Aug 04, 2021

Integration of a cargo brick

In 1 collection

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dx.doi.org/10.17504/protocols.io.bvk9n4z6

SEGA

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ABSTRACT

SEGA, the Standardized Genome Engineering Architecture, is a comprehensive strain collection that enables genome engineering by combining only two reagents: a DNA fragment that can be ordered from a commercial vendor and a stock solution of bacterial cells followed by incubation on agar plates. Recombinant genomes are identified by visual inspection using green-white colony screening akin to classical blue-white screening for recombinant plasmids. The modular nature of SEGA allows precise multi-level control of transcriptional, translational, and post-translational regulation. The SEGA architecture simultaneously supports increased standardization of genetic designs and a broad application range by utilizing well-characterized parts optimized for robust performance in the context of the bacterial genome

This protocol describes the process of integrating a SEGA cargo brick. A cargo brick is integrated using *tetA* counterselection. This protocol also applies to integration of other bricks using tetA counterselection, e.g. splitting *tetA*.

DOI

dx.doi.org/10.17504/protocols.io.bvk9n4z6

PROTOCOL CITATION

Carolyn N Bayer, Maja Rennig, Anja Ehrmann, Morten Norholm 2021. Integration of a cargo brick. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bvk9n4z6

COLLECTIONS

SEGA protocol collection

KEYWORDS

SEGA, genome engineering, recombineering, E. coli, synthetic biology

CREATED

Jun 08, 2021

LAST MODIFIED

Aug 04, 2021

PROTOCOL INTEGER ID

50561

PARENT PROTOCOLS

Part of collection

SEGA protocol collection

BEFORE STARTING

Transform a SEGA strain with pSIM19 (Spectinomycin resistance). From now on cultures have to be kept at **30 °C** to retain the plasmid (temperature-sensitive *ori*)

preculture and DNA fragment- Day 1 1d

1d

1 Prepare a PCR product of the cargo brick and purify it from an agarose gel.

2 Setup a preculture of the strain with pSIM19 in LB medium supplemented with Spectinomycin **0.05 mg/ml** and incubate at **250 rpm, 30°C** overnight

Recombineering- Day 2 1d

3 Prepare:

3.1 Cold sterile water

3.2 Cold Glycerol **15 % volume**

3.3 Pre-chilled centrifuge and tabletop centrifuge at **4 °C**

3.4 M9 agar plates supplemented with **50 Micromolar (µM)** $NiCl_2$

> Additionally, specific inducers to enable green-white screening can be added

4 Inoculate **50 mL** LB-Medium supplemented with Spectinomycin (**0.05 mg/ml**) with **500 µl** of the preculture from step 3

4.1 Incubate at **250 rpm, 30°C** until cultures reached an OD_{600} of 0.5

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5 Induce expression by transferring the culture to a shaking water bath at **150 rpm, 42°C, 00:20:00**

6 Transfer culture to prechilled **50 mL** falcon tubes and put on ice for **00:15:00** 15m

7 Spin the culture down at **4000 x g, 4°C, 00:05:00** and discard the supernatant 5m

8 Add **1 mL** of ice cold water, resuspend and transfer to a 1.5 ml tube

9 Spin at **11000 x g, 4°C, 00:00:30** in a tabletop centrifuge 30s

10 Wash pellet twice with **1 mL** ice cold water **go to step #9**

11 Resuspend the pellet in **600 µl** cold glycerol ([M] **15 % volume**)

11.1 Unused cells can be stored at **-80 °C**

> This is not possibe for *E. coli* Nissle

12 Electroporate **50 µl** of cells with **200 ng** of purified PCR product from step 2 or **2 µl** of a [M] **100 Micromolar (µM)** single stranded oligonucleotide

13 Recover cells **800 rpm, 30°C, 01:00:00** in a tabletop shaker using SOC medium.

14 Transfer the cells into **5 mL** LB medium supplemented with Spectinomycin

15 Incubate at **250 rpm, 30°C** overnight

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Plating- Day 2 1d

16 Wash **1 mL** of the recovered cells twice with sterile water. Centrifuge at **11000 rpm, 20°C, 00:00:30** 30s

17 Make a dilution series and plate **100 µl** of the 1:10 - 1:1000 dilution on M9 agar supplemented with **50 Micromolar (µM)** $NiCl_2$

the appropriate inducer can be added to the agar plates to enable green-white screening

18 incubate the plates at **30 °C** for **48:00:00** to **72:00:00** 5d

Cells can be incubated at **37 °C** if it is the last step of recombination and pSIM19 is no longer needed in the cells

Screening- Day 4-5 1d

19 Screen for positive colonies by "green-white screening"on a blue-light table and perform colony PCR on the colorless colonies to identify the correct recombinants.

Streaking out of the colonies at least twice is necessary to ensure a clean culture

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