Printing a Microwestern Gel

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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=0iUhoWL1IC0).

Pre-requisite Note: We use the GeSim Nanoplotter Model 2.1E; GeSim software NPC16 V2.15.53 and Short Manual for Version 2.15.50 and later.

Printing requires the choice of an appropriate work plate, transfer file, and pipetting program depending on the experiment. A work plate graphically defines all the objects (microtiter plate(s), gel(s), wash station, stroboscope) in x, y space on the Nanoplotter platform (See p.65, Short Manual). We use the work plate, "050415RK_24 Well.npw" when using the 24 well hybridization plate. A transfer file specifies which samples are spotted in a particular position on the gel (See p. 94 and p. 129, Short Manual). Transfer files generally vary, reflecting the number of samples to be printed and the positioning of those samples on the gel. The pipetting program determines how the pipette(s) pick up the sample(s) and spot the gel (See p. 129, Short Manual, we use "TransferSimMultiPlates_074" program). Our files are freely available upon request, and appropriate choices are described in the SOP. Creating new files is beyond the scope of our SOPs.

1) Load the appropriate work plate file and ensure the transfer file is properly set up.

    a) Click "Work Plate," open Work Plate settings and, in our set up, in "RK workplate files" folder choose "050415RK_24 Well.npw" and click "Load."

    b) Run the transfer file in Simulation mode (section 4.5, GeSim short manual). We recommend doing this the first time a transfer file is used.

c) Our transfer files are set up to deposit 15 drops per spot, and 10 cycles of spots. We draw 1.5 uL of lysate into the tip every cycle, and discard the unused lysate (most of it). There is a recycle option available but so far we have not optimized it.

2) Make 1.0 L 5X Gel Buffer (SOP#A-9.0—step 2a), if not already made up.

a) Note: 5X Gel Buffer must stand overnight so prepare this the day prior.

3) Turn on humidifier (Airwin UB1 CT) to 75%.

4) Prepare 100 mL of Rehydration buffer.

a) In a 125 mL Erlenmeyer flask add 57 mL MilliQ water (Millipore Advantage A10 system 18.2 MΩ); 20 mL of glycerol (Sigma #G5516-500ml) and 20 mL of 5X Gel buffer. Shake to completely mix glycerol.

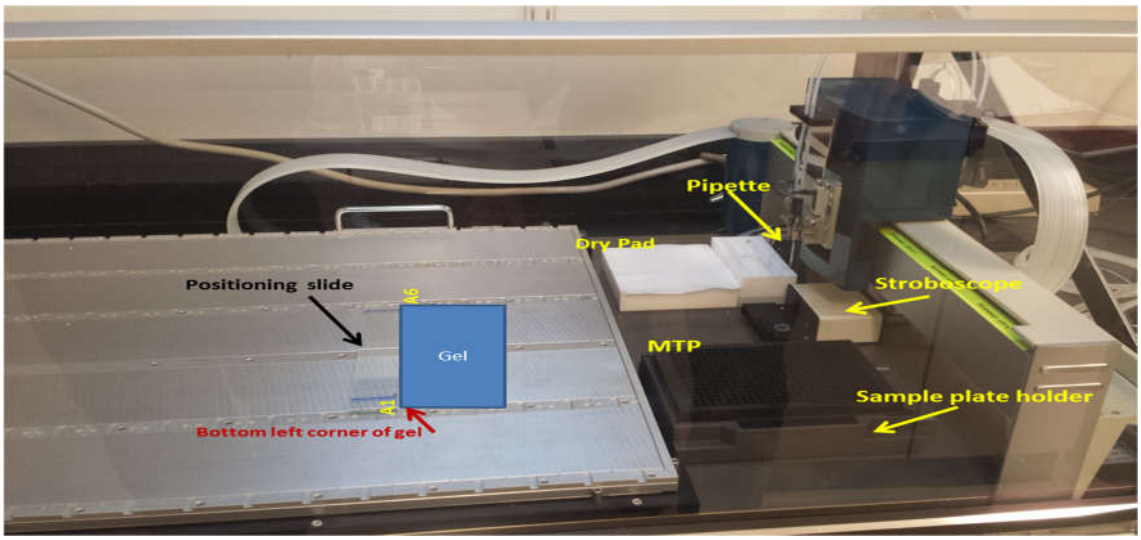
b) Add 1 mL 10% SDS (Fisher #BP 2436); 1 mL 1 M sodium bisulfate (Fluka #71656) and 1 mL 1M DTT (Fisher, #AC42638-100).

5) Place the 100 mL of Rehydration buffer into a clean glass tray (Fisher #15-242B). Take a gel from 4^0C storage (see SOP # A-9.0), remove one of the plastic sheets, and rehydrate with uncovered gel side facing up for 5 minutes in rehydration buffer in glass tray. The gel should be completely immersed.

6) Take gel out of tray and blot off excess buffer by touching gel edge to paper towels at an angle. Save buffer for step 2d in SOP A-13.0 (Gel Electrophoresis).

7) Place the gel with uncovered side up onto the Nanoplotter platform as shown in Figure 1 below. Use the Positioning slide to orient gel in the correct x,y coordinates (refer to Work plate for the positioning for your setup).

Fig. 1: Illustration of Gel Placed on GeSim Platform prior to Printing

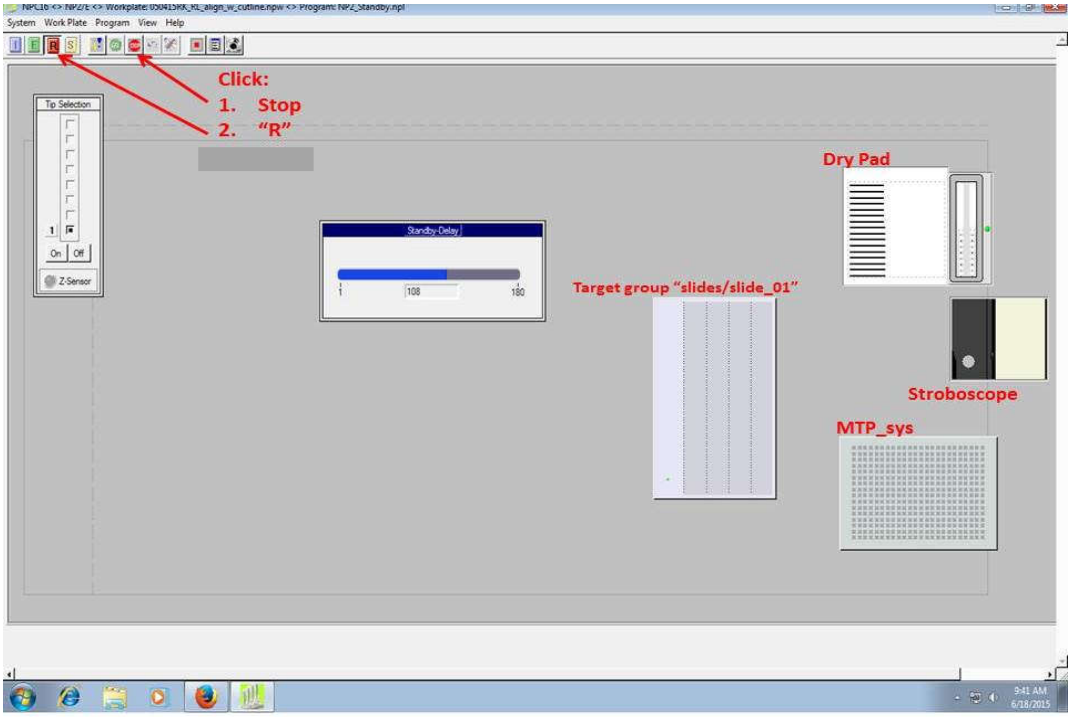


8) Gently roll out bubbles between gel and bottom plastic sheet using roller from BioRad Criterion blotter kit (BioRad#1704070), and allow gel to sit in the humidified chamber for 10 minutes before printing.

   a) Make sure there are no visible puddles of fluid on the gel. If so, repeat Steps 6-8.

9) Take nanoplotter out of “Standby” mode by first clicking “STOP”, and then clicking “R” (see Fig 2, below).

Fig 2: Screenshot of Standby Mode



10) QA/QC1: Check drop alignment.

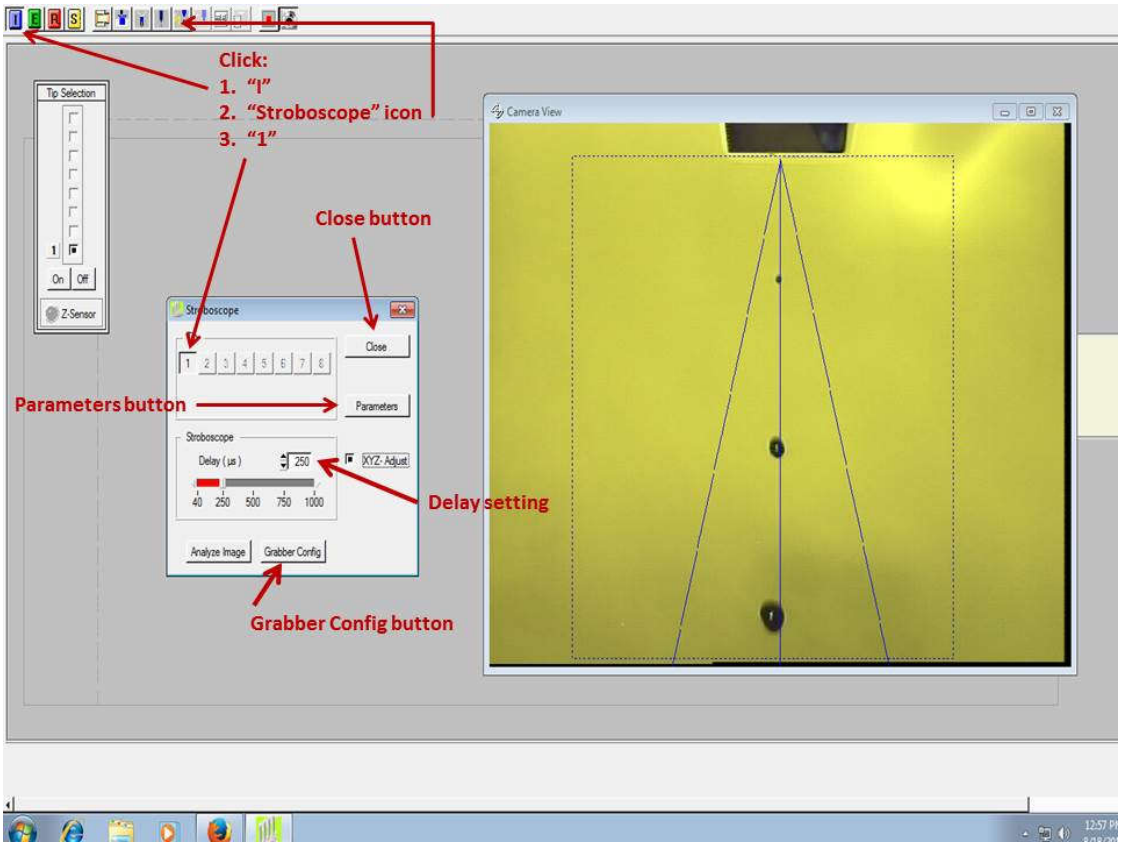
   a) Click “I” mode (see Fig 3 below). This permits water to be ejected from the nanoplotter tip when selected in step c.

   b) Click “Stroboscope” icon opening “Stroboscope” window (see Fig 3 below)

   c) Click on “Tip 1” box in stroboscope window (see Fig 3) to open camera view window and bring pipette into view.

   d) If droplets are aligned as shown below in Fig 3 “Camera View” window, click “Close” in Stroboscope window. (The 3 droplets shown are a “drop”, defined as what is ejected from the nanoplotter pipette due to a single pulse.)

Fig. 3: Screenshot After Opening Stroboscope and Camera View Windows



e) If droplet is not aligned this may be due to improper tip position for spotting the gel. The following steps correct the alignment.

i) Slide "Delay" (see Fig. 3) to 40 µs (from default of 250 µs) to see droplet ejected from the nozzle of the pipette tip as shown below in Fig.4.

ii) Click "Grabber Config" tab shown in Fig. 3 above which produces the "Grabber Config screen shot", below, Fig. 4.

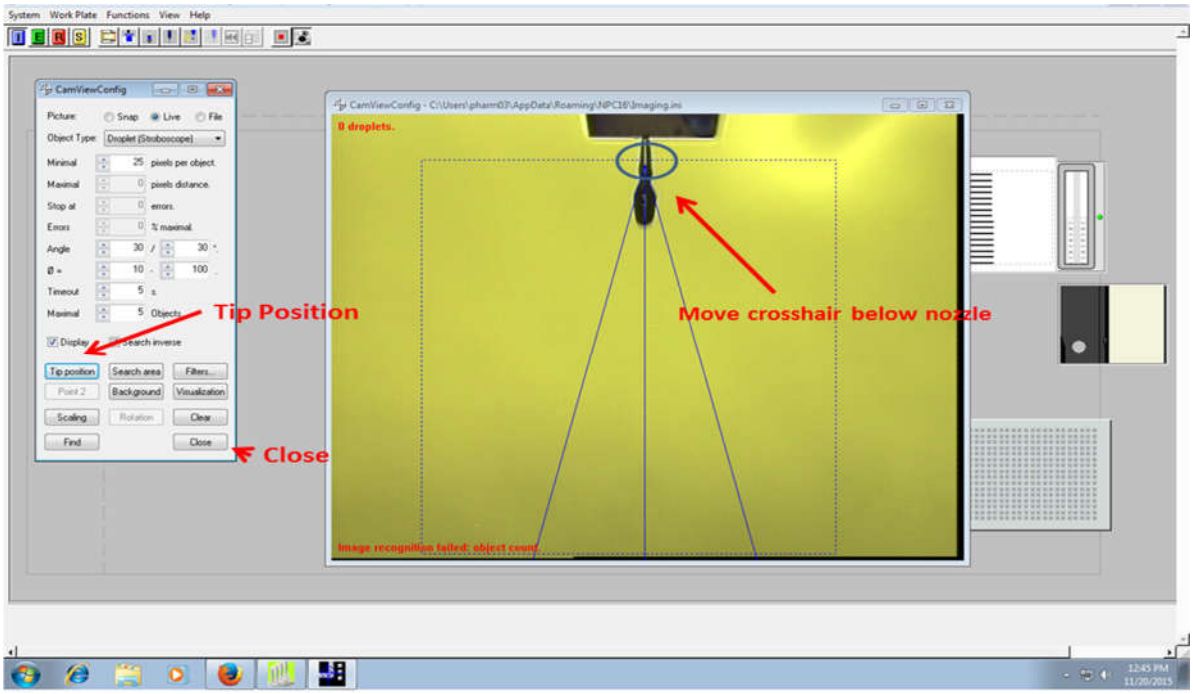
iii) In new window press "Tip Position." The cursor changes to a crosshair.

iv) Click on the spot just below the nozzle position.

v) Close the window. When new popup screen asks to "save the new setting" click ok.

vi) In Stroboscope window (see Fig. 3), click on "Parameters" and click "Set Defaults." Clicking "Set Defaults" will reset the "Delay" setting back to 250 µs. If droplet is now aligned properly close Stroboscope window.

Fig. 4: Grabber Config screen shot



11) Open the desired Work plate. Click “Work Plate” on tool bar (top row of Fig. 4 to the left). Click “Open Work Plate Settings …” and choose the appropriate work plate file from the folder. The nanoplotter may already have the file loaded. The work plate chosen depends on the eventual hybridization plate to be used. We use “091715RK_Target_24well.npw” for a 24 well plate.

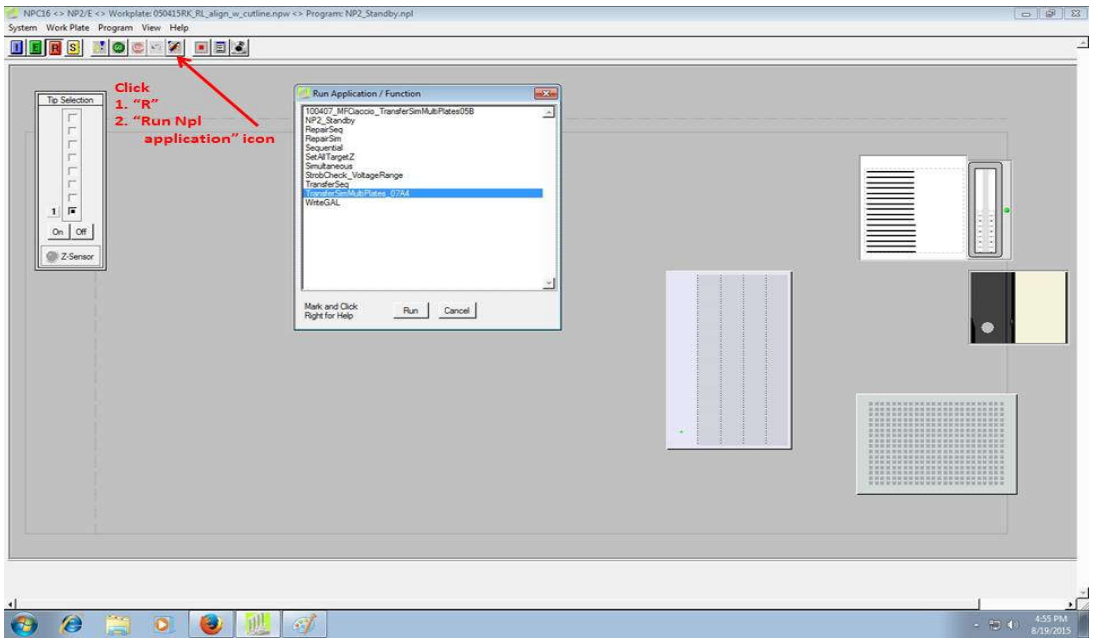
12) Before printing, allow sample lysates to thaw on ice. Mix 12 μL LI-COR protein molecular weight ladder (LiCor #928-70000), 1 to 1 with lysis buffer without inhibitors (see SOP A-10.0 Preparation of Adherent Cell Lysates Step 2a). Heat lysates at 95°C in a dry heat block for 2 minutes. Centrifuge at 14,000g for 2 minutes to collect condensation and settle debris (if present).

13) Place 24 μL of LI-COR protein ladder in lysis buffer in A1 of the 384-well black microtiter plate (Corning #4514). Load subsequent samples horizontally in the A2, A3, A4, etc. plate locations, 24 μl per well, according to the transfer file format being used. Load 24 μl of Bromophenol blue (0.1% in water) in a well after the samples.

14) Place the 384-well plate flat into the sample plate holder (see Fig. 1 above) in the GeSiM arrayer.

15) Click “R”, click “Run Npl application” icon and choose “TransferSimMultiPlates_07A4” in “Run Application/Function” window. (Fig 5, below.) This run program is used for all plate configurations.

Fig. 5 : “Run Application/Function:



16) Check that the parameters in "Settings" window (see Fig. 6, below) are set to the following and then click "OK.":

i) "Stroboscope check before spotting" checked.

ii) "passes" 4.

iii) "Stroboscope check after spotting" checked.

iv) "passes" 1.

v) "delay time" 1.0 sec

vi) "Dry before sample uptake" checked

vii) "Distance to target (spot) (mm)" 1.5.

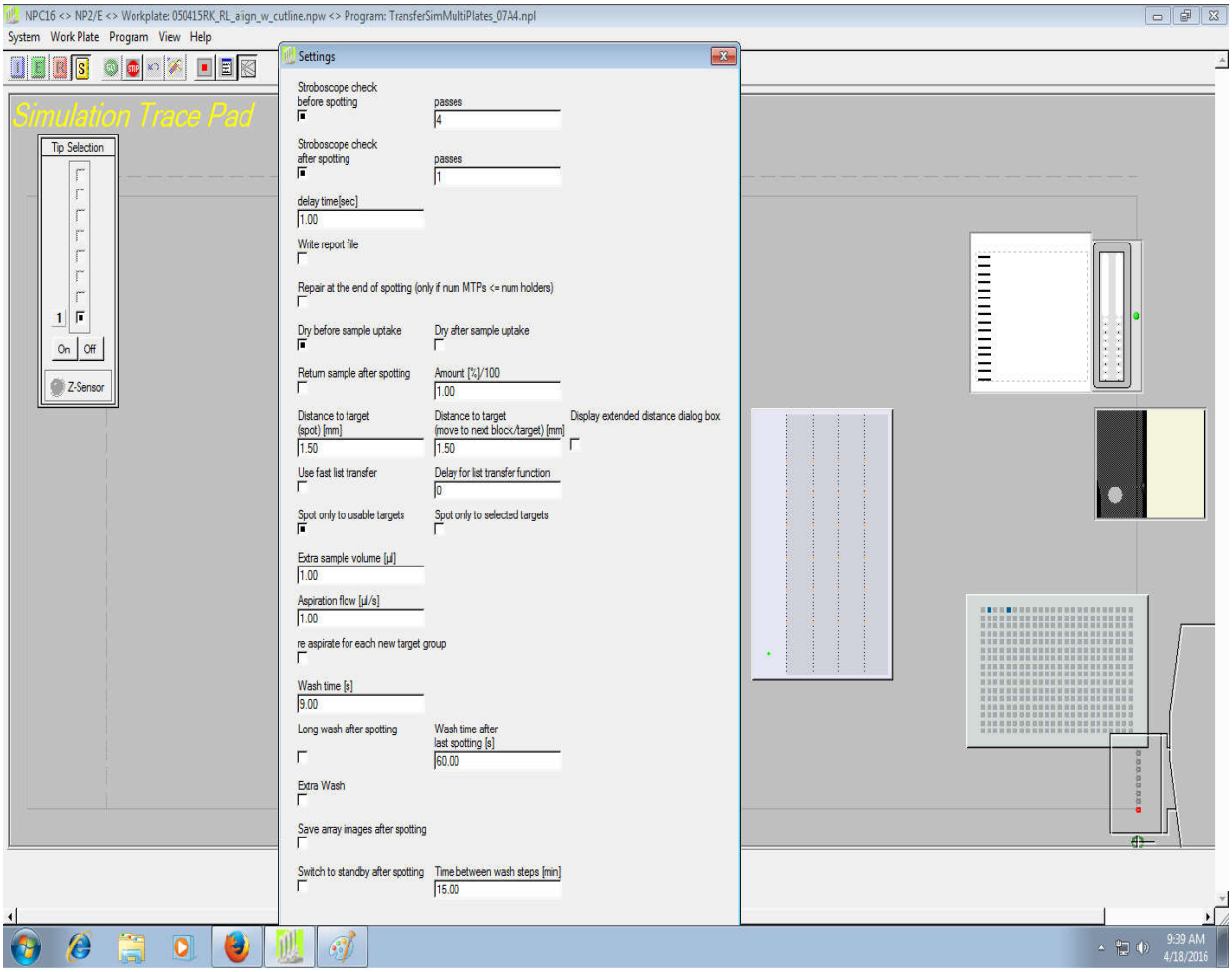
viii) "Distance to target (move) (mm)" 1.5

ix) "Extra sample volume" 1.0

x) "Aspiration flow [uL/s]" 1.0

xi) "Wash time [s]" 9

Fig. 6:"Settings Window"



17) After clicking “OK” in the “Settings” window above, the following windows pop up:

a) Workplate objects – click “OK”

b) Open File – under “Look in” choose “RK transfer files”, choose appropriate file based on number of samples and plate configuration and click “Select”

c) How to handle missing TG/TA/TI-com …” – all 4 options should be selected

d) Plate exchange type 16x24_45 – click “OK”

e) MTP content type 16x24_45-Plate 1 – click “No”

18) Printing of the gel will start using the transfer file chosen as the “Washing” window pops up.

a) Our transfer files include a line (“S9 TG[slides] BL1,2,3,4,25,26,27,28 15,1-15”) to allow bromophenol blue to be printed for measuring the distance samples travel during electrophoresis. (See SOP-A 13.0 “Gel Electrophoresis,” step 2k).

19) Printing can take several hours. Intermittently check (every ~30 min) that the printing is proceeding appropriately and that the chamber remains humidified for the duration of the print. Particularly long prints may require refilling the water tank used for tip washing.

20) After the printing is finished, leave the gel on the deck for ten minutes. This is to increase droplet adsorption.

21) Printed gel is ready for electrophoresis (see SOP-A 13.0 “Gel Electrophoresis")

22) QA/QC2. Following printing and between print runs, the NP2 standby program should be engaged to keep tips irrigated and hydrated until the next printing activity. Both the humidifier and arrayer water reservoir should be checked at least once a week, for adequate volumes of fluid. Turn off humidifier but keep computer, arrayer, and diluter on between prints.

Quality Assurance/Quality Control

QA/QC 1. If drop alignment is off then software will discard that particular drop. At the end of the print the software allows for running an “error” program to replace discarded drops. However, since the entire volume taken up by the tip for a drop is discarded after each cycle, there is a risk of not having enough sample in a well to replace the discarded drop.

QA/QC 2. Keeping tip irrigated and hydrated maximizes its accuracy in a print.