DeCyder 2D Software 中文操作手册



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DeCyder 6.5 分析軟體組織圖:



1. Image Loader 膠片影像載入模組

(1) 雙擊桌面上DeCyder圖示。



(2) 輸入帳號與密碼,若無帳號密碼請洽系統管理員。

DeCyder logi	n			
Username: Password:				
Database:	DECYDER			
More >>	_	Login	 Quit	1

(3) 出現主畫面, 雙擊Image Loader圖示



 (4) 開啟Image Loader模組後,點擊Add 加入膠片影像至載入列表, 影像之命名格式為"Gel xx Cyx XXXXX.gel" (x為數字; X為任 何字元)。

af Image Loader								
Me Edit Hop Invages to import L181 (path)	(4)					Import anapos tar	(6)	(5)
Gettere Get	De denatu	terge (p2	lings (2)	Freque Cull	Negat Unassigned	In # 10Me-rice Pr-6ht 2014 In # Anders In # Anders In # Anders In # Sublet In		

- (5) 點擊 🗾 增加新的分析專案(Project)。
- (6) 點擊Import 將膠片影像載入置分析專案中。

(7) 增加新的專案分析時,可依需求命名與註解。若分析結果不願與其他使用 者分享,可將Public access勾選取消。





2. Batch Processor 全自動批次分析模組

(1) 點選軟體主畫面Batch Processor的圖示。



 (2) 開啟Batch Processor模組後,執行 File -> Add DIA item,出現以下 畫面。



- (3) 點擊Add 或Add all 將分析專案中的可分析影像加入至分析列表。
- (4) 輸入蛋白質點數10000,以及過濾背景訊號Volume< 30000,如有需要可再設定其他濾點參數。</p>
- (5) 設定完成後點擊OK,進行BVA分析設定。

(6) 點擊Add 加入分組組別,並將Unassigned資料夾中的膠片影像拖曳至適當 的分組組別中。

🖶 BVA item settings		
Standard Group 1 Group 2 Unassigned (6)	Gel 01 Cy3 Control.ge	Experimental group Name Unassigned Description Default group for non-standard images Experimental conditions Value Condition1 0 Condition2 0
	(8	(6) Add Remove
▲		OK Cancel OK / next ▶ Help

- (7) 如要使用Paired test 統計分析,於Sample ID 欄位輸入樣品來源編號。
- (8) 組別分組設定完成後,將Setup protein statistics and filterd勾選選取,點
 擊Next 進行統計分析設定。



(9) 選擇統計分析種類:Independent tests 或 Paired tests。

若欲比較的樣品來源來自同一個體,則選擇Paired tests;反之則選擇 Independent tests。

🖋 Protein Statistics				
Type of statistical test for Independent tests (normal) for Paired tests (use sample ID)	" (9)			
Statistical tests				
Population 1	Population Z			
Select group(s)	Select group(s)			
Control Treated (10)	Control Tresked			
0 included spotmaps	0 included spotmaps			
One-way ANOVA between different groups Two-way ANOVA between condition 1 and condition 2 False discovery rate (12)				
INFO: Minimum Average Ratio Student's T-te 1-way ANOVA 2-way ANOVA				
Standard must be present for all	tests. (13) ancel OK / next ⊾ Help			

- (10) 為提供差別倍率計算目標, Population 1, 2中各選擇欲分析組別: Population1為分母; Population2為分子。
- (11) 勾選Average Ratio 以獲得平均差異倍數;勾選Student's T-test 以獲得 兩組差異可信度數據;勾選One-way ANOVA 以獲得單一變因多組分 析可信度數據;勾選Two-way ANOVA 以獲得雙變因多組分析可信度 數據。
- (12) 勾選False discovery rate (FDR) 以降低得到假真或誤報結果的機率。
- (13) 點擊Next 進行 興趣蛋白質點篩選設定。

(14) 依照需求設定有興趣蛋白質的條件門檻) 可依統計數據、差異倍數、在膠 片出現次數等條件設定,符合門檻的蛋白質標記成POI 或Pick。

f Protein filter		
Filter action Assign protein of interest Ass	ian pick status	in list
General filter settings		
T Select all		
Restrict to proteins present in	>= 1.0	no of spot maps
Select proteins with	-	
F Studentis T-test Value	s= 0.05	
F Average ratio	>= [4,5]	
C Average ratio	<= 15	
F Average ratio	>= 1.5	and <= 1.8
Concervay ANGVA value	>= 0	and <= 0.05
Two-way ANGVA - condition 1 value	>= 10	and <= 0.05
F Two-Way ANGVA - condition 2 value	>= 0	and <= 0.05
Two-way ANGVA - interaction value	×- [0	and <= 0.05
-Properties for proteins in pick spot	map	
✓ Volume ✓	>= 100000	and <- 10000000
C Location K-coordinate	>= 0	and <= 1000
🗖 Location <i>R</i> -coordinate	>= 0	and <= 1000
1. 66	-	
OK	Cancer	нер

(15) 執行 Process-> Run batch 後,選擇儲存的分析專案及分析結果檔案名稱。





(17) 分析狀態參閱下表:

Batch status	Description
Empty	New batch workspace can be created.
Workspace changed	Data is entered or changed.
Workspace saved	Current batch workspace is saved; an existing batch workspace is loaded from the database or batch processing has successfully completed.
Waiting	Batch process i scheduled to start at a set time.
Processing - Items completed: DIA 0/	Batch processing is running.
Failed	Processing has completed and one or several batch list items has entered failed state.
Aborting - Items completed: DIA 0/!	A started process is being aborted. The batch processing is halted once the currently running detection or analysis is completed.

(18) 直到出現Workspace saved 表示分析完成,此時可關閉Batch processor 模組,並可打開 DIA 或 BVA 模組以檢視分析結果。



3. DIA 膠內差異分析模組

(1) 點選軟體主畫面Batch Processor的圖示。



- (A) 執行File -> Open Workspace開啟已有的DIA分析檔案,或是
 (B) 執行File -> Create Workspace創建新的DIA分析。
- (3) (A) 選擇欲開啟的DIA檔案,或是
 - (B) 選擇欲創建DIA分析的膠片影像。

(A) 開啟DIA檔案

Jpen Workspace	Create Workspace	
□ □	□ □	el 1 Control (1 C el 1 Standard C el 1 Trealed (2
	Multiple selection of images is possible by pre pressing Shift + clicking	essing CTRL + clicking, or
Open Cancel	I Help Cre	ate Cancel Help

(B) 創建DIA檔案



(4) 開啟分析畫面如下,分為膠片影像(Image View)、分析圖(Histogram View)、
 3D立體圖(3D View)、分析數據表格(Table View):



- (5) 若要偵測蛋白質點,執行Process-> Process gel image,設定蛋白質點數, 建議值10000。
 - 若此膠片影像已包含自動取點機所需之參考定位點,則可勾選Autodetect Picking references。

Process Gel Images		
Algorithm Selection		
DeCyder detection algorithm		
Version: 6.0		
Description		
DeCyder detection algorithm, Performs spot detection in one or more images based on leatures in the image contrast.		
Estimated no. of spots 2500		
OK Cancel Help		



(6) 使用排除過濾器(Exclude filter)濾掉雜點,執行Process -> Exclude filter,依 蛋白質點的數值與分析需求來設定參數門檻,建議值Volume<30000。

Exclude Filter		? 🛛
Spot properties	spots	
🔽 Slope	> 1.1	1
🔽 Area	< 100	
Volume	< 100	
🔽 Peak Height	< 100	and > 64000
Current Area of Intera X-direction: 157 - 21 Y Position: 141 - 186	est 83 65	
	OK	Cancel Help

(7) 偵測出的蛋白質點資訊可參閱分析數據表格,表格內各欄數值代表意義如下:

Legend	Description
Status	Indicates whether a spot has been confirmed by the user (see section 4.6.2).
Spot No.	Spot reference number (unique to a spot pair on a set of images).
Abundance	"Decreased", "Similar" or "Increased" depending on thresholds set in DIA Histogram View
Excluded	Spot assigned by user or Exclude Filter for removal from analysis set. An excluded spot is never completely removed from the workspace and can be recovered by the user. Excluded spots are not copied into BVA at import of DIA workspaces.
Volume Ratio	Normalized Volume Ratio between co-detected spots in the primary and secondary images. The histogram is automatically recalculated if the primary and secondary images are changed.
Picked	"Pick" designation indicates that a spot has been selected for picking (see section 6.4)
POI	Indicates whether a spot has been selected as a protein of interest, denoted by the letter "I" in the column.
Max Slope	Largest gradient associated with co-detected spots.
Area	Number of pixels within the spot boundary.
Max Peak Height	Largest pixel value associated with co-detected spots.
Max Volume	Volume of the largest of the co-detected spots.
Protein ID	User defined protein identification (manually entered in the Protein ID text box in the Spot Control Panel at the bottom of the screen).
Comment	User defined comment (manually entered in the Comment text box in the Spot Control Panel at the bottom of the screen).
РТМ	Indicates whether a spot has been manually assigned as a protein with a post translational modification, denoted by the letters "PTM" in the column.



4. BVA 生物差異分析模組

(1) 點選軟體主畫面Batch Processor的圖示。



- (2) (A) 執行File -> Open Workspace 開啟已有的BVA分析檔案,或是
 (B) 執行File -> Create Workspace創建新的BVA分析。
- (3) (A) 選擇欲開啟的BVA檔案,或是
 - (B) 加入欲創建BVA分析的DIA檔案。

Create BVA Workspace	
Available Workspaces:	Add -> Remove
Multiple selection of workspaces possible by pressing CTRL + clicking, or pressing Shift + clicking,	Ereste Cancel Help



(4) 開啟分析畫面如下,分為膠片影像(Image View)、分析圖(Histogram View)、
 3D立體圖(3D View)、分析數據表格(Table View):



(5) BVA模組分析模式與功能如下所示,分析時依ST、MT、PT、AT順序分析:

	Mode	Function	Rows in table represent
ST	Spotmap Table	Set up experimental design	Spot maps, corresponding to gel images
мт	Match Table	Inter-gel matching	Matched/unmatched spots for Master Spot Map and Primary (match) Spot Map
PT	Protein Table	Examine statistics of all proteins	Proteins (spots in Master Spot Map)
AT	Appearance Table	Examine statistics of one protein	Spot maps (all spot maps where the protein is present)

(6) 在ST模式下,將膠片影像依實驗設計分組。按下Add加入新組別,再將未分 組膠片拖曳至適當組別。





(7) 在MT模式下,進行膠片間比對。可先執行View -> Display Multiple Gel Views 並將畫面轉換成只表現膠片影像模式,然後將膠片切換成Cy2 Internal Standard的影像。



- (8) 按著鍵盤的Ctrl選取不同膠片間相同的蛋白質點,點選Add Match加入地標點(Landmark)。或是點選Break Match取消已比對到的蛋白質點。
- (9) 執行Process -> Match 自動比對。選擇Match All 比對所有膠片蛋白質點, 包括已經比對過的;選擇Match Primary 將所選擇的膠片影像與Master 膠片相比對;選擇Match Pending and LandMarked 比對那些還未比對 過的膠片和那些有加入地標點的膠片。

- (10) 在PT模式下,進行每個蛋白質點的統計學分析並找出有差異表現的蛋白質點。執行Process -> Protein Statistics 設定所需的統計計算,請參考2. Batch Processor全自動分析模組中的第(9)~(12)項。
- (11) 在Protein Table中可以T-test 或 ANOVA 排序,找出組別表現趨勢一致的 蛋白質點,T-test 欄位數值顯示 0.05代表具有95%的信心水準,數值月 小信心水準越高。有關T-test等統計數值詳細的意義與說明,請參閱操 作手冊User Manual。
- (12) 再考慮Av. Ratio與Appearance等數值,找出有興趣的目標蛋白質。Av. Ratio 數值越大代表兩組表現差異倍數越大,建議倍數至少1.5 倍。

Protein Table T-test and Av.Ratio: 6 / 3										
Pos.	Master No.	Status	Protein ID	Protein AC	Name	Appea	arance	T-test	Av. Ratio	1-ANOVA
16	230	Unconfirmed				52 (60)	A. M. P	0.00007	-1.35	
17	1071	Unconfirmed				44 (60)	A, M	0.0010	1.93	
18	652	Unconfirmed				38 (60)	A, M	0.0011	-1.37	
19	1031	Unconfirmed				44 (60)	A, M	0.0015	1.97	
20	214	Uncontermed				50 (60)	6 M	0.0010	1.45	
21	601	Unconfirmed				52 (60)	A, M	0.0019	-1.36	
22	207	Unconfirmed				50 (60)	A, M	0.0021	1.42	
23	638	Unconfirmed				38 (60)	A, M, P	0.0021	-1.10	
24	430	Unconfirmed				46 (60)	A, M, P	0.0022	1.18	
25	525	Unconfirmed				50 (60)	A, M	0.0022	-1.05	
26	237	Unconfirmed				42 (60)	A, M, P	0.0025	-1.62	
27	728	Unconfirmed				60 (60)	A, M, P	0.0026	1.10	
28	1078	Unconfirmed				38 (60)	A, M	0.0026	1.32	
29	202	Unconfirmed				58 (60)	A, M, P	0.0027	1.88	
30	592	Unconfirmed				51 (60)	A, M, P	0.0028	1.10	
31	765	Unconfirmed				36 (60)	A, M	0.0028	-1.22	
32	1089	Unconfirmed				26 (60)	A, M	0.0028	1.24	
33	894	Unconfirmed				54 (60)	A, M	0.0035	1.08	
34	514	Unconfirmed				48 (60)	A, M, P	0.0040	1.09	
35	597	Unconfirmed				58 (60)	A, M, P	0.0040	1.09	
36	576	Unconfirmed				58 (60)	A, M, P	0.0046	-1.13	
37	715	Unconfirmed				38 (60)	A, M	0.0046	1.29	
38	730	Unconfirmed				38 (60)	A, M	0.0046	1.48	
39	391	Unconfirmed				38 (60)	Α, Μ	0.0048	-1.28	
40	541	Unconfirmed				48 (60)	A, M	0.0052	-1.14	
41	223	Unconfirmed				40 (60)	A, M	0.0054	-1.46	
42	624	Unconfirmed				40 (60)	A, M, P	0.0055	1.16	
43	115	Unconfirmed				30 (60)	А, М	0.0057	1.84	
44	1020	Unconfirmed				46 (60)	A, M	0.0057	-1.14	
45	203	Unconfirmed				56 (60)	A, M, P	0.0059	1.76	
46	625	Unconfirmed				68 (60)	A, M, P	0.0060	1.07	
47	860	Unconfirmed				50 (60)	A, M, P	0.0060	-1.15	
48	734	Unconfirmed				60 (60)	A, M, P	0.0061	-1.12	
49	341	Unconfirmed				58 (60)	A, M	0.0062	-1.07	
50	1035	Unconfirmed				36 (60)	A, M	0.0062	2.94	
51	691	Unconfirmed				54 (60)	A, M, P	0.0066	-1.13	
2	~~~	the ere firm and				40 1000			1.12	
10000										

(13) 檢查過的有興趣蛋白質如要使用自動取點儀(Spot picker)取點,則在畫面下 方勾選 Pick,為蛋白質標記。



5. Export Pick List

- (1) 將欲取點之膠片,依照Spot picker操作說明貼上Pick Reference,並 用掃描器擷取膠片影像。
- (2) 將膠片影像以Image Loader載入,並放入相同的分析專案中,請參考
 1. Image Loader說明。
- (3) 開啟DIA模組偵測蛋白質點,偵測時勾選自動偵測Pick Reference。偵 測後將欲取的蛋白質點標記成Pick。

EX DOLL		
I▼ PICK	I FIM	Confirm
👿 poi	E Evoludo	
IV FUI	Exclude	

- 執行 File -> Export Pick List 將取點列表輸出存檔。取點列表檔案即 可載入Spot picker 自動取點。
- (5) 若欲取點膠片需要與之前分析過的DIGE結果比較後再取點,則將DIA 分析過的膠片先存檔。
- (6) 開啟BVA 模組載入先前分析之DIGE 結果,然後執行File -> Add Template/DIA Workspace,載入欲取點膠片之DIA檔案。
- (7) 將欲取點膠片指定成為取點膠片(Pick gel)。

Function for Spot Ma	ap: Deep Purple.gel —			
Analysis (A)	Master (M)	Template (T)	Pick (P)	

- 執行 Process -> Match -> Match Pending and LandMarked,比對結 束後,所有標記上Pick 之蛋白質點皆會在欲取點之膠片上表現。
- 執行 File -> Export Pick List 將取點列表輸出存檔。取點列表檔案即 可載入Spot picker 自動取點。



需要更多資訊?



您可以參考: 1. 操作手册 28-4010-06 DeCyder User Manual

2. 連絡 GE Healthcare 產品專員。

3. 造訪網站 Healthcare website <u>www.gelifesciences.com</u> <u>www.ettandige.com</u>

