Analyzation of PFGE Gel Images, Linking Gel Lanes, and Entering Data



The National Molecular Subtyping Network for Foodborne Disease Surveillance

Angie Dixon Jen Castleman



May 2009



Overview

Copy a TIFF to the Database Analyze a TIFF Convert a TIFF to Gel Strips Define Curves Normalize the Gel Find Gel Bands Link Lanes to Database Entries Add Text Data for Isolates











Highlight the database of interest

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Databasi	Last accessed	Comment	Version 5.00
Databasi Campy-clien	Last accessed 2008-09-16 14:06	Comment	Version 5.00
Databasi Campy-clien Campylobacta	Last accessed 2008-09-16 14:06 2008-09-05 08:35	Comment	Version 5.00
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Databasi Campy-clien Campylobacta DemoBase DemoBase Ecoli-client	Last accessed 2008-09-16 14:06 2008-09-05 08:35 2008-09-03 15:28 2008-09-04 16:15 2008-09-16 12:58	Comment	Version 5.00
Databasi Campy-clien Campylobacte DemoBase 2D DemoBase Ecoli-client Ecoli	Last accessed 2008-09-16 14:06 2008-09-05 08:35 2008-09-03 15:28 2008-09-04 16:15 2008-09-16 12:58 2008-09-15 09:30	Comment	Version 5.00
Databasi Campy-clien Campylobacte DemoBase 2D DemoBase Ecoli-client Ecoli Ecoli	Last accessed 2008-09-16 14:06 2008-09-05 08:35 2008-09-03 15:28 2008-09-04 16:15 2008-09-16 12:58 2008-09-15 09:30 08-09-09 16:09	Comment	Version 5.00
Databasi Campy-clien Campylobacta Demobase 2D DemoBase Ecoli-client Ecoli ECtrainingSalmonella Listeria Monocytogenes	Last accessed 2008-09-16 14:06 2008-09-05 08:35 2008-09-03 15:28 2008-09-04 16:15 2008-09-16 12:58 2008-09-15 09:30 08-09-09 16:09 09-15 09:00	Comment	Version 5.00
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Click "Analyze"













Click on "Add new experiment file"

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Gel Size

- High resolution: file becomes 3 times larger (~1.2MB)
- Low resolution: fewer pixels, which makes the image smaller and file smaller...requires less space to save (~300Kb)
- Refer to the "Image Acquisition" document:
 CDC Team: CDC PulseNet » Image Acquisition » GelDoc XR Image Acquisition Instructions Amended





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Analyze a TIFF

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Analyze a TIFF





Analyze a TIFF







The "Fingerprint Data" window opens

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Strips Curves Normalization Bands				
Findeminit type: PEGE-Bini TIFF: 567 x 425 x 8 (x1.00)	.1			



NOTE: the TIFF should be gray with black bands



What if your TIFF is black with white bands?





Check the "Inverted values" box and click "OK"

Fingerprint conversion settings 🛛 🔀				
Raw data Densitometric curves Norr	nalization Bands			
Data source 2D TIFF imat Densitometric conves Bands tables Image strip extraction Thickness: Image pts Nodes: Image strip extraction Thickness: Image pts Background subtraction 60 pts Spot removal 4 pts Use bounding box curvature	Image coloring Inverted values Background color: 3 3 Foreground color: 3 Color scale: OD range 255 pts			
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Fingerprint data of CDC08445 Image: Strips Window	
	Place the top of the frame just below the wells
	Make sure bottom line includes all bands
Strips Curves Normalization Bands Fingerprint type: PFGE-Xbal TIFF: 640 x 480 x 8 (x1.00)	



TIFF will not normalize correctly otherwise



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If your TIFF has slanted lanes: hold down SHIFT as you adjust the green box



Fingerprint data of CDC08445 File Edit Lanes Strips Window	
	To define lane strips, click "Auto Search Lanes"
Search lanes	
Viting Commo Monustration Davida	
Finderprint type: PFGE-Xball TIFF: 640 x 480 x 8 (x1 00)	























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

Make sure the strip does not:
1. cut off the edges of the lane,
2. include too much space,
But instead the strip should
include all of the lane







To adjust the brightness of the TIFF, select "Edit Tone Curve" from the "Edit" menu

This step increases the contrast between the bands and the background









Click "Enhance weak bands" and/or "Enhance dark bands" to further optimize gel









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Analysis Step 2: Curves

Use blue node to drag strip to best area of each lane

Avoid artifacts, specks, etc.





*Peaks correspond with band intensity in gel lanes



Click "Next" arrow

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1) Click on standard lanes 🖆 Fingerprint data of CDC08445 File Edit References Normalization 日 酉 北 ■ 人 ▲ ▶ | ♀ ♀ | ▼ ▶ | 段 幅 ♡ 凸 Referenc... Image 3 18 2 4 53 7 <mark>۳</mark>۵ 668.9 452.7 398.4 336.5 244.4 138.9 104.5 78.2 54.7 33.3 28.8 > Strips Curves Normalization Bands Finderprint type: PEGE-Xbal TIFF: 640 x 480 x 8 (x1.00)

2) Click on the weight a constant of the standard lanes constant of the standard lanes constant of

NOTE: For proper normalization, the 1st, last, and every 4th or 5th lane should have a standard





Click "Auto assign reference positions" to assign bands in standard lanes

> NOTE: Only use this tool if gel has minimal artifacts and ghost bands!







If bands were marked before auto assignment, check the "Keep existing assignments" box

Auto assign reference bands	
Search method	
 Using bands 	
 Using densitometric curve (requires standard) 	
Keep existing assignments	
Alignment settings Cancel	













Verify band assignments and good normalization

Fingerprint data of CDC08445 Image: Colored CDC08445 File Edit References Normalization Image: Colored CDC08445 Assign reference position ENTER Image: Colored CDC08445 Delete assignment DEL Image: Colored CDC08445 Delete assignments Delete assignments Delete assignments Delete assignments (ourrent lane) 5% 6 7 8 9%	Click "Show distortion bars" to check normalization
Delete assignments (current position) Auto assign Show normalized view Update normalization Show distortion bars	Normalization
4527 386.4 336.5 310.1 244.4 216.9 138.9 104.5 78.2 54.7 33.3 28.8	Assign reference positionENTERDelete assignmentDELDelete all assignmentsDelete assignmentsDelete assignments (current lane)Delete assignments (current position)Auto assignDelete assignments (current position)
Strips Curves Normalization Bands	 Show normalized view Update normalization Show distortion bars







Results after proper normalization









Band assigned to wrong reference position

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

Save work then click "Next" arrow





Overview

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To add a new band assignment, select it and then press Enter

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To delete a band assignment, select it and then press Delete

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Lane 1 Values are shown on log scale	

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Add or delete bands where needed by selecting the band and pressing the ENTER or DELETE keys on keyboard











If you get this message, click "Yes"

Confirm	ation 🛛 🔀
2	Configuration has been changed. Do you want to save the changes?
	<u>Yes</u> <u>N</u> o Cancel



Click "No" to preserve default settings





Band Marking: Singlets vs. Doublets

Refer to SOP "PND04 Gel Analysis Guidelines" for marking bands





If there is an indentation, then it's marked as a doublet



If there's a difference in color (light/dark can be resolved), then it's marked as a doublet



If there is clear separation, then it's marked as a doublet



If none of the above cases hold true, then it's marked as a singlet





Band Marking: Ghost Bands



Ghost bands should not be markedThe isolate should be rerun





Tips for analysis

Use a printout of your gel to help identify bands
Use the zoom (in/out) buttons for ease of viewing bands

To give better control for band placement on the gel, de-select "snap to peaks" option from "Edit" menu, or hold down the <Tab> key while dragging the mouse within the gel strip When in doubt, mark bands MANUALLY Remember to refer to SOP "PND04 Gel Analysis Guidelines"





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 - Add Text Data for Isolates





If the gel contains lanes restricted with multiple enzymes, the fingerprint type for those lanes should be changed before linking













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NOTE: Do not link standard lanes





Overview

Copy a TIFF to the Database Analyze a TIFF Convert a TIFF to Gel Strips Define Curves Normalize the Gel Find Gel Bands Link Lanes to Database Entries Add Text Data for Isolates





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÷,	5	PFGE-Xbal [H9812Sal]					
•	6	PFGE-Bini [H9812Sal]			CDC 61-99		
•	7	PFGE-BInI [H9812Sal]			CDC 78-99		
4	8	PFGE-Bini [H9812Sal]			CDC 87-03		
-	9	PFGE-Xbal [H9812Sal]			CDC 98-03 (H9812)		
48	10	PFGE-Xbal [H9812Sal]		//			

To enter demographic information, double-click on the key





Click on "Edit database fields" to enter specific demographic information







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Isolate Date Friday , September	26, 2008 💉 Antibi	iotics Match with list OK		Type details	E. coli O100 E. coli O103	
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Serotype information				Traveled To	E. coli O111 E. coli O112	-
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O Group				Isolate status	E. coli O115 E. coli O117	-
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Other State





Add Text Data: Character Data

Entry properties - CDC 61-99			
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Add Text Data: Character Data

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To add Biochemical information, click "Biochemical..."







Add Text Data: Character Data

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Ornithine decarboxylase ?	Gelatin?	Esculin?		
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Malonate?	Trehalose?	Glycerol ?		
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Check info: make note of the format of some information

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Country	USA 🔽	Source Site			mf03320S,3	mf03320S,7	
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Summary

Copied a TIFF to the Database Analyzed a TIFF Converted a TIFF to Gel Strips Defined Curves Normalized the Gel ■ Found Gel Bands Linked Lanes to Database Entries **Added Text Data for Isolates**









Thank you **for your attention** The findings and conclusions in this presentation are those of the author and do not necessarily represent the views of the Centers for Disease Control and Prevention



