Pulsed Field Gel Electrophoresis

Protocol

Bulletin 6227

Image Analysis

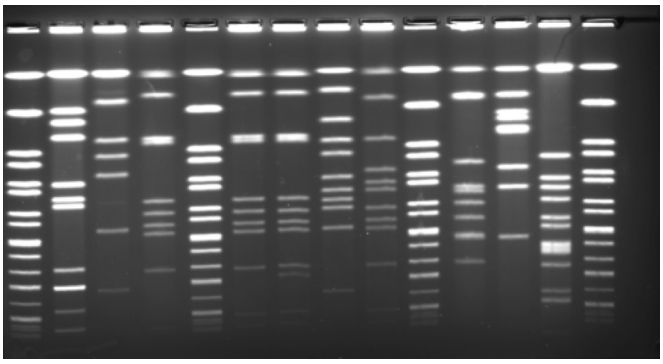
Imaging systems detect images, document and analyze stained gels, and produce publication-quality outputs. This protocol includes guidelines about gel staining and image acquisition for pulsed field gel electrophoresis (PFGE).

Gel Staining

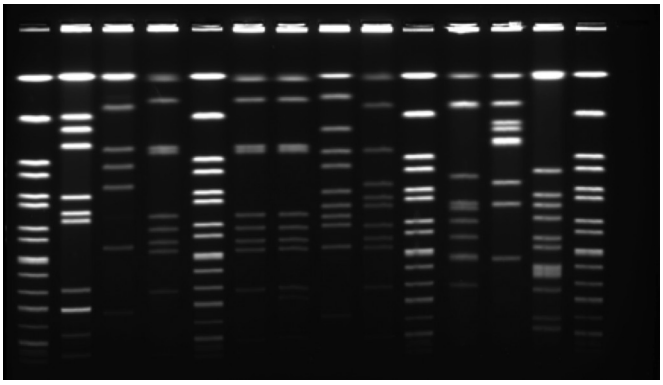
Process

1. Stain the gel in ethidium bromide solution (40 µl of 10 mg/ml stock solution per 400 ml) on a rocker or shaker for 25–30 min.
2. Remove the gel to a new container and destain with ~500 ml water.
3. Change the water every 15–20 min — three 20-min washes are recommended. It is important to use fresh water for each wash.
4. When stored properly, the staining solution can be used for 10–20 gels. As it ages, staining fades and background may increase.

Note: Ethidium bromide is mutagenic and must be disposed of properly according to institutional guidelines. Nonmutagenic stains are available and may be used with PFGE gels after validation.



Gel showing high background due to insufficient destaining.



Gel showing low background following destaining.

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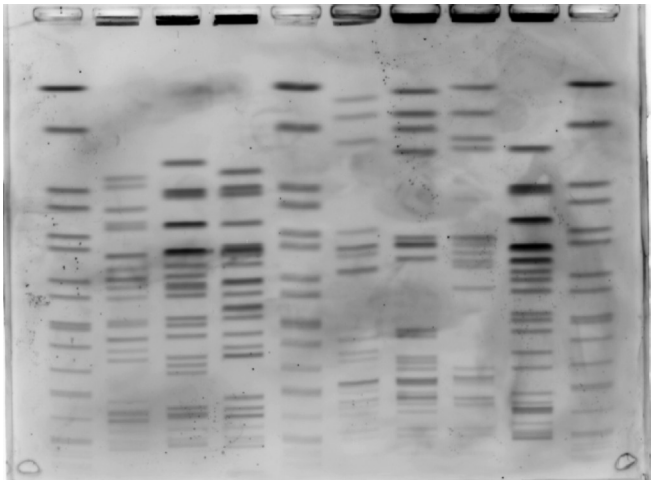
Potential Problems

Aloe or powder from treated gloves leaves a residue on the gel which is visible on the image.

Recommendation

Avoid using gloves that are treated with either aloe or powder. Untreated latex or nitrile gloves are acceptable.

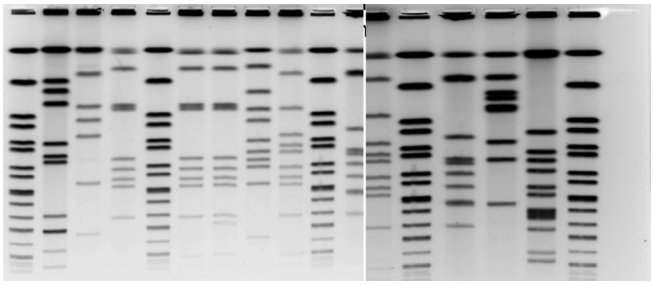


Gel showing cloudiness and darkened areas due to handling the gel while wearing nitrile gloves treated with aloe.

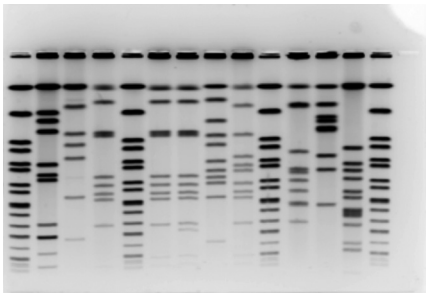
Image Acquisition

Process

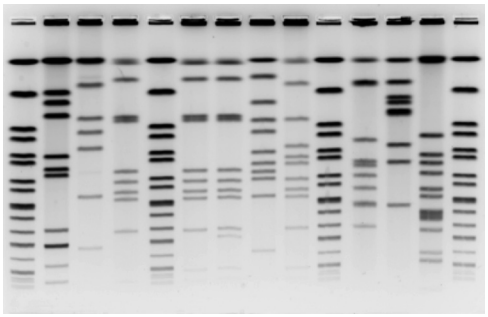
1. Center the gel and zoom as close as possible to eliminate blank space. Include wells and the bottom of the gel. Do not include the space above wells and do not cut off after the smallest band of the standard.



Do not take multiple images of the same gel.



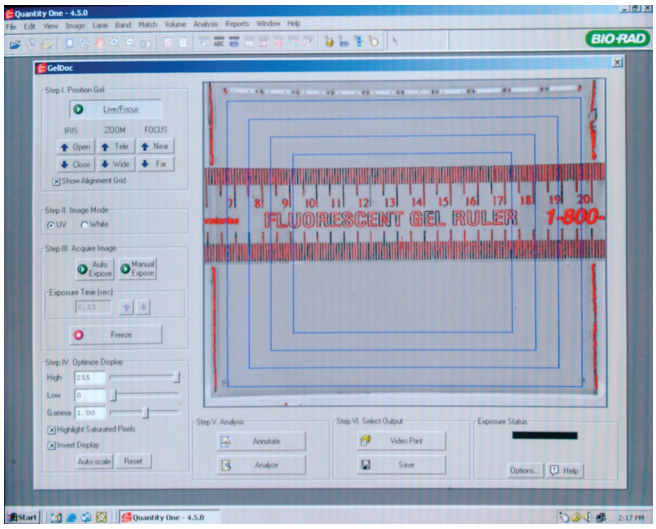
Zoom in to eliminate space around the gel.



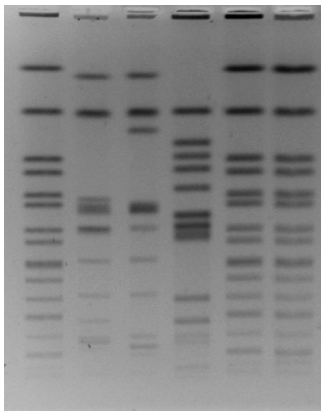
Gel showing appropriate image acquisition.

2. Note that the ability to focus may be automatic or manual depending on the imaging system used.

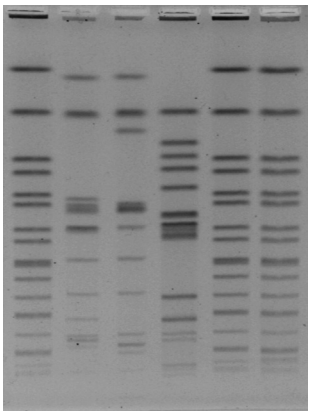
Tip: To make sure that the camera is in focus, use a fluorescent ruler to focus on lines so that they are sharp; lines will appear highly pixilated (red).



Example of a fluorescent ruler.



Unfocused gel image.

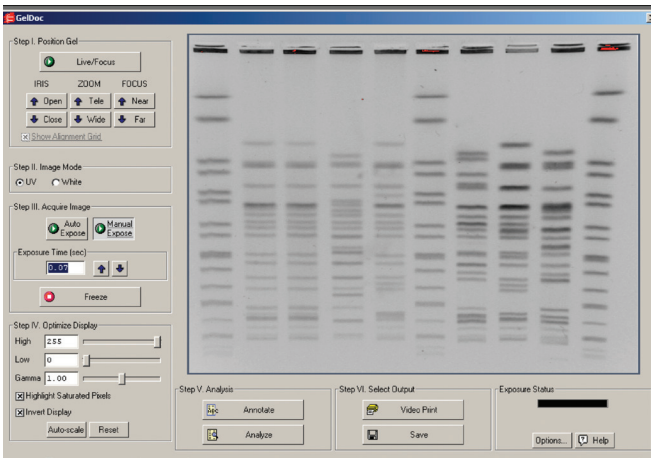


Focused gel image.

3. Avoid overexposure (overintegration) of the image. This increases background and makes discriminating closely migrating bands difficult. In a correctly imaged gel, background is low and closely migrating bands are distinct. Areas on the gel may appear slightly faint to the naked eye, but will be visible when analyzing the TIFF with BioNumerics software.

- If available, select **Highlight Saturated Pixels**. Saturated areas will appear red
- Adjust the integration time or the aperture until all red is removed
- If the image capture software allows it, collect numerous images over a range of exposure times (for example, 5 –25 sec)
- Some saturation in the wells is acceptable

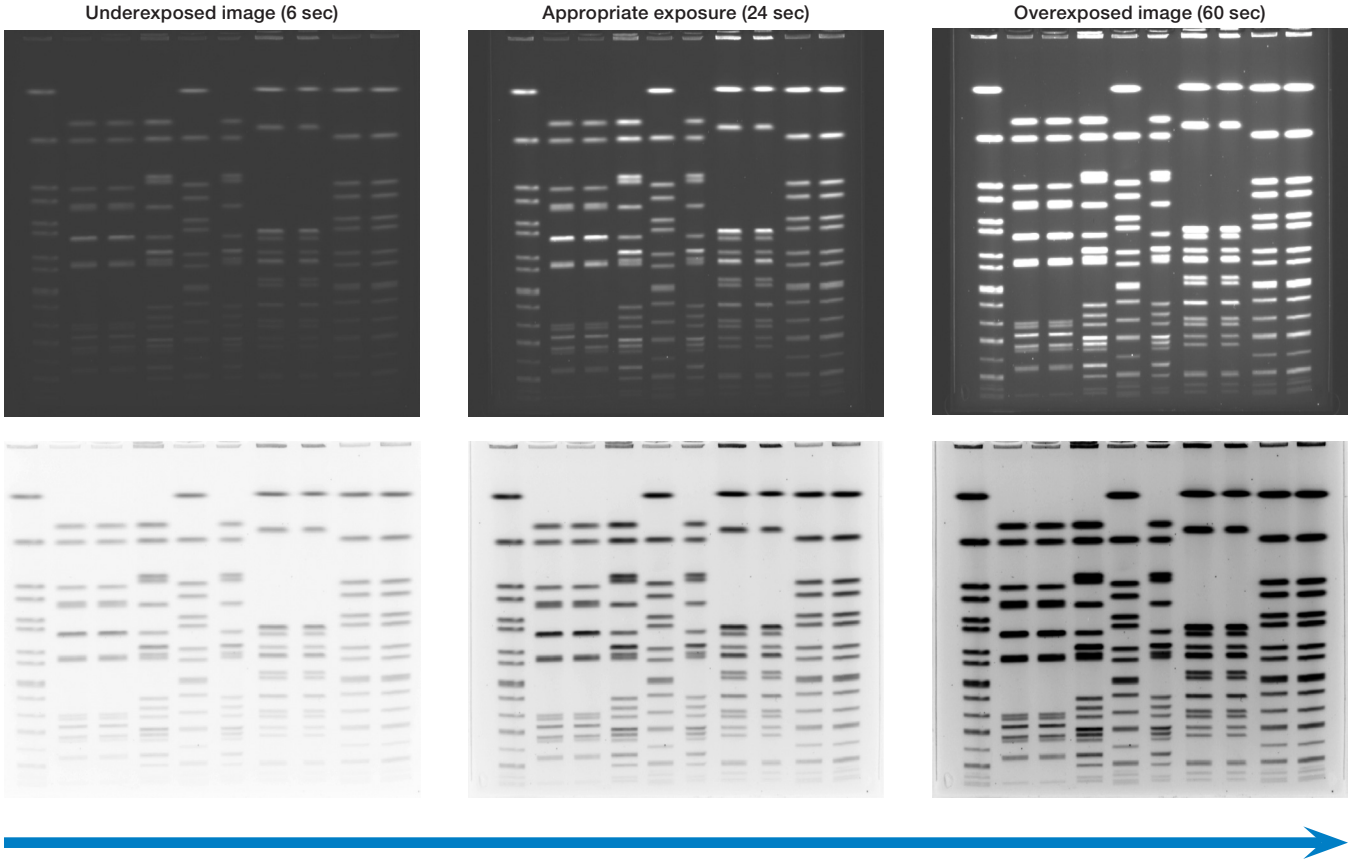
Gel image is overexposed.



Gel image is correctly exposed.

4. Exposure time:

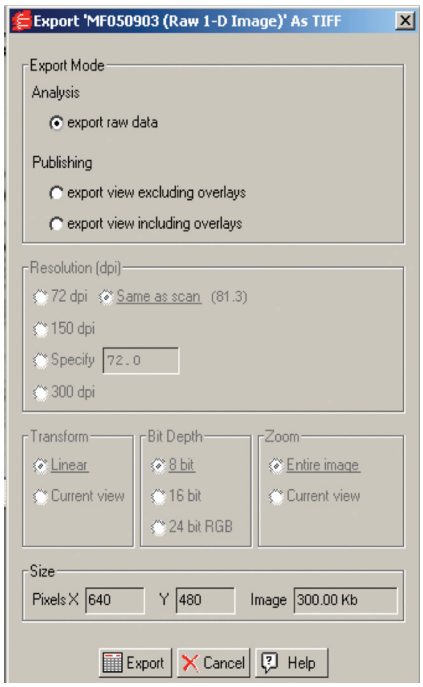
- If exposure time is too short, it is difficult to visualize faint bands at the bottom of the gel image
- If exposure time is too long, background is increased, resulting in loss of distinction between closely migrating bands



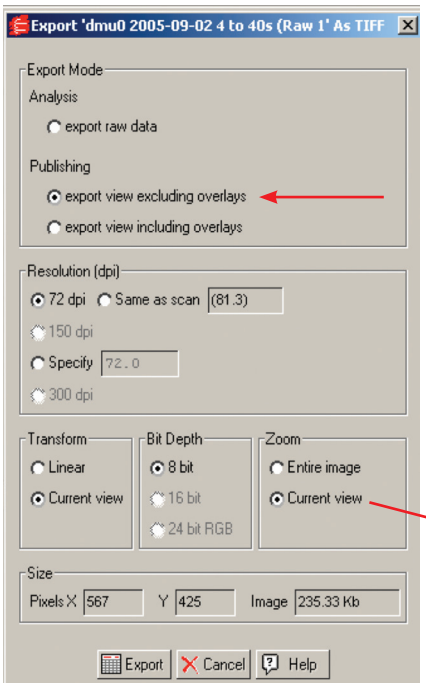
Effect of increasing exposure time on a gel image.

5. File Conversion

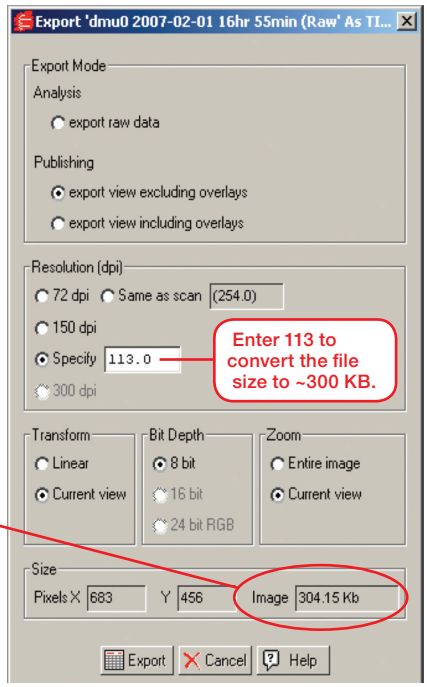
- File conversion is not necessary for the Molecular Imager® GelDoc™ XR+ system with Image Lab™ software. Select **Export to PulseNet** in the File menu
- File conversion steps for GelDoc EQ and XR systems and Molecular Imager® VersaDoc™ MP systems with Quantity One® software (version 4.5.0 or higher) are illustrated below



Export window in the Quantity One software.



Selecting "export view excluding overlays" under Publishing in the Export window.



Entering 113 in the Specify field under Resolution in the Export window produces a file size ~300 KB.

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