# Redesign and Construction of Bacteriophage Genomes in Yeast

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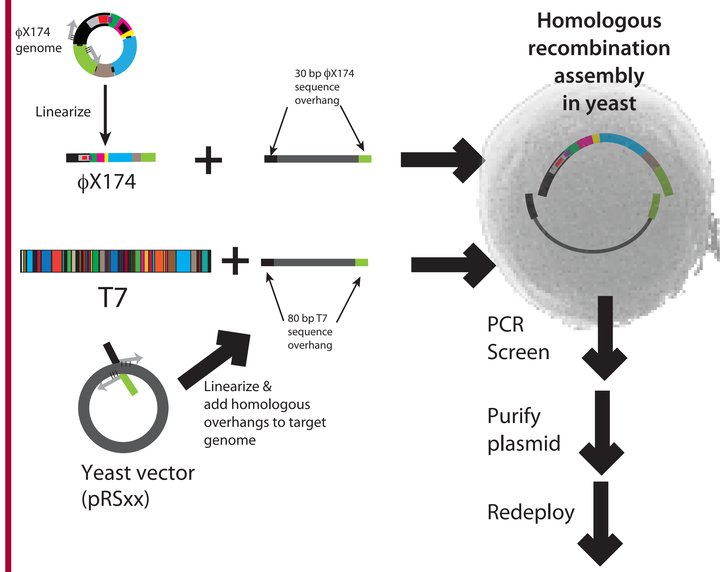
## Why redesign genomes?

- 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]

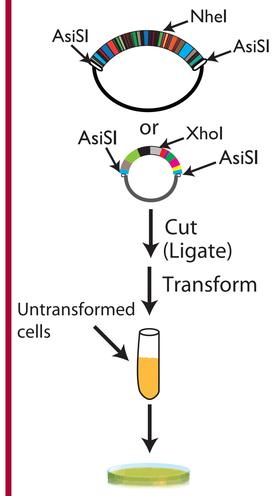
## Why use phage genomes?

- 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

## Yeast Assembly Platform



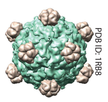

## ϕX174 & T7 Redeployment



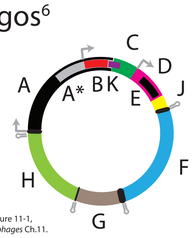

| | Cut with Restriction Enzymes | Ligation | Plaques |
|---|---|---|---|
| ϕX174 | wild type control | | |
| | AsiSI | Yes | |
| | AsiSI XhoI | Yes | |
| T7 | wild type control | | |
| | - | - | |
| | AsiSI | - | |
| | AsiSI NheI | - | |

**Both T7 and ϕX174 can be assembled within yeast and then redeployed into *E. coli***

## ϕX174

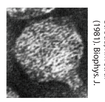



- *E. coli* host
- lytic
- circular ssDNA genome
- 5.4 kb
- uses host DNA, RNA polymerases[5]
- completely assembled *in vitro* from synthetic oligos[6]




Based on Figure 11-1, *The Bacteriophages* Ch.11.

## T7




- *E. coli* host
- lytic
- linear dsDNA genome
- 40 kb
- uses phage-encoded DNA, RNA polymerases
- partially refactored in past[4]


Based on Figure 20-2, *The Bacteriophages* Ch.20.

## References

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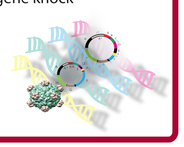
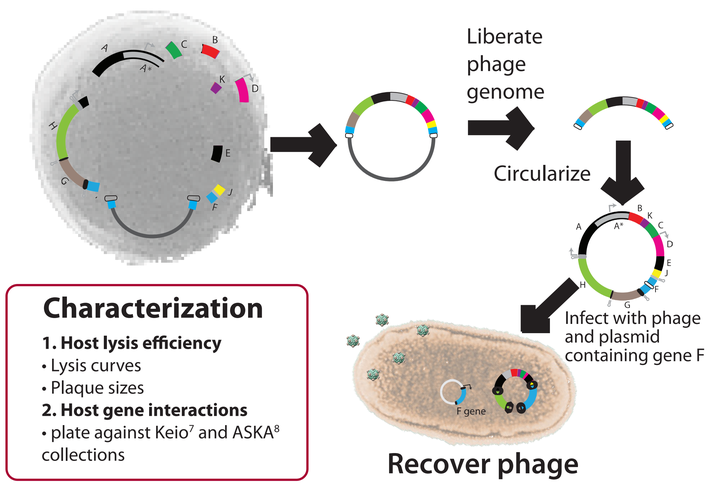


SynBERC Synthetic Biology Engineering Research Center
NSERC CRSNG
NSF

## ϕX174.1 Construction and Redeployment




### Characterization

**1. Host lysis efficiency**
- Lysis curves
- Plaque sizes

**2. Host gene interactions**
- plate against Keio[7] and ASKA[8] collections