Redesign and Construction of Bacteriophage Genomes in Yeast

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Why redesign genomes?
• Enables discovery and annotation of functional genetic elements at the genome scale
• Develops the capacity to rapidly create new genomes not found in nature\(^1,2\)
• Creates genomes that more closely reflect models\(^2,3,4\)

Why use phage genomes?
• Small genome size compared to cells
• Self-contained genetic program
• Venue to study cryptic regulation and gene overlap
• Develop methods to assemble E. coli-toxic DNA

\(\phi X174\) • E. coli host
• lytic
• circular ssDNA genome
• 5.4 kb
• uses host DNA, RNA polymerases\(^5\)
• completely assembled in vitro from synthetic oligos\(^6\)

\(T7\) • E. coli host
• lytic
• linear dsDNA genome
• 40 kb
• uses phage-encoded DNA, RNA polymerases
• partially refactored in past\(^4\)

References
8. Kitagawa, M. et al. (2001) Complete set of ORF clones of Escherichia coli ASKA library (a complete set of E. coli K-12 ORF clones) unique resources for biological research. DNA Research. 12, 297-6

Yeast Assembly Platform

Homologous recombination assembly in yeast

PCR
Screen
Purify plasmid
Redeploy

Yeast vector (pRSxx)

Linearize \(\phi X174\) genome

Linearize & add homologous overhangs to target genome

\(T7\) & \(\phi X174\) Recombination

\(\phi X174\) & \(T7\) Redeployment

\(\phi X174\) Cut with Restriction Enzymes Ligation Plaques

AsiSI Yes
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AsiSI -
AsiSI NheI -

野生型 control

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Both \(T7\) and \(\phi X174\) can be assembled within yeast and then redeployed into E. coli

\(\phi X174.1\) Construction and Redeployment

Liberate phage genome

Circularize

Infect with phage

Recoup phage

Characterization
1. Host lysis efficiency
   • Lysis curves
   • Plaque sizes
2. Host gene interactions
   • plate against Keio\(^*\) and ASKA\(^*\) collections