

Dissecting the molecular mechanisms regulating epithelial to mesenchymal transition (EMT) using Mass Spectrometry

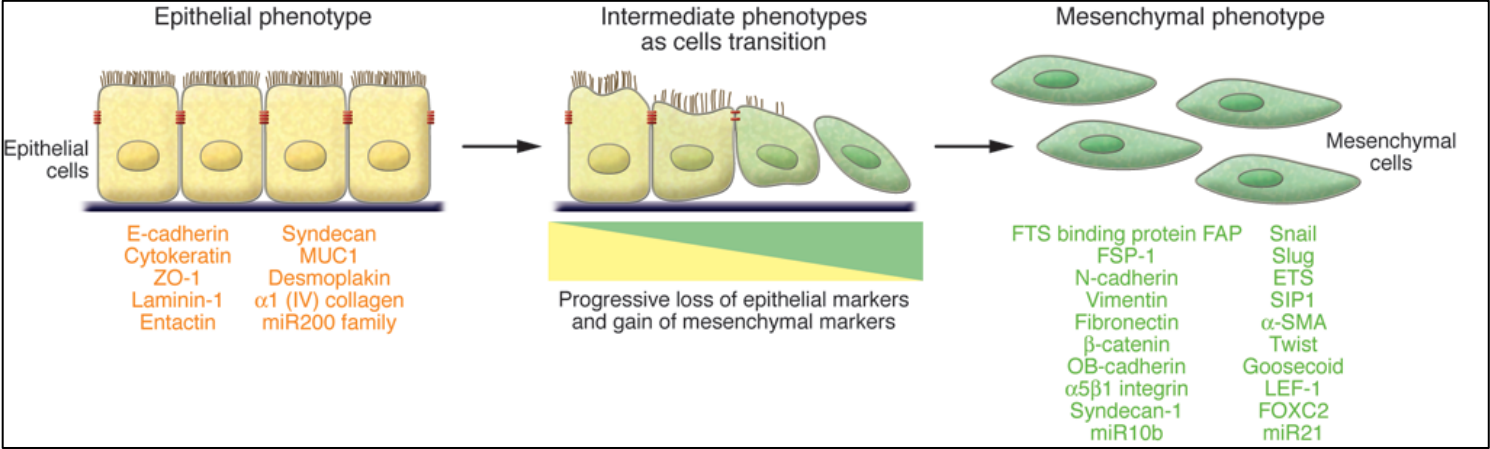
Hackathon

Paola Cavaliere

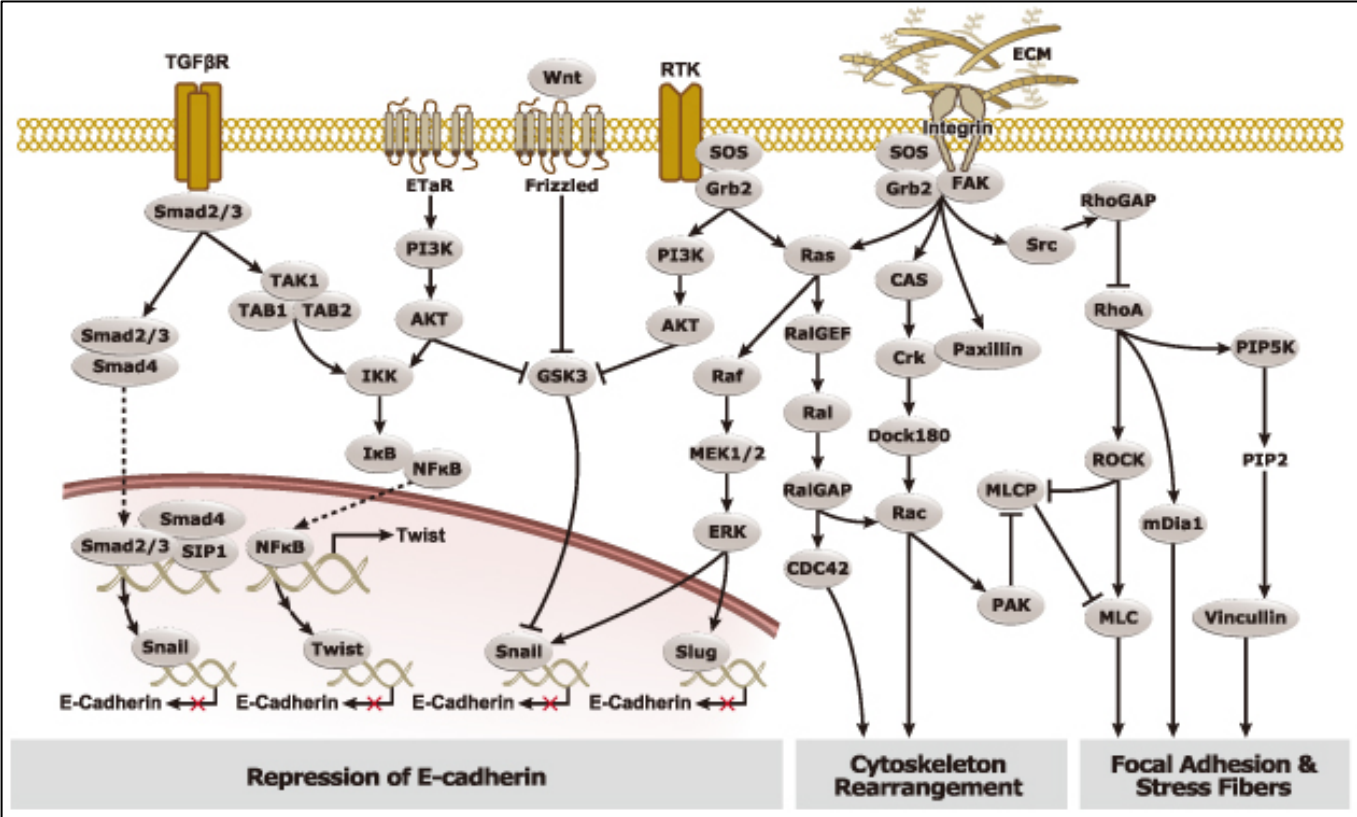
Dephoure's lab

06/19/2017

Epithelial-mesenchymal transition (EMT)



Several oncogenic pathways
(TGF- β , Wnt/ β -catenin,
Integrins, Notch)



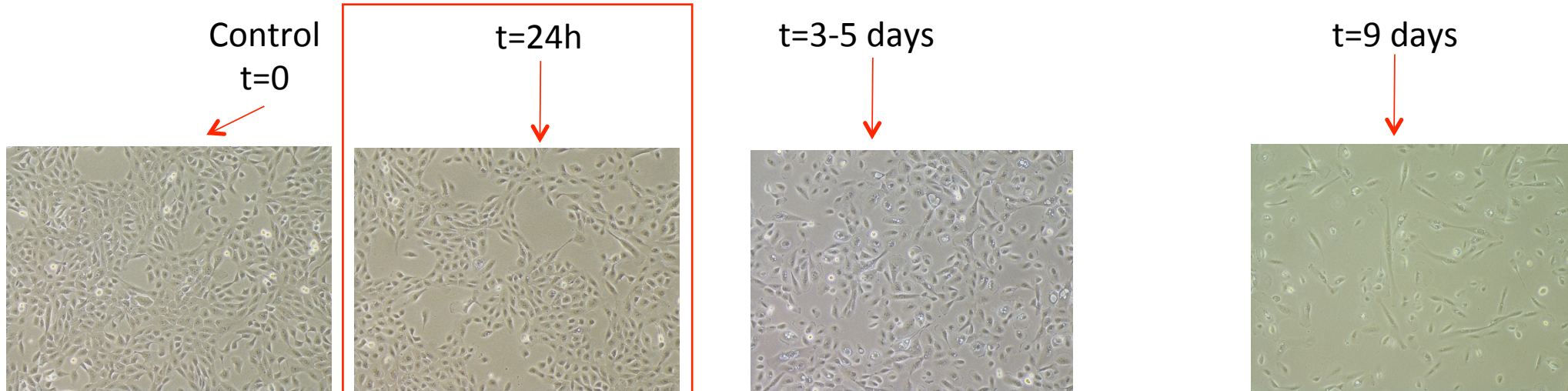
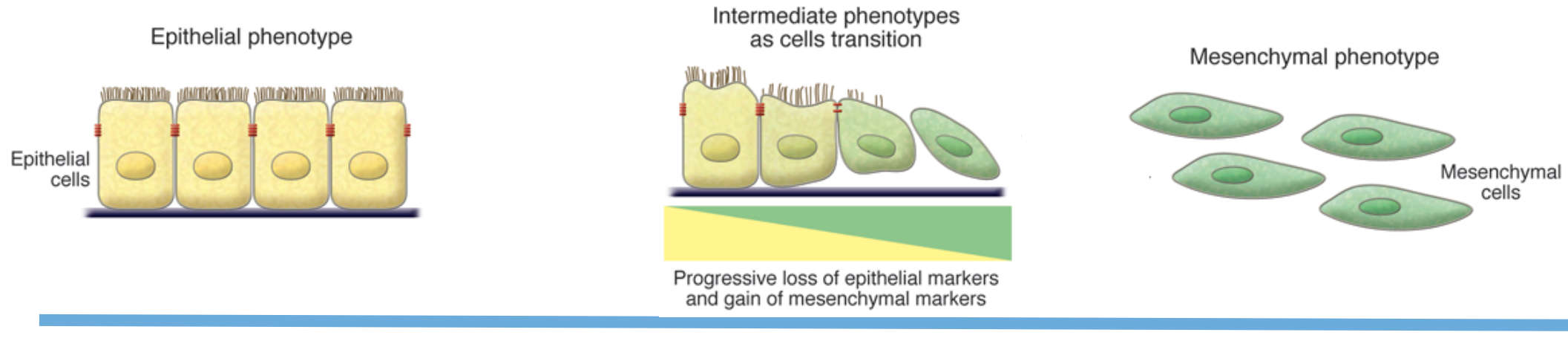
Proteomic strategies for EMT discovery

- ✓ Determination of protein translocation using detergent-based subcellular fractionation
- ✓ Determination of temporal changes in the interactome using size exclusion chromatography
- ✓ Characterization of key markers to define an EMT state in an easy and quickly way using targeted proteomics

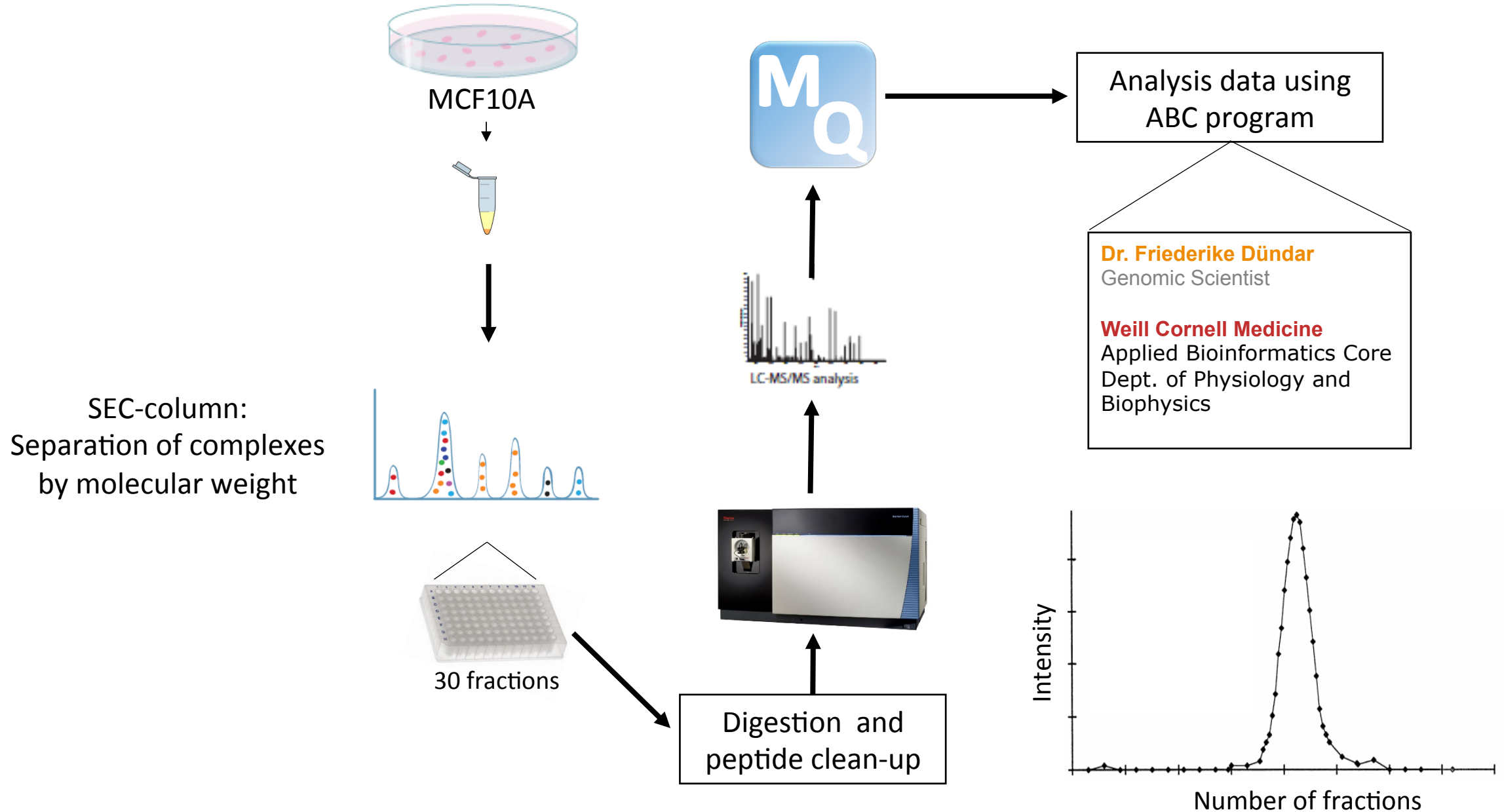
Epithelial to mesenchymal transition (EMT)

Model used for EMT: overexpression of ERK2 in MCF10A

MCF10A Empty vector (EV)
MCF10A ERK2 wild type (WT)
MCF10A ERK2 D319N (DN)



Workflow for SEC-MS



Output file from maxquant

proteinGroups_ERK-DN_chrom_1trial - Excel (Product Activation Failed)

FILE

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PAGE LAYOUT

FORMULAS

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REVIEW

VIEW

Ablebits Data

Ablebits Utilities

Ablebits Quick Tools

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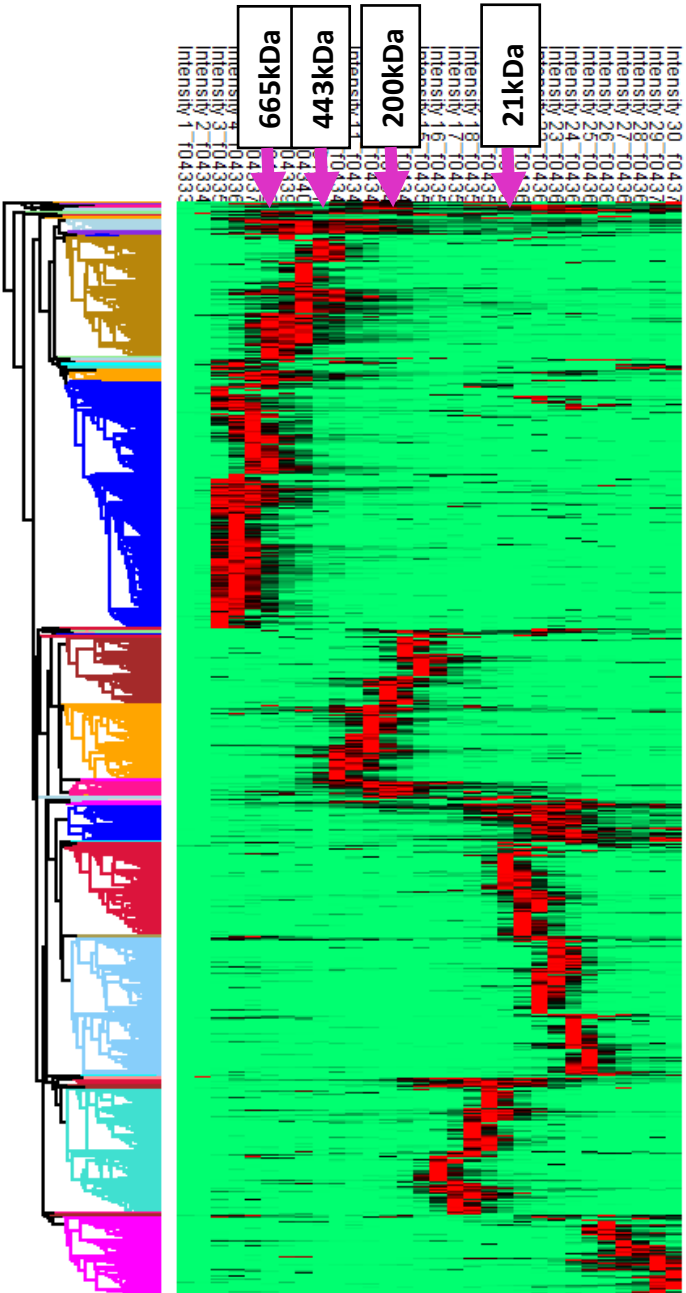
fx

Protein IDs

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	Pe
1	Protein ID	Majority p	Peptide c	Peptide c	Peptide c	Protein n	Gene nam	Fasta hea	Number o	Peptides	Razor + un	Unique p	Peptides	Peptides	Peptides	Peptides	Peptides	Peptides	Peptides	Peptides	Peptides	Peptides	Peptides	Peptides	Peptides	Peptides	Peptides	Peptides	Peptides	Pe
2	A0A0B4J2	A0A0B4J2	1	1	1	PIGBOS1			1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	
3	A0AV96	A0AV96	1	1	1	RNA-bind RBM47			1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	
4	A0FGR8	A0FGR8	11	11	11	Extended ESYT2			1	11	11	11	1	1	0	0	0	0	0	0	1	0	0	0	4	0	2	1	0	1
5	A0JLT2	A0JLT2	2	2	2	Mediator MED19			1	2	2	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	A0MZ66	A0MZ66	2	2	2	Shootin-1 KIAA1598			1	2	2	2	0	2	2	2	1	1	0	1	1	1	1	1	1	0	0	0	0	

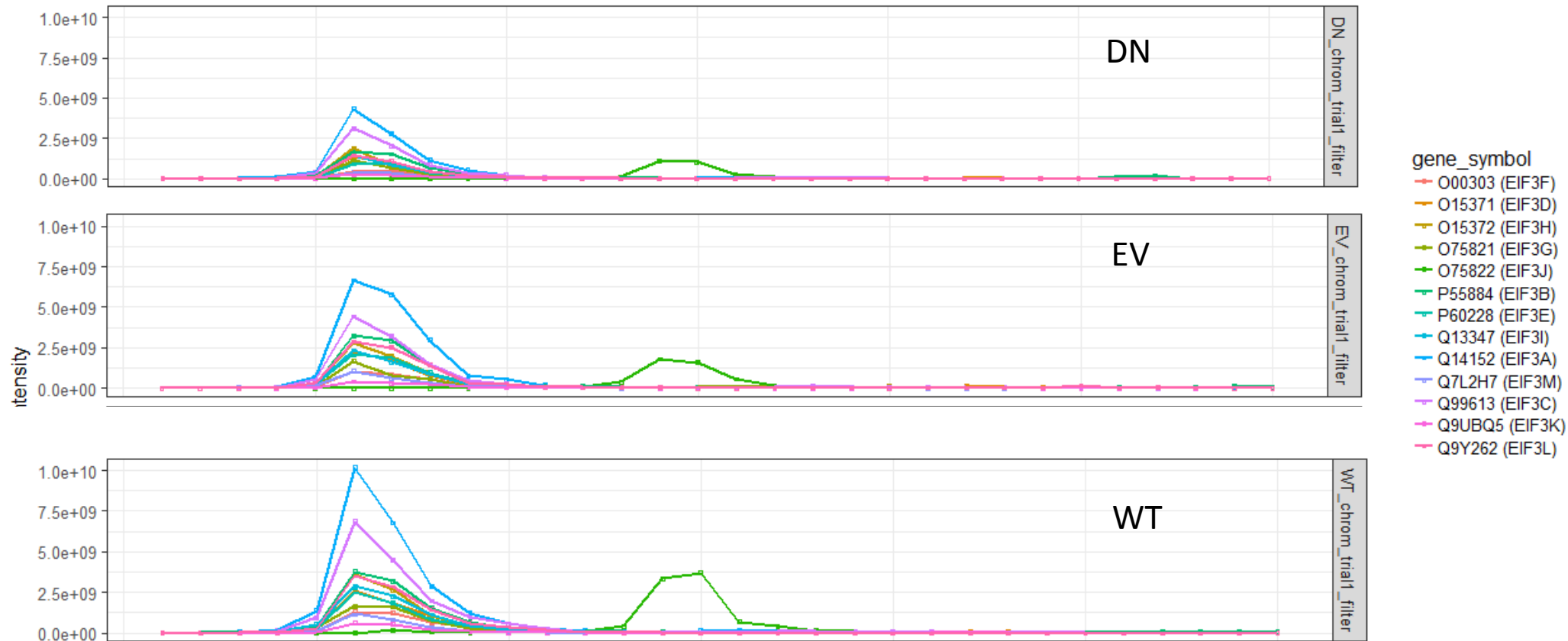
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Heatmap overview of SEC-MS data from MCF10A



Examples of protein complexes

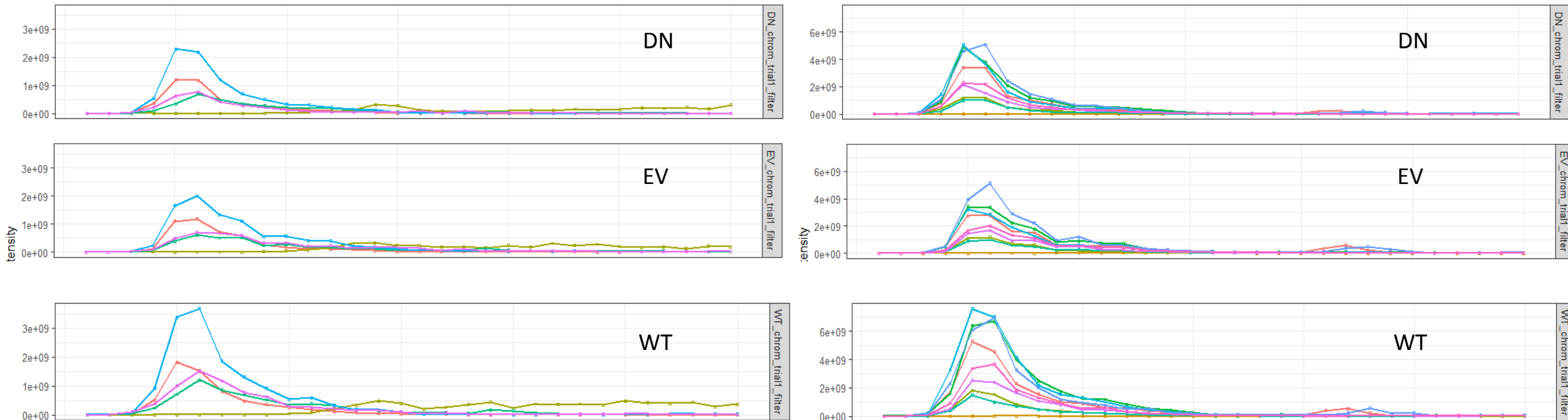
complex	total	missing	present
EIF3 complex (EIF3S6, EIF3S5, EIF3S4, EIF3S3, EIF3S6IP, EIF3S2, EIF3S9, EIF3S12, EIF3S10, EIF3S8, EIF3S1, EIF3S7, PCID1)	13	NA	13



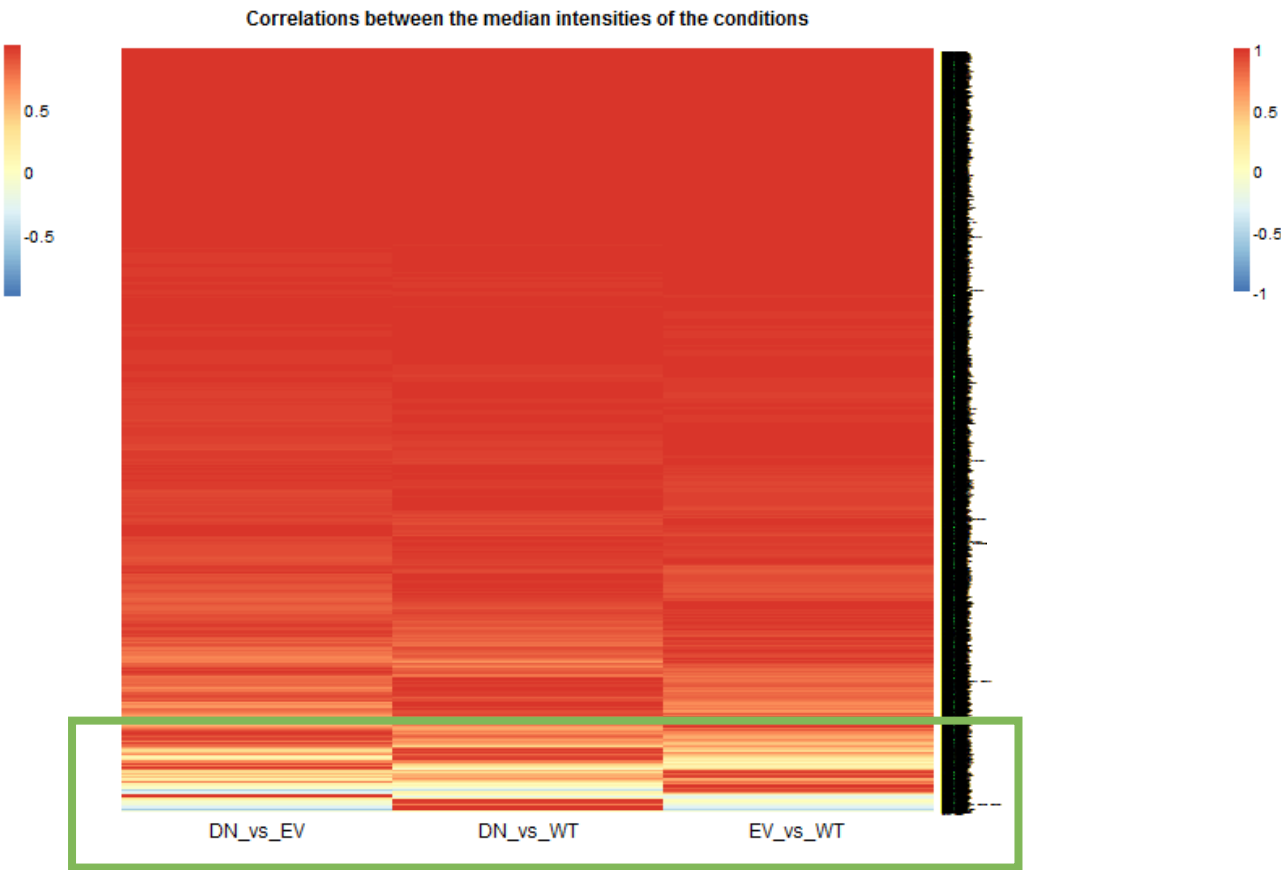
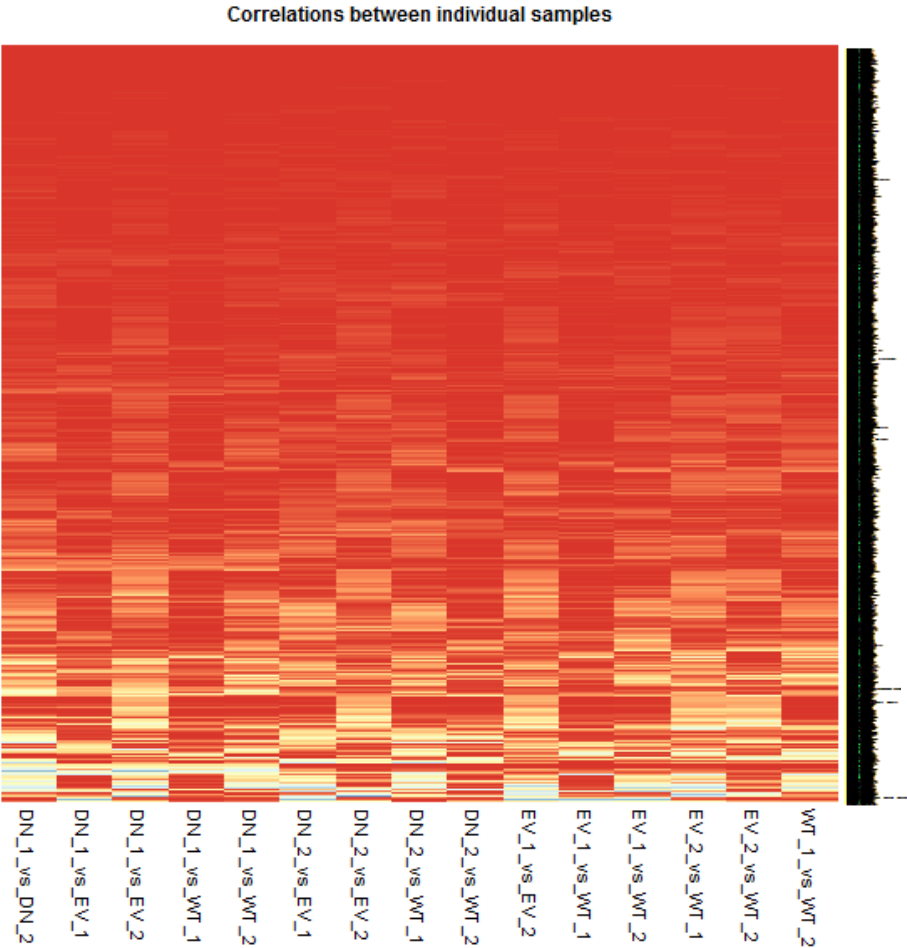
Examples of protein complexes

complex	total	missing	present
SWWSNF chromatin-remodeling complex	5	NA	5

complex	total	missing	present
SWI-SNF chromatin remodeling-related-BRCA1 complex	9	NA	9

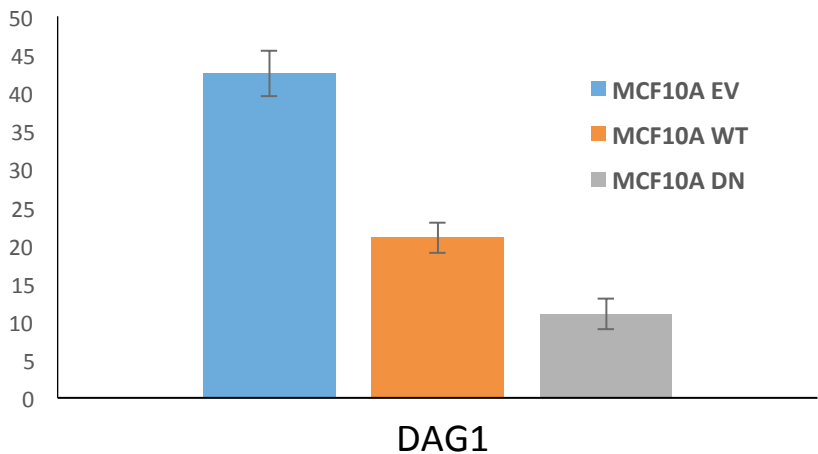
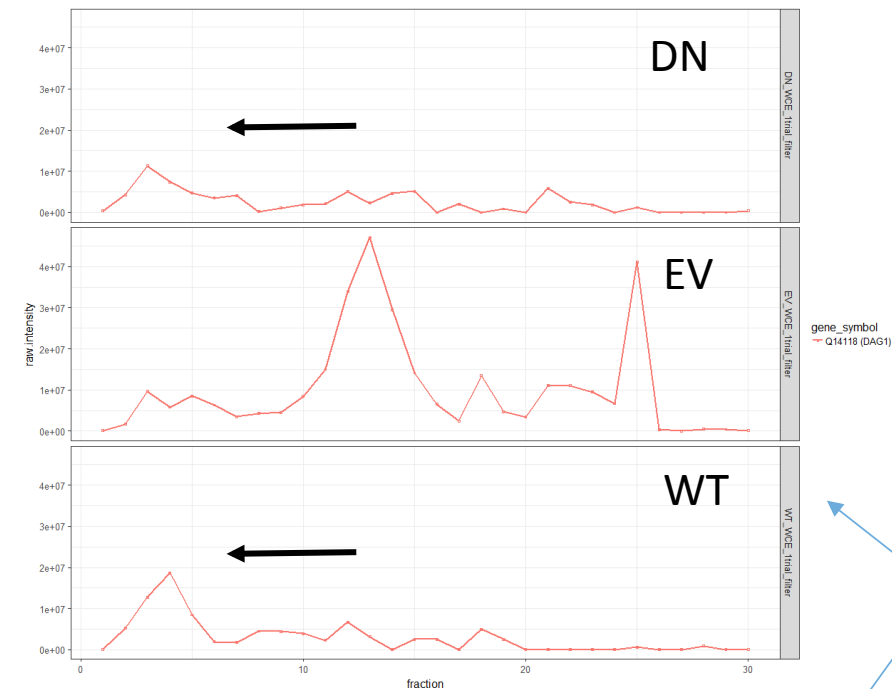


Correlation coefficient profile: ABC program



Pairwise comparison for each protein between replicates and cell lines

Example of a protein that moves between experimental conditions



Its expression is downregulated in cells undergoing EMT

Nakaya, Y, *et al.* (2011)Cells Tissues Organs, 193:64–73

DAG1= Dystroglycan

Involved in laminin and basement membrane assembly, cell survival, and epithelial polarization.

EV1	WT1	DN1	EV2	WT2	DN2	EV3	WT3	DN3
memb	memb	nucl	memb	memb	nucl	memb	memb	nucl

In tumour tissue, there is a tyrosine phosphorylation-dependent translocation of β -dystroglycan to the nucleus, altering the transcription of relatively few genes, the most unregulated being the transcription factor ETV1.

Mathew, G, *et al.* (2013) Sci. rep, 3 : 2792

What is needed in the ABC program:

- More powerful filtering process and statistical analysis
 - Filtering according to quality of the profiles, number of peptides
 - Filtering according to the profile between replicates
 - Statistical analysis to assess the quality of the data within and between replicates
- Using CORUM (database of known complexes), scoring how good these complexes are represented in our database
- Obtain automatically a list of proteins that change their profile (shift of the peaks) between experimental conditions
- Given a protein X, obtain a list (with scoring) of all the proteins with matching profile
 - list of protein that match protein X
 - also the list of complexes (from CORUM) that match protein X