

“PulseNet: Under the Microscope”

Volume 1

Capturing a Gel and Adjusting the Settings for Analysis

Every PulseNet knows that generating a high-quality PFGE gel requires solid laboratory technique, quality reagents and enzymes, and the ability to capture the image quickly. We all know that there are *many* places where problems can arise in producing sharp, well-resolved bands. Every one of us has experienced the frustration that comes along with seeing the GelDoc light up and a failed gel appear. Knowing that so many pitfalls exist through the process, it is most unfortunate when an excellent gel is generated, but the captured image does not reflect the quality of the gel produced.

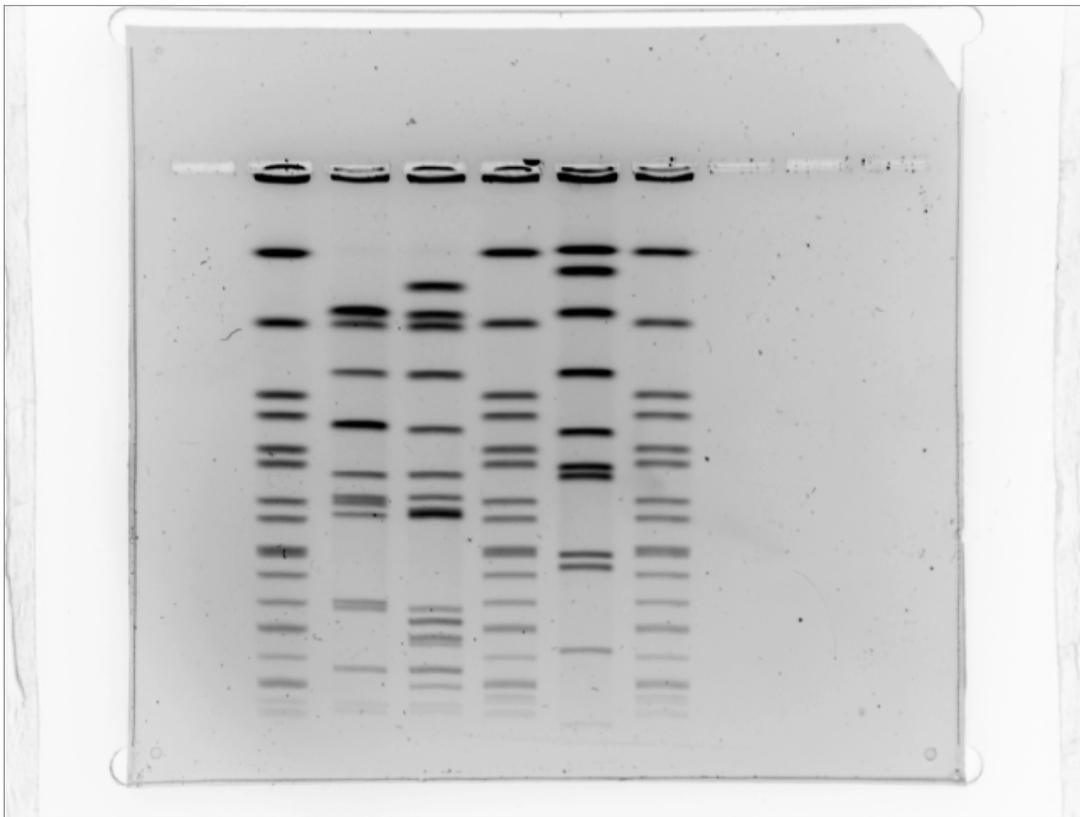
The PulseNet standardized protocols detail the procedure for capturing the gel image within the “Staining and Documentation of PFGE Agarose Gel” section. The steps include staining and destaining the gel, capturing the image at its optimum size, and what to look for to determine if the gel meets PulseNet standards. In addition to the defined steps from the protocol, there are the refined techniques, the little tricks and the benefit of years of experience that affect the quality of the gel captured.

The gels in this document illustrate one of the most common issues associated with capturing a gel and the issues associated with analyzing these gels within BioNumerics: a gel image that does not fill the “field of view”. The size of the initial gel image captured affects the results of analysis, the comparability of patterns, and the ability to compare lanes within the national database with confidence. Additionally, the settings used within BioNumerics should be adjusted to the size of the lane, not left unaltered from gel to gel with the assumption that all gels are equal. The following gels will highlight these two issues and illustrate how different settings within BioNumerics can affect the analysis of the resulting gel. Each gel includes an explanation of the problem and steps one can take to address each issue. The Massachusetts lab has provided several examples of *Salmonella* gels that specifically illustrate certain issues and each gel has room for improvement.

Gel image MA10029A is below. This is a *Salmonella* gel with 6 lanes and standards run in lanes 1, 4, and 6. The initial image shows a gel with space on either side of the edge of the gel, plus space at the top and bottom.

The wells are visible and the last band of the standard is approximately 1.5 centimeters from the bottom of the gel. This is a good raw image of a gel. There are many instances within the National Database when this gel will be uploaded exactly as it is seen below. Admittedly, this is a high quality gel that any database manager would gladly work with given the sharp band resolution. However, not all submissions to the National Database look as clear as this gel. The tools available in BioNumerics can be used to improve upon this gel. If BioNumerics can improve upon an excellent raw image, it can certainly improve upon images that are considered less than excellent.

MA10029A

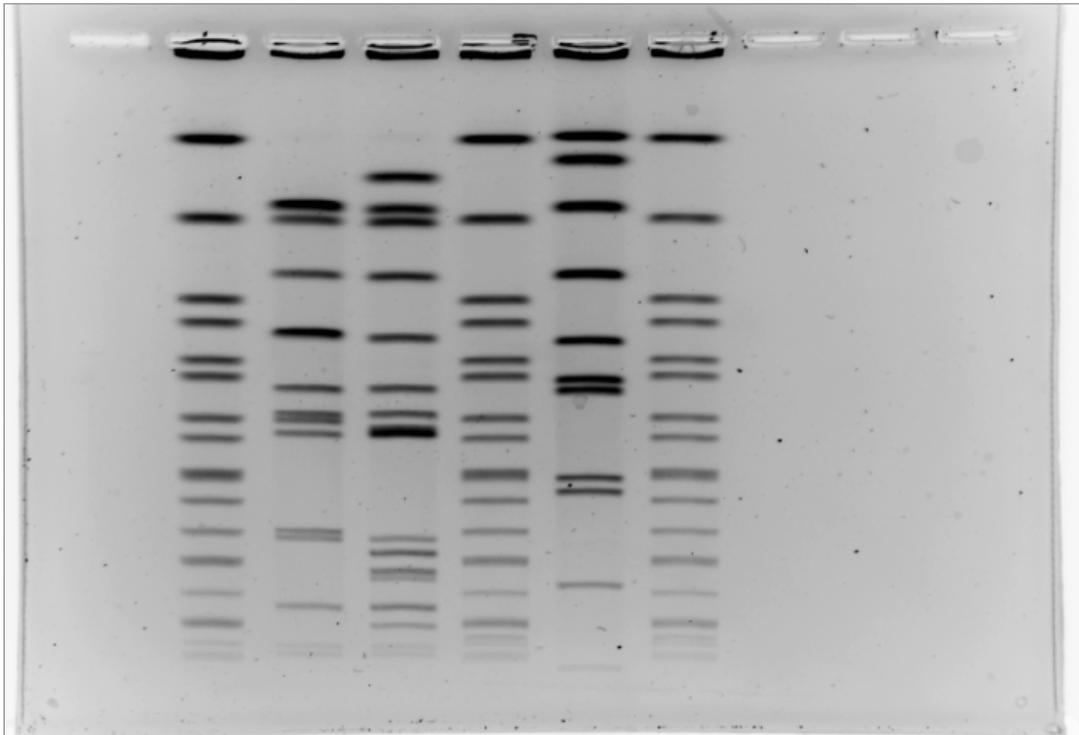


(Above) Raw, unaltered TIFF image of gel MA10029A.

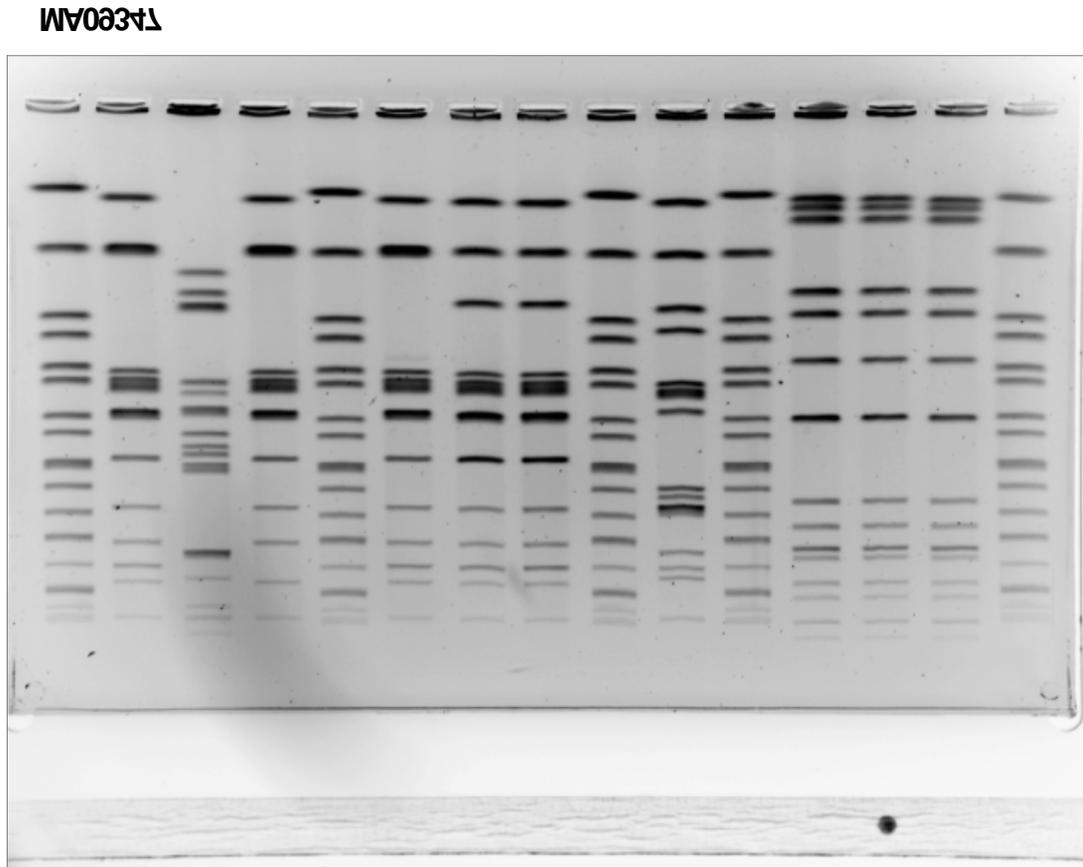
The next image is the same gel, MA10029, with the TIFF enhanced using the GelDoc 2000. Tiff MA100029 is darker. On this gel all the bands can be marked with confidence, including the lowest molecular weight bands.

Prior to fine focusing the camera, the lab zoomed in on the image, filling the TIFF frame (see image below). Best practices for capturing an image recommend that the acquired image show the bottom of the gel. This way the operator can assess whether or not the standard has run the appropriate distance down the gel. The bottom edge of the gel should always be included when capturing gel images. The gel should not be cropped below the last band of the standard and the wells should be visible. The gel image can be cropped at the top, but the wells must always be included. Likewise, empty lanes may be cropped out of the image as long as the wells and the bottom of the gel remain clearly visible. The image acquisition software (imager) provides a visible field that is square-shaped and therefore the relatively square 10-well gels will completely fill the screen. However, since the 15-well gels are rectangular in shape there will be empty space on either the top or the bottom of the gel.

MA10029



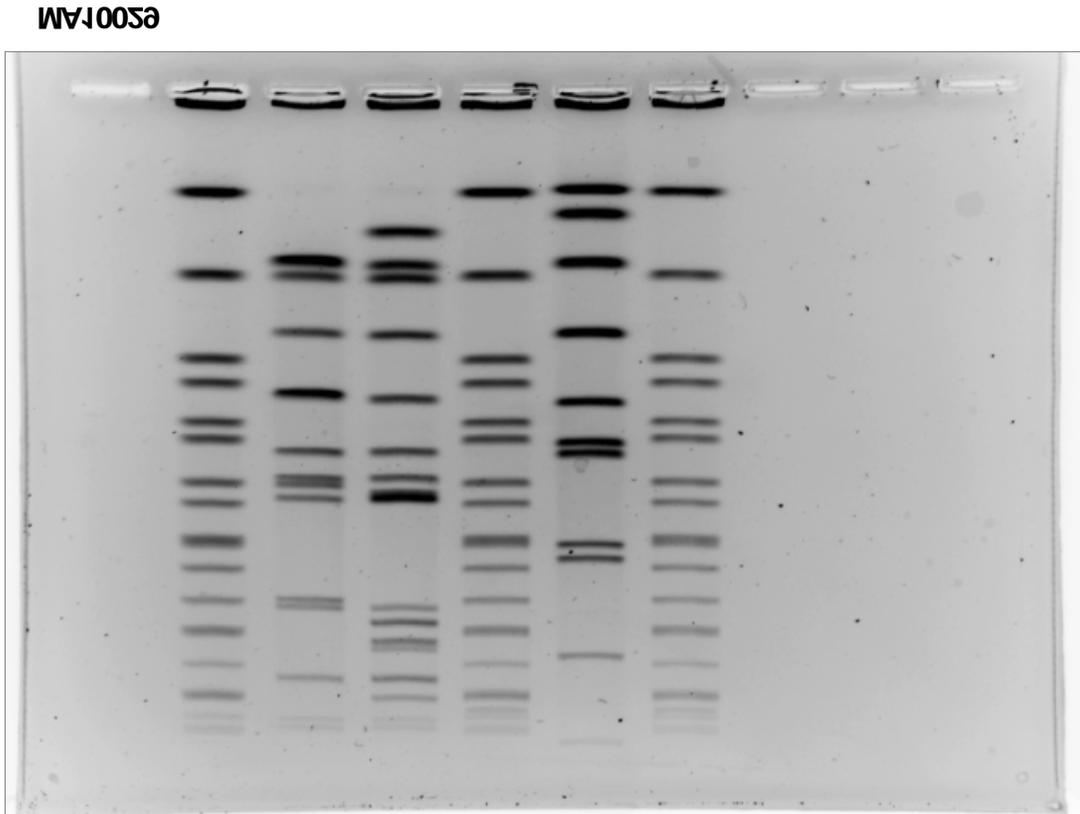
The previous gel illustrates that a 10-well gel fills the visible field at the top and bottom. Despite the fact that there are several blank lanes, there is very little cropping to do because the image already fills the frame at the top and bottom. Zooming in closer would cut off necessary information at the top (the wells) or the bottom (the bottom of the gel).



The 15-well gel above shows how the shape limits the ability to zoom in closer. While there is space at the bottom of the gel, both sides and the top of the gel are at the edge of the visible field.

Zooming in on the gel affects the overall image. The top and the bottom outlines are not independent adjustments as seen in the analysis feature of BioNumerics. Capturing the best possible image is a matter of

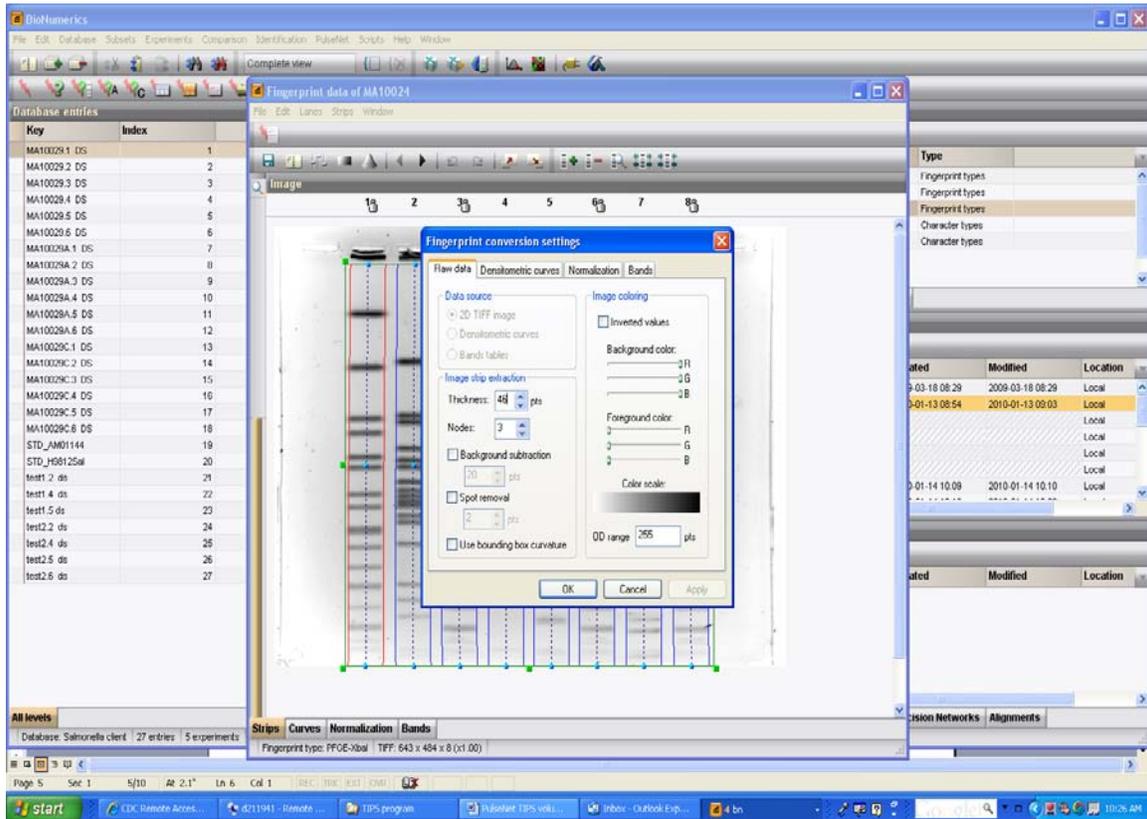
finding the balance between maximizing the size of the gel without “cutting off” any necessary data. Once the TIFF frame is filled (or is as large as possible without cutting off any information), the fine focus tool should be utilized to sharpen the image resolution.



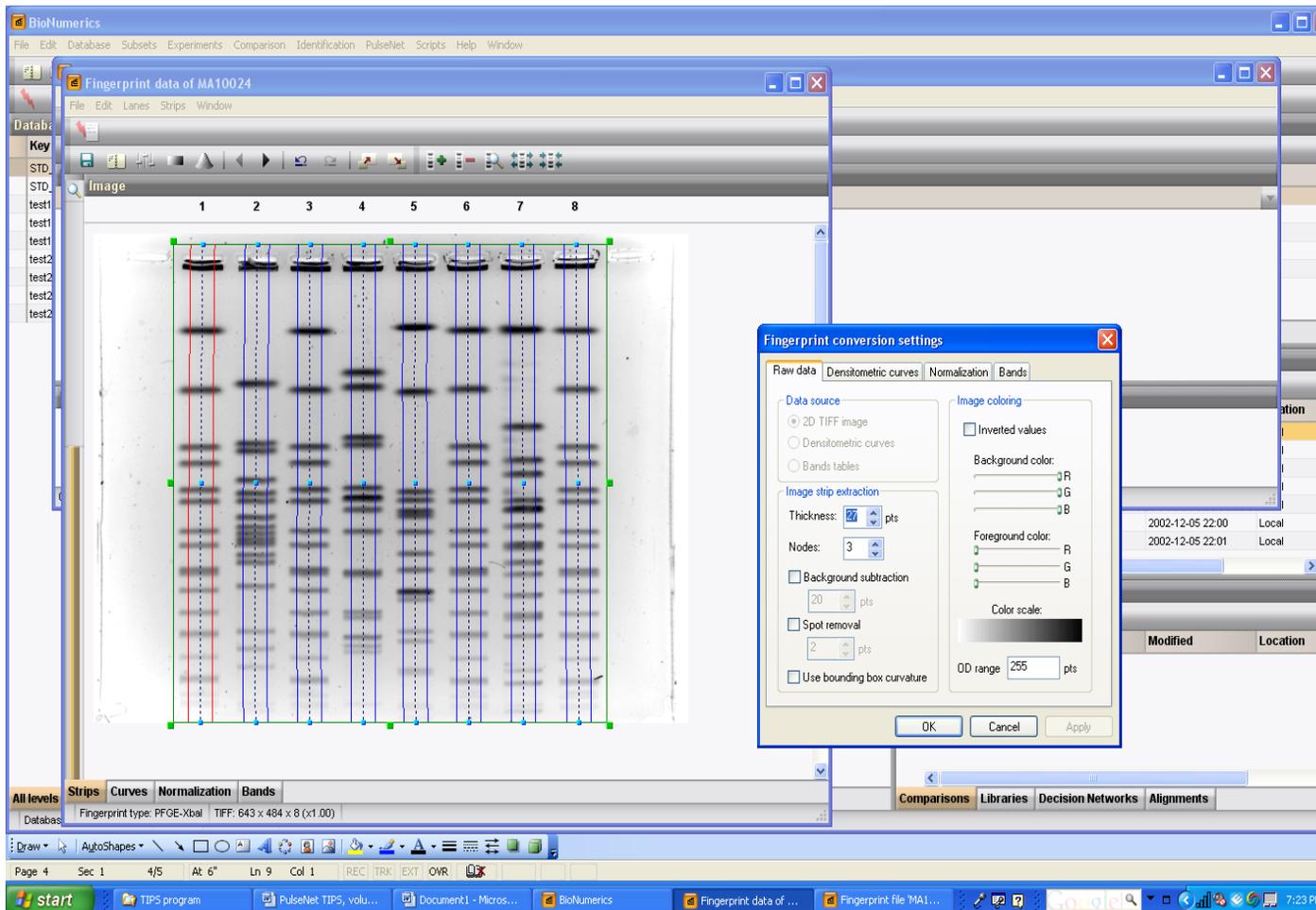
After the image is captured, it is analyzed in BioNumerics. There are several settings that should be adjusted prior to analyzing a gel. It is important to keep in mind that settings established in the first step can impact the quality of the lane available for band-marking in the fourth step.

In the first step of the analysis process, labeled “Strips” at the bottom, the settings can be adjusted to accommodate each individual gel. For the best possible results in band-marking, it is necessary to adjust the strips so that each strip hugs the edge of the band on either side. If the strips are too wide, there will be measurable white space on either side of the band in the final step of analysis and the corresponding lane strip in the national database. The band resolution can be affected by strips that are set too wide.

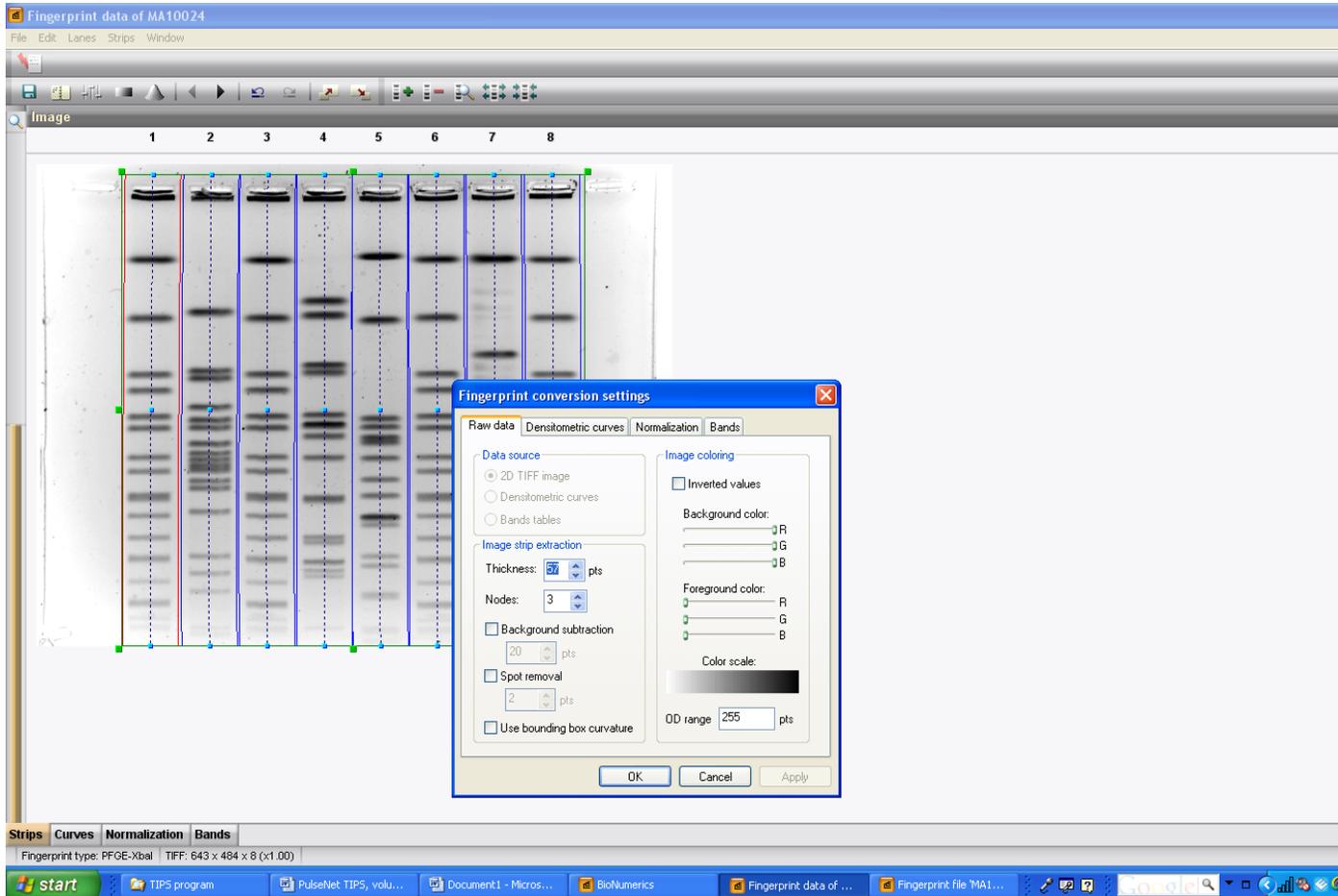
Bands will appear thicker, making it more difficult to accurately and consistently mark the bands. If the strips are too narrow, valuable shoulder data will be cut off. This makes it more difficult to mark multiple bands (doublets, triplets, etc...) because of the data lost. Below the screenshot shows where these strip settings can be adjusted for the entire gel (follow 'edit' → 'edit settings' → 'Image strip extraction'). The appropriate setting for the gel below is 46 pts.



Below is the same gel image with a smaller set spline thickness. This is set at 27 points. The edges of the bands clearly extend beyond the edge of the spline. Such a narrow setting will cut off any 'shoulder data' or the edges of close bands that can indicate the presence of a tight doublet or triplet.



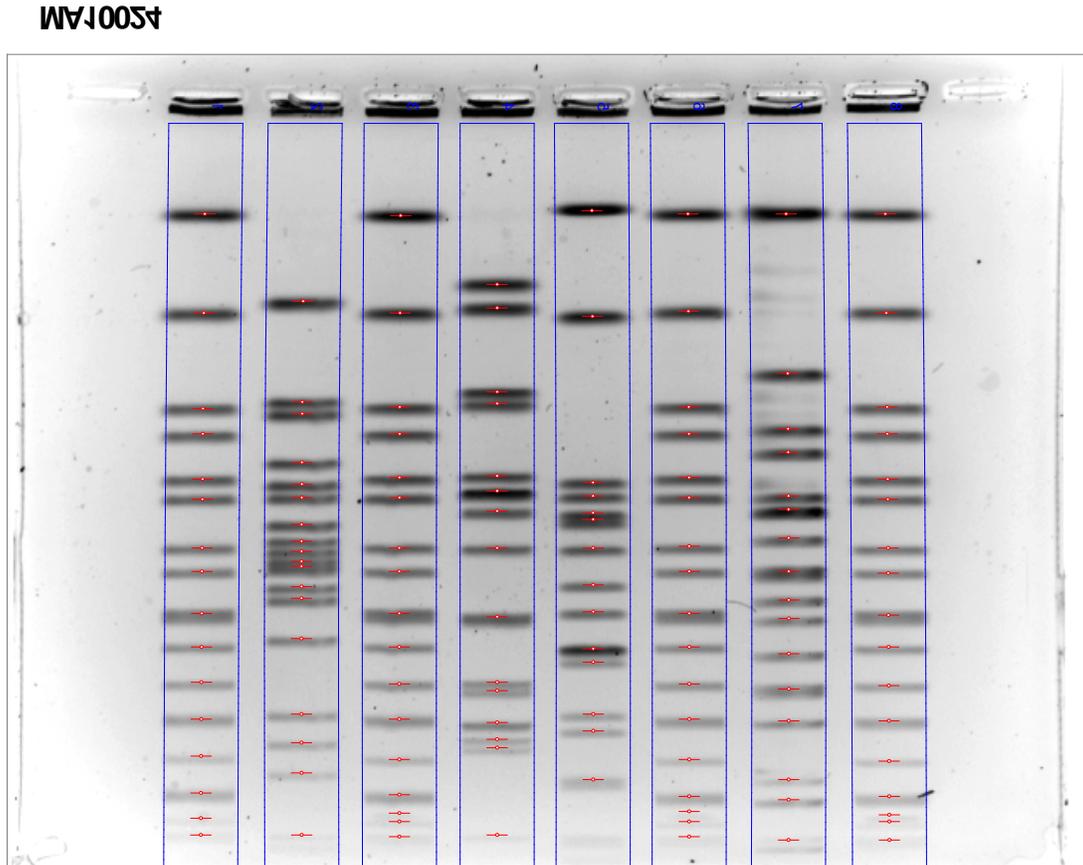
The third version of this gel illustrates spline thickness much wider than the bands in the lane.

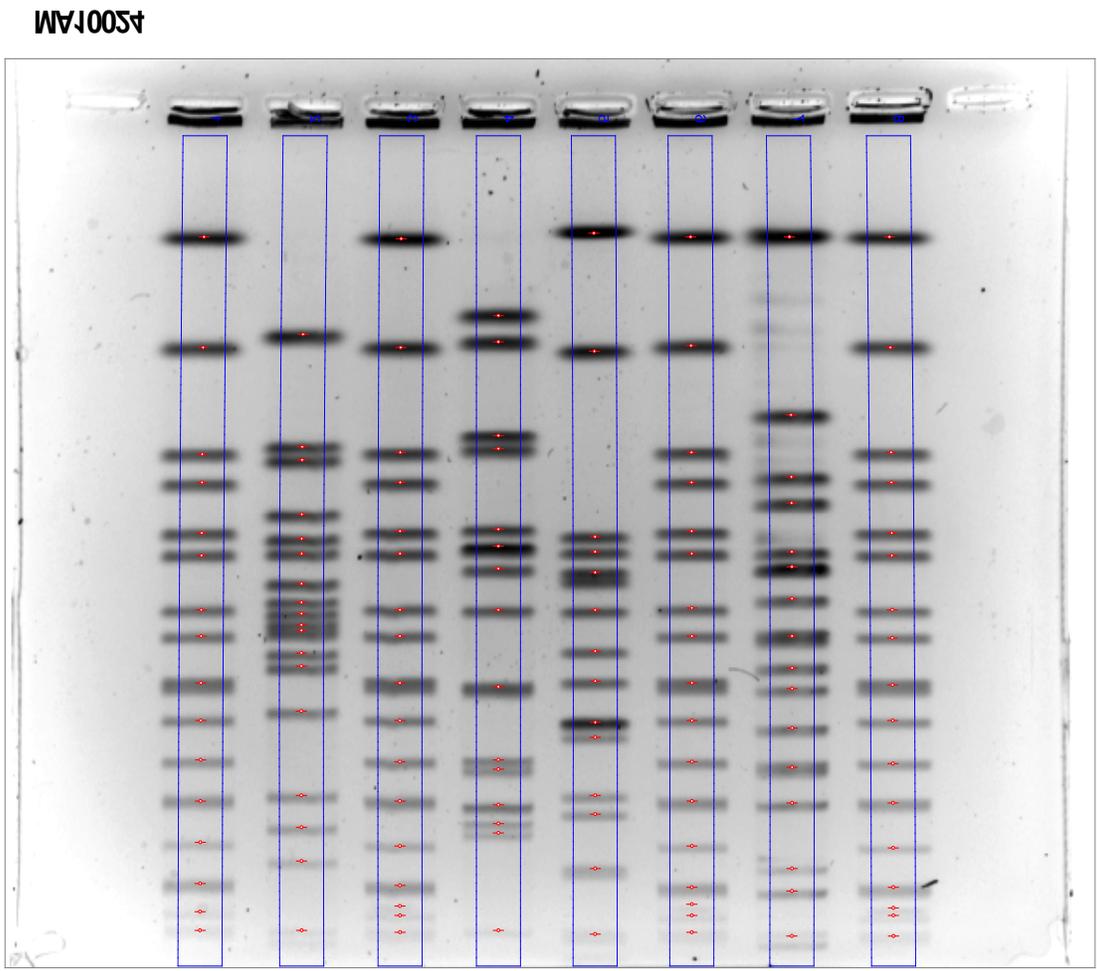


How does this setting really affect the end result? If the gel is good enough, does it really make a difference? Below is the corresponding set of images from the band-marking step, using the settings detailed above. The image set at 46 pts shows the gel with sharp, thin bands and all doublets are visible and easy to mark. The doublet in lane 5 is a point of difference for these three images.

When the settings are set at 27 pts, the shoulders of the doublet are cut off, making it more difficult to see the resolved doublet. It should be noted that Automatic Band Marking did not pick up that doublet under any of the settings. Manual review of the band marking is essential to catching missed bands and removing any debris marked as a band.

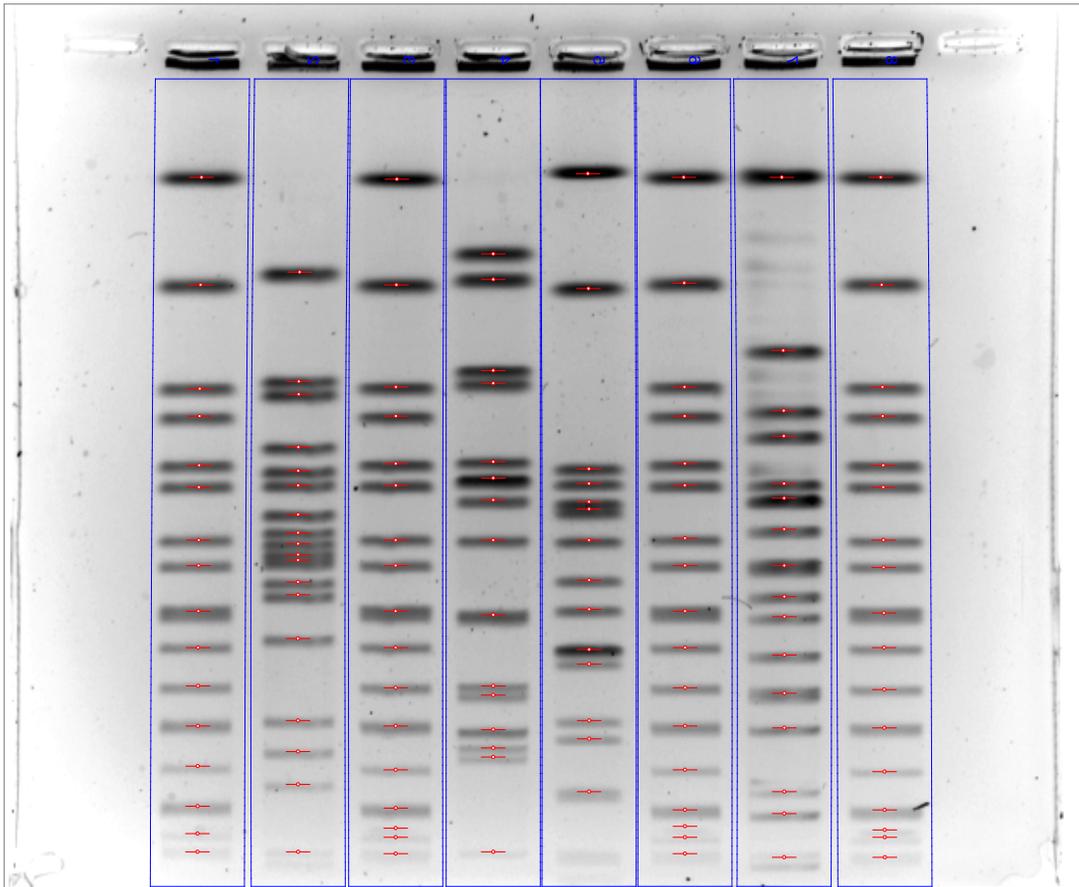
The gel with settings at 57 points shows a considerable amount of space on either side of the bands. If the gel is excellent, the impact of this setting will not be so great. For gels that range from mediocre to good, this setting makes a considerable difference, causing bands to look very fat and closer together than they actually are on the gel. The image below has a spline thickness of 46pts.





This image has the spline thickness set at 27 points.

45001AM



The image above has a spline thickness set at 57 points. Such a wide setting will result in white space on either side of the lane and make the bands look fatter in a dendrogram.

Below is a dendrogram of the MA gel. The three different versions of the clinical isolates all clustered correctly. The nine standard lanes demonstrated a small amount of difference in the dendrogram. If a lab is only considering three isolates, these will all cluster correctly. But when a lab considers 300 isolates, the small differences will be compounded. Over time, all the databases will grow. The more isolates there are to compare, the greater the likelihood that these small differences between the same isolate will appear to be legitimate differences instead of variations in BioNumerics settings or differences in the size of the gel used for analysis.



When the next gel comes off, take an extra moment to consider if the TIFF frame is filled as much as possible. When the gel is analyzed, look at the spline thickness to see if it hugs the edges of the bands. If not, take a moment to adjust the width accordingly. These seem like very small steps to take, but when you consider that all fifty states contribute to the National Database and that can amount to over 3000 isolates a month in the summer time, small steps make a big difference.