

Oct 24, 2020

NLP screening (dot blot)

DOI

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



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iGEM Groningen 2020



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Protocol Citation: Andreea S 2020. NLP screening (dot blot). protocols.io <https://dx.doi.org/10.17504/protocols.io.bkbfksjn>

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Protocol status: Other

The protocol is developed based on literature, but has not been tested yet.

Created: August 25, 2020

Last Modified: October 24, 2020

Protocol Integer ID: 41031

Keywords: dot blot procedure, detecting protein, dot blot, nlp screening, immunological technique, bacteria, protein, tagged nlp, testing different secretion signal, nlp, gel separation

Abstract

In order to determine if the His-tagged NLP is expressed and secreted by the bacteria, we plan to use the dot blot procedure with anti-His tag anti-bodies. Dot blot is an immunological technique used for detecting proteins directly from the culture supernatant (without gel separation). Thus, the samples are directly spotted on the membrane, making it a high throughput procedure ideal for testing different secretion signals.

Materials




MATERIALS

⊗ TBS: 20 mM Tris-HCl 150 mM NaCl pH 7.5

⊗ TBS-T: 0.05% Tween20 in TBS

⊗ BSA/TBS-T: 0.1% BSA in TBS-T

Troubleshooting

- 1 On nitrocellulose membrane indicate the blotting region by drawing a grid by pencil.
- 2 Slowly apply  2 μL of sample on the nitrocellulose membrane at the center of the grid.
- 3 Use purified NLP-His and apply 5 points of increasing concentration of them on the nitrocellulose membrane in order to create a standard concentration curve.
- 4 Dry the membrane.
- 5 Block non-specific binding sites by washing the membrane in 5% BSA in TBS-T for 1h at room temperature.
- 6 Incubate with primary antibody diluted at the concentration recommended by the producer for 30 min. This step may require optimizing of the concentration.
- 7 Wash 3 times with TBS-T for  00:05:00
- 8 Incubate with the secondary antibody conjugated with HRP for  00:30:00 (concentration recommended by the producer).
- 9 Wash 3 times with TBS-T (15 min, 5 min, 5 min)
- 10 Wash with TBS
- 11 Incubate with ECL reagent for 1 min. Develop the blot.
- 12 Compare the intensity of the sample with the standard curve in order to estimate the concentration.