

GelCompar II training

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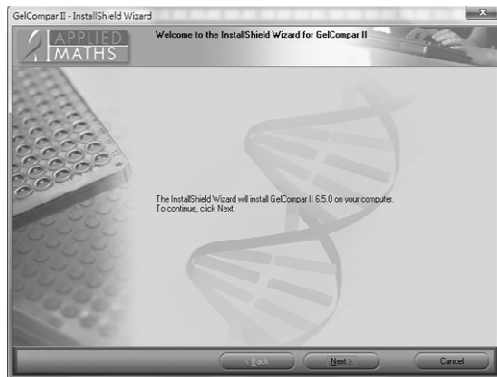


Outline

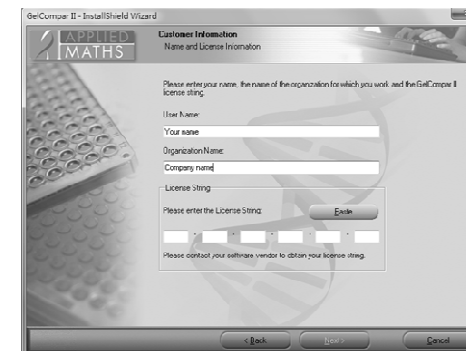
- **Software installation & creating a database**
- Analyzing Gel Images, Entering Data and Linking Images to a Database
- Local comparisons in GelCompar II



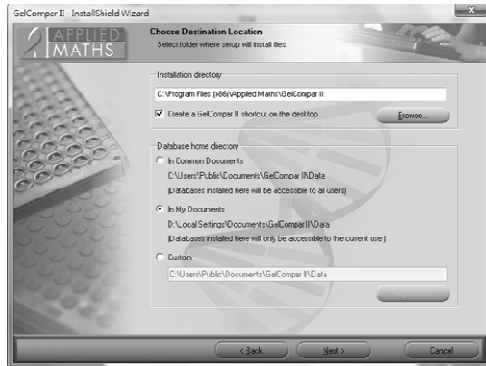
Running install CD



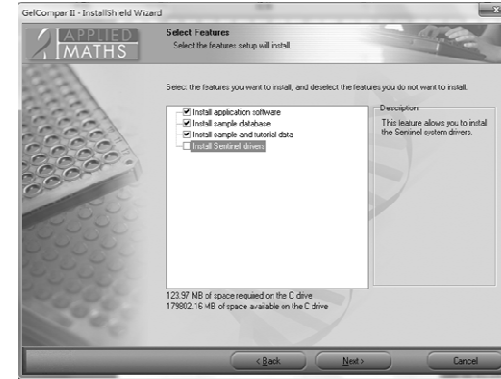
Input Customer information



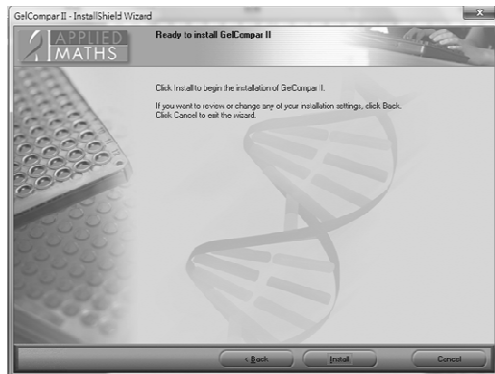
The Choose Destination Location



Install application with software



Install software



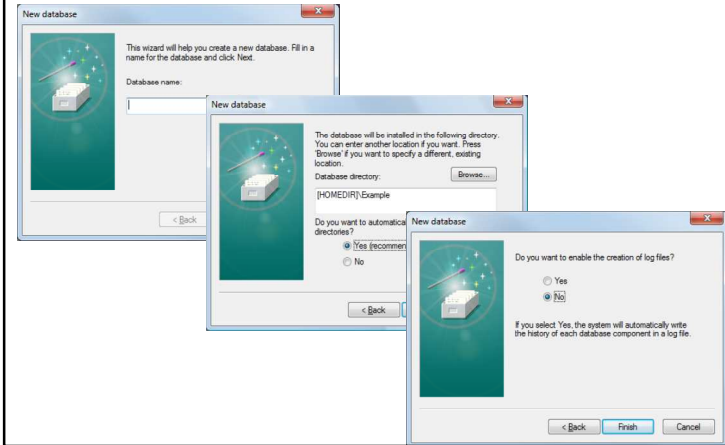
The Startup program appears



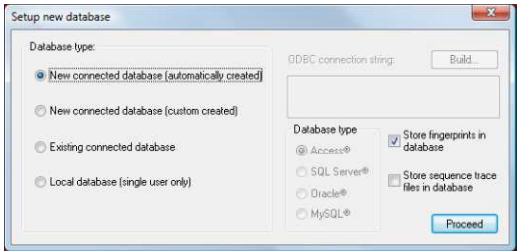
Opening the Database



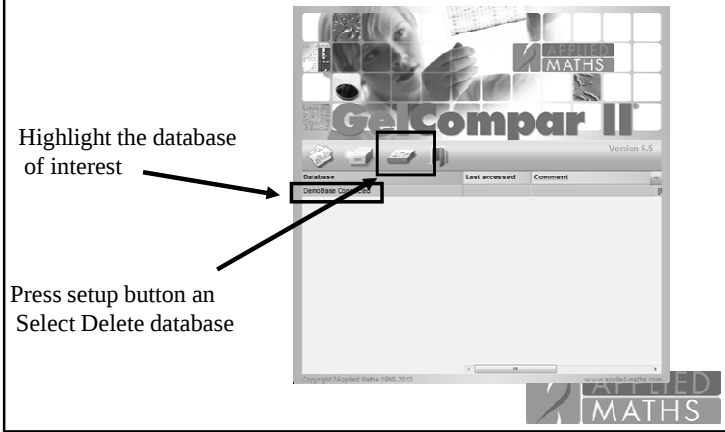
Creating new databases



Selected Database type

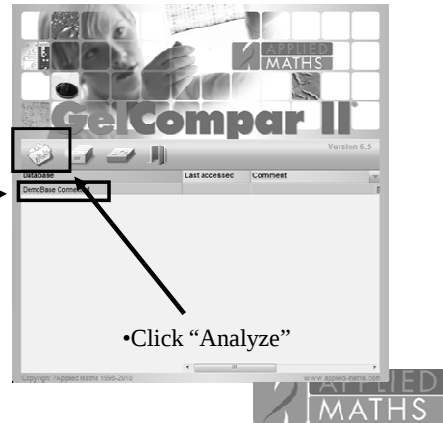


Removing Database

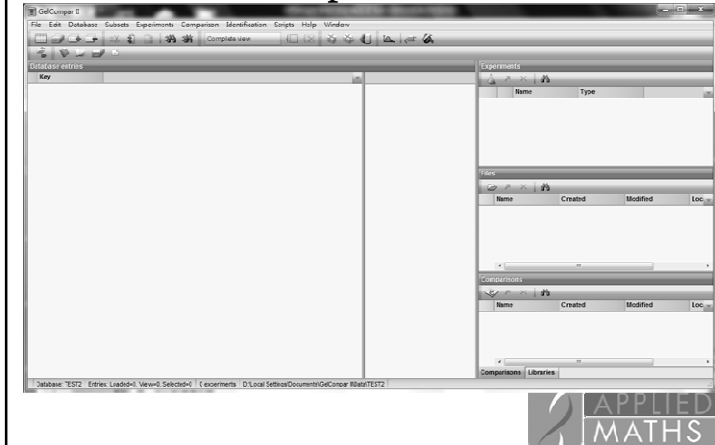


Opening the Database

- Open GelCompar
- Highlight the database of interest



The GelCompar main window



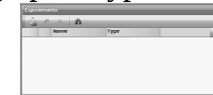
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Creating a new fingerprint type

1. Experiments > Create new fingerprint type from the main menu
2. Enter a name, for example "PFGE-XbaI" and press
3. Press "Next" to next step until "Finish"



Objectives

- I. **Copying a TIFF to the database**
- II. Analyze the TIFF
 - a. Converting a TIFF to gel strips
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 - c. Finding gel bands



Two Ways of Copying a TIFF to the database

- 1) Click on the “folder icon”



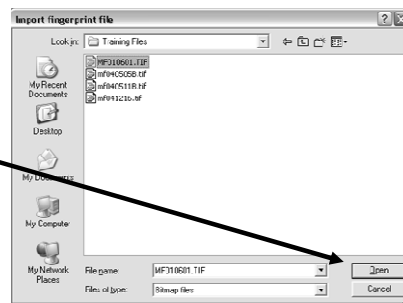
OR

- 2) Right-click under the “Files” area and select “Add new experiment file”



Copying a TIFF to the database

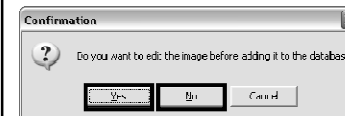
Navigate to the desired TIFF, select it and click “Open”



•Important: No Chinese & symbol in file's name.



Copying a TIFF to the database



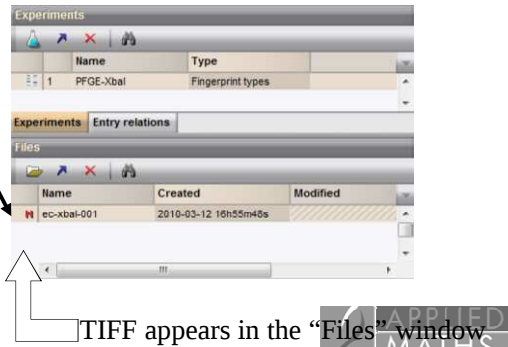
Fingerprint Image Import screen

- crop the TIFF
- alter color and orientation



Copying a TIFF to the database

Red “N” denotes
an unanalyzed
TIFF (= New)



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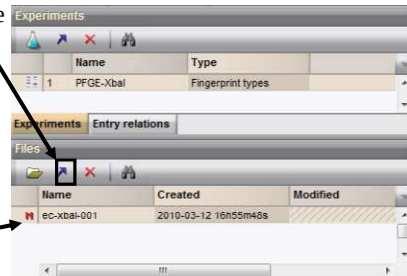


Opening an Imported TIFF

- 1) Highlight the TIFF and click the arrow in the Files window

OR

- 2) simply double-click on the TIFF

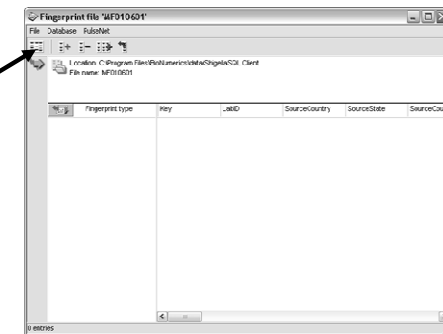


Analysis: Step 1

Click on the



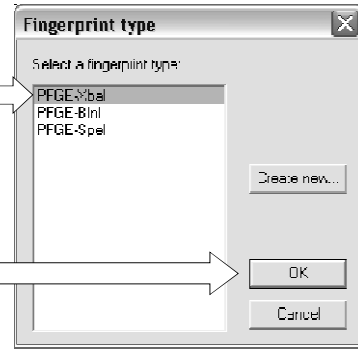
“Edit Fingerprint Data” icon to analyze the TIFF



Analysis: Step 1 Select Fingerprint Type

Select enzyme that was used on that gel (if multiple, select the predominant one)

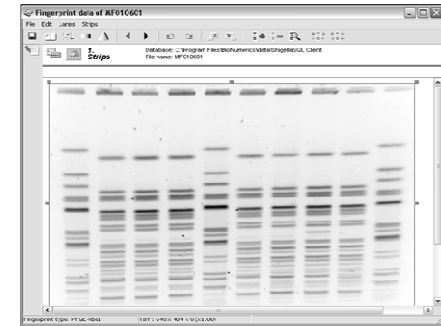
Click "OK"



APPLIED
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Analysis: Step 1

A "Fingerprint Data" window should appear for your TIFF

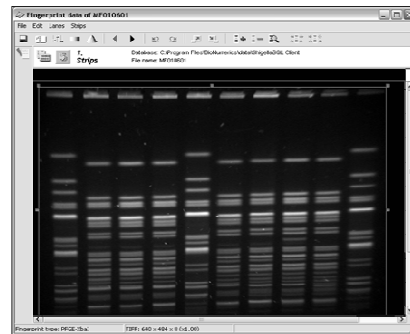


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Analysis: Step 1

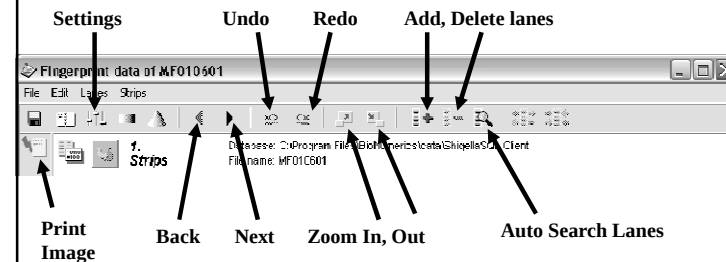
What if your gel looks like this?

Adjust your settings in the Toolbar!!



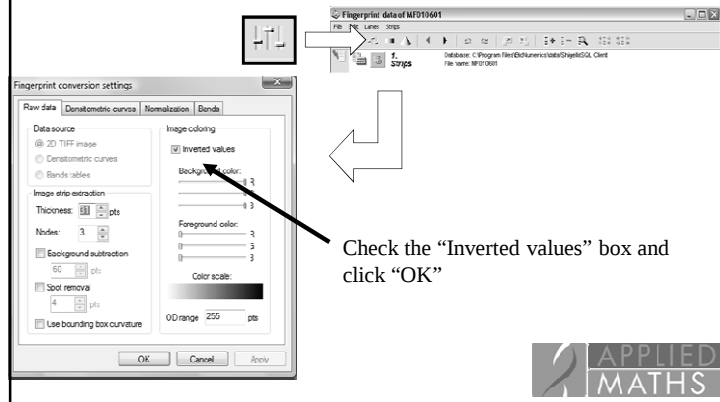
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Analysis: Step 1 Toolbar Overview

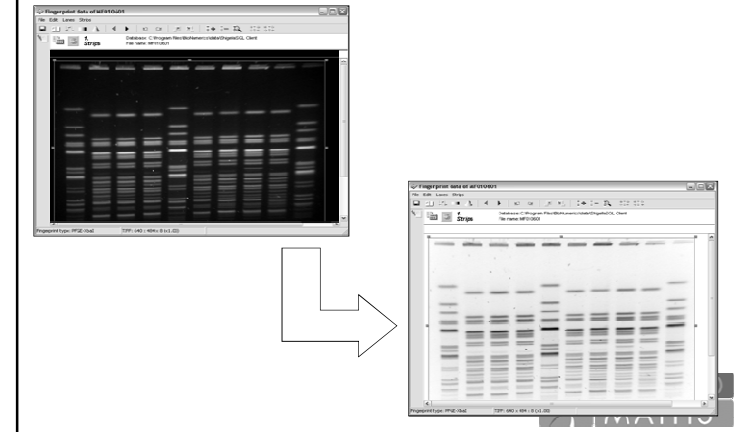


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MATHS

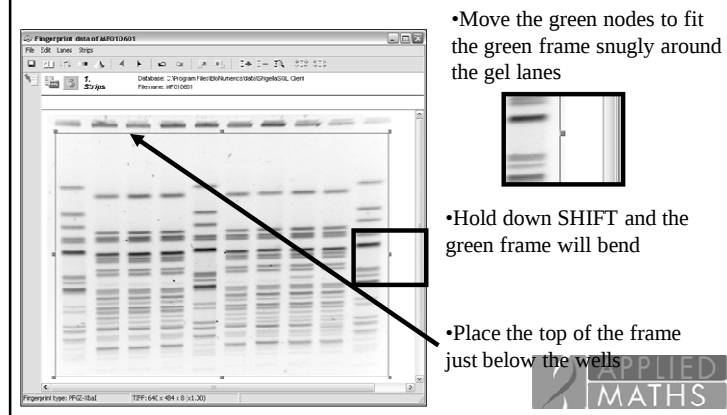
Analysis: Step 1 Adjust your Settings



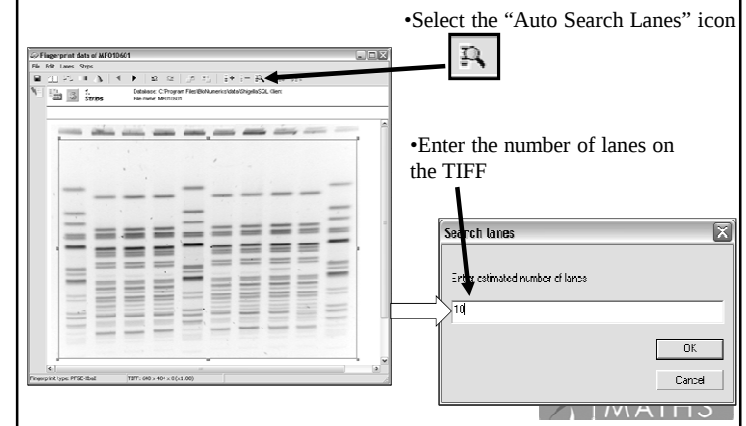
Analysis: Step 1



Analysis: Step 1 Defining area of gel

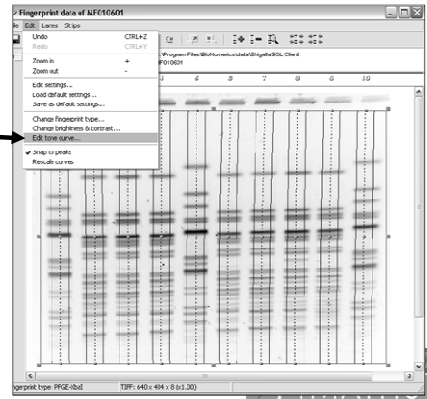


Analysis: Step 1 Auto search for lanes



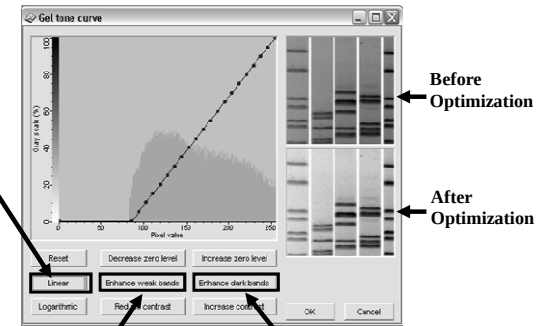
Analysis: Step 1

In the “Fingerprint Data” window under the “Edit” menu select “Edit Tone Curve”



Analysis: Step 1

On the “Gel Tone Curve” screen click the “Linear” button to optimize image



The “Enhance weak bands” and “Enhance dark bands” buttons can be used to further optimize gel with weak or dark bands

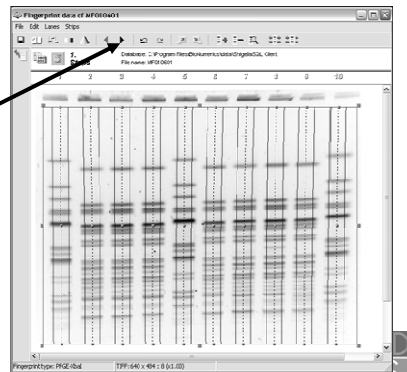


Analysis: Step 1

Click the “Next” arrow

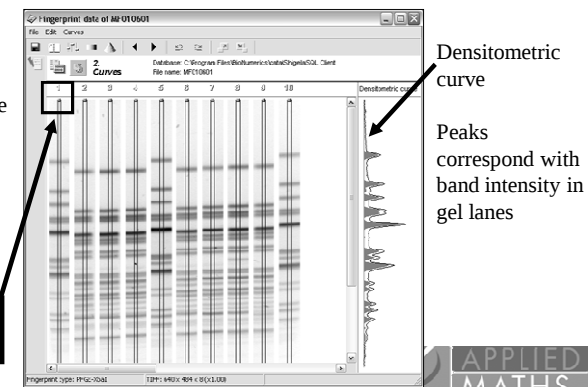


On to Step 2!!



Analysis: Step 2 Defining densitometric curves

Use the blue node to drag the strip to the best area of each lane, avoiding artifacts (specks, etc.) on the gel



Densitometric curve

Peaks correspond with band intensity in gel lanes



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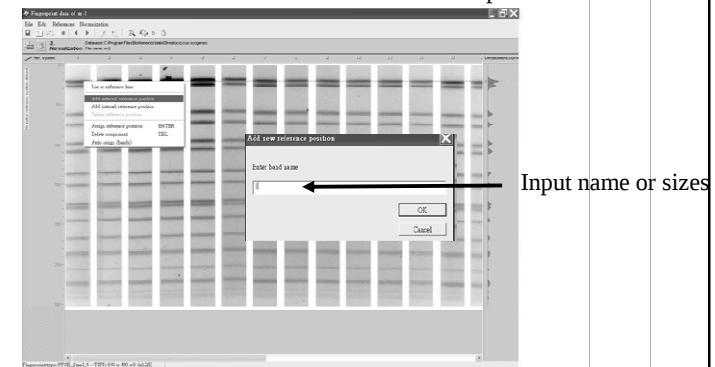
The Experiment with reference system or not



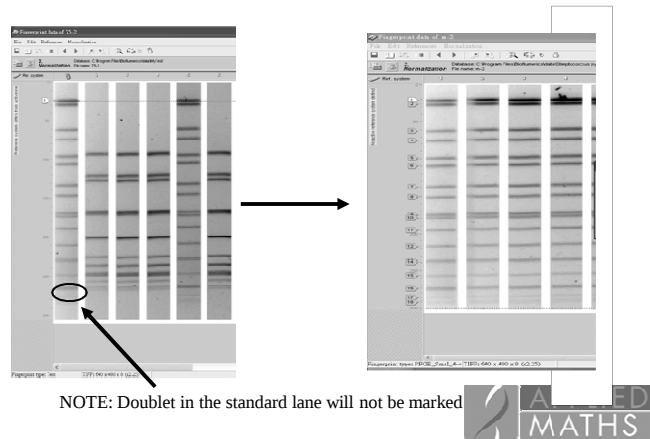
Setup a new Reference system



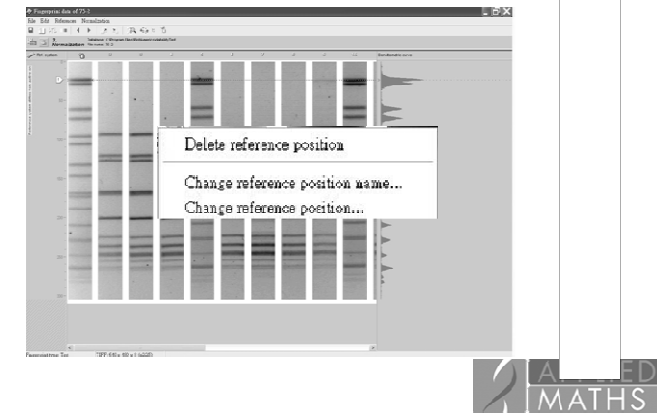
Right click on reference bands →
add external reference position



Setup up as more as possible bands for external reference bands



Right click on reference position →
to Delete or change external reference

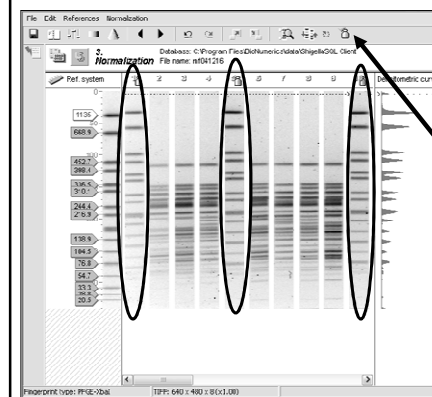


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Analysis: Step 3 Selecting Standard Lanes



1) Select the standard or reference lanes

2) Select the icon to designate the standard lanes

Note: At minimum for proper normalization, the first, last, and every 4th or 5th lane should be a standard lane!!



Analysis: Step 3 Marking to the Global Standard

3 Ways to mark reference bands

1. Auto band assign only
2. Manual band assign only
3. Combination Auto and Manual



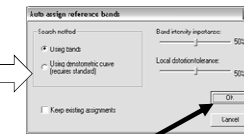
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Analysis: Step 3 Auto band assign only



NOTE: Doublet in the standard lane will not be marked

a) Click the  icon to auto assign reference bands

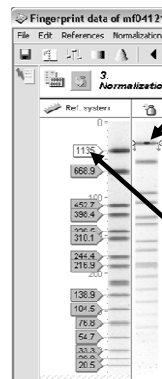


b) Click "Ok" and standards will be automatically marked

c) Check band assignments

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Analysis: Step 3 Manual band assign only



a) Click on its corresponding global standard lane band marker

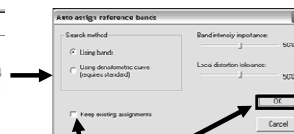
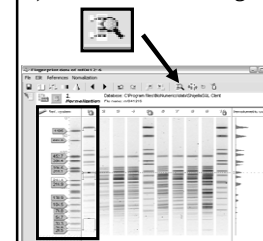
b) Click on the corresponding band in the reference lane and press "Enter"

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Analysis: Step 3 Combination Auto and Manual band assignment

a) Mark top, bottom and middle bands

b) Click the Auto assign icon



c) Keep existing band assignments by checking the box and clicking "OK"

NOTE: Doublet in Standard Lanes are marked differently for different organisms

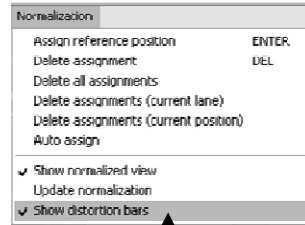
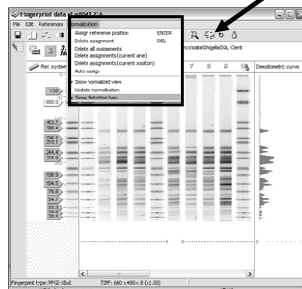
APPLIED
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Analysis: Step 3 Showing Distortion Bars

•Click on the normalization icon to see the normalized view



SAVE YOUR WORK!!!

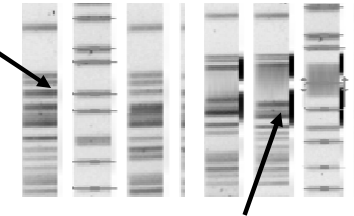


Click on "Show distortion bars" to check normalization

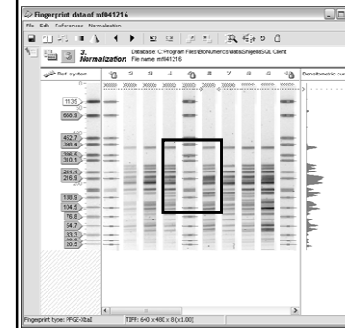


Analysis: Step 3 Showing Distortion Bars

Light color distortion bars in the gel indicate good normalization



Dark colors, especially in one part of the gel indicates poor normalization, possibly due to an incorrect band assignment



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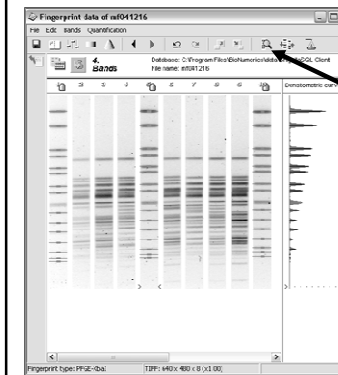


Analysis: Step 4 2 Ways to Mark Gel Bands

1. Auto Band assign



2. Manual Band assign



Analysis: Step 4 Auto Assign Bands

Adjust to alter band detection

Preview band assignments

Assign bands to all or one lane

Click "Yes" to preserve band assignments

Analysis: Step 4 Manual Band Assignment

Click on each individual band and mark it by pressing the ENTER key

Analysis: Step 4 Verify Bands

- Check over your work
- Add or delete bands where needed by selecting the band(s) and pressing the ENTER or DELETE keys

Single Band

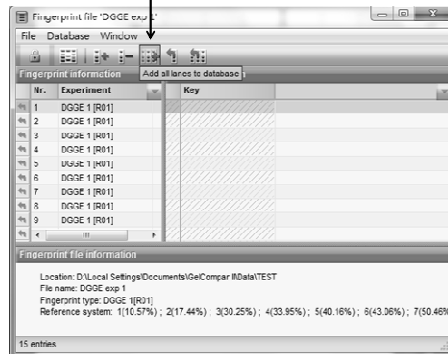
Double Band

• Double, overlapping bands are denoted with arrows on the ends of the band

Analysis: Step 4 Save and Close

- Click on the "Save" icon
- Click "Yes" to save changes
- Click "No" to preserve Default settings

Add all lanes to database



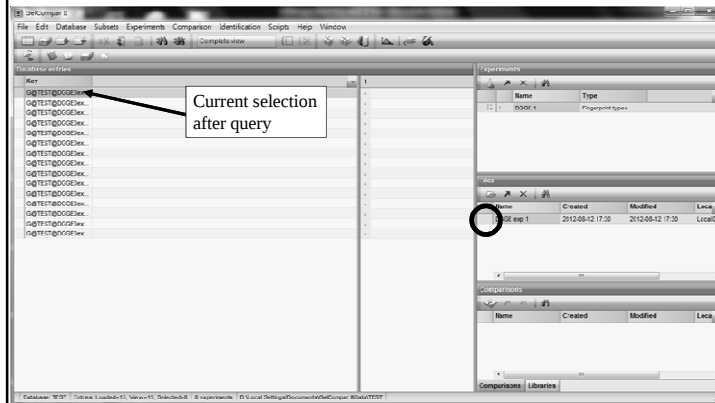
APPLIED
MATHS

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- **Local comparisons in GelCompar II**

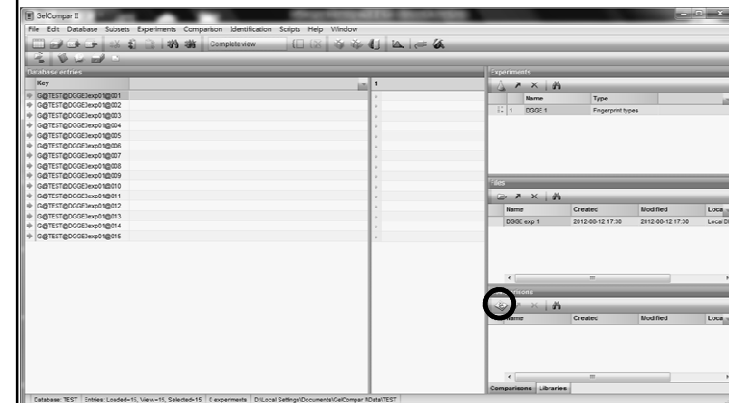
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Create a comparison with the current selection



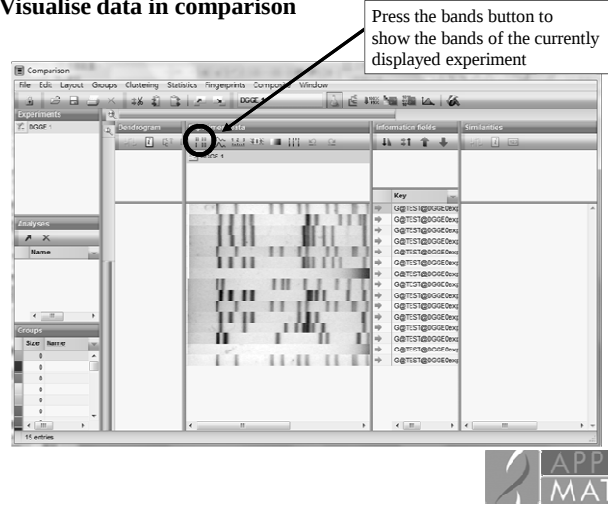
APPLIED
MATHS

Choose a comparison with the current selection

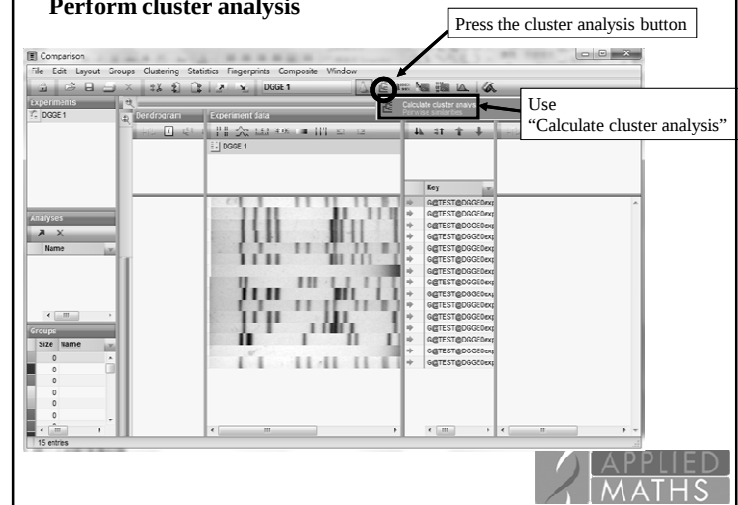


APPLIED
MATHS

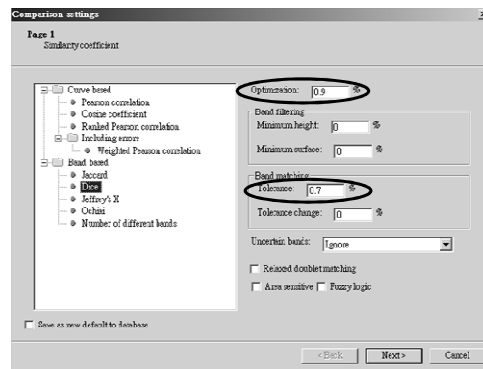
Visualise data in comparison



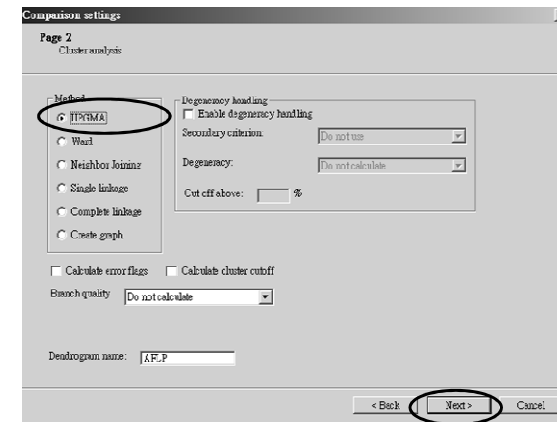
Perform cluster analysis



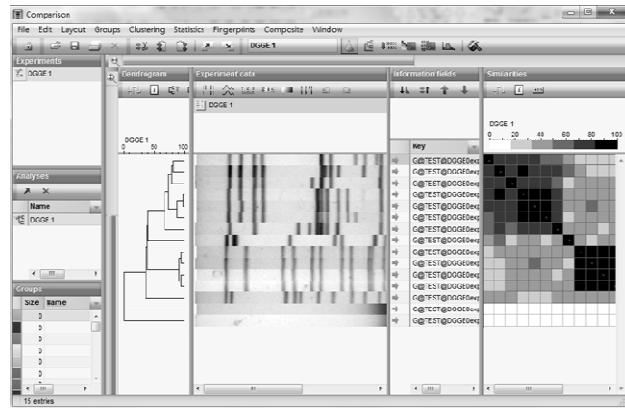
Perform cluster analysis



Perform cluster analysis

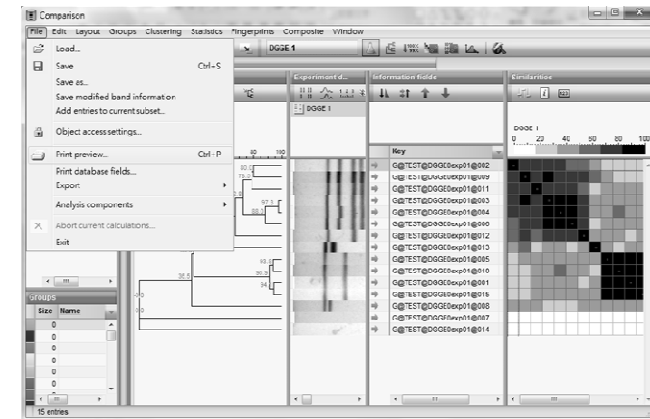


Perform cluster analysis



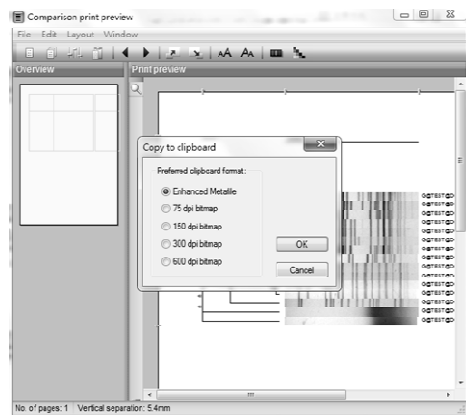
APPLIED
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Export Data



APPLIED
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Export Data



No. of pages: 1 Vertical separator: 5.4mm

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Thanks for your attention !!

