Analyzing ISSR Gels using Kodak 1D

1. Open Adobe Photoshop LE and open your TIFF file

2. adjust gel so that it is straight, by selecting:
   Image → Rotate → Arbitrary

   NOTE: it may also help to draw a straight line across a specific band on your ladders, then you can easily tell if your gel is straight (hold **Shift** down while you draw your line).

3. **Edit → Undo Line Tool** (to remove the line)

4. Once gel is straight, you can save your file: **File → Save As**
   Erase the file extension on the name (.TIF) and press **OK**. You should then be prompted on how to save your file. Select **Macintosh** and press **OK**.

5. Quit Photoshop

6. Open Kodak 1D 2.0.2 and open your new file

7. Maximize the window if you have an especially long gel

8. Select the button (on left floating toolbar - "Mark Lanes") and position your cursor at the top left of the 3054 bp band on the left ladder. While dragging the cursor, draw a box around the bands you want to analyze. Remember to end at the right top corner of the 3054 bp band on the right ladder. It is important to begin and end your box at the outermost edges of the 3054 bp bands – this will ensure that your lane lines are equidistant from one another. You will next be asked how many lanes you have in your gel – fill in this information.

9. Now you need to make sure that all of your lane lines match up with your lanes. First, click on the arrow on the floating toolbar. it might be helpful to enlarge the image using the button on the bottom toolbar (1.5X is good). If you move the location of a line, click on it and drag it to the proper location (you will see a hand). If the lane line needs to be slanted, click on the red square on the top or bottom of the lane line and drag to desired position. To delete a line, click on it to highlight (it should be red) and press delete (the backspace key, not the small delete key).

10. Next, select the **Find Bands** button on the left for the software program to find all the bands in the specified area of your gel. You should eventually see all the bands highlighted in blue across the gel.

11. If a large number of bands need to be deleted (e.g., on the bottom of the gel where it was sliced), draw a box around the bands using your arrow cursor and press delete.

12. To designate ladder lanes: Holding down the **Shift** key, click on each of your ladder lanes (you can also do them individually). Double-click on the last lane – you should now see a window. Under "Lane Type", select **Standard** and then press the **Select** button. You

should see a menu of possible ladders – select **1 KB Ladder**, and press **Select** and **OK** buttons. All the ladder lanes should now be a bright pink.

13. Next you need to adjust the individual bands on your gel. Select the ← button in the lower left to view the chromatograph. If you wish to see the ladder peaks on the display (or if you don't want to), click on the "Std:A" button on the lower left and select the appropriate action.

14. For each band, you need to click on it (if will appear red) and then drag the middle line to the middle of the peak (you can do this either directly on your gel or on the chromatograph). On the chromatograph, click on each of the outside lines and drag them as close as possible to the center line on your band (this is so it will be easier to add a new band, if needed).

15. If you need to add a new band, click on the red band icon on the floating toolbar and then click on your gel where you want the band to appear. You can then adjust the position of the band as in #12.

16. Once you have checked all of your bands, it is time to do the final analysis. First, make sure that all the bands are highlighted on your gel (you may have to select the → button in the lower left). Select **Show** → **Lanes/Bands** → **Lane Analysis Data**

17. You should now see a new window showing all of the fragment sizes for each of your lanes. Copy and paste this file into Excel, and then save your Excel file.

18. If you wish to print your gel image, it is sometimes best to first make sure that your bands are not highlighted in red. Click anywhere on the gel to return the bands to their normal blue color. If you wish to change how your image will be printed (with a white or black background), select: **File** → **Page Setup** and press the **Options** button. Check or uncheck the "Invert Image" box until you have the image type you want. To print the image, select: **File** → **Print** → **Print**

19. Before exiting Kodak 1D, remember to save your Kodak file: **File** → **Save As**
    - Enter in your file name and make sure it will be saved as a Kodak 1D file.

NOTE: At the end of the analysis of one gel, you should have four files saved: (1) your original JPG file, (2) the Kodak 1D file, and (3) your Excel file. I prefer to save all my Excel files separately and them combine them into one large file at the end (or you can choose to just cut and paste the information from each gel into one large Excel file as you go along).

Organizing Band Positions

Once you have successfully used Kodak 1D to find and analyze your bands, you should have one (or several) Excel files containing the molecular weight information. The next step is to convert this mass of information into a binary table that you can import it into a software package for final analysis. This is perhaps the most difficult part of the ISSR analysis because it is very tedious and involves some subjective reasoning on your part. Here are the steps:

1. For each primer, place the printouts of your gels on a table. On each gel printout, begin drawing horizontal lines through the bands in the different lanes that you believe are homologous, using a clear ruler and a pencil. Also draw lines through a band even if it is the only one in that location. Do this for all gels of the same primer (I like to concentrate on one primer at a time).

2. Now line up the printouts of all your gels (for one primer) next to one another and place their replicates below. Examine all of your gels and determine which band is the highest (i.e., largest). This band will be #1, and write Its number next to the appropriate line in the margin. Examine all other gels to see if this band also appears (it's helpful to compare its location against that of the ladder). Look for the next lowest band and that will be band #2. Continue until you have assigned all bands to a number. Remember that you will probably have bands present on one gel that are not found on another (which is the reason this is so difficult).

   NOTE: On gels with an especially large number of bands, I have found it helpful to first mark the bands (with a symbol, not a number yet) that appear common on all gels. Then you can concentrate on lining up the bands between these locations.

3. Check your replicates and make sure that bands marked on primary and replicate gels are in identical positions. If any band is missing from either your primary or replicate gel, delete it (unless you have a very strong reason to believe that it is truly there – e.g., it is very bright on one gel, but you can only see a very small peak on the replicate).

4. If you haven't already, it may be helpful to combine your Excel files for each primer (so that each individual lane is a column). For now, leave the molecular weight of the ladders in your file.

5. Open Excel on your computer. Insert a column at the beginning of your matrix, and number it according to the number of band locations you will have. For the first gel, place your printouts of the primary and replicate gels in front of you. For each individual lane, move the band molecular weights to the corresponding location in the data matrix.

   NOTE: You may find it helpful to first insert a number of blank rows to the beginning of your Excel file and then move the molecular weights up into those appropriate blank spots.

6. Continue for the remaining gels for the primer.

7. Continue for the gels from all other primers.

8. For each primer, you should now have a large Excel file that mirrors your gel, with each band being represented by its corresponding molecular weight. Print out this Excel file and tape the various pages together.

9. Place your Excel printout on a table and carefully examine it, making sure that the distribution of the molecular weights makes sense. If not (e.g., a larger molecular weight is placed below a smaller one), you will need to look back at your gel photocopies and see if an error was made. Occasionally you will find that molecular weights are not even across the gel – this usually occurs if your gel did not run perfectly straight (lanes sometimes slant towards the right side).

10. Next, examine your Excel printout once again against the gel photocopies, making sure you are satisfied with the scoring. Remember that this entire step of the ISSR analysis is very important and so it should be as accurate as possible.

11. Remove the ladder lanes, and negative (and positive) controls from your data matrix.

12. Transpose all data in each Excel file (see instructions) such that the band loci are in columns and the lane data are in rows.

13. Now combine your Excel files from all your primers into one large file. You may wish to strip off the header row(s) at this time.

14. You are now ready to convert your file into binary format.

NOTE: I prefer to use the above method rather than blindly distributing molecular weights and then comparing them against the photocopies, because some of the homologous bands on my gels had slightly different or sometimes very different molecular weights (because of what was mentioned in #10).