# biotechne

## COMPASS 软件介绍 数据分析

Xianting Wang

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## PROTEIN SIMPLE -- 创新蛋白质分析技术专家





biotechne<sup>®</sup> protein simple

#### MEET SIMPLE WESTERN

JESS ABBY WES





## 超微量样品+自动化+定量

1 Simple Western 工作原理

2 Simple Western 优势及应用

3 Simple Western 实验操作简介

GEL-RUNNING AUTO TRANSFER-FREE BLOT-FREE HANDS-FREE

#### 您需要做的工作





\*图片来源于网络

UNE PAIR OF

Pants LATer ...





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# 01 02 03

## Compass 介绍





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## Compass 介绍





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#### COMPASS 软件



#### COMPASS 有三个主要界面

- Assay 实验前的设置
- Run summary 运行过程中的实时情况
- Analysis 分析界面

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12–230 kit: 1 kDa, 29 kDa, 230 kDa	
66–440 kit: 57 kDa, 280 kDa	
2–40 kit: 1 kDa, 26 kDa	
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- 峰型图
- 峰形图信息量最大,
   数据质量分析推荐
   用峰形图
- 选择不同的通道,
   将有不止一个Y轴
- 点击 Auto Scale 将自动调整所有毛 细管的量程



峰形图信息量最大,
 数据质量分析推荐
 用峰形图

峰型图

- 选择不同的通道,
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峰形图信息量最大, 数据质量分析推荐 用峰形图

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- •选择不同的通道, 将有不止一个Y轴
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- 峰型图
- 峰形图信息量最大,
   数据质量分析推荐
   用峰形图
- 选择不同的通道,
   将有不止一个Y轴
- 点击 Auto Scale 将自动调整所有毛 细管的量程





成像图

- 毛细管实际曝光结
   果
- 选择 "Show All Images" 后若信号 过曝,毛细管曝光 图中将出现红色像 素点 (Compass v6.1.0)

Probe 1, CHEMI, 4s, Sample	
Probe 1, CHEMI, 4s, Sample	jes

大部分报告的结果形

式

泳道图

可以任意显示或横向
 移动泳道

- 可以单独调整每个通 道的对比度
  - 对比度调整仅改变图片
     中条带呈现效果, 峰定
     量结果不变





泳道图

- 大部分报告的结果形
   式
- 可以任意显示或横向
   移动泳道
- 可以单独调整每个通 道的对比度
  - 对比度调整仅改变图片
     中条带呈现效果, 峰定
     量结果不变







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大部分报告的结果形 式

- 可以任意显示或横向 移动泳道
- 可以单独调整每个通 道的对比度
  - 对比度调整仅改变图片 \_ 中条带呈现效果, 峰定 量结果不变

泳道图 大部分报告的结果形

式

可以任意显示或横向
移动泳道

- 可以单独调整每个通 道的对比度
  - 对比度调整仅改变图片
     中条带呈现效果, 峰定
     量结果不变





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可以单独调整每个通 道的对比度

对比度调整仅改变图片 \_ 中条带呈现效果, 峰定 量结果不变



大部分报告的结果形 式

泳道图

- 可以任意显示或横向 移动泳道



















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## Compass 介绍





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- 请确保在查看数据前,先通过几个部分的检查确认实验没有问题,包括荧光内参确认、
   Ladder确认、数据质量确认
  - 荧光内参整齐并识别良好
  - Ladder 识别良好
  - 化学发光信号无过曝
  - 荧光通道相机无饱和 (Jess)
  - 背景无过高
  - 峰面积正常




		🔚 Assay 🕒 🕒 Run Summary 🛲 Analysis
Run: ERK 1 Wes run results rev 001	■ Separation 😫 IV Plot	
🕑 Status 🖺 History		
run Wes Installation run path <u>C:\</u> assay Wes-25 Size kit info Regular: 12-230 kDa		
instrument Wes : Wes WS2002 - WS2002 plate S/N 7751502228		
started 星期日 4:10 下午 一月 10, 2016 CST         completed 星期日 6:48 下午 一月 10, 2016 CST         Sample Sep B 1° 2° Detect Results <u> <u> </u> <u> </u></u>		
<	>	





		🖽 Assay 🕒 🖓 Run Summary 🚛 Analysis
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O Status History		
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plate S/N 7751502228		
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### 1. 荧光内参整齐



## 1. 荧光内参整齐





1. 荧光内参整齐



File Edit View Instrument Window	Help	
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Hela L. ERKI 12	40	
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HeLa L ERK1 25	sHela L., ERK., 2 3 646.0 24.0	

1. 荧光内参整齐



#### Compass 软件一般能自动识别校正荧 光内参,但是偶尔也会识别不正确

#### 荧光内参识别不正确,将导致<mark>分子</mark>量 <mark>计算错误</mark>



1. 荧光内参整齐



#### Compass 软件一般能自动识别校正荧 光内参,但是偶尔也会识别不正确

#### 荧光内参识别不正确,将导致<mark>分子</mark>量 <mark>计算错误</mark>



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### 2. LADDER 识别良好





### 2. LADDER 识别良好

- 如果 ladder 峰识别错误, 点击右键进行校正 - 注意您的 ladder 通道要选择正确
- 2. 如果有一个 ladder 峰不可见, 可能调整下视野 - 点击 View→ View Region,选择 Full Range, 可查看整个毛细管的视野数据
- 3. 如果不能通过识别来校正某个 ladder 峰 点击 Edit → analysis →ladders.
  - 如有必要,移除某个Ladder; 或选择 "none" 关闭 ladder 选项
- \* 在没有 ladder 识别的情况下, Compass 软件依赖于每个毛细管内的荧光内参进行分子量计算

Standards	Ladders						
Standards Ladders Images Normalization Peak Names Peak Fit Lane Contrast Signal to Noise Advanced	Ladder Channel: CHEMI ~						
Standards Ladders Images Normalization Peak Names Peak Fit Lane Contrast Signal to Noise Advanced	MW (kDa) 12 40						
	66						
Standards Ladders Images Normalization Peak Names Peak Fit Lane Contrast Signal to Noise Advanced	180						
	230						



### 3. 化学发光信号无过曝





### 3. 化学发光信号无过曝





### 3. 化学发光信号无过曝



- 化学发光信号实际上是信号/时间的比值.
  - 在长曝光时间中, 信号衰减, 所以峰高下降
- 如果您看到在前几个曝光时间中,某个峰的信号明
   显下降,说明:
  - 化学发光底物消耗完
  - 该信号超出了动态范围
- •保持化学发光的峰高小于300,000









## 4. 荧光通道相机无饱和 (JESS)



- 在 NIR 通道中,信号太强有可能导致相机 像素饱和
- 一旦观察到信号开始下降,说明相机饱和, 最小的两个曝光时间峰应该重叠,否则说明 相机较快饱和
- 分析数据时,建议选择HDR模式
- 保证荧光检测的峰高低于 60,000



### 5. 背景无过高——基线拟合



- 软件默认显示扣除背景后的信号曲线 (背景设为0)
- 要观察基线, 需要勾选 "Baseline fit"; 基线参数设置在 Edit→Analysis…中
- 软件根据基线下面的点拟合出基线, 可添加或删除基线点



#### 5. 背景无过高——基线拟合



- •为什么高背景是个问题?
  - 信号饱和风险
  - 显著减弱动态范围
  - 降低检测灵敏度

	Ξ	毛细管	号						相	对峰面	积		
样品	一抗		峰序号	上 峰名	迁移位置	計分子量		峰高	峰面积	Į	峰宽	信噪比	上 基线拟合
Sample	Primary	Сар	Peak	Name	Position	MW (kDa)		Height	Area	% Area	Width	S/N	Baseline
Biot. Ladder	Blocking	1	1	Ldr 12	355	12	4	4279.4	66444.9		14.6	1667.0	22.5
Biot. Ladder	Blocking	1	2	Ldr 40	476	40	3	3095.9	49008.2		14.9	1180.9	35.6
Biot. Ladder	Blocking	1	3	Ldr 66	549	66	7	7139.7	109106.0		14.4	2831.7	40.0
Biot. Ladder	Blocking	1	4	Ldr 116	593	116	4	4454.0	97617.1		20.6	1743.1	41.4
Biot. Ladder	Blocking	1	5	Ldr 180	633	180	7	7471.2	111393.5		14.0	2652.4	41.8
Biot. Ladder	Blocking	1	6	Ldr 230	654	230	(	6921.9	137776.2		18.7	2454.0	41.8
HeLa Lysate	ERK1 RTU	2	1	ERK1	486	48		5940.1	80205.3	100.0	12.7	412.8	183.9

#### 5. 峰面积正常





#### 5. 峰面积正常



- 灰色部分的面积即峰面积.
- 需确认拟合峰跟实际峰形拟合良好
- 右键单击可通过 "add peak" 或 "remove peak" 增加或去除峰
- 在 analysis 菜单中,可选择拟合方式,包
   括 gaussian fit (高斯拟合) vs dropped
   lines (垂直拟合,推荐总蛋白定量时使用)





#### 5. 峰面积正常



- 灰色部分的面积即峰面积.
- 需确认拟合峰跟实际峰形拟合良好
- 右键单击可通过 "add peak" 或 "remove peak" 增加或去除峰
- 在 analysis 菜单中,可选择拟合方式,包
   括 gaussian fit (高斯拟合) vs dropped
   lines (垂直拟合,推荐总蛋白定量时使用)

Standards Ladders	Peak Fit				
Images	Analysis Groups	A	nalysis Groups: fit	t	
<ul> <li>Peak Names         <ul> <li>Standard Curves</li> <li>Loading Controls</li> </ul> </li> <li>Peak Fit         <ul> <li>Lane Contrast</li> <li>Signal to Noise</li> <li>Advanced</li> </ul> </li> </ul>	fit Add Re Apply Default: fit	emove T	Range Minimum Maximum /iew Baseline Fhreshold Window	Analysis	1.0 250.0 ) Full 1.0 15.0
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Import Ex	port	OK	Cancel	Ар	ply

### 数据分析前检查



- 确认完成了以下检查
  - ✓ 荧光内参整齐并识别良好
  - ✓ Ladder 识别良好
  - ✓ 化学发光信号无过曝
  - ✓ 荧光通道相机无饱和 (Jess)
  - ✓ 背景无过高
  - ✓ 峰面积正常

### 数据分析前检查



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- ✓ 背景无过高
- ✓ 峰面积正常



#### 可以开始分析数据了



# 01 02 03

# Compass 介绍

## 数据分析前检查



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#### 编辑实验信息



- 样品及抗体信息等在 Assay 界面输入
  - Assay template 里输入的信息可以随时更改(运行前/后)

- 右键点击可隐藏泳道
- 如果要显示隐藏的泳道, 点击:
  - View→Show Hidden
  - 右键点击 unhide 恢复

💿 ERK 1 W	es run results rev 001 - Comp
File Edit \	/iew Instrument Window I
🗄 Standard	s 🚔 Samples 🛛 🚍 🗏
Experime	nt 🛛 🗖 🗖
Sample	Primary C
Biot. La	Blocki 1
HeLa L	ERK × Hide
HeLa L	ERK Clear
HeLa L	ERKT 4
HeLa L	ERK1 5







- 毛细管/泳道可选择性浏览或者全局浏览
- 检测通道可进行开/关
- 选择不同的通道,将有不止一个Y轴

File Edit Vie	ew Instrument	Window	Help	
🗄 Standards	会 Samples		$\bullet \circ \bullet$	
Experiment		🗷 Graph	🕲 Image	Ξ Lane





#### 当您查看数据时,标记您的峰值很有帮助

选项 1: 右键点击峰形图
 中的峰,选择 Name
 Peak → New...进行命名

- 选项 2: Analysis Options
   窗格里
  - 在 Name 中: 下拉选中 [New]
  - 输入峰名称等
  - 单击 Create

1			
	Zoom Out		1
	Remove Peak		
×	Hide		
	Name Peak	>	ERK1
	Add Peak		New
	Add Baseline Point	l	
	Remove Baseline Point		
	Clear		
	Сору		

Analysis Options Annotations
Images
Exposures High Dynamic Range 4.0
Peak Names
Name
MW
Color
Caps
Modify

选项 3: 右键点击峰
 统计表中的峰,选择
 Name Peak →
 New...进行命名



 选项 4: 在Edit > Analysis 菜单栏中
 在 Peak Names
 栏中进行峰命名

Peak Names								
Analysis Groups	Analysis Groups: Protein 2							
Protein 2 Add Remove	Name ERK1	M 48	Color	or Range ( 10				
Apply Settings								
Apply To Group [1:2-25] Protein 2								
Add Remove			Ad	d Remove				
	Peak Names Analysis Groups Protein 2 Add Remove Apply Settings Apply To Group [1:2-25] Protein 2 Add Remove	Analysis Groups     Analysis Groups       Protein 2     Name ERK1       Add     Remove       Apply Settings     Apply To       Apply To     Group       [1:2-25]     Protein 2       Add     Remove	Peak Names  Analysis Groups: Analysis Groups: Analysis Groups: Analysis Groups: Analysis Groups: Analysis Groups: Apply Settings  Apply Settings  Apply To Group [1:2-25] Protein 2  Add Remove  Add Remove	Analysis Groups     Analysis Groups: Protein 2       Protein 2     Name       Add     Remove       Apply Settings     Image: Comparison of the set of the se	Analysis Groups     Analysis Groups: Protein 2       Protein 2     Name       Add     Remove       Apply Settings     FRK1       Apply Settings     Apply To       Group     Group       [1:2-25]     Protein 2       Add     Remove			







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峰命名





ANALYSIS 界面



泳道图

- 大部分报告的结果形 式
- 可以任意显示或横向
   移动泳道
- 可以单独调整每个通
   道的对比度
  - 对比度调整仅改变图片
     中条带呈现效果, 峰定
     量结果不变







bio-techne<sup>®</sup> protein simple

Linearity of Total Protein Assay



#### **Total Protein**

HeLa/Erk with RePlex and TP Lane\_annotation\_training\_file.cbz



🗲 Erk

🗕 Erk

**Linearity of Total Protein Assay** 



**Total Protein** 

HeLa/Erk with RePlex and TP Lane\_annotation\_training\_file.cbz

bio-techne<sup>®</sup> protein simple

**Linearity of Total Protein Assay** 



Total Protein

HeLa/Erk with RePlex and TP Lane\_annotation\_training\_file.cbz



# COMPASS 6.0.0 版本

#### 适用于 WES/JESS/ABBY








・添加了Lane 结果注释





**Total Protein** 

HeLa/Erk with RePlex and TP Lane\_annotation\_training\_file.cbz



・添加了Lane 结果注释

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- ・添加了Lane 结果注释
  - "New Assay" 仅适用于 (Abby/Jess/Wes)

ile	Edit	Instrument	Wind	ow	Help	
	New Assay		>	1	Abby	
	Open Assay Save Save As Import Protocol Import Template		>		Jess	
					Wes	
						×





















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### LANE 注释 只需在 COMPASS 内即可进行注释





### LANE 注释 只需在 COMPASS 内即可进行注释





### LANE 注释 只需在 COMPASS 内即可进行注释









#### 只需在 COMPASS 内即可进行注释





• 注释结果名, 单击可更改









#### 只需在 COMPASS 内即可进行注释



bio-techne<sup>®</sup> protein simple

#### 只需在 COMPASS 内即可进行注释





bio-techne<sup>®</sup> protein simple



💩 Analysis Options 🛒 Annotations	- 8
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Lane Labels	*
Band Labels	*
Title & Notes	*
Settings	*



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Band Labels		*				
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39-213	CHEMI P1,CHEMI P2					
Re-crop						



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Crop Regions Caps 3,7,11,2,6	可以自定义分子量范围	*				
MW Range	Channel Contrast					
39-213	CHEMI P1, CHEMI P2					
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Re-crop						

















### bio-techne<sup>®</sup> protein simple

Crop Re	Crop Regions			
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Link to	Sample			
Chula	Sample			
style	Sample Attribute			
	Primary			
	Primary Attribute			
1	Secondary			
	Secondary Attribute			
2	Probe:Capillary			
3	None			
4	HeLa			
5	HeLa			

## bio-techne<sup>®</sup> protein simple

Crop Regions						
Lane Labels						
Group	SampleGroup					
Link to	Sample					
<b>Style</b> 1 2 3	Sample Sample Attribute Primary Primary Attribute Secondary Secondary Attribute Probe:Capillary None	Lane 标签可使用 Assay 界面实验设计, 也可以自己重新编辑				
4	HeLa					
5	HeLa					

### bio-techne<sup>®</sup> protein simple

Crop Re Lane La	gions bels							
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			5 HeLa 6 HeLa 7 HeLa 8 HeLa 9 HeLa 10 HeLa	I	自定义标签	位置,分组	,旋转角度等	F

### bio-techne<sup>®</sup> protein simple

Crop Re Lane Lab	gions bels							
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2 3 4	Probe:Capillary None HeLa		Crop Regions Lane Labels					~
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### bio-techne<sup>®</sup> protein simple

						HeLa		
Crop Re	gions				kDa	0.08 ug/ul	0.1 ug/ul	
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只需在 COMPASS 内即可进行注释



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			_				
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Band Lab	■      展示形式	3				
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		Lane Labels	×
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#### 只需在 COMPASS 内即可进行注释



Assay Pan Summary Analysis Analysis Options Annotations		STINGTING LOT ACT		
Assay Analysis Options Annotations Crop Regions S Anal Labels S Title & Notes S				
<ul> <li>Analysis Options Annotations</li> <li>Figure-1</li> <li>Crop Regions</li> <li>Lane Labels</li> <li>Band Labels</li> <li>Title &amp; Notes</li> <li>Annotations</li> <li>I</li> </ul>	💾 Assay 🕒 Run Summary 🏨	Analysis		
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Lane Labels*Band Labels*Title & Notes*	Crop Regions	*		
Band Labels*Title & Notes*	Lane Labels	×		
Title & Notes ×	Band Labels	×		
	Title & Notes	×		
Settings ×	Settings	×		

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#### 只需在 COMPASS 内即可进行注释





# LANE 注释功能目前的局限

只需在 COMPASS 内即可进行注释



- Add Run 同时打开多个运行文件结果不能使用 Lane 注释功能
- Access Control 受管控的运行文件不能使用 Lane 注释功能
- Graph view 不能注释电泳图,只能注释泳道图
- Fonts Lane 工具暂不支持所有类型的字体







#### • 图像拷贝

- 在峰形图或者泳道图中,右键点击Copy复制或保存图像

### • 点击 File→export tables

- 软件将建立一个文件夹导出各类统计数据

#### • 数据拷贝

– 选中峰统计表中的行(一行或多行),右键
 点击 Copy 复制数据

### • 点击 File→run report

- 软件将建立一个 PDF 文档, 详细报告图和表

## 公众号咨询/报修流程







### PROTEINSIMPLE SIMPLE YOUR PROTEIN ANALYSIS



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## QUESTIONS