Image analysis: profiles analysis and interpretation

Step 4: DEEP INTO THE ANALYSIS

- Analyze a TIFF
- 1. Convert a TIFF to Gel Strips
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- 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»		
. 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

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Fingerprint data of DPCF0709gelpfg	File	Edit	References	Normalization							
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	Q	Referen	ice system	_	_	- 6	Auto a	ssign reference positions	Densitometric	rcurve	
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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

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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

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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

