Aug 04, 2021

Integration of a landing pad brick

In 1 collection

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dx.doi.org/10.17504/protocols.io.bviwn4fe

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 to process of integrating a SEGA landing pad brick. A landing pad brick is integrated using *tetA* positive selection. This protocol also applies to integration of other bricks using tetA positive selection, e.g. complementation of split *tetA*.

DOI

dx.doi.org/10.17504/protocols.io.bviwn4fe

PROTOCOL CITATION

Carolyn N Bayer, Maja Rennig, Anja Ehrmann, Morten Norholm 2021. Integration of a landing pad brick. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bviwn4fe

COLLECTIONS

SEGA protocol collection

KEYWORDS

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

CREATED

Jun 04, 2021

LAST MODIFIED

Aug 04, 2021

Citation: Carolyn N Bayer, Maja Rennig, Anja Ehrmann, Morten Norholm (08/04/2021). Integration of a landing pad brick. https://dx.doi.org/10.17504/protocols.io.bviwn4fe

PROTOCOL INTEGER ID

50486

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 landing pad brick and purify it from an agarose gel.

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

Recombineering- Day 2 1d

3 Prepare:

3.1 Cold sterile water

3.2 Cold Glycerol **[M]15 % volume**

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

3.4 LB agar plates supplemented with **[M]0.025 mg/ml** or **[M]0.05 mg/ml** Tetracycline

> Using **[M]0.0025 mg/ml** tetracycline will increase chances to get positive colonies in case of difficult to integrate constructs. However, less background is to be expected at higher tetracycline concentrations

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

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

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 [M] **100 Micromolar (µM)** single stranded oligonucleotide

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

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

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14 Plate cells on LB agar plates supplemented with [M]**0.025 mg/ml** or [M]**0.05 mg/ml** Tetracycline

Using [M]**0.0025 mg/ml** tetracycline will increase chances to get positive colonies in case of difficult to integrate constructs. However, less background is to be expected at higher tetracycline concentrations

15 Incubate at **30 °C** for **24:00:00** up to **48:00:00** 3d

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 3-4 1d

16 Screen for positive colonies by "green-white screening"on a blue-light table and perform colony PCR on the fluorescent 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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