IMAGE ANALYSIS: PROFILES ANALYSIS AND INTERPRETATION

Step 4: DEEP INTO THE ANALYSIS

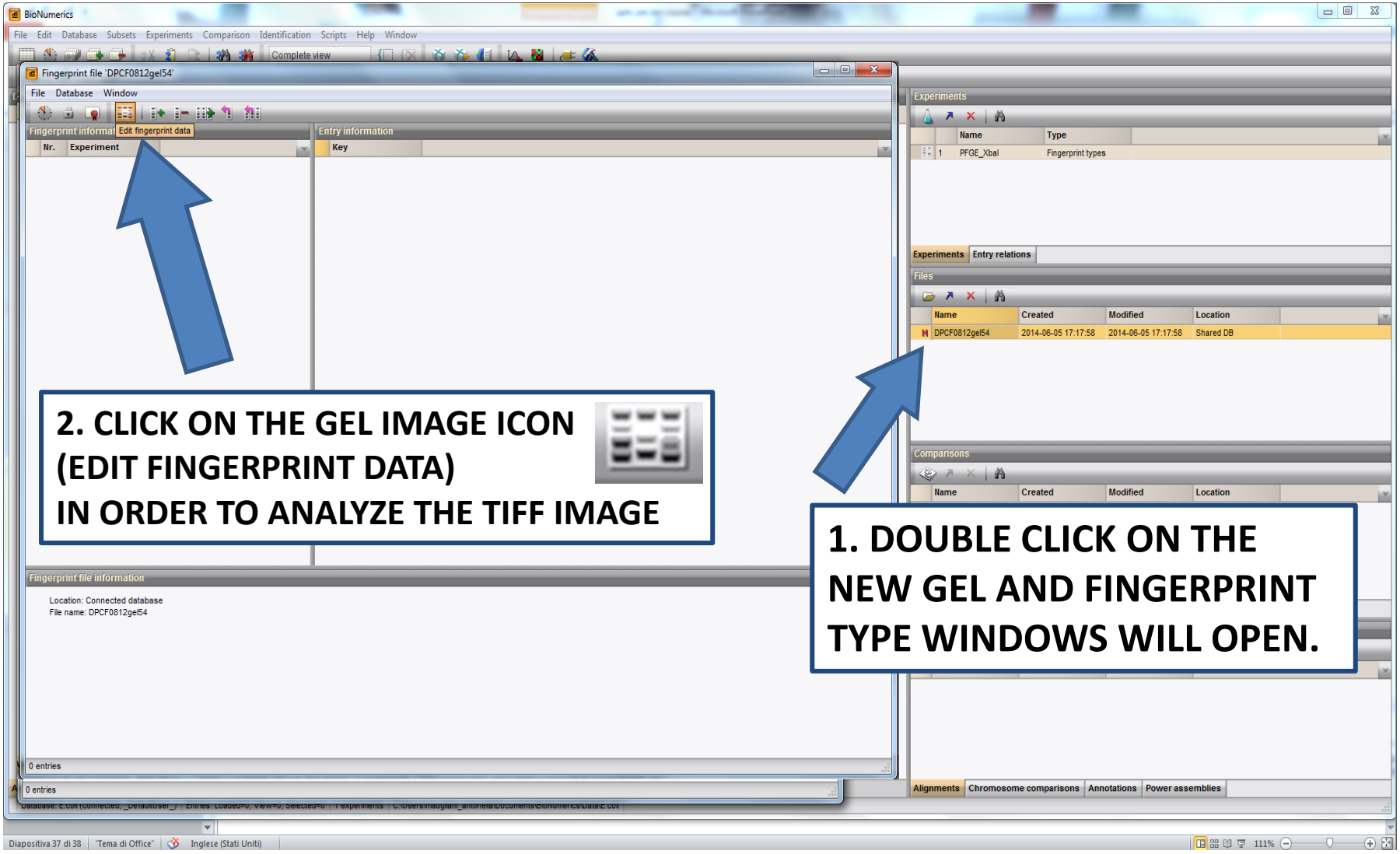
- **Analyze a TIFF**

1. **Convert a TIFF to Gel Strips**
2. Define Curves
3. Normalize the Gel
4. Find Gel Bands

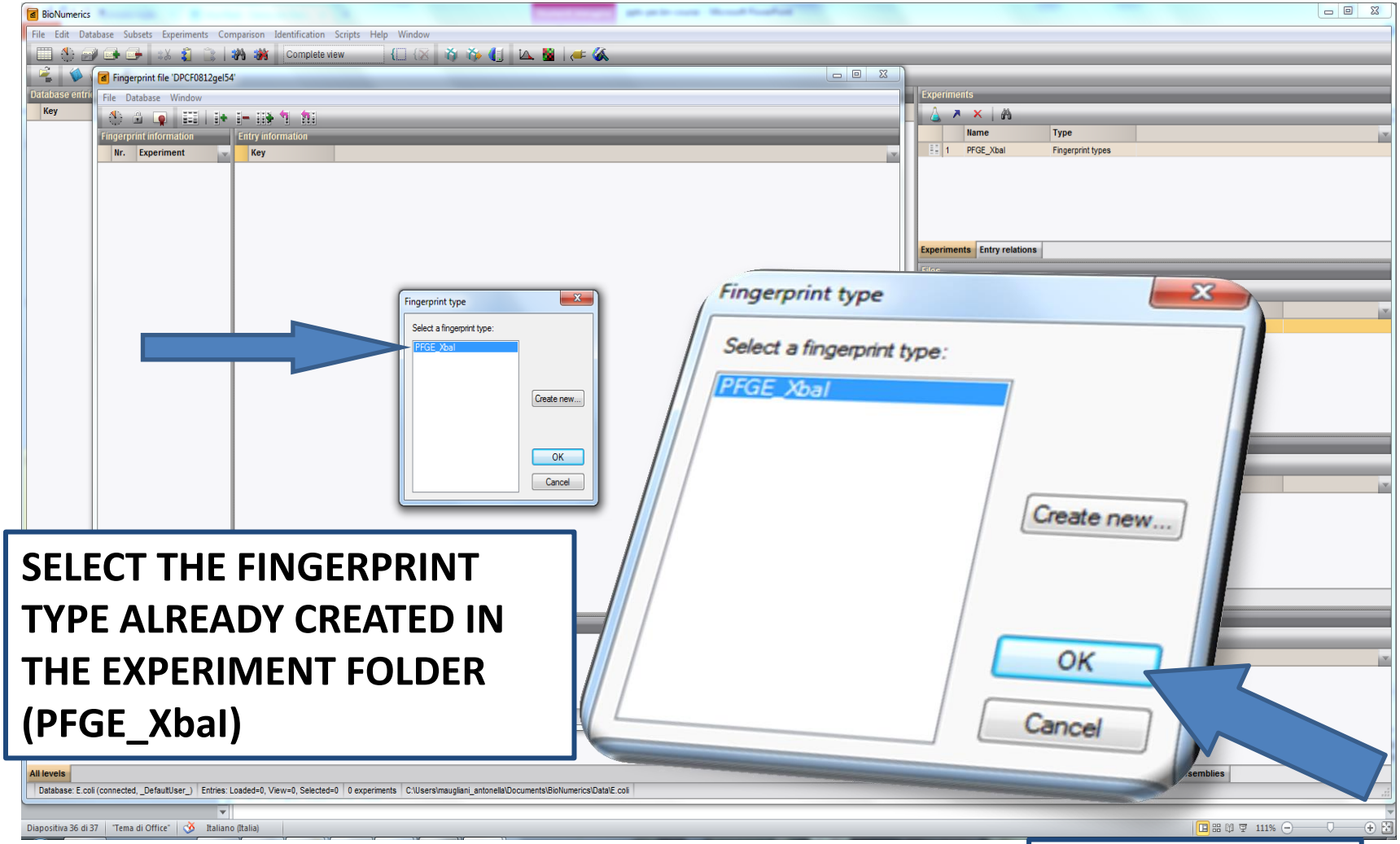
- **Link Lanes to Database Entries**
- **Add Text Data for Isolates**

Image analysis: profiles analysis and interpretation

Step 4: ANALYZE A TIFF - LET'S START



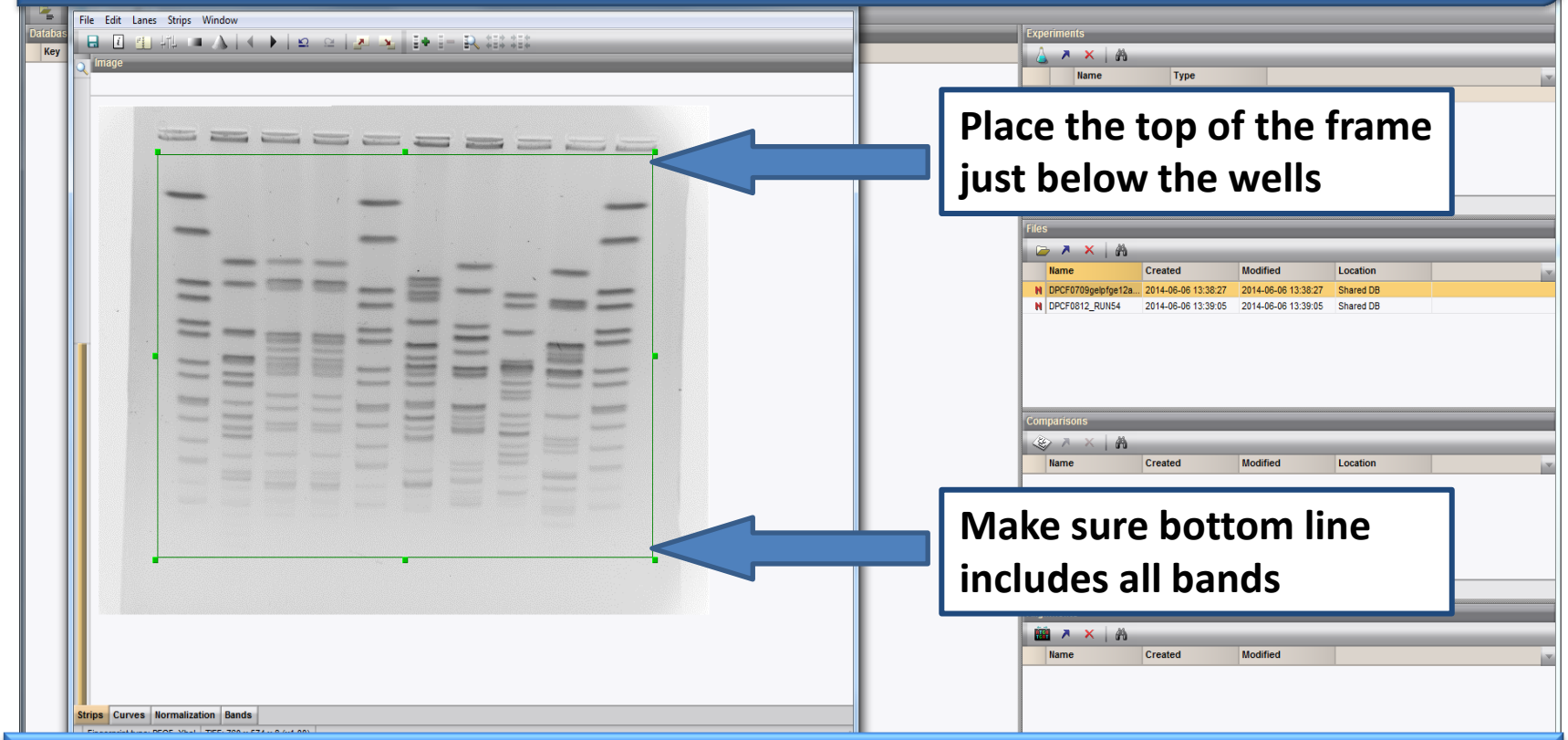
Step 4: Select the experiment created



TIFF ANALISYS AND PFGE PROFILE INTERPRETATION

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

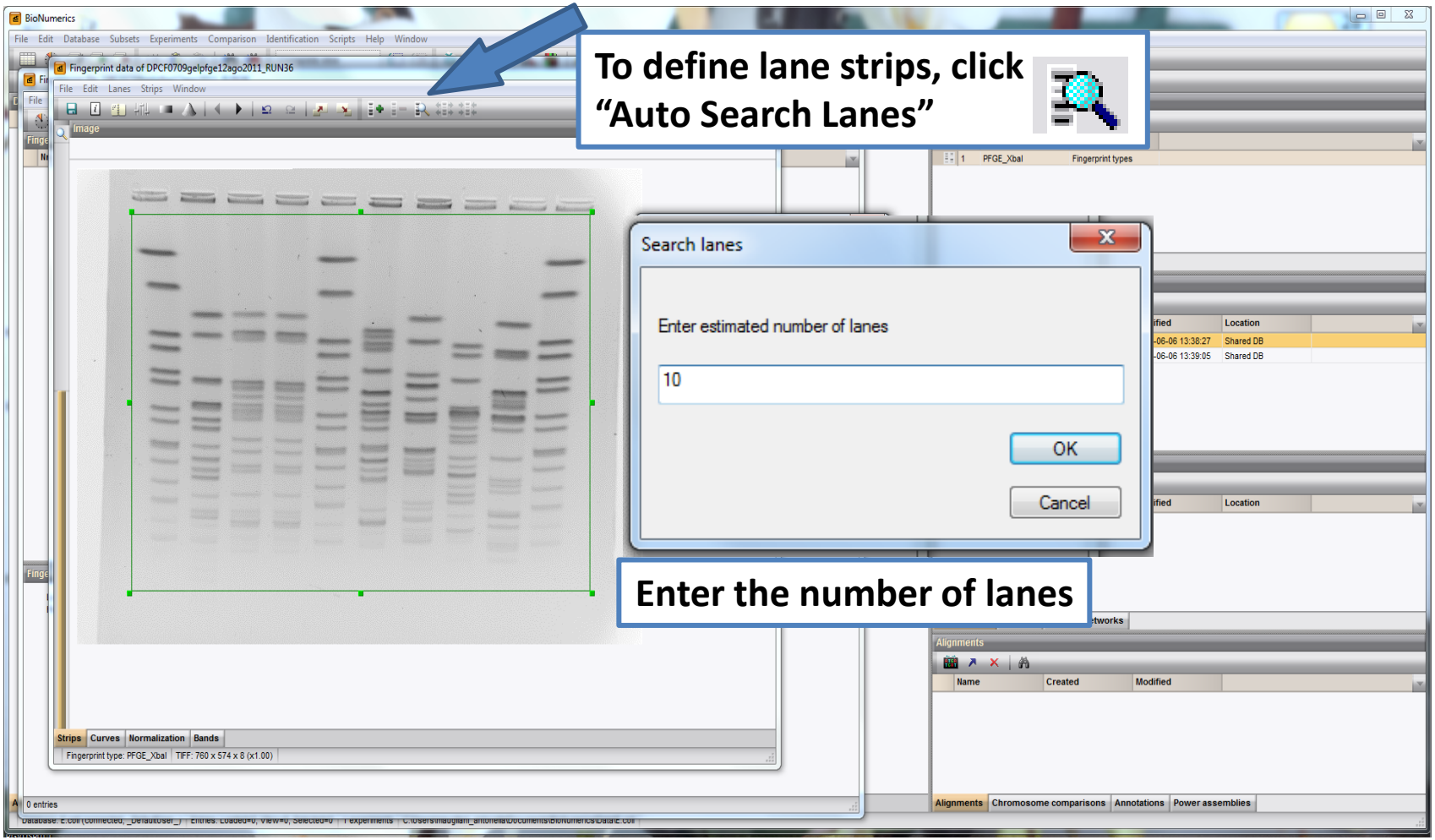
NOTE: IF THE WELLS ARE NOT INCLUDED IN THE TIFF THE GEL CANNOT BE ANALYZED!



normalization process will not be done correctly otherwise!

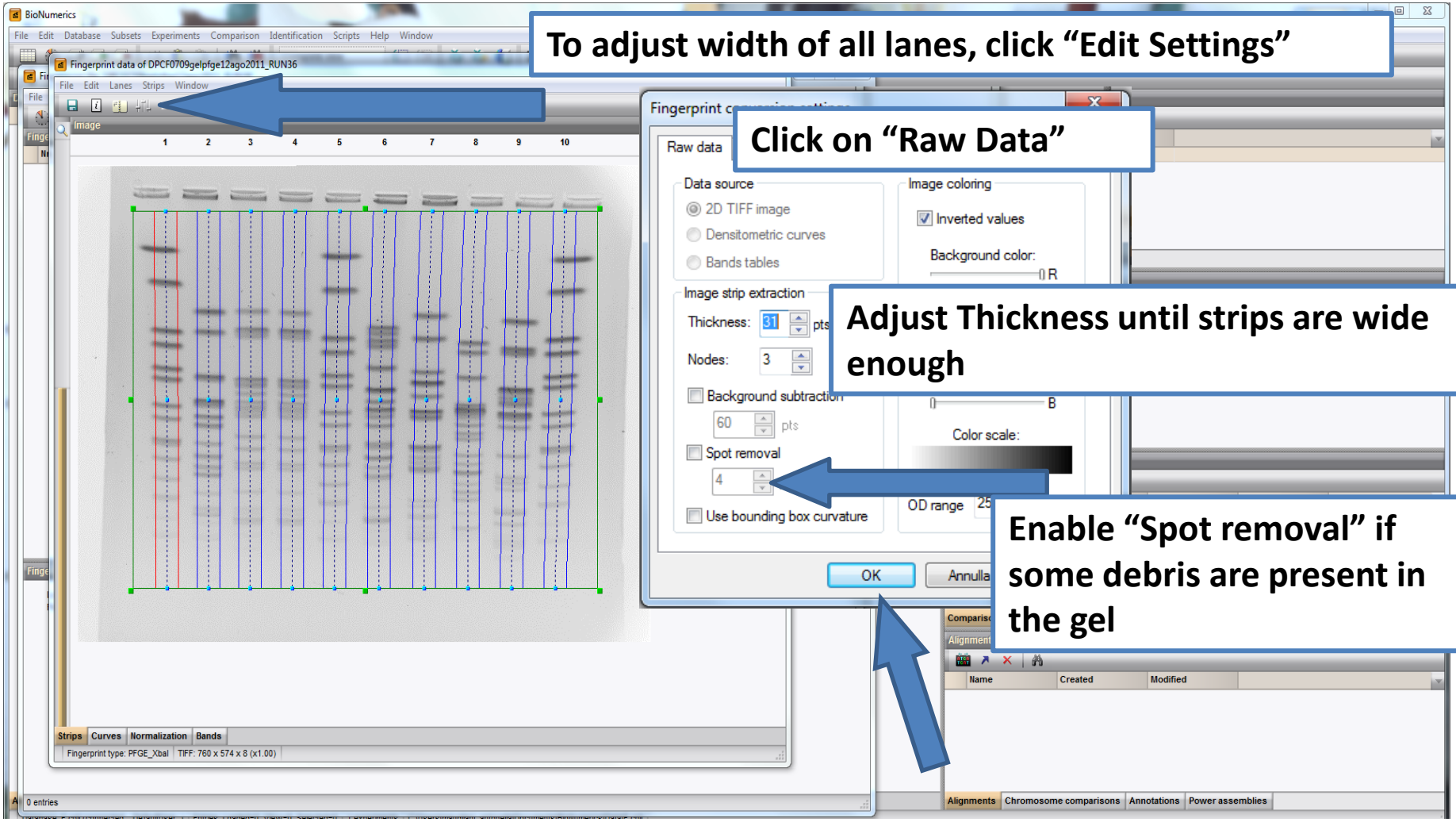
TIFF ANALISYS AND PFGE PROFILE INTERPRETATION

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



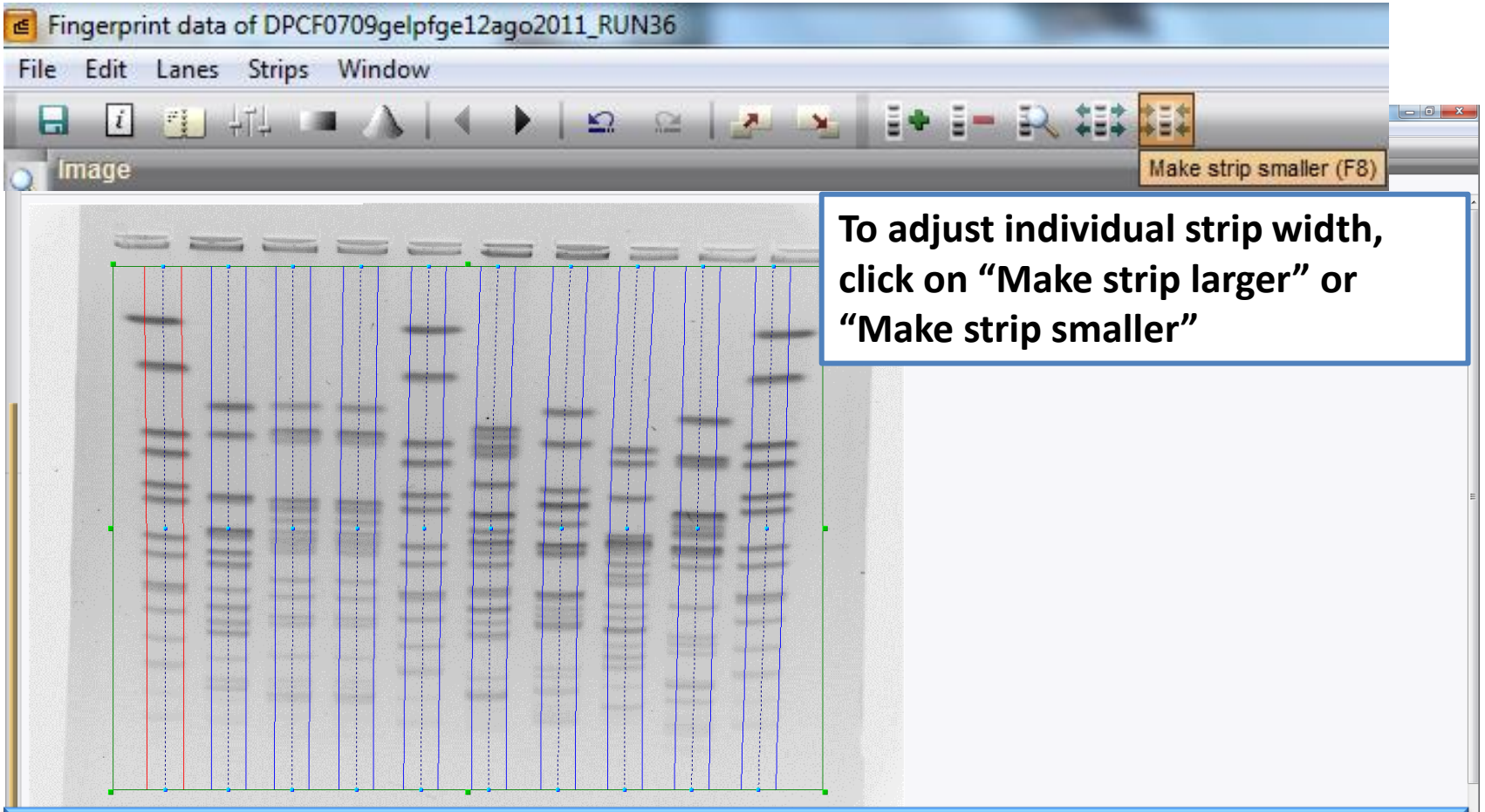
TIFF ANALISYS AND PFGE PROFILE INTERPRETATION

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



TIFF ANALISYS AND PFGE PROFILE INTERPRETATION

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



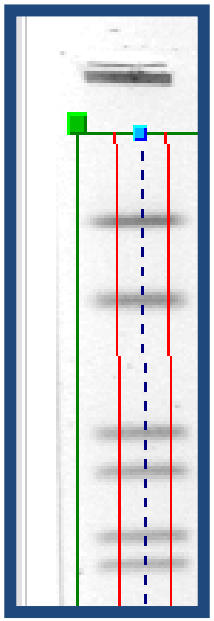
To adjust individual strip width, click on “Make strip larger” or “Make strip smaller”

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

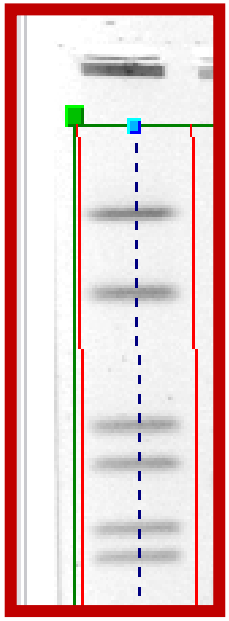
TIFF ANALISYS AND PFGE PROFILE INTERPRETATION

Step 4: CORRECT ASSIGNMENT OF STRIPS WIDTH

MAKE SURE THE STRIP DOES NOT:

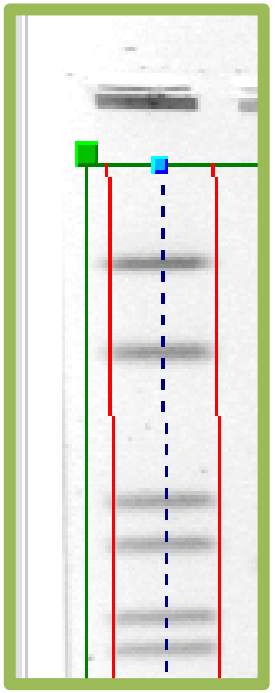


… cut off the edges of the lane



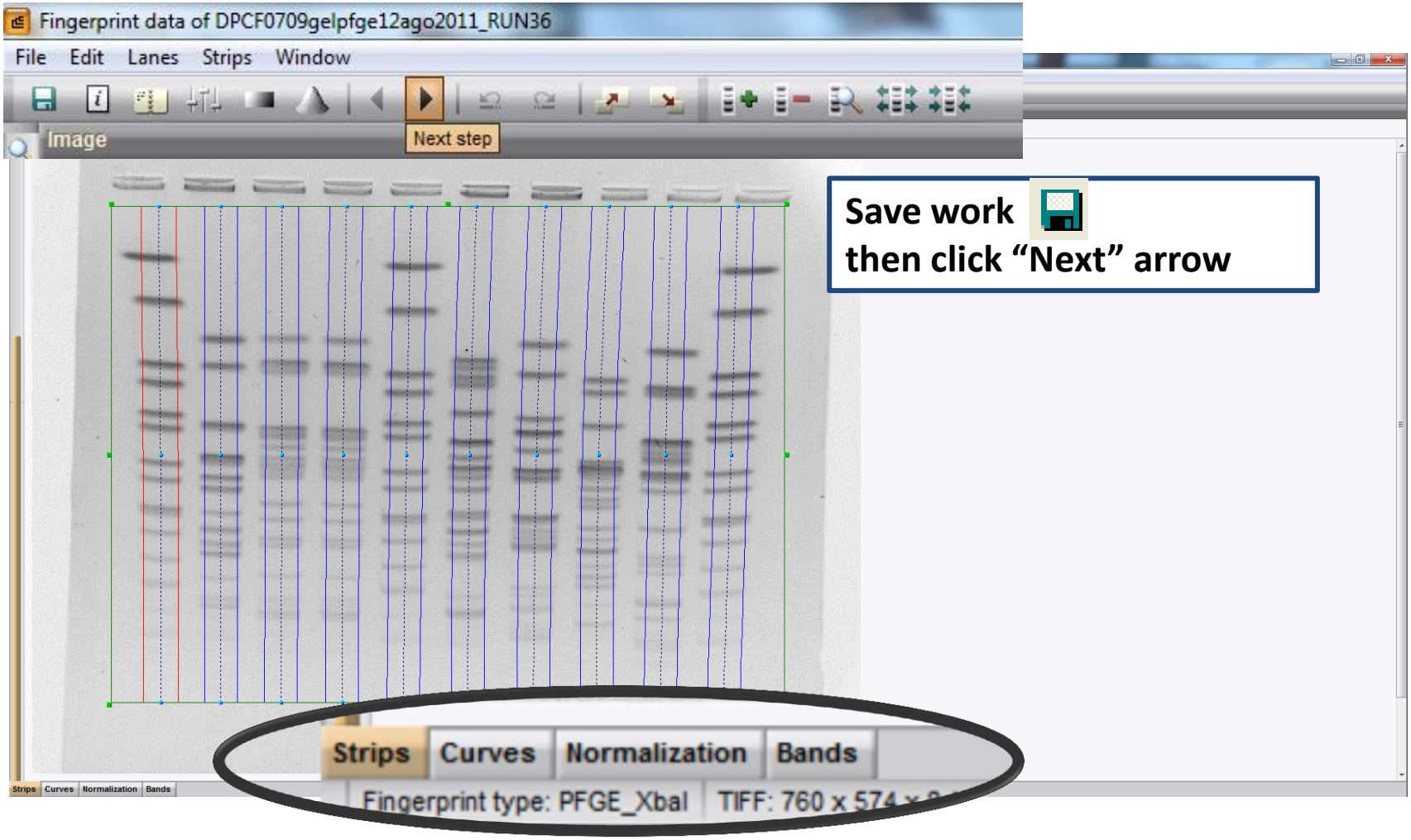
… include too much space

THE STRIP SHOULD INCLUDE ALL OF THE LANE



TIFF ANALISYS AND PFGE PROFILE INTERPRETATION

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



DEEP INTO THE ANALYSIS

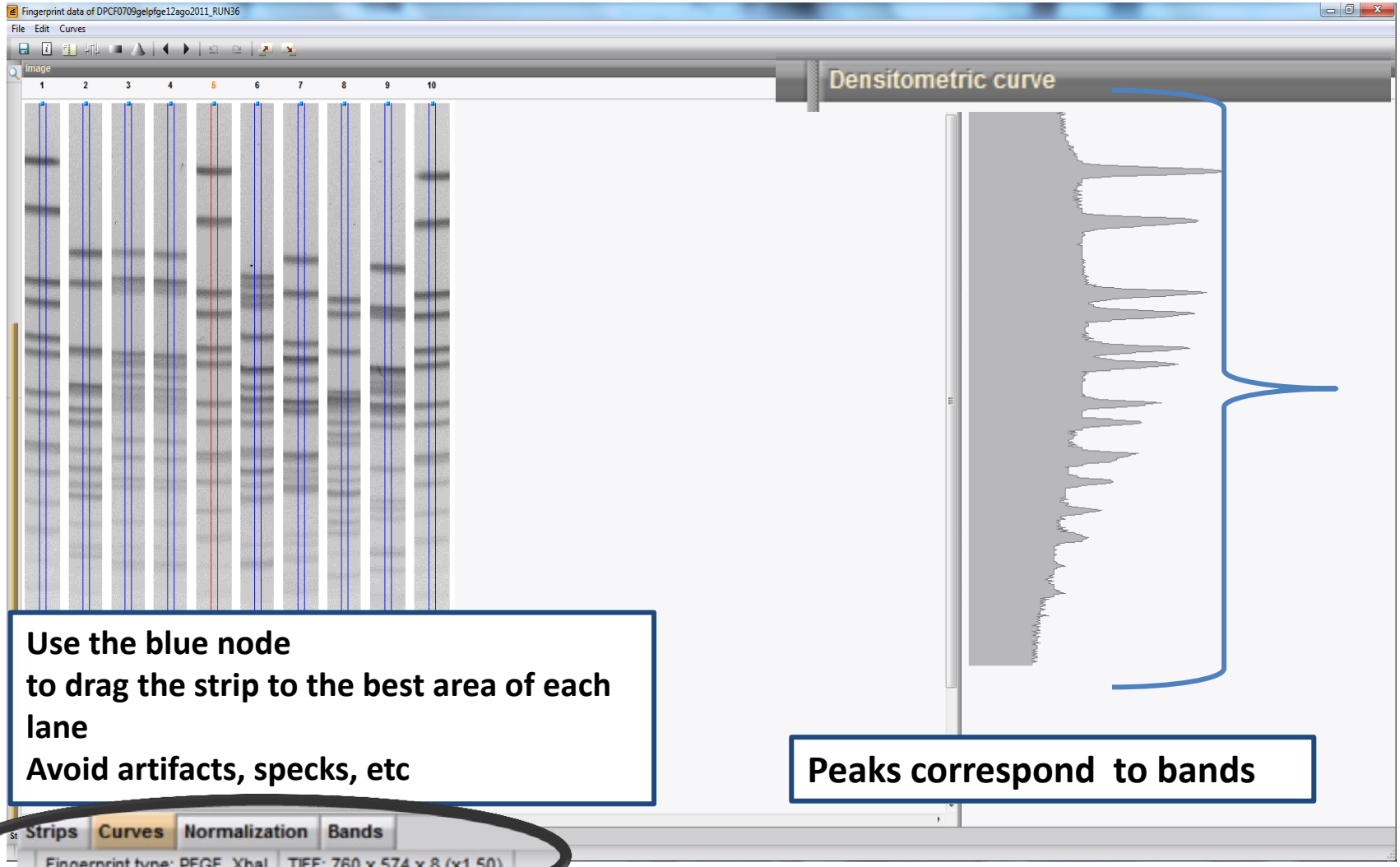
- **Analyze a TIFF**

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**

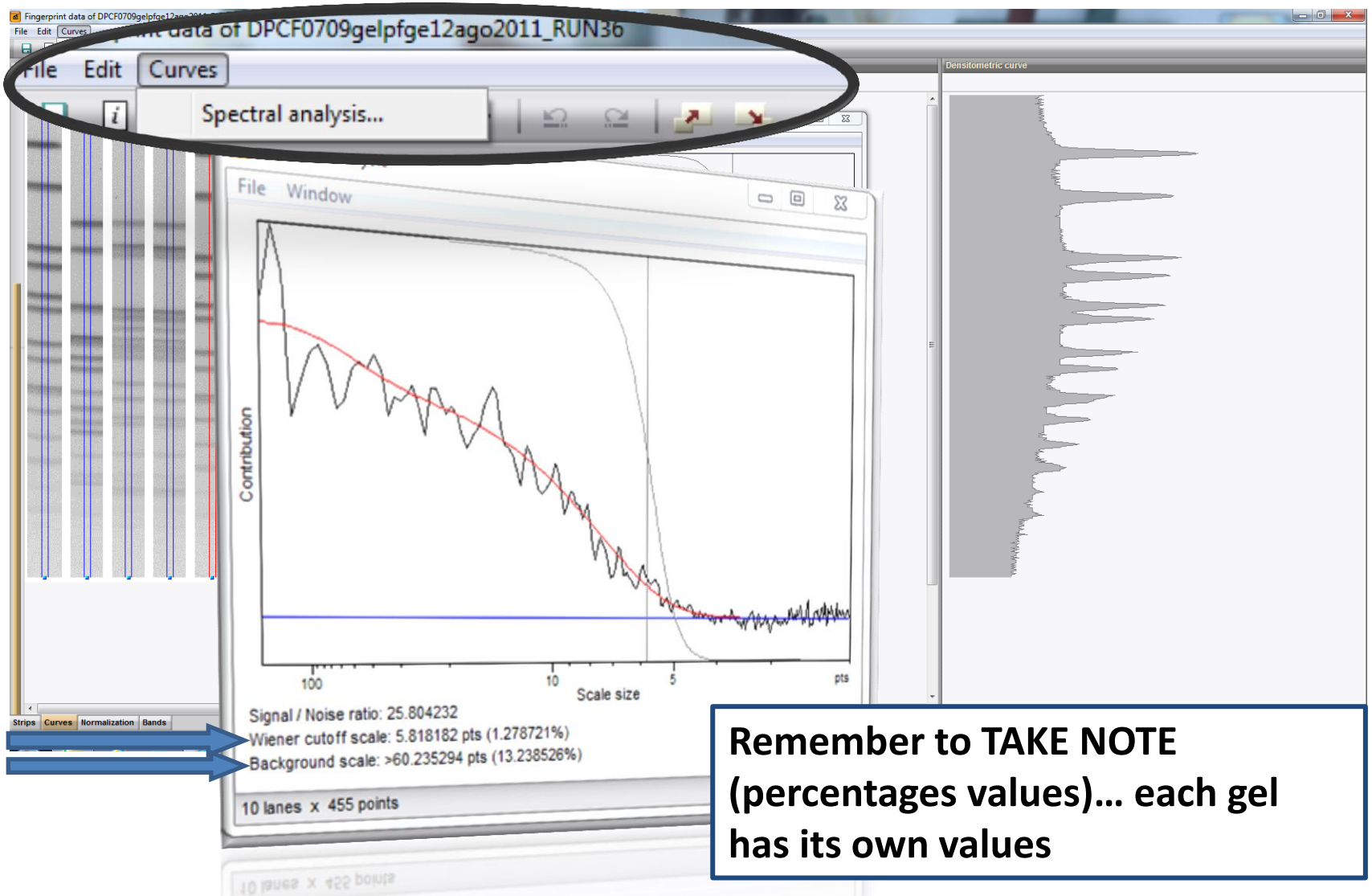
TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

Step 4: DEFINE CURVES



TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

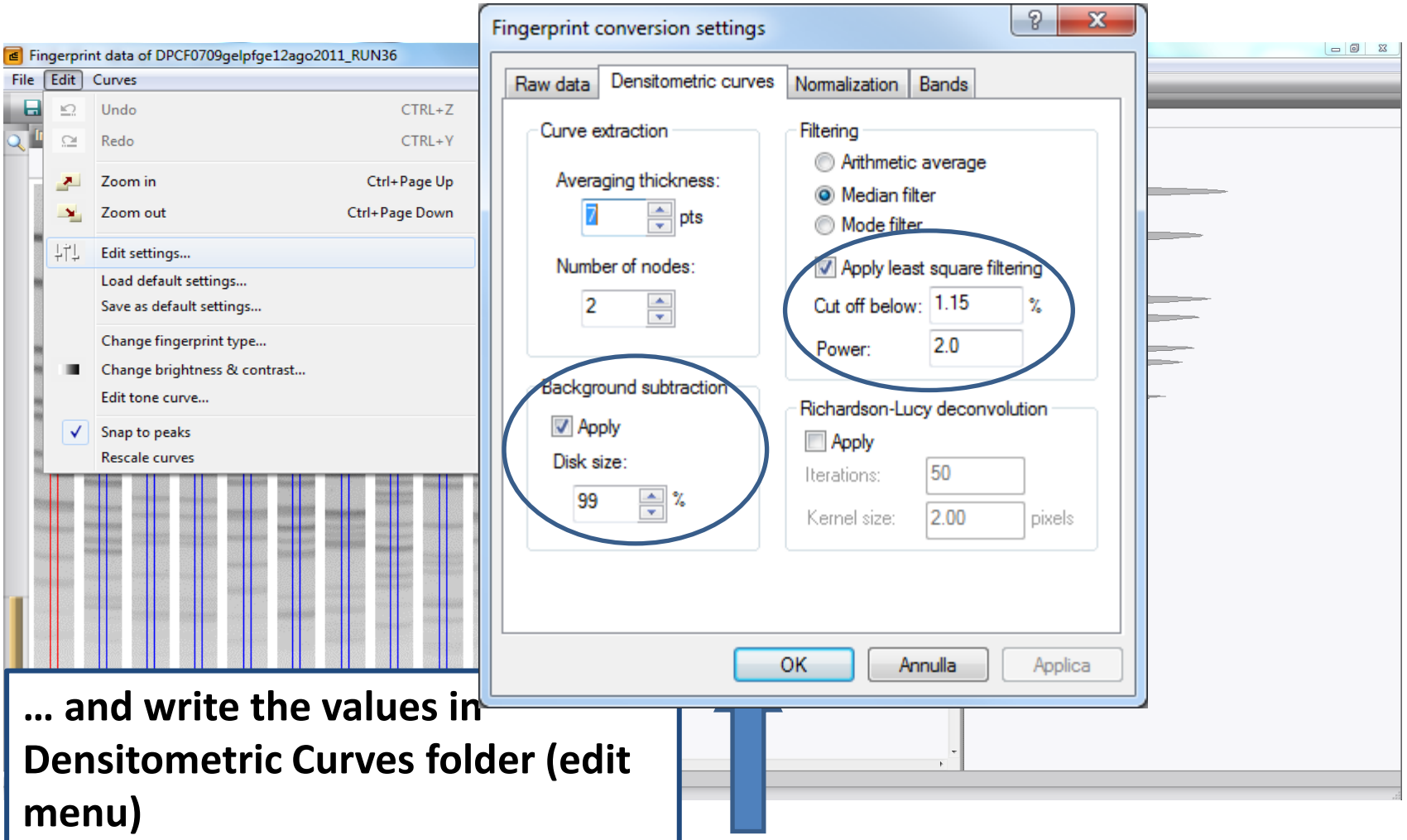
Step DEFINE CURVES



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

TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

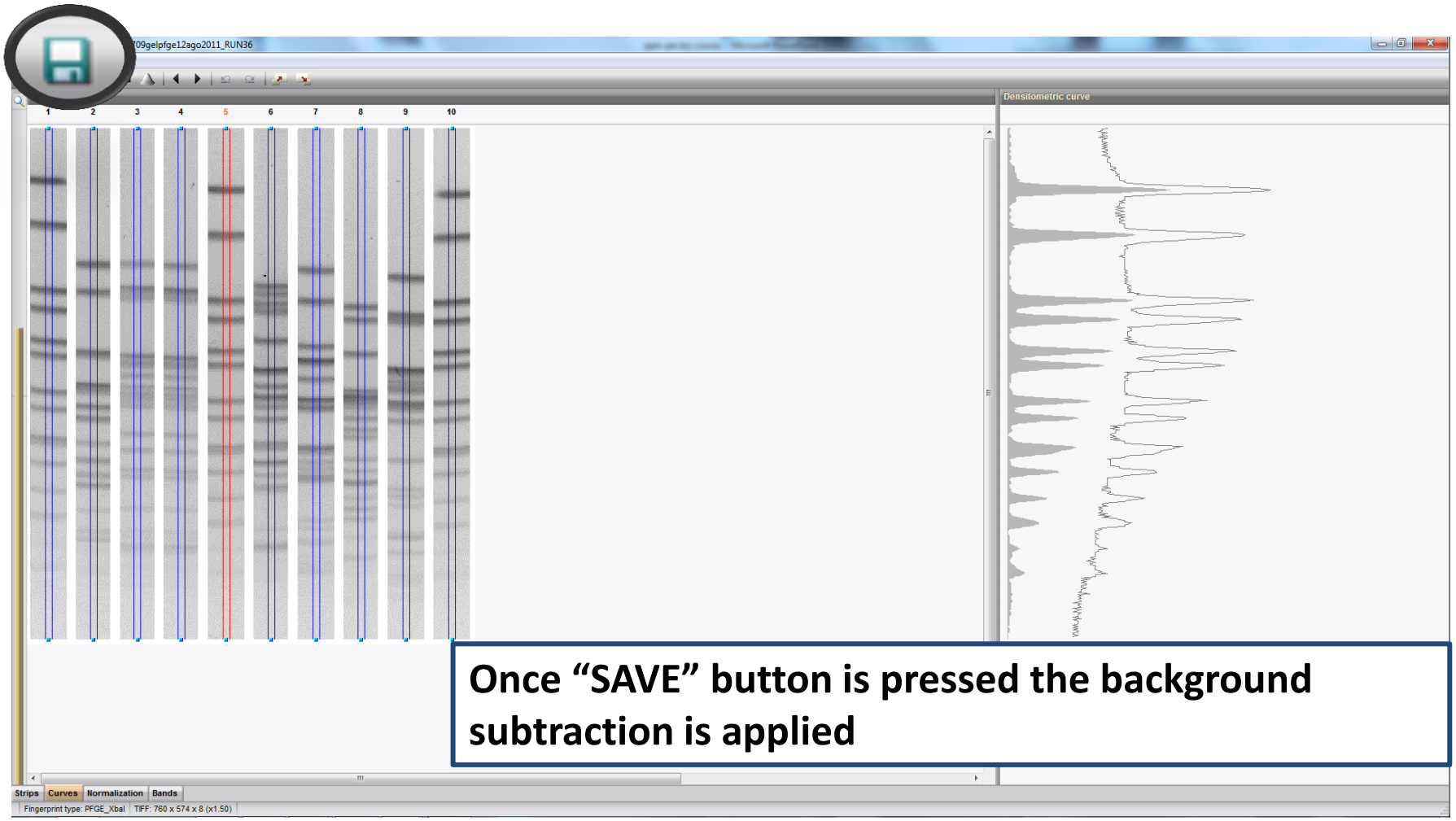
Step 4: DEFINE CURVES



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

TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

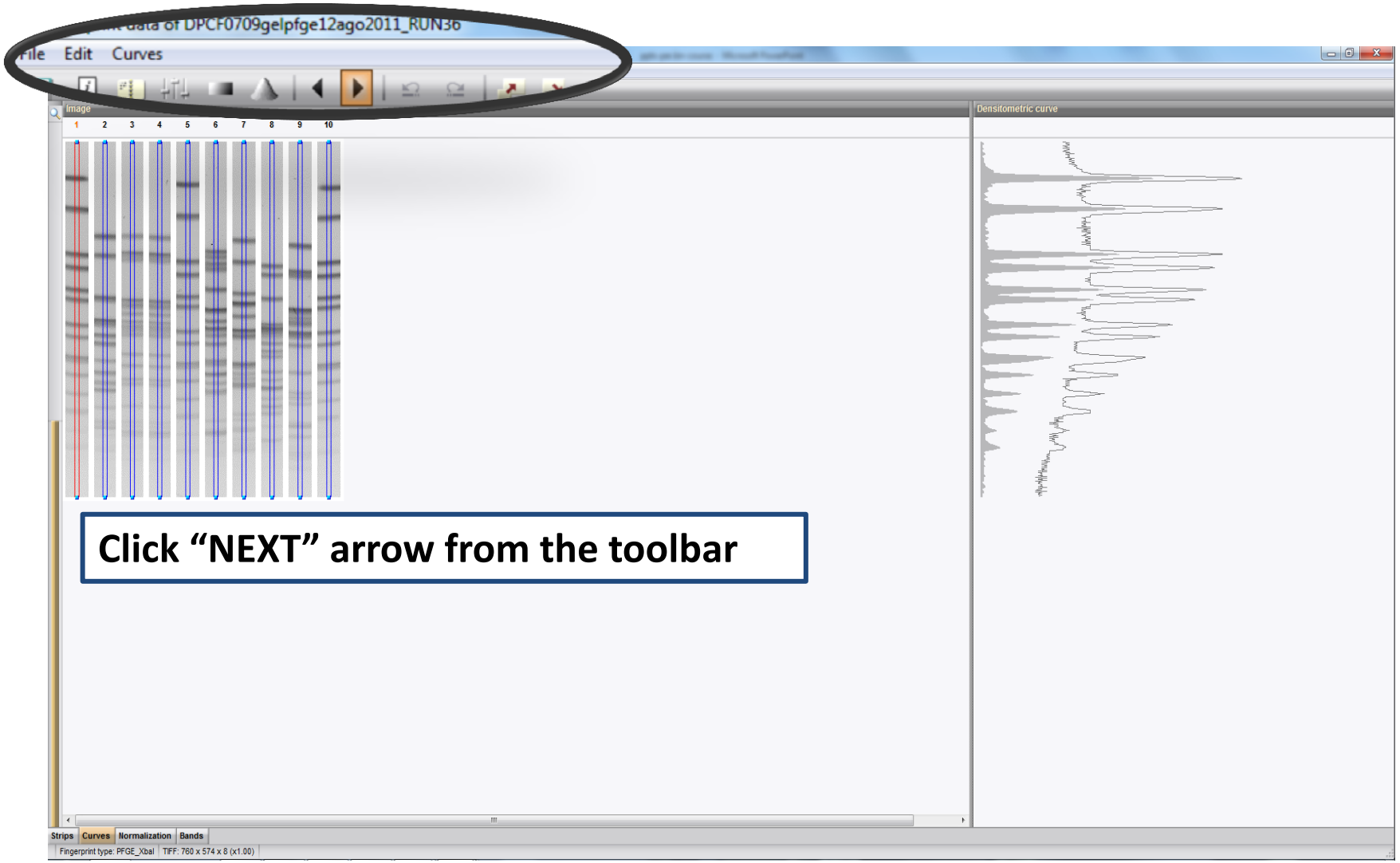
Step 4: Setting up the experiment – DEFINE CURVES



Once "SAVE" button is pressed the background subtraction is applied

TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

Step 4: DEFINE CURVES



TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

- **Analyze a TIFF**

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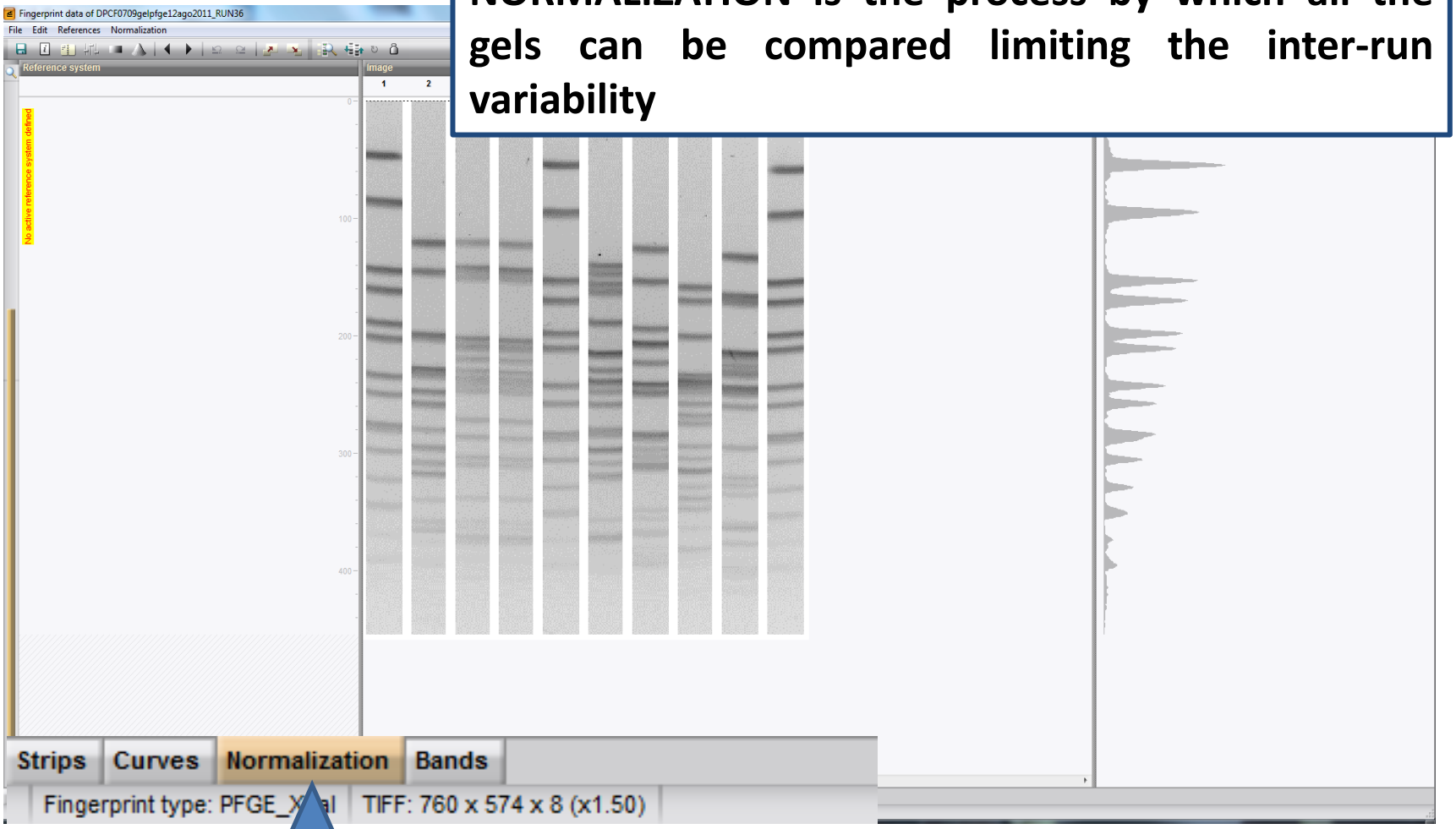
TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

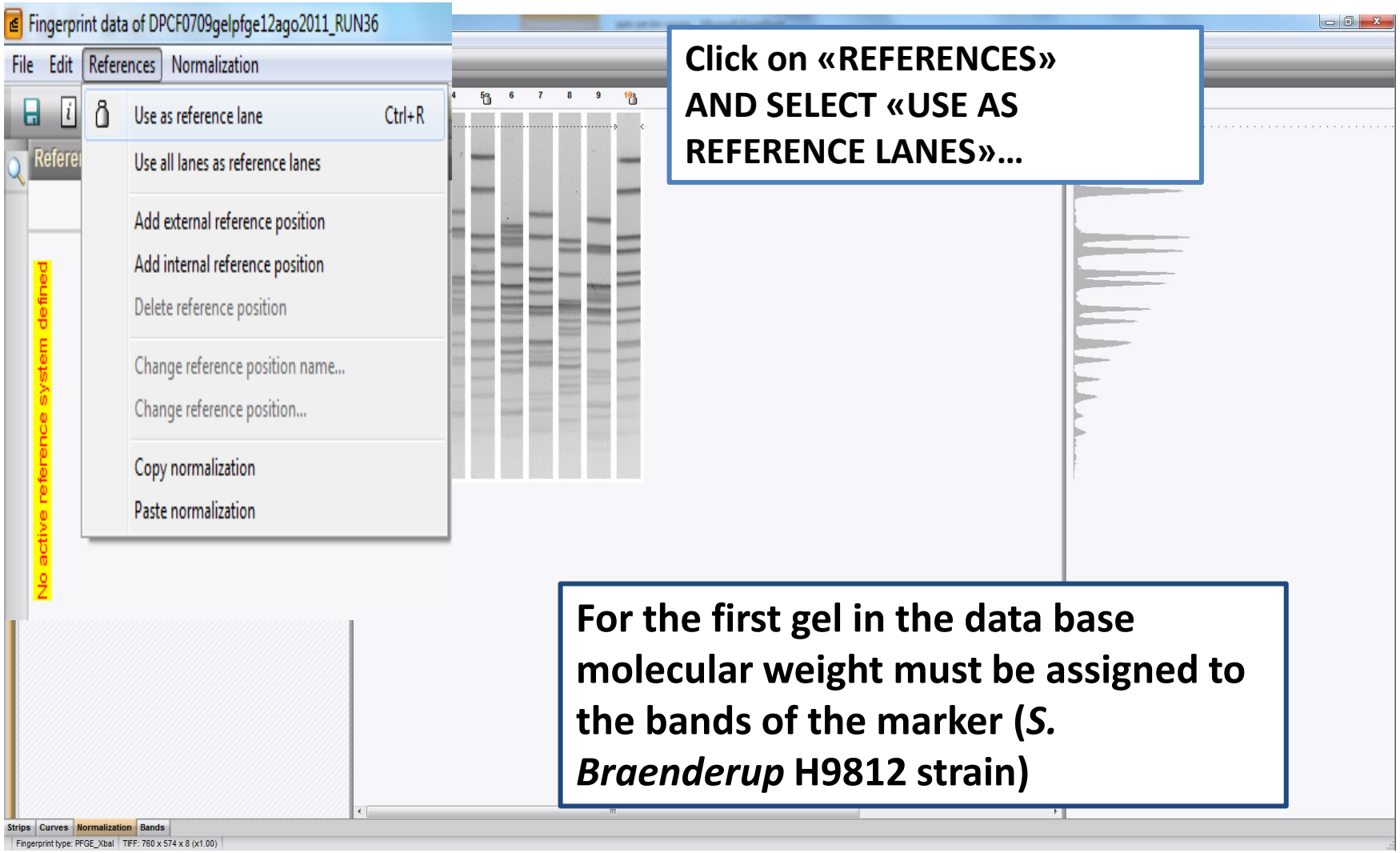
Step 4: NORMALIZATION

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

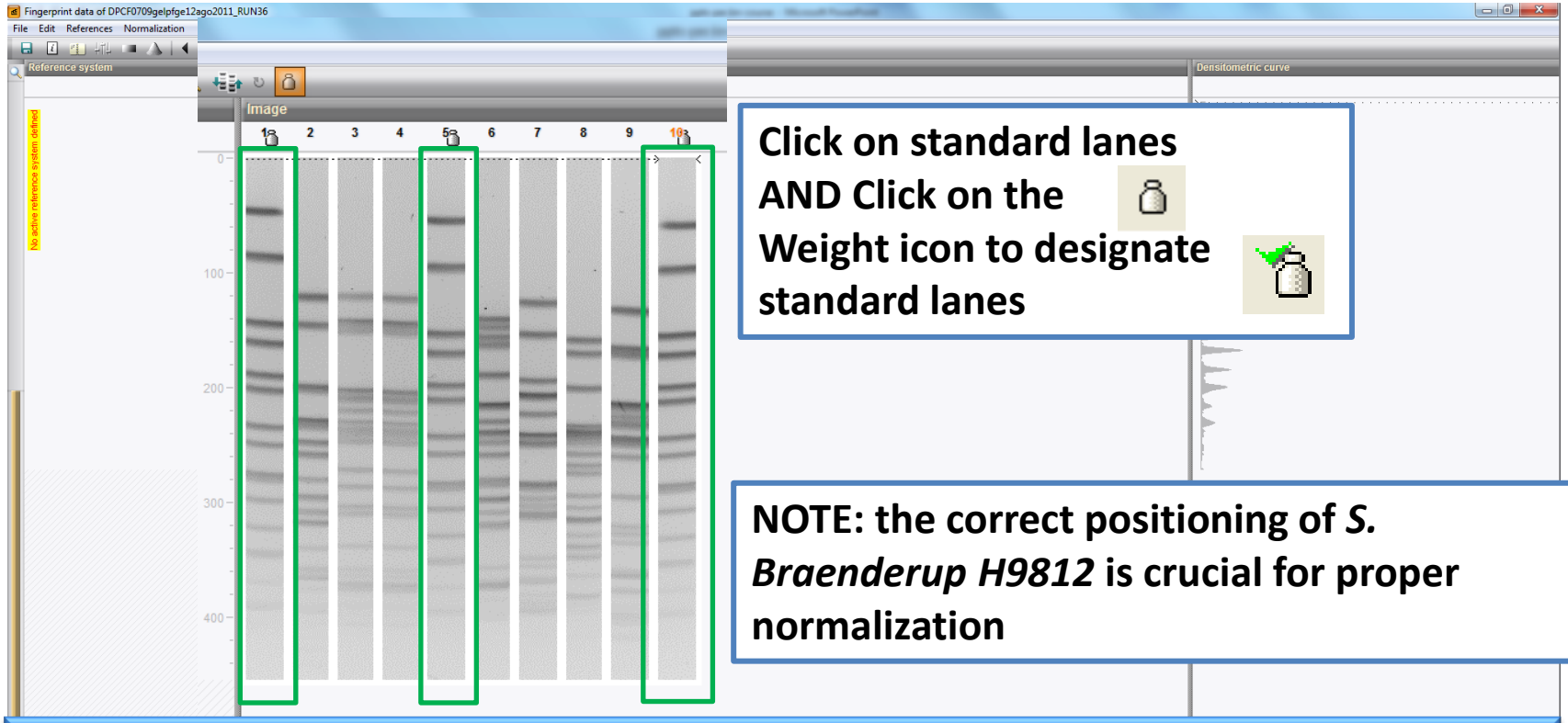


Click on «REFERENCES» AND SELECT «USE AS REFERENCE LANES»…

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

TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

Step 4: ASSIGNING THE REFERENCES BAND POSITIONS - NORMALIZATION



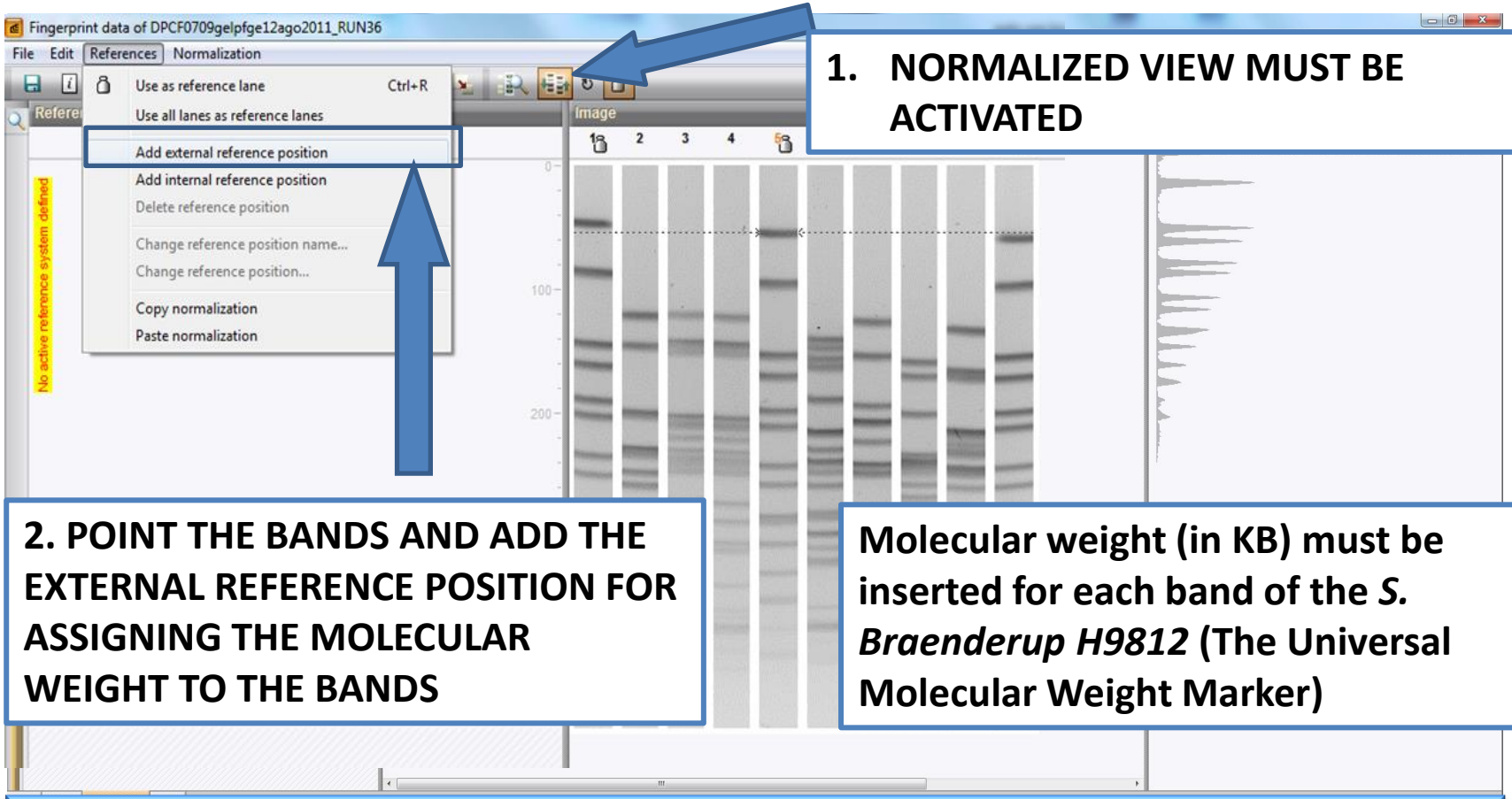
Click on standard lanes AND Click on the Weight icon to designate standard lanes

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



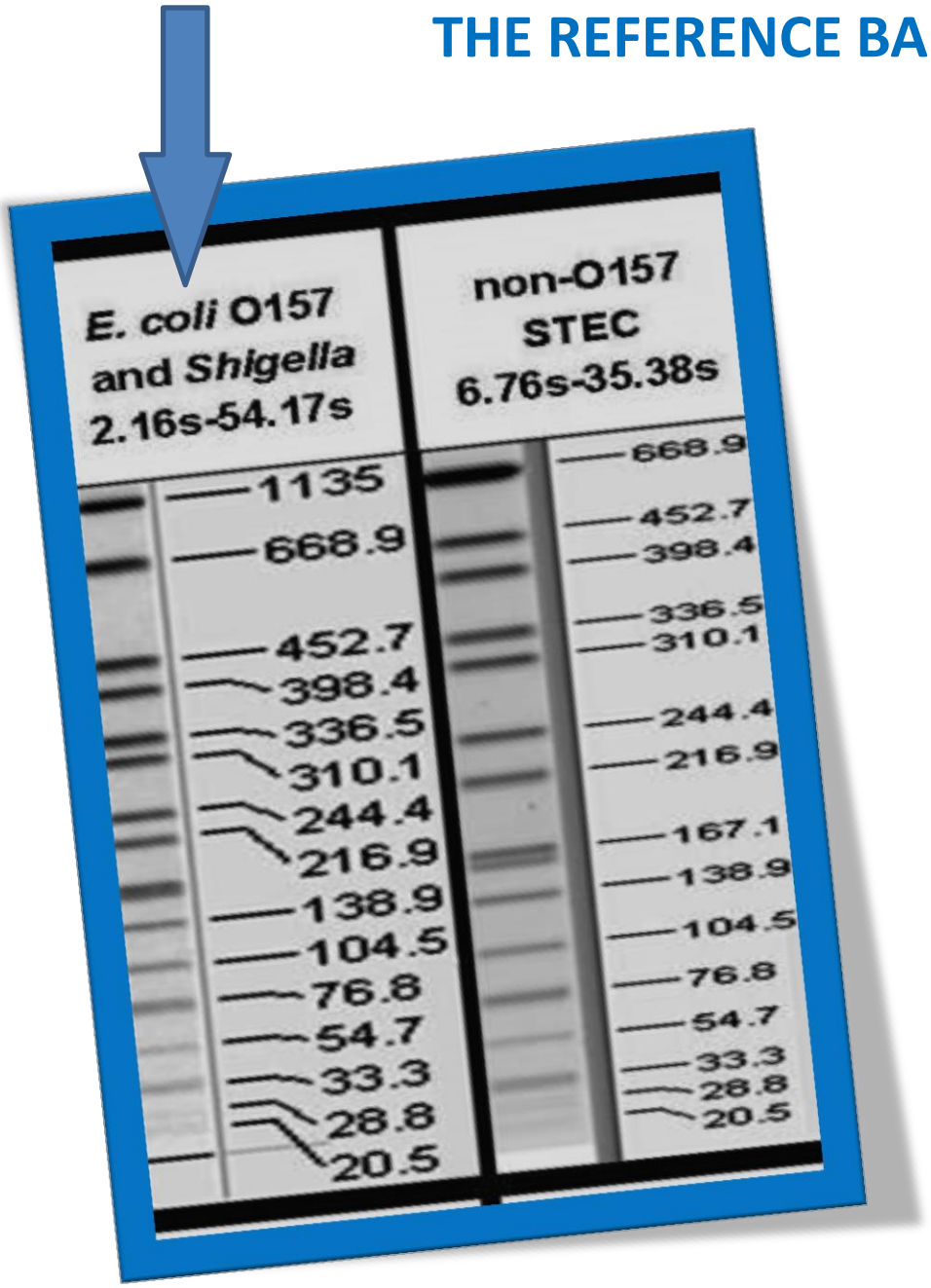
1. NORMALIZED VIEW MUST BE ACTIVATED

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

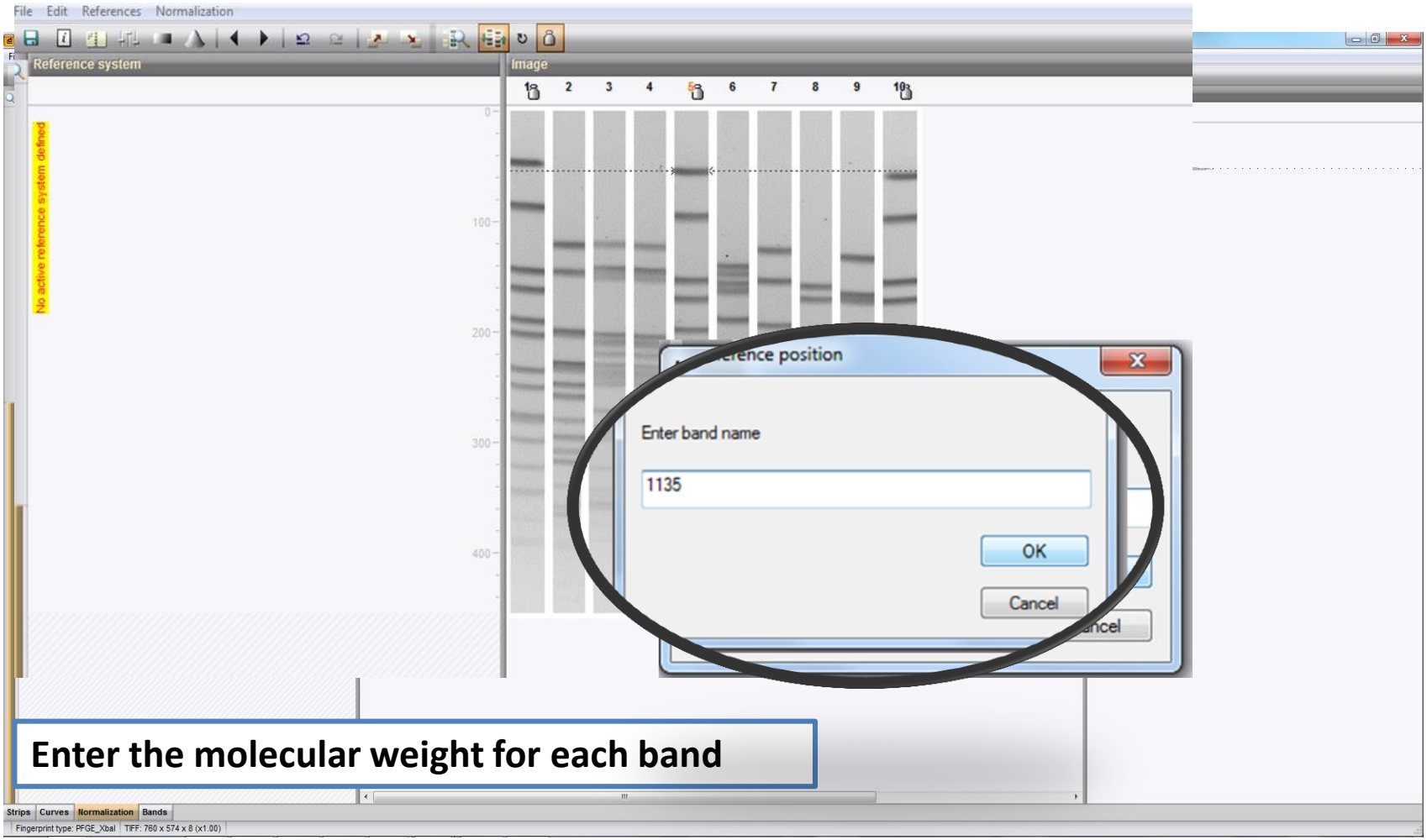


Band Number	Molecular weight (Kb)
1	1135
2	668,9
3	452,7
4	398,4
5	336,5
6	310,1
7	244,4
8	216,9
9	138,9
10	104,5
11	76,8
12	54,7
13	33,3
14	28,8
15	20,5

MANDATORY

TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

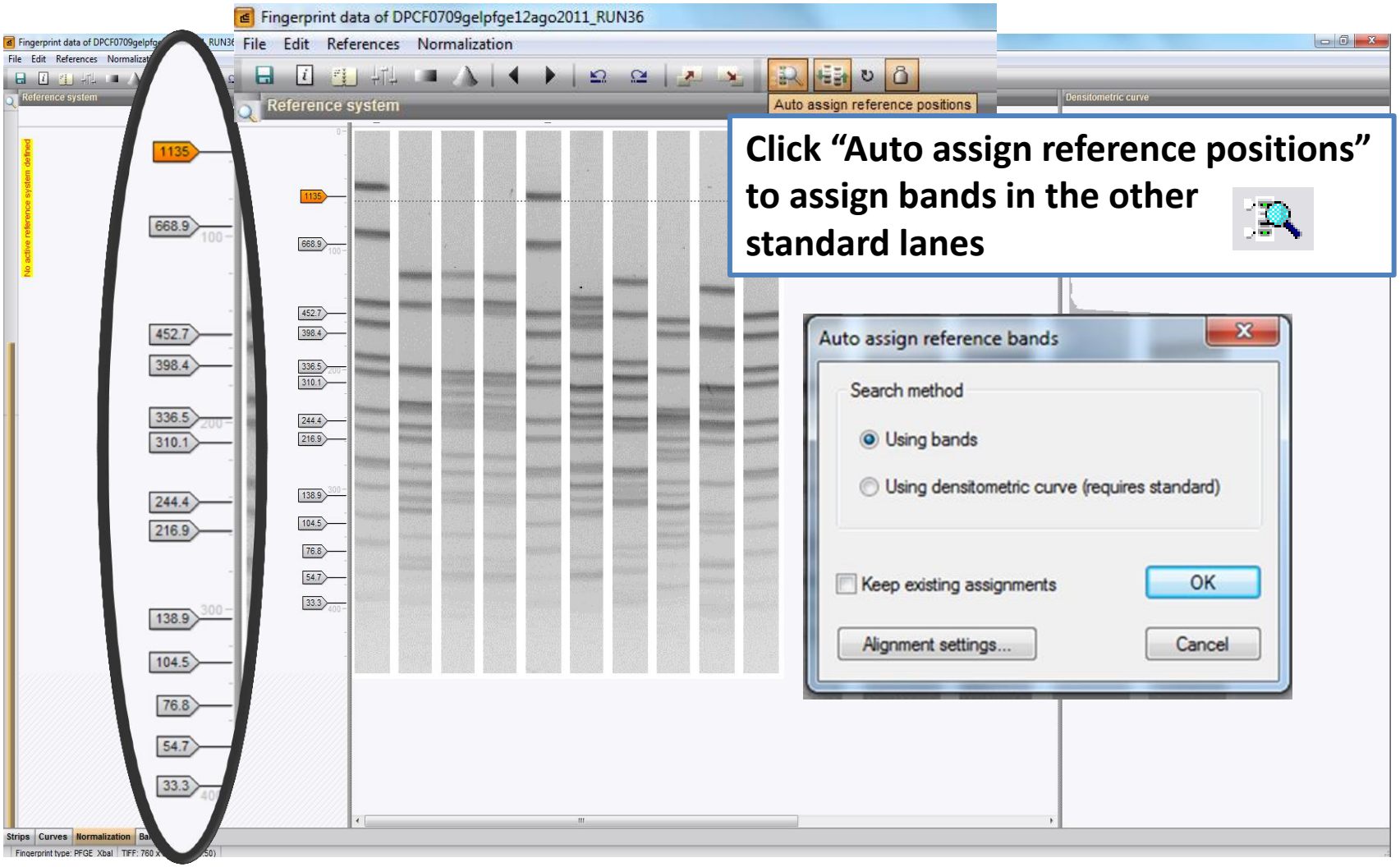
Step 4: ASSIGNING THE REFERENCE BANDS POSITIONS - NORMALIZATION



Enter the molecular weight for each band

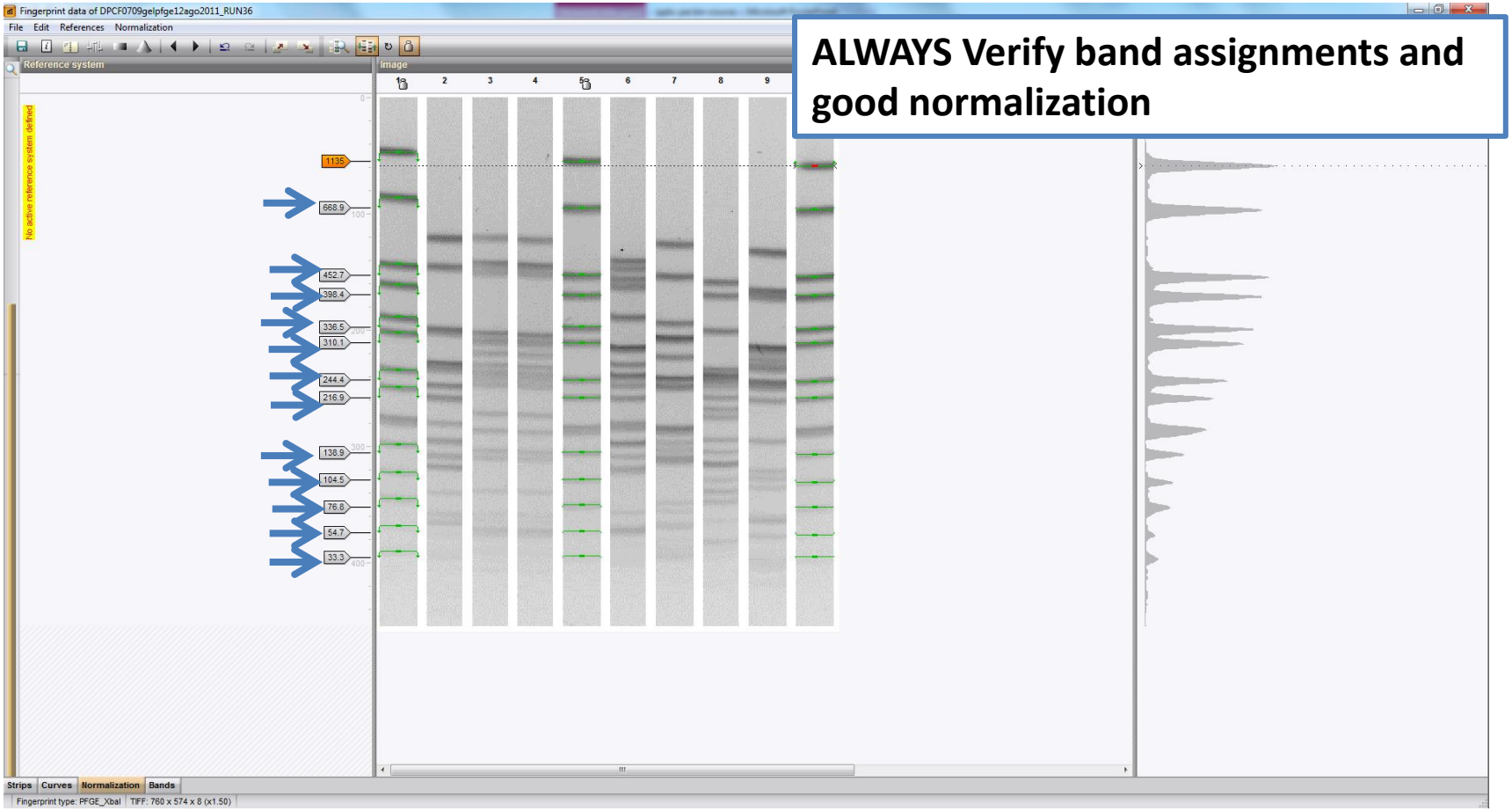
TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

Step 4: ASSIGNING THE REFERENCES BAND POSITIONS - NORMALIZATION



TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

Step 4: ASSIGNING THE REFERENCES BAND POSITIONS - NORMALIZATION



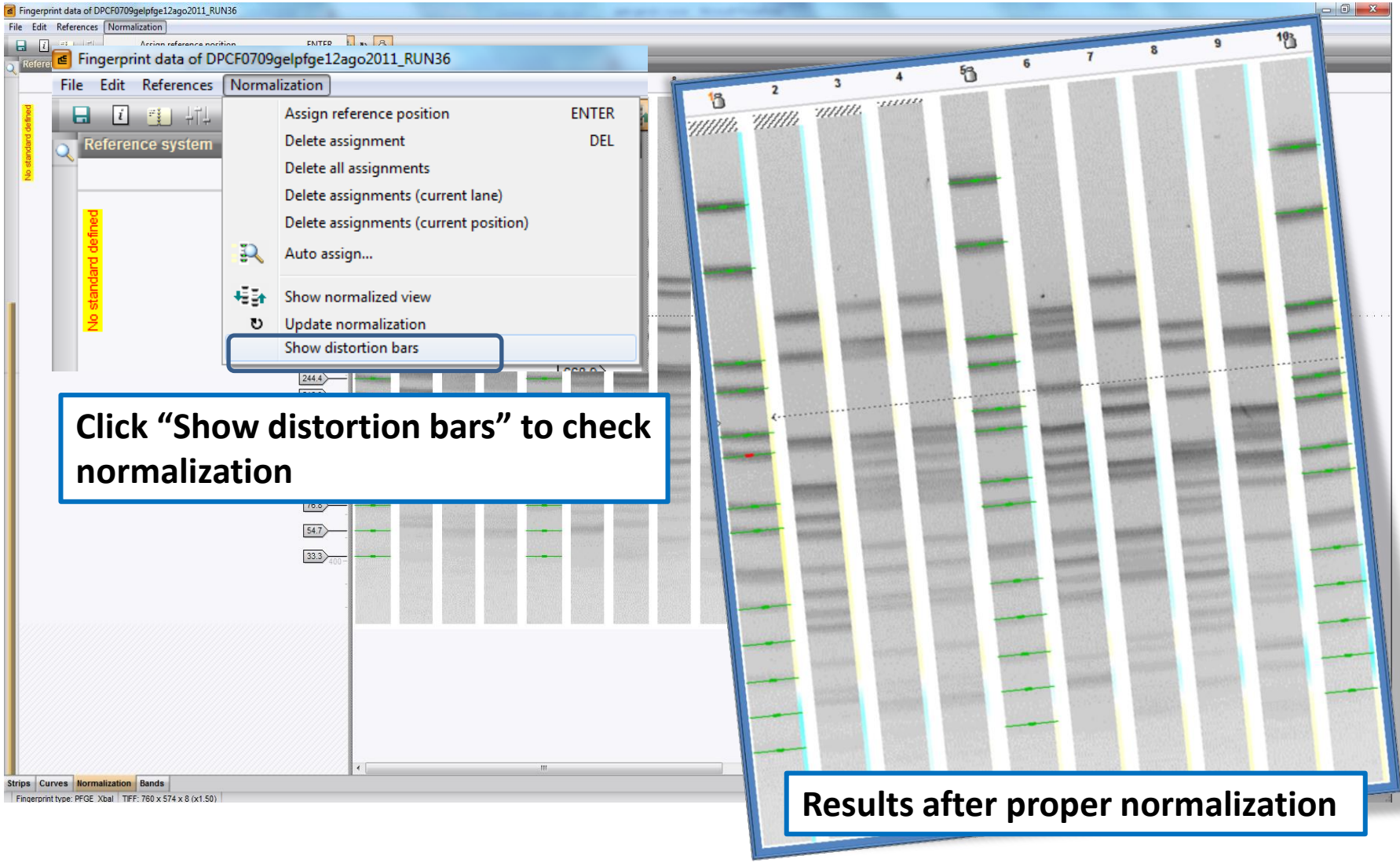
ALWAYS Verify band assignments and good normalization

TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

Step 4: NORMALIZATION

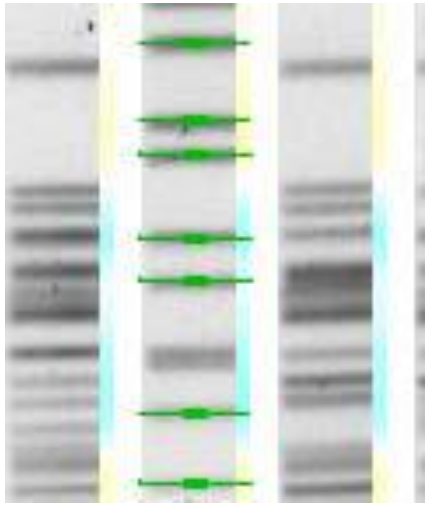
TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

Step 4: NORMALIZATION

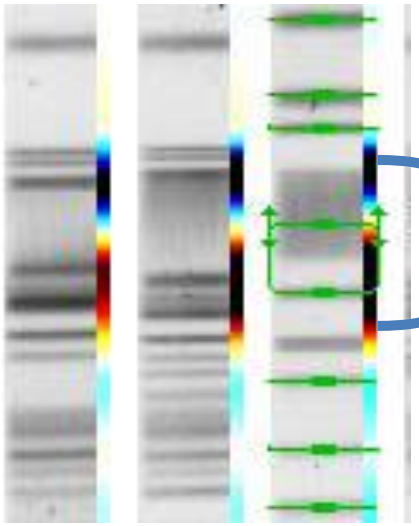


TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

Step 4: NORMALIZATION



Light colors indicate good normalization

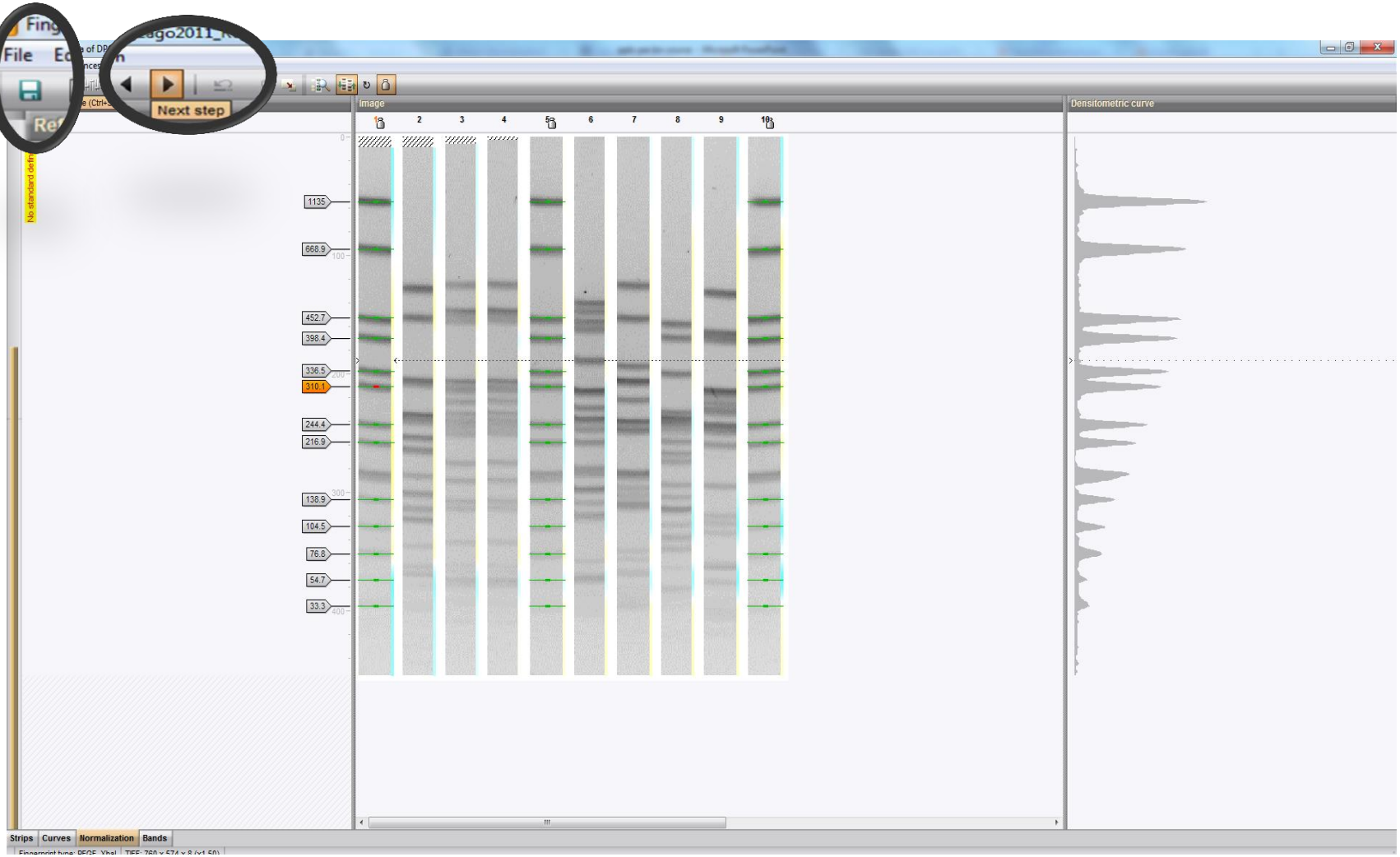


Band assigned to wrong reference position

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

TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

Step 4: NORMALIZATION



TIFF ANALISYS AND PFGE PROFILE INTERPETATION

- **Analyze a TIFF**

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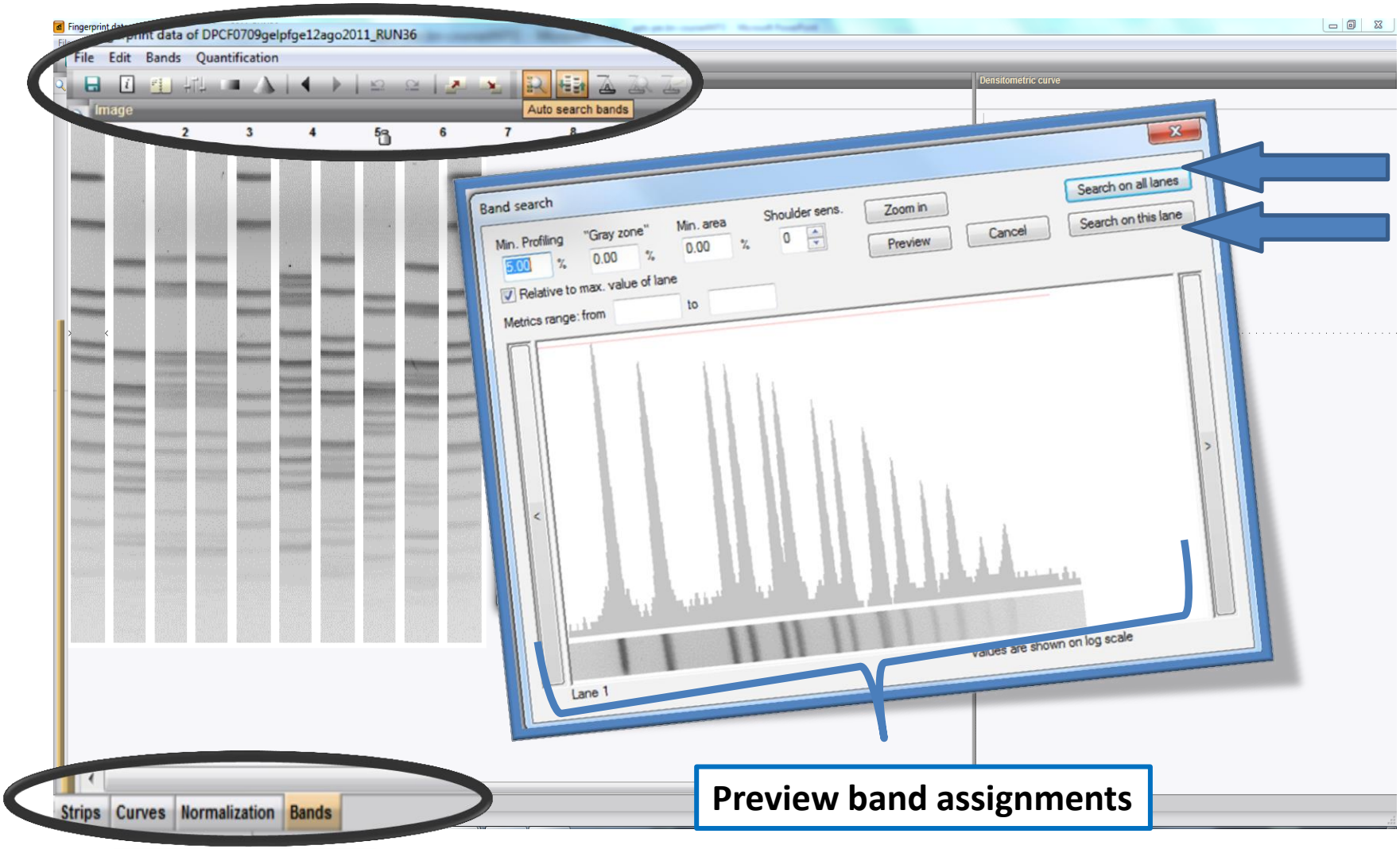
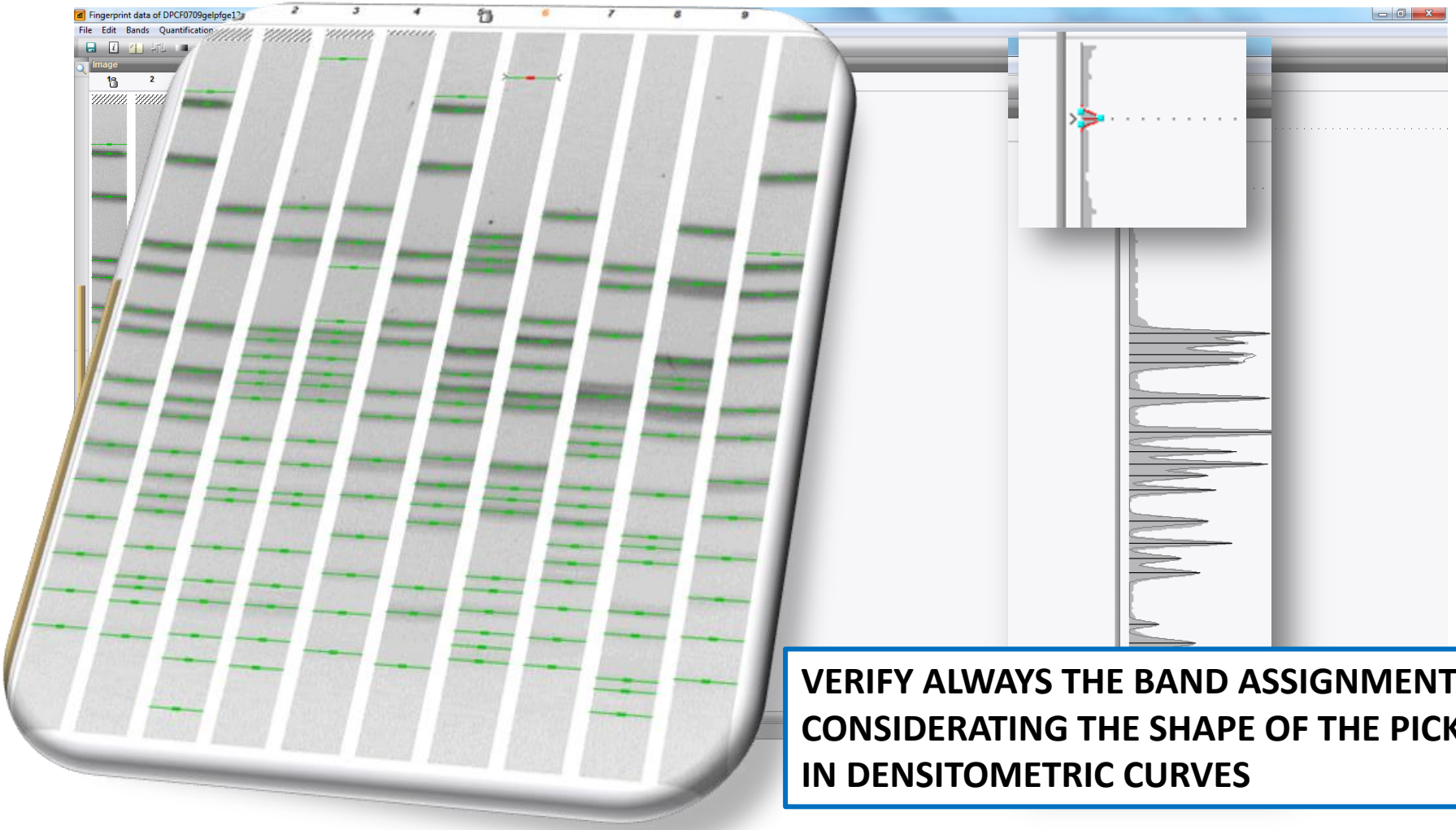
TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

Step 4: BAND ASSIGNMENT



TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

Step 4: BAND ASSIGNMENT

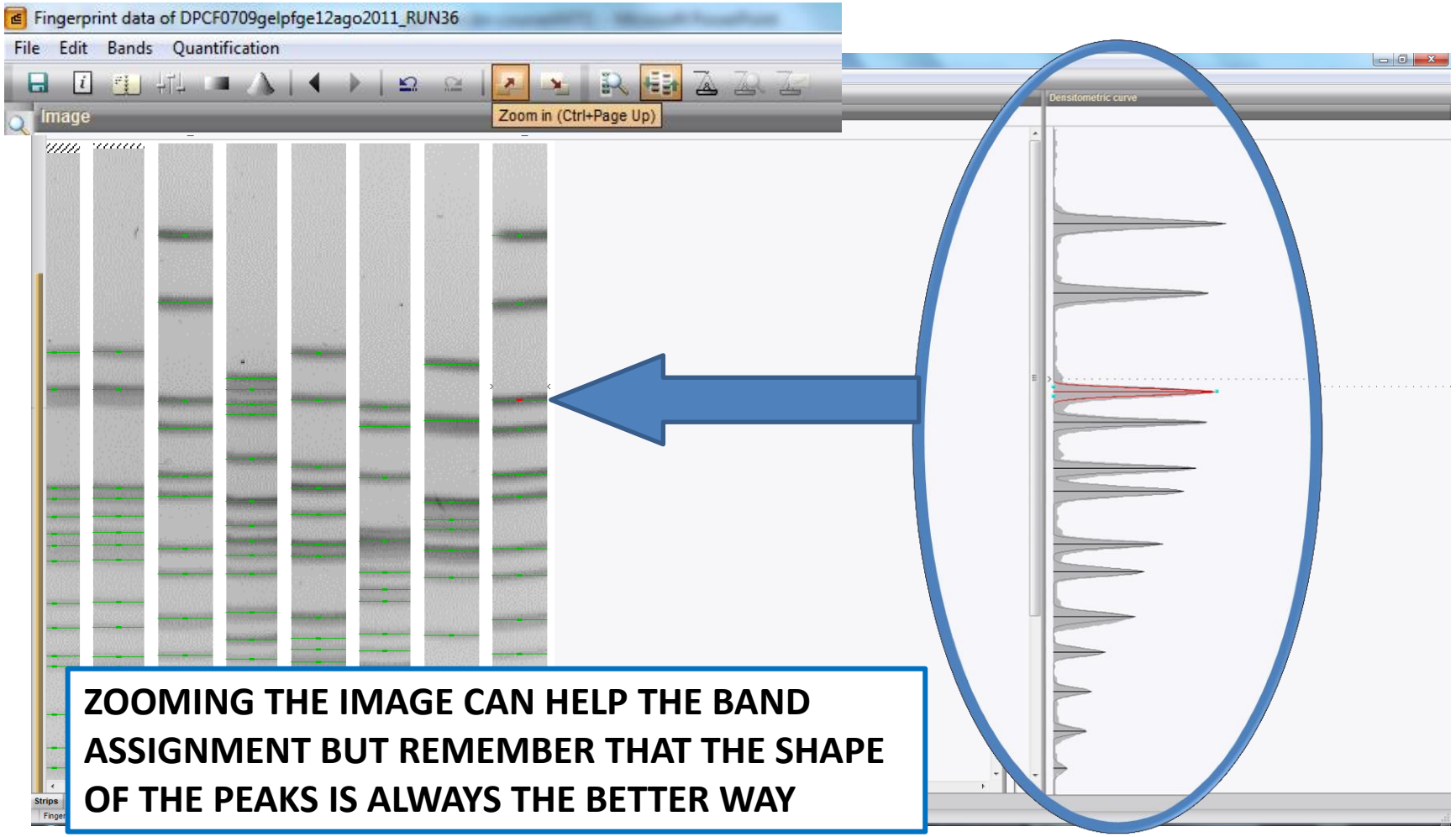


VERIFY ALWAYS THE BAND ASSIGNMENT CONSIDERATING THE SHAPE OF THE PICKS IN DENSITOMETRIC CURVES

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

TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

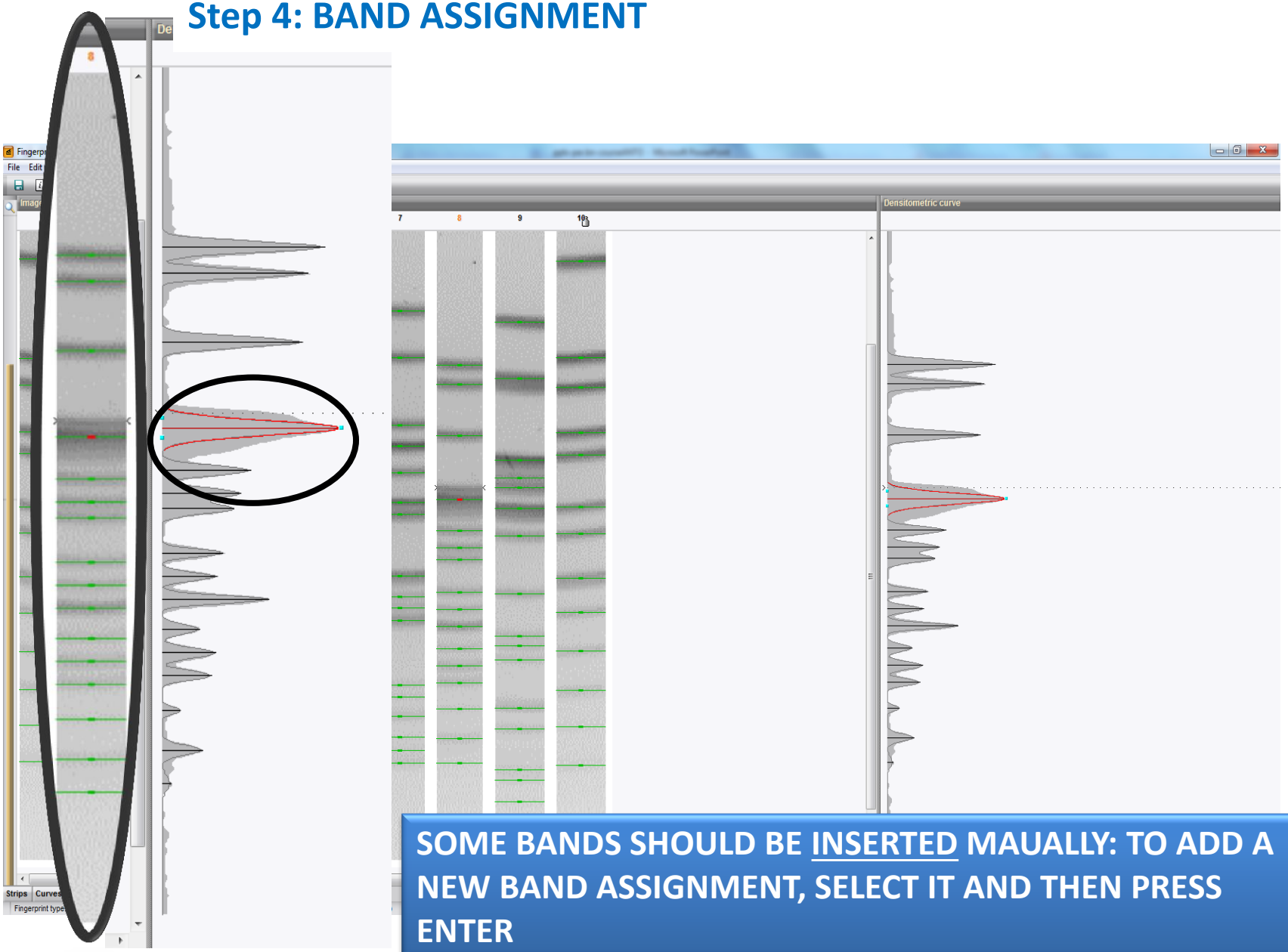
Step 4: BAND ASSIGNMENT



ZOOMING THE IMAGE CAN HELP THE BAND ASSIGNMENT BUT REMEMBER THAT THE SHAPE OF THE PEAKS IS ALWAYS THE BETTER WAY

TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

Step 4: BAND ASSIGNMENT



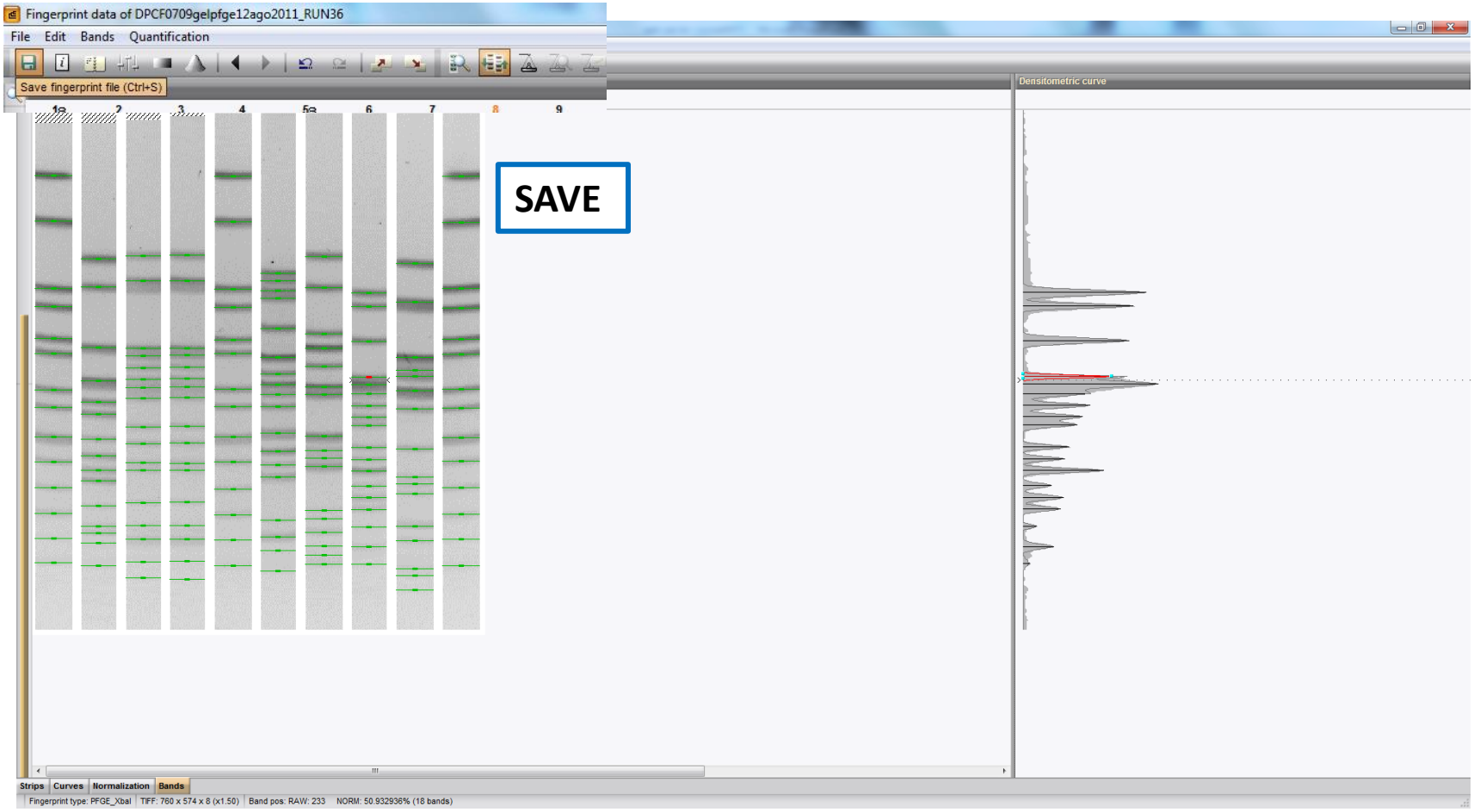
SOME BANDS SHOULD BE INSERTED MAUALLY: TO ADD A NEW BAND ASSIGNMENT, SELECT IT AND THEN PRESS ENTER

TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

Step 4: BAND ASSIGNMENT

IN DEPTH OF THE EXPERIMENT: TIFF ANALISYS AND PFGE PROFILING INTERPETATION

Step 4: Setting up the experiment – BAND ASSIGNMENT



Step 4: BAND ASSIGNMENT

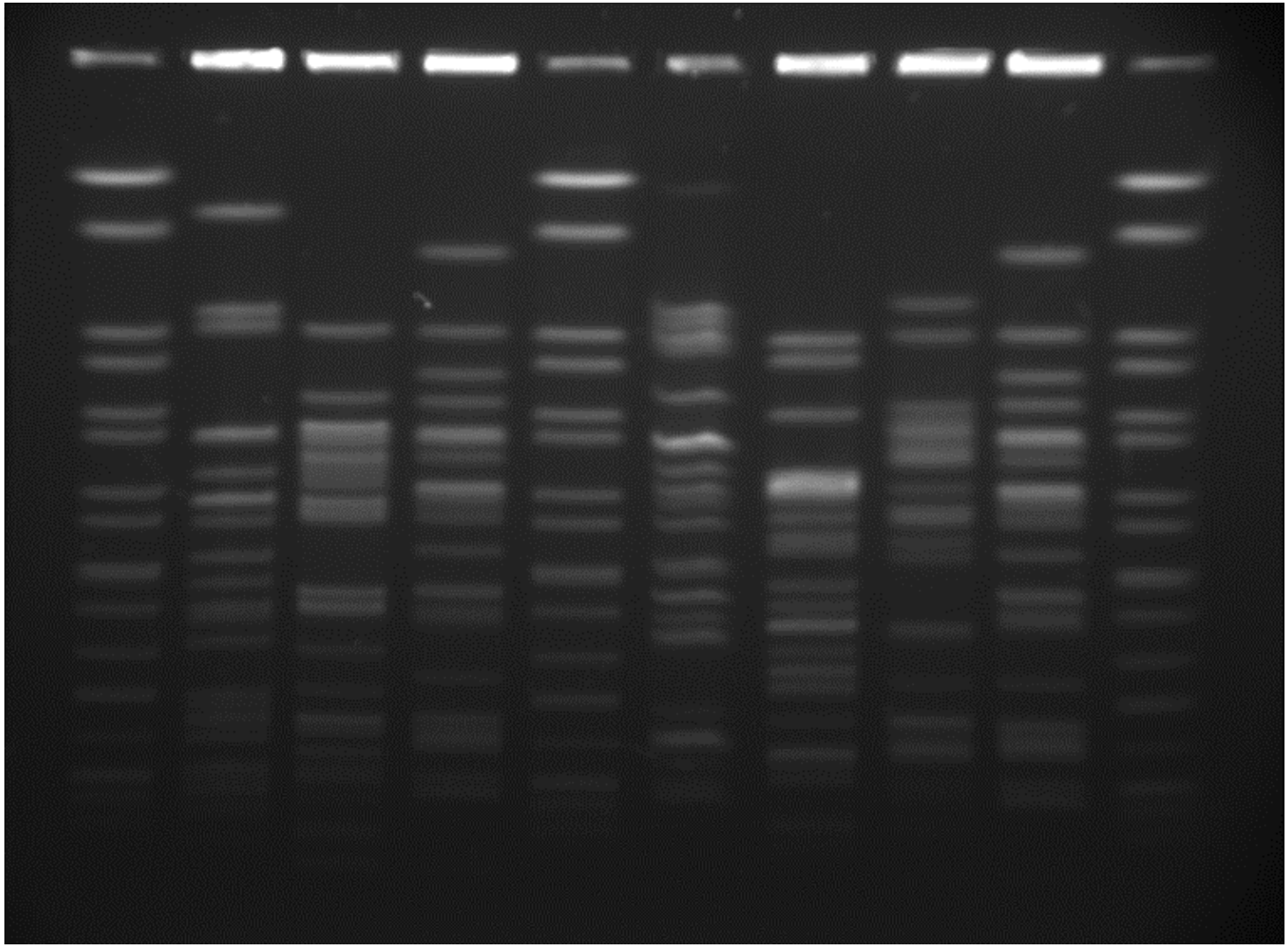
INTERNAL CONTROL

- 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 *XbaI* 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.

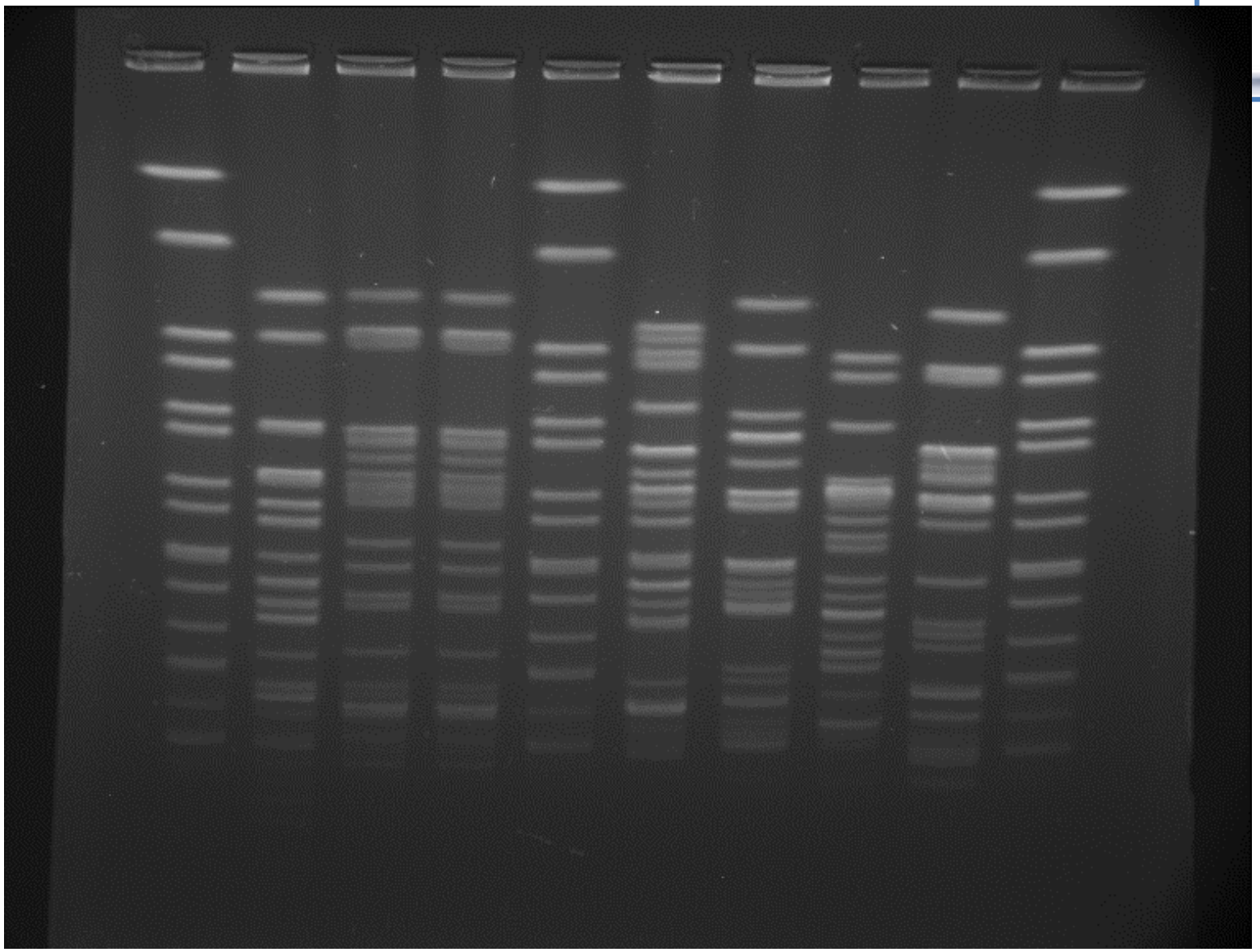
ANALYSABLE PFGE PROFILES DISPLAY ALL THE BANDS IN THE INDICATED INTERVAL CLEARLY VISIBLE. THEREFORE, ANY PROFILE, WHICH DOES NOT HAVE THE BANDS CLEARLY VISIBLE IN THIS INTERVAL, SHOULD BE REJECTED.

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

IS THIS GEL SWITABLE?

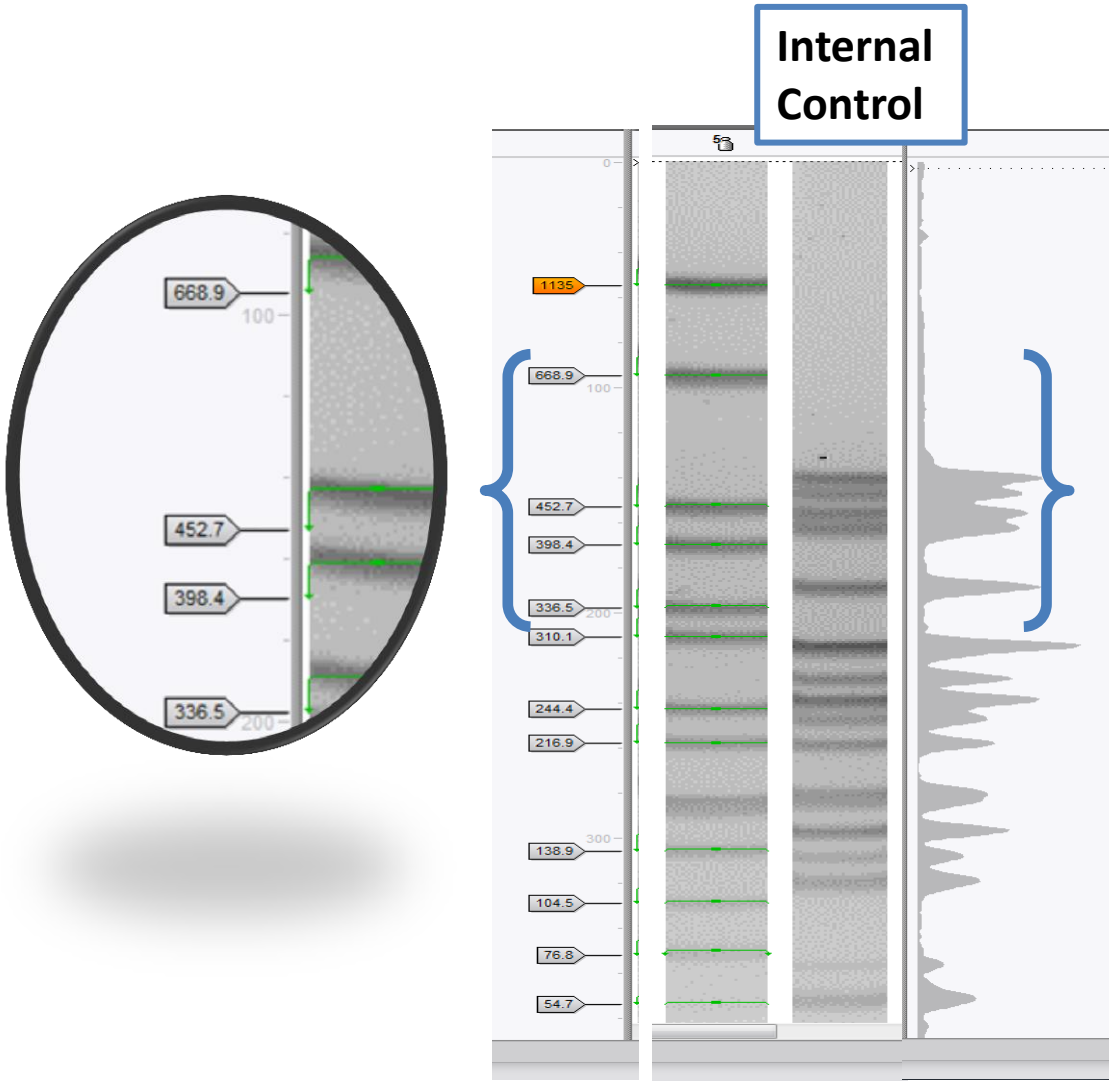


AND THIS ONE?



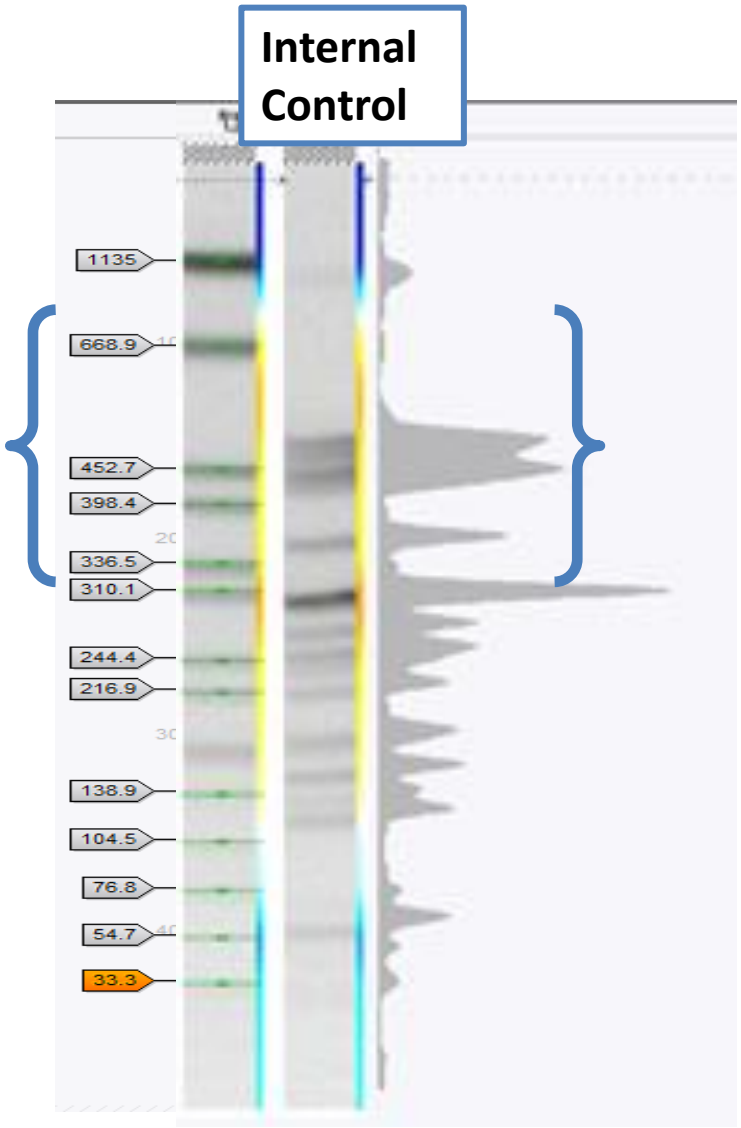
TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

Step 4: BAND ASSIGNMENT



IN DEPTH OF THE EXPERIMENT: TIFF ANALISYS AND PFGE PROFILING INTERPETATION

Step 4: Setting up the experiment – BAND ASSIGNMENT



TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

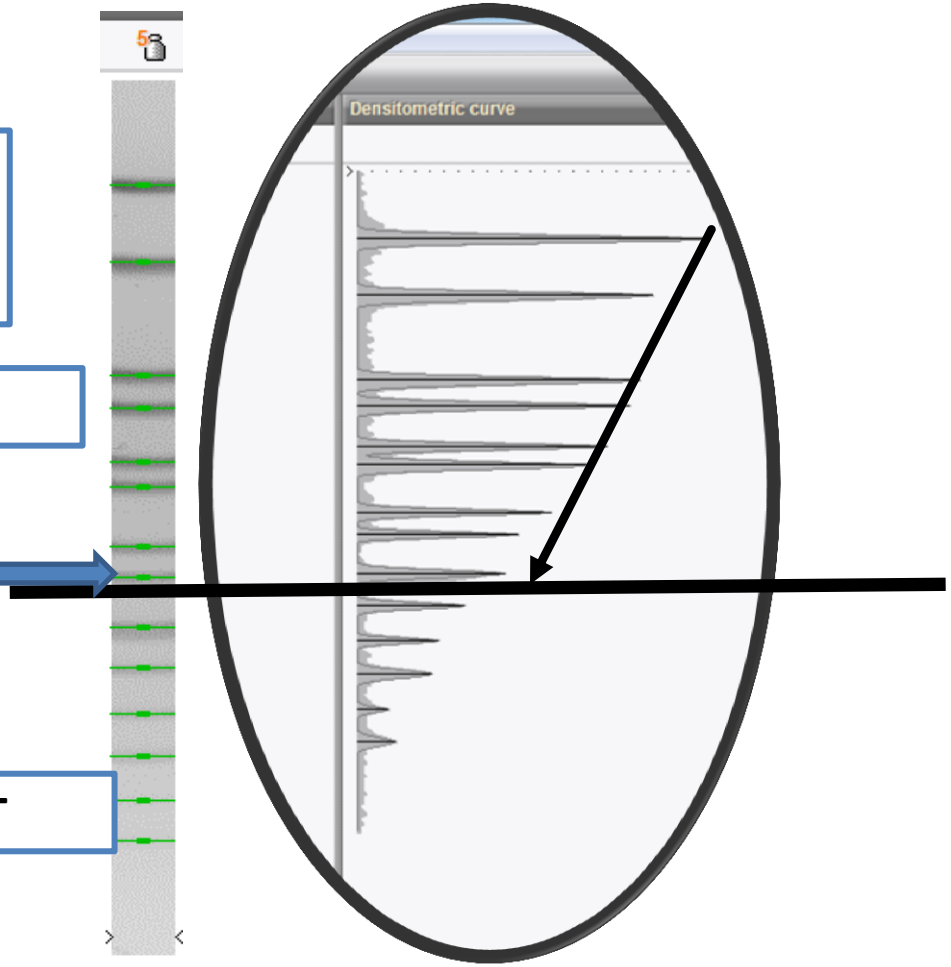
Step 4: BAND ASSIGMENT - Validation of doubtful bands

DISCENDING ORDER OF BAND INTENSTITY 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**

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- **Link Lanes to Database Entries**
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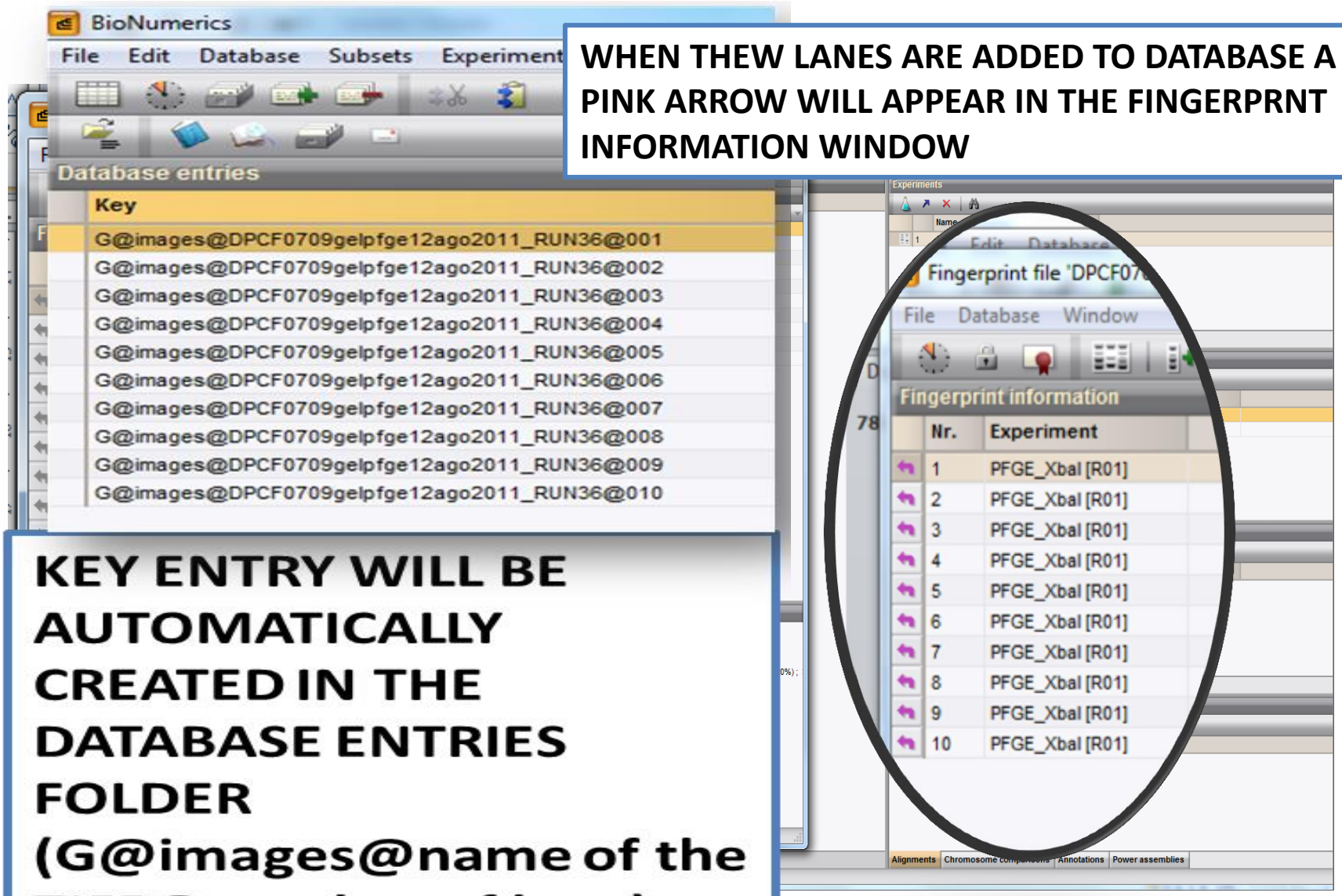
Step 5: LINK LANES TO DATABASE ENTRIES

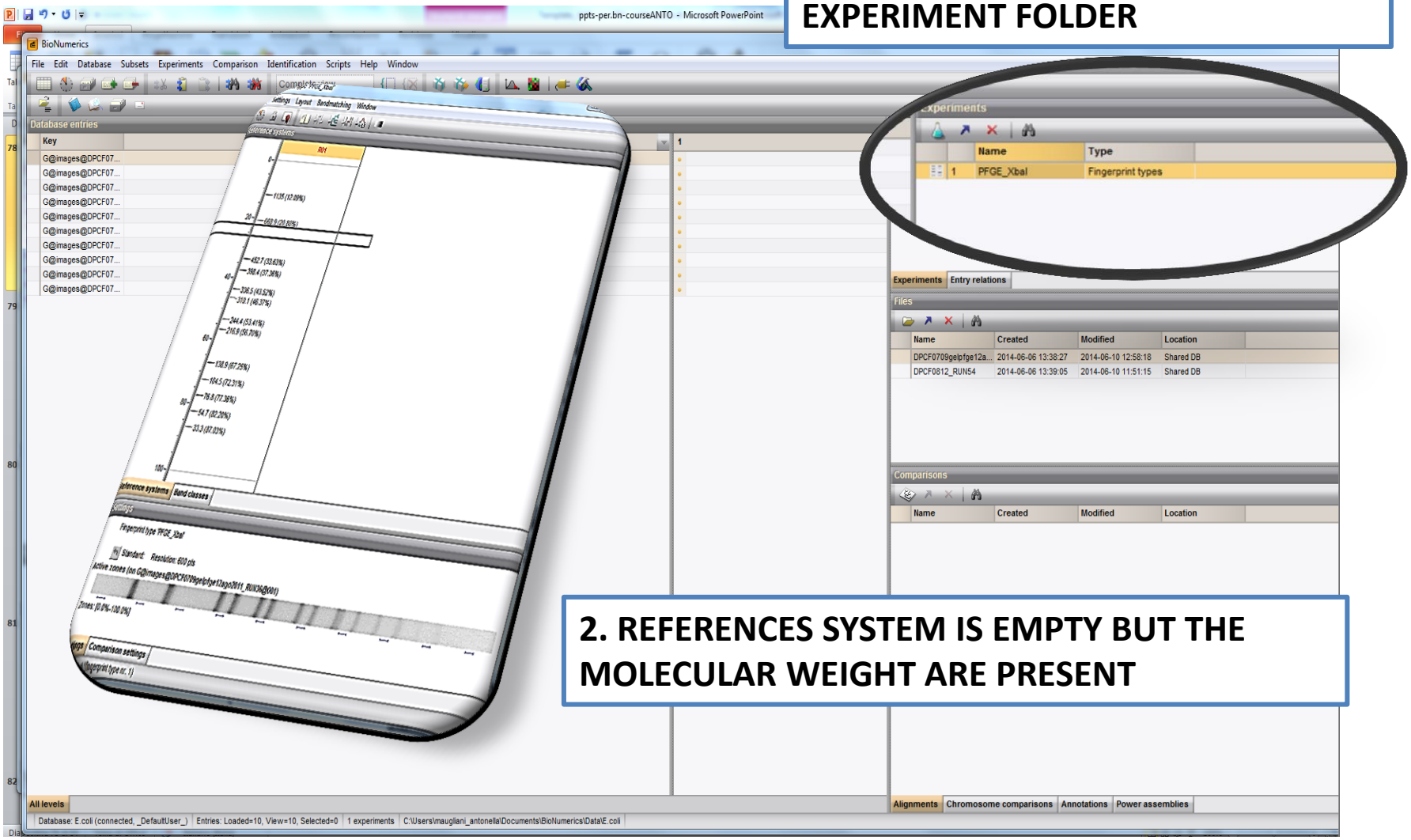


WHEN THEW LANES ARE ADDED TO DATABASE A PINK ARROW WILL APPEAR IN THE FINGERPRNT INFORMATION WINDOW

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

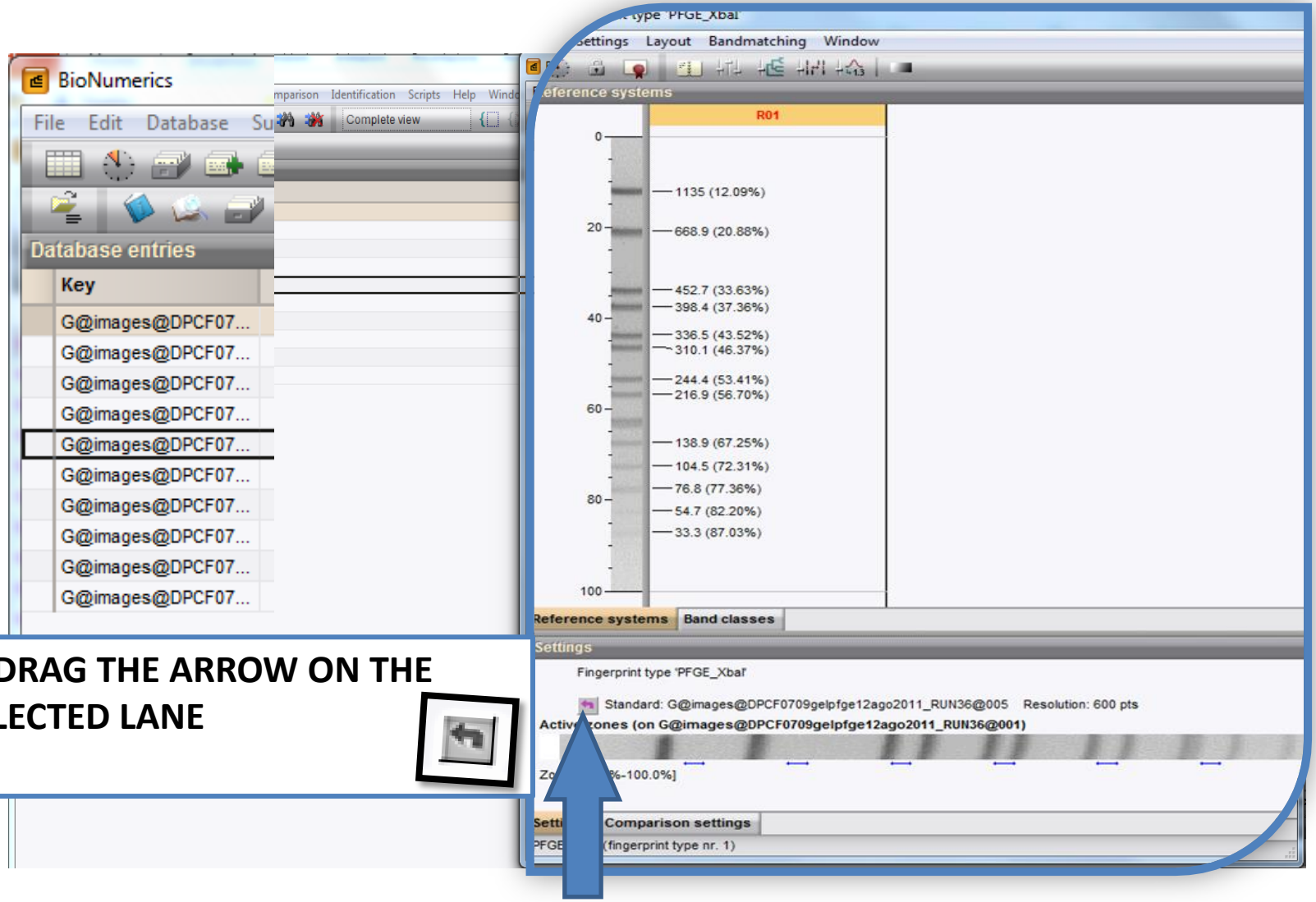
Step 5: SETTING UP THE MOLECULAR STANDARD

1. DOUBLE CLICK IN THE EXPERIMENT FOLDER



2. REFERENCES SYSTEM IS EMPTY BUT THE MOLECULAR WEIGHT ARE PRESENT

Step 5: SETTING UP THE UNIVERSAL MOLECULAR STANDARD



3. DRAG THE ARROW ON THE SELECTED LANE

TIFF ANALYSIS AND PFGE PROFILE INTERPETATION

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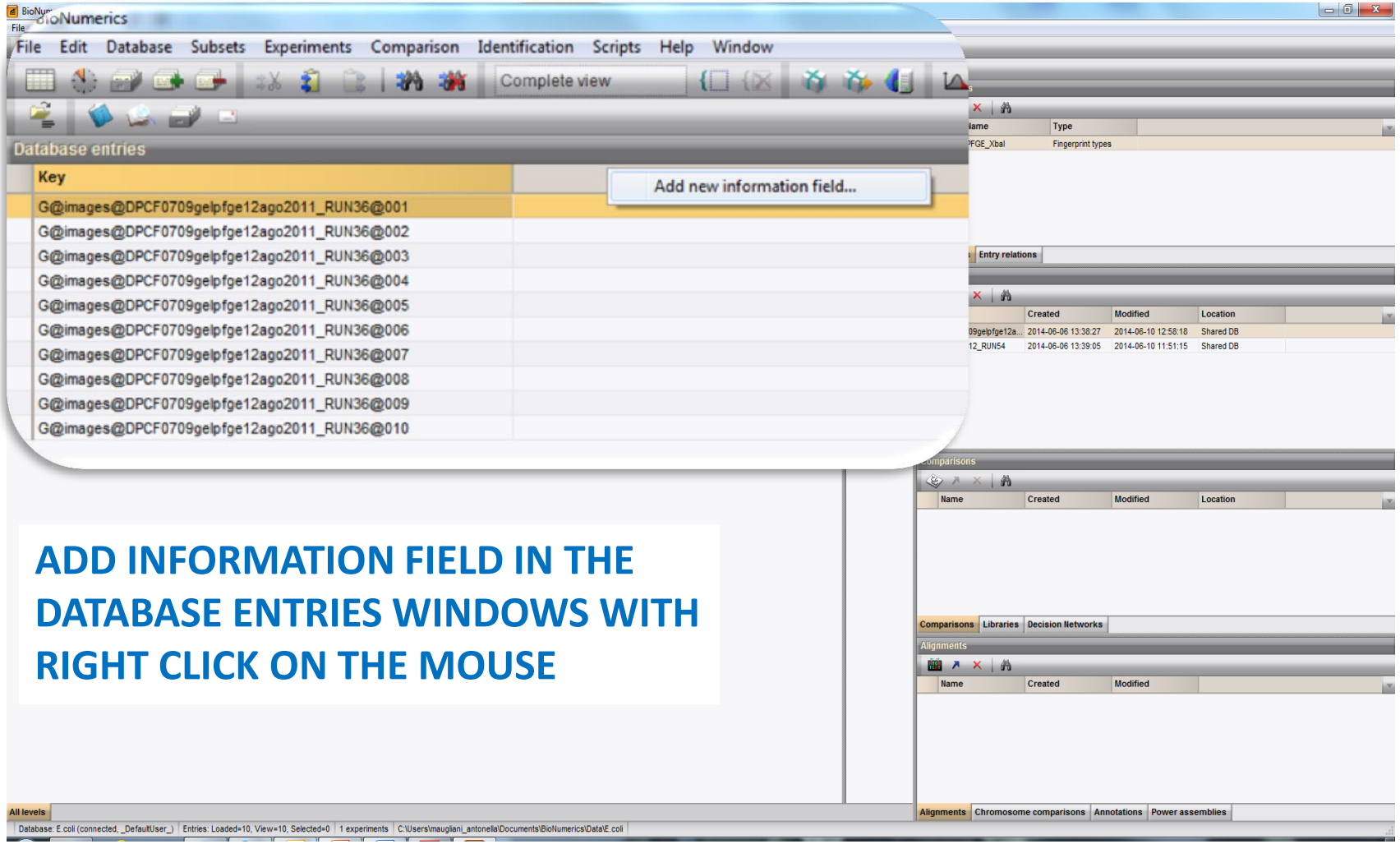
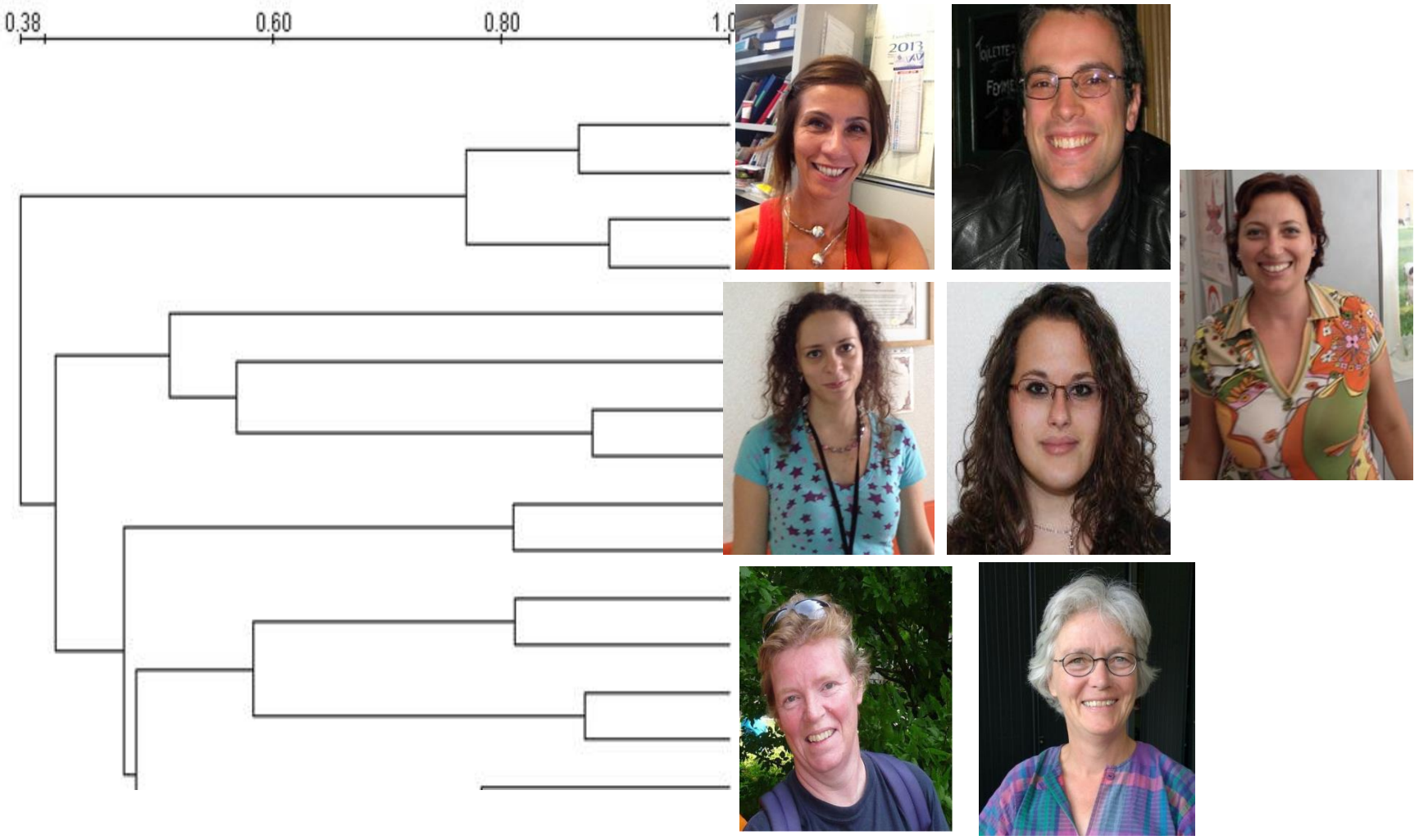
IN DEPTH OF THE EXPERIMENT: TIFF ANALISYS AND PFGE PROFILING INTERPETATION

Step 6: Add Text Data for Isolates



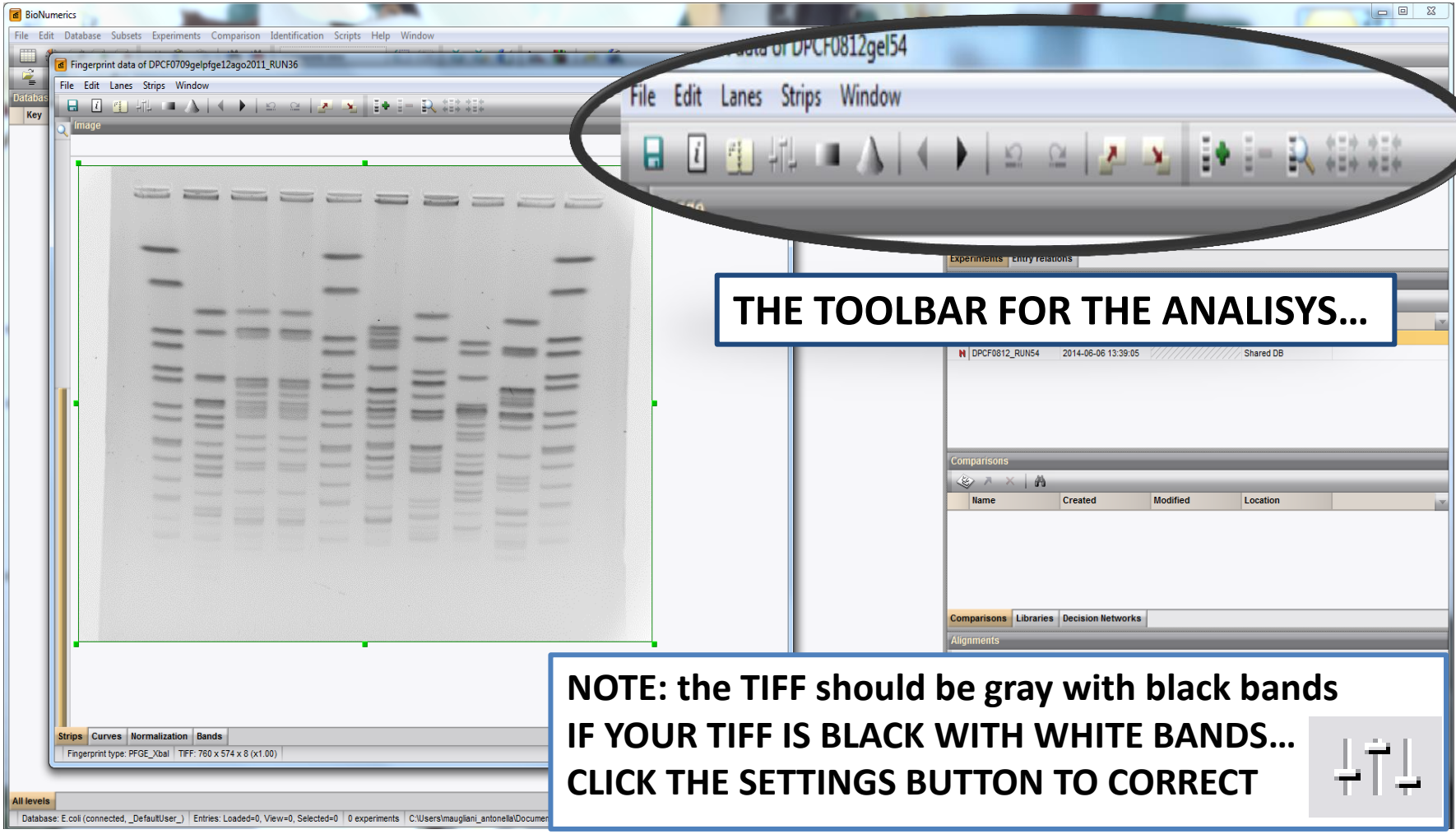
ADD INFORMATION FIELD IN THE DATABASE ENTRIES WINDOWS WITH RIGHT CLICK ON THE MOUSE

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

Step 4: Setting up the experiment