Dot to dot: Troubleshooting spotty or patchy western blots

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Dark spots and white patches on western blot

You came in early today, as you really needed the results of this western blot. It'll determine what happens next, and if the last week's work was wasted.

You patiently and diligently followed the protocol, finishing off paperwork during incubations and running back into the lab for each subsequent step. And, now it's finished.

You look at your blot.

You're reminded of the grainy image from an early 1800s camera at the dawn of photography – white gaps and dark spots cover it. It's hard to make out what you're looking at. You sigh.

We've all been there, and it is frustrating.

There are a wide range of things that can go wrong during a blot, or any experiment with so many steps. We are therefore creating a series of posts concerning issues you may encounter with your western blot and the ways to fix them. This post will highlight some of the more common reasons for patchy or uneven spots all over a blot.

Below we list some of the more common problems, which may appear alone or in combination, and the possible solutions to them.

As a general tip, if you have problems and need to repeat a blot, it's good practice to start again with freshly cleaned equipment and freshly made buffers and reagents stored appropriately, where possible.

Uneven white spots or patches across western blot

There's a couple of reasons this can happen.

Possible Cause 1: Air Bubbles

This generally indicates that there were air bubbles trapped against the membrane during the transfer step. The air bubbles prevent a complete transfer, meaning there's nothing on the membrane for the detection antibody to detect.

Solution:

Be careful to remove, or roll out, any bubbles when assembling the transfer sandwich. Using extra transfer buffer may help too.

You can also use Ponceau S, which reversibly stains proteins on western blots, to check how well the transfer has gone before moving on to the detection antibody steps.

Possible Cause 2: Detection Antibody

- Another possibility is that the detection antibody didn't fully cover the membrane, or it wasn't incubated with gentle agitation.

Solution:

Ensure that you're using enough detection antibody to cover the blot with gentle agitation during the incubation, or that you're using an appropriately sized container for the size of the membrane.

Black dots randomly scattered across western blot

Possible Cause 1: Blocking Agent

This could be due to the blocking agent (BSA, milk, etc) having not dissolved properly prior to being used, and sticking to the membrane in clumps. These clumps can trap or bind the detection antibodies, leading to the black spots.

Solution:

Make sure that the blocking agent is thoroughly dissolved in your blocking buffer. Adding a little (0.1%) tween-20 can also help this out, as can filtering buffers through a 0.2 µm filter.

Possible Cause 2: Antibody storage

Sometimes, if antibodies haven't been stored incorrectly, are getting old, or have seen multiple freeze-thaw cycles, they can begin to aggregate forming the clumps which can cause dark spots.

Solution:

Ideally use a fresh aliquot of the antibody. If this is not possible, you may be able to filter the antibody buffer through a 0.2 µm filter to help remove aggregates. Adding a little tween-20 (0.1%) may also help to break them up.

Possible Cause 3: Contaminated buffer

- Rarely can anyone tell how or why a buffer has become contaminated, but sometimes they do.

Solution:

If repeating a western blot, make up fresh buffers for all steps, if possible. Again, filtering the buffer may be helpful if this is not possible.