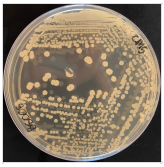

Oct 27, 2020

Transformation of heterolous DNA in *Bacillus subtilis*

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1 Works for me dx.doi.org/10.17504/protocols.io.bdmti46n


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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.

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KEYWORDS

B. subtilis, Bacillus, Bacillus subtilis, Transformation, Natural competence

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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 TEXT

MATERIALS

Glucose **P212121 Catalog #Glucose**

Sodium citrate **P212121**

Ammonium Sulfate **P212121**

Potassium phosphate (dibasic) **P212121**

Calcium Chloride **Contributed by users**

Potassium dihydrogen phosphate **Sigma – Aldrich Catalog #NIST200B**

Magnesium Sulfate **Amresco Catalog #0338**

Yeast extract **Contributed by users**

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.

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.

BEFORE STARTING

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

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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

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

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

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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

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**

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

The protocol can be paused at this point, by adding **250 µl** **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

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

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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

The longer duration of incubation the better

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

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**

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