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Streptavidin dot blot with aptamers V.1

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

The aim of this protocol is to check the efficiency of the detection system that will be present in the strips. The result expected is a good binding between the streptavidin and the aptamers, great hybridization between both aptamers and a clear visible result when yielding with peroxidase.

Materials

MATERIALS

- Pipette Tips
- Glass Petri dishes 90 × 15 cm
- Streptavidin, 1mg **Promega Catalog #**Z7041
- PBS
- nitrocellulose membrane sheets size 210 m × 297 mm thickness 200 µm **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**Whatman® FF170HP Din A
- Wax crayons (non water-soluble)
- Ultrospec 1100 pro

Preparing the nitrocellulose strip

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Set the hot plate to 100ºC or at least warm enough to melt the wax. Cut a small amount from one of the wax pencils and place it on a Petri dish. Set the Petri dish on the hot plate and wait for the wax to melt.

Cut a strip from the nitrocellulose sheet with the desired size.

Once the wax is melt, grab a 200µL pipette tip from the pointy side and place the broad end in the wax. Check the whole circle contains enough wax and carefully place it on the nitrocellulose strip. Apply a little pressure and remove the pipette tip. You should be able to see the wax circle on the strip.

Cover the nitrocellulose strip with paper (one sheet on top, another underneath). Place the sandwich on the hot place with a Petri dish on top (this is just to apply some pressure, any temperature-resistant flat object is valid here). Allow the strip to warm for 5 minutes. This will allow the wax to penetrate all through the strip.
Remove the strip from the hot plate and let it cool down again. You should see a circle of wax but the center must be clear, otherwise the protein will not have anywhere to bind. If the circle is too thick, consider repeating these steps.

Adding the streptavidin and blocking the membrane

2 Pipette 5 µL of Streptavidin. If the protein is too diluted, you might not see a staining with the Ponceau. We ended using the protein suspended in PBS but without any dilutions.

Allow it to dry completely. Room temperature is adequate.

Once the membrane is completely dry, pipette 5 µL of the blocking solution (BSA, powder milk and Tween 20) to block the empty spaces left in the membrane. Allow it to dry completely again.

Hybridization of the aptamers

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