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## Integration of a cargo brick

In 1 collection

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**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*.

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COLLECTIONS (i)

SEGA protocol collection

**KEYWORDS** 

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

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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 Prepare a PCR product of the cargo brick and purify it from an agarose gel. Setup a preculture of the strain with pSIM19 in LB medium supplemented with Spectinomycin [M]0.05 mg/ml and incubate at \$\triangle 250 \text{ 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 at § 4 °C 3.4 M9 agar plates supplemented with [M]50 Micromolar (μM) NiCl<sub>2</sub> Additionally, specific inducers to enable green-white screening can be added Inoculate ⊒50 mL LB-Medium supplemented with Spectinomycin ([M]0.05 mg/ml) with ⊒500 µl of the preculture from step 3 Incubate at \$\alpha 250 \text{ rpm, 30°C} until cultures reached an OD<sub>600</sub> of 0.5

PROTOCOL INTEGER ID

PARENT PROTOCOLS

SEGA protocol collection

Part of collection

50561

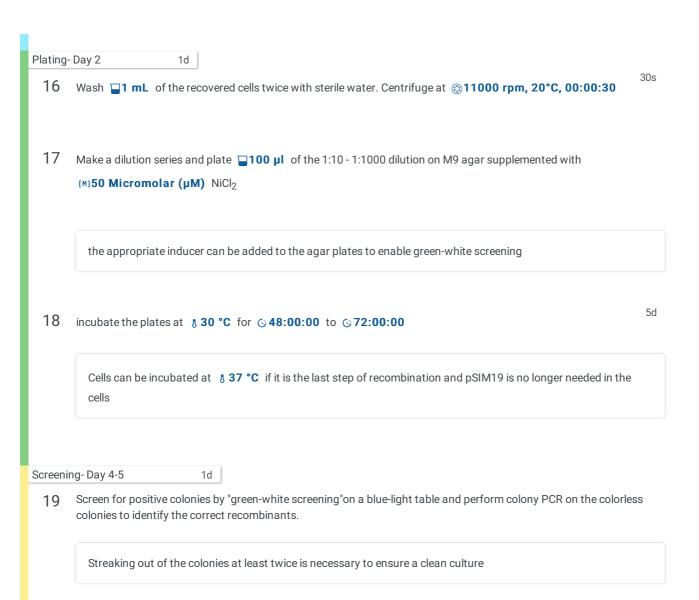
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Induce expression by transferring the culture to a shaking water bath at $\triangle 150 \text{ rpm, 42°C, 00:20:00}
                                                                                                                         15m
      Transfer culture to prechilled $\sum_50$ mL falcon tubes and put on ice for $\infty$ 00:15:00
                                                                                                                          5m
      Spin the culture down at 34000 x g, 4°C, 00:05:00 and discard the supernatant
      Add 11 mL of ice cold water, resuspend and transfer to a 1.5 ml tube
                                                                                                                          30s
 9
      Spin at 311000 x g, 4°C, 00:00:30 in a tabletop centrifuge
      Wash pellet twice with ■1 mL ice cold water ◆ go to step #9
11
      Resuspend the pellet in \Box 600 \ \mu I cold glycerol ( [M] 15 % volume )
                      Unused cells can be stored at 8 -80 °C
                         This is not possibe for E. coli Nissle
12
      Electroporate \square 50 \,\mu l of cells with \square 200 \,ng of purified PCR product from step 2 or \square 2 \,\mu l 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.
      Transfer the cells into 5 mL LB medium supplemented with Spectinomycin
      Incubate at $\rightarrow$250 rpm, 30°C overnight
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