DNA Analysis by Native Polyacrylamide Gel Electrophoresis and Infrared Fluorescence Imaging

Michael Van Dyke

Abstract

Short DNA probes, for example, those used in characterizing protein-DNA complexes, have historically been radiolabeled with ^{32}P to allow their detection and quantitation following native polyacrylamide gel electrophoresis (nPAGE). For reasons of economy or safety, alternative means of DNA detection need to be used. Described here is the resolution of 5' IRD700 end-labeled DNA by nPAGE and visualization by IR fluorescence.

Citation: Michael Van Dyke DNA Analysis by Native Polyacrylamide Gel Electrophoresis and Infrared Fluorescence Imaging. **protocols.io**
dx.doi.org/10.17504/protocols.io.k6eczbe
Published: 17 Dec 2017

Guidelines

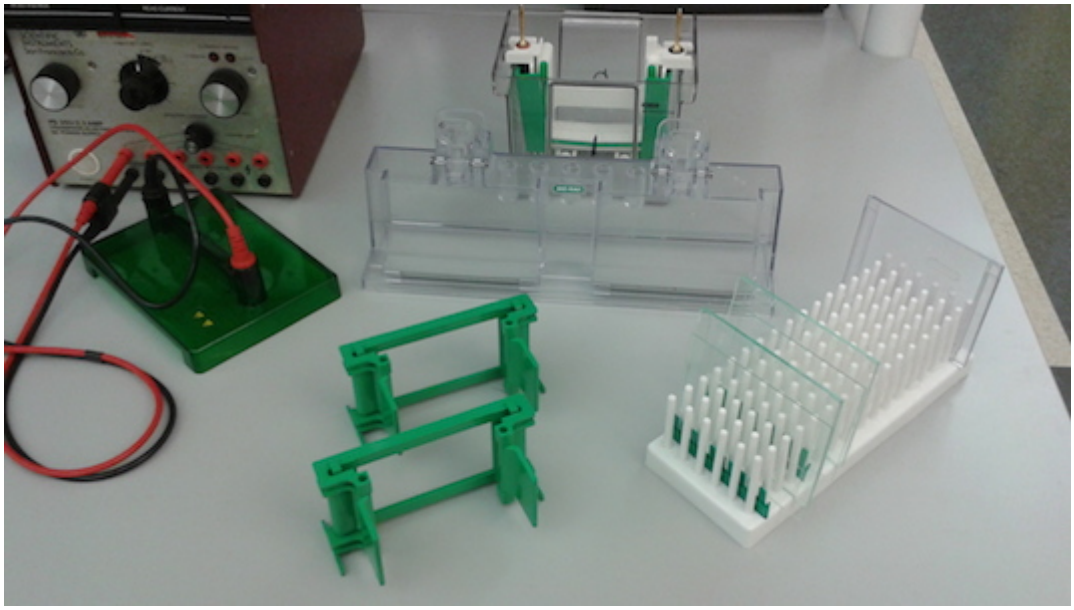
Protocol development supported by grants from the NIH (1R15GM104833-01) and NSF (MCB-1714778).

Protocol

Handcast native polyacrylamide gels

Step 1.

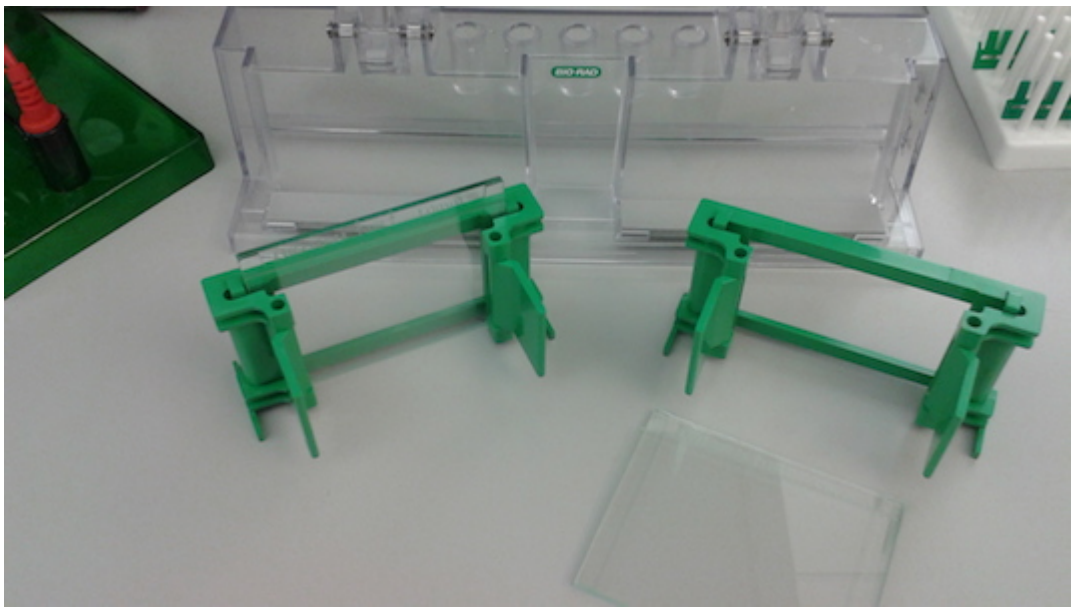
We routinely use the Bio-Rad Mini-PROTEAN® vertical electrophoresis system to cast and run 10-well 1.0 mm-thick 9.5%:0.5% polyacrylamide:bisacrylamide minigels in ½X TBE buffer [44.5 mM Tris base, 44.5 mM boric acid, 1 mM EDTA]. Needed materials: Bio-Rad casting stand, two casting frames, two short plates, two 1.0 mm spacer plates, two 1.0 mm 10-well combs, and one Mini-PROTEAN® vertical electrophoresis cell with buffer dam. Needed reagents: 5x TBE buffer, 38:2 acrylamide:bisacrylamide, 10% ammonium persulfate (APS), and tetramethylenediamine (TEMED), all stored at 4 ºC.

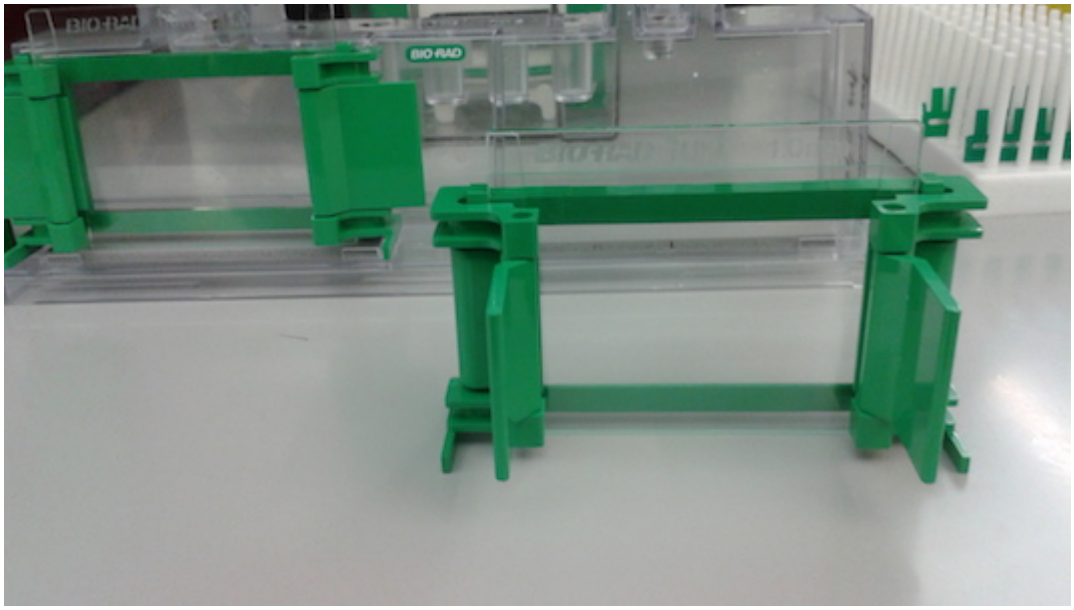


Handcast native polyacrylamide gels

Step 2.

Assemble one short plate and spacer plate sandwich in each casting frame. Make sure spacers are between both plates, that the short plate is towards the front of the casting frame, and that both plates are flush with the bottom of the casting frame before closing clamps. Secure plates/casting stand assembly into casting stand with top spring clamp, making sure bottom is properly sealed.

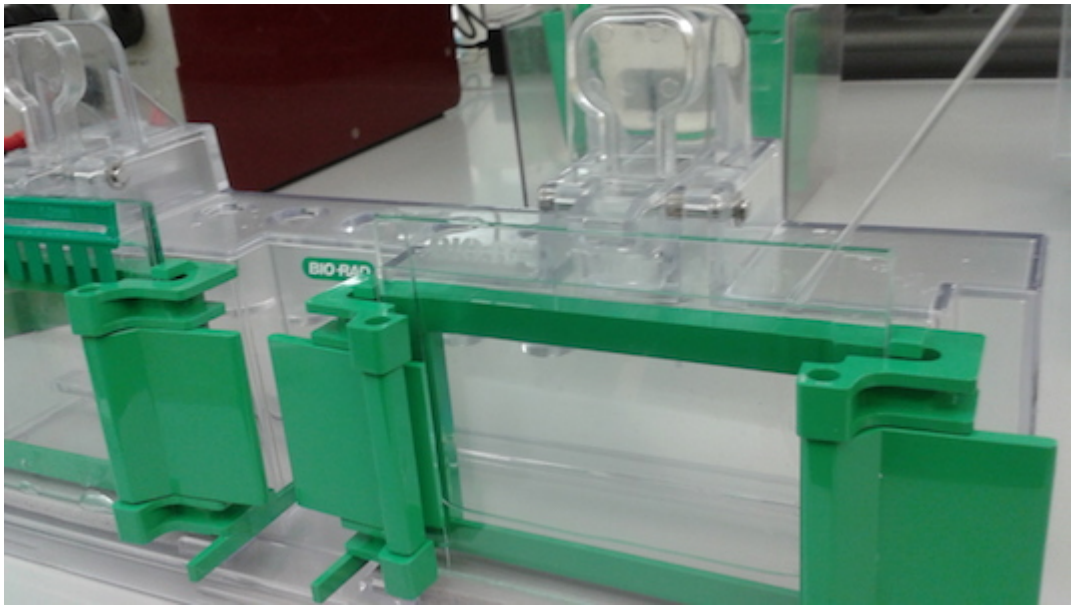


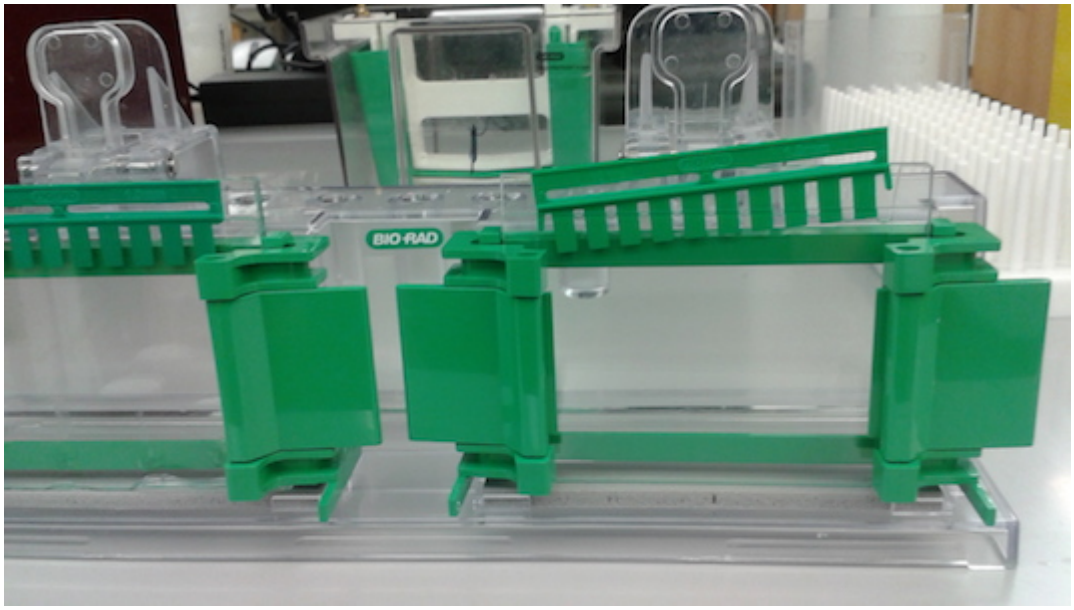


Handcast native polyacrylamide gels

Step 3.

To cast two minigels, add in order: 7.8 mL water, 1.2 mL 5X TBE, 3.0 mL 38:2 acrylamide:bis, and 45 µL 10% APS to a 15 mL screw cap centrifuge tube. Mix by vortexing briefly. Once everything is ready, add 15 µL TEMED and vortex briefly to mix before loading gel solution into each of the two casting assemblies. A 9” Pasteur pipette works well for this purpose. Stop before filling (2 mm from top). Carefully insert 10-well comb, using a slight angle to avoid trapping any air, and seat completely. Free-radical polymerization of these gels should be complete in 15 min at room temperature, and these minigels should be ready for use immediately after that.





Handcast native polyacrylamide gels

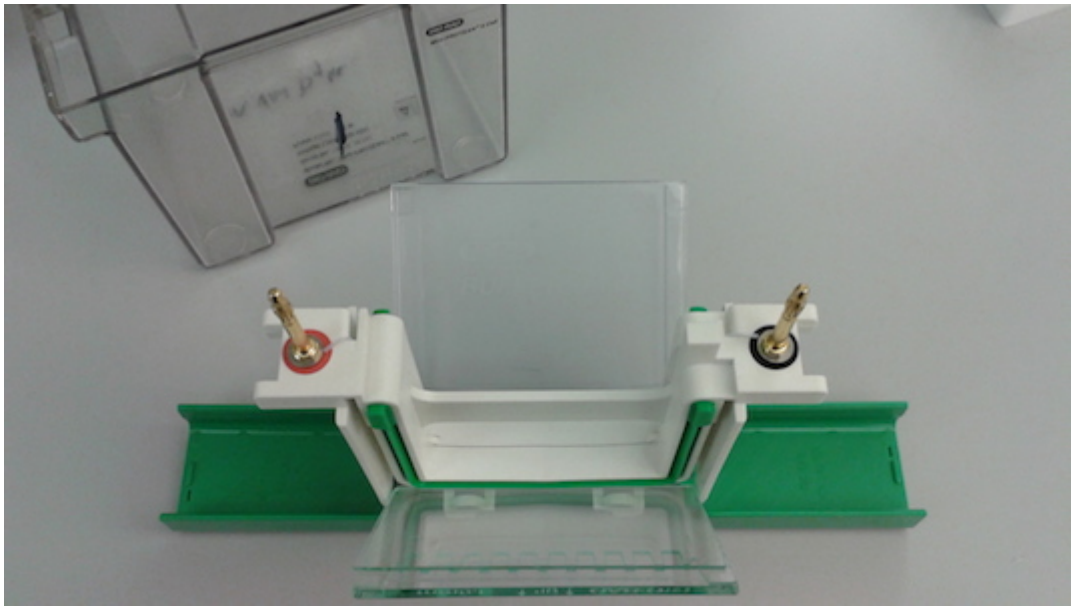
Step 4.

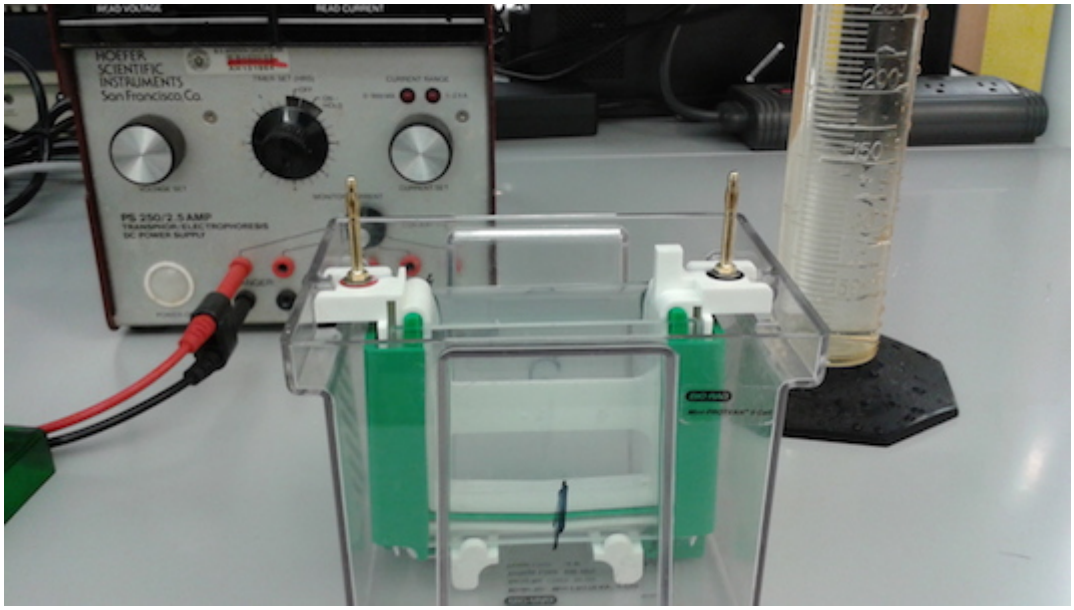
Note: polyacrylamide minigels in their casting stands can be kept at room temperature/humidity for several hours without any problem. Long-term storage is best when hydrated with a small quantity of water (200 µL on comb/small plate junction), wrapped in plastic and/or sealed in an airtight container, and stored at 4 ºC. Such minigels have effective lifetimes of at least 1 month if stored properly.

Polyacrylamide gel electrophoresis

Step 5.

With care, secure one polyacrylamide minigel and one buffer dam to each side of the Mini-PROTEAN® electrode assembly. Ensure the green gasket “ears” fully seal with the small plate/spacer junction of the minigel and corresponding plastic lip of the buffer dam before closing side clamps. Place assembly into the tank. Note: we routinely use smaller Mini-PROTEAN® II tanks for buffer economy; adjust subsequent volumes accordingly. Prepare 500 mL ½X TBE buffer. Fill the central chamber with ½X TBE buffer and observe if any leakage occurs. If so, drain and repeat seating minigel and buffer dam. Note: if two gels are to be electrophoresed simultaneously, substitute buffer dam for a second minigel.





Polyacrylamide gel electrophoresis

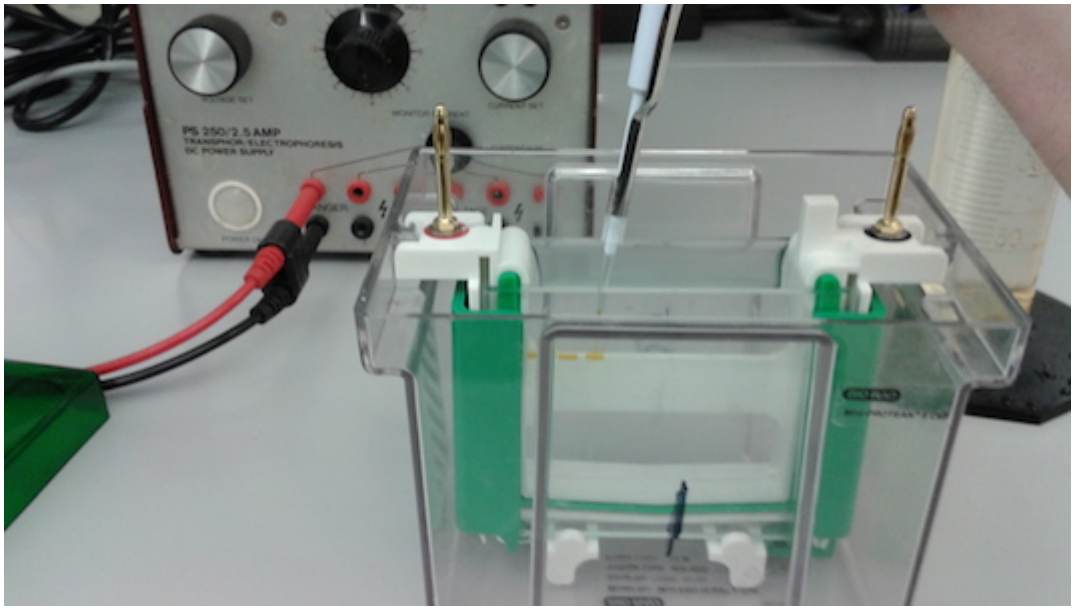
Step 6.

Prepare DNA samples for electrophoresis. Total volume should not exceed 12 µL and contain at least 3.3% glucose and 0.025% Orange G dye. **Note**: do not use blue or cyan loading dyes, as many fluoresce in the infrared. Thus, for a 5 µL sample, we add 2 µL 6X Orange Loading Dye (20% glucose, 0.15% Orange G, stored –20 ºC), and mix thoroughly by repeated pipetting.

Polyacrylamide gel electrophoresis

Step 7.

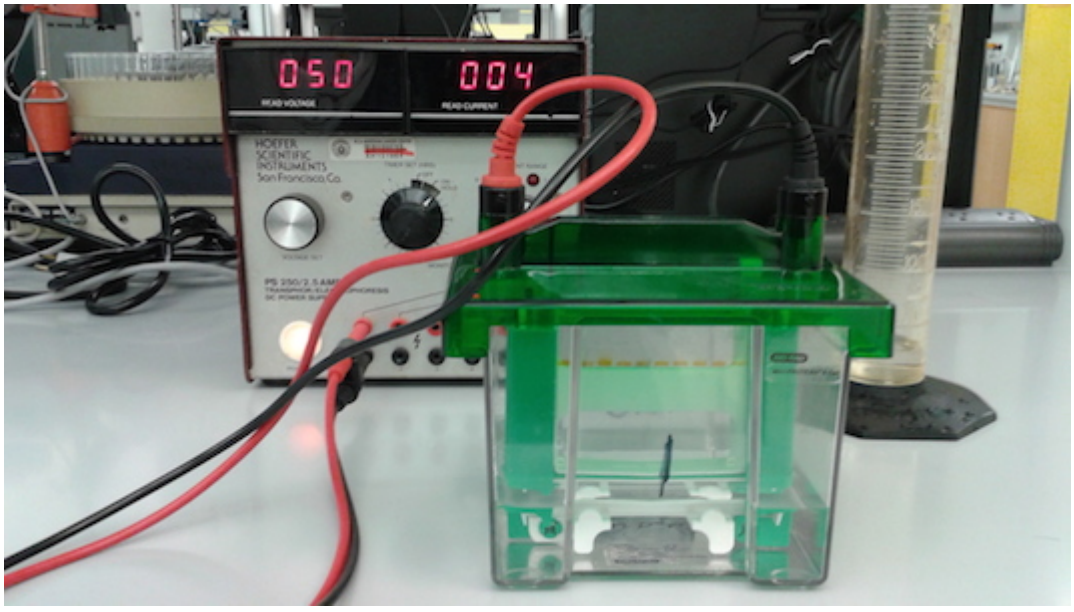
Samples are loaded in order into their respective wells using a P10 Pipetman and 0.1 - 10 µL standard tips. Once all samples are loaded, ½X TBE buffer is added to the bottom chamber, the tank lid seated, and leads attached to a power supply. **Note**: be aware of lid-to-electrode assembly and lead-to-power supply socket orientations to maintain proper electrode polarity.



Polyacrylamide gel electrophoresis

Step 8.

Electrophorese samples at constant voltage 5 V/cm (50 V) for 5 min to allow the DNA to enter gel, then increase the voltage to 10 V/cm (100 V) for the duration of the run. One hour is sufficient to deliver the Orange G dye to near the bottom of the minigel, which corresponds to approximately 10 bp dsDNA. Once the run is completed, drain tank and electrode assembly, wash with water and disassemble. **Do not take apart gel**, as they can be directly imaged while between glass plates. Note: use of a power supply with integrated timer is a helpful convenience, preventing sample overruns and facilitating reproducibility between runs. However, do not let gels remain at room temperature for extended periods of time, as diffusion of shorter DNAs can occur.

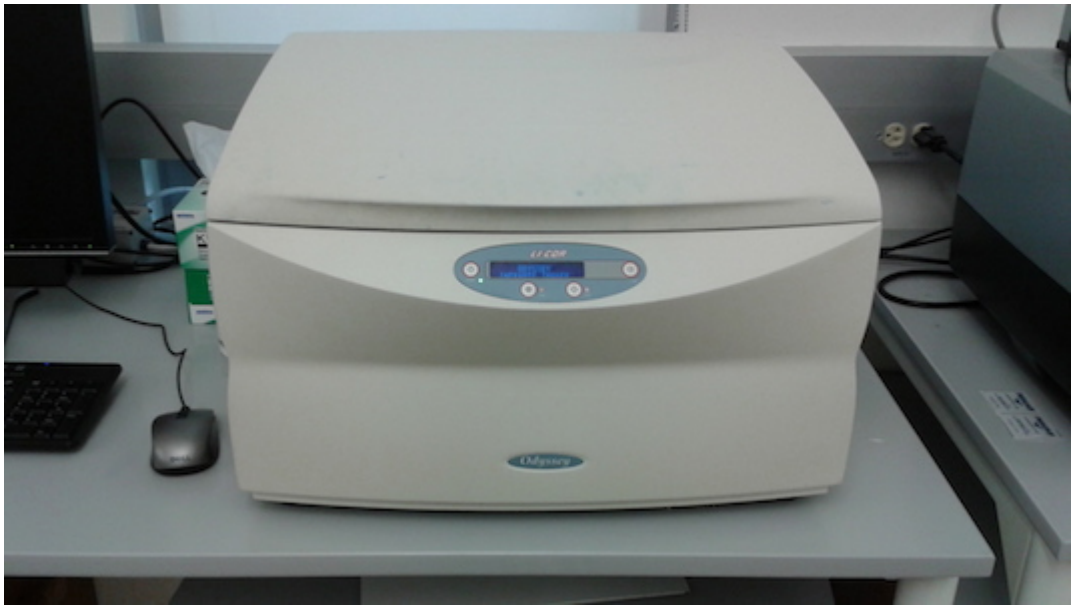


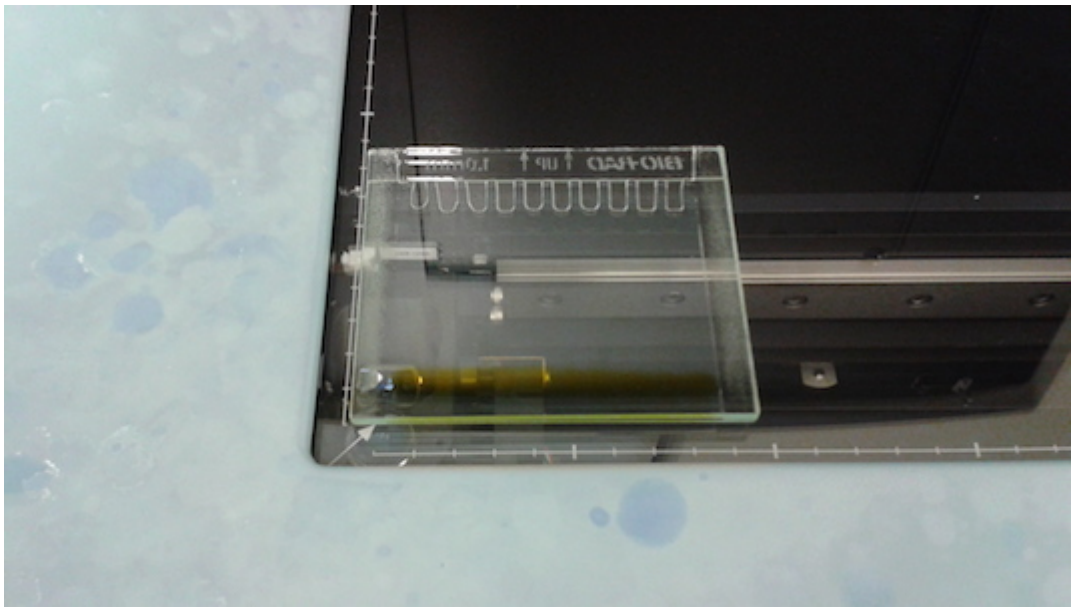
Infrared fluorescence imaging

Step 9.

To perform infrared fluorescence (IRF) imaging of PAGE minigels, we use a LI-COR Odyssey imager and LI-COR Image Studio™ 5.2 software. The DNA preset is used with Scan Controls settings:

Scan Resolution (μ): 84 μm, Scan Quality (Q): Medium, Focus Offset (⊕): 1.5 mm, Flip Image. A 7 × 9 cm grid is selected corresponding to the minigel, which is placed onto the image surface with the short plate face down.

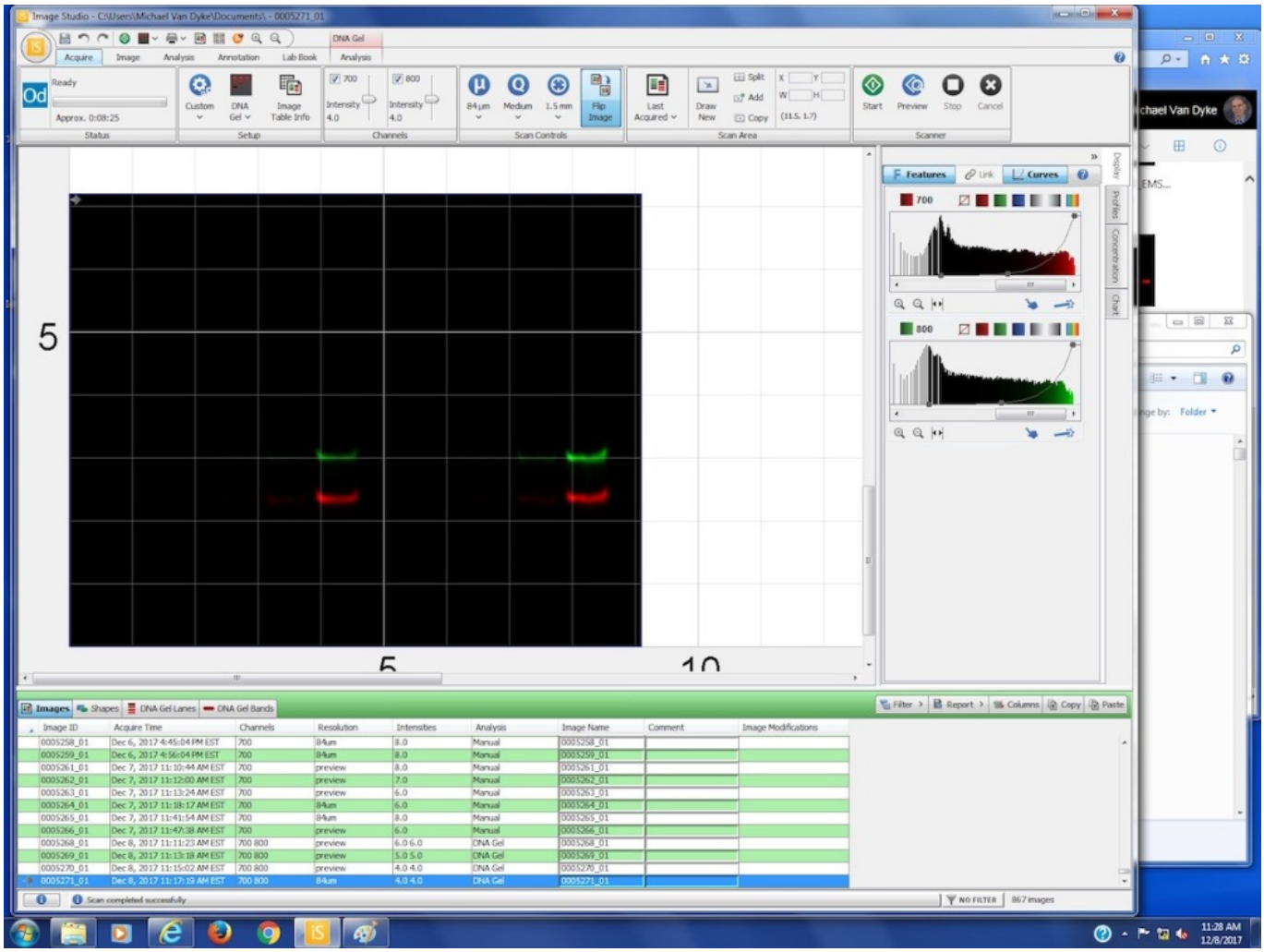




Infrared fluorescence imaging

Step 10.

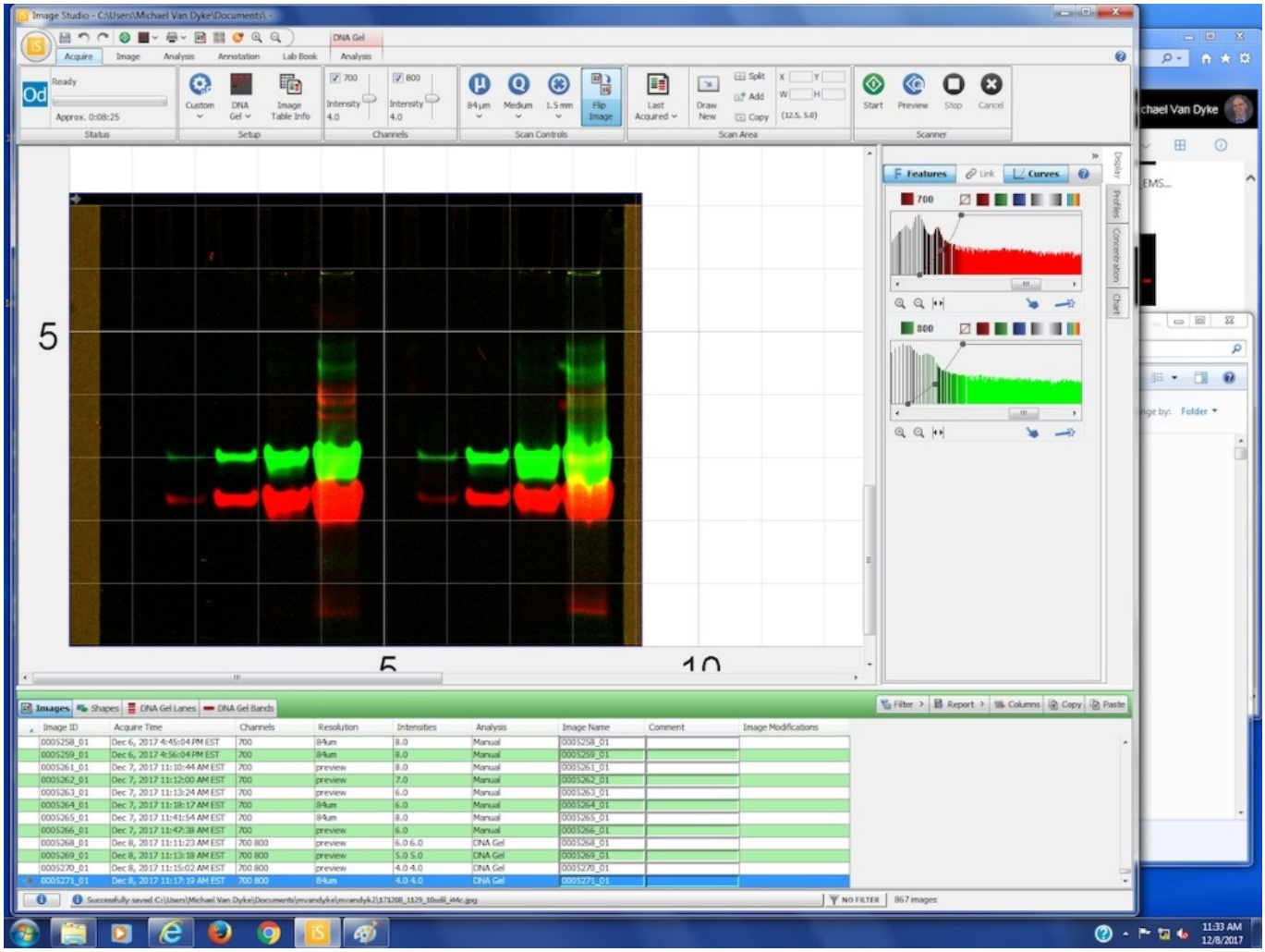
Initially, a Preview scan at Intensity 4 for both 700 and 800 channels (as needed) is performed, which takes approximately 30 s to run. Additional Preview runs at increasing or decreasing intensity are performed, as needed, until no saturated pixels (white) are observed. Once maximal Intensity values have been determined, a full run may be initiated (Start). Such takes approximately 8.5 min to run.



Infrared fluorescence imaging

Step 11.

Images can be saved as tif, png, jpg files and at 150, 300, 600 dpi resolution. Our standard is a 600 dpi jpg file. Gain can be adjusted for each channel using the Curves box, at right. While this allows observation of weaker bands, it is an image manipulation that may not be suitable for publication.



Infrared fluorescence imaging

Step 12.

Image Studio™ software provides the capability to quantitate image bands. Note that gain adjustments do not affect these values, so long as no saturated (white) pixels are present. Bands may be automatically identified using the DNA Analysis tab or manually, using the Analysis tab. Note: Analysis requires identifying bands one channel at a time. Remember after quantitation to save final images, thereby providing a record of pixel numbers for each band. Note: we routinely find that a densitometric analysis of IR fluorescent DNAs by this method can yield useful data over a 1000-fold dynamic range.