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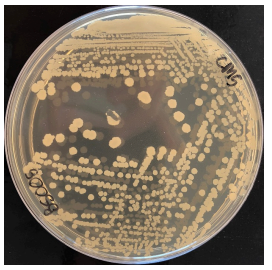
Transformation of heterolous DNA in *Bacillus subtilis*



In 1 collection

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Protocol status: Working
We use this protocol routinely and it works for us

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Abstract

B. subtilis is a gram-positive bacteria used by both academia and industry as a protein production workhorse. This is due to its' excellent fermentation properties, high production titers, and capacity to secrete proteins into the extracellular medium.

This protocol describes transformation of *B. subtilis* by natural competence. The method utilizes the natural stress-induced competence of *B. subtilis* to take up heterologous DNA. The protocol requires the cells to be grown for a specific amount of time in starvation media (SM). The protocol is adapted from Vojcic, L., Despotovic, D., Martinez, R., Maurer, K., & Schwaneberg, U. (2012). An efficient transformation method for *Bacillus subtilis* DB104. *Applied Microbiology and Biotechnology*, *94*(2), 487–493. https://doi.org/10.1007/s00253-012-3987-2.

Guidelines

This protocol works for a range of *B. subtilis* strains (e.g. 168, WB800, PY79, KO7, KO7-S, and derivatives), although the protocol might need optimization for heavily growth impaired strains. For protein production, it is generally recommended to use a protease deficient strains such as WB700, WB800, KO7 or KO7-S.

Materials

MATERIALS

- Glucose **P212121** **Catalog #**Glucose
- Sodium citrate **P212121**
- Ammonium Sulfate **P212121**
- Potassium phosphate (dibasic) **P212121**
- Calcium Chloride
- Potassium dihydrogen phosphate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**NIST200B
- Magnesium Sulfate **Amresco** **Catalog #**0338
- Yeast extract
- Bacto™ Casamino Acids **Thermo Fisher** **Catalog #**223020

Safety warnings

This protocol describes the construction of GMO classified organisms. Make sure that the local GMO and safety legislations are respected.

Before start

Make sure you have your recipient strain freshly streaked on an agar plate.

Preparation of stock solutions

1 Mix 10xSM1 stock

1.1 Weigh the following in a 100mL blue cap bottle:
- 2 g ammonium sulphate
- 14 g dipotassium hydrogen phosphate
- 6 g potassium dihydrogen phosphate
- 0.7 g sodium citrate
- 0.2 g magnesium sulfate heptahydrate
- 2 g yeast extract
- 0.25 g casamino acids

1.2 Add MQ water to 100 mL and mix to dissolve the ingredients

2 Mix 1xSM2 stock

Note

SM2 is made as a 1x stock since it otherwise precipitates

2.1 Weigh the following in a 1L blue cap bottle:
- 2 g ammonium sulfate
- 14 g dipotassium hydrogen phosphate
- 6 g potassium dihydrogen phosphate
- 0.7 g sodium citrate
- 0.8 g magnesium sulfate heptahydrate
- 1 g yeast extract
- 0.1 g casamino acids

2.2 Add MQ water to 1 L and mix to dissolve the ingredients

3 Autoclave the stocks and store at Room temperature or 4 °C

4 Prepare a 200g/L glucose and a 1M $CaCl_2$ stock, separately

Note

Glucose is prepared separately to avoid Millard's reaction. $CaCl_2$ is prepared separately since it makes the SM2 stock precipitate.

Overnight culture

5 Prepare fresh SM1 media from the stock by mixing 1 part 10xSM1 media stock with 9 parts sterile MQ water and 25uL 200g/L glucose stock per mL media (to final concentration of 5g/L).

6 Inoculate 10mL SM1 media in a 50mL falcon tube with a single colony from a freshly streaked plate

7 Incubate Overnight at 37 °C with 250RPM shaking

Note

Make sure to not incubate the overnight culture for longer than 16:00:00 . Using an overnight culture that has been incubating for longer than this, often results in low transformation efficiencies

Transformation

8 Measure OD_{600} of the O/N culture

9 Dilute the O/N culture in 10 mL SM1 media to a final OD_{600} of 0.5 in a 250 mL Erlenmeyer flask

10 Grow culture at 37 °C at 250 RPM shaking for 03:00:00

11 Add 10 mL SM2 medium to the culture

12 Add 250 µL 200g/L glucose stock to the culture

13 Swirl the culture around, and add 45 µL 1M $CaCl_2$ stock while the media is still in motion

Note

The swirling is done since high local concentrations of $CaCl_2$ makes the media precipitate. This way the $CaCl_2$ is mixed in the media before it precipitates

14 Grow culture at 37 °C at 250 RPM shaking for 02:00:00

Note

After this step the cells are competent for approximately 01:00:00

15 Distribute the cells in 500 µL aliquotes in 2 mL eppendorf tubes

Note

The protocol can be paused at this point, by adding 250 µL [M] 50 % volume glycerol to the aliquotes and freezing them in a -80 °C freezer. The protocol can be restarted by thawing the cells on ice and moving on the next step. This can also be used to prepared stocks of strains that are used often

16 Add 250ng - 1pg of plasmid DNA to the aliquotes

Note

Some protocols call for linearized plasmids or PCR products, although we have found that circular plasmids work as well

17 Incubate the aliquotes in a thermoblock for 00:30:00 at 37 °C with 800 RPM shaking

18 Add 300 µL LB media and recover the cells for at least 02:00:00 at 37 °C with 800 RPM shaking

Note

The longer duration of incubation the better

19 Plate up to 200 µL of each aliquot on LB agar plates with appropriate antibiotics

Note

In order to increase the number of colonies on the transformation plates, the aliquotes can be centrifuged for 6000 x g, 00:02:00 and reinoculated in a smaller volume.

20 Incubate the plates Overnight at 37 °C