OCT 24, 2020

Transcriptomics

Andreea S¹

¹University of Groningen

iGEM Groningen 2020

a.stan.6

OPEN ACCESS

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>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Other
 The protocol is developed based on literature and has not been tested yet.

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 dependency 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. (lglycine 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.
- 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-Grade™ 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.
- 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 μ L 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 μ L 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 μ L 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 μ L 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.