

Scout2.1.10 软件数据分析指南

- 1) Scout软件兼容64bit, 16GB RAM的Windows7以上的电脑。
- 2) 软件下载地址: <u>http://www.proteinsimple.com/scout/downloads/</u>
- 3) 安装完成后,双击警图标,打开Scout软件。
- 4) 如需打开保存的 .tiff 文件有以下两种方法: File-Add scan to current

chip-New auto registration或者点击 D 图标Add new scanned

image.		
👈 Scout		* Scout
File Edit Tools Help		File Edit Tools Help
Add scan to current chip >	New auto registration	
Open existing .zcp file	New manual registration	
Save all to .zcp file	Copy registration from current scan	Add new scanned image (.tif) file
New chip	Add scans from .zcp file	
Exit		

5) 如果您对.tiff的文件进行了修改,可以将它保存为.zcp文件(File-Save all to .zcp file),方便下次直接调取,否则需重新分析结果,且该软件 没有返回键。另外您也可以将其导出成.csv和.fcs文件(Tools-Export CSV/FCS),用其他软件打开,进行后续分析。



6) 如需打开.zcp文件有以下两种方法: File-Add scan to current chip-Add scans from .zcp file或者File-Open existing .zcp file.

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 File
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 Tools
 Help

 Add scan to current chip
 New auto registration

New auto registration	Add scan to current chip >
New manual registration	Open existing .zcp file
Copy registration from current scan	Save all to .zcp file
Add scans from .zcp file	New chip
	Exit
	New auto registration New manual registration Copy registration from current scan Add scans from .zcp file

7) 判定蛋白迁移方向。可通过查看最上面一排样品的迁移方向判断,如 果信号在最上面一排孔的上面,选择 Up; 如果在最上面一排孔的下





8) 如果自动校正失败,会弹出如下对话框,此时您需要选择Manual手动 校准。



9) 手动校准时您可以选择电泳方向,以及 选择采用哪两个block的第一排和最后 一排作为基准,然后点击Start Registration, block之间距离越远,校 准效果越好。

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Select registration points		
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Start Registration	8	





 在弹出的对话框中,调整上面的直方图或单击并拖动图像上的鼠标, 进行图片对比度调整。

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12)Scout软件将自动将有信号的泳道加绿框,无信号的泳道加蓝框。



13)如需改变某张图片目的峰识别的参数设置,可在Edit-Scan properties 修改,如下图。包括泳道起点,终点,宽度;电泳方向;峰宽,信噪 比;基线计算方法等。

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Changes dimensions of	Lane width (um)	125
lanes used for detection	Lane start (um)	75
	Lane end (um)	825
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direction in image		Down
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Cata imaga proprocessing	Enable preproces	ssing
(typically leave as default)	Rotation (degrees)	90
(typically leave as default)	Filter radius (pixels)	5
	Filter threshold	500
Parameters used in	Peak SNR Threshold	3
peak detection	Peak width factor (um)	150
algorithm	Peak slope threshold	0.05

Area ignore threshold

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OFlat

Save as Default

Different methods of setting peak baseline

Baseline Method Two point Re-detect Peaks

14)如果信号太强或者跑的太慢太快,超出了绿色边框,可通过lane width/start/end调整泳道的宽度,起点和终点,如下图。

	• •	• •	• • •	• •	• •
👈 Scan P 🗕			👈 Scan P —		
Lane width (um)	200		Lane width (um)	125	
Lane start (um)	75		Lane start (um)	75	
Lane end (um)	825		Lane end (um)	825	
Electrophoresis Direc	tion		Electrophoresis Direc	tion	
	Down			Down	

15) 通过调整Peak width factor可更好的识别宽的或者狭长的目的信号。 16) 如果目的信号跑入下一个泳道,上一个微孔正好位于斑点内,为了准 确定量,您需要将Baseline Method由默认的Two-point baseline改为 Flat baseline。如下图所示,红线即为baseline(基线),阴影部分为峰 面积(表达量)。同一个泳道,采用不同的方法扣背景,最终Area大不 相同。





17)如果采用滤纸轻压的方式将芯片分成不同区域,用于同时分析不同细胞样品或者利用三抗体孵育槽,孵育不同的抗体,那么有些区域属于无效区域,可通过 □选中该区域,此时被选中的泳道会自动高亮,点击鼠标右键Mark as Rejected,将其剔除,此时该区域的泳道变为红色。

	Reset and Autodetect [a]	
	Mark as Rejected [r]	
	Mark as Empty (Manual) [m]	
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19)如果异常的泳道太多,手动修改太麻烦,您可以通过修改SNR/信噪比 进行批量修改: Edit-Scan properties,在弹出的对话框中修改SNR 值,然后点击Re-detect Peaks.



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 20)如果不知道SNR值设置为 多少比较合适,您可以选 择某个有代表性的泳道, 点击鼠标右键Plot
 Selected Correlations-Curves,如右图。



21) 弹出该泳道相对的SNR值, 见右图,如果您需要把该泳 道剔除,则SNR值需要设置 高于其Max值,即41,这时 其他比该泳道信号弱的泳道 也一并被剔除了。



22) 泳道核对之后,点击 Tools-Peak tables-Show/Update peak table。





23)出现信号散点图后,点击 Peak Table-Auto Tag或者点击
 Point框进去,然后 Peak Table-Apply Tag To Selected Peaks进行靶信号的命名。

to Peak Table: Chip 463,AML1_5_um − □ ×	1	Peak Table: Chip 463_AML1_5_um
Auto Tag	File	Peak Table
Apply Tag To Selected Peaks [t] Remove Tag From Selected Peaks [u] axis Variable: Index Global Col Row	SA	
Edit Peak Tag agged	9	Auto Tag
Delete a Peak Tag Replace Peak Tag		Apply Tag To Selected Peaks [t]
Clear All Peak Tags		Remove Tag From Selected Peaks [u]
Scan Image Selection		Edit Peak Tag
Tag Duplicates Within a Lane Tag Matching Peaks / Stripping Efficiency		Delete a Peak Tag
		Replace Peak Tag
	1	Clear All Peak Tags
2300 State S		Peak Table Selection >
200	Ě	Scan Image Selection >
100		Tag Duplicates Within a Lane
0 1000 2000 3000 4000 5000 6000 Index Global Col:Row		Tag Matching Peaks / Stripping Efficiency

24) 散点图聚类表示同一个靶点信号。右图 Num expected peaks 选择 1,表示泳道中有1个靶点。左图聚类散点图的上下两侧表示分子量 太大或太小的非靶点信号。或者您也可以通过参数设置进行筛选, 一般选用Peak Center和Peak Fill Factor两个参数。



25)点击<Create New Tag>,然后点击Apply或者直接双击<Create New Tag>给靶点命名。



X

Cancel

Select a tag to Apply:

< Create New Tag >

Apply

Excluded Target <Name> NoiseLike AutoExcluded

26)例如Name设置为AML1,点击OK。

Peak lable: Chip 463_AML1_5_um — 🗆 X		🙁 Peak Tag Properties 🚽 🗆	×
🔍 ् 🖑 🐔 🖬 🎗 🖉 🕶 🚥 😽 🕹 🐘 🗶 🗡	Excluded	-	
Y-axis Variable: PeakCenter V X-axis Variable: Index Global Col.Row V	Target <name></name>	Name: AMI 1	
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	< create new tag >	Color: Marker:	~
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$\begin{array}{cccccccccccccccccccccccccccccccccccc$		OK Cancel	
0 1000 2000 3000 4000 5000 6000 Index Global Col:Row			

28)此时散点图如图所示,每一个点相当于传统 WB中的一条Band,同一个Lane中可能包含 多个Band,即多个Point,当同一个泳道中多 个点被命名为一个Tag时,即为 Duplicate。 Peak Table-Tag Duplicate Within a Lane将

duplicate lane显示出来。此时您需要将同一个泳道中的非特异性 Point删除,我们可以看到靶蛋白的Peak center基本在300-400之间, 显然绿色方框指示的这个Point是杂带,您可以利用 II 或 3 选中该 点,此时它会变成绿色高亮,然后点击 4 进行删除。



²⁷⁾ 在弹出的对话框中选择之前命名的靶点,并 点击Apply。



29)如果Duplicate比较多,且与Target Point重叠或者离得较近,不易操作,此时您可以在芯片上点击鼠标右键Select-Select by Peak Tags,在弹出的对话框中选择Duplicate-OK,然后在芯片上点击鼠标右键Apply Selection-Select on Peak Table



- 31) 在扫描芯片图上右键, Select-Select by Peak Tags, 选择之前命名的 靶点名称。
- 32) 在扫描芯片图上右键, Occupancy-Set Occupancy[o]并将值设为 1。





33) 点击 Tools-Data
Visualization,通过五种不同的方式展示结果。如直方图,散点图,统计表格等。

Reset and Autodetect [a] Mark as Rejected [r] Mark as Empty (Manual) [m]	
Select >	
Deselect >	
Apply Selection	Set Occupancy [o]
Count Selected Lanes	Reset Occupancy
Lane Properties	
Plot Selected [p] Plot Selected Correlations	
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🖏 Data Visualization Dataset Selector 🛛 🗌 😒	×							
Subset of Lanes to use:								
Il Lanes with Tagged Peaks								
◯ Lanes with Occupancy = 1								
◯ Lanes with Selected Peak Tag(s):								
Select Tags								
◯ Lanes Selected in Active Tab								
751 lanes selected.								
Lane Plot Histogram 1D Scatter 2D Scatter Enumerate								
Close								

34)例如用直方图展示表达分 布,在弹出的对话框中选择 Peak Area 代表考察的参数为 表达丰度,右侧选择命名的 靶点。

Select Data:		Selec	t Tag:	
PeakCenter PeakHeight PeakFWHM PeakArea PeakFillFactor PeakSize PeakSignalToNoise		<name> AML1 AutoExcluded Duplicate Excluded NoiseLike Target</name>		
	~			~
Histogram Type: C Linear Scale C Linear Scale, Missing = 0 C Log10 Scale		ОК	Cance	I

35)示例数据: AML1平均表达量为2.58X10⁵。您也可以点击[∞] 直接平移 或者点击[∞] 在弹出的对话框中对X-Y轴进行编辑。



- A 🖑		×
x-axis lower 141262	limit	
x-axis upper 1077118	limit	
y-axis lower 0	limit	
y-axis upper 91.3	limit	
Font size 10		
	OK	Cancel

proteins

×

36) 如果同一张芯片采用相同荧光 Peak Table: Chip 463_BTUB_GAPDH_5_um П 通道检测了两个不同的靶蛋 File Peak Table ९, ९, 🖑 🖳 🖬 🍃 🥔 💆 🚥 🚥 👶 👶 🐘 🗶 🌂 白,此时点击 ^三把一群Point Y-axis Variable: PeakCenter ~ X-axis Variable: Index Global Col:Row Tubulin GAPDH untagged 框进去,点击 Peak Table-800
 Peak Center (microns from well center)

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 00 Apply Tag To Selected Peaks进 行靶信号的命名,重复此操作 直至所有靶蛋白均已成功命 名,然后点击Peak Table-Tag Duplicate Within a Lane, 查看 一下是否有Duplicate Lane,如 100 0 1000 2000 3000 4000 5000 6000 果有,按照(27-29)处理即可。 Index Global Col:Row

37)此时您可以直方图,一维散点图,二维散点图,统计表等多种形式展示Peak Area的结果。点击 Tools-Data Visualization-Histogram/1D Scatter Plot/2D Scatter Plot/Enumeration,通过直方图展示表达分布。







👈 GAPDH vs. Tubulin (by PeakArea)

File							
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	Tubulin+	Tubulin-	Total				
GAPDH+	737	11	748				
GAPDH-	114	34	148				
Total	851	45	896				
	Tubulin	Tubulin	Total				
CARDUL	1000111+	1000	10101				
GAPDH+	82.3%	1.2%	83.5%				
GAPDH-	12.7%	3.8%	16.5%				
Total	95.0%	5 0%	100 00/				



38) 如果同时检测了三个蛋白: GAPDH, β-Tubulin, AML1, 只想在表达 了两个内参蛋白的基础上分析AML1的变化,此时选择Lanes with Selected Peak Tag, 弹出的对话框中选择BTUB和GAPDH, Selection type选择OR或者AND, 在双击选择以哪种方式展示结果, 如 Histogram, 然后参照(33-34)即可。

	👈 Data Vi	sualization Dataset Se	lector	-	×		
	Subset of Lanes to use: All Lanes with Tagged Peaks Lanes with Occupancy = 1 Lanes with Selected Peak Tag(s): Select Tags Lanes Selected in Active Tab				Select — AML1 AutoExcluded BTUB Duplicate Excluded GAPDH NoiseLike Target		
	Lane) lanes selected	I. ID Statter Close	2D Scatter	Selection mera О ОК ОК	on Type:	×
Nistogram Plot		- Coloct 1		File			
Select Data: PeakCenter PeakHeight PeakFWHM PeakArea PeakFillFactor PeakSize PeakSignalToNoise		Select T <name> AML1 AutoExcluded Duplicate Excluded NoiseLike Target</name>	ag:	- 00 - 00 - 00 - 00 - 00 - 00 - 00 - 00	Histogram of AML1 Peak (721 AM Mean: 2.78Ε Median: 2.37	Area for the subset o L1+/13 AML1-)	f 734 wells

OK

Cancel

Histogram Type:

○ Linear Scale, Missing = 0

O Linear Scale

Log10 Scale

39) 如果您想查看哪些泳道在表达了GAPDH的基础上也有AML1的表达, 先选中表达内参的泳道,然后鼠标右键, Apply Selection-Select Across All Scans,这样就可以实现Merge了。

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3.64

3.93

4.23

4.52

4.81

Log AML1 PeakArea

5.10

5.40

5.69

5.98



Reset and Autodetect [a]		
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Mark as Rejected [r]		
Mark as Empty (Manual) [m]		
Select	>	
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Invert Selection	_	we get the the second
Apply Selection	>	Select Across All Scans
Occupancy	>	Select on Peak Table [s]
Count Selected Lanes		Deselect on Peak Table [d]
Lane Properties	>	
Plot Selected [p]		
Plot Selected Correlations	>	

40) 可点击 Export CSV-Tagged peaks, one row per lane 导出原始数据, 进一步通过第三方统计软件进行数据处理, 作图展示等等。

👈 Scout				
File Edit	Tools	Help		
🗅 😂 🖬	Re	-detect peaks		
Chip 463_B	Peak tables Calculate size coefficients			_5_um
	Ins	pect	>	· · · · · · · · · · · · · · · · · · ·
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	Ex	port CSV	>	Standard: (one row per lane for selected tags)
n sin as	Ex	port FCS		Advanced: (one row for each peak)
	Ov	erview Image	>	

- 41)如果同一张芯片采用不同荧光通道检测了两个不同的靶蛋白,您可以 将分析一个荧光通道,然后点击File-Add scan to current chip-Copy registration from current scan将该荧光通道的Registration and Alignment 应用于另一个通道。
- 42)如果利用Scout软件分析芯片剥离再杂交的效率,您需要在Stripping 之前先分析结果,并把Target蛋白命名为"Target Before",然后再加 载这张芯片剥离之后的结果。之后点击Peak Table -Tag Matching Peaks/Stripping Efficiency,在弹出的对话框中选择"Target Before", 点击"OK",在新弹出的对话框中选择"Create New Tag",点 击"Apply",把Target蛋白命名为"Target After",选择"Target After",点击"Apply",弹出的对话框中选择Yes,Scout软件会自动进 行剥离效率的统计,如下图所示:





43)如果想知道某个泳道在peak table中具体是哪个点,可以通过鼠标选 中该泳道,然后点击右键Apply Selection-Select on Peak Table,此时 弹出一个新的窗口,如下,红色框内的那个黑色高亮的点即为目的泳 道。



Peak Table: no_Name_2019-05-16_09h43m28_5µm_5-16-10_0023



44)如果想要展示某个泳道的峰型图,可选中该泳道,鼠标右键-Plot Selected,即出现右边对应的峰型图。其中横坐标为迁移率,峰面积 A=832512.

