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

# Image analysis: profiles analysis and interpretation

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



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

# **Step 4: Select the experiment created**



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

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

File     Edit     Lanes     Strips     Window       Databas     Image     Image     Image     Image	Experiments
	Place the top of the frame just below the wells
	Name         Created         Modified         Location           N         DPCF0709gebrye12a         2014-06-06 13:38:27         2014-06-06 13:38:27         Shared DB           N         DPCF0812_RUN54         2014-06-06 13:39:05         2014-06-06 13:39:05         Shared DB
	Comparisons Rev A Manager Created Modified Location
	includes all bands
normalization process will not be d	one correctly otherwise!

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

BioNumerics           File         Edit Database Subsets Experiments Comparison Identification Scripts Help Window	
Image       File       Edit       Lanes       Strips       Window         Image       Image       Image       Image       Image       Image	To define lane strips, click "Auto Search Lanes"
	Finderprint types
	Search lanes
	Enter estimated number of lanes     fiel     Location       10     06-06 1339/05     Shared DB
	OK Cancel Ified Location
Finge	Enter the number of lanes
	Alignments       Image: Name     Created     Modified
Strips         Curves         Normalization         Bands           Fingerprint type:         PFGE_Xbal         TFF: 760 x 574 x 8 (x1.00)	
D entries preparatese: c.cor (comected, _perantoser_/ = nimes: coaper/v, vew/v, percept/v = 1 experiments = c.rosers/marytain_antoneia.cocoments/bonome	Alignments Chromosome comparisons Annotations Power assemblies

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



# 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

## **Step 4: CORRECT ASSIGNMENT OF STRIPS WIDTH**



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



# **DEEP INTO THE ANALYSIS**

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# **Step 4: DEFINE CURVES**



## **Step DEFINE CURVES**



# **Step 4: DEFINE CURVES**



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



# **Step 4: DEFINE CURVES**

and data of DPCF0709gelpfge12ago2011_RUN36	
Edit Curves	
	Densitometric curve
Click "NEXT" arrow from the toolbar	
Trips Curves Normalization Bands	· · ·

- Analyze a TIFF
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## **Step 4: NORMALIZATION**



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

Fingerprir	nt data	of DPCF0709gelpfge12ago2011_RUN36	all or it	and the state of t		- 0 - X
File Edit	Refere	nces Normalization		Click on «REFERENCES»		
<b>.</b> 1	Ô	Use as reference lane Ctrl+R	4 5g 6 7 8 9 10g	AND SELECT «USE AS		
Q Referei		Use all lanes as reference lanes		REFERENCE LANES»		
		Add external reference position				
7		Add internal reference position				
defin.		Delete reference position			-	
stem.		Change reference position name			-	
s s		Change reference position				
eferenc		Copy normalization				
<u>ve</u>		Paste normalization				
o <mark>act</mark>	_					
			For t mole the k <i>Brae</i>	he first gel in the data k cular weight must be a oands of the marker ( <i>S.</i> <i>nderup</i> H9812 strain)	base ssigned	to

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

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

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



**REFERENCE SYSTEM** 

# **THE REFERENCE BANDS POSITIONS**

coli O157 nd Shigella 16s-54.17s	non-0157 STEC 6.76s-35.38s	N
	$     \begin{array}{c}       -452.7 \\       -398.4 \\       -336.5 \\       -310.1 \\       -244.4 \\       -216.9 \\       -167.1 \\       -138.9 \\       -104.5 \\       -76.8 \\       -54.7 \\       -333.3 \\       -28.8 \\       -28.8 \\       -20.5 \\    \end{array} $	

E.

ar 2.

Band	Molecular weight
Number	(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

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



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

-	🖻 Fi	ngerpri	nt data of DP	CF0709gelpfge12ago	2011_RUN36						
Fingerprint data of DPCF0709gelpfg	File	Edit	References	Normalization							
		i			2 2 2	<u> </u>	R	Han U C	Development		_
	Q	Referen	ice system	_	_	- 6	Auto a	ssign reference positions	Densitometric	rcurve	
1135			- 0 -				Click	( "Auto assi	gn refer	ence posit	ions"
ty stem d		4400	-	,		-	to 20	scian handa	in tha c	thor	
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100-	Ν.	668.9	100-			•	stan	dard lanes			•
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244.4	н	104.5		and the second s	and and a second s	and the second second					
210.9		76.8		Same second second second	a and a second second		and the second second				
	H.	54.7		and the second second	and the second second	in and the second		Keep existing assig	gnments	ОК	
138.9 300-	11	33.3	400-								
104.5								Alignment settings		Cancel	
				inter protocol andorete automot							
76.8											
54.7											
33.3											
					m				ь		
Strips Curves Normalization Ban Fingerprint type: PFGE Xbal TIFF: 760 x											

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



# **Step 4: NORMALIZATION**



# **Step 4: NORMALIZATION**



## **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

# **Step 4: NORMALIZATION**



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# **Step 4: BAND ASSIGNMENT**

File Edit	Bands	f DPCF0709ge Quantification	lpfge12ago2(	011_RUN	36 Ali 🔁	Auto search bands	
strips Cur	ves No.	3	Bands	5	6	Image: The search       Search on all longs         Image: The search on this lange       The search on this lange         Image: The search on this lange       The search on this lange         Image: The search on this lange       The search on this lange         Image: The search on this lange       The search on this lange         Image: The search on this lange       The search on this lange         Image: The search on this lange       The search on this lange         Image: The search on this lange       The search on this lange         Image: The search on this lange       The search on this lange         Image: The search on this lange       The search on this lange         Image: The search on this lange       The search on this lange         Image: The search on this lange       The search on this lange         Image: The search on the search on the search on this lange       The search on t	

# Step 4: BAND ASSIGNMENT



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

# Step 4: BAND ASSIGNMENT





## **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 *Xbal* 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

# THIS GEL IS IT SWITABLE?





# AND THIS ONE?





# 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 ASSIGMENT - Validation of doubtful bands**



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# **Step 5: LINK LANES TO DATABASE ENTRIES**

#### **BioNumerics** WHEN THEW LANES ARE ADDED TO DATABASE A Experiment Edit Subsets File Database 21 indo. PINK ARROW WILL APPEAR IN THE FINGERPRNT **INFORMATION WINDOW** Database entries Key G@images@DPCF0709gelpfge12ago2011\_RUN36@001 G@images@DPCF0709gelpfge12ago2011 RUN36@002 Fingerprint file 'DPCF0' G@images@DPCF0709gelpfge12ago2011\_RUN36@003 File Database Window G@images@DPCF0709gelpfge12ago2011 RUN36@004 G@images@DPCF0709gelpfge12ago2011 RUN36@005 G@images@DPCF0709gelpfge12ago2011 RUN36@006 Fingerprint information G@images@DPCF0709gelpfge12ago2011 RUN36@007 Nr. Experiment G@images@DPCF0709gelpfge12ago2011 RUN36@008 G@images@DPCF0709gelpfge12ago2011 RUN36@009 PFGE\_Xbal [R01] **n** 1 G@images@DPCF0709gelpfge12ago2011 RUN36@010 **a** 2 PFGE\_Xbal [R01] PFGE Xbal [R01] **h** 3 4 4 PFGE\_Xbal [R01] **KEY ENTRY WILL BE h** 5 PFGE Xbal [R01] AUTOMATICALLY PFGE\_Xbal [R01] 6 PFGE\_Xbal [R01] **n** 7 CREATED IN THE PFGE\_Xbal [R01] **a** 8 9 PFGE\_Xbal [R01] DATABASE ENTRIES 10 PFGE\_Xbal [R01] FOLDER (G@images@name of the Alignments Chromosome com TIFF@number of lane)

# Step 5: SETTING UP THE MOLECULAR STANDARD



# **Step 5: SETTING UP THE UNIVERSAL MOLECULAR STANDARD**



- Analyze a TIFF
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# IN DEPTH OF THE EXPERIMENT: TIFF ANALISYS AND PFGE PROFILING INTERPETATION

# Step 6: Add Text Data for Isolates

Bie File	one Hononerics	Scripts Help Window						
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	😤 🔇 🏟 🔐 🗉			× A	Туре			×
Da	atabase entries			PFGE_Xbal	Fingerprint type	S		
	Кеу	Add new information field						
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	G@images@DPCF0709gelpfge12ago2011_RUN36@002							
	G@images@DPCF0709gelpfge12ago2011_RUN36@003			Entry relat	ons			
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	G@images@DPCF0709gelpfge12ago2011_RUN36@007			12_10104	2014-00-00 10.00.00	2014-00-10 11.31.13	Shared DD	
	G@images@DPCF0709gelpfge12ago2011_RUN36@008							
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# LETS' 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**

