

Siphoviridae Phage Assembly TX/TL

Introduction

This protocol is used to assembly phage in vitro method by iGEM Munich 2018 made cell extract

Materials

- Buffer B (iGEM Munich 2018)
- Cell Extract E (iGEM Munich 2018)
- GamS (iGEM Munich 2018)
- dNTPs (NEB) (iGEM Munich 2018)
- Phage DNA
- Nuclease-free H₂O

Procedure

- Day 1

Assembly

1. Keep the reagents on ice. Extract and Buffer must thaw on ice
2. Mix the reagents in the following order:

Concentration	Reagents
8.9 µl	Buffer B
3.3 µM	GamS
0.5 mM	dNTPs
Fill to 20 µl	Nf H ₂ O

7.1 μ l	Extract E
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3. Incubate for 10 min on ice
4. Add 0.5 nM of Phage DNA
5. Incubate at 29°C for at least 5 hours (overnight possible)
6. Following: Measure the Phage titer
7. Prepare also the negative control: mix Buffer B, dNTPs, Extract E and fill it with water (do not add GamS and Phage DNA)