

# IMAGE ANALYSIS: PROFILES ANALYSIS AND INTERPRETATION

## Step 4: DEEP INTO THE ANALYSIS

- Analyze a TIFF
  1. **Convert a TIFF to Gel Strips**
  2. Define Curves
  3. Normalize the Gel
  4. Find Gel Bands
- Link Lanes to Database Entries
- Add Text Data for Isolates

# Image analysis: profiles analysis and interpretation

## Step 4: ANALYZE A TIFF - LET'S START

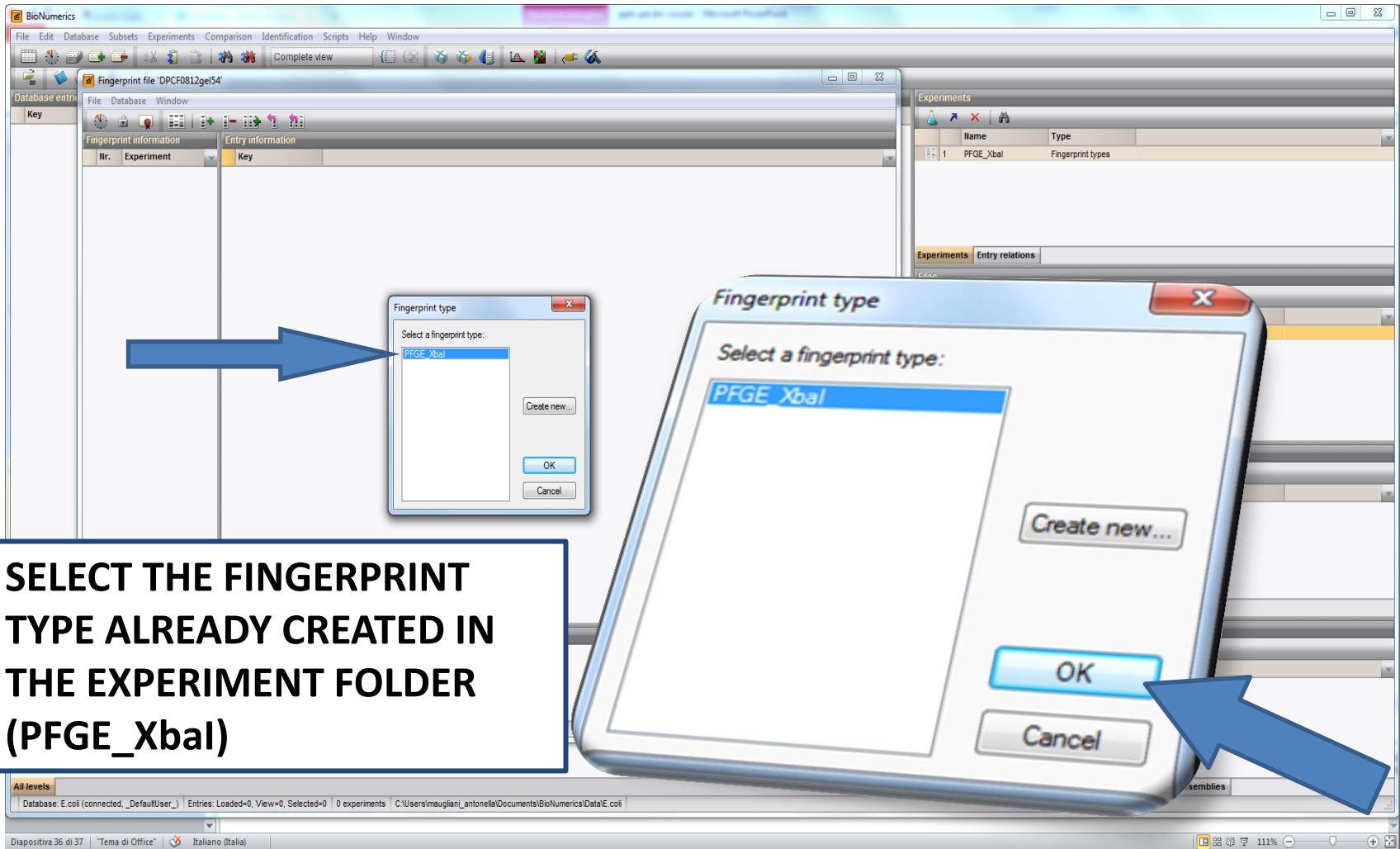
**2. CLICK ON THE GEL IMAGE ICON (EDIT FINGERPRINT DATA) IN ORDER TO ANALYZE THE TIFF IMAGE**

**1. DOUBLE CLICK ON THE NEW GEL AND FINGERPRINT TYPE WINDOWS WILL OPEN.**

The screenshot shows the BioNumerics software interface. The main window is titled 'Fingerprint file 'DPCF0812geI54''. It has a menu bar (File, Database, Subsets, Experiments, Comparison, Identification, Scripts, Help, Window) and a toolbar. Below the menu bar, there are two tabs: 'Fingerprint information' and 'Edit fingerprint data'. The 'Edit fingerprint data' tab is active, showing a table with columns 'Nr.' and 'Experiment'. A blue arrow points to the 'Edit fingerprint data' button. The 'Experiments' panel on the right shows a table with columns 'Name' and 'Type'. It contains one entry: 'DPCF0812geI54' with type 'Fingerprint types'. A blue arrow points to this entry. The 'Files' panel below 'Experiments' shows a table with columns 'Name', 'Created', 'Modified', and 'Location'. It contains one entry: 'DPCF0812geI54' with created and modified dates of '2014-06-05 17:17:58' and location 'Shared DB'. The 'Comparisons' panel below 'Files' is empty. The status bar at the bottom shows 'Database: C:\cor\connected\_\defaultoset\ Entries: loaded=0 View=0 Selected=0' and 'Experiments: C:\users\magnum\documents\biometrics\data\c\cor\'. The system tray at the bottom shows 'Diapositiva 37 di 38', 'Tema di Office', 'Inglese (Stati Uniti)', and a zoom level of '111%'.

The BioNumerics Software: database creation, experiment type, import of TIFF files, and setting up experiments

## Step 4: Select the experiment created



# TIFF ANALYSIS AND PFGE PROFILE INTERPRETATION

## Step 4: CONVERT A TIFF TO GEL STRIPS (I)

**NOTE: IF THE WELLS ARE NOT INCLUDED IN THE TIFF THE GEL CANNOT BE ANALYZED!**

The screenshot shows a software interface with a gel image in the center. A green rectangular box is drawn around the gel lanes. Two blue arrows point to the top and bottom of this box. The top arrow points to the top edge of the box, and the bottom arrow points to the bottom edge. To the right of the gel image, there are two text boxes with instructions. The top box says 'Place the top of the frame just below the wells' and the bottom box says 'Make sure bottom line includes all bands'. The software interface also shows a menu bar (File, Edit, Lanes, Strips, Window) and a toolbar. On the right side, there are panels for 'Experiments', 'Files', and 'Comparisons'. The 'Files' panel shows a table with columns: Name, Created, Modified, and Location. The 'Comparisons' panel shows a table with columns: Name, Created, Modified, and Location.

Place the top of the frame just below the wells

Make sure bottom line includes all bands

**normalization process will not be done correctly otherwise!**



# TIFF ANALYSIS AND PFGE PROFILE INTERPRETATION

## Step 4: CONVERT A TIFF TO GEL STRIPS (II)

The screenshot shows the BioNumerics software interface. A gel image is displayed in the center, with a green rectangular box highlighting a portion of it. A blue arrow points from the 'Auto Search Lanes' dialog box to the 'Image' menu in the top toolbar. The dialog box is titled 'Search lanes' and contains the text 'Enter estimated number of lanes' followed by a text input field containing the number '10'. Below the input field are 'OK' and 'Cancel' buttons. A blue box with the text 'Enter the number of lanes' is positioned below the dialog box. The background shows the main software window with various panels and a table of data.

To define lane strips, click “Auto Search Lanes”

Enter the number of lanes

Modified	Location
06-06 13:38:27	Shared DB
06-06 13:39:05	Shared DB

Name	Created	Modified
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Alignments Chromosome comparisons Annotations Power assemblies

# TIFF ANALYSIS AND PFGE PROFILE INTERPRETATION

## Step 4: CONVERT A TIFF TO GEL STRIPS (III)

To adjust width of all lanes, click “Edit Settings”

Click on “Raw Data”

Adjust Thickness until strips are wide enough

Enable “Spot removal” if some debris are present in the gel

OK

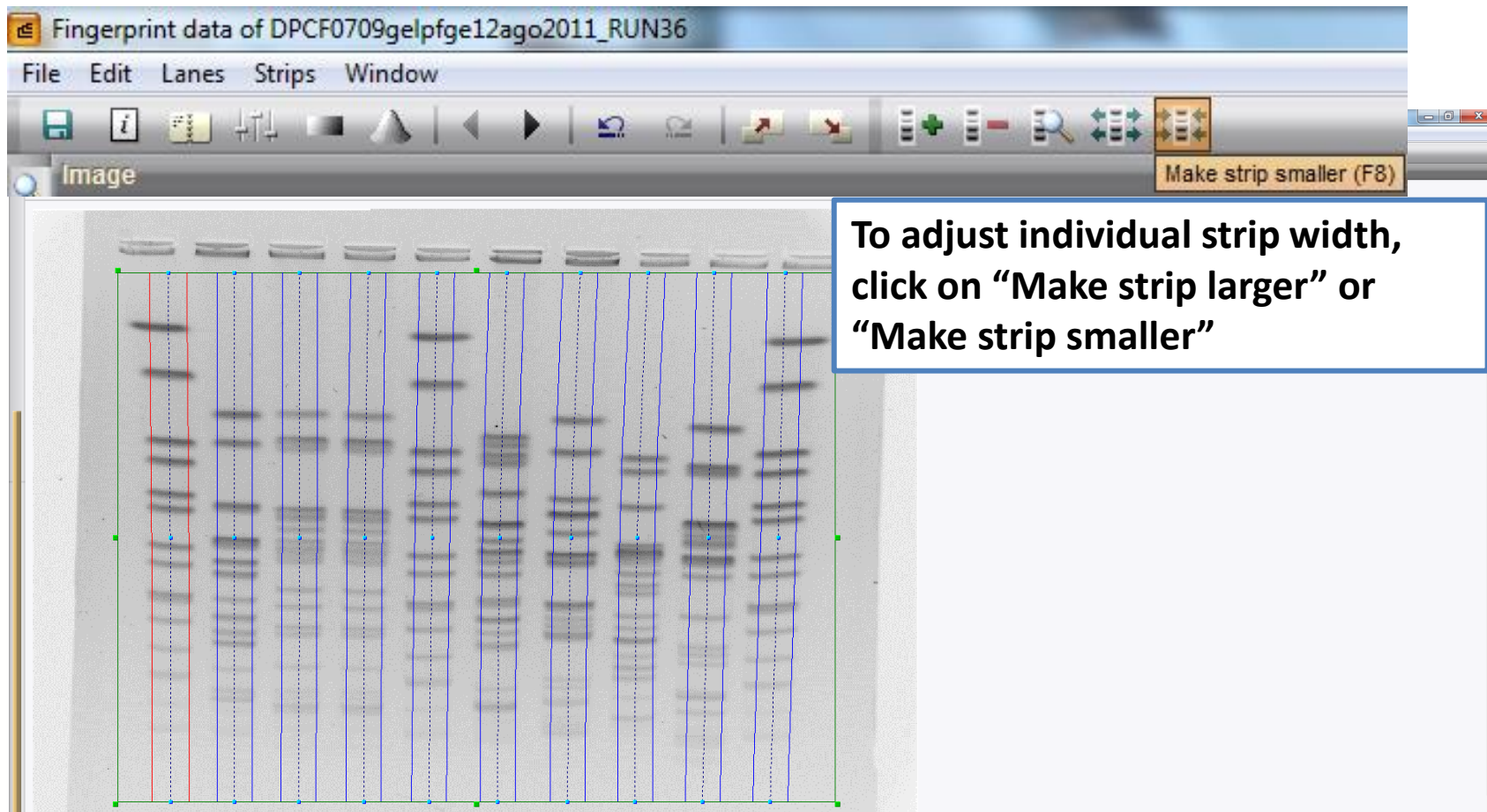
Strips Curves Normalization Bands

Fingerprint type: PFGE\_XbaI TIFF: 760 x 574 x 8 (x1.00)

Alignments Chromosome comparisons Annotations Power assemblies

# TIFF ANALYSIS AND PFGE PROFILE INTERPRETATION

## Step 4: CONVERT A TIFF TO GEL STRIPS (IV)



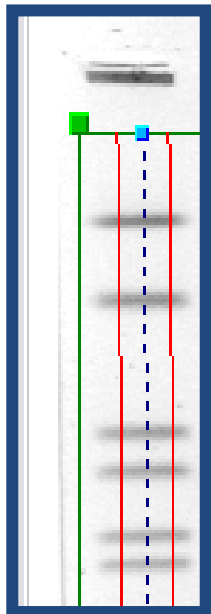
**NOTE: This is an important step to help determine doublet or triplet resolution during band marking**

# TIFF ANALYSIS AND PFGE PROFILE INTERPRETATION

## Step 4: CORRECT ASSIGNMENT OF STRIPS WIDTH

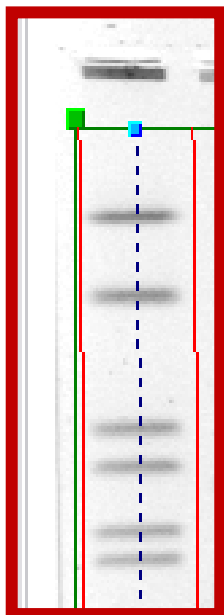
MAKE SURE THE STRIP DOES NOT:

... cut off the edges of the lane



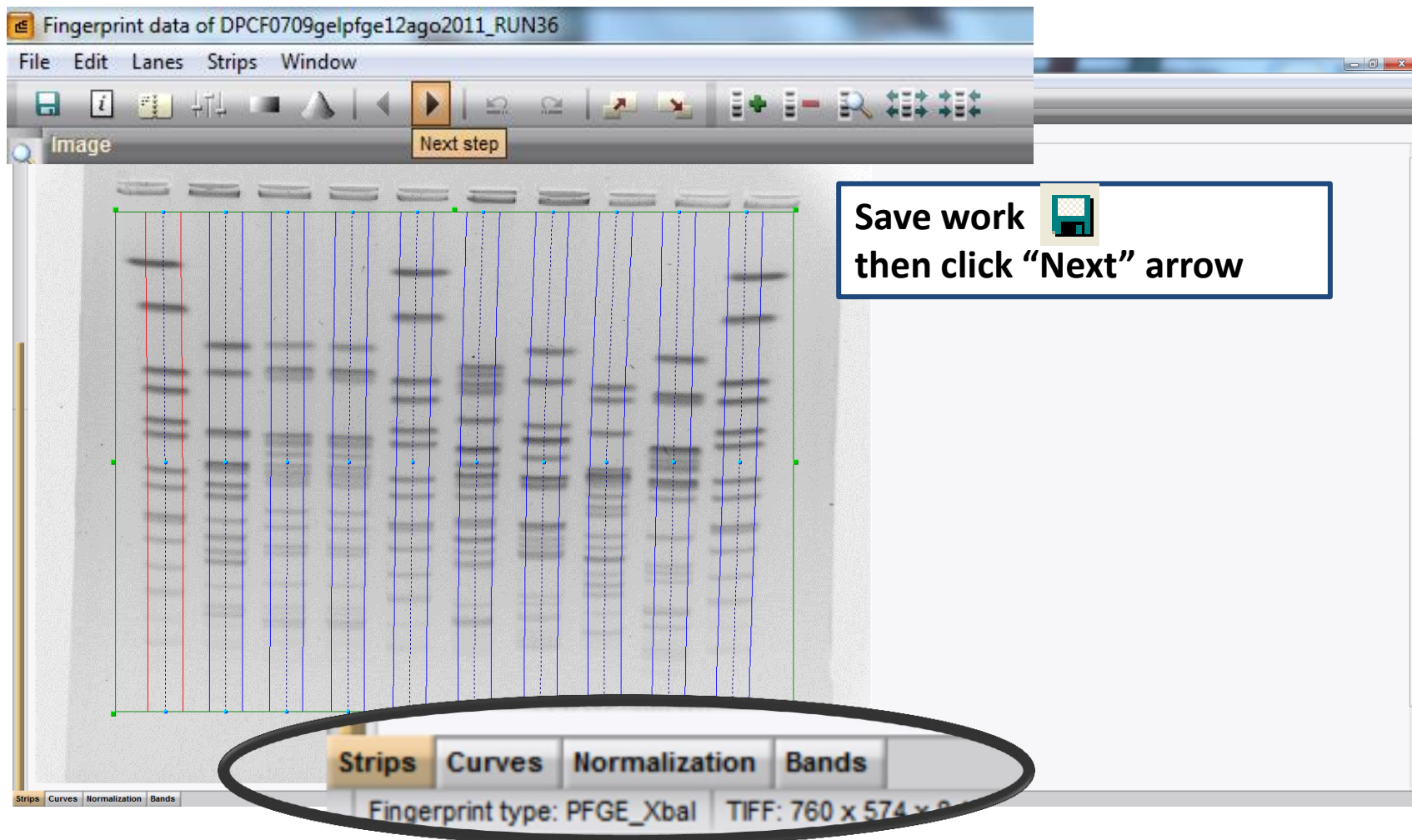
THE STRIP SHOULD INCLUDE ALL OF THE LANE

... include too much space



# TIFF ANALYSIS AND PFGE PROFILE INTERPRETATION

## Step 4: CONVERT A TIFF TO GEL STRIPS (V)



## DEEP INTO THE ANALYSIS

- Analyze a TIFF

1. Convert a TIFF to Gel Strips

- 2. Define Curves**

3. Normalize the Gel

4. Find Gel Bands

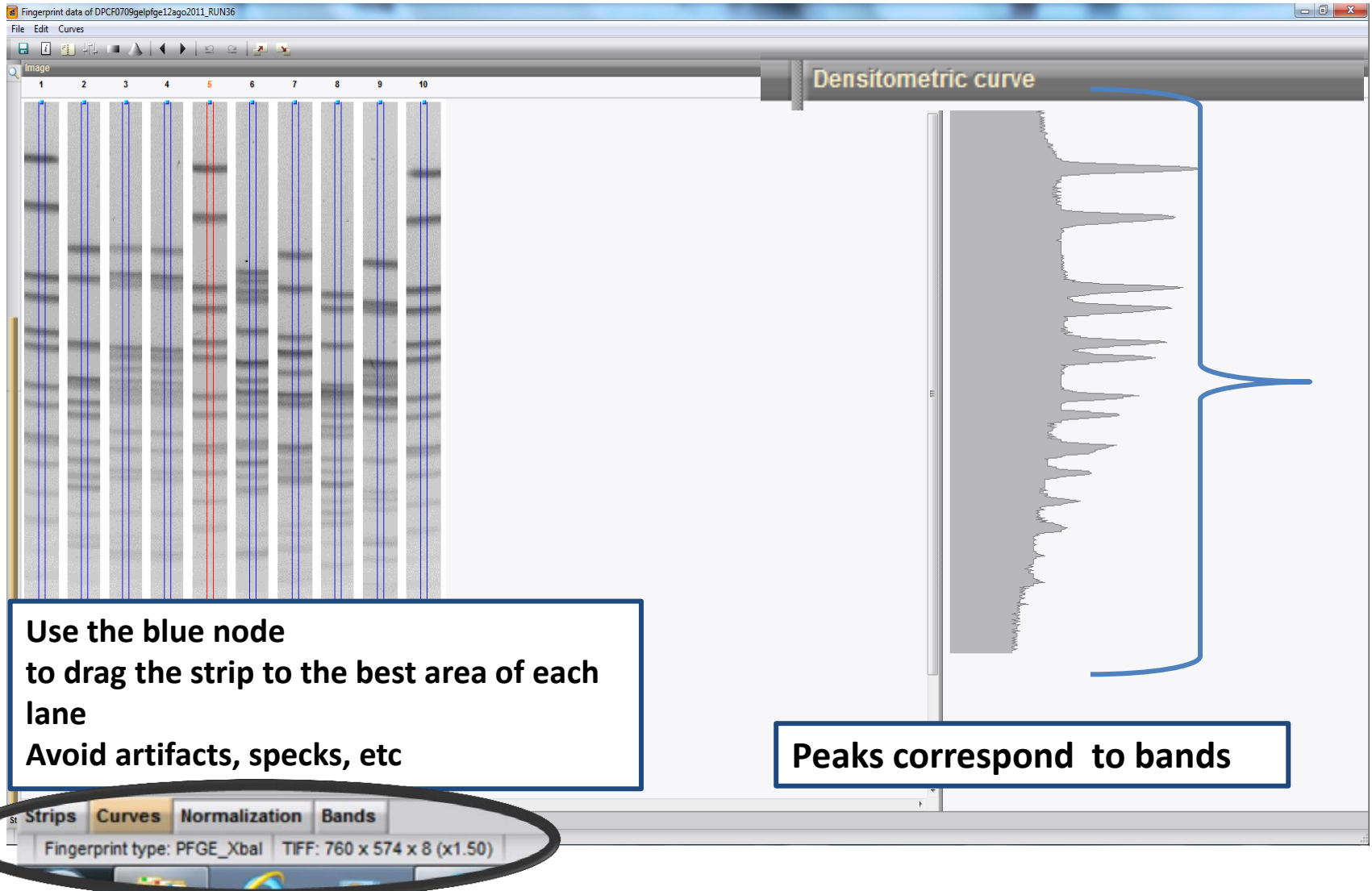
- Link Lanes to Database Entries

- Add Text Data for Isolates



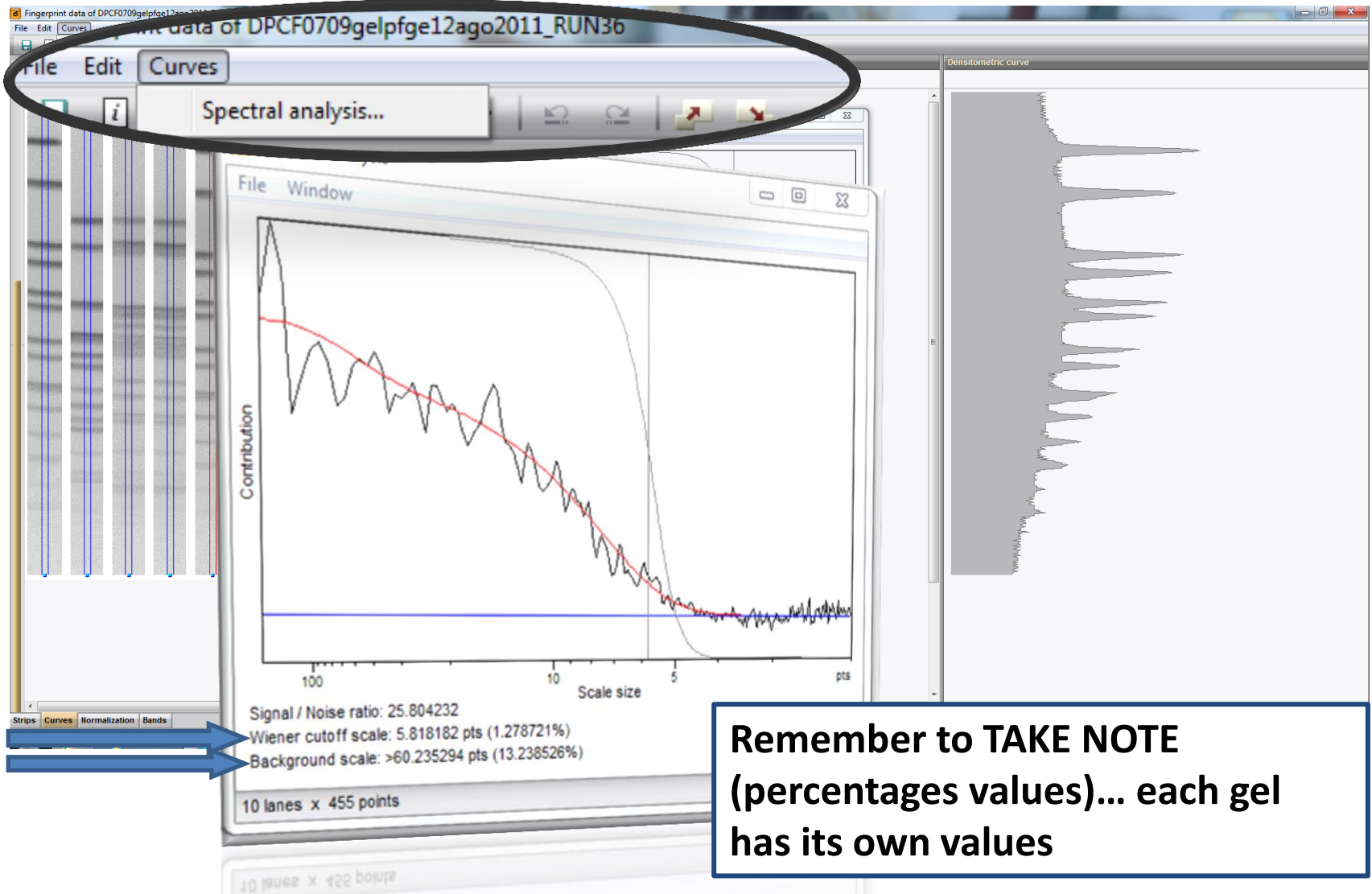
# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

## Step 4: DEFINE CURVES



# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

## Step DEFINE CURVES

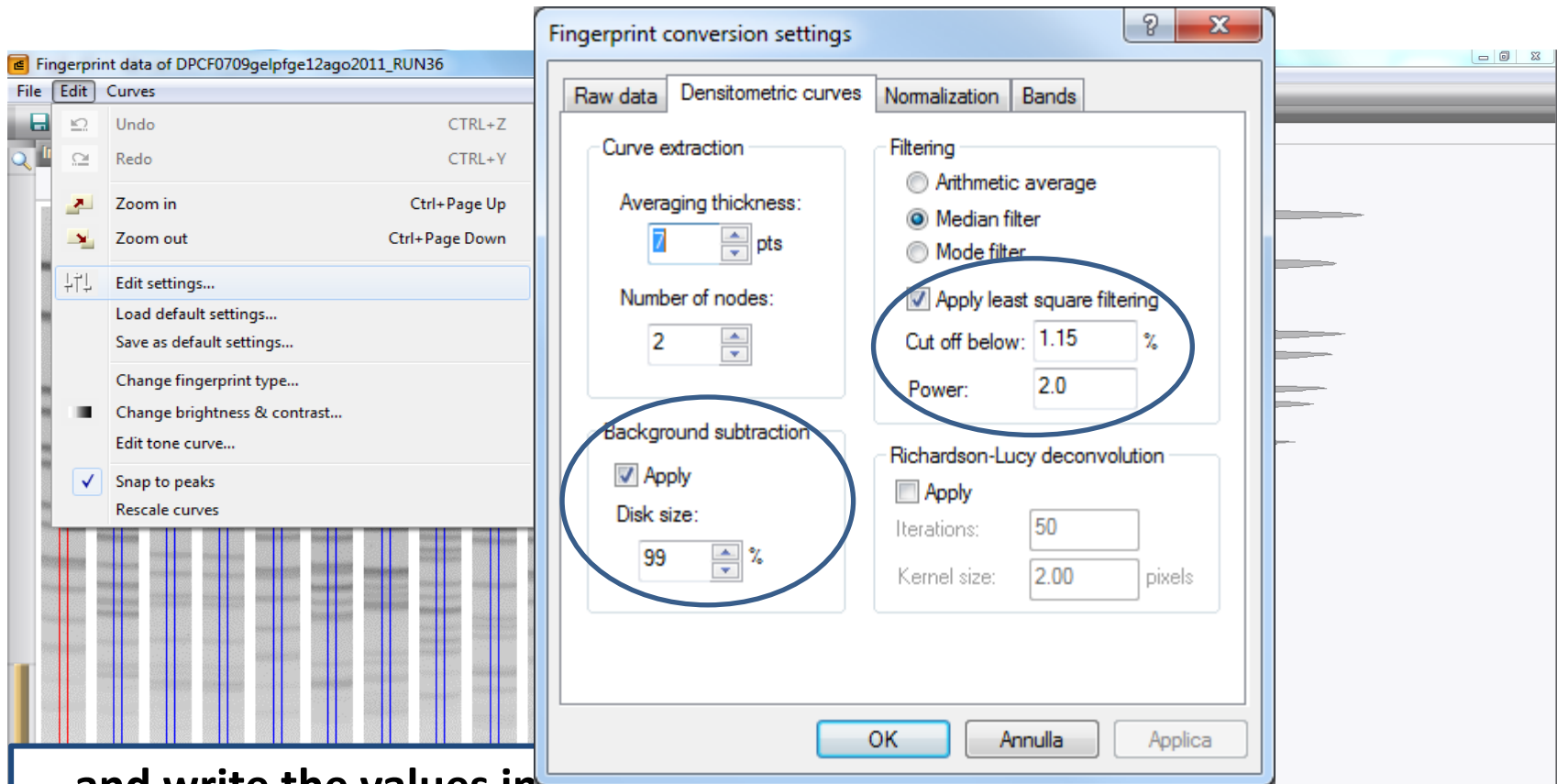


**Remember to TAKE NOTE**  
(percentages values)... each gel  
has its own values



# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

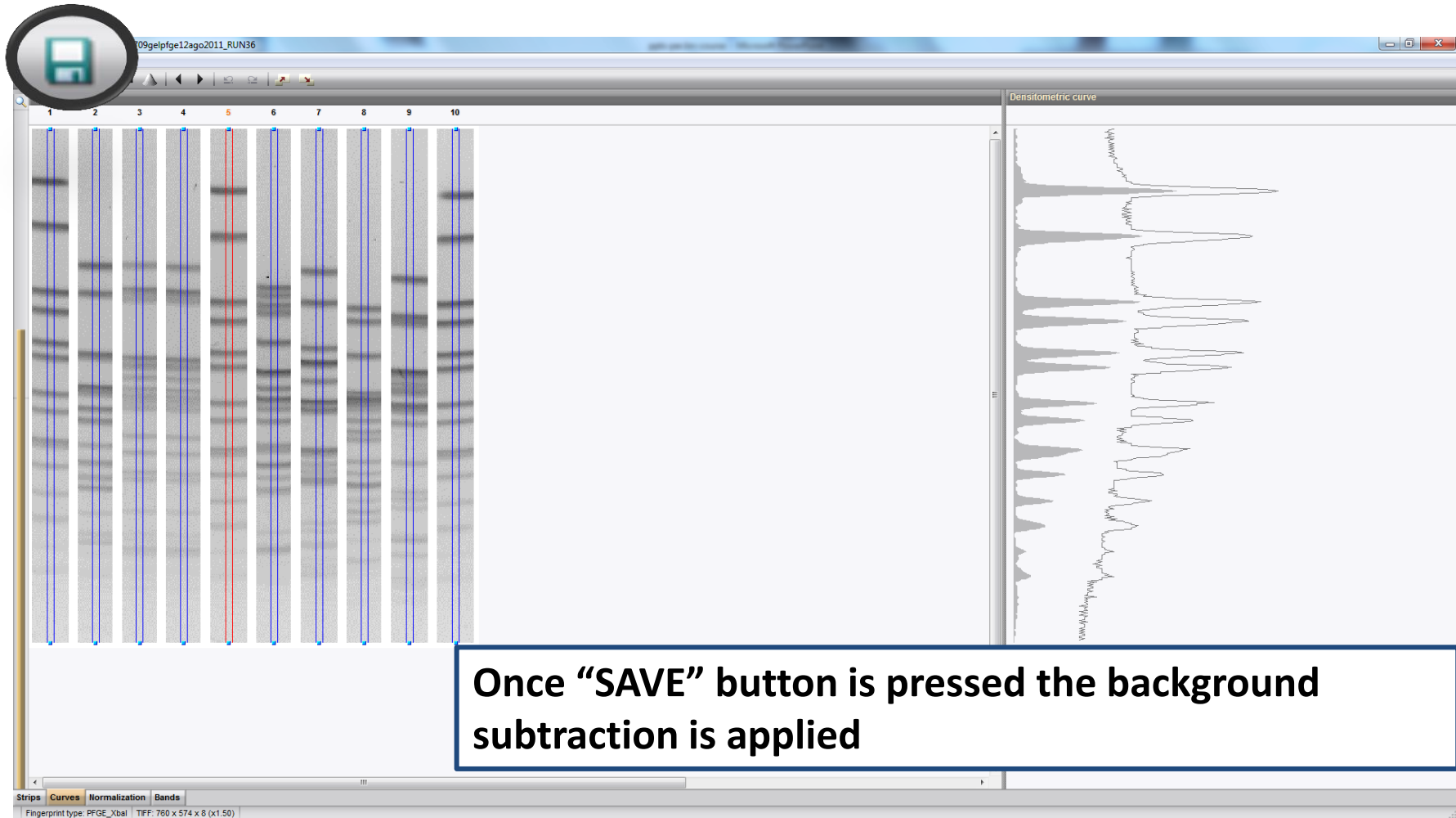
## Step 4: DEFINE CURVES



... and write the values in  
Densitometric Curves folder (edit  
menu)

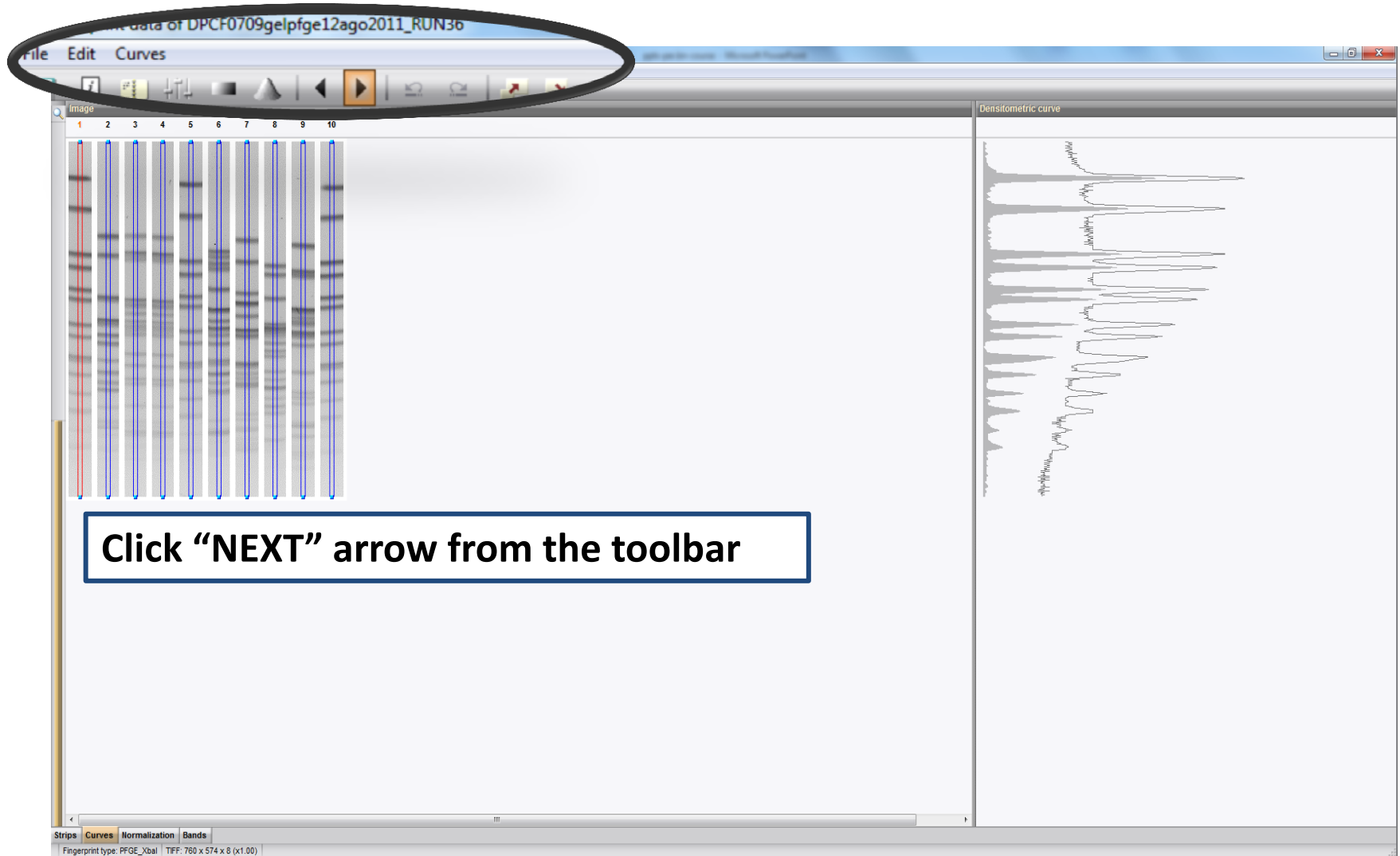
# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

## Step 4: Setting up the experiment – DEFINE CURVES



# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

## Step 4: DEFINE CURVES



# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

- Analyze a TIFF

1. Convert a TIFF to Gel Strips

2. Define Curves

- 3. Normalize the Gel**

4. Find Gel Bands

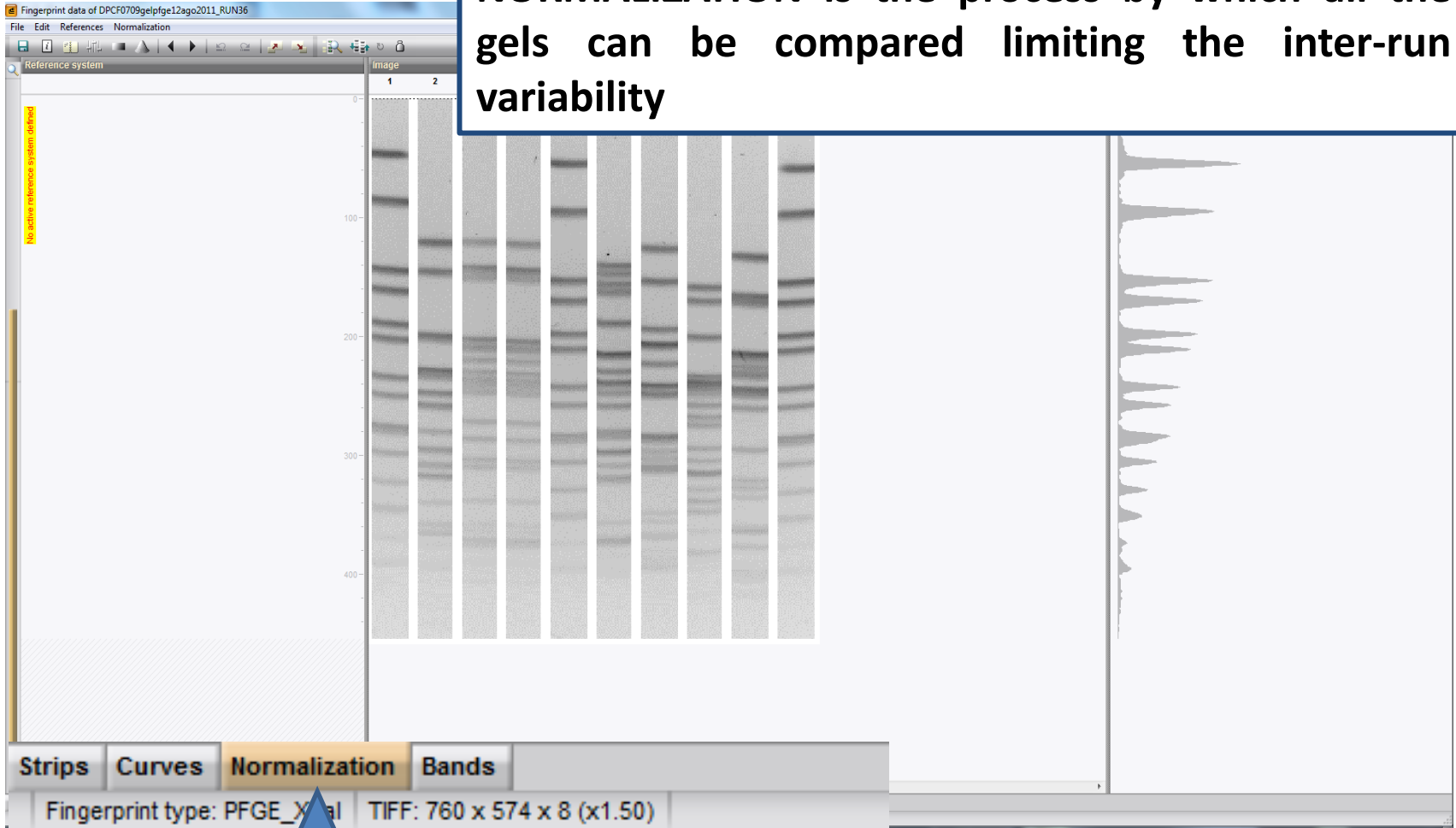
- Link Lanes to Database Entries

- Add Text Data for Isolates

# TIFF ANALYSIS AND PFGE PROFILE INTERPRETATION

## Step 4: NORMALIZATION

**NORMALIZATION** is the process by which all the gels can be compared limiting the inter-run variability



# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

## Step 4: ASSIGNING THE REFERENCES LANES AND BAND POSITIONS - NORMALIZATION

Fingerprint data of DPCF0709gelpfge12ago2011\_RUN36

File Edit References Normalization

Refer

No active reference system defined

Use as reference lane Ctrl+R

Use all lanes as reference lanes

Add external reference position

Add internal reference position

Delete reference position

Change reference position name...

Change reference position...

Copy normalization

Paste normalization

Click on «REFERENCES»  
AND SELECT «USE AS  
REFERENCE LANES»...

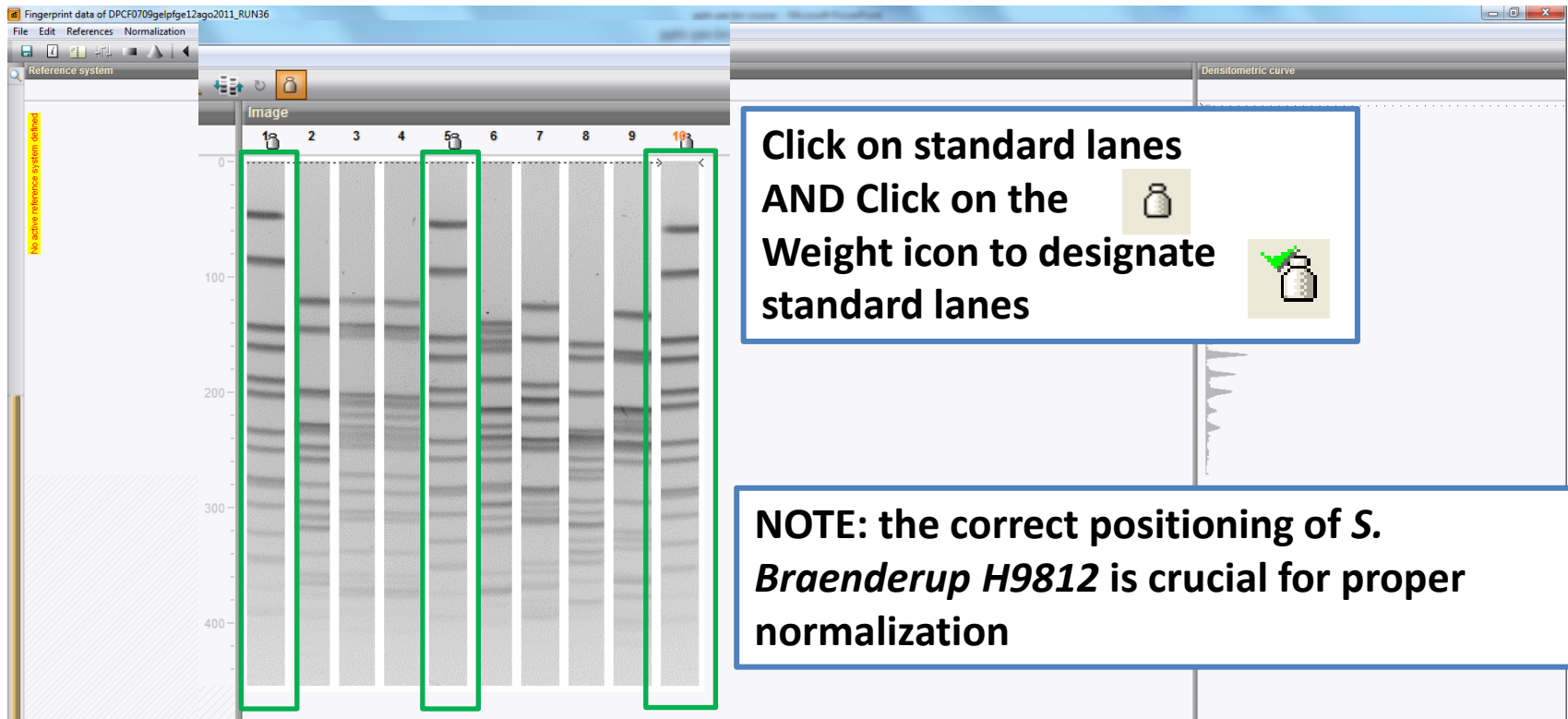
For the first gel in the data base  
molecular weight must be assigned to  
the bands of the marker (S.  
*Braenderup* H9812 strain)

Strips Curves Normalization Bands

Fingerprint type: PFGE\_Xbal TFF: 760 x 574 x 8 (x1.00)

# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

## Step 4: ASSIGNING THE REFERENCES BAND POSITIONS - NORMALIZATION



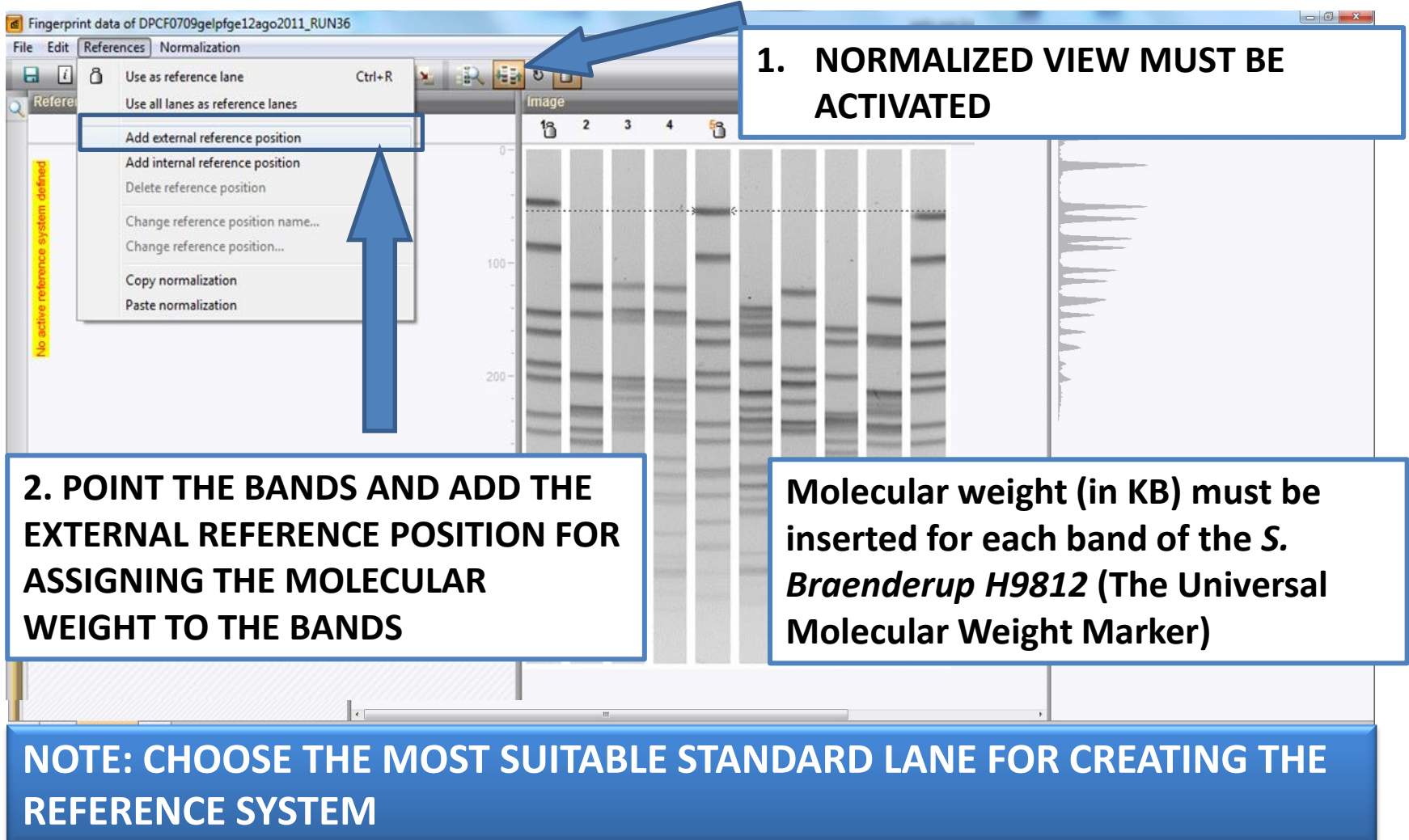
Click on standard lanes  
AND Click on the  
Weight icon to designate  
standard lanes

NOTE: the correct positioning of *S. Braenderup H9812* is crucial for proper normalization

- STANDARD LANES 1,5,10 FOR THE 10 WELL GEL
- STANDARD LANES 1,5,10,15 FOR THE 15 WELL GEL
- STANDARD LANES 1,5,10,15,20 FOR THE 20 WELLS GEL

# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

## Step 4: ASSIGNING THE REFERENCES BAND POSITIONS - NORMALIZATION



**1. NORMALIZED VIEW MUST BE ACTIVATED**

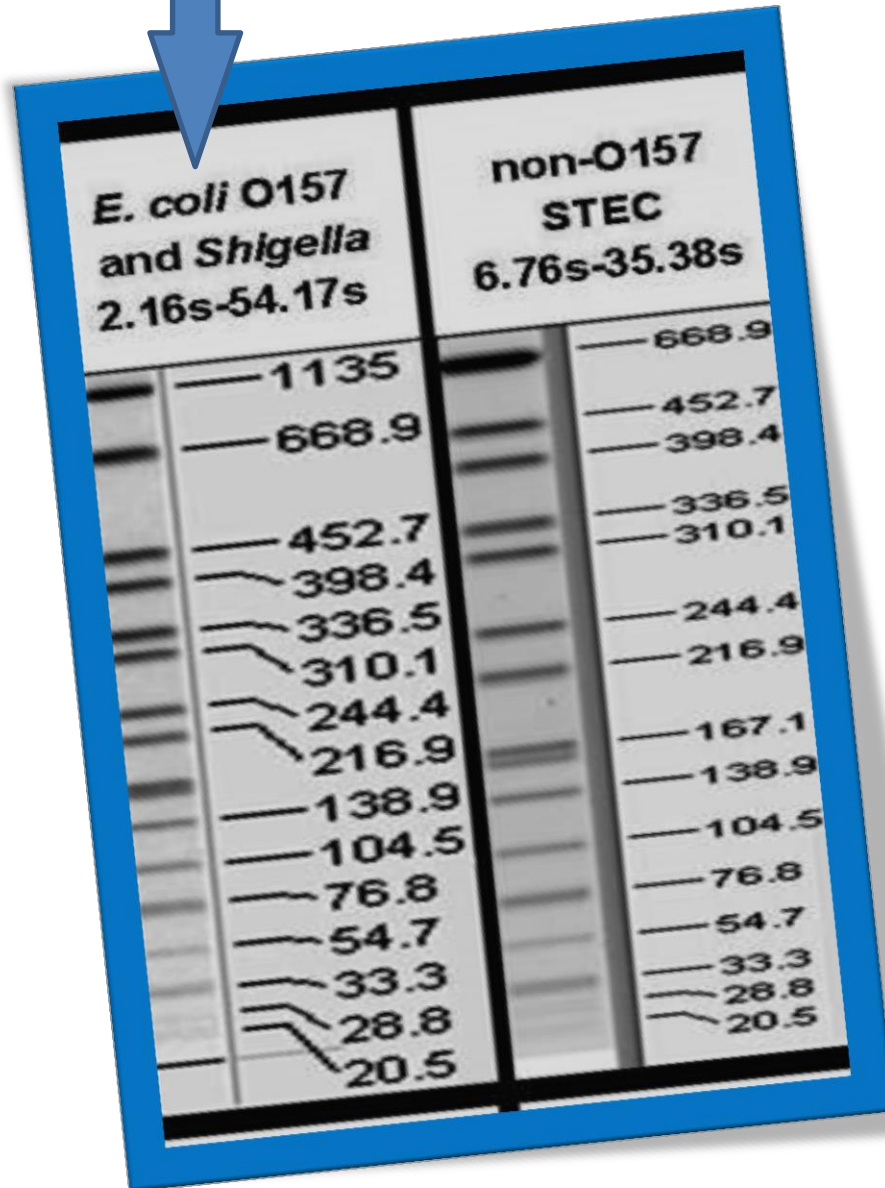
**2. POINT THE BANDS AND ADD THE EXTERNAL REFERENCE POSITION FOR ASSIGNING THE MOLECULAR WEIGHT TO THE BANDS**

Molecular weight (in KB) must be inserted for each band of the *S. Braenderup H9812* (The Universal Molecular Weight Marker)

**NOTE: CHOOSE THE MOST SUITABLE STANDARD LANE FOR CREATING THE REFERENCE SYSTEM**



## THE REFERENCE BANDS POSITIONS



Band Number	Molecular weight (Kb)
1	1135
2	668,9
3	452,7
4	398,4
5	336,5
6	310,1
7	244,4
8	216,9
9	138,9
10	104,5
11	76,8
12	54,7
13	33,3
14	28,8
15	20,5

M  
A  
N  
D  
A  
T  
O  
R  
Y

# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

## Step 4: ASSIGNING THE REFERENCE BANDS POSITIONS - NORMALIZATION

The screenshot displays the 'Normalization' window of a PFGE analysis software. The main area shows a gel image with 10 lanes, numbered 1 to 10. A vertical scale on the left indicates band positions from 0 to 400. A dialog box titled 'Reference position' is overlaid on the image, with a text field containing '1135' and 'OK' and 'Cancel' buttons. A yellow vertical bar on the left side of the gel image indicates 'No active reference system defined'. At the bottom, a blue callout box contains the text 'Enter the molecular weight for each band'. The software interface includes a menu bar (File, Edit, References, Normalization) and a toolbar with various icons. The status bar at the bottom shows 'Fingerprint type: PFGE\_Xbal | TIFF: 760 x 574 x 8 (x1.00)'.

Enter the molecular weight for each band

# TIFF ANALYSIS AND PFGE PROFILE INTERPRETATION

## Step 4: ASSIGNING THE REFERENCES BAND POSITIONS - NORMALIZATION

The screenshot displays the 'Fingerprint data of DPCF0709gelpfge12ago2011\_RUN36' software interface. The 'Reference system' panel on the left shows a list of reference band positions (1135, 668.9, 452.7, 398.4, 336.5, 310.1, 244.4, 216.9, 138.9, 104.5, 76.8, 54.7, 33.3) and a corresponding gel image with multiple lanes. A black oval highlights the reference band positions list. The 'Auto assign reference bands' dialog box is open, showing the 'Search method' section with 'Using bands' selected. The 'Keep existing assignments' checkbox is unchecked. The 'Alignment settings...' button is visible. A text box with a magnifying glass icon instructs the user to click 'Auto assign reference positions' to assign bands in the other standard lanes.

**Click "Auto assign reference positions" to assign bands in the other standard lanes**

**Auto assign reference bands**

Search method

- ☒ Using bands
- ☐ Using densitometric curve (requires standard)

☐ Keep existing assignments

Alignment settings...

OK

Cancel

# TIFF ANALYSIS AND PFGE PROFILE INTERPRETATION

## Step 4: ASSIGNING THE REFERENCES BAND POSITIONS - NORMALIZATION



# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

## Step 4: NORMALIZATION





# TIFF ANALYSIS AND PFGE PROFILE INTERPRETATION

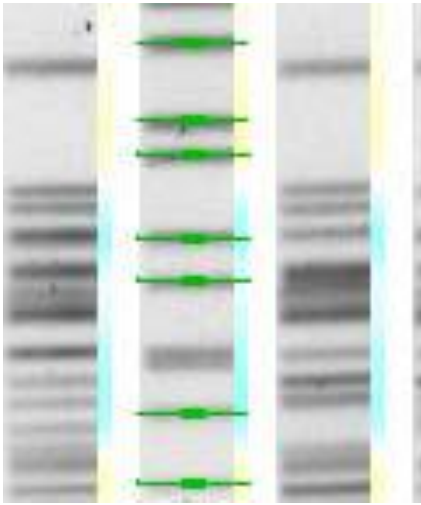
## Step 4: NORMALIZATION

The screenshot displays the 'Fingerprint data of DPCF0709gelpfge12ago2011\_RUN36' window. The 'Normalization' menu is open, showing options such as 'Assign reference position', 'Delete assignment', 'Delete all assignments', 'Delete assignments (current lane)', 'Delete assignments (current position)', 'Auto assign...', 'Show normalized view', 'Update normalization', and 'Show distortion bars'. The 'Show distortion bars' option is highlighted. A callout box points to this option with the text: **Click "Show distortion bars" to check normalization**.

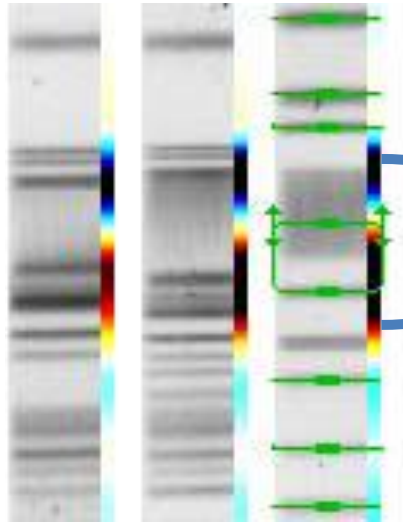
The inset image shows the results after proper normalization, displaying a gel profile with distortion bars. The lanes are numbered 1 through 10. The distortion bars are shown as vertical lines with green markers indicating the normalized positions. A callout box points to the results with the text: **Results after proper normalization**.

# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

## Step 4: NORMALIZATION



Light colors  
indicate good  
normalization



Dark colors, especially in one  
part of the gel indicate poor  
normalization

Band assigned to wrong  
reference position

# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

## Step 4: NORMALIZATION





# TIFF ANALYSIS AND PFGE PROFILE INTERPRETATION

- Analyze a TIFF

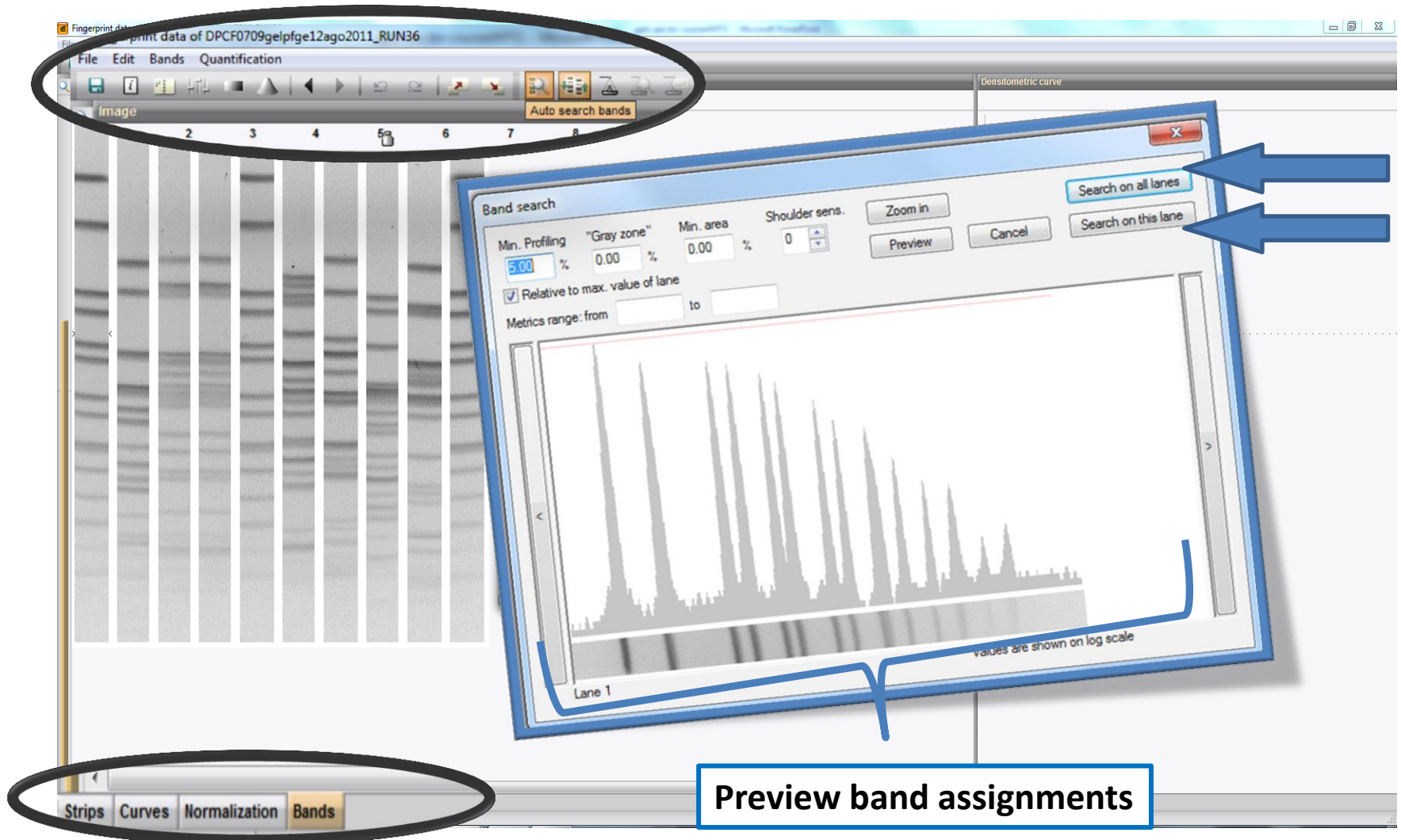
1. Convert a TIFF to Gel Strips
2. Define Curves
3. Normalize the Gel

## 4. Find Gel Bands

- Link Lanes to Database Entries
- Add Text Data for Isolates

# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

## Step 4: BAND ASSIGNMENT



# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

## Step 4: BAND ASSIGNMENT

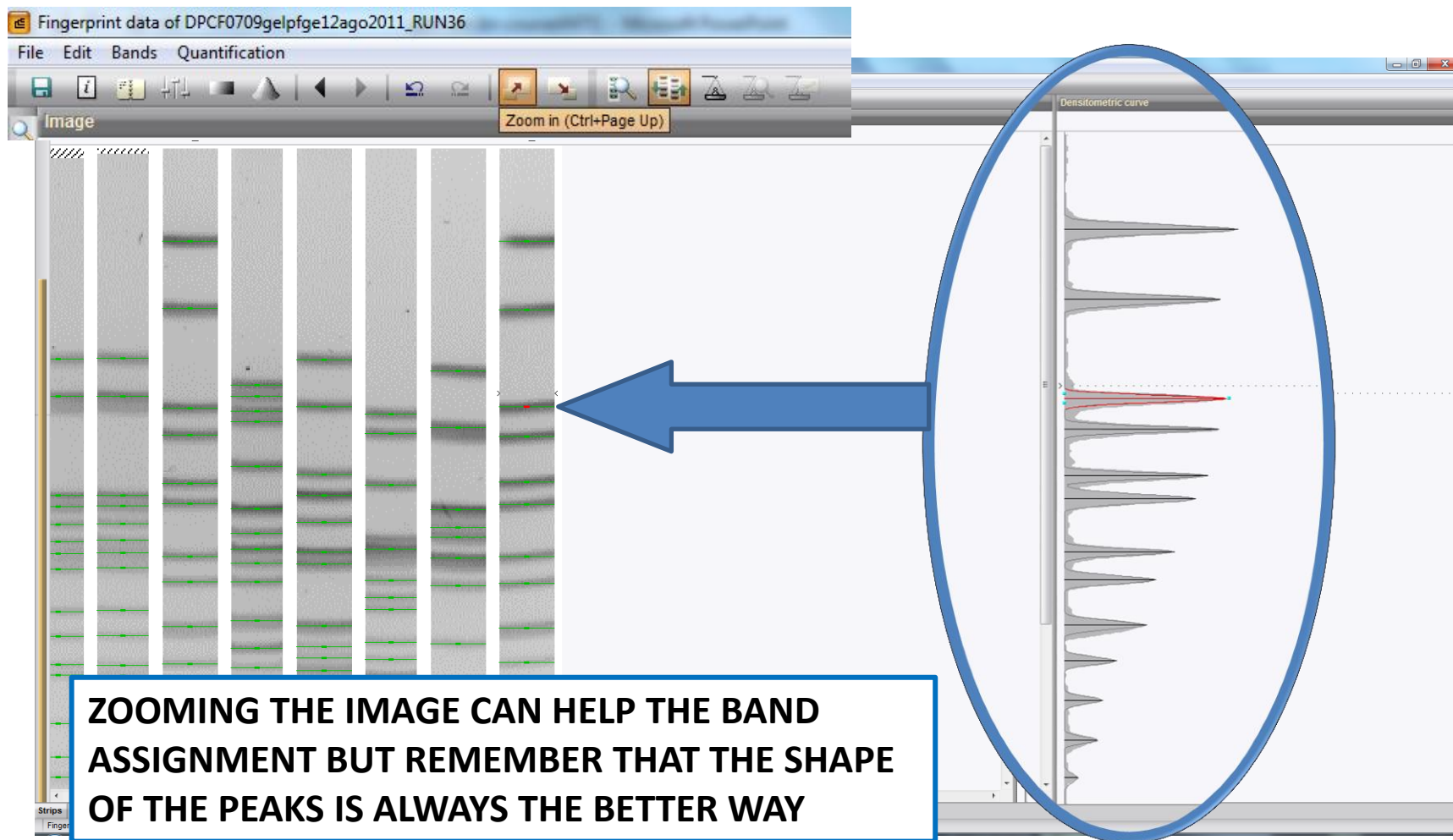


**VERIFY ALWAYS THE BAND ASSIGNMENT  
CONSIDERING THE SHAPE OF THE PICKS  
IN DENSITOMETRIC CURVES**

**SOME BANDS SHOULD BE DELETED MAUALLY:  
TO DELETE A BAND ASSIGNMENT, SELECT IT AND THEN PRESS DELETE**

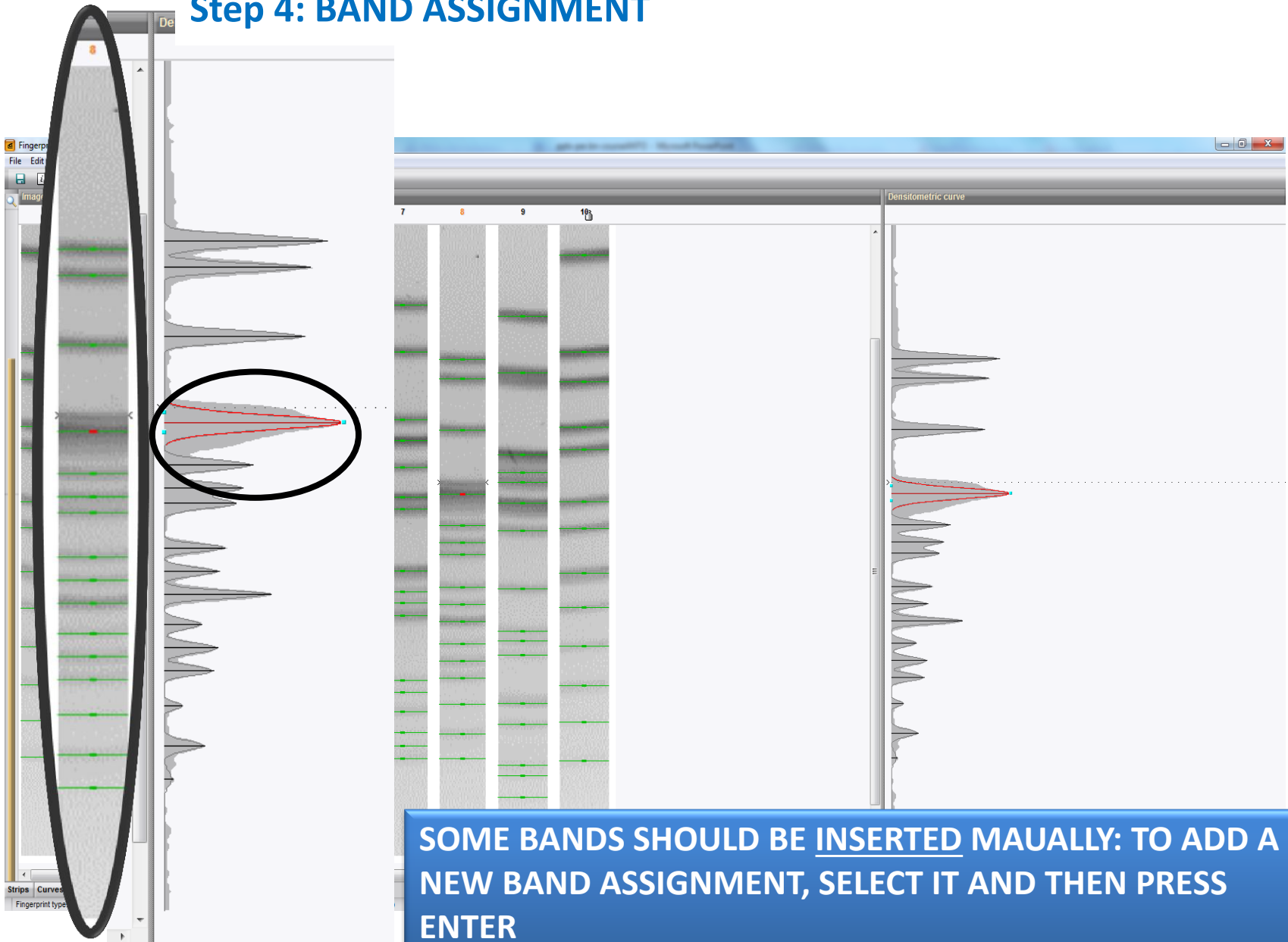
# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

## Step 4: BAND ASSIGNMENT



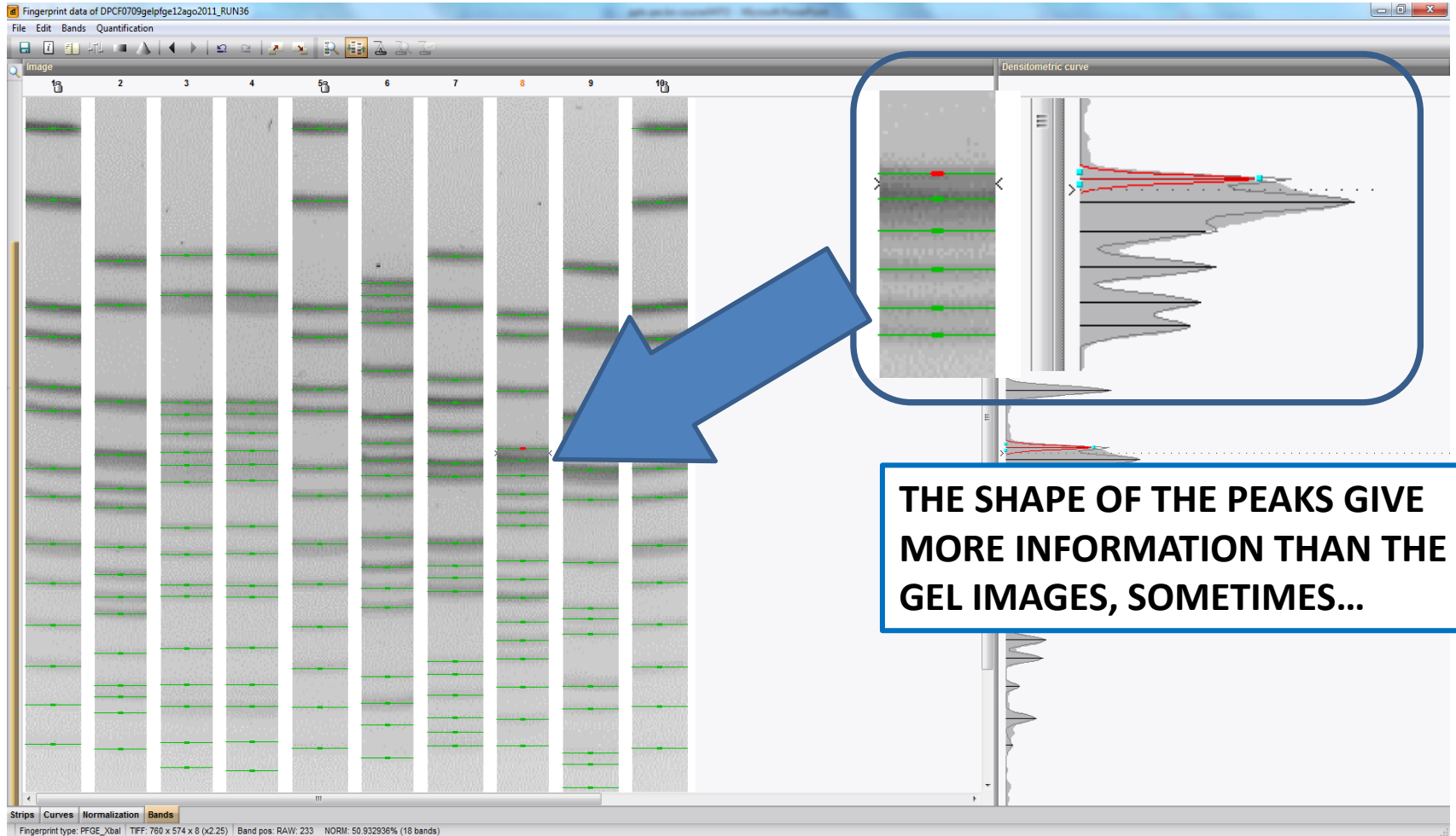
# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

## Step 4: BAND ASSIGNMENT



# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

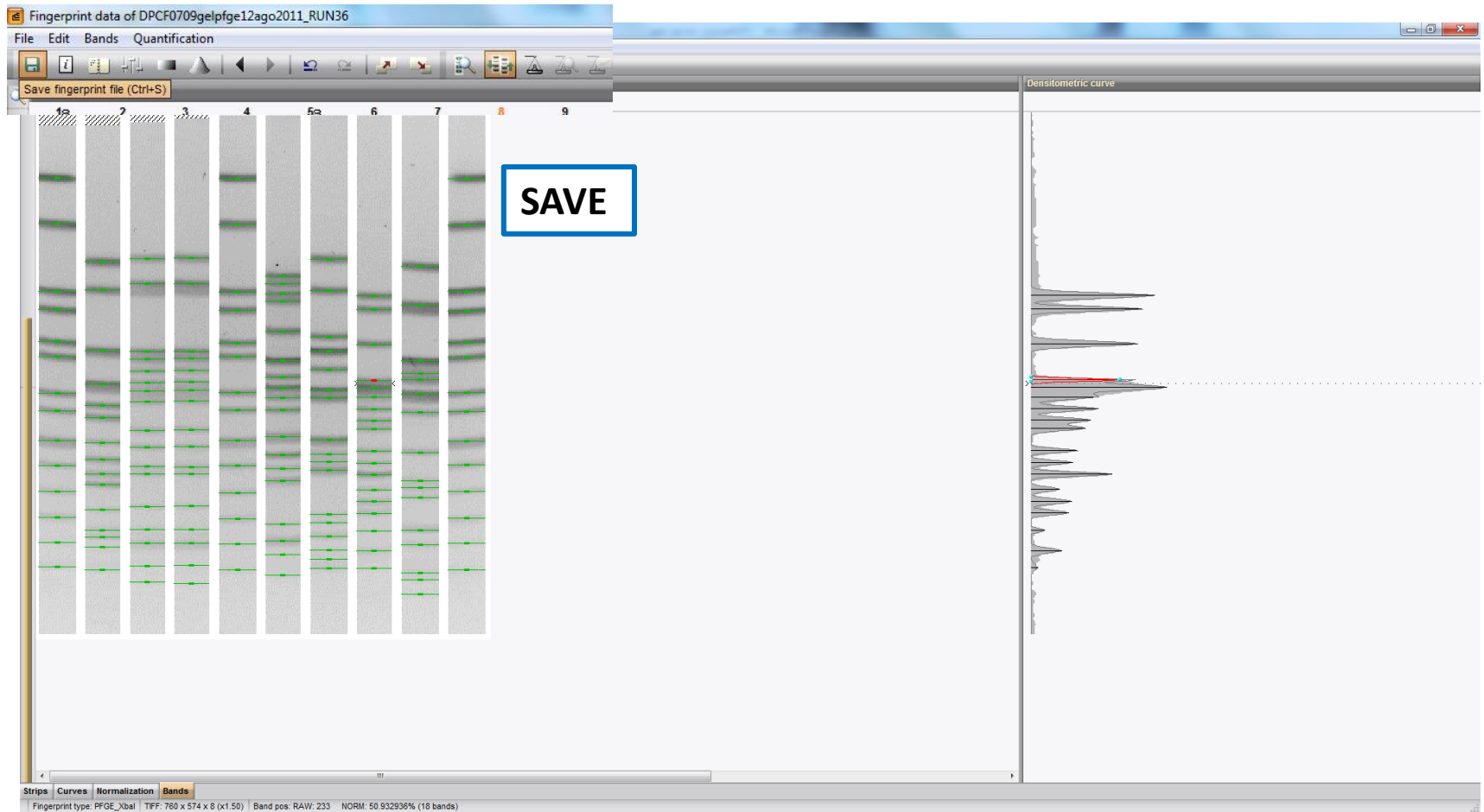
## Step 4: BAND ASSIGNMENT





# IN DEPTH OF THE EXPERIMENT: TIFF ANALYSIS AND PFGE PROFILING INTERPETATION

## Step 4: Setting up the experiment – BAND ASSIGNMENT



## TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

### Step 4: BAND ASSIGNMENT

#### INTERNAL CONTROL

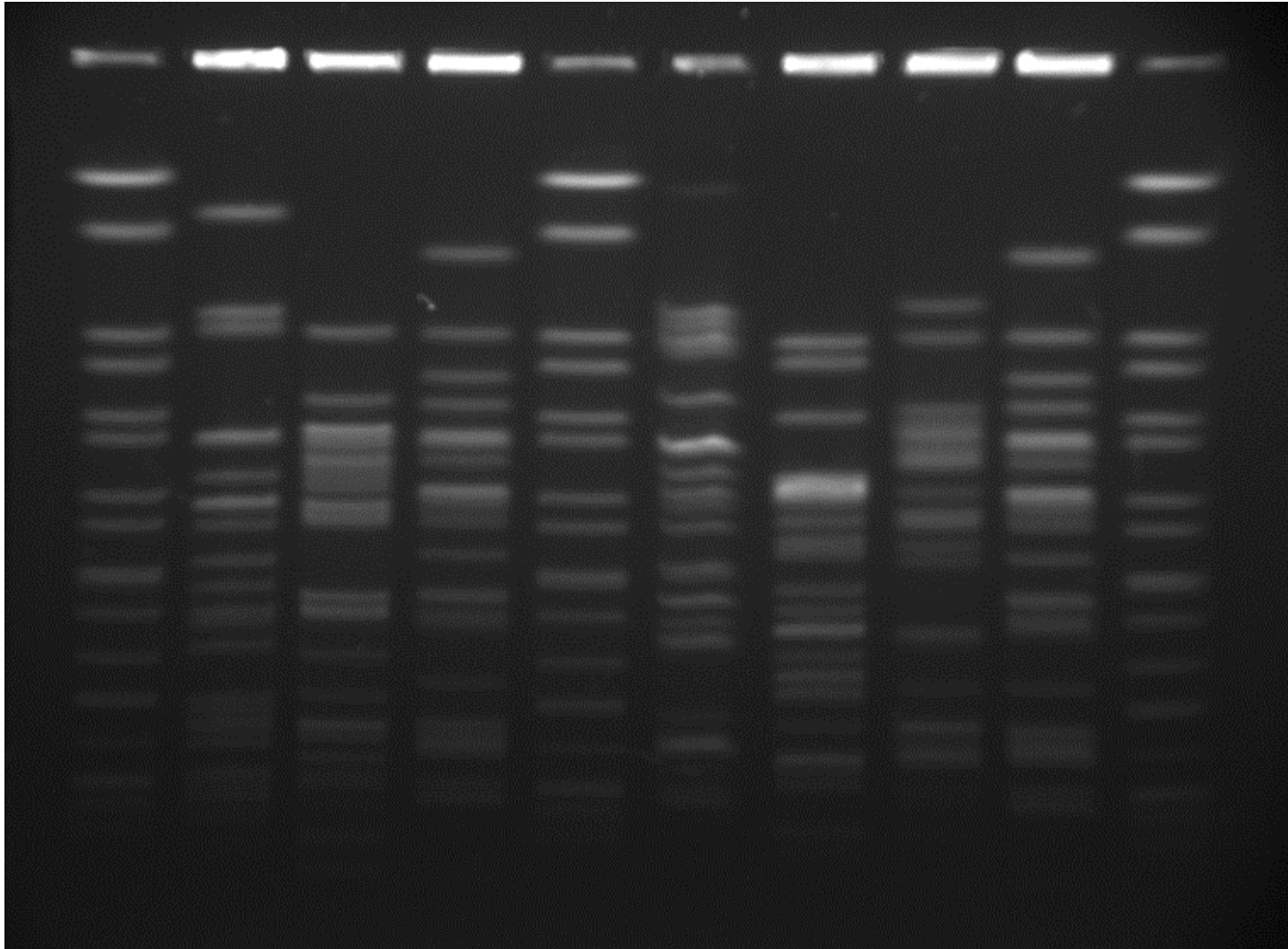
- In each gel at the extreme right (just before the last Braenderup)
- The bands of the IC should be well visible and the intensity of the band peaks should not be at the background level
- In order to define the acceptance of the PFGE profiles in a gel image, an analysis of the bands in the *Xba*I restriction of the IC that fall in the range of the bands 2 to 5 ( 668.9 Kb to 336.5 Kb) of the *S. Braenderup* standard is performed.

**ANALYSABLE PFGE PROFILES DISPLAY ALL THE BANDS IN THE INDICATED INTERVAL CLEARLY VISIBLE. THEREFORE, ANY PROFILE, WHICH DOES NOT HAVE THE BANDS CLEARLY VISIBLE IN THIS INTERVAL, SHOULD BE REJECTED.**

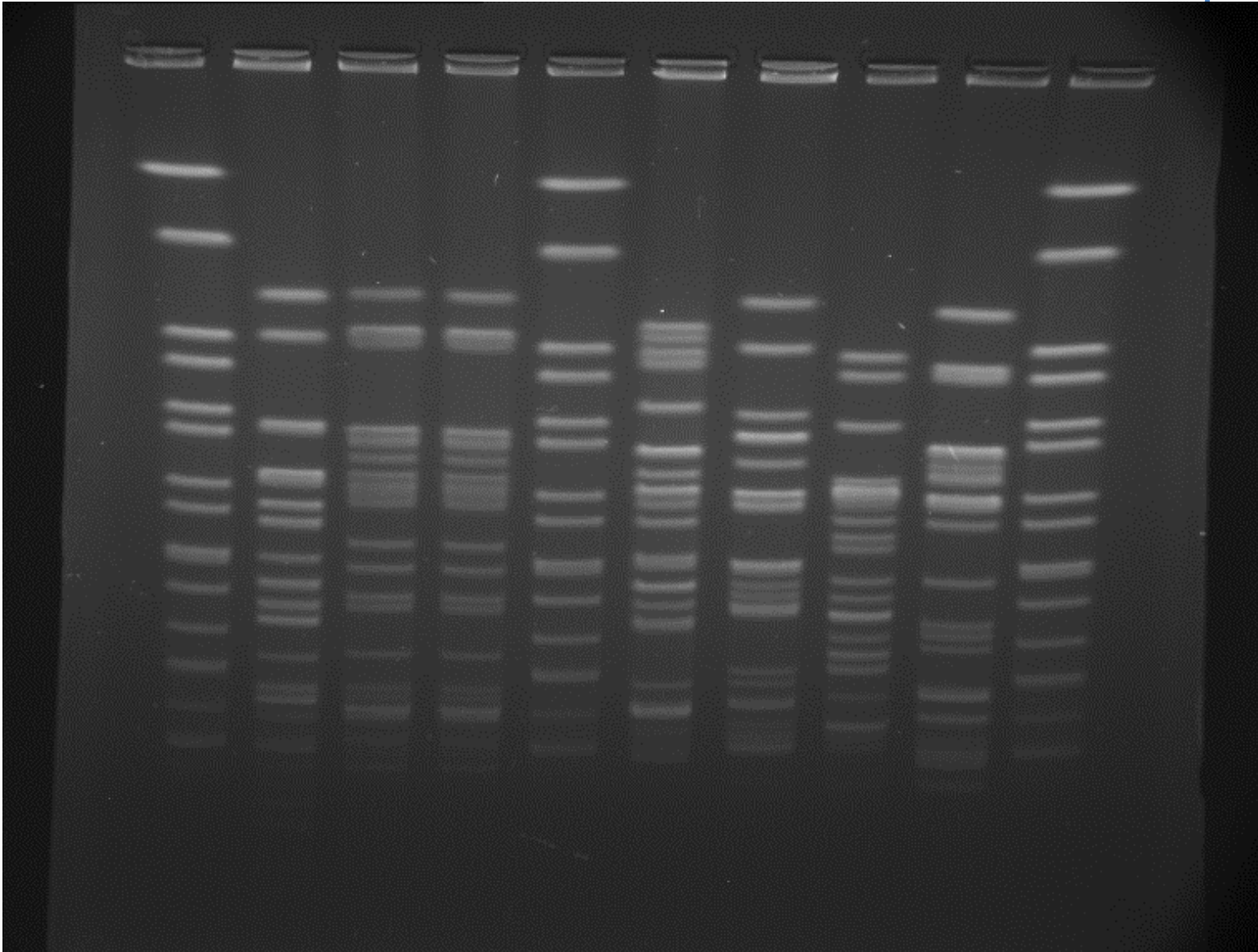
**EACH BAND SHOULD BE EASILY ASSIGNED BY PLACING THE CORRECT MARKER IN THE DENSITOMETRIC CURVE**



# IS THIS GEL SWITABLE?

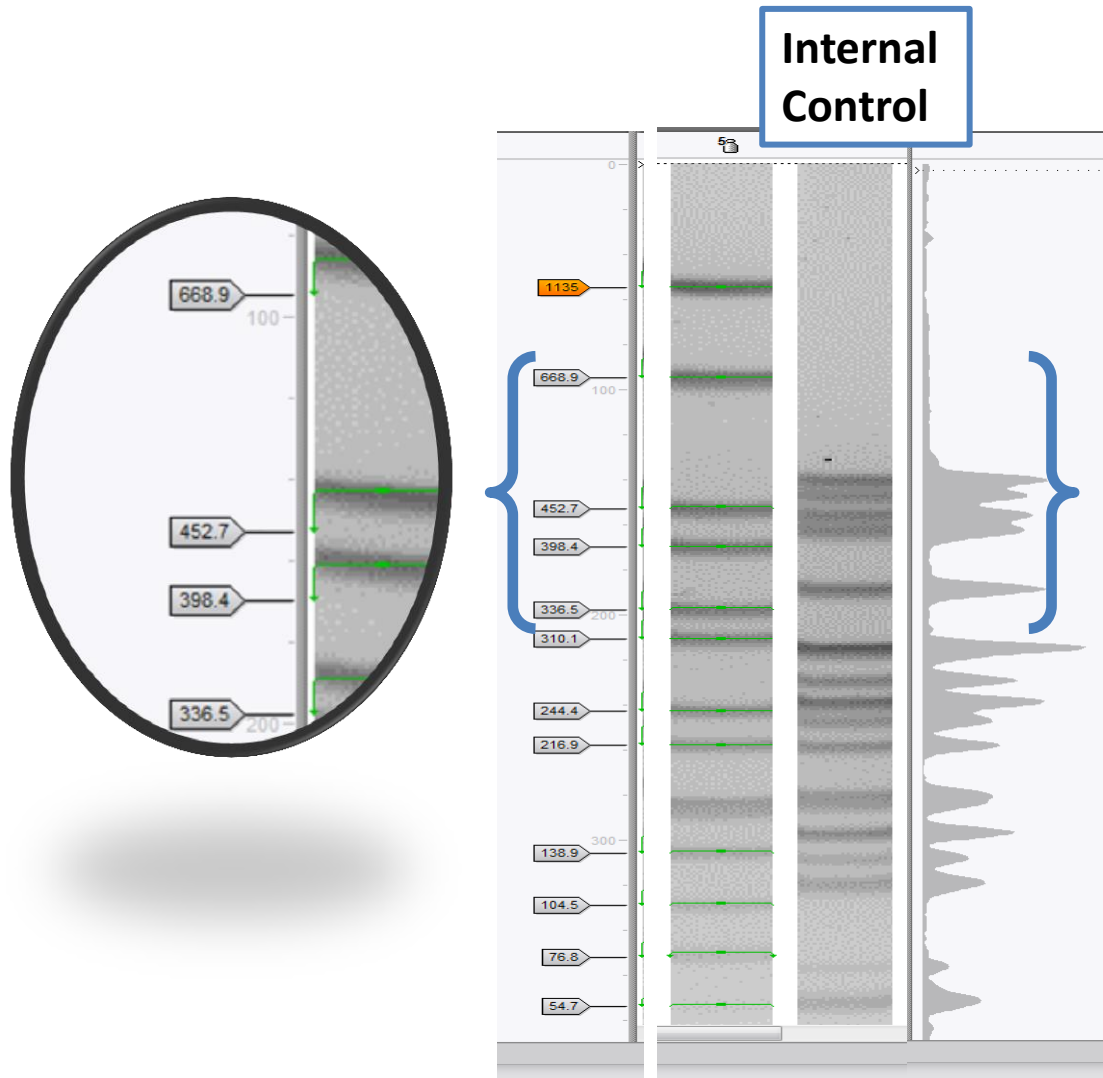


# AND THIS ONE?



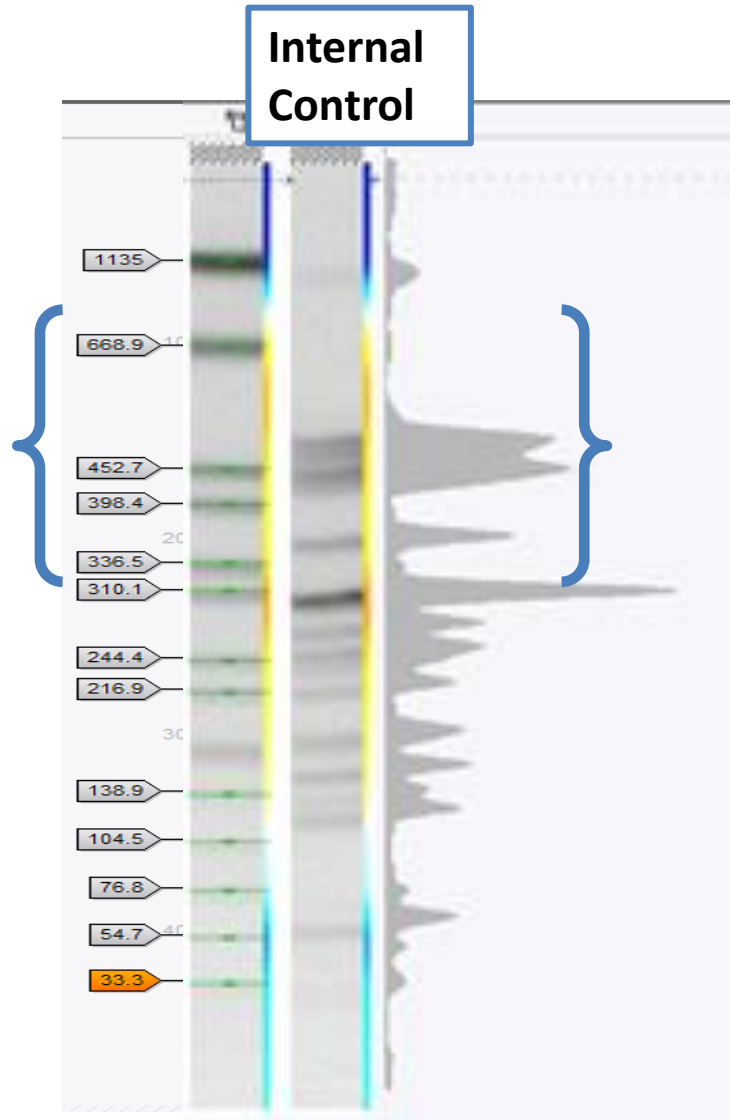
# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

## Step 4: BAND ASSIGNMENT



# IN DEPTH OF THE EXPERIMENT: TIFF ANALYSIS AND PFGE PROFILING INTERPRETATION

## Step 4: Setting up the experiment – BAND ASSIGNMENT



# TIFF ANALYSIS AND PFGE PROFILE INTERPRETATION

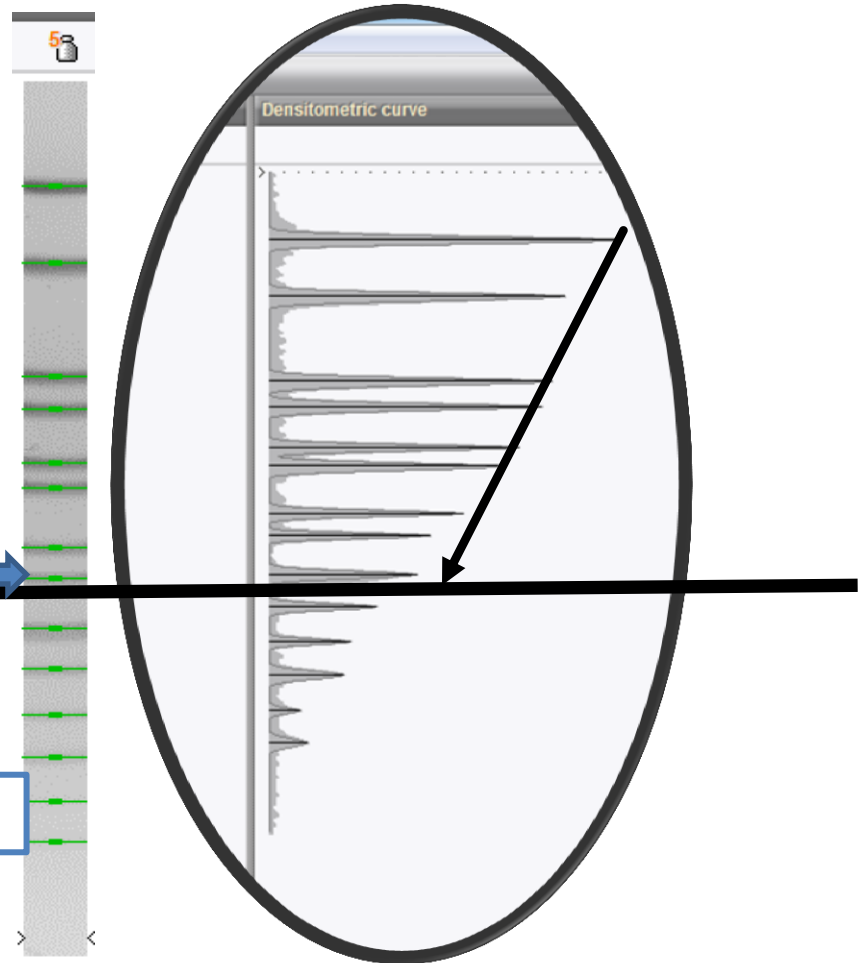
## Step 4: BAND ASSIGNMENT - Validation of doubtful bands

DISCENDING ORDER OF BAND INTENSITY IN THE UPPER PART OF THE GEL SHOULD BE OBSERVED

UPPER PART

216,9 KB

LOWER PART

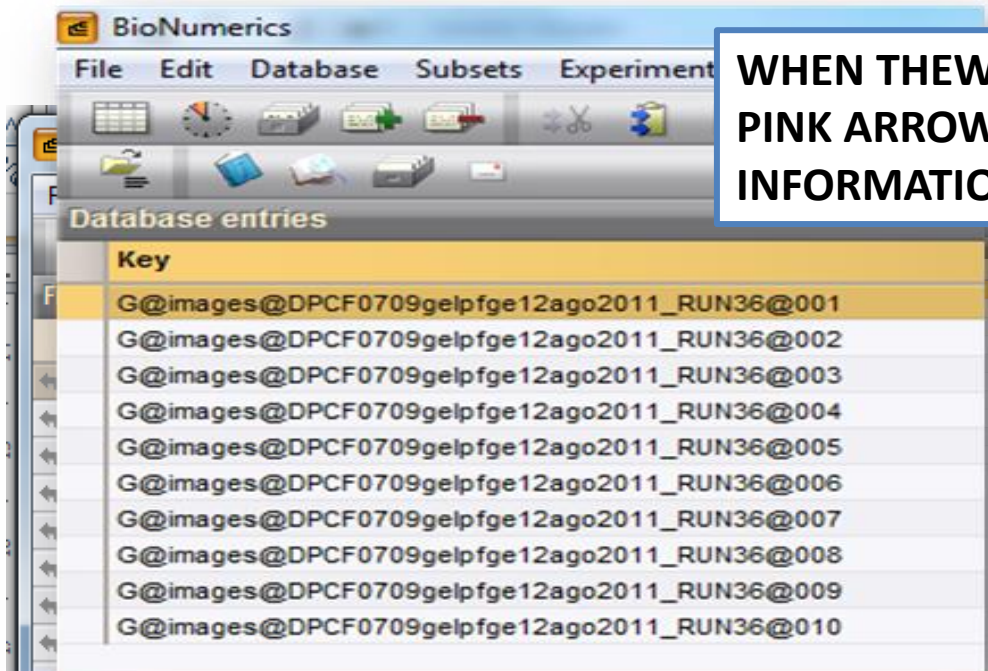


## TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

- Analyze a TIFF
  1. Convert a TIFF to Gel Strips
  2. Define Curves
  3. Normalize the Gel
  4. Find Gel Bands
- Link Lanes to Database Entries
- Add Text Data for Isolates

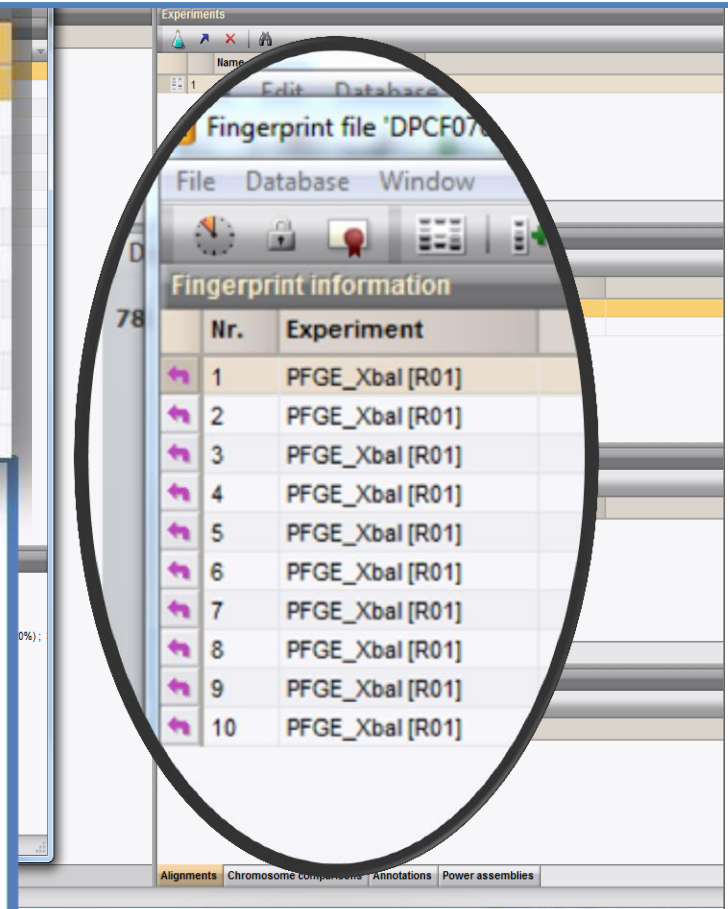


# Step 5: LINK LANES TO DATABASE ENTRIES



WHEN THE LANES ARE ADDED TO DATABASE A PINK ARROW WILL APPEAR IN THE FINGERPRINT INFORMATION WINDOW

**KEY ENTRY WILL BE  
AUTOMATICALLY  
CREATED IN THE  
DATABASE ENTRIES  
FOLDER  
(G@images@name of the  
TIFF@number of lane)**



# Step 5: SETTING UP THE MOLECULAR STANDARD

1. DOUBLE CLICK IN THE EXPERIMENT FOLDER

Database entries

Key
G@images@OPCF07...
G@images@OPCF07...
G@images@OPCF07...
G@images@OPCF07...
G@images@OPCF07...
G@images@OPCF07...
G@images@OPCF07...
G@images@OPCF07...
G@images@OPCF07...

References system

Key
G@images@OPCF07...
G@images@OPCF07...
G@images@OPCF07...
G@images@OPCF07...
G@images@OPCF07...
G@images@OPCF07...
G@images@OPCF07...
G@images@OPCF07...
G@images@OPCF07...

Experiments

Name	Type
1 PFGE_Xbal	Fingerprint types

Experiments Entry relations

Files

Name	Created	Modified	Location
DPCF0709gepfpe12a...	2014-06-06 13:38:27	2014-06-10 12:58:18	Shared DB
DPCF0812_RUN54	2014-06-06 13:39:05	2014-06-10 11:51:15	Shared DB

Comparisons

Name	Created	Modified	Location
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2. REFERENCES SYSTEM IS EMPTY BUT THE MOLECULAR WEIGHT ARE PRESENT

# Step 5: SETTING UP THE UNIVERSAL MOLECULAR STANDARD

The screenshot shows the BioNumerics software interface. On the left, the 'Database entries' list contains multiple entries with the key 'G@images@DPCF07...'. The main window displays the 'Reference systems' tab, which shows a gel image with bands and their corresponding molecular weights and percentages. A blue arrow points to the 'Standard' entry in the 'Active zones' section.

**Reference systems**

Molecular Weight (kDa)	Percentage (%)
1135	12.09%
668.9	20.88%
452.7	33.63%
398.4	37.36%
336.5	43.52%
310.1	46.37%
244.4	53.41%
216.9	56.70%
138.9	67.25%
104.5	72.31%
76.8	77.36%
54.7	82.20%
33.3	87.03%

**Reference systems** **Band classes**

**Settings**

Fingerprint type 'PFGE\_XbaI'

Standard: G@images@DPCF0709gelpfge12ago2011\_RUN36@005 Resolution: 600 pts

Active zones (on G@images@DPCF0709gelpfge12ago2011\_RUN36@001)

Zo... %~100.0%]

**Comparison settings**

PFGE... (fingerprint type nr. 1)

**3. DRAG THE ARROW ON THE SELECTED LANE**

# TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

- Analyze a TIFF

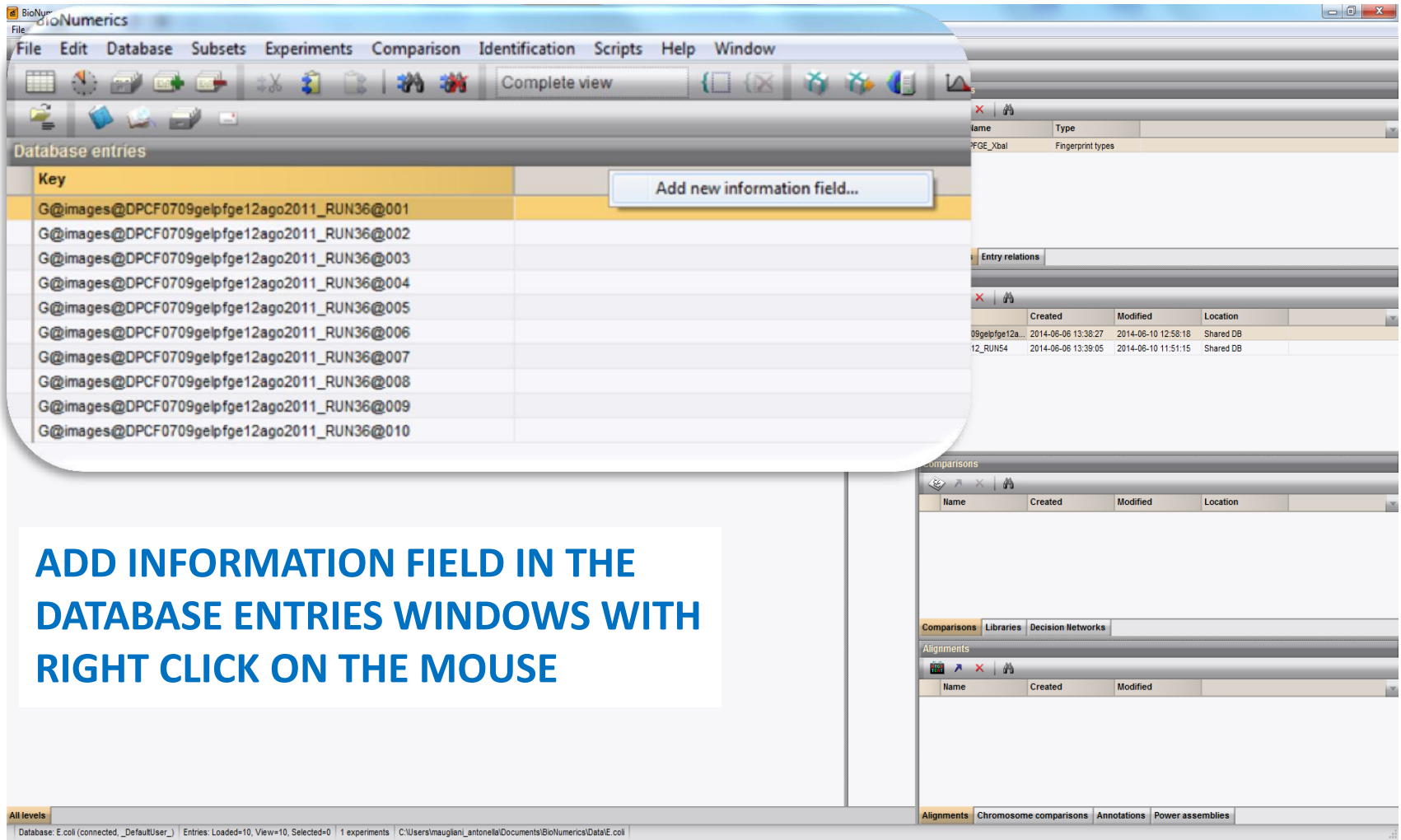
1. Convert a TIFF to Gel Strips
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- Link Lanes to Database Entries

- Add Text Data for Isolates

# IN DEPTH OF THE EXPERIMENT: TIFF ANALYSIS AND PFGE PROFILING INTERPRETATION

## Step 6: Add Text Data for Isolates

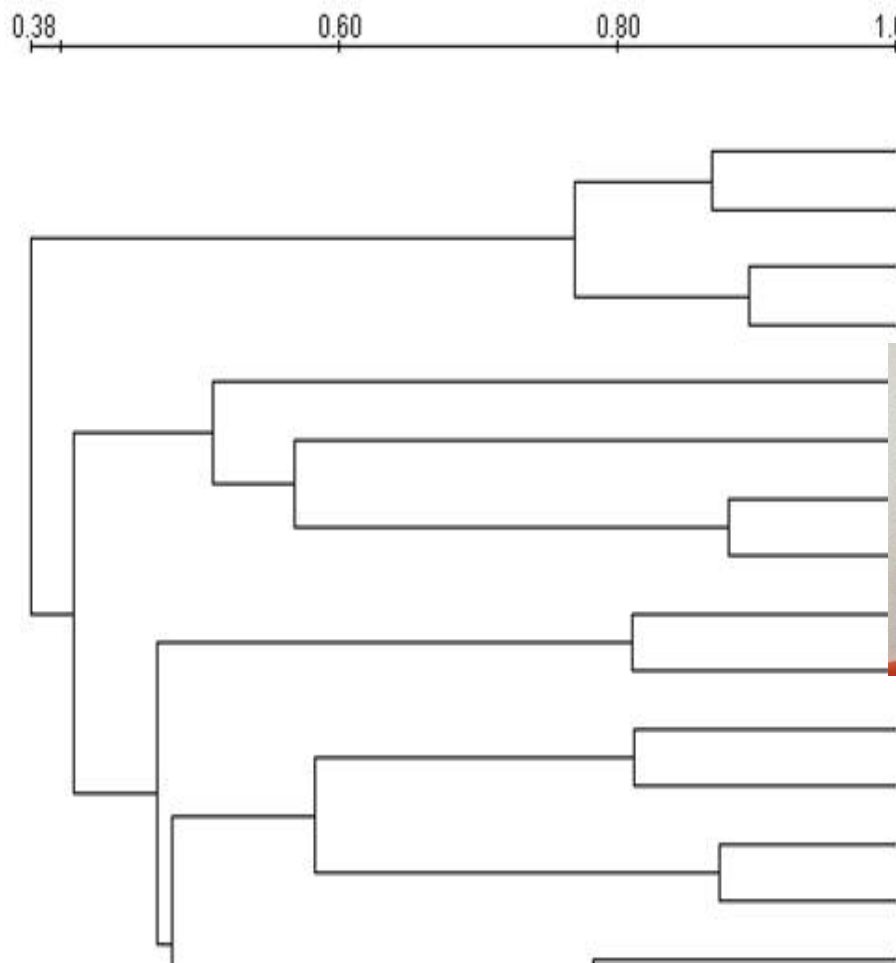


The screenshot displays the BioNumerics software interface. The 'Database entries' window is active, showing a list of 10 entries with keys starting from 'G@images@DPCF0709gelpfge12ago2011\_RUN36@001' to '@010'. A right-click context menu is open over the first entry, with the option 'Add new information field...' highlighted. The background shows other panels like 'Entry relations', 'Comparisons', and 'Alignments'.

**ADD INFORMATION FIELD IN THE DATABASE ENTRIES WINDOWS WITH RIGHT CLICK ON THE MOUSE**



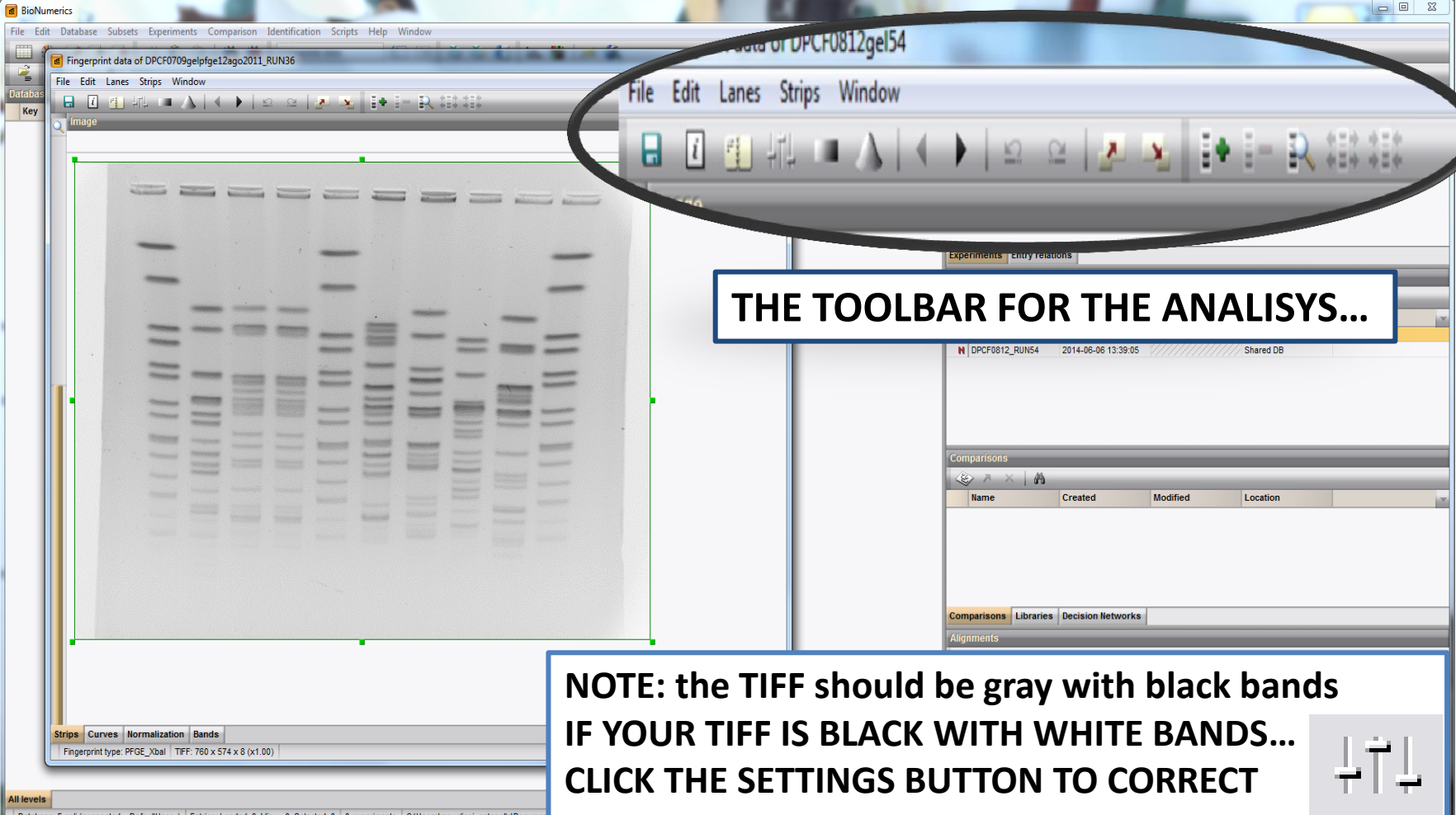
# LET'S START WITH CLUSTER ANALYSIS





# The BioNumerics Software: database creation, experiment type, import of TIFF files, and setting up experiments

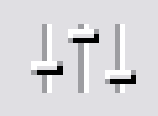
## Step 4: Verify



The screenshot displays the BioNumerics software interface. The main window shows a gel image with multiple lanes. A toolbar is highlighted with a black oval, containing various icons for file operations, navigation, and analysis. Below the gel image, there are tabs for 'Strips', 'Curves', 'Normalization', and 'Bands'. The 'Strips' tab is currently selected. The status bar at the bottom indicates the database is 'E.coli (connected, \_DefaultUser\_)', with 'Entries: Loaded=0, View=0, Selected=0' and '0 experiments'.

**THE TOOLBAR FOR THE ANALYSIS...**

**NOTE: the TIFF should be gray with black bands  
IF YOUR TIFF IS BLACK WITH WHITE BANDS...  
CLICK THE SETTINGS BUTTON TO CORRECT**



The settings icon is a small square button with a vertical double-headed arrow in the center, used to toggle the image from black/white to gray/black.

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## Step 4: Setting up the experiment

**Fingerprint conversion settings**

Raw data | Densitometric curves | Normalization | Bands

Data source

- ☒ 2D TIFF image
- ☐ Densitometric curves
- ☐ Bands tables

Image strip extraction

Thickness: 31 pts

Nodes: 3

☐ Background subtraction

60 pts

☐ Spot removal

4 pts

☐ Use bounding box curvature

Image coloring

☒ Inverted values

Background color:

— R

— G

— B

Foreground color:

— R

— G

— B

Color scale:

OD range 255

**AND CLICK OK**

**SELECT INVERTED VALUES IN ORDER TO HAVE BLACK BANDS**

**Experiments**

Name	Type
1 PFGE_Xbal	Fingerprint types

**Comparisons**

Name	Created	Modified	Location
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**Alignments**

Name	Created	Modified
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