

a

Task Name: 

Import parameters from other Tasks

**Is DIA file**

**Search Engine Parameters**

Parameter Set: (883) APMS\_BioID

Search engines:  Mascot  XTandem  Comet  MS-GF+

Database: 16--HEK293Ref57cRapRevG

**Fixed Modifications**

selected modifications:

available modifications: Carbamidomethyl (C), Phospho (ST), Phospho (Y), Acetyl (N-term), GG (K)

**Variable Modifications**

Deamidated (NQ), Oxidation (M)

**Proteowizard Parameter set**

Parameters required for converting raw/wiff files: (885) APMS\_BioID

**Run TPP**

TPP can be run after the search task is finished in search results page.

TPP Name:

TPP Parameter set: (886) APMS\_BioID

**Data Files (total: 0)**

[Folder ID] / File Name

Select search parameters, search engines, sequence database, modifications.

Navigate through folder structure to add files to be searched

b

**Search Engine Parameter Set**

New Set  Modify Set  Set by: Brett Larsen Set date: 2015-05-30

Set name: APMS\_BioID for machine: TRIPLETOF1

**Mascot Parameters**

**XTandem Parameters**   not saved

**Comet Parameters**   [online help]

**Enzyme**

Enzyme:

Max missed cleavages: 2

Decoy:

**Advanced options**

Parent Mass Type:  Monoisotopic  Average

Fragment Mass Type:  Monoisotopic  Average

Peptide Mass Tolerance: 35 ppm

Binning to use on fragment ions: 1,0005 amu offset position to start the binning 0.4

Theoretical\_fragment\_ions: 1

Neutral Losses (H2O/NH3):

Isotope error: 0

Peptide Max Charge: 2+, 3+ and 4+

**MSGF+ Parameters**   not saved

**MS-GFDB Parameters**   not saved

c

### List of databases

- If a database cannot be seen from a new task, the database has been hidden by ProHits admin. A hidden database cannot be used for a new task, but old tasks will still be able to use the database.
- The database information is manually added by Prohits administrator ("pop\_dbs\_info.txt").

#### 01--BuddingYSTandREV [Download](#)

Time files compressed : Mon Mar 4 15:13:42 2013  
 Number of sequences : 11668  
 Version of Mascot : 2.3.02  
 Reverse sequence gi|999

#### 02--HEK293V57cRapRevTag [Download](#)

[Homo sapiens]  
 Number of residues : 39921702  
 Number of sequences : 72477  
 Human adenovirus C(RefseqV57)txid:129951(146 proteins)  
 http://maxquant.org/contaminants.zip for contaminants from MPI  
 ftp://ftp.thegpm.org/fasta/cRAP/crap.fasta for cRAP from GPM  
 Reverse sequence >DECOY###  
 Strep or some tags (GFP, BirA\*, GST, mCherry) List from Payman.  
 >BirA-R118G\_H0QFJ5  
 >Streptavidin\_P22629  
 >GST26\_P08515  
 >mCherry\_V9VHH0  
 >sp|GFP\_AEQVI|

#### 03--HEK293RefV57cRapRev [Download](#)

d

### Set Default Modifications

The modification list is from file "/Prohits/autoSearch/mod\_file" which originated from Uniprot and was converted to the Mascot style. It can be manually modified.

**All modifications:**

2-dimethylsuccinyl (C)  
 2-monomethylsuccinyl (C)  
 2-nitrobenzyl (Y)  
 2-succinyl (C)  
 2HPG (R)  
 3-deoxyglucosone (R)  
 3-phosphoglyceryl (K)  
 3sulfo (N-term)  
 4-ONE (C)  
 4-ONE (H)  
 4-ONE (K)  
 4-ONE+Delta-H(-2)O(-1) (C)  
 4-ONE+Delta-H(-2)O(-1) (H)  
 4-ONE+Delta-H(-2)O(-1) (K)  
 4AcAllyl(Gal (C)  
 a-type-ion (C-term)  
 AccQTag (K)  
 AccQTag (N-term)  
 Acetyl (C)  
 Acetyl (H)  
 Acetyl (K)  
 Acetyl (Protein N-term)  
 Acetyl (S)  
 Acetyl (T)  
 Acetyl (Y)

**Default fixed modifications**

**Default variable modifications**

Deamidated (NQ)  
 Oxidation (M)

**User selectable modifications**

Carbamidomethyl (C)  
 Phospho (ST)  
 Phospho (Y)  
 Acetyl (N-term)

**Modifications only for user**

-- --

## Supplementary Fig. 1. Initiating searches for Data Dependent Acquisition.

a) Search Task overview. Use drop-down menus and clickable options to select parameters and options or import from a previously-defined task. Generic parameter sets for each instrument and search engine can be selected (see panel b), the search engine(s) to be used specified, and the database and modifications chosen. c) To keep the interface clean and avoid human error, an administrator can define which sequence databases will be visible to users. d) An administrator can set the list of modifications which all users are allowed to use in setting up their tasks. Additional modifications can be added at any time by an administrator and made available for all users or for selected users. ProHits tracks all of these parameters: once a task has been started, these parameters cannot be further modified, though new files can be added to the search task.

**a**

Task Name: DIA\_MEPCE



Import parameters from previous Tasks

Is DIA file    Run **DIA-Umpire**

Convert Raw file → Run DIA-Umpire SE → Search pseudo MS/MS files → Run Peptide Prophet → Run Protein Prophet → Run DIA-Umpire Quant

**Search Engine Parameters**

Parameter Set: (901) SWATHTOF1    View

Search engines:  Mascot     XTandem     Comet     MS-GF+

Database: 16--HEK293Ref57cRapRevG    View

**Fixed Modifications**

selected modifications: [empty]

available modifications: Carbamidomethyl (C), Phospho (ST), Phospho (Y), Acetyl (N-term), GG (K)

**Variable Modifications**

Deamidated (NQ), Oxidation (M)

Set default modifications

**DIA-Umpire Parameter set**

Parameters required for SWATH raw file to generate pseudo ms/ms.  
870 (870) 25DaWindowFixed    View

**Proteowizard Parameter set**

Parameters required for converting raw/wiff files  
(828) SWATH\_TOF1    View

**Run TPP**

TPP can be run after the search task is finished in search results page.  
TPP Name: DDA\_MEPCE  
TPP Parameter set: (829) SWATH\_umpire    View

Select DIA-Umpire for SWATH file

Select:

- database search parameters,
- search engines,
- sequence database,
- fixed and variable modifications.

Select conversion and TPP parameters

Specify DIA-Umpire parameters

**Data Files (total: 0)**

[Folder ID] / File Name

[23713] / LongSwath\_EIF4aJune7-Biorep1.wiff (27851)

[23713] / LongSwath\_EIF4aJune7-Biorep2.wiff (27854)

[23713] / LongSwath\_EIF4aJune7-Biorep3.wiff (27869)

[23713] / LongSwath\_GFPJune7-Biorep1.wiff (27788)

[23713] / LongSwath\_GFPJune7-Biorep2.wiff (27791)

[23713] / LongSwath\_GFPJune7-Biorep3.wiff (27794)

[23713] / LongSwath\_MEPCEJune7-Biorep1.wiff (27761)

[23713] / LongSwath\_MEPCEJune7-Biorep2.wiff (27773)

[23713] / LongSwath\_MEPCEJune7-Biorep3.wiff (27818)

Add Files

Navigate through folders to add files to be analyzed

**b** **DIA-Umpire Parameters**

Untargeted peptide and protein identification and quantitation using DIA data, that also incorporates a targeted extraction approach to reduce missing quantitation.

PrecursorRank	25	FragmentRank	300	CorrThreshold	0.2	DeltaApex	0.6
MS1PPM	30	MS2PPM	40	SN	2	MS2SN	2
MinMSIntensity	1	MinMSMSIntensity	0.1	MaxCurveRTRange	1.5	Resolution	17000
StartCharge	2	EndCharge	4	MS2StartCharge	2	MS2EndCharge	4
NoMissedScan	1	MinFrag	10	EstimateBG	Yes <input checked="" type="radio"/> No <input type="radio"/>		
WindowType	SWATH	Fixed window size	25				

SWATH window setting ==window setting begin

(Start m/z, end m/z separated by space)

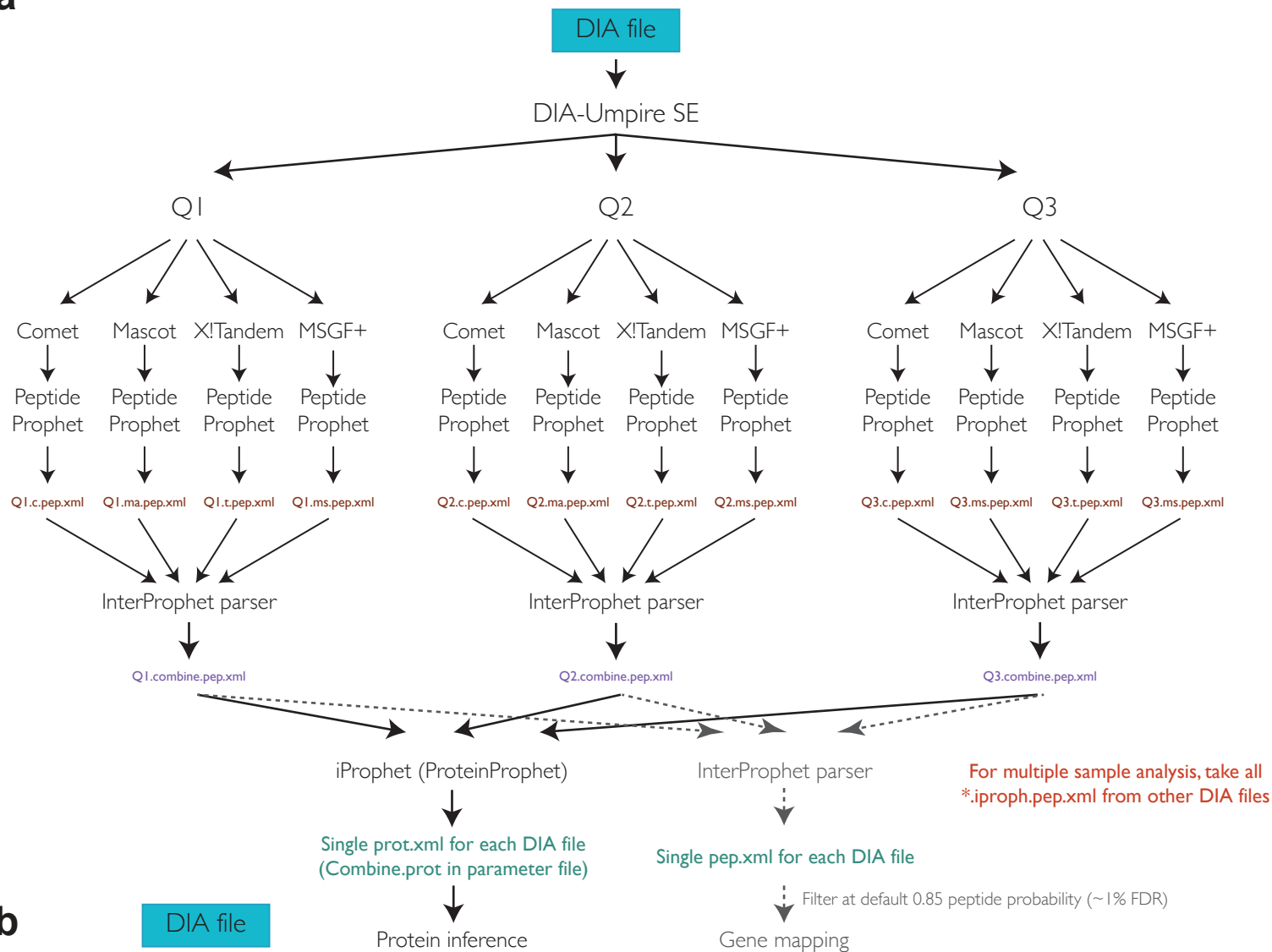
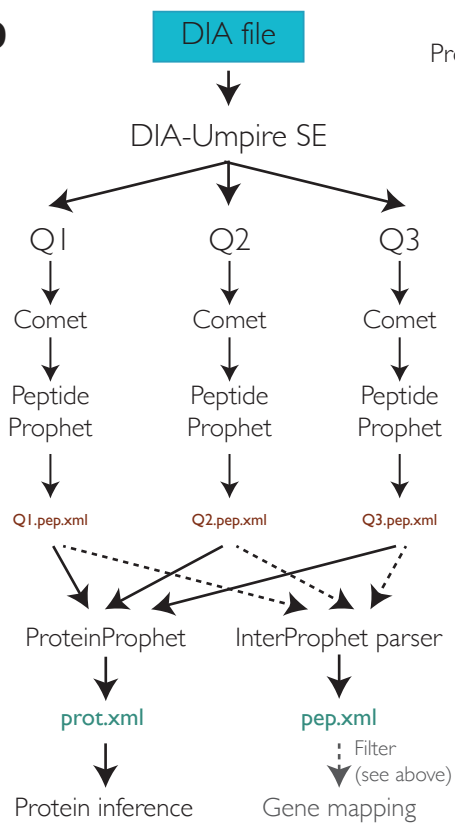
V\_SWATH (variable SWATH window)

==window setting end

For variable windows, enter each of the windows on a separate line (leave blank for fixed sized windows)

### Supplementary Fig. 2. Running DIA-Umpire untargeted identification through the ProHits interface.

a) Select "DIA file" and "MS-Umpire". In this Data Management module of ProHits, DIA-Umpire SE (Signal Extraction) generates pseudo-MS/MS spectra from precursor-fragment group data. These spectra can be searched using standard tools designed for DDA analysis, including the iProphet pipeline; note that DIA-Umpire SE extracts three quality tiers of precursor-fragment group data that are each searched and modeled separately (Supplementary Fig. 3). The final iProphet ProteinProphet result merges all quality groups for all search engines used. Currently, Comet, MSGF+, XTandem and Mascot are supported. Select the desired database search engines, protein database and search parameters that will be used to search the pseudo MS/MS spectra. b) Select the DIA-Umpire parameters. DIA-Umpire supports a variable window design (see Tsou et al., Nature Methods, 2015). In this case, specify each individual window's boundaries. Leave blank if fixed windows are used.

**a****b**

**Supplementary Fig. 3. Detailed process for combining search results in the DIA-Umpire SE module.**

a) iProphet pipeline. DIA-Umpire separates the precursor-fragment groups in three quality tiers. Q1 corresponds to MS1 clusters of 3 or more isotope peaks, Q2, to those with 2, and Q3 to those that have been detected only as clusters of at least 2 unfragmented precursor ion peaks in MS2. Because of the different ratios of correct versus incorrect identifications across these tiers, PeptideProphet is run separately for each of the tiers (see details in DIA-Umpire original paper by Tsou et al., Nature Methods 2015). To combine results from multiple search engines into one final report, searches using each search engine are first performed on individual quality tiers, and PeptideProphet is performed on those results. InterProphet parser is used to combine the results into a single pep.xml file for each of the quality tiers, and once again to combine across all search engines and all quality tiers (this is the version used for extraction of gene level information). iProphet (ProteinProphet) is then applied to these three combined pep.xml files to yield the final protein list. Note that the gene level viewer instead extracts information directly from the pep.xml to perform mapping at the gene level (grey); see Supplementary Fig. 6 for additional information. b) In addition to providing an iProphet view of the data, pep.xml and prot.xml files are generated for each search engine. In this case, PeptideProphet is run on the individual files, which are combined into a single pep.xml using the InterProphet parser (gene level information is extracted from the resulting file). ProteinProphet runs directly on the Q1, Q2 and Q3 pep.xml outputs to generate the prot.xml file.

a

Select instrument and search engine

Machine: TRIPLETOF1 | XTandem | MSGFPL | Mascot | Comet | IProphet

Process files

**Searched tasks**  
Tasks name [Tasks ID]  
 UMPIRE\_MEPCE\_EIF\_50ppm\_XTandem\_MSGF\_n2 (237)  
 Umpire\_MEPCE (269)

Select at least 1 task  
(only tasks with the same search parameters can be re-extracted jointly)

**Raw files**  
Raw file name [Raw file ID, Sample ID, Tasks ID]  
 LongSwath\_GFPJune7-Biorep1.wiff [27788] [17333] [269]  
 LongSwath\_GFPJune7-Biorep2.wiff [27791] [17334] [269]  
 LongSwath\_GFPJune7-Biorep3.wiff [27794] [17335] [269]  
 LongSwath\_EIF4aJune7-Biorep1.wiff [27851] [17577] [269]  
 LongSwath\_EIF4aJune7-Biorep2.wiff [27854] [17578] [269]  
 LongSwath\_EIF4aJune7-Biorep3.wiff [27869] [17579] [269]

**Selected raw files**  
Raw file name [Raw file ID, Sample ID, Tasks ID]  
 LongSwath\_MEPCEJune7-Biorep1.wiff [27761] [17321] [269]  
 LongSwath\_MEPCEJune7-Biorep2.wiff [27773] [17322] [269]  
 LongSwath\_MEPCEJune7-Biorep3.wiff [27818] [17323] [269]

b

### DIA-Umpire Quant parameters

DIA-Umpire Quant				
Task Name	MEPCE_EIF4A_SAIN			
Task Description	Biological triplicates			
TargetedExtraction	true: <input checked="" type="radio"/> false: <input type="radio"/>			
PeptideFDR	0.05			
ProteinFDR	0.05			
ProbThreshold	0.9			
FilterWeight	GW: <input checked="" type="radio"/> PepW: <input type="radio"/>			
MinWeight	0.9			
TopNFrags	6 Suggested values SAINT=6; mapDIA=20			
TopNPep	6 Suggested values SAINT=6; mapDIA=20			
Freq	0.5 Suggested values SAINT=0.5; mapDIA=0			
Run SAINT:	<input checked="" type="radio"/> Run mapDIA: <input type="radio"/> Only Run DIA-Umpire-Quant: <input type="radio"/>			
Raw File ID	Sample Name	Bait Name/Label	Use sample name	Is control
27761	MEPCE_SWATH_LongMS1_BR1_June	MEPCE	<input type="checkbox"/>	<input type="checkbox"/>
27773	MEPCE_SWATH_LongMS1_BR2_June	MEPCE	<input type="checkbox"/>	<input type="checkbox"/>
27818	MEPCE_SWATH_LongMS1_BR3_June	MEPCE	<input type="checkbox"/>	<input type="checkbox"/>
27788	GFP_SWATH_LongMS1_BR1_June	GFP	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
27791	GFP_SWATH_LongMS1_BR2_June	GFP	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
27794	GFP_SWATH_LongMS1_BR3_June	GFP	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
27851	EIF4A2_SWATH_LongMS1_BR1	EIF4A2	<input type="checkbox"/>	<input type="checkbox"/>
27854	EIF4A2_SWATH_LongMS1_BR2	EIF4A2	<input type="checkbox"/>	<input type="checkbox"/>
27869	EIF4A2_SWATH_LongMS1_BR3	EIF4A2	<input type="checkbox"/>	<input type="checkbox"/>
Next				

Select desired parameters for targeted re-extraction

(Here, the suggested values for SAINT scoring are shown)

Options available for scoring:  
 SAINT (shown here)  
 mapDIA (see Sup Fig 6)  
 none (run only DIA-Umpire Quant)

If running SAINT, select which files are "control" runs

c

### SAINT parameters

SAINT express (exp3.3)	SAINT (2.5.0)
Use SAINT with controls	You have selected 3 control sample(s) in previous step. How many compressed controls: 3
Compress baits	3 replicates in each interaction with the highest counts is involved in the computation of the scores
<b>SAINT (2.5.0) parameters</b>	
Burn-in period	nburn: 2000 Iterations: niter: 5000
exclude extremely high counts	lowMode: 0 forcing separation: minFold: 1
divide spectral counts by the total spectral counts of each IP normalize: 1	
Run DIA-Umpire Quant & SAINT	

Specify SAINT parameters.

By default, ProHits will run both MS1 and MS2 data.  
 ProHits will run both SAINT and SAINTexpress.

The options shown here (other than compression) are only used by standard SAINT.

## Supplementary Figure 4. Running the semi-targeted re-extraction module of DIA-Umpire (DIA-Umpire Quant).

a) To be able to perform targeted extraction using DIA-Umpire's Quant, a DIA-Umpire SE task must first have been run in the Data Management module. All files to be re-extracted jointly must have been searched with the same database and search parameters. Select the files for re-extraction. b) Specify the parameters for DIA-Umpire extraction, and whether you want to only run DIA-Umpire or to also analyze the data with SAINT (panel c) or mapDIA (Supplementary Fig. 7). If running SAINT, specify here which samples should be used as negative controls. Note that in SAINT, all samples listed with the same name (here, the gene name by default) will be combined as one SAINT bait (with n replicates): If a separation of the samples is desired, either click the "Use Sample Name" box, or manually force separation by giving each sample a different name. c) By default, ProHits will initiate the following SAINT tasks: SAINTexpress, intensity model, with both MS1 and MS2 quantification, and standard SAINT (currently SAINT 2.5.0), also with both MS1 and MS2 quantification. In each case, the controls can be compressed (to the highest values, a more stringent parameter for scoring), and the baits can also be compressed (meaning that for each prey, the x highest values will be considered). The parameters for standard SAINT can be specified. Each of these four SAINT variations is then associated with a SAINT report that can be viewed online or downloaded for further analysis.

a

Task Name: MSPLIT\_MEPCE



Import parameters from previous Tasks

Is DIA file  Run MSPLIT-DIA

Workflow: Convert Raw file → Search DDA file → **MSPLIT-DIA** (Create spectral library → Perform library search for DIA files → Filter search results → Create quantification Input files)

Search Engine Parameters

Parameter Set: (883) APMS\_BioID View

Search engines:  MS-GFDB

Database: 18--HEK293RefV57cRAPg View

Fixed Modifications: selected modifications (empty), available modifications: Carbamidomethyl (C), Phospho (ST), Phospho (Y), Acetyl (N-term), GG (K)

Variable Modifications: Deamidated (NQ), Oxidation (M)

MSPLIT-DIA Parameter set

Parameters required for MSPLIT-DIA: (810) MSPLIT\_fixed\_window View, Search existing Library  Archive-Search View

Proteowizard Parameter set

Parameters required for converting raw/wiff files: (828) SWATH\_TOF1 View

**\*\*TPP is not currently supported**

Data Files (total: 0)

[Folder ID] / File Name	is DDA	<input checked="" type="checkbox"/>
[23713] / IDA_EIF4aJune7-Biorep1.wiff (27845)	is DDA	<input checked="" type="checkbox"/>
[23713] / IDA_EIF4aJune7-Biorep2.wiff (27848)	is DDA	<input checked="" type="checkbox"/>
[23713] / IDA_EIF4aJune7-Biorep3.wiff (27866)	is DDA	<input checked="" type="checkbox"/>
[23713] / IDA_GFPJune7-Biorep1.wiff (27767)	is DDA	<input checked="" type="checkbox"/>
[23713] / IDA_GFPJune7-Biorep2.wiff (27782)	is DDA	<input checked="" type="checkbox"/>
[23713] / IDA_GFPJune7-Biorep3.wiff (27785)	is DDA	<input checked="" type="checkbox"/>
[23713] / IDA_MEPCEJune7-Biorep1.wiff (27758)	is DDA	<input checked="" type="checkbox"/>
[23713] / IDA_MEPCEJune7-Biorep2.wiff (27770)	is DDA	<input checked="" type="checkbox"/>
[23713] / IDA_MEPCEJune7-Biorep3.wiff (27815)	is DDA	<input checked="" type="checkbox"/>
[23713] / LongSwath_EIF4aJune7-Biorep1.wiff (27851)	is DDA	<input type="checkbox"/>
[23713] / LongSwath_EIF4aJune7-Biorep2.wiff (27854)	is DDA	<input type="checkbox"/>
[23713] / LongSwath_EIF4aJune7-Biorep3.wiff (27869)	is DDA	<input type="checkbox"/>
[23713] / LongSwath_GFPJune7-Biorep1.wiff (27788)	is DDA	<input type="checkbox"/>
[23713] / LongSwath_GFPJune7-Biorep2.wiff (27791)	is DDA	<input type="checkbox"/>
[23713] / LongSwath_GFPJune7-Biorep3.wiff (27794)	is DDA	<input type="checkbox"/>
[23713] / LongSwath_MEPCEJune7-Biorep1.wiff (27761)	is DDA	<input type="checkbox"/>
[23713] / LongSwath_MEPCEJune7-Biorep2.wiff (27773)	is DDA	<input type="checkbox"/>
[23713] / LongSwath_MEPCEJune7-Biorep3.wiff (27818)	is DDA	<input type="checkbox"/>

Add Files Run Task

Select MSPLIT-DIA for DIA file

These are the search parameters to create the spectral library from DDA files.

Select:

- database search parameters,
- search engine (only MSGFDB is supported at this time),
- sequence database,
- fixed and variable modifications.

Indicate whether a specific spectral library, the facility archive or the archive + active searches should be used by MSPLIT-DIA

Specify MSPLIT-DIA parameters

Select conversion parameters

b

**MSPLIT-DIA Parameters**

MSPLIT-DIA is a spectral library search tool that identify multiplexed MS/MS spectra in DIA data (e.g. SWATH)

Creating library from MSGFDB search results: FDR 0.01  
Creating library from DDA runs: picks a representative PSM (currently the one with lowest MSGF probability) for each unique peptide precursor (i.e. peptide sequence and charge state combination) and ensure that the overall peptide-level FDR for the combined search results is low

Creating decoy spectral library: fragment mass tolerance 0.05 Da  
appends a decoy version of the spectral library to the original library and also performs some noise filtering of the target spectrum based on the fragment ion annotation from the identified peptides.

Performing spectral library search: parent mass tolerance 25 Da, fragment mass tolerance 50 PPM, number of scans in one cycle 33  
This is the number of MS scans per cycle of the DIA run (e.g. in SWATH, 1 MS1 is followed by 32 MS/MS to cover a mass range of 400 -- 1200, thus the number of scans in one cycle is 33)

Filtering search results: max RT used to build RT correlation 5, min RT used to build RT correlation 5, use retention time to filter result

SWATH window setting: #Scan windowBegin windowEnd  
MS1 0 1250  
MS2 [ ]  
separated by space (variable SWATH window)

For variable windows, enter each of the windows on a separate line (leave blank for fixed sized windows)

Supplementary Fig. 5. Running MSPLIT-DIA through the ProHits interface.

a) Select "DIA file" and "MSPLIT-DIA". If using DDA files to create a library, select DDA search parameters (only MSGFDB is currently supported as a search engine). Add both DDA and DIA (SWATH) files to the list of files and select which of those are the DDA files that will be used for library generation. Existing libraries can also be searched: individual external libraries are listed (here Human\_Swath\_Atlas\_v1 from the Aebersold group). In addition, ProHits automatically generates a spectral library from all previously-searched DDA files that it appends to externally generated libraries ("Archive"). These can be searched in isolation, or alongside the newly searched results. In all cases, ProHits will take the highest MSGFDB spectrum for spectral matching with MSPLIT-DIA. b) Select the parameters to be used for creating the spectral library from MSGFDB results, creating the decoy spectral library, performing the spectral library search and filtering the search results using retention time correlation. MSPLIT-DIA supports a variable window design (this was not specifically described in Wang et al., Nature Methods, 2015). In this case, specify each individual window's boundaries. Leave blank if fixed windows are used.

**a**

**TRIPLET0F1 Search Results**

Task ID: 268  
 Task Name: MEPCE\_EIF4A\_MSPLIT\_forHW  
 Task Project Name: Gingras\_Lab\_Public  
 Folder ID: 23713  
 Folder Name: Zhen 4(MB) 2012-12-12  
 Folder Project: Gingras\_Lab\_Public

Start Time: 2015-11-20 13:58:29  
 Set By: James Knight

Search Engine: MSGFDB-SWATHTOF1  
 Converter=SWATH\_TOF1  
 MSPLIT=MSPLIT\_default  
 Database=HEK293RefV57cRAPg

Set Search Results to Run TPP  
 TPP not yet supported for MSPLIT-DIA

Parse Hits to ProHits Analyst database  
 Navigation box to transfer results to "Analyst" module

Parsed Hits Status: Completed 2015-12-04 18:19:05  
 Parsed By: Anne-Claude Gingras

Download an assay library for targeted extraction (outside of ProHits)  
 Openswath, Peakview, Skyline

File ID	File Name	Size(KB)	Search Results	TPP
27764	[23713] / Swath_MEPCEJune7-Biorep1.wiff	8,848	MSPLIT	
27776	[23713] / Swath_GFPJune7-Biorep1.wiff	8,848	MSPLIT	
27797	[23713] / Swath_GFPJune7-Biorep2.wiff	8,848	MSPLIT	
27800	[23713] / Swath_GFPJune7-Biorep3.wiff	8,848	MSPLIT	

Tracked parameters

**Search Engine Parameters**

**MSGFDB**

Database\_name: HEK293RefV57cRAPg  
 Enzyme\_number: 1  
 Multiple\_select\_str: frm\_variable\_MODS(Oxidation (M):Acetyl (N-term)&&frm\_fixed\_MODS(Carbamidomethyl) (C)  
 Num\_enzyme termini: 1  
 Decoy\_search: 1  
 Peptide\_mass\_tolerance\_start: 50  
 Peptide\_mass\_tolerance\_end: 50  
 Peptide\_mass\_units: 2  
 C13: 1  
 MinPeptLength: 8  
 MaxPeptLength: 30  
 MinPrecursorCharge: 2  
 MaxPrecursorCharge: 4  
 MegId: FragmentMethodID: 0  
 MegId\_InstrumentID: 2  
 NumMatchesPerSpec: 1  
 UniformAAProb:

**MSPLIT**

FDR: 0.01  
 Decoy\_fragment\_mass\_tolerance: 0.05  
 Parent\_mass\_tolerance: 25  
 Fragment\_mass\_tolerance: 50  
 Number\_scans: 33  
 MaxRT: 5  
 MinRT: 5  
 Dia\_win\_ms1\_start: 0  
 Dia\_win\_ms1\_end: 1250  
 Dia\_SWATH\_window\_setting:

**Modifications**

Fixed Modifications: Carbamidomethyl (C)  
 Variable Modifications: Oxidation (M)/Acetyl (N-term)

**Converter**

--32 --mz32 --intn32 --filter "peakPicking true 2" --filter "msLevel 2" --gl --centroid /singleprecision

Files manually linked to a Sample in the "Analyst" module

Clickable boxes to select files to be "parsed" to Analyst module

**b**

**Samples (Project 94: Gingras\_SWATH\_development)**

Experiment status color keys [+]  
 Group and exported versions [+]

Sample ID	Sample Name	BaitID	BaitGene	SB.Tag	User	Date	Show groups	Exp. Status	Options
17335	GFP_SWATH_LongMS1_BR3_June	5037	GFP	N-Flag	Brett Larsen	2013-06-09	33743	Experiment	
17334	GFP_SWATH_LongMS1_BR2_June	5037	GFP	N-Flag	Brett Larsen	2013-06-09	33675	Experiment	
17333	GFP_SWATH_LongMS1_BR1_June	5037	GFP	N-Flag	Brett Larsen	2013-06-09	33914	Experiment	
17332	GFP_SWATH_ShortMS1_BR3_June	5037	GFP	N-Flag	Brett Larsen	2013-06-09	5765	Experiment	
17331	GFP_SWATH_ShortMS1_BR2_June	5037	GFP	N-Flag	Brett Larsen	2013-06-09	5772	Experiment	
17330	GFP_SWATH_ShortMS1_BR1_June	5037	GFP	N-Flag	Brett Larsen	2013-06-09	5806	Experiment	

Protein level Report  
Gene level Report (MSPLIT-DIA)**c**

**Gene level MSPLIT Hits**

ID	Gene	Redundant	# SpectralCount	# Unique Group Peptide	Subsumed	Project Frequency	Links	Filter	Option
201168	56257 / MEPCE		3038	114		100%	(Gene) BioGRID		
1030353	708 / C1QBP		2562	60					
1030417	2194 / FASN		292	55					
1030569	5591 / PRKDC		212	49					

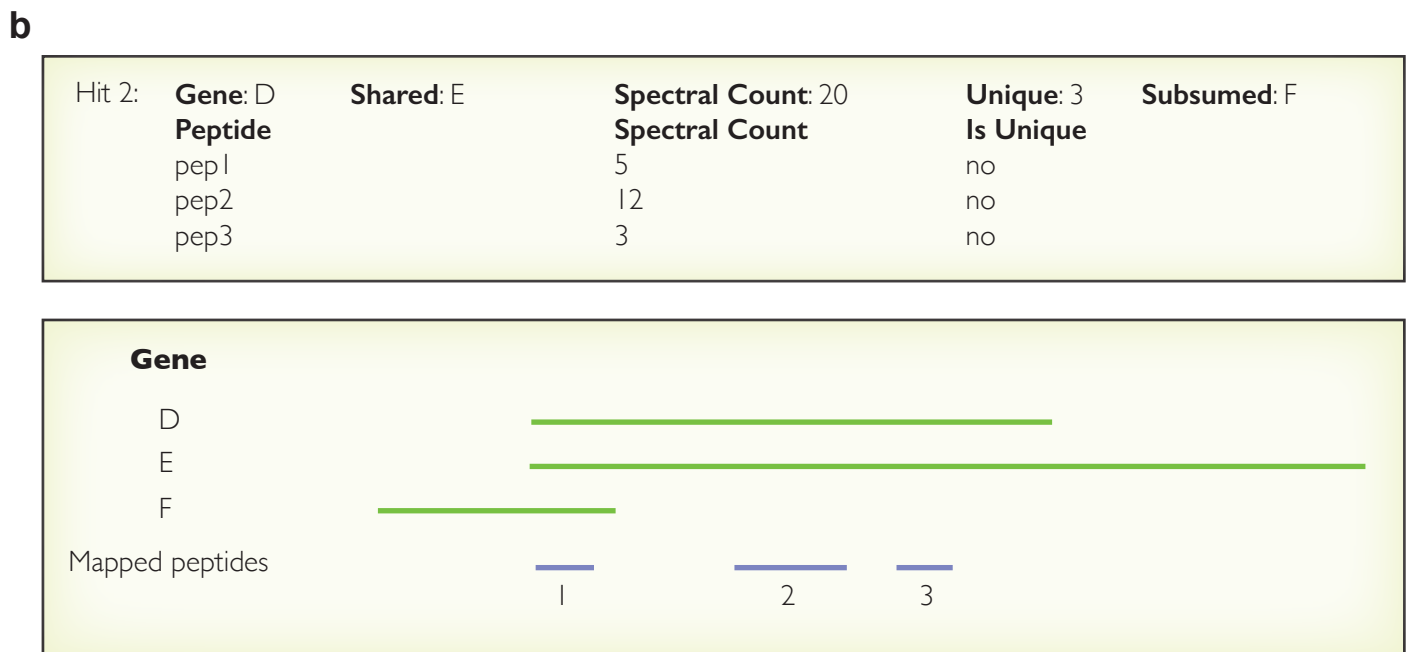
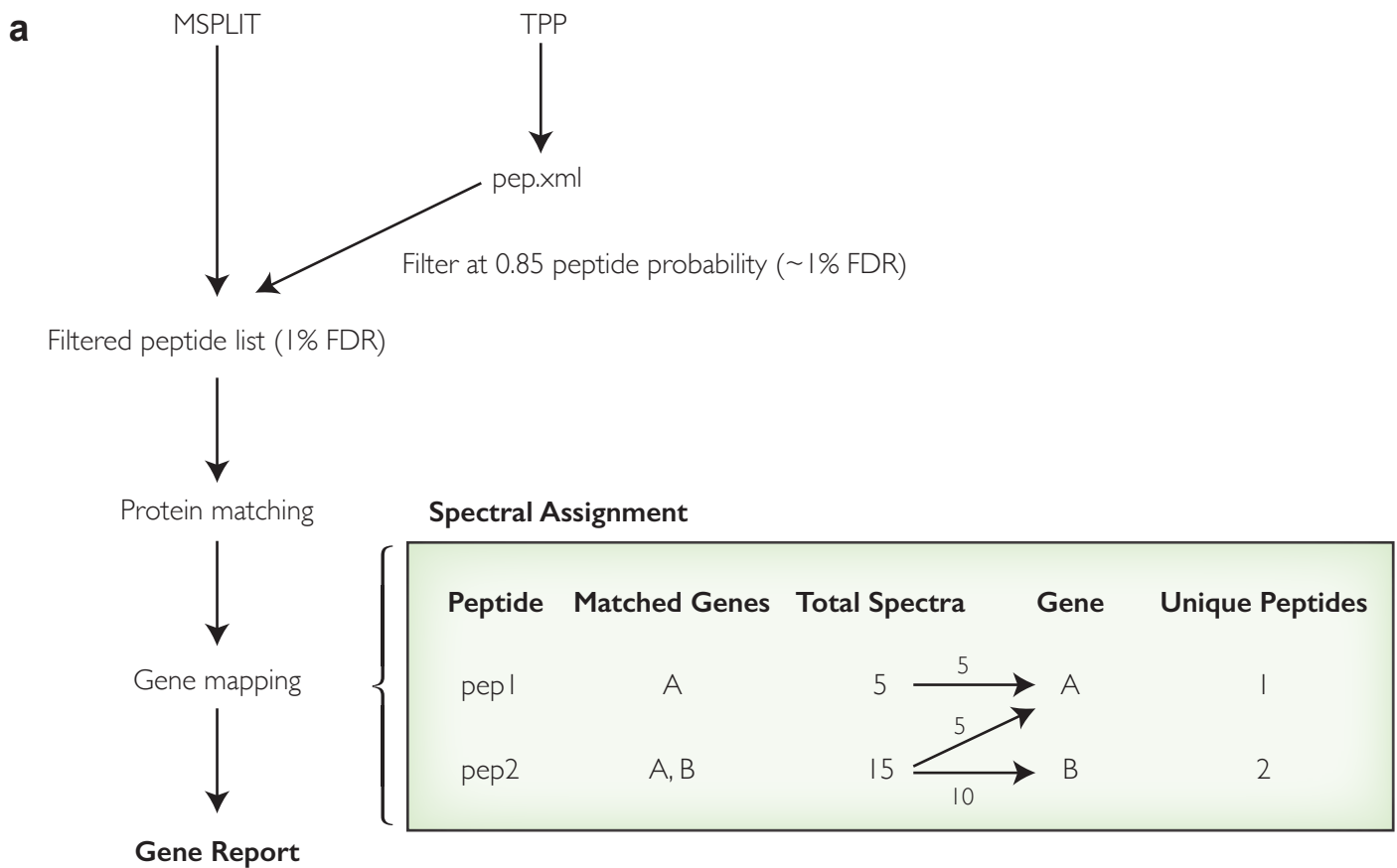
35. Gene: CCNT2  
 GeneID: 905  
 SpectralCount: 21  
 Unique: 5  
 Subsumed: -

Peptide	SpectralCount	IsUnique
DHYIAAQVEGQHK	4	yes
EQLENTPSR	3	yes
HHGPISITPGIIPQK	4	yes
SPVGLSSDGISSSSSSR	5	yes
TEQLYSQK	5	yes
LNVSQLTINTAIYVMHR	2	no

Detailed Gene level report

Supplementary Fig. 6. Results page for MSPLIT-DIA in the Data Management module.

a) ProHits tracks all search parameters to facilitate reporting. The identification results for each file can be downloaded as a tab delimited file (download icon by each file name). Alternatively, the results can be parsed to the "Analyst" module of ProHits. ProHits also facilitates the generation through MSPLIT-DIA of "assay libraries" for targeted extraction by the popular tools OpenSWATH, PeakView and Skyline. b) Gene level reports (e.g. here for MSPLIT-DIA) are distinguishable from protein level reports through color-coded icons. c) Gene level views. Peptides are first matched to proteins and those identifications are then used for mapping peptides to genes (using Refseq for protein and gene IDs). For peptides shared between multiple genes, spectral counts are assigned exclusively to those genes with unique peptides in proportion to the existing evidence for those genes. If a peptide matches exclusively to genes that have no unique peptides, then spectral counts are divided equally between the genes. After all spectra have been assigned, counts for each gene are rounded to the nearest integer. The final report contains all genes to which peptides have been matched. For each gene, "shared genes" match the exact same set of peptides. "Subsumed genes" are also reported that include genes that match to a subset of the current gene's peptides and nothing outside of this set. In addition to total spectral counts, unique peptide counts are also reported for each gene. In the case of genes that match to the exact same set of peptides, this "unique" number will refer to the number of unique peptides for the corresponding group.



**Supplementary Fig. 7. Gene inference.**

a) Peptides are first matched to proteins and those identifications are then used for mapping peptides to genes (using Refseq for protein and gene IDs). For peptides shared between multiple genes, spectral counts are assigned exclusively to those genes with unique peptides in proportion to the existing evidence for those genes. If a peptide matches exclusively to genes that have no unique peptides, then spectral counts are divided equally between the genes. b) The final report contains all genes to which peptides have been matched. For each gene, "shared genes" are reported if they exist. These encompass genes that match to the exact same set of peptides, i.e. we have no evidence to determine which of the corresponding proteins is in the sample although the peptides tell us a least one is. "Subsumed genes" are also reported that include genes that match to a subset of the current gene's peptides and nothing outside of this set. In addition to total spectral counts, unique peptide counts are also reported for each gene. In the case of genes that match to the exact same set of peptides, this "unique" number will refer to the number of unique peptides for the corresponding group.

## a DIA-Umpire Quant parameters

DIA-Umpire Quant			
Task Name	MEPCE EIF4A_mapDIA		
Task Description			
TargetedExtraction	true: <input checked="" type="radio"/> false: <input type="radio"/>		
PeptideFDR	0.05		
ProteinFDR	0.05		
ProbThreshold	0.9		
FilterWeight	GW: <input checked="" type="radio"/> PepW: <input type="radio"/>		
MinWeight	0.9		
TopNFrags	20 Suggested values SAINT=6; mapDIA=20		
TopNPep	20 Suggested values SAINT=6; mapDIA=20		
Freq	0 Suggested values SAINT=0.5; mapDIA=0		
Run SAINT:	<input type="radio"/>		
Run mapDIA:	<input checked="" type="radio"/>		
Only Run DIA-Umpire-Quant:	<input type="radio"/>		
Raw File ID	Sample Name	Bait Name/Label	Is control
		<input checked="" type="checkbox"/> Use sample name	
27788	GFP_SWATH_LongMS1_BR1_June	GFP	<input type="checkbox"/>
27791	GFP_SWATH_LongMS1_BR2_June	GFP	<input type="checkbox"/>
27794	GFP_SWATH_LongMS1_BR3_June	GFP	<input type="checkbox"/>
27761	MEPCE_SWATH_LongMS1_BR1_June	MEPCE	<input type="checkbox"/>
27773	MEPCE_SWATH_LongMS1_BR2_June	MEPCE	<input type="checkbox"/>
27818	MEPCE_SWATH_LongMS1_BR3_June	MEPCE	<input type="checkbox"/>
27851	EIF4A2_SWATH_LongMS1_BR1	EIF4A2	<input type="checkbox"/>
27854	EIF4A2_SWATH_LongMS1_BR2	EIF4A2	<input type="checkbox"/>
27869	EIF4A2_SWATH_LongMS1_BR3	EIF4A2	<input type="checkbox"/>

Select desired parameters for targeted re-extraction

(Here, the suggested values for mapDIA scoring are shown)

Options available for scoring:

- SAINT (see Sup. Figure 3)
- mapDIA (here)
- none (run only DIA-Umpire Quant)

Use arrows to organize order as desired

## b mapDIA parameters

<http://xtandemserver.mshri.on.ca/thegpm-cgi/>

mapDIA (version:2.0.5)																			
Experimental design	<input type="text" value="replicatedesign"/> <p>REP design: For example, if the design is a time course experiment with 3 time points (t1,t2,t3) across 2 biological replicates (A,B), then the conditions are time points and thus the samples should be organized in the following order (t1-A, t1-B) (t2-A, t2-B) (t3-A, t3-B)</p>																		
Normalization	<input type="text" value="none"/> none/TIS/rt 30																		
Filter	<input type="text" value="SDF 2"/> <input type="text" value="MIN_CORREL 0.2"/> <input type="text" value="MIN_FRAG_PER_PEP 3"/> <input type="text" value="MAX_FRAG_PER_PEP 5"/> <input type="text" value="MIN_PEP_PER_PROT 1"/>																		
Sample information	<table border="1"> <thead> <tr> <th>Group #</th> <th>Raw file ID</th> <th>LABEL</th> <th>MIN_OBS</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>27794 27791 27788</td> <td>GFP</td> <td>2</td> </tr> <tr> <td>2</td> <td>27818 27773 27761</td> <td>MEPCE</td> <td>2</td> </tr> <tr> <td>3</td> <td>27869 27854 27851</td> <td>EIF4A2</td> <td>2</td> </tr> </tbody> </table>			Group #	Raw file ID	LABEL	MIN_OBS	1	27794 27791 27788	GFP	2	2	27818 27773 27761	MEPCE	2	3	27869 27854 27851	EIF4A2	2
Group #	Raw file ID	LABEL	MIN_OBS																
1	27794 27791 27788	GFP	2																
2	27818 27773 27761	MEPCE	2																
3	27869 27854 27851	EIF4A2	2																
DEPs	<input type="text" value="MIN_DE 0.01"/> <input type="text" value="MAX_DE 0.99"/>																		
Protein_level	<input type="text" value="MAX_PEP_PER_PROT 5"/>																		
Contrast matrix for group comparison	<table border="1"> <thead> <tr> <th></th> <th>1</th> <th>2</th> <th>3</th> </tr> </thead> <tbody> <tr> <td>1</td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> <tr> <td>2</td> <td><input checked="" type="checkbox"/></td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> <tr> <td>3</td> <td><input checked="" type="checkbox"/></td> <td><input checked="" type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> </tbody> </table>				1	2	3	1	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	3	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
	1	2	3																
1	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>																
2	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>																
3	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>																

Link to the mapDIA user manual

Specify experimental design and parameters

Suggested parameters are listed by default

Run DIA-Umpire Quant & mapDIA

mapDIA "analysis output"

## c

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	Protein	nPeptide	nFragment	Label	Label2	log2FC	score	FDR	log_oddsDE	log2FC_1	log2FC_2	log2FC_3	nUp	nDown	mcr	min.mcr	max.mcr	PreyGeneID	PreyGene
2	Q13347	8	41	2 to 1	EIF4A2/GFP	2.33989	1	0	164.192	2.1362	2.42028	2.46321	3	0	0.756243	0.702197	0.785021	EIF3I	8668
3	O75821	5	28	2 to 1	EIF4A2/GFP	2.31502	1	0	169.756	2.5796	1.94332	2.42214	3	0	1	1	1	EIF3G	8666
4	O00303	4	15	2 to 0	EIF4A2/MEPCE	2.23984	1	0	54.1806	2.03122	2.6019	2.08639	3	0	0.864434	0.594655	0.999517	EIF3F	8665
5	P60842	7	32	2 to 1	EIF4A2/GFP	2.0113	1	0	67.3016	1.53731	2.43739	2.0592	3	0	0.599081	0.514769	0.714154	EIF4A1	1973
6	P22626	3	12	2 to 1	EIF4A2/GFP	1.99311	1	0	69.5806	1.92326	1.90409	2.15198	3	0	0.81223	0.775	0.884661	HNRNPA2B1	3181
7	P62753	4	20	2 to 1	EIF4A2/GFP	1.79617	1	0	134.034	1.97417	1.69194	1.72239	3	0	0.969364	0.942671	0.983032	RP56	6194
8	O00571	7	39	2 to 1	EIF4A2/GFP	1.7518	1	0	121.191	1.85427	1.9021	1.49904	3	0	0.83772	0.75	0.964453	DDX3X	1654
9	P60842	5	22	2 to 0	EIF4A2/MEPCE	1.67667	1	0	52.961	1.48226	1.39583	2.15192	3	0	0.599081	0.514769	0.714154	EIF4A1	1973

### Supplementary Fig. 8. Running mapDIA from DIA-Umpire through ProHits.

a) When initiating the DIA-Umpire semi-targeted extraction, select to run mapDIA. Use arrows to organize the data as desired to accommodate experimental design. b) Select mapDIA parameters (see linked mapDIA user manual for detailed explanation of the experimental design and parameters). c) mapDIA returns a folder with several files, including this "analysis output" file which contains quantitative information and statistical parameters. Note that this can be used as an input for visualization tools (e.g. Fig. 2) that are currently run outside of ProHits.



**a**

# Saint Comparison

Automatically add all files associated with a search task for Export

**b**

Manually add Raw files to export list

**c**

## Export Raw Files

Raw file download and export options. For ProteomeXchange, select MassIVE

FTP Server Download to Local Computer Copy files to other folder Export to MassIVE (FTP)

You can select files into a temp package, then download the package to your local computer.

- Select raw files from different folder in Storage.
- Select search result files from different search tasks.
- Exported raw file logs fileList\_2015-12-06.txt [LOG]

Files in the exporting list [remove all]

ID	TaskID	Mass Spec	File Type	Raw File Name	Size(MB)
27760		TRIPLETOF1	RAW	IDA_MEPCJune7-Biorep1.wiff.scan	779.23
27758		TRIPLETOF1	RAW	IDA_MEPCJune7-Biorep1.wiff	11.81
27772		TRIPLETOF1	RAW	IDA_MEPCJune7-Biorep2.wiff.scan	732.61
27770		TRIPLETOF1	RAW	IDA_MEPCJune7-Biorep2.wiff	11.79
27817		TRIPLETOF1	RAW	IDA_MEPCJune7-Biorep3.wiff.scan	723.42
27815		TRIPLETOF1	RAW	IDA_MEPCJune7-Biorep3.wiff	12.03
27769		TRIPLETOF1	RAW	IDA_GFPJune7-Biorep1.wiff.scan	723.91
27767		TRIPLETOF1	RAW	IDA_GFPJune7-Biorep1.wiff	11.42
27784		TRIPLETOF1	RAW	IDA_GFPJune7-Biorep2.wiff.scan	721.44
27782		TRIPLETOF1	RAW	IDA_GFPJune7-Biorep2.wiff	11.95
27787		TRIPLETOF1	RAW	IDA_GFPJune7-Biorep3.wiff.scan	722.44
27785		TRIPLETOF1	RAW	IDA_GFPJune7-Biorep3.wiff	11.9
27847		TRIPLETOF1	RAW	IDA_EIF4aJune7-Biorep1.wiff.scan	694.39
27845		TRIPLETOF1	RAW	IDA_EIF4aJune7-Biorep1.wiff	12.14
27850		TRIPLETOF1	RAW	IDA_EIF4aJune7-Biorep2.wiff.scan	714.02
27848		TRIPLETOF1	RAW	IDA_EIF4aJune7-Biorep2.wiff	11.84
27868		TRIPLETOF1	RAW	IDA_EIF4aJune7-Biorep3.wiff.scan	707.81
27866		TRIPLETOF1	RAW	IDA_EIF4aJune7-Biorep3.wiff	12.16

Enter MassIVE FTP address and credentials

Remote FTP Address (IP or domain name): massive.ucsd.edu

User Name: write name Password: \*\*\*\*\*

Connection Protocol: ftp Upload to Folder: new folder

Test Connection

Close Window

**d**

ProHits will retrieve peak lists and search results associated with the selected RAW file. If multiple tasks are available, override ProHits selection through drop-down menu.

Files in the exporting list [remove all]

ID	TaskID	Mass Spec	File Type	Raw File Name	Size(MB)
27760		TRIPLETOF1	RAW	IDA_MEPCJune7-Biorep1.wiff.scan	779.23
27758	task153_tpp43_iProphet	TRIPLETOF1	RAW	IDA_MEPCJune7-Biorep1.wiff	11.81
27772		TRIPLETOF1	RAW	IDA_MEPCJune7-Biorep2.wiff.scan	732.61
27770	task153_tpp43_iProphet	TRIPLETOF1	RAW	IDA_MEPCJune7-Biorep2.wiff	11.79
27817		TRIPLETOF1	RAW	IDA_MEPCJune7-Biorep3.wiff.scan	723.42
27815	task153_tpp43_iProphet	TRIPLETOF1	RAW	IDA_MEPCJune7-Biorep3.wiff	12.03
27769		TRIPLETOF1	RAW	IDA_GFPJune7-Biorep1.wiff.scan	723.91
27767	task153_Mascot	TRIPLETOF1	RAW	IDA_GFPJune7-Biorep1.wiff	11.42
27784	task153_tpp43_COMET	TRIPLETOF1	RAW	IDA_GFPJune7-Biorep2.wiff.scan	721.44
27782	task153_tpp43_iProphet	TRIPLETOF1	RAW	IDA_GFPJune7-Biorep2.wiff	11.95
27787	task153_COMET	TRIPLETOF1	RAW	IDA_GFPJune7-Biorep3.wiff.scan	722.44
27785	task158_MSPLIT_DDA	TRIPLETOF1	RAW	IDA_GFPJune7-Biorep3.wiff	11.9
27847	task195_MSPLIT_DDA	TRIPLETOF1	RAW	IDA_GFPJune7-Biorep3.wiff	11.9
27845	task250_MSPLIT_DDA	TRIPLETOF1	RAW	IDA_EIF4aJune7-Biorep1.wiff.scan	694.39
27848	task254_MSPLIT_DDA	TRIPLETOF1	RAW	IDA_EIF4aJune7-Biorep1.wiff	12.14
27850	task258_MSPLIT_DDA	TRIPLETOF1	RAW	IDA_EIF4aJune7-Biorep2.wiff.scan	714.02
27848	task261_MSPLIT_DDA	TRIPLETOF1	RAW	IDA_EIF4aJune7-Biorep2.wiff	11.84
27868	task262_MSPLIT_DDA	TRIPLETOF1	RAW	IDA_EIF4aJune7-Biorep3.wiff.scan	707.81
27866	task268_MSPLIT_DDA	TRIPLETOF1	RAW	IDA_EIF4aJune7-Biorep3.wiff	12.16
27868	task153_tpp43_iProphet	TRIPLETOF1	RAW	IDA_EIF4aJune7-Biorep3.wiff	12.16

Select fasta file: HEK293V57cRapRevTag

A full submission to ProteomeXchange requires the FASTA database  
• Select it here via a dropdown menu

**e**

- RAW FOLDER: Raw files (e.g. thermo RAW; AB Sciex wiff / wiff.scan)
- PEAKS FOLDER: Converted peaks lists (mzML; mzXML)
- RESULTS FOLDER: Search results converted to .mzid
- OTHER FOLDER: FASTA file selected by users

**f**

## Supplementary Fig. 9. ProHits facilitates the deposition of data in ProteomeXchange via MassIVE.

Files to be exported to MassIVE can be selected as a batch from the SAINT Comparison page (a), or manually added to the export list by clicking the download buttons located by individual files or folders in Data Management or individual Samples in the Analyst module (b). All files will be added to a "Export Raw files" list in Data Management (c). To export the data for complete ProteomeXchange submission at MassIVE, select the appropriate button, and login using your credentials. d) ProHits will retrieve both the Peaks list and the search results associated with the selected Raw files: ProHits uses by default the "Search Task" for which the results were parsed to the Analyst; this can be overridden by the user through the dropdown menu selection. For iProphet results, ProHits uses the TPP converters and renames the files for direct recognition in MassIVE. Complete submissions also require deposition of the FASTA sequence database that can be selected via the dropdown menus. e) ProHits generates at MassIVE subdirectories containing the Raw, Peak List, Results and FASTA files. They are named according to the MassIVE nomenclature and can easily be selected. f) MassIVE should recognize automatically all the files and associate them.

a

## Saint Comparison

Color code: Hit property color code  Shared hits color code

Sort by: SpecSum  Sample Name  EIF4A2  Descending  Ascending

[Click to remove filters]

Experiment Filters

SaintScore <  SpecSum <

AvgP <  maxSpec <

MaxP <  NumReplicates <

BFDR > 0.02   Background Set  background list

Frequency >  %

Bio Filters

Heat Shock  Ribosomal  Cytoskeleton  Bait

Keratin  Artifact Protein  Translation Elongation Factor  DEAD/H Box

Rib Nucleoprotein  Histone  Albumin

BioGRID BioGRID overlap

Physical HTP  Physical NON-HTP  Genetic HTP  Genetic NON-HTP

Select all desired filters, then hit "Go"

Cytoscape [Export (table)] [Export (matrix)] [Export to PSI-MI] [Send by email] [Export (Prohits web)] [Export (raw files)]

EIF4A2	MEPCE	Gene Name	Protein ID	Remove
<input checked="" type="checkbox"/>	<input type="checkbox"/>	EIF4A1 [BioGRID]	4503529	<input type="checkbox"/>
<input checked="" type="checkbox"/>	<input type="checkbox"/>	EIF4G1 [BioGRID]	302899237	<input type="checkbox"/>
<input checked="" type="checkbox"/>	<input type="checkbox"/>	EIF3A [BioGRID]	4503509	<input type="checkbox"/>
<input checked="" type="checkbox"/>	<input type="checkbox"/>	PDCD4 [BioGRID]	21735596	<input type="checkbox"/>
<input checked="" type="checkbox"/>	<input type="checkbox"/>	EIF4G2 [BioGRID]	289577060	<input type="checkbox"/>
<input checked="" type="checkbox"/>	<input type="checkbox"/>	EIF4G3 [BioGRID]	311771714	<input type="checkbox"/>
<input checked="" type="checkbox"/>	<input type="checkbox"/>	EIF3C [BioGRID]	4503525	<input type="checkbox"/>

Multiple export and visualization options are available: they will display / report the visible (post-filter) list

d

Bait List Experiment List Sample List Mascot TPP COMET TPP Xtander TPP MSGF+ TPP Mascot TPP iProphet

Samples

BaitID GeneName(Tag) SampleID SampleName

Selected Samples

BaitID GeneName(Tag) SampleID SampleName

User: All users

Search:

Group type:  Bait  Experiment  Sample

Show group:

Sort by: Bait ID

Experiment Filters

iProphet Probability <  %

Coverage <  %

Peptide Frequency <  %

min XPRESS Ratio:  %

max XPRESS Ratio:  %

Bio Filters

Heat Shock  Ribosomal  Cytoskeleton  Bait

Keratin  Artifact Protein  Translation Elongation Factor  DEAD/H Box

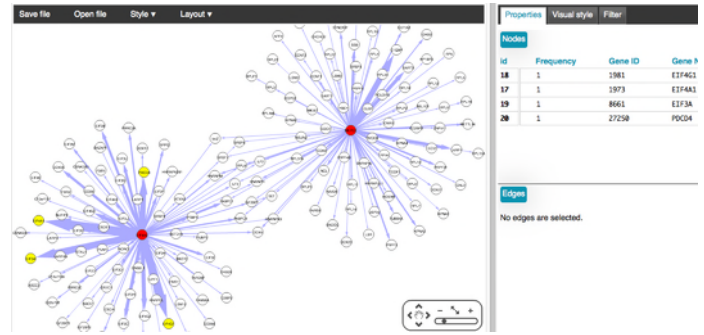
Rib Nucleoprotein  Histone  Albumin

Apply Filters

To use the customizable export function, first select which files are to be exported, as well as the filters when applicable.

b

## Cytoscape view of the data



c

## email / web link to a static html view of the data

Sent link: [gingras\\_94\\_seajudgh3dpp0m189pnnrjd14\\_2D\\_1449443980.html](http://prohits-web.lunenfeld.ca/prohits_report/gingras_94_seajudgh3dpp0m189pnnrjd14_2D_1449443980.html)

To:

Subject:

Contents:

[Send from email account of your choice]

- Option 1: Save the **FILE** to your local computer by select "SAVE LINK/TARGET AS"(shown upon right click). Then attach the saved file to you email.
- Option 2: Include the following URL link in your email: [http://prohits-web.lunenfeld.ca/prohits\\_report/gingras\\_94\\_seajudgh3dpp0m189pnnrjd14\\_2D\\_1449443980.html](http://prohits-web.lunenfeld.ca/prohits_report/gingras_94_seajudgh3dpp0m189pnnrjd14_2D_1449443980.html)

e

## Export Sample Report (Project: Gingras\_SWATH\_development)

Export rows as: CSV  Preview  Generate Report

Please select columns to be included in the export file

Pre-defined export format

[new]

**Selected columns**

Bait ID  
Bait Gene ID  
Bait Gene Name  
Sample ID  
Sample Name  
Protein Gene Name  
Protein ID  
Protein Gene ID  
Protein Probability  
Total Number Peptide  
Unique Number Peptide  
Coverage Percentage

Bait:

Bait ID  Bait Tax ID

Bait Gene ID  Bait Acc

Bait Gene Name  Bait Acc Type

Bait Locus Tag  Bait MW

Bait Clone  Bait Vector

Bait Description  Is Gel Free

Experiment:

Sample ID  Sample MW

Sample Intensity  Sample Name

Instrument  Raw File Name

Raw File Date  Raw File Size

Raw file path  Task ID

TPP task ID  Search parameters

TPP Protein:

TPPID  Protein Gene Name

Protein Acc Type  Protein Gene ID

Protein ID  Protein Acc

Protein Locus Tag  Protein Probability

PCT Spectrum IDs  Indistinguishable Protein

Protein Dec.  Total Number Peptide

Unique Number Peptide  Coverage Percentage

Xml File  Search Engine

Searched Database  Xpressratio Mean

Xpressratio SStandard Dev.  Xpressratio Number Peptide

Project Frequency  Filters

Hit Protein Length

TPP Protein Group Peptide:

## Supplementary Fig. 10. Selected visualization and export options in ProHits.

a) Multiple export and visualization options are available from any SAINT Comparison page. First select all desired filters and apply them by pressing "Go". Manual removal of selected hits can also be performed. The final view of the data can be downloaded as a table or matrix format, converted to the PSI-MI format, directly exported to the quantitative interaction proteomics repository [prohits-web.lunenfeld.ca](http://prohits-web.lunenfeld.ca), viewed as a Cytoscape figure (c), or emailed in a static html format with functional links (d). Additional export options are also available, e.g. that allow for the selection of any file (d) and download of selected values (e).