Icahn School of Medicine at Mount Sinai LINCS Center for Drug Toxicity Signatures

Standard Operating Procedure: Gel Electrophoresis for Microwestern Array

DToxS SOP Index: SOP A 13.0
Last Revision: 4/3/2017
Written By: Mark Ciaccio and Rick Koch

Approvals (Date): Joseph Goldfarb (5/11/2017)
Marc Birtwistle (4/3/2017)
Ravi Iyengar (4/3/2017)

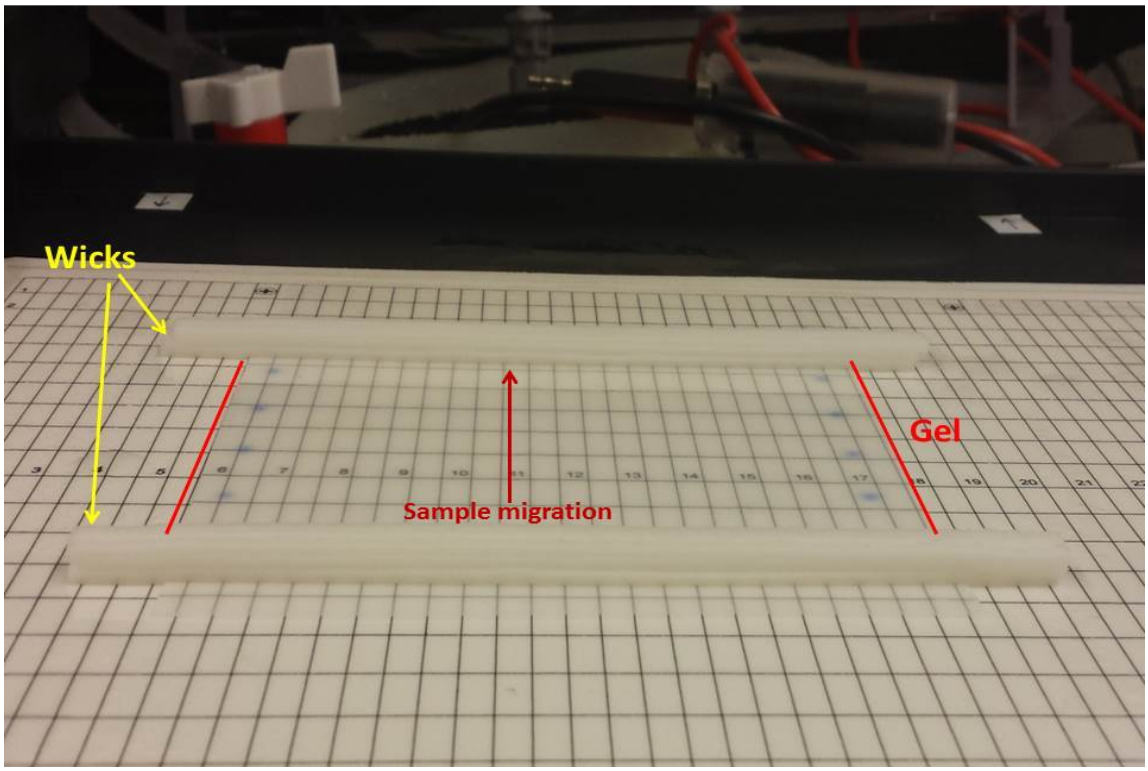
Quality Assurance/Control (QA/QC) steps are indicated with green highlight.

Metadata recording is highlighted with yellow highlight and superscript indices

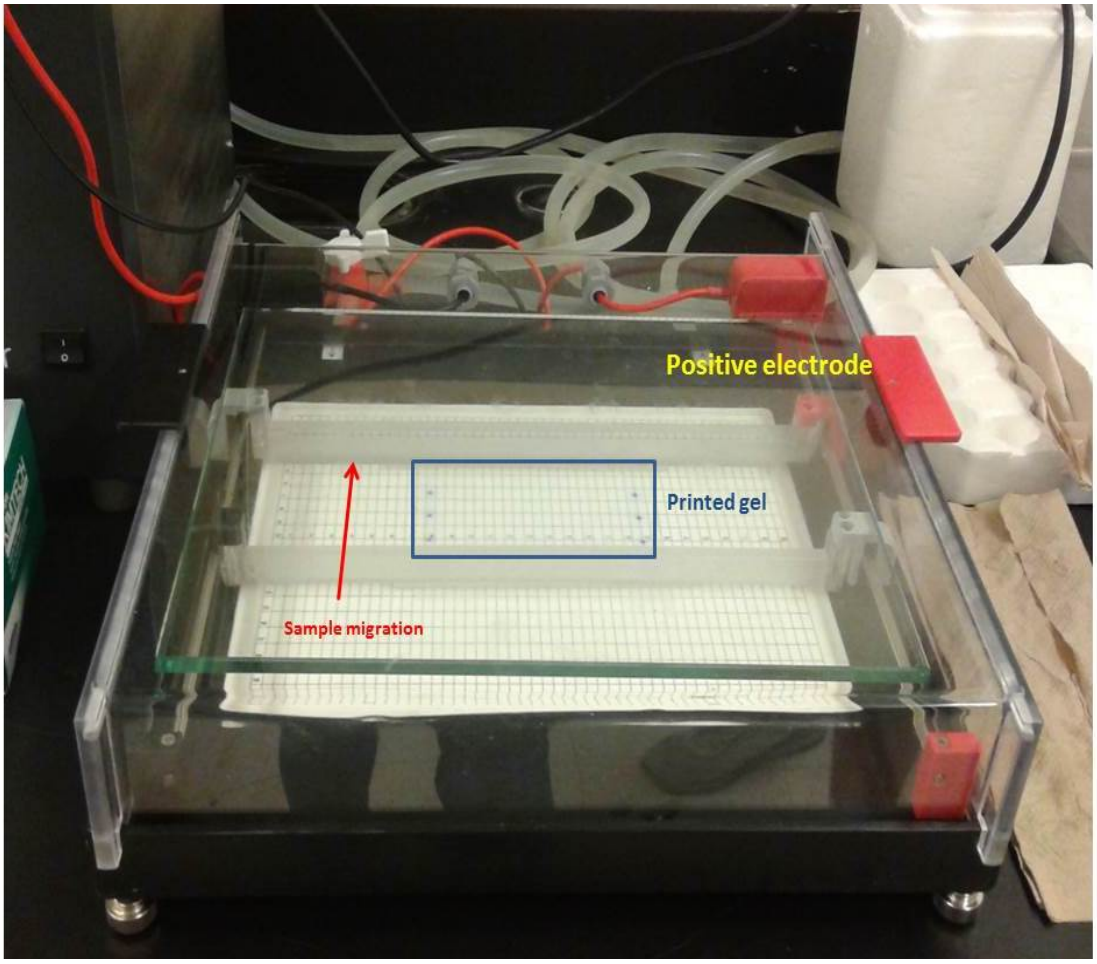
1) Preparation of reagents
   a) Rehydration buffer
      i) Combine 20 mL 5x Gel Buffer (SOP A 9.0, step *2a*), 20 mL glycerol (Sigma #G5516[1]) and 57 mL MilliQ water (Millipore Advantage A10 system 18.2 MΩ) in a 125 mL Erlenmeyer flask (Fisher, #FB-500-125).
      ii) Cover with parafilm (Fisher, #S37440) and mix vigorously by hand, shaking to ensure glycerol is evenly mixed
      iii) Add 1 mL 10% SDS (Fisher #BP 2436[2]) and gently mix by swirling.
      iv) Add 1 mL 1M NaBisulfate (6.00 g NaBisulfate (Fluka, #71656[3]) in a final volume of 50 mL of MilliQ water).
      v) Add 1 mL 1M DTT (Fisher, #AC42638[4]).
   b) Electrode buffer
      i) Combine 20 mL 5x Gel Buffer (SOP A 9.0, step *2a*), 29.5 mL of MilliQ water and 0.5 mL 10% SDS. Keep at 4°C. Prepare the day of electrophoresis.
2) Electrophoresis of printed gel
   a) Pre-chill the electrophoretic box (Gel Company #FC-EDCProf-2836) to 10°C with the Huber Minichiller (Huber, #3006.0029.99).
   b) While wearing gloves lift gel from the Nanoplotter deck (see SOP A 11.0, step 21) avoiding the printed area.
   c) Remove bottom plastic sheet from the printed gel by slowly peeling gel off sheet keeping the gel from curling in on itself.
   d) Place gel sample-side-up gently into 100 mL of Rehydration buffer in a glass tray (Daigger, #EF25365B) for 5 minutes.
      i) Gently submerge the entire gel with the sample side up.

ii) Wearing gloves, gently remove gel from rehydration buffer and vertically dab the gel bottom edge (along the D row of a gel used for a 24 well plate) on a paper towel for up to 5 seconds to remove excess buffer.
iii) Avoid pools of rehydration buffer on top of gel. If visible fluid is present leave gel on the electrophoresis box for 5 – 10 minutes to allow rehydration buffer to absorb into gel.
iv) QA/QC1 For prints that take 5 hours or longer (see SOP#A-11 0, Printing gel) the hydration time in step 2)d) should be increased to 12 minutes to avoid sample not migrating (sometimes referred to as “clumping” post-blotting).

e) Place the hydrated gel on the bed of the electrophoretic box with the sample side on top. Orient the gel so the proteins migrate to the red (positive) electrode.



f) Cut 3 filter wicks (Fisher, #14056, Serva wicks, 300 x 6 x 1 mm) in half to give 6 150 mm long wicks and soak all in electrode buffer for several seconds.
g) Stack 3 wicks on top of one another and place on the top of the gel (above spots) perpendicular to the eventual voltage. Repeat for the bottom of the gel.
i) Wicks should cover the length of the gel.
h) Run a gloved finger gently over the length of the wicks to make sure thorough (uniform ?) contact is made with the gel.



i) Place electrodes onto wicks and secure them with the heavy glass plate. Close the cover of the electrophoresis box.

j) Run the power supply at 60 V, constant voltage, for 5 min.

k) Turn the voltage up to 300 V (still at constant voltage). Stop the run when the bromophenol blue has run about 14 mm, approximately 30 minutes. (See SOP 11.0, Printing of Samples, step 18b).

i) Note: The time at 300V refers to a 24 well plate. This time will be shorter and should be independently determined for a 48 well or 96 well plate. Time will depend on the particulars of your equipment and setup so should be independently optimized. Particulars include the size of the wells (48 well size is 9mm x 18 mm vs the 18 mm x 18 mm for a 24 well plate) and the size of the proteins measured.

Metadata
Record lot # for the following:

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

1. Glycerol (Sigma #G5516) Lot # SHBH0231V

2. 10% SDS (Fisher #BP 2436) Lot #121099

3. NaBisulfate (Fluka, #71656) Lot #BCBD7791V

4. 1M DTT (Fisher, #AC42638) Lot #A0345819

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Quality Assurance/Quality Control

QA/QC 1. If gel is not properly hydrated sample will remain in the spot originally printed, failing to cleanly migrate down the gel as shown in the figure below (i.e. “clumping”).