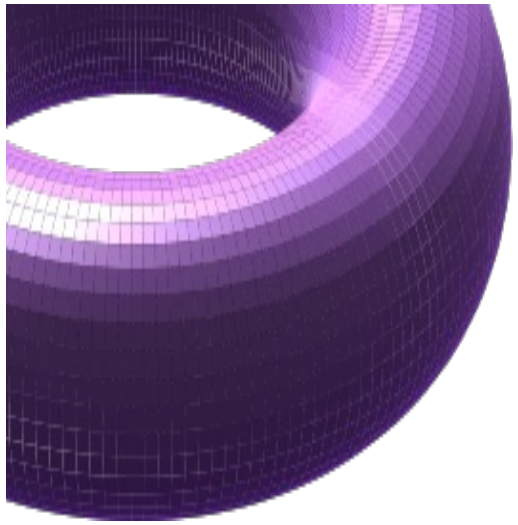
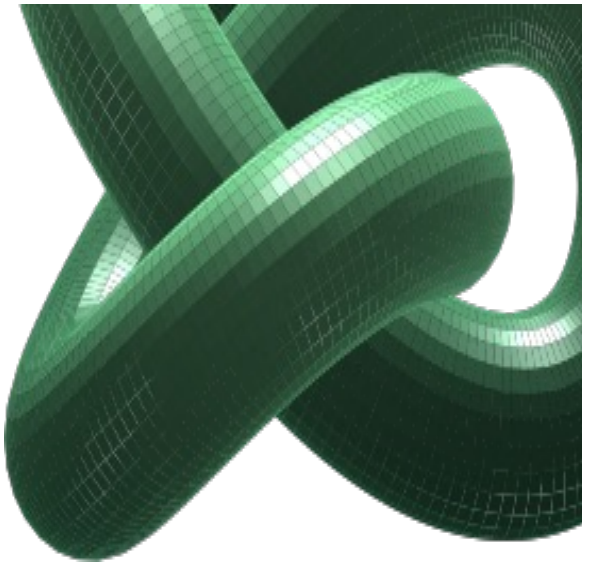
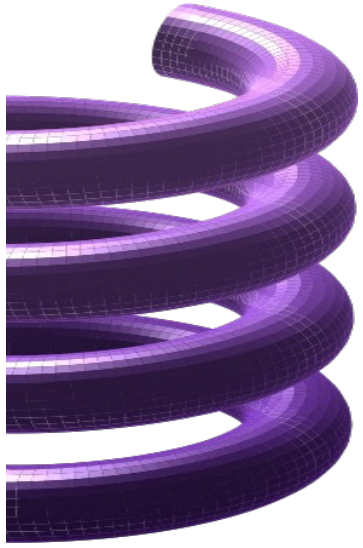




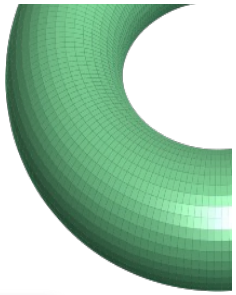
Transformation Workshop

Imperial iGEM



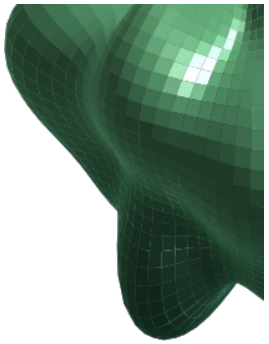


Key terms



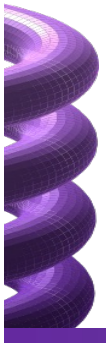
Transformation	Competent cell	Recovery
Beta-lactamase	Com / type IV pilus	Vector
Construct	Origin (ori)	Selection

Why do bacteria take up DNA?

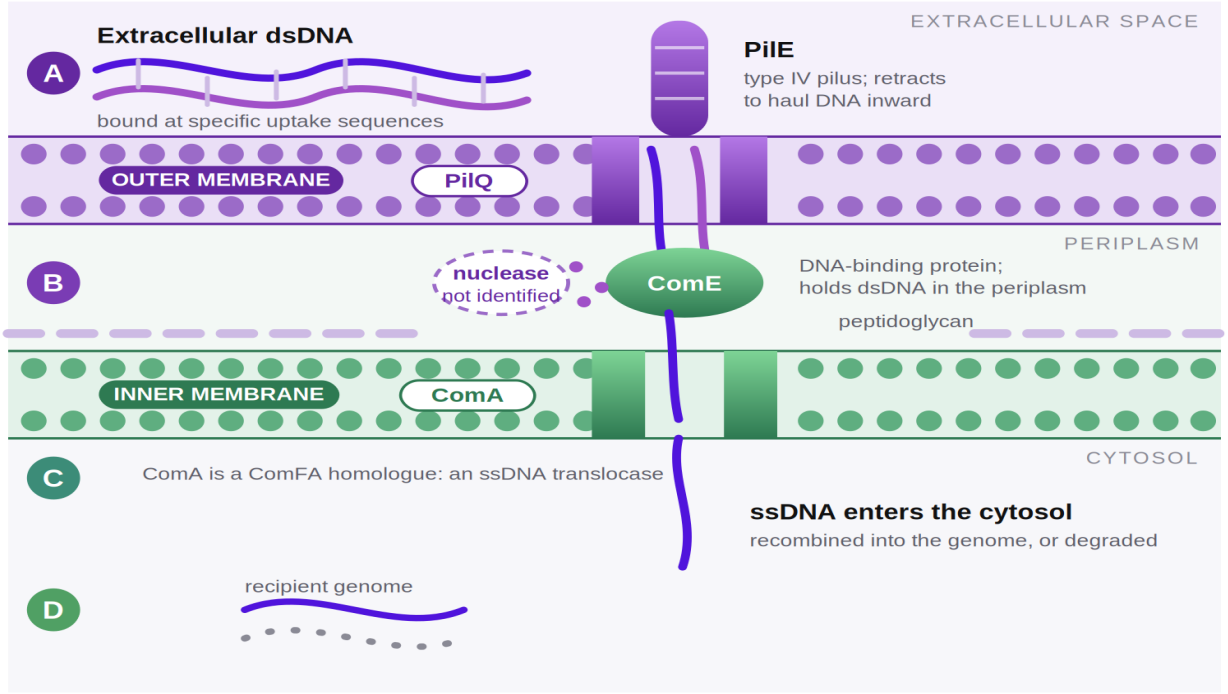


- Nutrition — DNA as a source of nucleotides, carbon and phosphate
- Genome repair — a homologous template to patch damaged chromosome
- Genome diversification — new alleles, including antibiotic resistance

What are the potential disadvantages of taking up foreign DNA?



Natural competence in Gram-negative bacteria

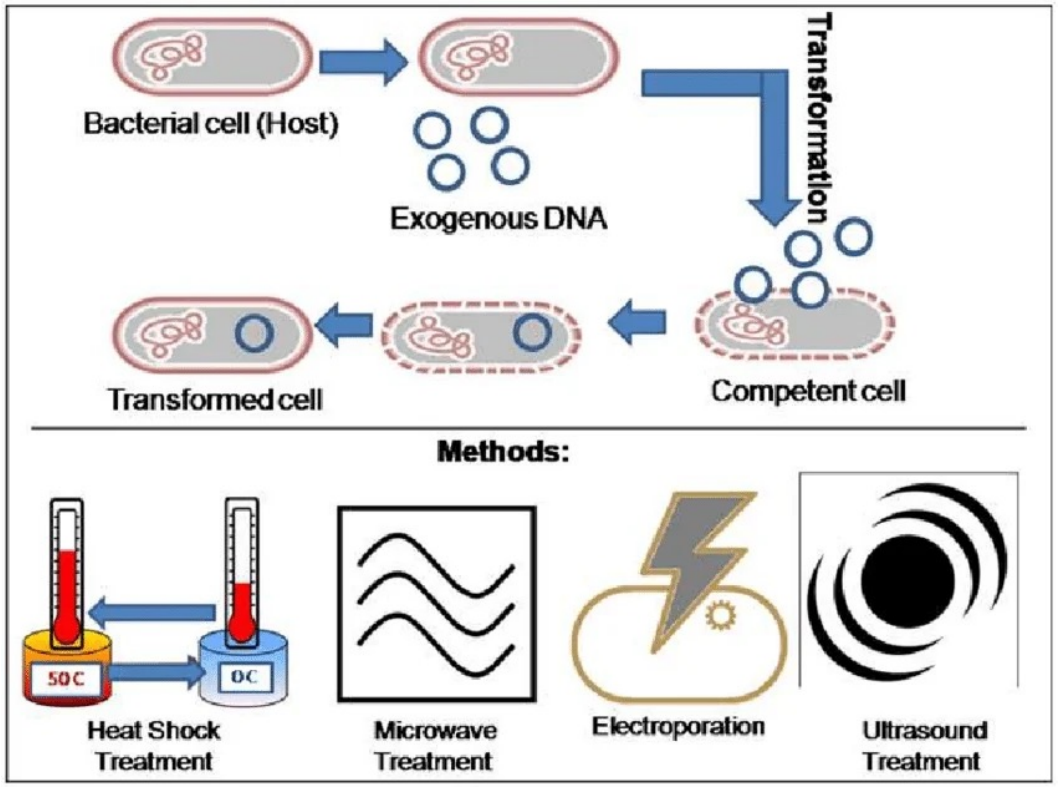


Uptake in four steps

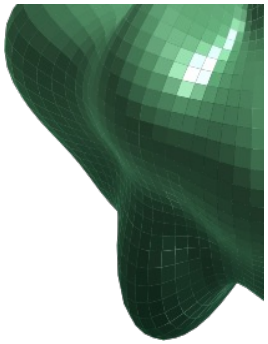
- **A** Pilus binds dsDNA at uptake sequences, then retracts it through PilQ.
- **B** ComE binds the dsDNA in the periplasm.
- **C** ComA, a ComFA homologue, pulls one strand across the inner membrane.
- **D** The other strand is degraded; the nuclease is still unidentified.

Johnston et al. (2014),
Nat. Rev. Microbiol. 12(3), 181–196

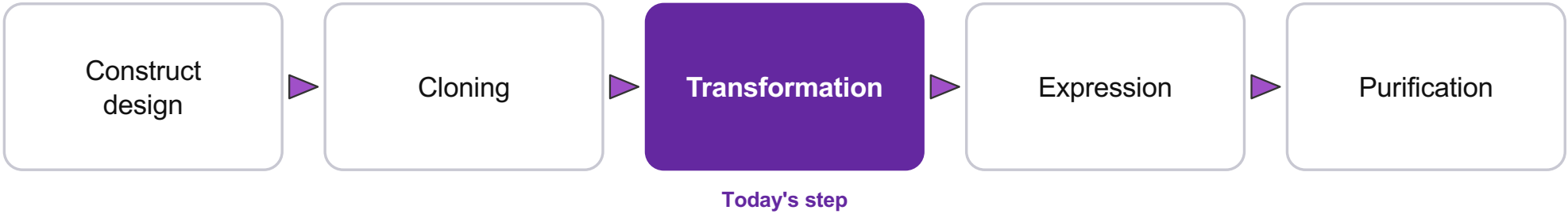
Artificial transformation



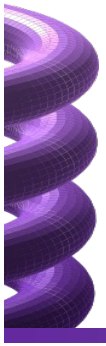
Why we do it: making human insulin



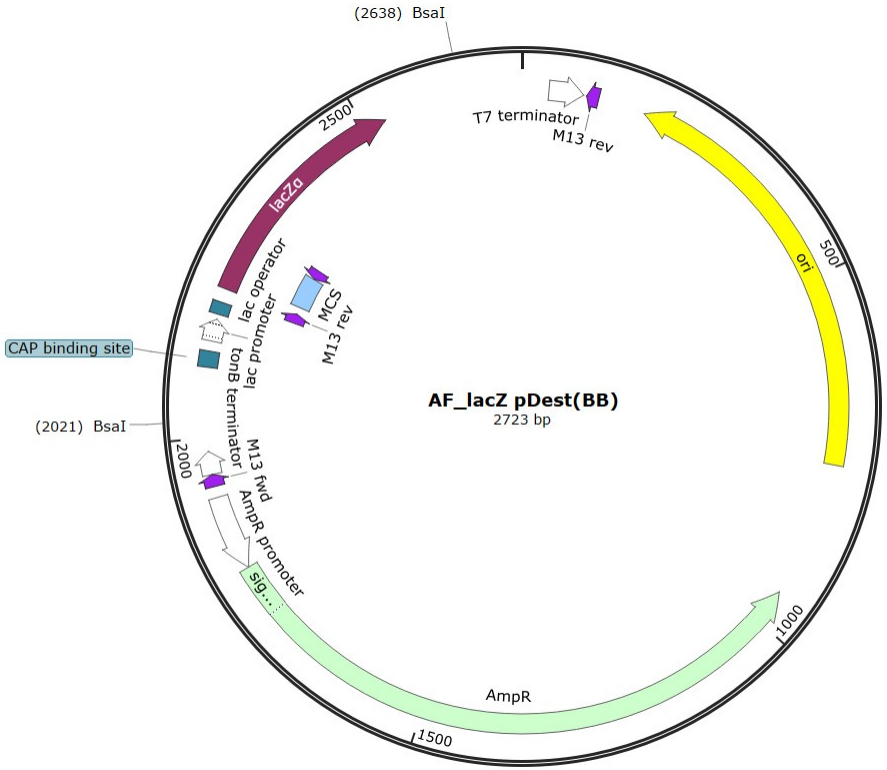
Scenario You want human insulin, in bulk, without a human.



- The insulin gene is human, but *E. coli* reads the same genetic code
- Introduce construct with transformation

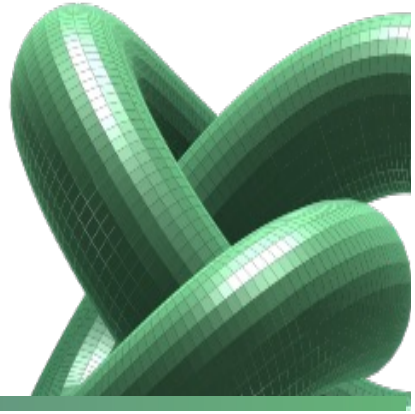


Our plasmid: AF_lacZ pDest (BBa_J435500)

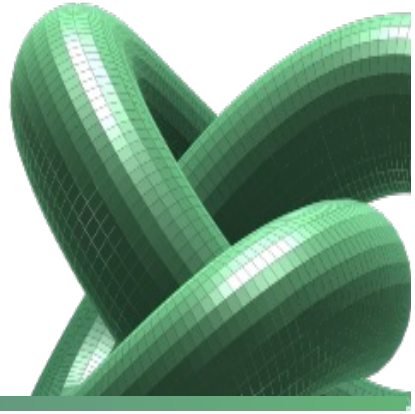


ori	895–1483 (rev)
bla (AmpR)	1674–2639 (rev)
lac promoter	95–188
lacZ-alpha	206–529
MCS	223–279

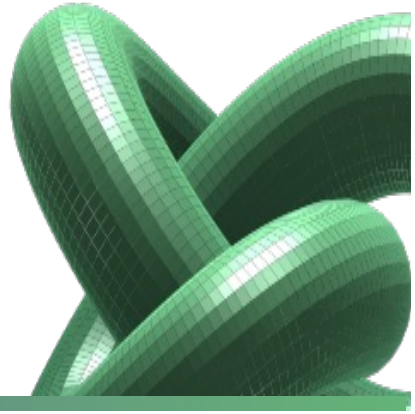
Why incubate on ice?



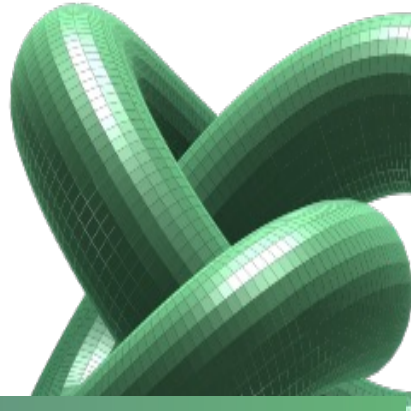
What happens if heat shock runs too long?



Why the long recovery incubation?

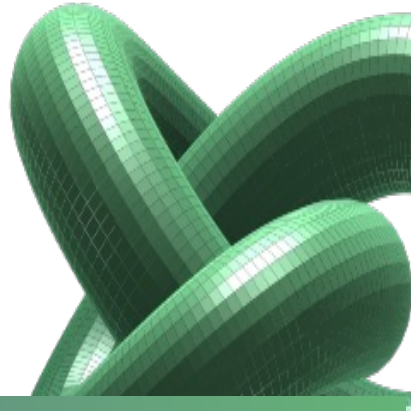


Why include AmpR in a construct?

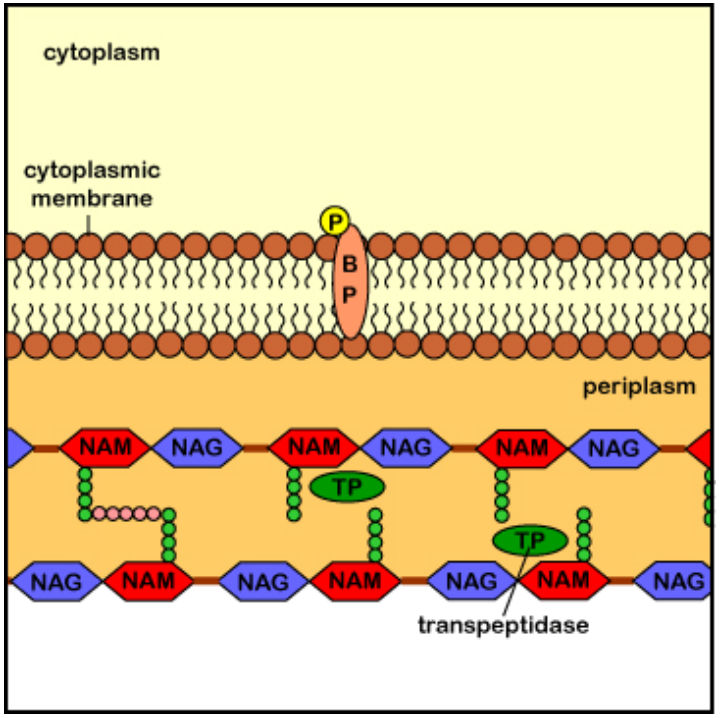


Bonus question

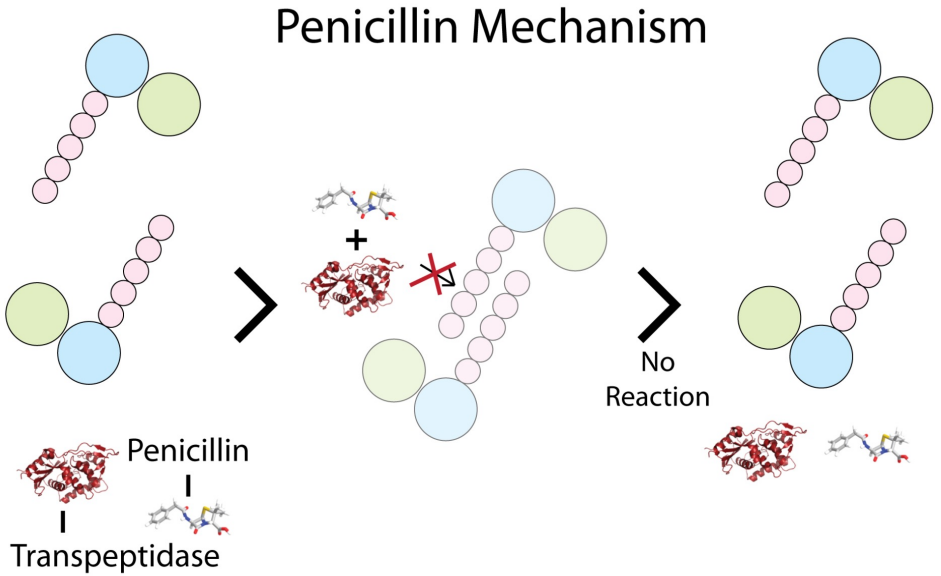
Skipping recovery for ampicillin



Ampicillin mode of action



Transpeptidase cross-links peptidoglycan



Ampicillin mimics the substrate and blocks the active site