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Protein expression in Bacillus subtilis

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1 Works for me dx.doi.org/10.17504/protocols.io.bdmui46w



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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' their excellent fermentation properties, high production titers, and capacity to secrete proteins into the extracellular medium

This protocol describes how to express proteins in B. subtilis. The protocol is developed using KO7-S, although it might also work for other strains as well. The method is adapted from Rasmussen, M. D.; Bjoernvad, M. E.; Diers, I. Pectate Lyase Fusion for Expression and Secretion of Polypeptides. WO 00/75344, 2000 and Jensen, K.; Østergaard, P. R.; Wilting, R.; Lassen, S. F. Identification and Characterization of a Bacterial Glutamic Peptidase. BMC Biochem. 2010, 11 (1), 47. https://doi.org/10.1186/1471-2091-11-47.

DOI

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PROTOCOL CITATION

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KEYWORDS

B. subtilis, Bacillus, Bacillus subtilis, Protein expression

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Mar 13, 2020

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Oct 27, 2020

PROTOCOL INTEGER ID

34196

MATERIALS TEXT

MATERIALS

Sodium molybdate dihydrate Contributed by users

manganese sulfate Contributed by users

users Catalog #236489

Aldrich Catalog #204986

Sodium Phosphate dibasic Fisher

Scientific Catalog #S373-500

⊠ Copper (II) sulfate pentahydrate Sigma −

Aldrich Catalog #209198

🔯 Nalgene™ Rapid-Flow™ Sterile Disposable Bottle Top Filters with PES Membrane, 150mL, 0.45μm pore, 45mm neck **Thermo**

Fisher Catalog #296-4545

mprotocols.io

10/27/2020

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Maltodextrin (DE 13.0-17.0) Sigma
Aldrich Catalog #419680

Pluronic L-61 Sigma
Aldrich Catalog #435422

SAFETY WARNINGS
Be sure to wear protective equipment
ABSTRACT

B. subtilis is a gram-positive bacteria is due to its' their excellent fermentatic extracellular medium.

This protocol describes how to expressible also work for other strains as a

Be sure to wear protective equipment when adjusting the pH of the media. Follow local safety regulations are protective equipment when adjusting the pH of the media. Follow local safety regulations are protective equipment when adjusting the pH of the media. Follow local safety regulations are protective equipment when adjusting the pH of the media. Follow local safety regulations are protective equipment when adjusting the pH of the media. Follow local safety regulations are protective equipment when adjusting the pH of the media. Follow local safety regulations are protective equipment when adjusting the pH of the media. Follow local safety regulations are protective equipment when adjusting the pH of the media. Follow local safety regulations are protective equipment when adjusting the pH of the media. Follow local safety regulations are protective equipment when adjusting the pH of the media are protective equipment when adjusting the pH of the media are protective equipment and physical equipment are protected as a physical equipment and physical equipment are protected as a physical equipment and physical equipment are protected as a physical equipment and physical equipment are protected as a physical equipment and physical equipment are protected as a physical equipment and physical equipment are protected as a physical equipment and physical equipment are protected as a physical equipment and physical equipment are protected as a physical equipment are protected as a physical equipment and physical equipment are protected as a physical equipment and physical equipment are protected as a physical equipment and physical equipment are protected as a physical equipment and physical equipment are protected as a physical equipment and physical equipment are protected as a physical equipment and physical equipment are physical

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BEFORE STARTING

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

Cal18-2 media preparations

1 Prepare a stock solution of 2.0g/L Na₂MoO₄. Sterilize by filtration

- 2 Prepare a trace metal solution consisting of
 - 4.48g/L MnSO₄·H₂O
 - 3.33g/L FeCl₃·6H₂O
 - 0.625g/L CuSO₄·5H₂O
 - 7.12g/L ZnSO₄·7H₂O

Sterilize by filtration

- 3 Fill a blue cap bottle to \sim 80% of the desired final volume with MQ water.
- 4 Add a magnetic stirrer to the blue cap bottle and place the bottle on a stirring plate. Turn on the stirring, and make sure it's mixing well.
- 5 Add the following to the bluecap bottle:
 - 40g/L yeast extract
 - 1.3 g/L MgSO₄·7H₂O
 - 50 g/L maltodextrin (DE \sim 12)
 - $\hspace{0.4in} \bullet \hspace{0.4in} 20 \hspace{0.4em} g/L \hspace{0.4em} \text{NaH}_2 \text{PO}_4 \cdot 2\text{H}_2 \text{O} \\$
 - 6.7mL/L 2.0g/L Na₂MoO₄ stock solution
 - 6.7mL/L Trace metal solution
 - 100μL/L Pluronic L-61
- 6 Make sure that all of the ingredients are dissolved
- 7 Adjust to pH 6 with 5M NaOH
- 8 Add MQ water to the desired final volume
- 9 Sterilize by filtration



The media easily clogs filters, so choose a 0.45μM vacuum bottle top filter for this step and be prepared to

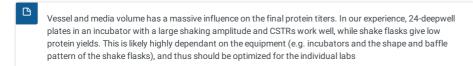
 10 Store the media at 8 4 °C until needed

Overnight culture - Day 1

- 11 Inoculate between 3 mL to 50 mL LB media with a single colony of the expression strain. Depending on the expression volume and overnight OD. The culture can be grown in in a 24-deepwell plate, a falcon tube or a shake flask
- 12 Grow the strain at § 37 °C © Overnight
 - Make sure to not incubate the overnight culture for longer than \odot **16:00:00**. Using an overnight culture that has been incubating for longer than this, often results in non-reproducible results

Expression - Day 2 - 4/5

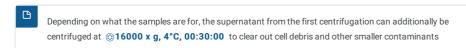
13 Prepare the desired volume of expression media in the desired vessel



- 14 Inoculate the expression media to an OD₆₀₀ of 0.1
- 15 Incubate the expression culture at § 20 °C with 250 RPM shaking between © 48:00:00 and © 72:00:00
 - The expression temperature and duration is dependant on the target protein, although the specified values seem to be a good starting point in our experience

Harvesting - Day 4/5

16 Harvest the culture by centrifuging at **60000 x g, 4°C, 00:05:00**



17 Keep the sample & On ice when working with it and at & -20 °C for storage