Gel Alignment and Band Scoring for DNA Fingerprinting Using Adobe® Photoshop®

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**Jeffrey McDaniel and
Suresh D. Pillai**
Texas A&M University,
College Station, TX, USA

Understanding the genetic relatedness among bacterial strains has a variety of practical applications, including the identification of possible sources of food-borne disease outbreaks and tracking the dissemination of specific bacterial genotypes. The genetic relatedness of bacterial strains is usually identified using molecular DNA fingerprinting techniques such as pulse-field gel electrophoresis, repetitive sequence-based PCR, and ERIC-PCR analysis (1,3,4). A key step common to all of these methods is the banding pattern analysis of DNA bands in electrophoresis gels. By interpreting these banding patterns, genetic relatedness “dendograms” or “trees” are created that allow for the identification of genetically related strains (2).

Commercial software programs are available that will facilitate almost all of the steps involved in DNA banding pattern analysis, including image capture, digitization, image analysis, and interpretation. Some of these programs

are sold either as stand-alone programs or as parts of the electrophoresis or image capture equipment. The prices can vary between \$2000 and \$15 000. Very often, because of cost considerations, many laboratories (especially those at academic institutions that have only modest operating budgets) are forced to rely on the visual examination and interpretation of the banding patterns to identify genetic relatedness among bacterial strains. The differences in the running conditions between gels on different days and by different individuals who may use different electrophoresis equipment and power supplies can create differences in the migration patterns of the DNA size markers (ladders) between different gels. This prevents an accurate gel-to-gel comparison, thereby compromising the quality of the data analysis and interpretation.

We rationalized that if commonly available and relatively inexpensive software such as Adobe® Photoshop® (version 5.5 or higher) is used for the standardization and alignment of DNA banding patterns obtained in different gels, then it will help reduce human errors when aligning the DNA banding patterns on different gels. There are two critical steps for comparing DNA banding patterns, (i) the alignment (standardization) of the bands across multiple gels and (ii) the “scoring” (presence/absence) of specific bands across the different gels. The approach presented here will permit the align-

ment of DNA bands across multiple gels and create a grid that will facilitate the scoring of the various bands. Gel running conditions such as run times, voltage, and staining need to be rigorously standardized before electrophoresis. We recommend the use of pre-made gels and the loading of DNA size markers on the two outermost lanes and a center lane to help in gel alignment. This method can be used to compare as many gels as possible; however, factors such as fatigue and eye strain can play a role. Given below is a standardized protocol for the alignment of banding patterns and the creation of a grid that will facilitate the scoring of the banding patterns.

GEL ALIGNMENT

The electrophoresis gel images are initially saved as JPEG files because this file format requires less disk space. Other file formats can be used but should be kept constant for all the gels being compared. The images are then imported into Adobe Photoshop using the File → Open commands of the software. The image is then converted to a negative image using the Image → Adjust → Invert commands. (This creates a negative of the original image that facilitates the manual scoring of the bands on the gel.) The view in the “navigator box” (located on the top right corner of the screen) is changed to fit

the entire gel image on the screen. The image is then adjusted to ensure that the loading wells between the different gels are evenly oriented in the vertical direction using the Image → Rotate → Canvas → Arbitrary commands. The Rectangular Marquee Tool is selected, and

a box encompassing the DNA size marker is created. The distance is obtained using the navigator box and clicking “information”. The value of H is the height measured from the Rectangular Marquee Tool (Figure 1). Note that the first gel image (termed “base

image”) will serve as the base value for H. To standardize subsequent images, the following formula is used:

$$\frac{\text{Height}(H)\text{ofbaseimage}}{\text{Height}(H)\text{ofimage}} \times 100 \quad [\text{Eq. 1}]$$

Using this value, standardize the image by adjusting the vertical proportion. To perform this, use the Image → Image Size commands. From this dialog box, locate the Height under “pixel dimensions” and select *percent* from the “image size” window. Input the calculated value as the height value and click OK. This will standardize your image. (Opening the computer program “Calculator” will allow for easy calculations, and you can copy the exact value calculated from the formula. This will allow you to copy and paste the value directly into Adobe Photoshop.)

BAND SCORING

Adjust the image to maximize band visibility by adjusting the Brightness and Contrast. To perform this function, Image → Adjust → Brightness/Contrast adjusts the image to your own preference.

Before beginning the scoring process, one must ensure that the lane one is scoring is always in a complete vertical orientation. One can check for vertical orientation by observing the positioning of the DNA ladder located on the ends and in the middle of the gel. To rotate the image to obtain a vertical orientation, use Image → Rotate Canvas → Arbitrary. Rotate the image using the appropriate values until the lane is vertically oriented. This process should be done for each and every lane scored. Change the image zoom to 100%, which is located in the navigator window on the right-hand side of the screen, to maximize band assessment. The grid lines are placed over the image. To turn on the grids, use View → Show → Grid. The grid’s values also need to be standardized. To standardize, use the Edit → Preferences → Guides and Grids commands. Under the grid dialog, values for the grid are selected and used across all the gels. Note that the grid line placement must remain standard between gels to assure proper

Figure 1. Screen image showing where the height (H) is measured. The dotted Rectangular Marquee Tool is used to create a box encompassing the DNA size marker. The value of H for the box is obtained from the navigator box data (circled).

Figure 2. Screen image indicating examples of “1” and “0” band scoring. The standardized grid lines are placed over the image. A value of 1 is given when a grid line passes through a band on the gel, while a value of 0 is given when the grid line does not pass through a band.

comparison. It is not necessary for the grid lines to cross each and every band on a gel, but an attempt must be made to cover the majority of the bands. It is critical to determine before gel scoring the number of grid lines that one can accurately score, and this grid pattern should not be changed during the course of the scoring process. (In our work, the DNA size marker was used to help place the grid lines over the image in a standardized method.)

Once standardized, the grid lines must be placed over the image in a standard manner. The grid should be positioned so that the grid lines travel through the center of the bands. Careful attention should be taken to make proper adjustments to any "smiling" of the gel. If "gel smiling" is encountered, the image itself can be rotated to align the three size marker bands to one another. Attention should be paid so that the image is not distorted. The grid line position may be adjusted by the Select All command (Ctrl + A) and selecting the Move Tool.

The banding patterns can then be scored. Use a spreadsheet to facilitate the scoring. To score a gel, the value "1" is marked when a grid line travels through a band on the gel. The value zero (0) is marked if the grid line does not travel through a band on the gel (Figure 2). Continue the scoring until the end of the range selected for scoring is reached.

The binary values (1 and 0) can then be used for statistical comparisons (2). We have used the statistical analysis package NTSYS-pc (Exeter Software, Setauket, NY, USA) for these comparisons. The SIMQUAL and SAHN clustering subroutines were used to generate the genetic similarity trees or dendograms.

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Address correspondence to:

Dr. Suresh D. Pillai
2472 TAMUS
418D Kleberg Center
Texas A&M University
College Station, TX 77843-2472, USA
e-mail: spillai@poultry.tamu.edu

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