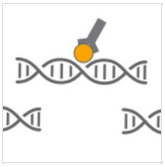

Oct 24, 2020

Transcriptomics

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Other dx.doi.org/10.17504/protocols.io.bme9k3h6

iGEM Groningen 2020

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ABSTRACT

The potato root secretes root exudates, a mixture of organic molecules out of which solanine is found in the highest concentration (range of ug/ml). Also, solanine is a molecule specifically found in the root exudate of potato plants, which makes it a good candidate for a dependecy molecule which will ensure that *Bacillus mycoides* doesn't escape the designated action area. Studies have shown that there are soil bacteria able to metabolize solanine as a carbon source. Unfortunately, we weren't able to find an already described solanine-inducing promoter. Because the natural habitat of *B. mycoides* is the potato rhizosphere, we have strong reasons to believe that if such a promoter may already be present in its genome.

In order to find this promoter, we designed a CHIP-Seq experiment. B. mycoides will be incubated with a different range of solanine concentrations. The DNA will be extracted and the CHIP-Seq technique will be used to detect potential binding sites of solanine operators using monoclonal anti-solanine antibodies.

DOI

dx.doi.org/10.17504/protocols.io.bme9k3h6

PROTOCOL CITATION

Andreea S 2020. Transcriptomics. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bme9k3h6

CREATED

Sep 16, 2020

LAST MODIFIED

Oct 24, 2020

PROTOCOL INTEGER ID

42177

ABSTRACT

The potato root secretes root exudates, a mixture of organic molecules out of which solanine is found in the highest concentration (range of ug/ml). Also, solanine is a molecule specifically found in the root exudate of potato plants, which makes it a good candidate for a dependecy molecule which will ensure that *Bacillus mycoides* doesn't escape the designated action area. Studies have shown that there are soil bacteria able to metabolize solanine as a carbon source. Unfortunately, we weren't able to find an already described solanine-inducing promoter. Because the natural habitat of *B. mycoides* is the potato rhizosphere, we have strong reasons to believe that if such a promoter may already be present in its genome.

In order to find this promoter, we designed a CHIP-Seq experiment. B. mycoides will be incubated with a different

range of solanine concentrations. The DNA will be extracted and the CHIP-Seq technique will be used to detect potential binding sites of solanine operators using monoclonal anti-solanine antibodies.

Sample preparation

1 Dilute an overnight culture of B. mycoides to OD600=0.05 in LB. Divide the culture in 2 parts. Supplement one of them with solanine (concentration range from [M]**0.0001 mg/ml** to [M]**1 mg/ml**) . Keep the other one as a negative control.

2 Culture the cells for **200 rpm, 30°C, 01:00:00**

3 Collect cells by spinning down at **500 x g, 4°C, 00:05:00**

4 Wash cells with PBS at room temperature.

5 Remove the PBS and add freshly made basic cell culture media containing [M]**1 % volume** formaldehyde to cross-link the DNA-protein complexes.

6 Incubate for **00:10:00** **Room temperature** with gentle agitation on a rocking platform.

7 Remove the fixation solution by spinning down at . **500 x g, 4°C, 00:05:00**

8 To quench the reaction, for cell count of 1-5 million, add **3 mL** [M]**0.65 Molarity (M)** glycine solution. (!glycine solution concentration dependent on cell count)

9 Incubate at **Room temperature** **00:05:00** with gentle agitation on a rocking platform.

10 Remove the glycine solution by **500 x g, 4°C, 00:05:00** . Discard the supernatant. The pellet can be frozen at **-80 °C** after adding **1 µl** of Protease Inhibitor Cocktail (PIC).

11 To lyse the cells, for cell count of 1-5 million, add **0.4 mL** of Hypotonic Buffer; Resuspend the cells and incubate at **4 °C** **00:05:00** .

12 Centrifuge the hypotonic slurries at **5000 x g, 4°C, 00:05:00** to collect the nuclei.

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13 For cell count of 1-5 million, add **0.3 mL** of Digestion Buffer to the nuclei, immediately followed by adding **2 µl** of PIC to each sample.

14 Add Micrococcal Nuclease to each sample to digest the DNA. Mix by inverting the tube several times and incubate at **37 °C** **00:20:00** . Mix by inversion every 3-5 minutes. The amount and incubation time of Micrococcal Nuclease required to digest the genomic DNA to an optimal 150 900 bp length may need to be determined empirically for individual cell types.

15 Stop digestion by adding **10 µl** of **0.5 Molarity (M)** EDTA per sample and place the sample on ice.

16 Pellet nuclei by centrifugation at **12000 x g, 4°C, 00:01:00** .
Discard the supernatant.

17 Resuspend nuclear pellet in Lysis Buffer and incubate the sample **On ice** **00:10:00** to lyse the nuclei. Alternatively, sonicate to shear the DNA. The time and strength for sonication may need to be determined empirically.

18 Centrifuge the sample at **16000 x g, 4°C, 00:10:00** .
Transfer the supernatant to a clean dry microcentrifuge tube.

19 Add **1 µl** of PIC to each sample and mix.

20 Chromatin sample is now ready for ChIP Assay. If the sample is not to be used immediately, store at **-80 °C** .
It is recommended that the shearing efficiency is analyzed at this stage to ensure that 150-900bp fragments are obtained during shearing the DNA to increase the ChIP efficiency.

21 Mix the Chromatin Sample, Protease Inhibitor Cocktail (PIC), optimal quantity of BioLegend's Go-ChIP-GradeTM Purified antibodies and add Column Conditioning Buffer to make final **1 mL** slurries.
Gently rotate at **4 °C** for **01:00:00**
incubation can also be extended **Overnight .

Chromatin Immunoprecipitation Assay:

22 Prepare the high-throughput (HT) Protein A or G 96 well plate or Spin Column by adding **600 µl** of Column Conditioning Buffer in each well or column and allow it to flow through via gravity (~ **00:15:00**).

23 Discard the flow-through and repeat steps 18 and 19.

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24 Remove the slurries from the rotator following **4 °C** incubation and briefly spin down to remove residual liquid from the caps.

25 Load the entire **1 mL** slurries and allow to flow completely through the high-throughput (HT) Protein A or G 96 well plate or Spin Column at room temperature (approximate **00:15:00**).

26 Add **600 µl** of Wash Buffer 1 to each well or column and centrifuge at **2.000 x g** for plate, or **4.000 x g** for column, for **00:01:00** at **Room temperature** . Discard the flow through and repeat once.

27 Add **600 µl** of Wash Buffer 2 to each well or column and centrifuge at **2.000 x g** for plate, or **4.000 x g** for column, for **00:01:00** at **Room temperature** . Discard the flow through and repeat once.

28 Add **600 µl** of Wash Buffer 3 to each well or column and centrifuge at **2.000 x g** for plate, or **4.000 x g** for column, for **00:01:00** at **Room temperature** . Discard the flow through and repeat once.

29 Spin dry at **4.000 x g** for the plate or **16.000 x g** for the column for one minute at room temperature to remove any remaining liquid from the membrane of the plate or column. Place a clean 96 well collection plate or a collection tube beneath the plate or column.

30 Add **50 µl** of Elution Buffer to each well or column. Incubate at room temperature for **00:15:00** .

31 Centrifuge the plate or column at **4.000 x g** for the plate, or **16.000 x g** for the column, for **00:01:00** at **Room temperature** to collect the eluted chromatin-protein complex.

32 To each eluted sample, add **5 µl** of 1M NaHCO3, **5 µl** **5 Molarity (M)** NaCl and **50 µl** of Distilled Water. Mix thoroughly and incubate at **65 °C** on the heat block for two hours or the incubation time can be extended to overnight.

33 Remove the sample from the heat block, add **1 µl** of Proteinase K to each sample, vortex briefly and perform a short spin down. Incubate at **37 °C** for **01:00:00** .

34 Add **2 µl** of Proteinase K Stop Solution to each sample, vortex briefly and perform a short spin down.

35 Purify the DNA by QIAquick™ PCR purification kit, according to manufacturer's manual. Purified DNA is ready for downstream real-time qPCR analysis.

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