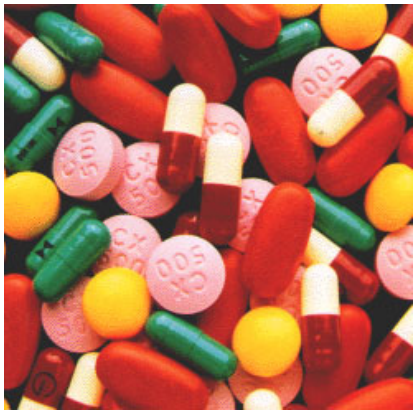
Learning objectives

You should be able to:

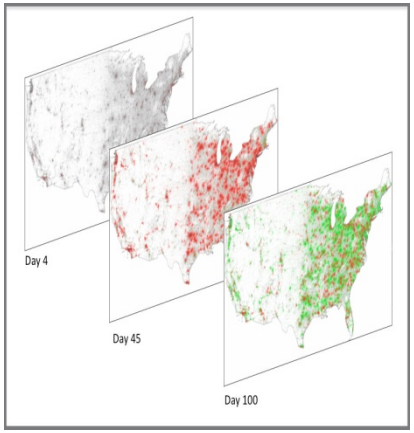
(1) Give examples of methods used to identify virulence factors

(2) Show how application of such methods can lead to new therapies

Why is understanding virulence important?



Antibiotics



Epidemiology

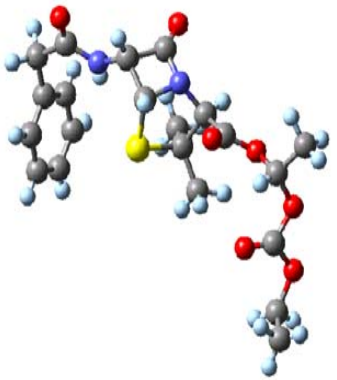
Vaccines

What type of virulence factor should we look for?

- Involved in host interaction
  - adhesion, invasion, toxins etc
- Essential
- Important for survival and/or expressed *in vivo*

Why look for genes important for survival or expressed *in vivo*?

- Antibiotic targets



- Vaccines
  - attenuated
  - subunit

- Understanding pathogenesis

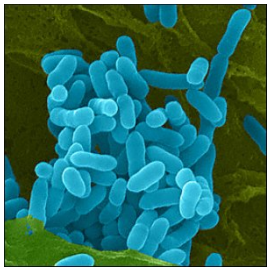
some methods

some examples…

(1) Screen for gain of function

(2) Screen for loss of function

Burkholderia pseudomallei



shear genomic DNA
and clone into a plasmid

Transform
E. coli

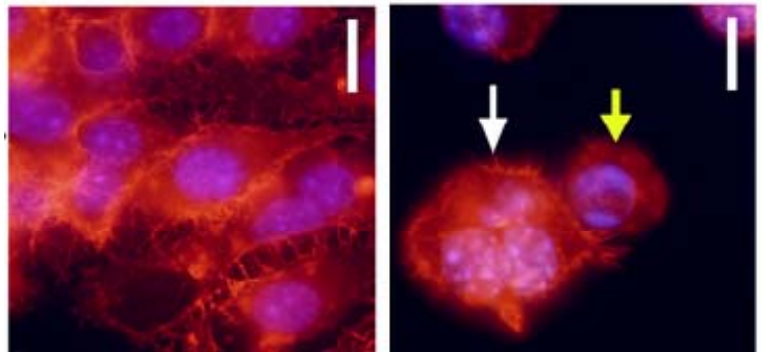
Grow each clone
in broth

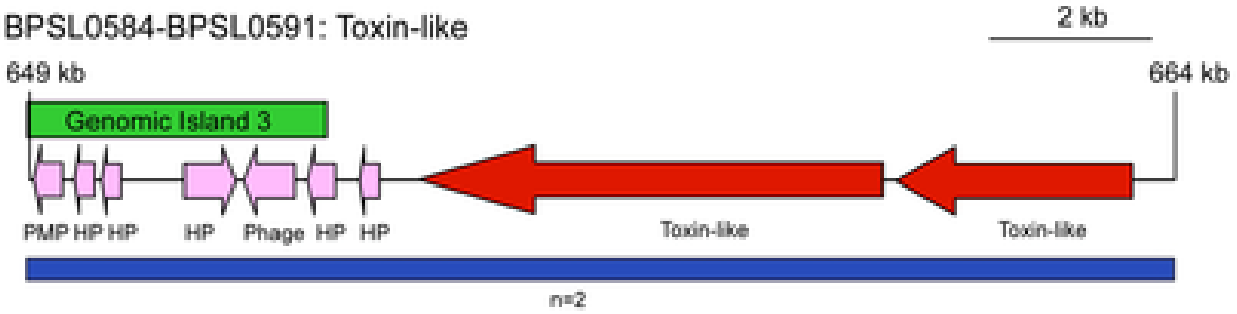
Test supernatant
for toxin activity

Determine
identity
of toxin

Dowling AJ et al. (2010) Genome-Wide analysis reveals loci encoding anti-macrophage factors in the human pathogen *Burkholderia pseudomallei* K96243. PLoS ONE 5: e15693.

Normal Toxin-like





(1) Screen for gain of function

(2) Screen for loss of function

Signature-tagged mutagenesis

Hensel M et al. (1995)

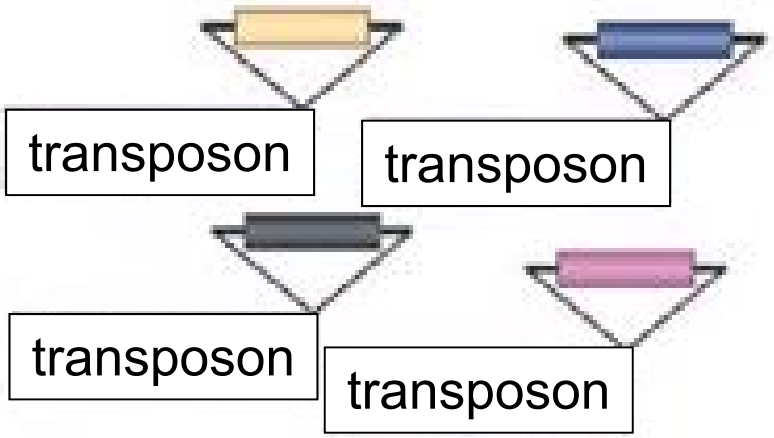
Simultaneous identification of bacterial virulence genes by negative selection

Science 269:400-403.

Transposons

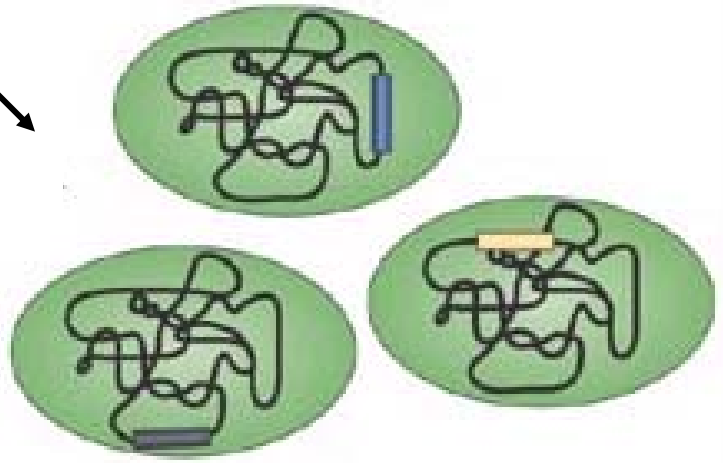
- Inverted repeats
- Antibiotic resistance
- Useful for making random mutants

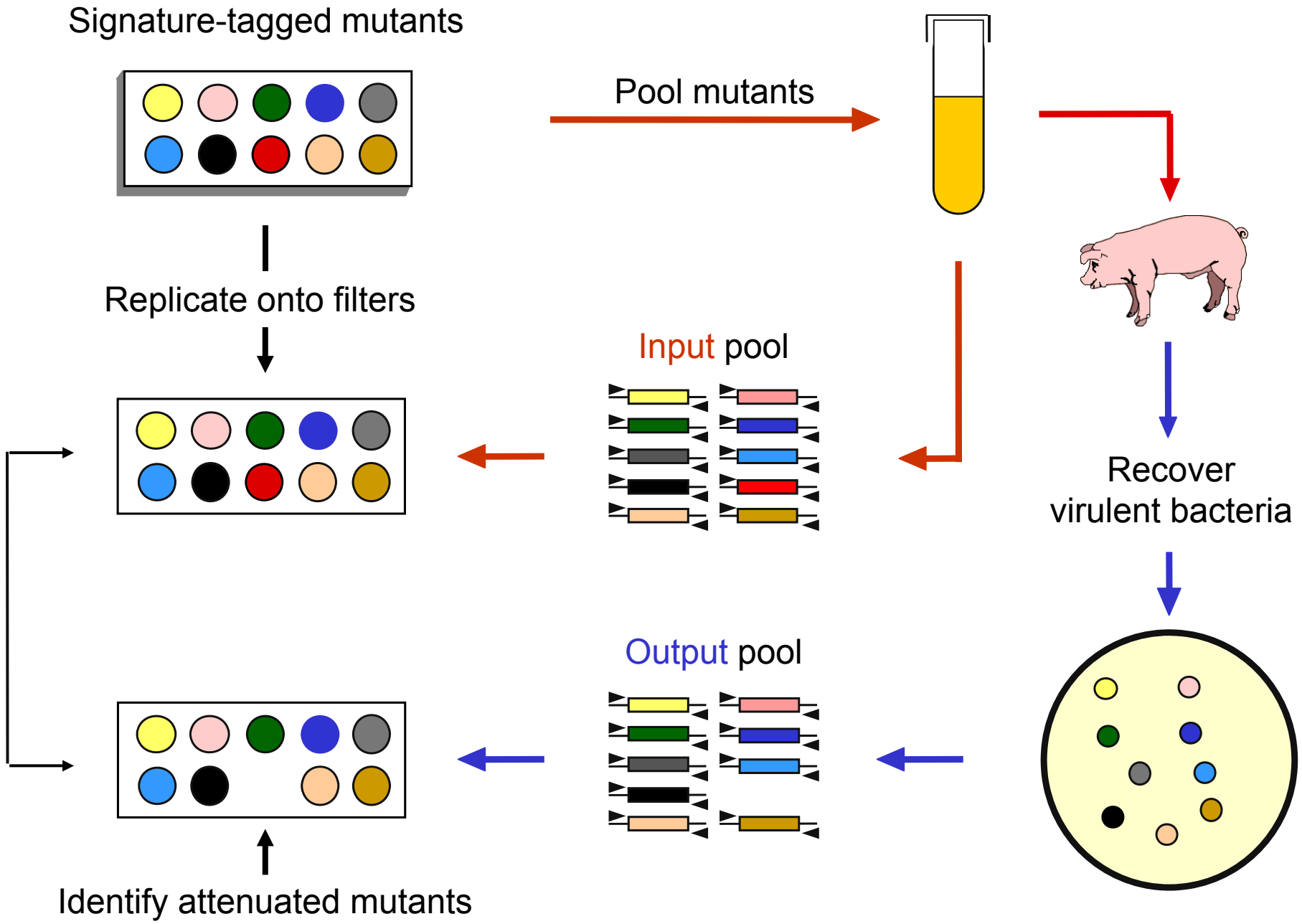
  e.g. Tn*10, mariner*



incorporate tags
into transposon

transposon mutagenesis
of bacteria

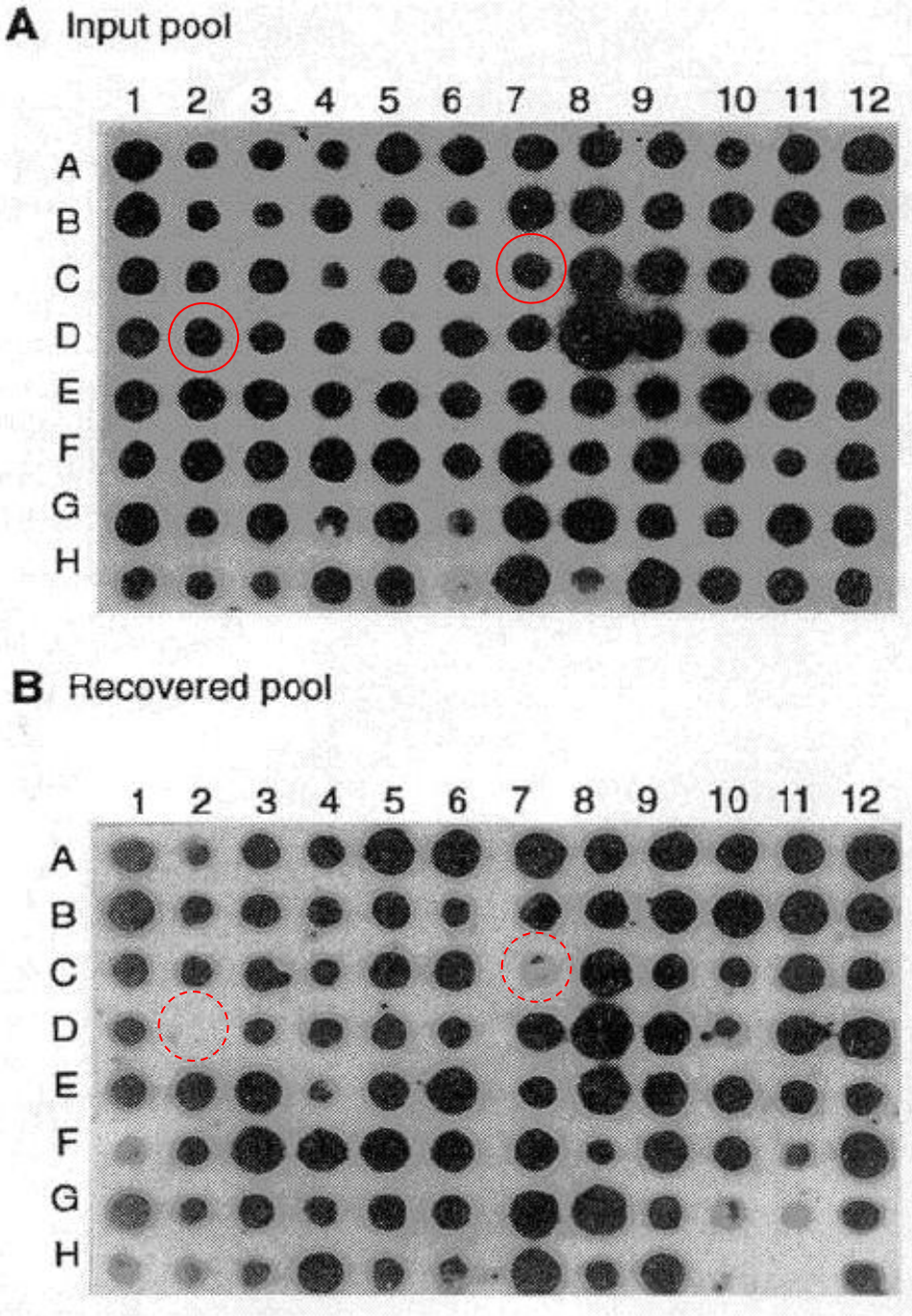



Signature-tagged mutants
Pool mutants
Replicate onto filters
Input pool
Recover
virulent bacteria
Output pool
Identify attenuated mutants

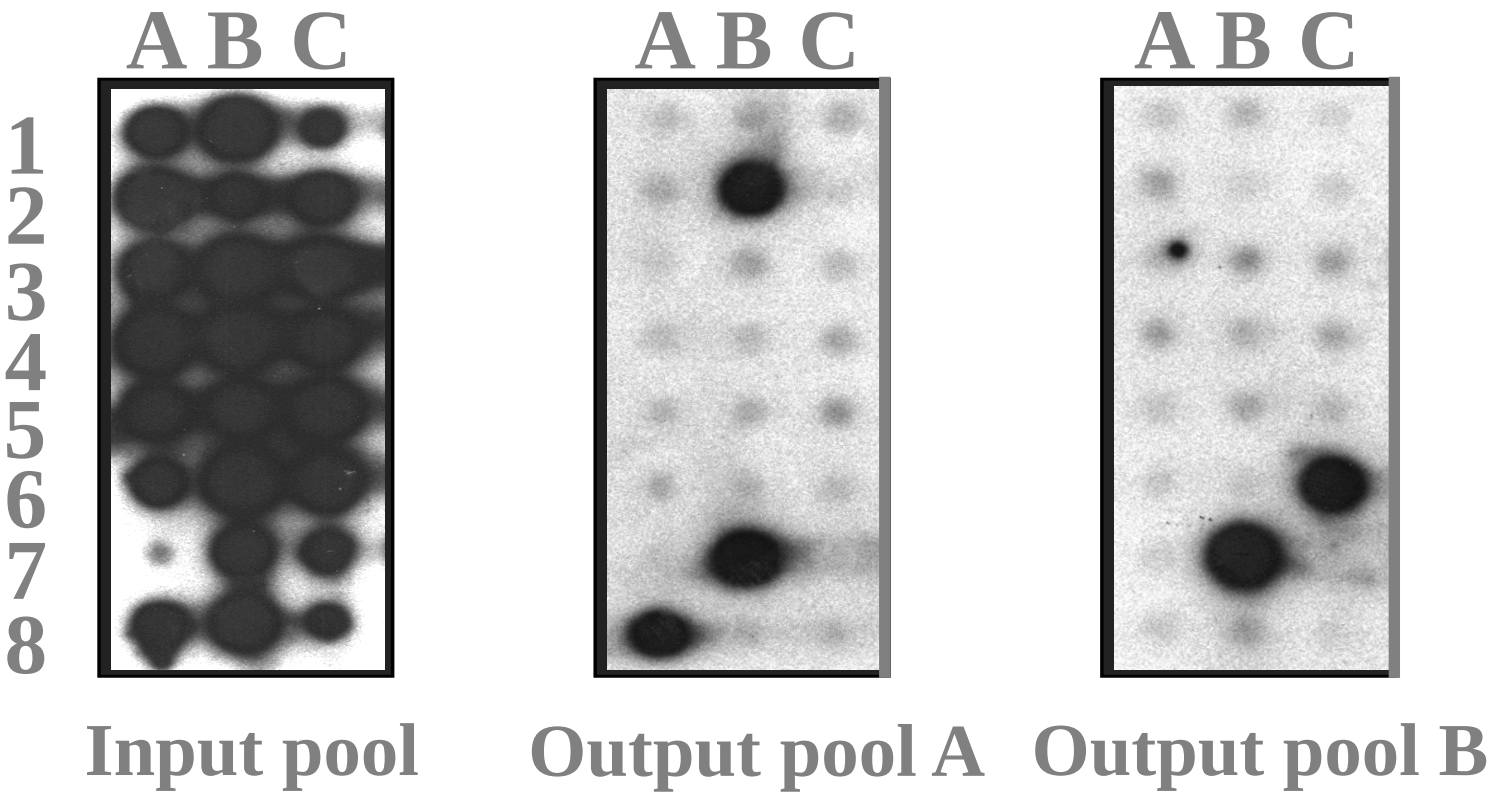
Salmonella typhimurium recovered from infected mice

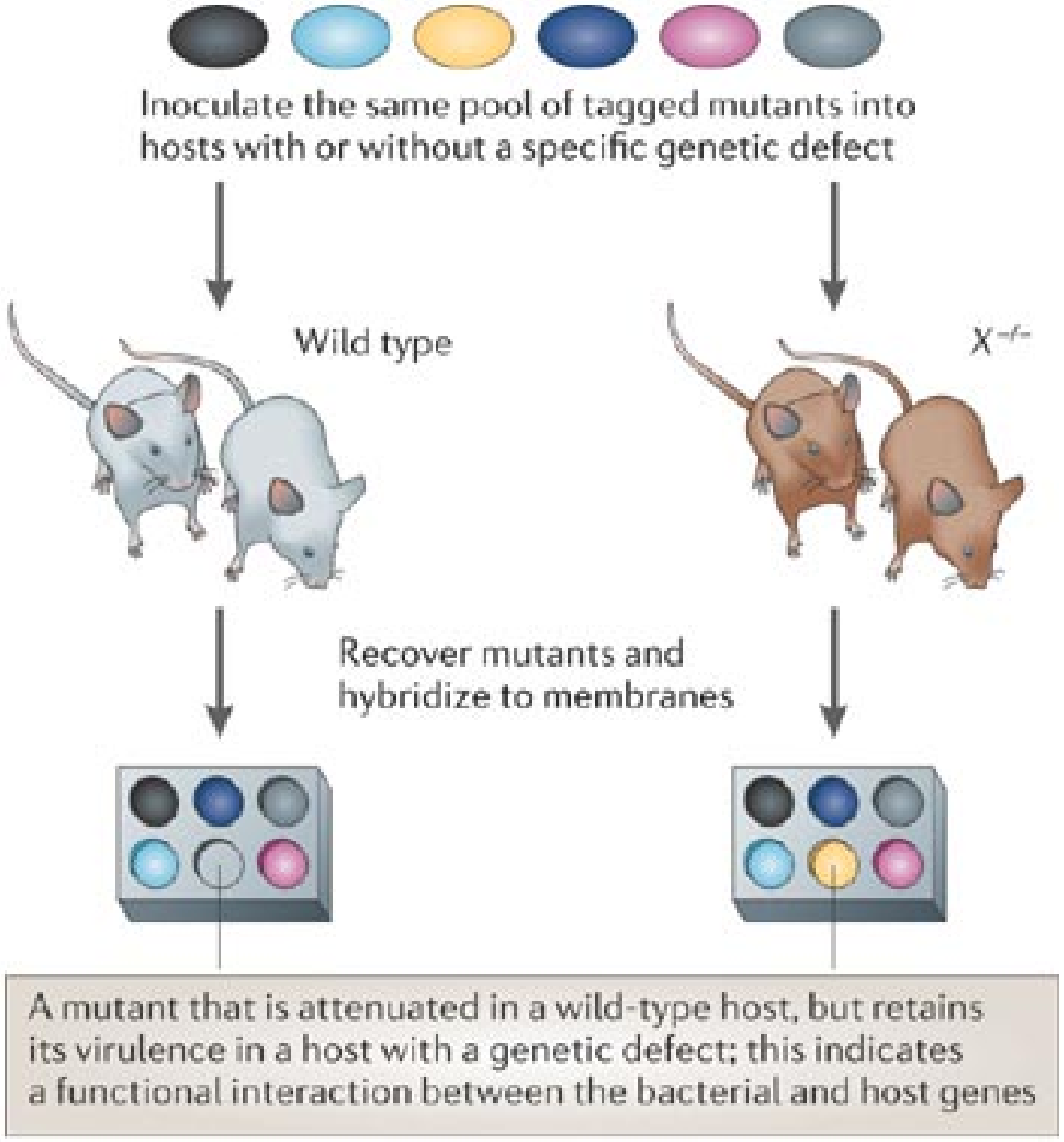
↓

Led to identification of T3SS



STM does NOT work if you have clonal expansion

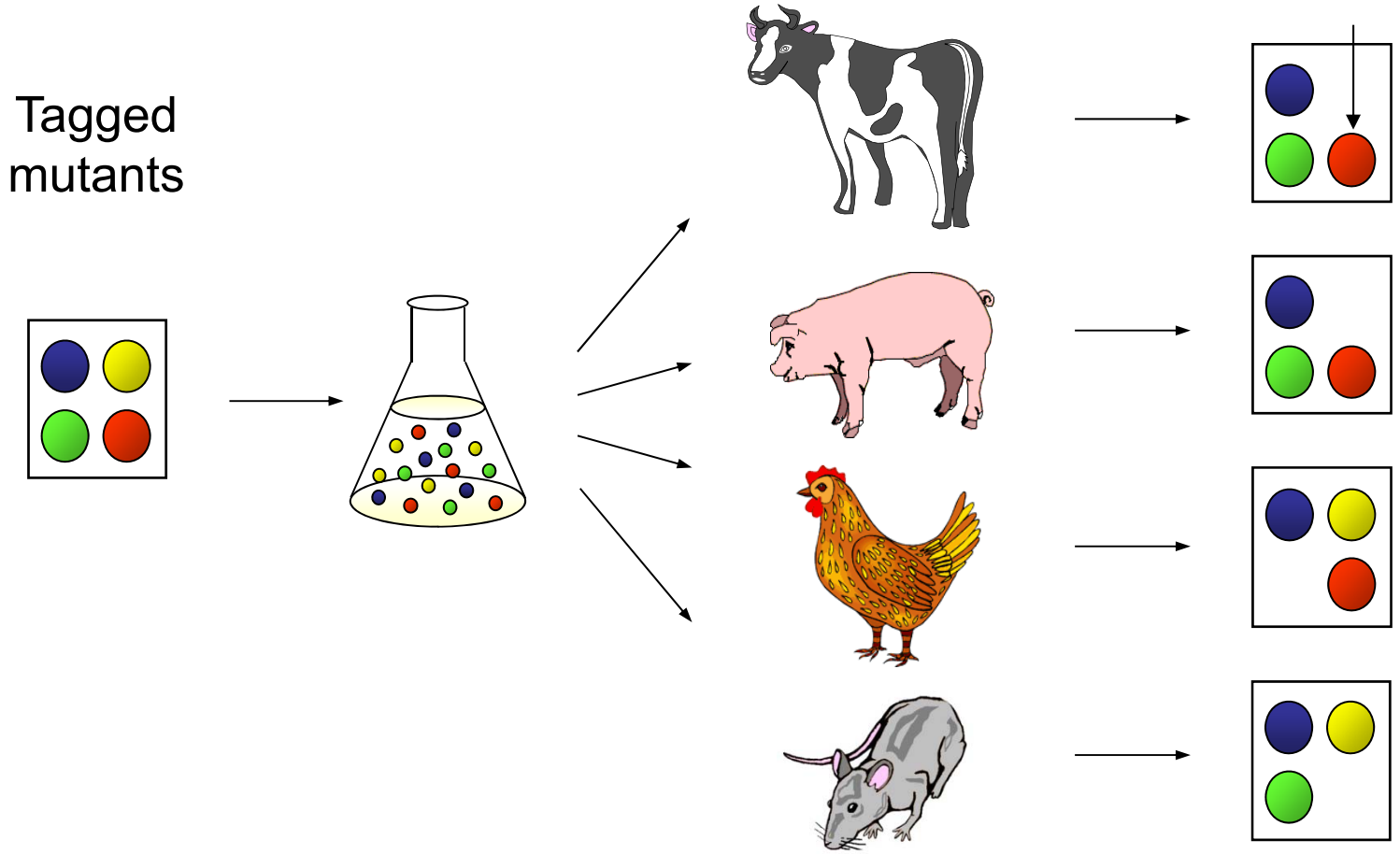




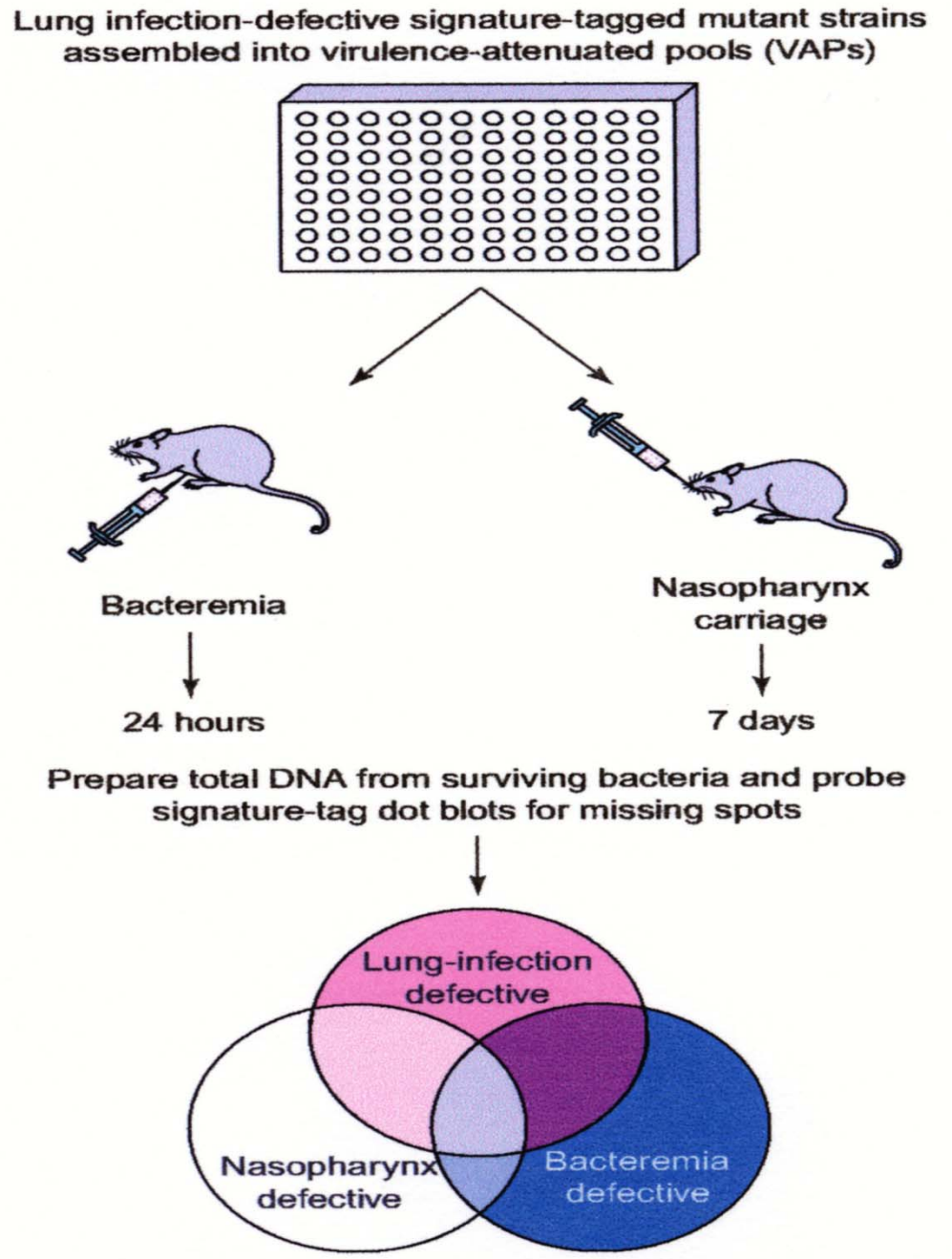
Mazurkiewicz et al. (2006) Nature Reviews Genetics **7**:929–939

Attenuated mutant

Tagged mutants

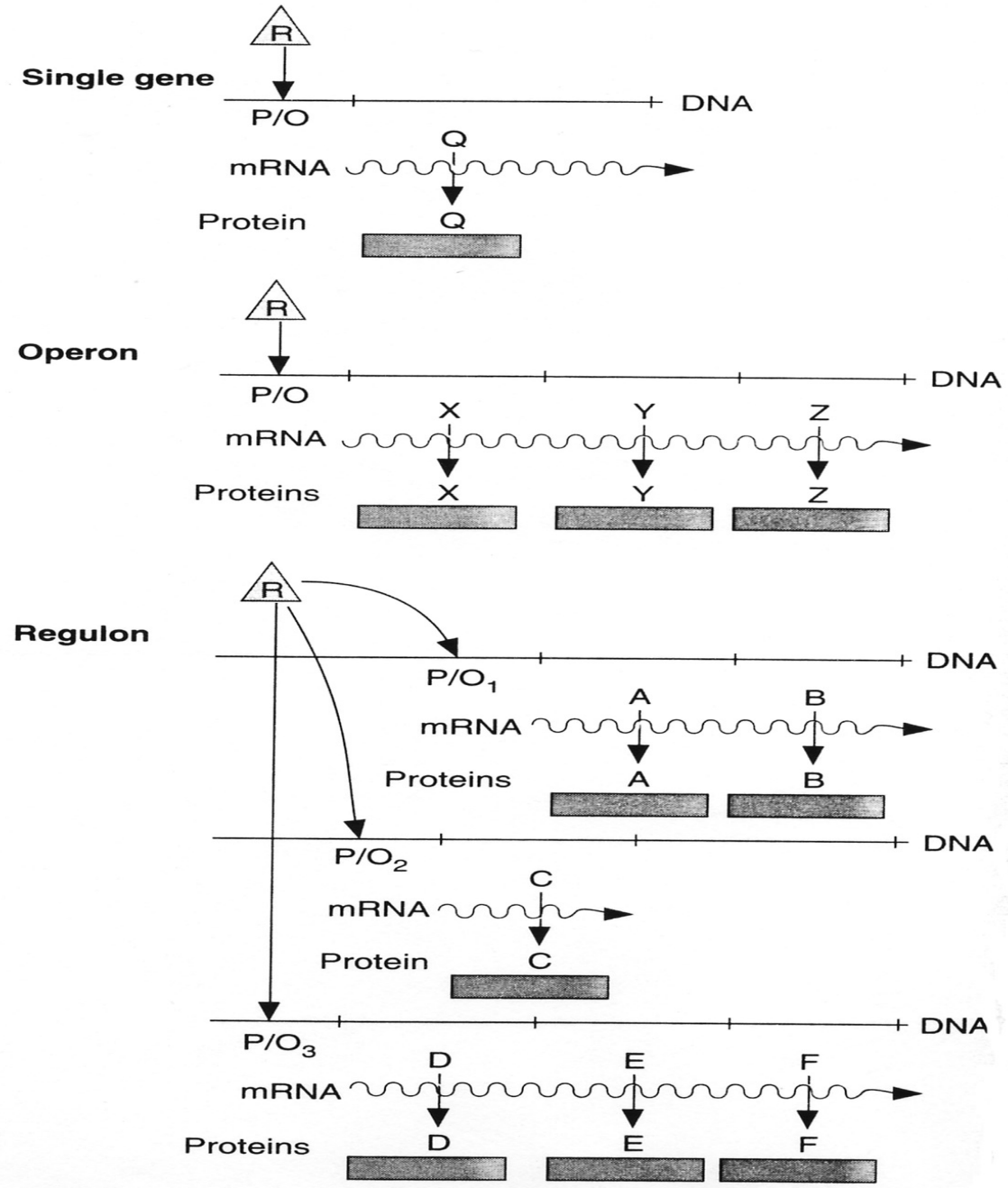


Virulence attenuated pools (VAPs)



Gene order is not always simple

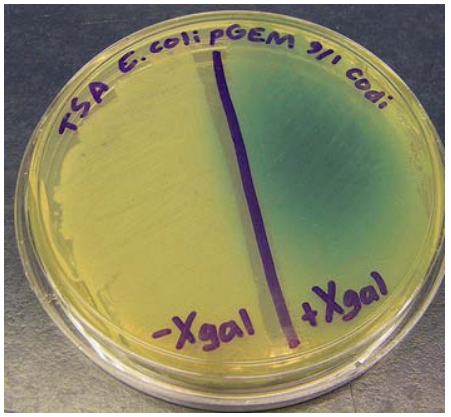
Care!



R, Regulatory protein or protein complex;
P/O, promoter-operator

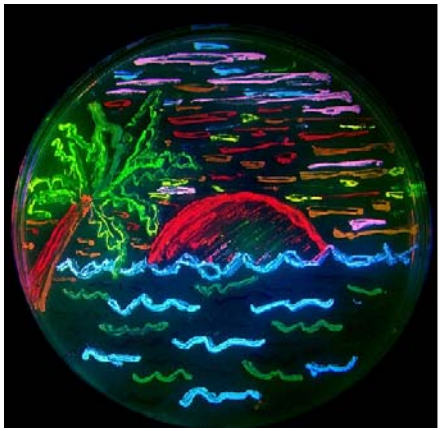
Reporter genes

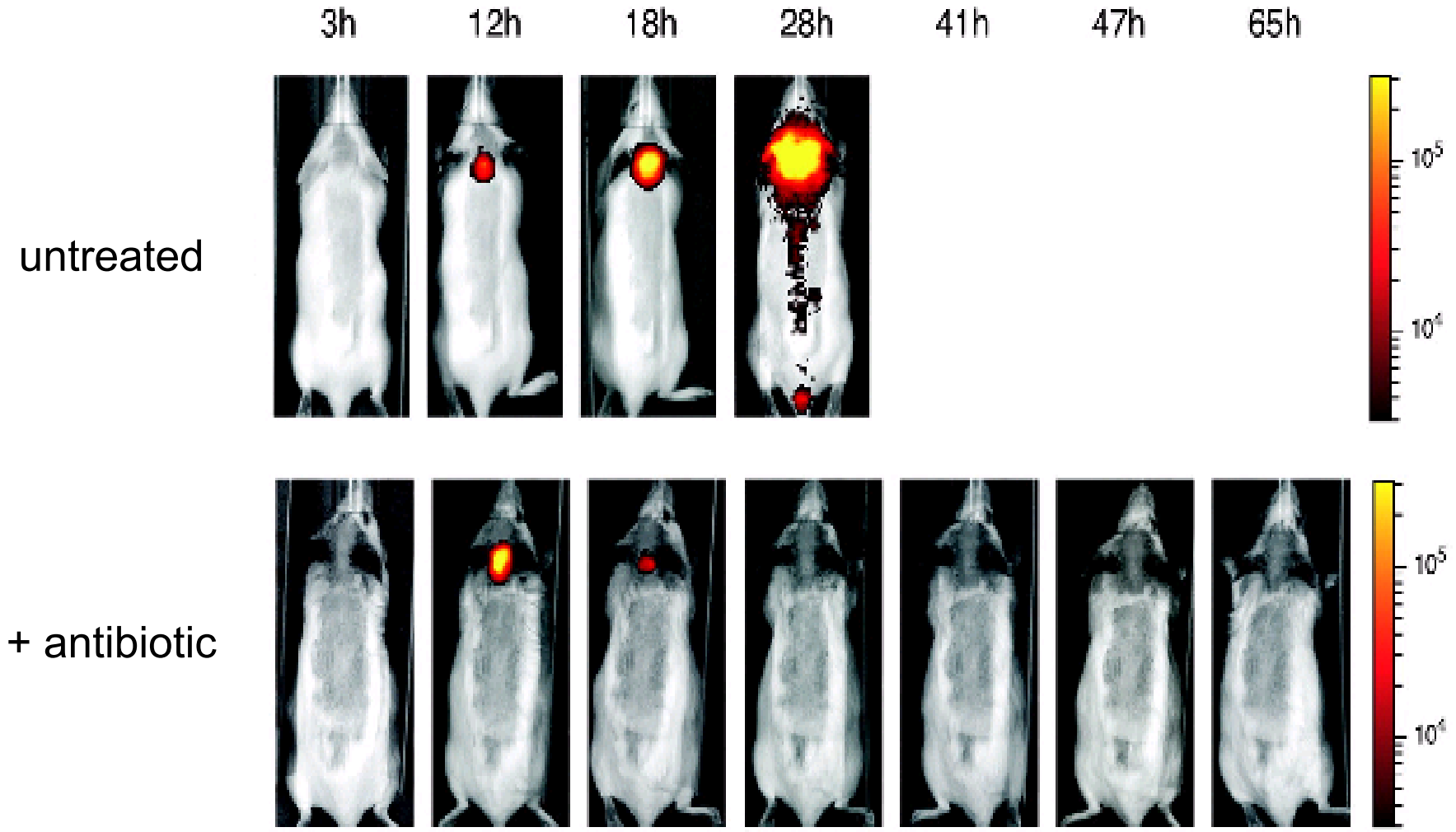
- Colour change and activity
  - β-galactosidase (LacZ)



- Light
  - Luciferase (Lux)

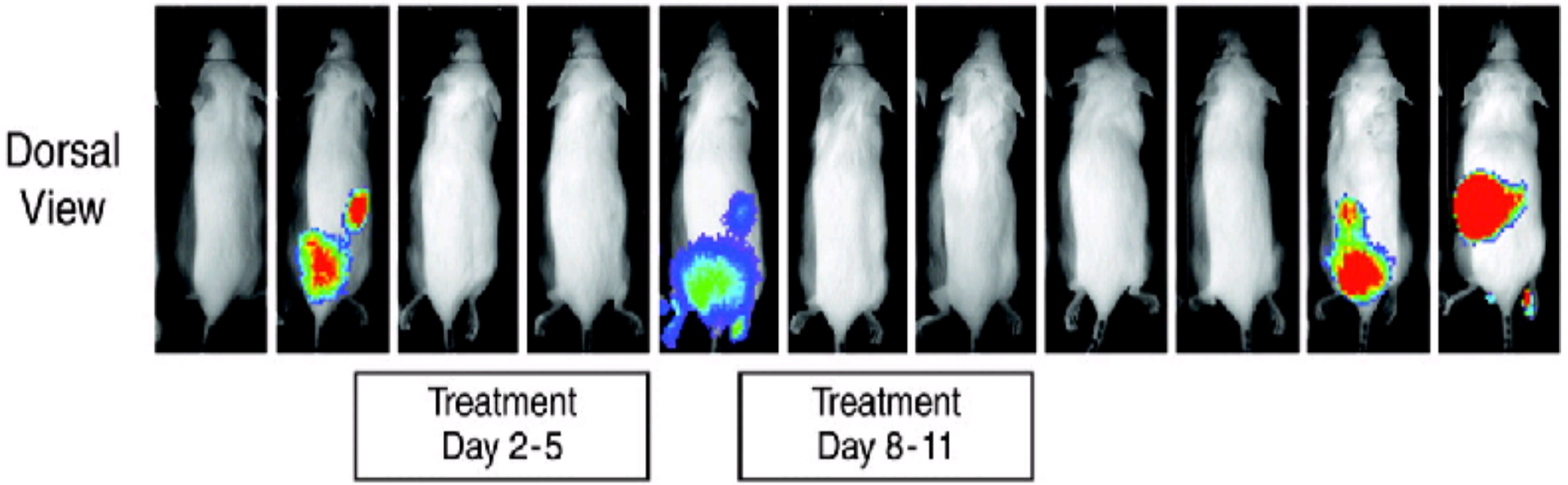
- Fluorescence
  - Green Fluorescent Protein (GFP)





S. pneumoniae expressing *lux*

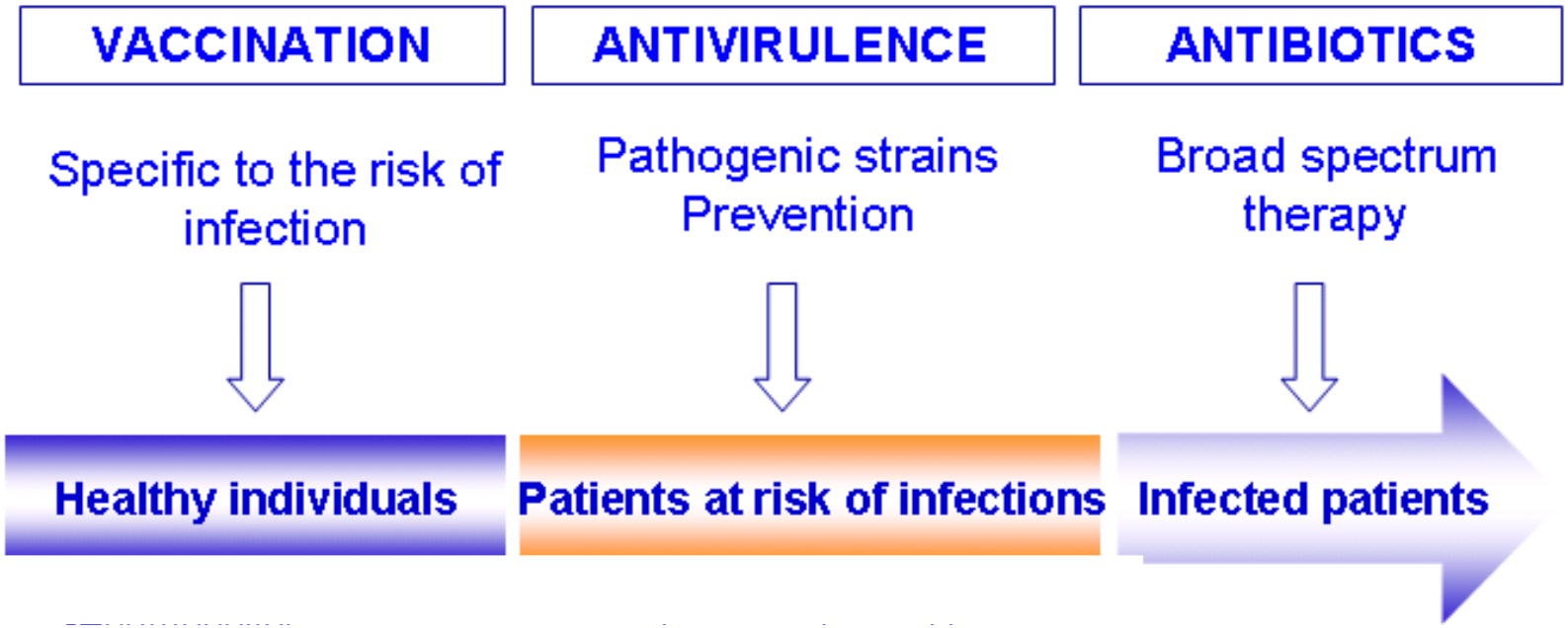
Kadurugamuwa JL et al. (2005) Infect Immun 73:7836-43



Proteus mirabilis expressing *lux* - catheter infection

Kadurugamuwa JL et al. (2005) Infect Immun 73:3878-87.

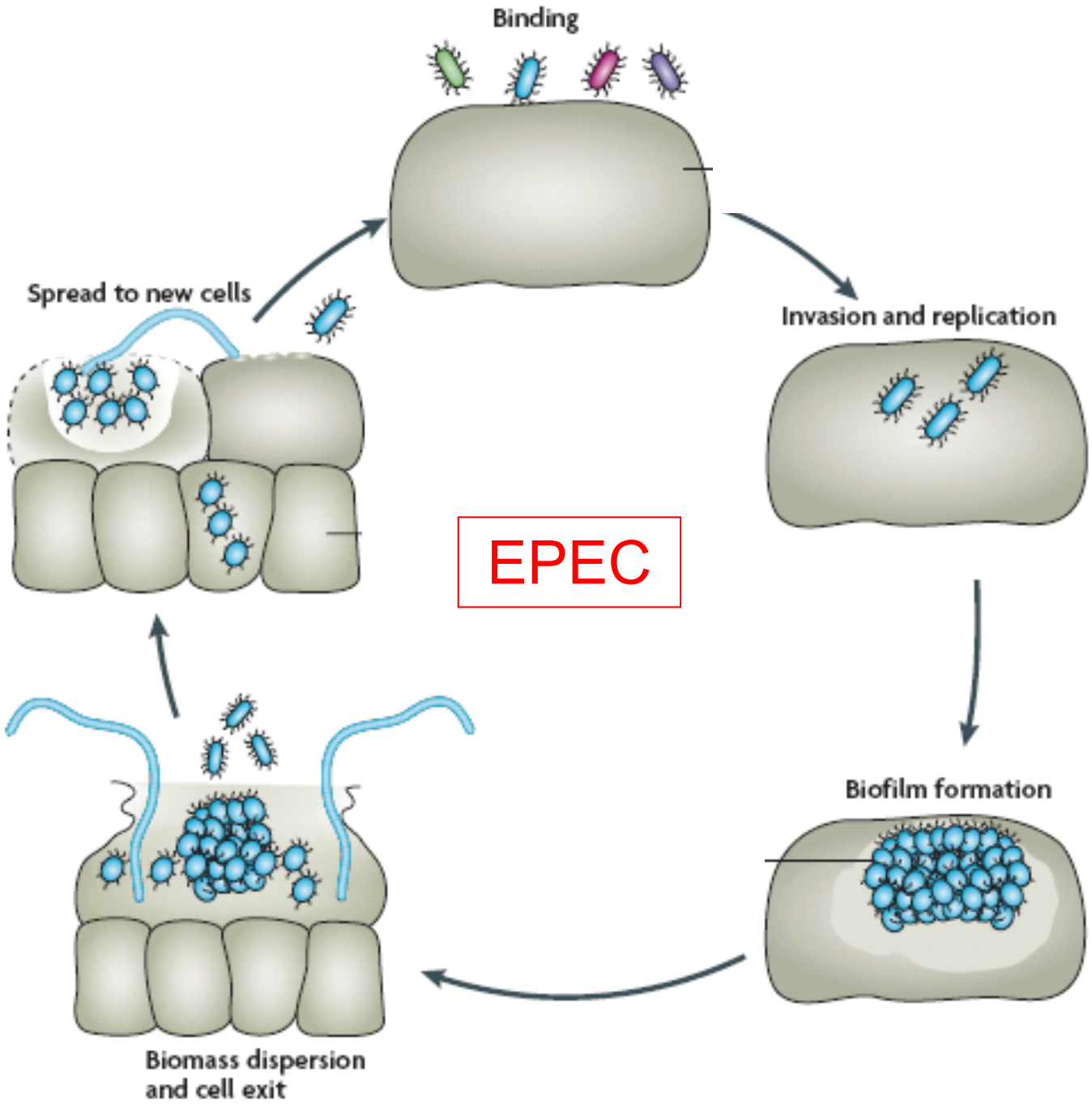
Concept of anti-virulence therapy



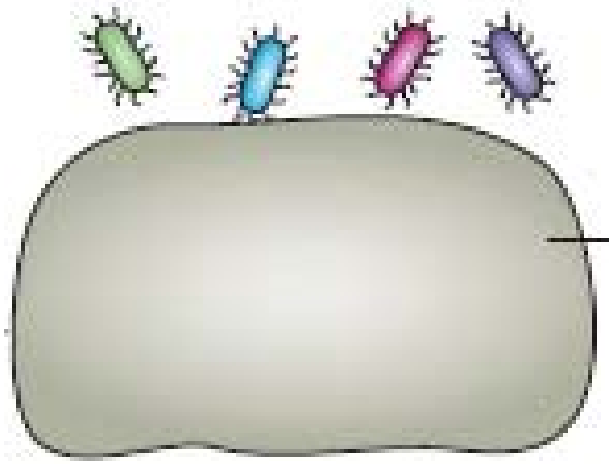
Hib
Meningococcus
Pneumococcus
Pertussis

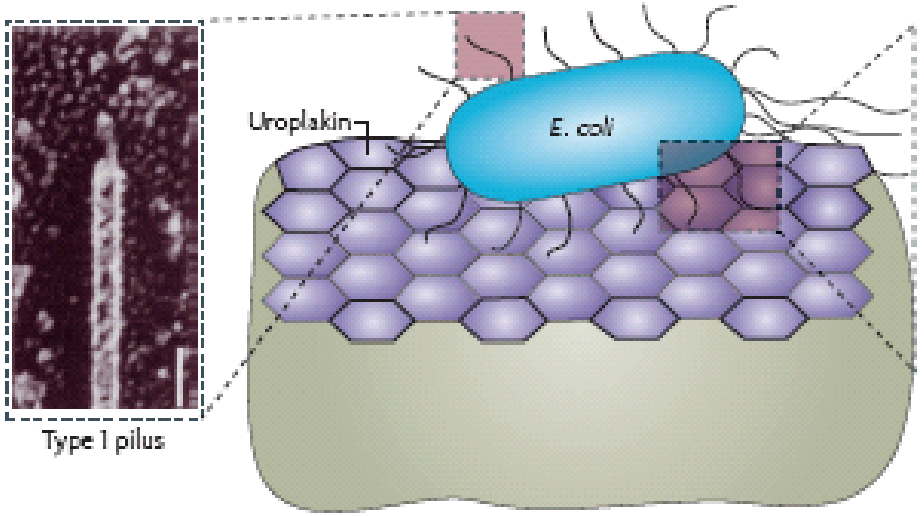
Intensive care
Kidney failure
Transplantees
Cancer therapy

Cegelski et al. (2008) Nat Rev. Microbiol 2008, 6:17-27

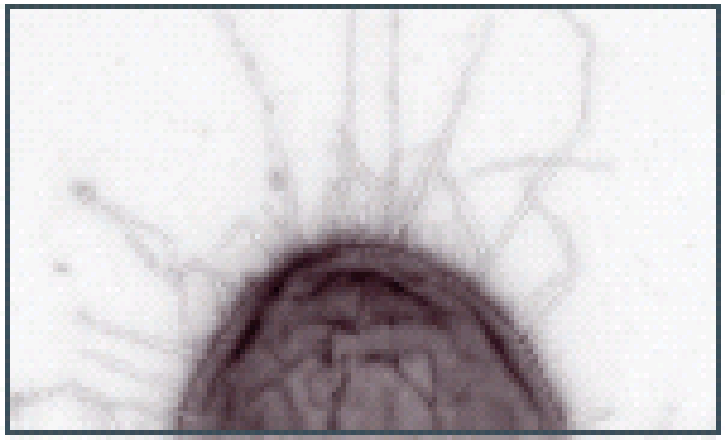

Binding
Invasion and replication
Biofilm formation
Biomass dispersion
and cell exit
Spread to new cells
EPEC

binding

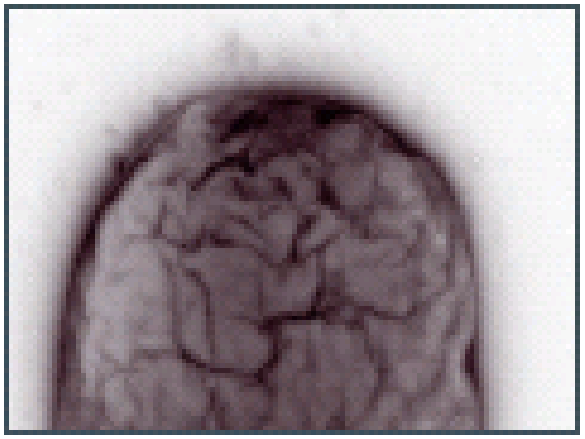




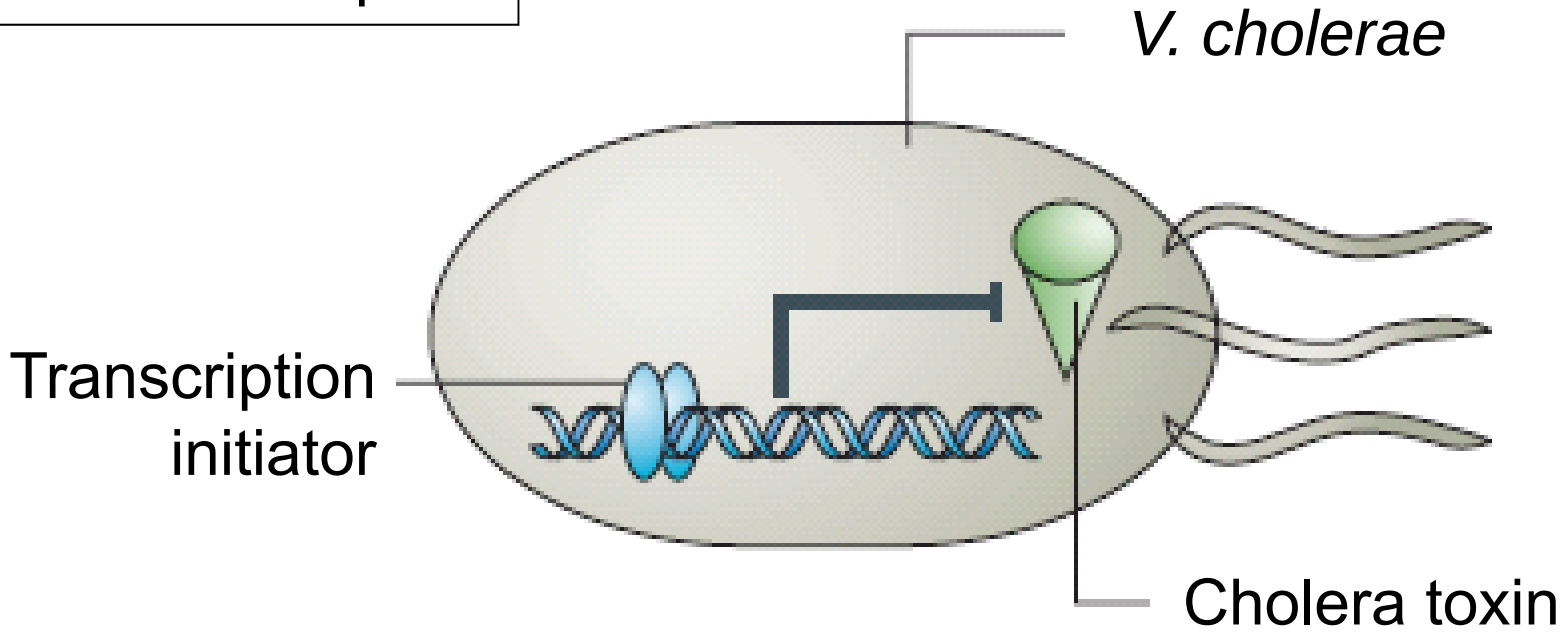
No compound



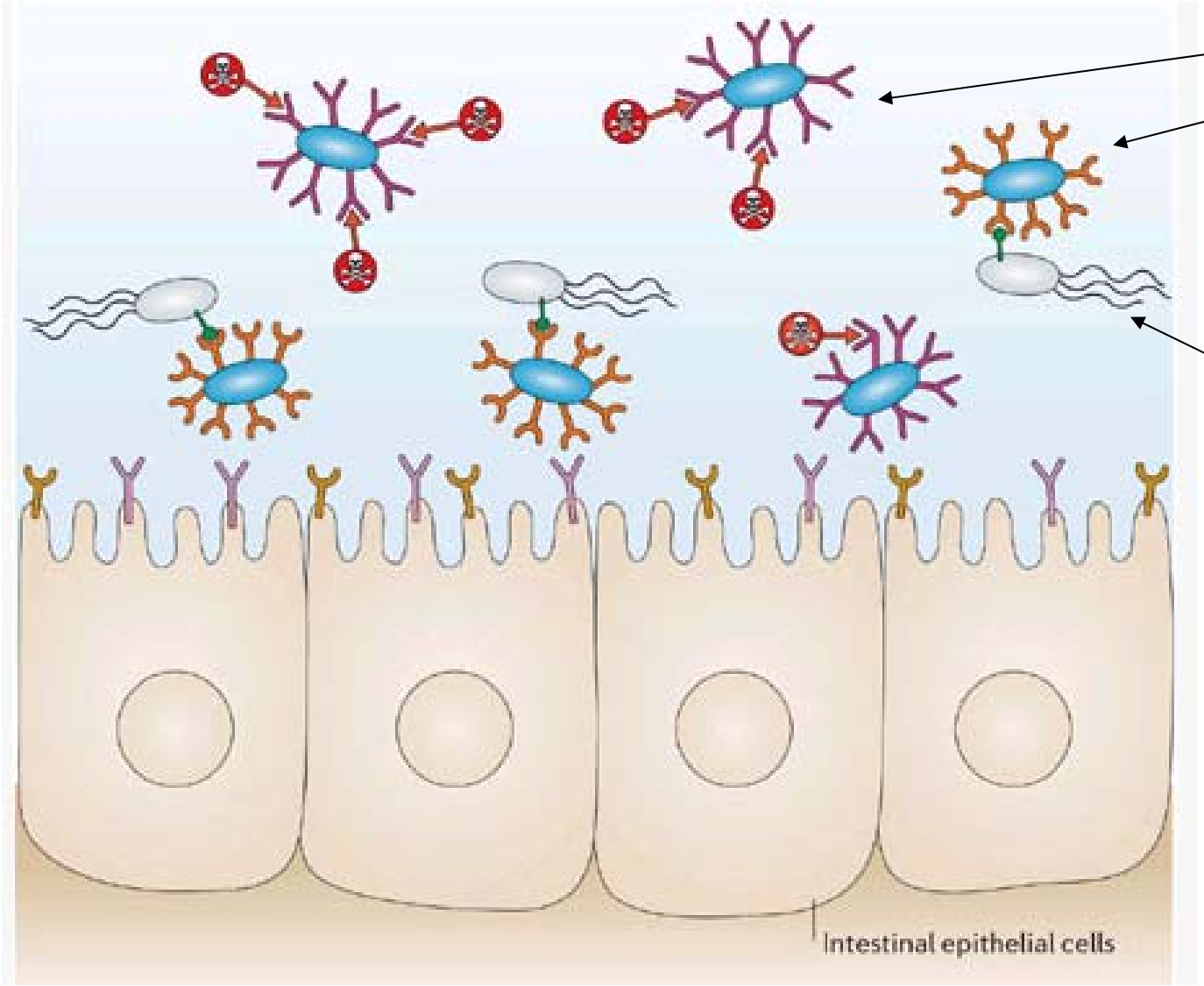
pilicide



Toxin transcription



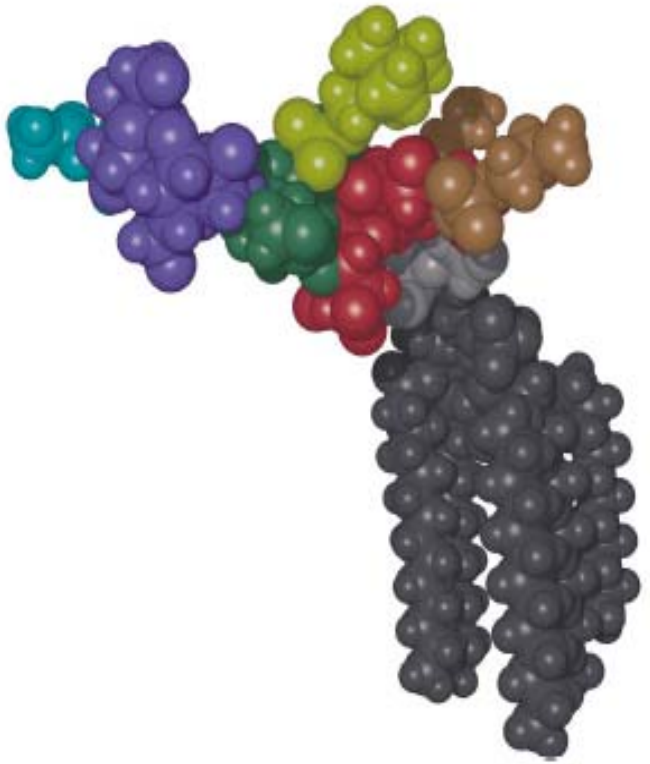
Virstatin inhibits cholera toxin formation



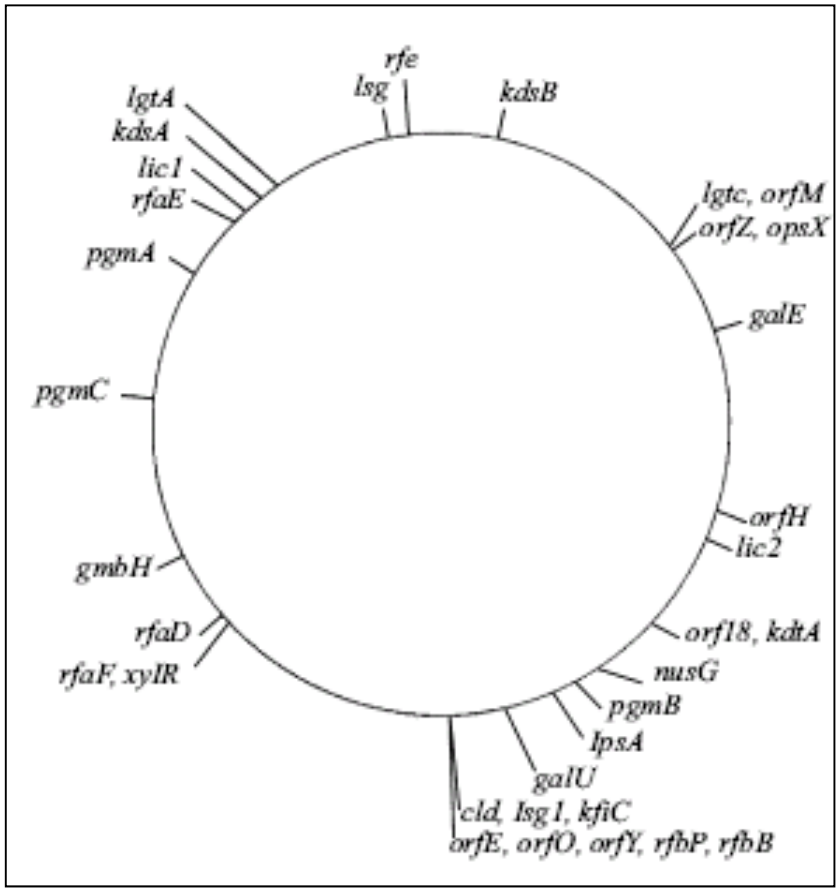
Paton AW et al. (2006) Nature Reviews Microbiology 4, 193-200

Having a genome helps…

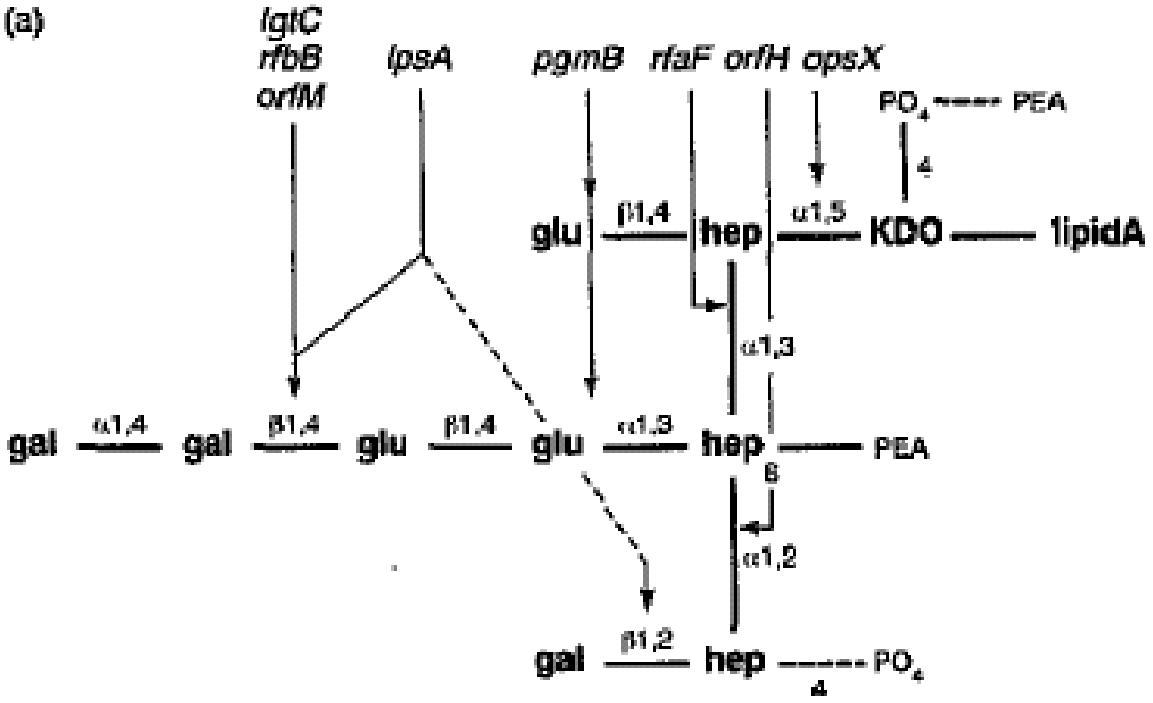
Haemophilus influenzae Rd



LPS



Fold less virulent than Eagan	gene
5 - 100 X	*kfiC, lgtC*
	lpsA, ribF
100 -1000 X	*pgmB, orfH*
	opsX, galU
>1000 X	*rfpB*



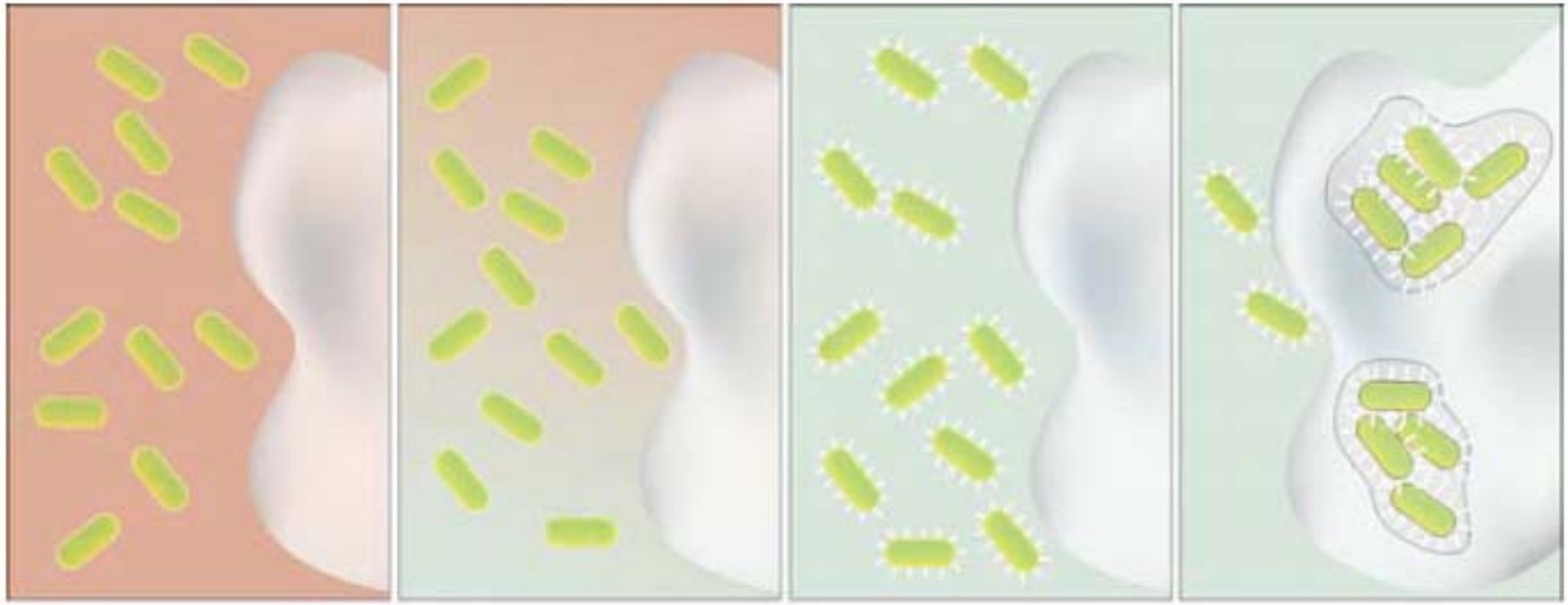
identify LPS pathways

↓

vaccine developmemt

Some cool stuff…

(The future?)



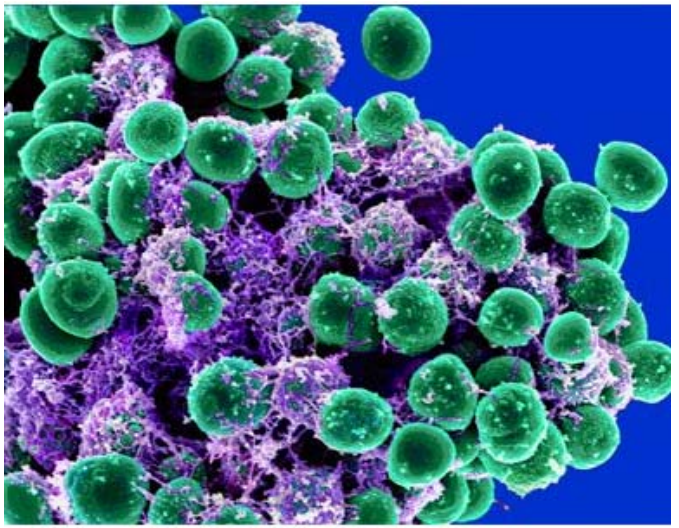
Anderson JC et al. (2005) Environmentally controlled invasion of cancer cells by engineered bacteria. J Mol Biol 315:699-627

Disruption of biofilms using dispersin B

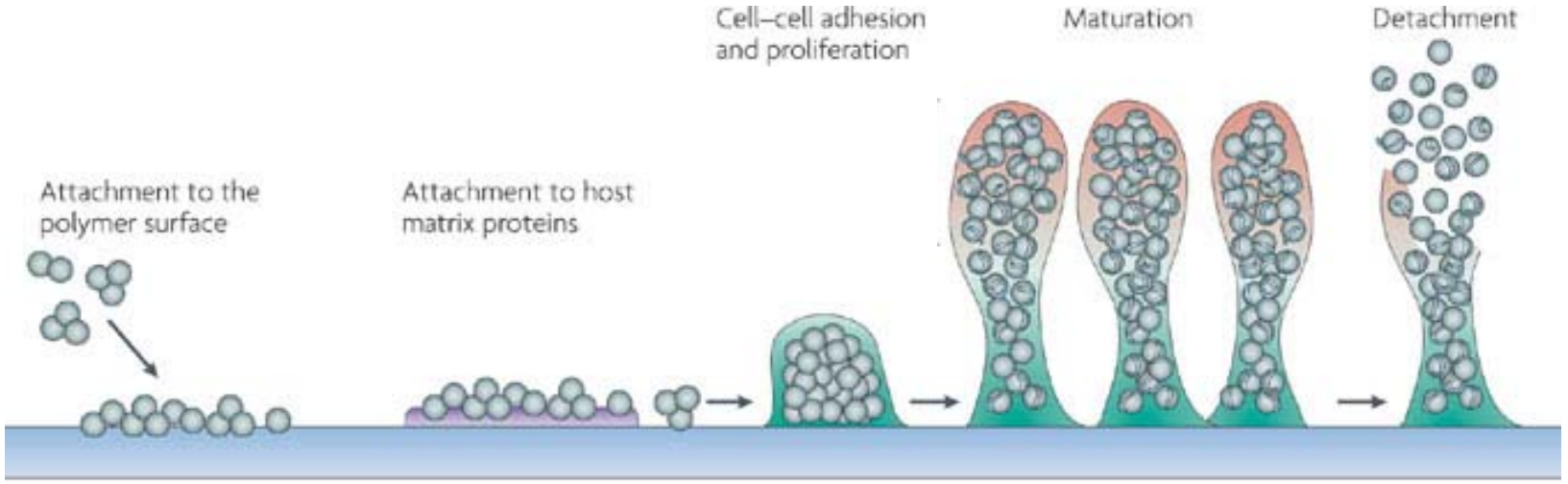
- *S. epidermidis*

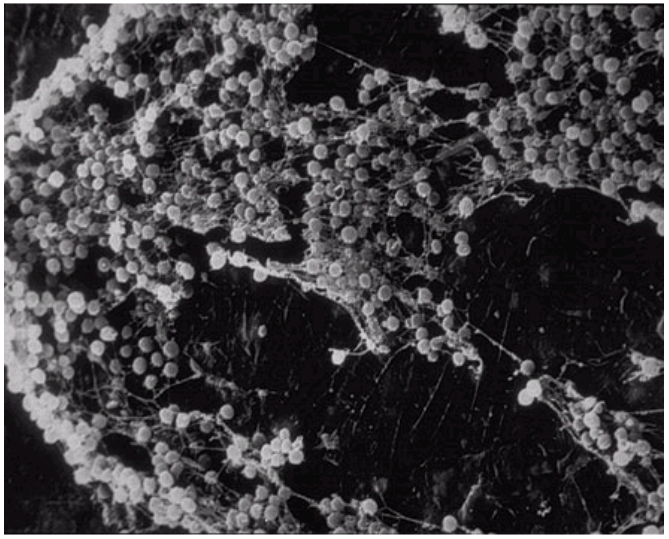
  S. aureus

- Catheter infection



- poly-gamma-DL-glutamic acid (PGA) is required for biofilm formation

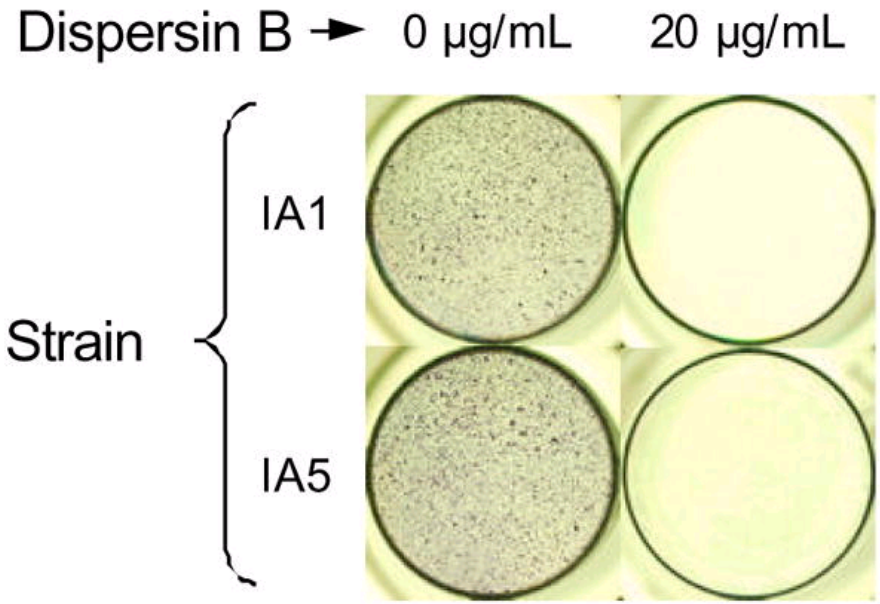




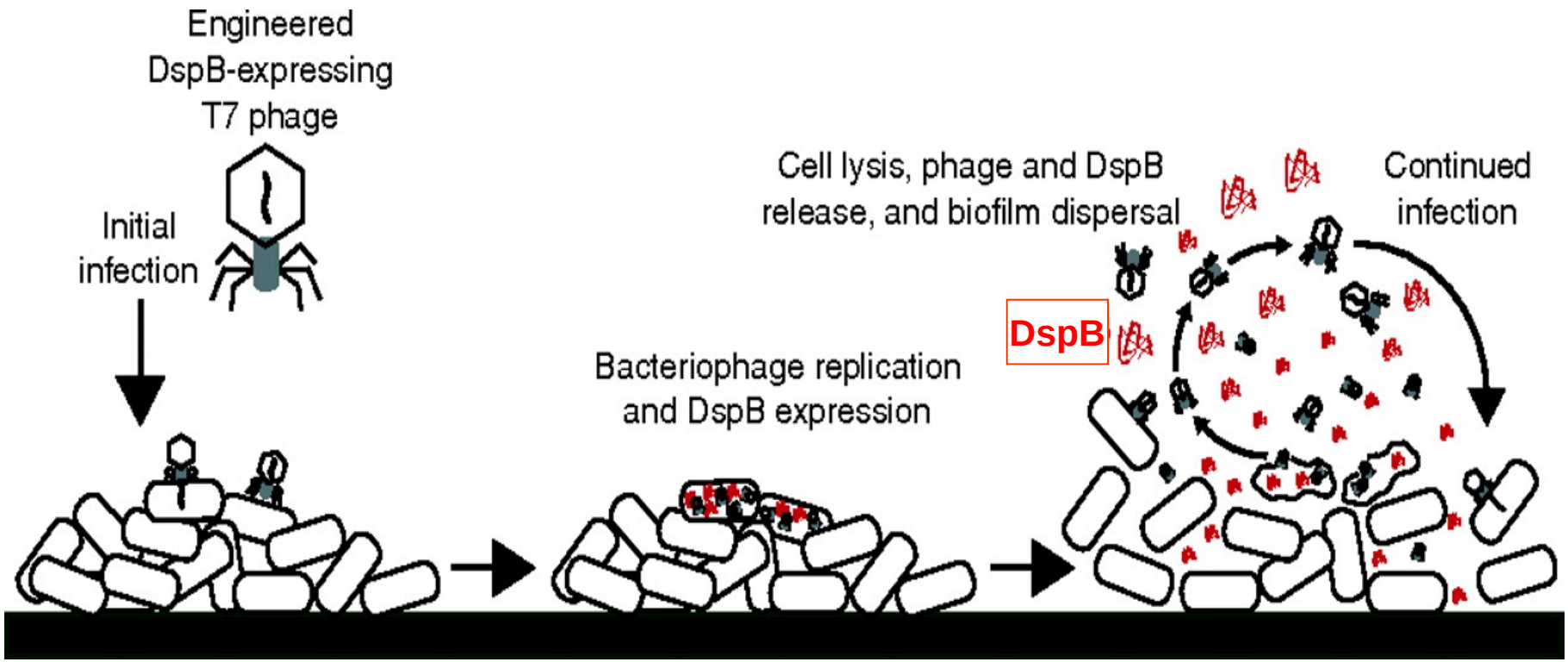
SEM of *S. epidermidis* on a catheter

dispersin B

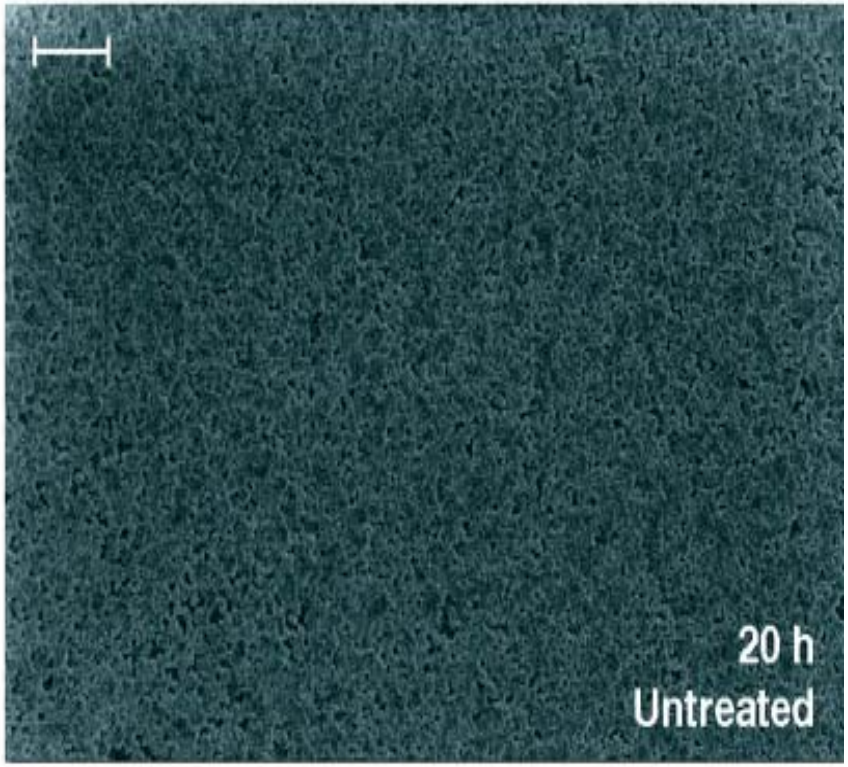
PGA → depolymerisation



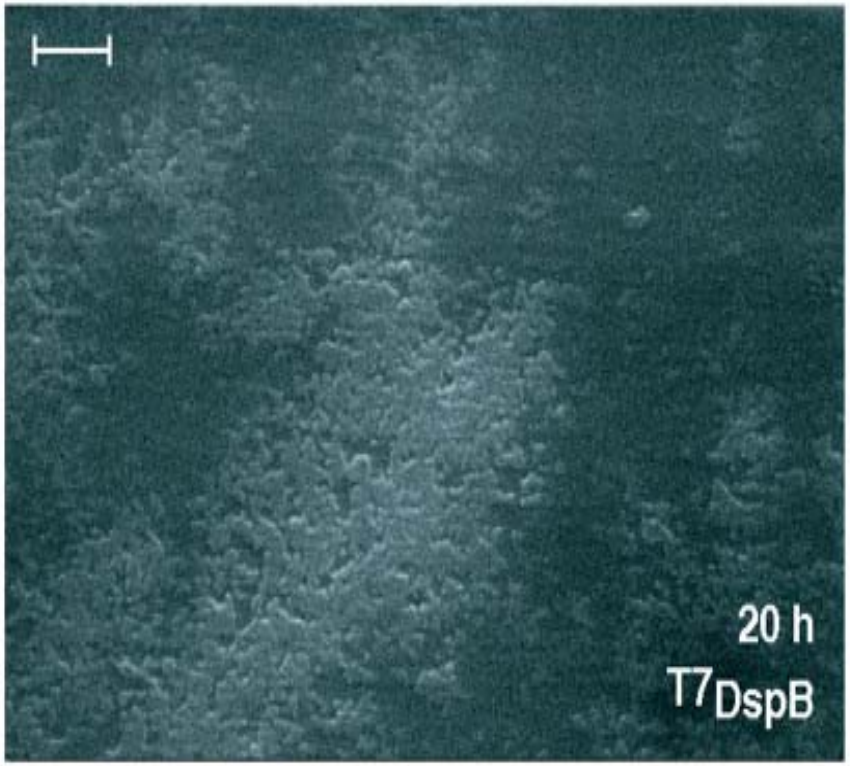
Two-pronged attack strategy for biofilm removal with enzymatically active DspB-expressing phage



Lu TK & Collins JJ (2007) PNAS 104:11197-11202



Intact biofilm



Disrupted biofilm

Messages

- Lots of strategies possible
- Know the genetics of your organism
- Consider time and effort
- Different techniques give you different genes
- Know the advantages and disadvantages of the technique applied to your pathogen and system