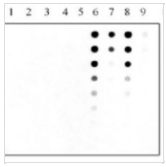

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NLP screening (dot blot)

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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.

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MATERIALS TEXT

MATERIALS

TBS: 20 mM Tris-HCl 150 mM NaCl pH 7.5 **Contributed by users**

TBS-T: 0.05% Tween20 in TBS **Contributed by users**

BSA/TBS-T: 0.1% BSA in TBS-T **Contributed by users**

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.

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.

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