Aug 04, 2021

Integration of a control brick

In 1 collection

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dx.doi.org/10.17504/protocols.io.bvman42e

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 control brick. A control brick is integrated using *galK* counterselection.

DOI

dx.doi.org/10.17504/protocols.io.bvman42e

PROTOCOL CITATION

Carolyn N Bayer, Maja Rennig, Anja Ehrmann, Morten Norholm 2021. Integration of a control brick. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bvman42e

COLLECTIONS

SEGA protocol collection

KEYWORDS

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

CREATED

Jun 08, 2021

LAST MODIFIED

Aug 04, 2021

Citation: Carolyn N Bayer, Maja Rennig, Anja Ehrmann, Morten Norholm (08/04/2021). Integration of a control brick. https://dx.doi.org/10.17504/protocols.io.bvman42e

PROTOCOL INTEGER ID

50562

PARENT PROTOCOLS

Part of collection

SEGA protocol collection

BEFORE STARTING

Transform a SEGA strain, harbouring the upstream gadget *galK*, 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

1 Prepare a PCR product of the control elements that need to be integrated and purify it from an agarose gel. 1d

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

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 at **4 °C**

3.4 M63 agar plates supplemented with [M]**0.2 Mass / % volume** 2-deoxy-galactose, [M]**0.2 % volume** glycerol and [M]**5 Milimolar (mM)** L-rhamnose

4 Inoculate **50 mL** LB-Medium supplemented with Spectinomycin ([M]**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

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

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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 Transfer cells into **50 mL** LB medium in a 250 ml baffled conical shake flask and recover overnight at **250 rpm, 30°C**

Alternatively, cells can be recovered in **10 mL** medium for **04:30:00**

Plating- Day 3 1d

14 Wash **1 mL** of the recovered cells twice with 1X M9 salts. Centrifuge at **11000 rpm, 20°C, 00:00:30** 30s

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15 Make a dilution series and plate **100 µl** of the 1:100 - 1:1000 dilution on M63 agar supplemented with **0.2 Mass / % volume** 2-deoxy-galactose, **0.2 % volume** glycerol and **5 Milimolar (mM)** L-rhamnose.

In case the cells were recovered for **04:30:00** dilution 1:10 and 1:100 need to be plated

5d

16 incubate the plates at **30 °C** for **48:00:00** to **72:00:00**

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

17 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

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