Analyzing a TIFF, Linking Lanes, and Entering Data



The National Molecular Subtyping Network for Foodborne Disease Surveillance

Beth McGlinchey April 2011





Overview

Copy a TIFF to the Database Analyze a TIFF Convert a TIFF to Gel Strips Define Curves Normalize Mark Bands Link Lanes to Database Entries Add Demographic Information for Isolates





Open the Database







Open the Database







Copy a TIFF to the Database

Click on "Add new experiment file"

TIFFs should be named according to PulseNet protocol: LabID *Two Digit Year* <u>Three digit number (ex.</u> **GA**10001)

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Copy a TIFF to the Database

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Desktop					Navigate to the
My Documents					desired TIFF, select it
My Computer					and click "Open"
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Places	File name: Files of type:	Bitmap files	•	<u>U</u> pen Cancel	

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Copy a TIFF to the database



TIFF Size

- High resolution: file becomes 3 times larger (~1.2MB)
- Low resolution: fewer pixels, which makes the image 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





Analyze a TIFF







Analyze a TIFF

Click "Edit Fingerprint Data" to analyze TIFF	
	E Fingerprint file 'CDC08444'
	File Database PulseNet Window
	Fingerprint information Entry information
	Nr. Experiment Index Key Level Labib SourceCountry SourceState
	Fingerprint file information
	Location: \kodc\project\CCID_NCZVED_DFBMD_PulseNet\Data\Salmonella-client File name: CDC08444
SERVICES: 10	0 entries





Analyze a TIFF







The "Fingerprint Data" window opens

Fingerprint data of CDC10008	
File Edit Lanes Strips Window	
or limage	
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	×
Strips Curves Normalization Bands	
Fingerprint type: PFGE-Xbal TIFF: 643 × 481 × 8 (×1.00)	

HUNNY SERVICES. Co.

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 Norm	nalization Bands						
Data source 2D TIFF imate Densitometric conves 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: 3 5 Foreground color: 3 Color scale: 0D range 255 pts						
ОК	Cancel Apply						



















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



File Edit Lanes Strips Window	To define lane strips, click "Auto Search Lanes'
Search lanes	
Enter estimated number of lanes	Enter the number of lanes on the TIFF and click "OK"

























The strips can be moved by clicking a blue node and dragging the mouse. If the lane is crooked press the SHIFT key while dragging the node to move a portion of the strip.





1.

2.

3.

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,
- 3. but instead the strip should include all of the lane





To adjust the brightness of the TIFF, select Edit→"Edit tone curve"



This step increases the contrast between the bands and the background













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





2. Click on the weight a local standard lane

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 ghost bands and artifacts are present manually assign bands.







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

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













Verify band assignments and good normalization

File Edit References Normalization Reference Assign reference position ENTER Delete assignment Delete assignments Delete assignments (current lane) Delete assignments (current position) Auto assign * Show normalized view Update normalization Show distortion bars	Click Normalization→"Show distortion bars" to check normalization		
452.7	Normalization		
	Assign reference position ENTER		
	Delete assignment DEL		
	Delete all assignments Delete ansienseets (summet less)		
	Delete assignments (current lane)		
	Delete assignments (current position)		
20.5	Auto assign		
	Show normalized view		
Strins Curves Normalization Bands	Update normalization		
Fingerprint type: PEGE-Xhal TIFE: 640 x 480 x 8 (x1.00)	Show distortion bars		







Results after proper normalization









PFGE Patterns of *E. coli* O157:H7









To add a new band assignment, select the band and then press the ENTER key



Hold down the TAB key while dragging the mouse to get better control of band placement or de-select "Snap to peaks" under the "Edit" menu





To delete a band assignment, select the band and then press the DELETE key









To mark a band as uncertain select "Mark band(s) as uncertain" from the "Bands" menu



NOTE: use this function sparingly







Fingerprint data of CDC10008_Demo		🔳 🖬 🔳
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		>
Strips Curves Normalization Bands		
Fingerprint type: PFGE-Xbal TIFF: 643 x 481 x 8 (x1.00) 0 ba	ands	























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







Ghost bands should not be marked
The isolate should be rerun











Confirmation 🛛
Configuration has been changed. Do you want to save the changes?
<u>Yes</u> <u>No</u> Cancel
Click "Yes" to save changes
Confirmation
Settings have been changed Do you want to use the current settings as new defaults?
<u>Y</u> es <u>No</u>
Click "No" to preserve default settings





Tips for Analysis

- Use a printout of your TIFF 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"





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

	+ - + 1 11			_		Select/unselect entry	
Finger Nr.	rprint information	Entry information Index Key	LabID	SourceCoun		Add lane to database Add all lanes to database	
 1 2 3 4 5 6 7 8 9 10 	PFGE-Xbal [H9812Sal] PFGE-Xbal [H9812Sal] PFG H9812Sal] PFG H9812Sal] PFGE-Xbal [H9812Sal] PFGE-Xbal [H981 PFGE-Xbal [H9812Sal] PFGE-Xbal [H9812Sal]	Open entry Select/unselect entry Add lane to database Add all lanes to databas Link lane Remove link Change fingerprint type	e of lane.			Link lane Remove link > Change fingerprint type of lane	
*		Righ "Cha	t-cl	ick on e finge	the lan erprint t	e and select ype of lane"	Ċ/







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4	5	PFGE-Xbal [H9812Sal]								2	
4	6	PFGE-Xbal [H9812Sal]				///////				2	
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Lane 2 now has BlnI as the enzyme used during restriction





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9 PFGE-Xbal [H9812Sal]				
	<u> </u>			
Fingerprint file information				









Reminders: Duplicate Isolates

- Do not upload duplicate database entries
- If there are multiple picks from one culture, we recommend labeling them 123a, 123b, 123c, or in a similar fashion
 - Only upload those with different PFGE patterns
- When a repeat isolate is added into your database, BioNumerics automatically adds "/#" to the end of the key
- If you are re-uploading an unsatisfactory pattern, contact CDC before uploading so the previous pattern can be deleted beforehand and the better pattern confirmed
- If you upload a pattern that has already been named and confirmed, you will receive an error message. Contact CDC for assistance in completing this process.







File Dat	erprint file 'MF030627' tabase PulseNet Window It IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	У		
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n 3	PFGE-Xbal [H9812Sal]		CDC 78-99	
† 4	PFGE-Xbal [H9812Sal]		CDC 87-03	To enter
n 5	PFGE-Xbal [H9812Sal]			
1 6	PFGE-Bini [H9812Sal]		CDC 61-99	demographic
🐀 7	PFGE-Bini [H9812Sal]		CDC 78-99	information
1 8	PFGE-BInI [H9812Sal]		CDC 87-03	Information,
🐀 9	PFGE-Xbal [H9812Sal]		CDC 98-03 (H9812)	double-click on
🐀 10	PFGE-Xbal [H9812Sal]			
				the key





Fingerprint file 'MF030627' File Database PulseNet Window **Click on "Edit database** 133 | i+ i- iik 11 fii fields" 😼 to enter $\langle \varphi \rangle$ Fingerprint information Entry information specific demographic Nr. Experiment Key PFGE-Xbal [H9812Sal] **m** 1 information **h** 2 PFGE-Xbal [H9812Sal] CDC 61-99 • 3 PFGE-Xbal [H9812Sal] CDC 78-99 **t** PFGE-Xbal [H9812Sal] CDC 87-03 1 5 PFGE-Xbal [H9812Sal] 🝯 Entry edit PFGE [H9812Sal] File Edit Attachments Window 1 **h** 9 PFG (119812Sal) Database fields Experiments 10 PFGE-Xbal [H9812Sal] 🔋 🏂 🗙 🖳 🔿 🗸 Ok 🗡 Cancel FGE-BIN Key CDC 61-99 PFGE-Spel - ^ PFGE-Xbal antibio iochem Experime... Attachme... Fingerprint file information





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			Other State Isolate number	,		City	Mobile	~	Serotype	Typhimurium
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Add Text Data: Character Data



Add Text Data: Character Data

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SSS	?	*					
SULFONAMIDES							

Use pull down menus to enter appropriate data

AMK	?	<	
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AMPC	?	*	
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AMPICILLIN	Susceptible		
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AZITHROM	YCIN		





Check info: make note of the format of some information

Entry properties - CD	C 61-99			<u> </u>	
Source	Seroty	pe Typhimurium		•	
City Atlanta	Outbre	ak 0706PAJPX-1			
County Deklalb	Source	e Type Food	Patterns		
State GA	👻 Туре [Details Chicken	Xbal Mf030319,3	Bini Mf030319,7	
Country USA	Source	e Site CSF 🗸	mf03320S,3	mf03320S,7	
Patient	Travel	ed To Jamaica 💌	CDC08445,2		\sim
Age 12 yr 3	mn 6 dy Expos	ure Restaurant 🗸			
Sex MALE	V Isolate	status Confirmed 💌			
	Other Isolate	State number			
		D:			
Upload Date:	NA Foo	RMS-EB odNet			
Isolate Date 🔽 Fr	iday , October 10,2008	·		1	
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HUMAN SE	RVICES-IN	ReceivedDate, etc.) Ex: 2011-03-04			

Reminders: Text Data for Isolates

Provide as much information as possible

- Species/Serotype: do not leave this field blank (use serotype pending, undetermined, isolate to CDC) pattern name cannot be "confirmed" until this is entered
- Source State: extremely important for initial investigation
- Source City, County if known
- Source Type: *must* be one of these five: Animal, Human, Environment or Environmental, Food, Unknown
- Source Site: i.e. stool, urine, blood, etc.
- Patient Age and Sex
- Dates: Isolation and Received
- Type Details: use to provide more information about source site
- If importing, remember to check all data for accuracy









The National Molecular Subtyping Network for Foodborne Disease Surveillance

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





Exercise 1