Microwestern Wet Transfer

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Validated by / Date:

NOTE: This protocol was inspired by that written by Mark Ciaccio (Ciaccio MF, Wagner JP, Chuu CP, Lauffenburger DA, Jones RB (2010) Systems analysis of EGF receptor signaling dynamics with microwestern arrays. Nat Methods 7:148–155; Youtube videos on MWA, www.youtube.com/watch?v=0iUhoWL1lC0).

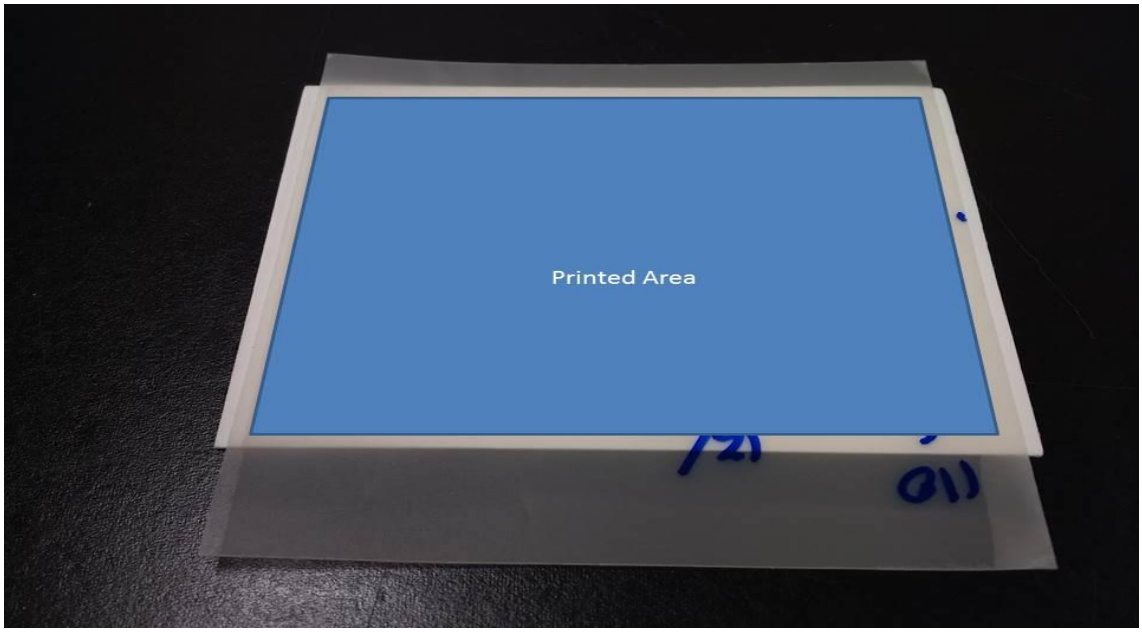
Quality Assurance/Control (QA/QC) steps are indicated with green highlight.

Metadata recording is highlighted with yellow highlight and superscript indices.

1) Prepare transfer buffer
    a. 10X Tris/Glycine
        i. Dissolve 30.2 g Tris (BioRad, #161-0719[1]) and 150.1g Glycine (BioRad, #161-0718[2]) in a final volume of 1 L MilliQ water (Millipore Advantage A10 system 18.2 MΩ). This can be kept at room temperature for several months (at least).
        ii. Measure pH; should be ~8.5. If less than 8.2 or greater than 8.8 start over with fresh Tris and Glycine.
    b. To make transfer buffer combine 300 mL methanol (VWR, # BDH1135-4LP[3]), 150 mL 10X Tris/Glycine and bring up to a final volume of 1.5 L with MilliQ water. Keep at 4°C.
2) Remove one "filter paper/Nitrocellulose (NC)/filter paper" sandwich (BioRad, #162-0233[4]) with gloves from box. Leave NC on top of one of the pieces of filter paper on clean bench surface. Immerse the other filter paper piece in 25 mL of transfer buffer.. Allow excess buffer to drain off and place filter paper on lab bench that has been cleaned with 70% ethanol.

3) Place a printed gel that has been subjected to electrophoresis (see Gel Electrophoresis SOP A 13.0) onto the damp filter paper, samples up, so that the sample region overlays filter paper as shown in Figure 1 below:

Figure 1. Printed gel overlaying blot



   a. If an air bubble forms between the filter paper and gel, lift the gel and place back down so that gel lies evenly on the filter paper.
4) Wet the NC sheet of the sandwich in the same 25 mL transfer buffer, drain excess buffer and place onto gel. Wear gloves and take care not to fold or bend the NC.
5) Carefully roll out any bubbles (BioRad #1704070, Criterion blotter kit; roller is part of package) without moving the NC. This step is extremely important. Moving the NC after placing it on the gel may create a double or smeared image. Precise transfer is more critical than for a normal Western blot because much of the protein is on the surface of the gel after electrophoresis and will transfer instantaneously when touching the nitrocellulose.
6) Immerse second filter paper from the sandwich into the same transfer buffer, drain excess as before, and place on top of the NC. Carefully roll out any bubbles as in Step 5.
7) Place filter/gel/NC/filter sandwich between 2 foam pads and then into blotter gel holder cassette with NC closer to the red side (BioRad #1704070, Criterion blotter w/plate electrode kit which includes foam pads, ice block and gel holder cassettes).
8) Add ice block to Criterion Blotter tank, slide cassette into tank with red side of cassette facing red side of tank, and fill with transfer buffer; about 1.5 L is needed. Add stir bar.
9) Transfer sample from gel to nitrocellulose overnight with power supply set at 150 mA at 4^{o}C while stirring (we place apparatus in the cold room).

Metadata

Record lot # for the following:

The specific control and lot #'s used in our experiments (as of July 2016) are:

1. Tris (BioRad, #161-0719) Control # 210003844

2. Glycine (BioRad, #161-0718) Control #210012087

3. Methanol (VWR, # BDH1135-4LP) Lot # 081916E

4. (BioRad, #162-0233) Control #1201323