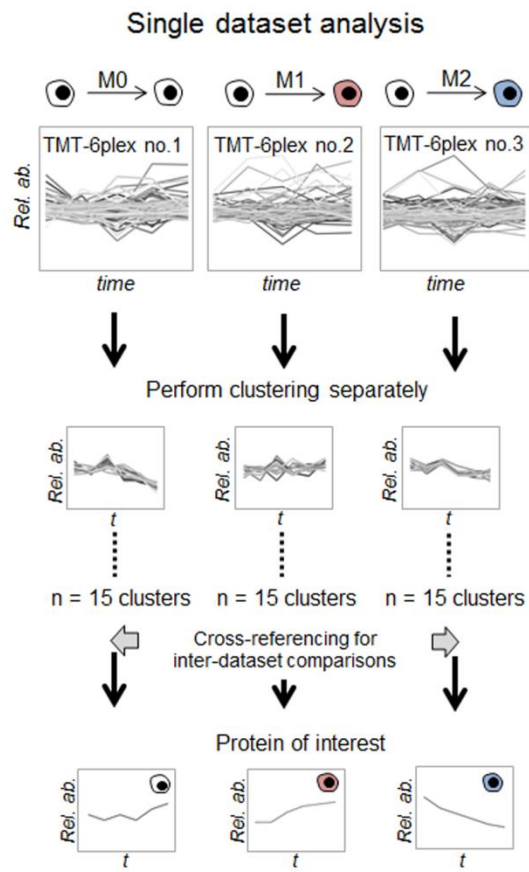


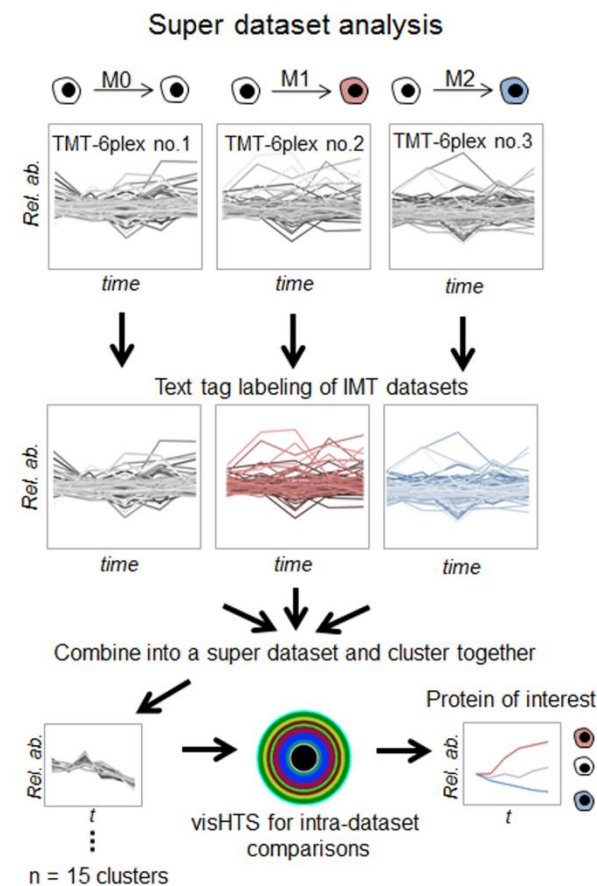
A brief tutorial on using the mIMT-visHTS software

mIMT-visHTS is a software for multiplexing time-resolved protein abundance profiles, typically analyzed by isobaric mass tagging strategies.

A typical multi-dataset comparison workflow










The mIMT-visHTS workflow



Files types

The mIMT-visHTS
package

Name	
 RebuildSuperDataSet	
 DynamicInterop.dll	
 RDotNet.dll	
 RDotNet.NativeLibrary.dll	
 Generic_Dataset1	
 Generic_Dataset2	
 Generic_Dataset3	

The application – PC version only

Dynamic link libraries to run some features of the application

Reference normalized protein abundance profiles in .csv format (example datasets); mIMT-visHTS performs the sum-normalization during the clustering step

The required dataset input file headers

Accession	Description	# Unique Peptides	Channel_1	Channel_2	Channel_3	Channel_4	Channel_5	Channel_6
AS-6699	GN=6699 Generic Protein Description	3	1	1.135	1.221	1.131	1.055	0.942
AS-4417	GN=4417 Generic Protein Description	4	1	1.03	1.322	0.965	1.099	1.033
AS-1190	GN=1190 Generic Protein Description	3	1	1.359	0.708	1.206	1.304	1.937
AS-2163	GN=2163 Generic Protein Description	3	1	0.935	1.219	1.068	1.049	0.83
AS-1994	GN=1994 Generic Protein Description	3	1	0.856	0.967	0.963	0.786	0.764
AS-4385	GN=4385 Generic Protein Description	3	1	1.275	0.715	1.442	1.254	2.149

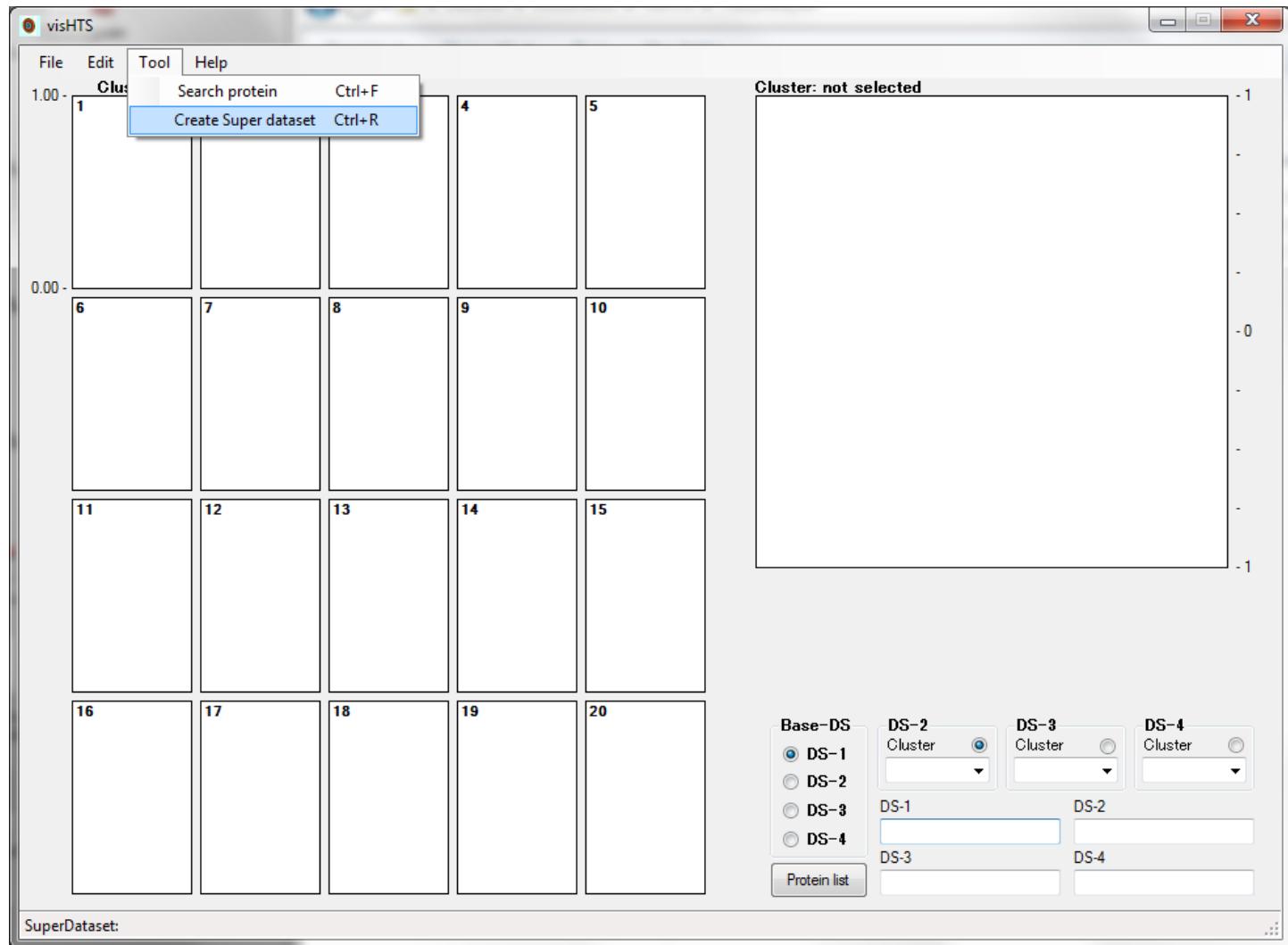
Notes

The GN=IDs must be included in the Description

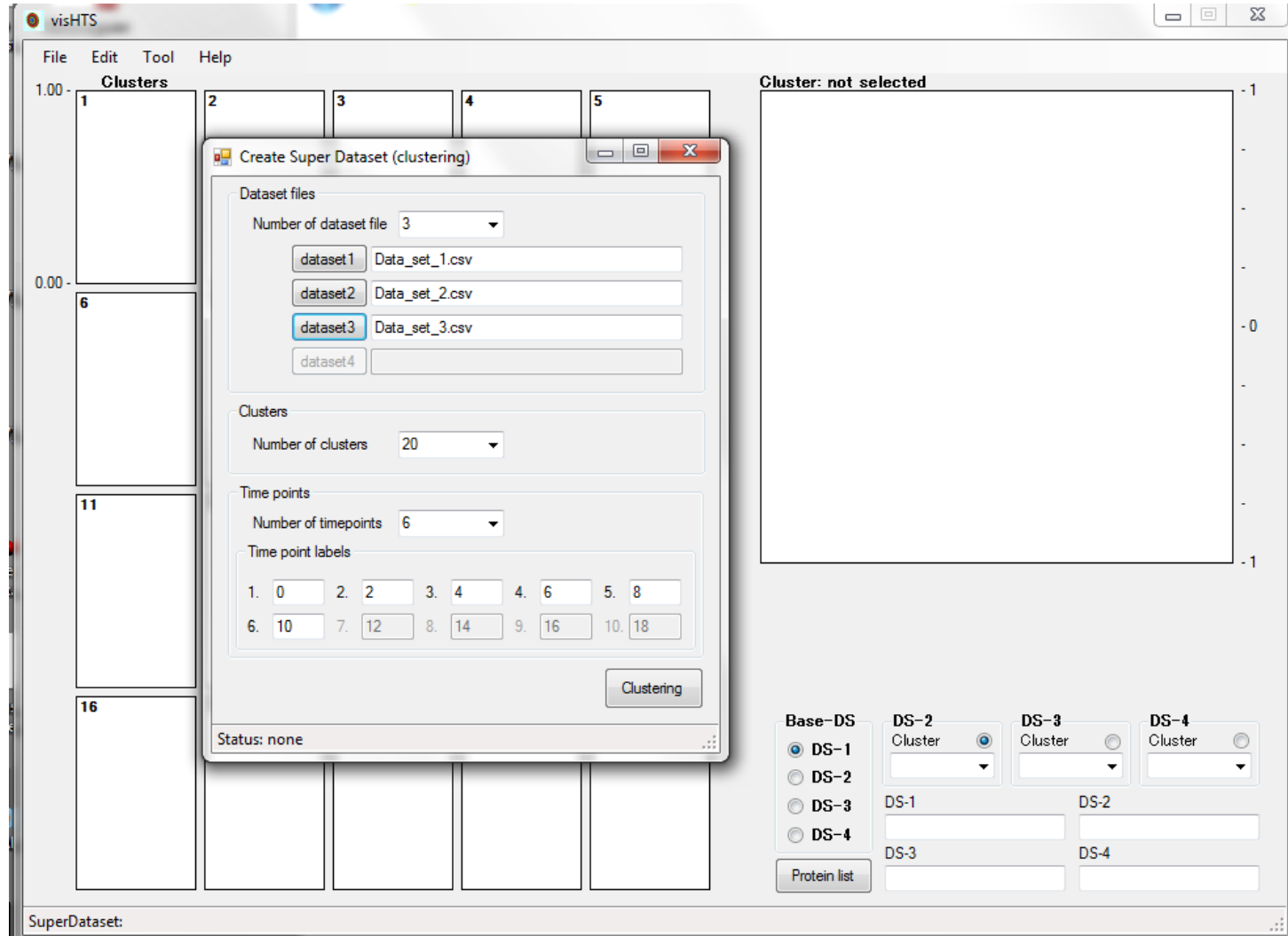
The data must be normalized prior to clustering

These profiles are our real data, however we have replaced the Accession and Description values with generic ones.

Begin with Model-based clustering steps



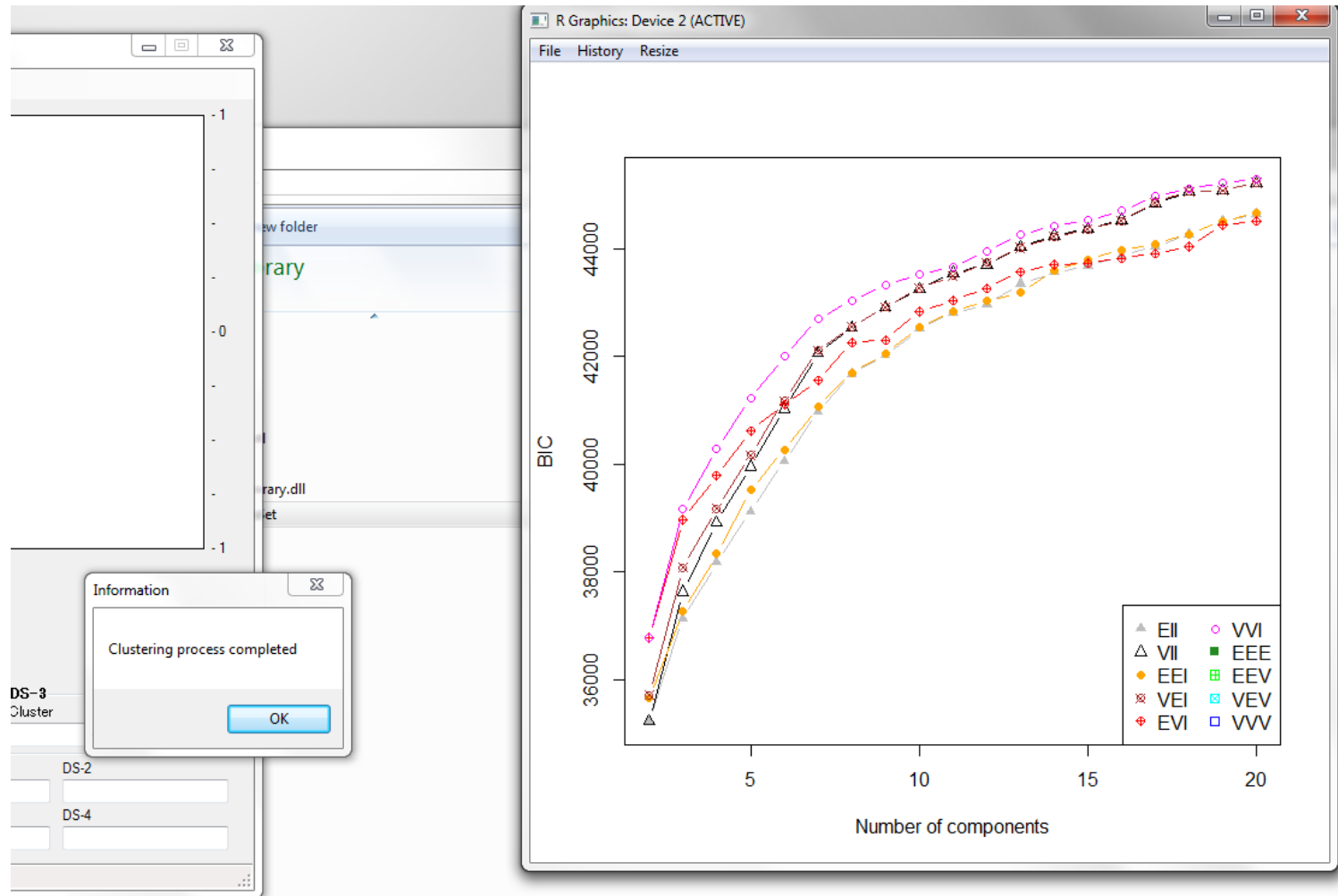
Upload normalized datasets



Notes

1. visHTS outputs are not optimized for 4 datasets (use with caution), however the clustering works.
2. If 2 or more datasets are entered, a super dataset will be created.
3. Select the number of clusters and (time point) labels.
4. Press "Clustering" to cluster. Clustering takes between 1 to 30 minutes, depending on the dataset size

The BIC plot appears at the end of clustering






Notes

1. The BIC plot disappears after you close the notification. Please grab a screen shot to preserve this output

visHTS creates the “mIMToutput” directory that contains the clustered data











Documents library
mIMT-visHTS

Arrange by: Folder ▾

Name	Date modified	Type	Size
 Website	5/21/2015 11:12 AM	File folder	
 Previous_visHTS	5/21/2015 11:07 AM	File folder	
 mIMToutput	5/21/2015 10:54 AM	File folder	


Documents library
Website

Input Directory

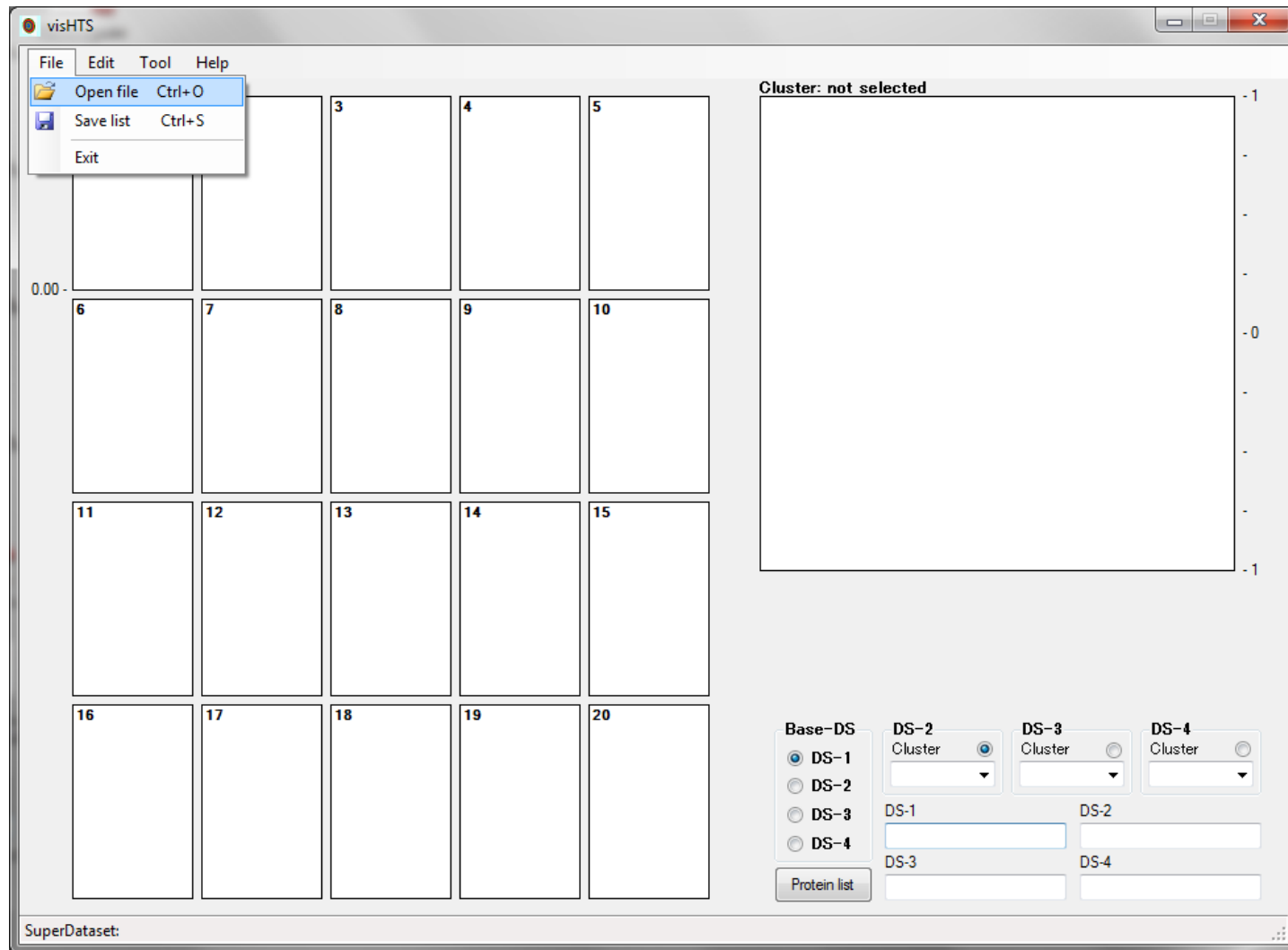
Name
 ScreenShots_visHTS_ss
 ScreenShots_visHTS
 RebuildSuperDataSet
 DynamicInterop.dll
 RDotNet.dll
 RDotNet.NativeLibrary.dll
 HeaderInputExample
 Generic_Dataset1
 Generic_Dataset2
 Generic_Dataset3

Documents library
mIMToutput

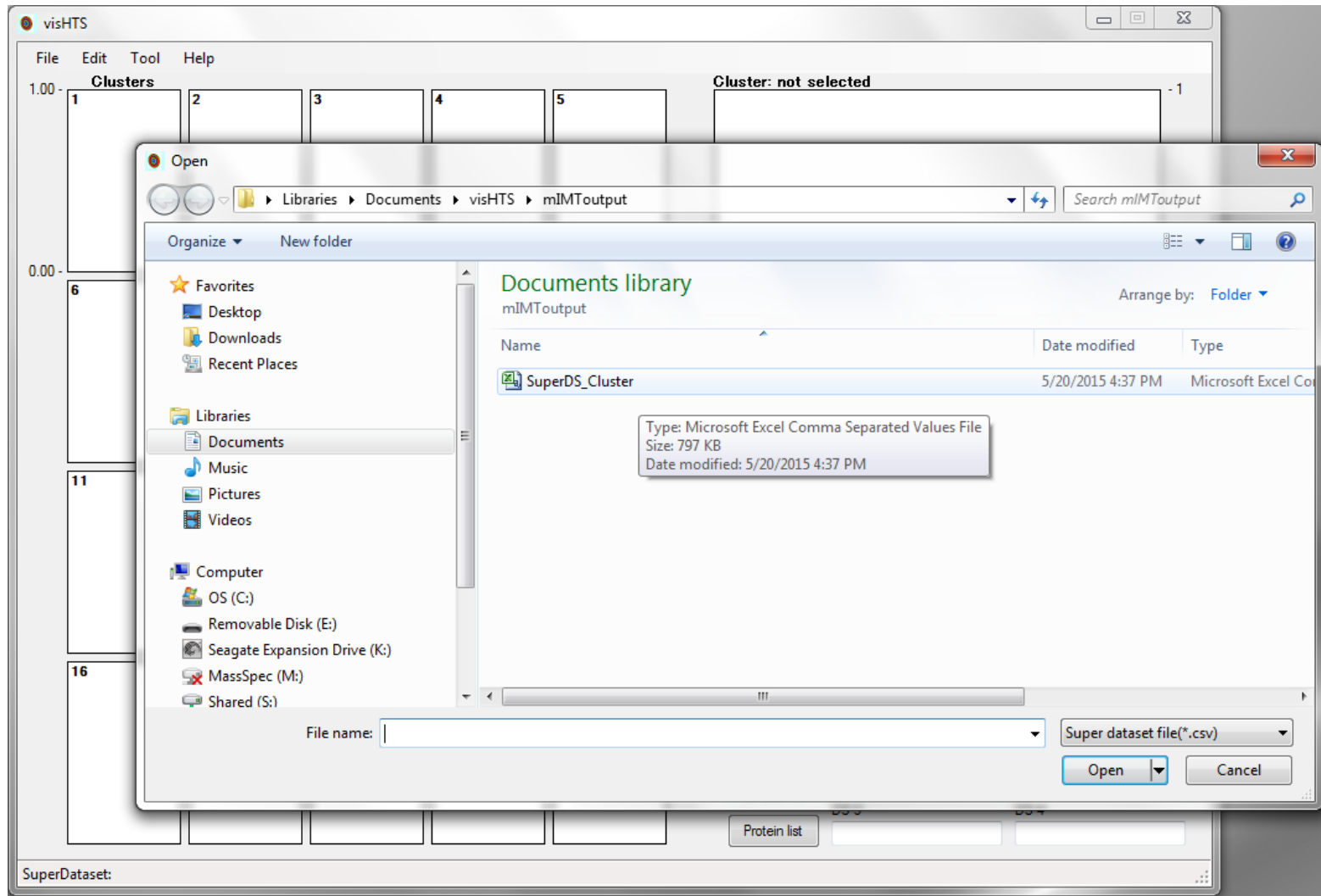
Output Directory

Name
 SuperDS_Cluster

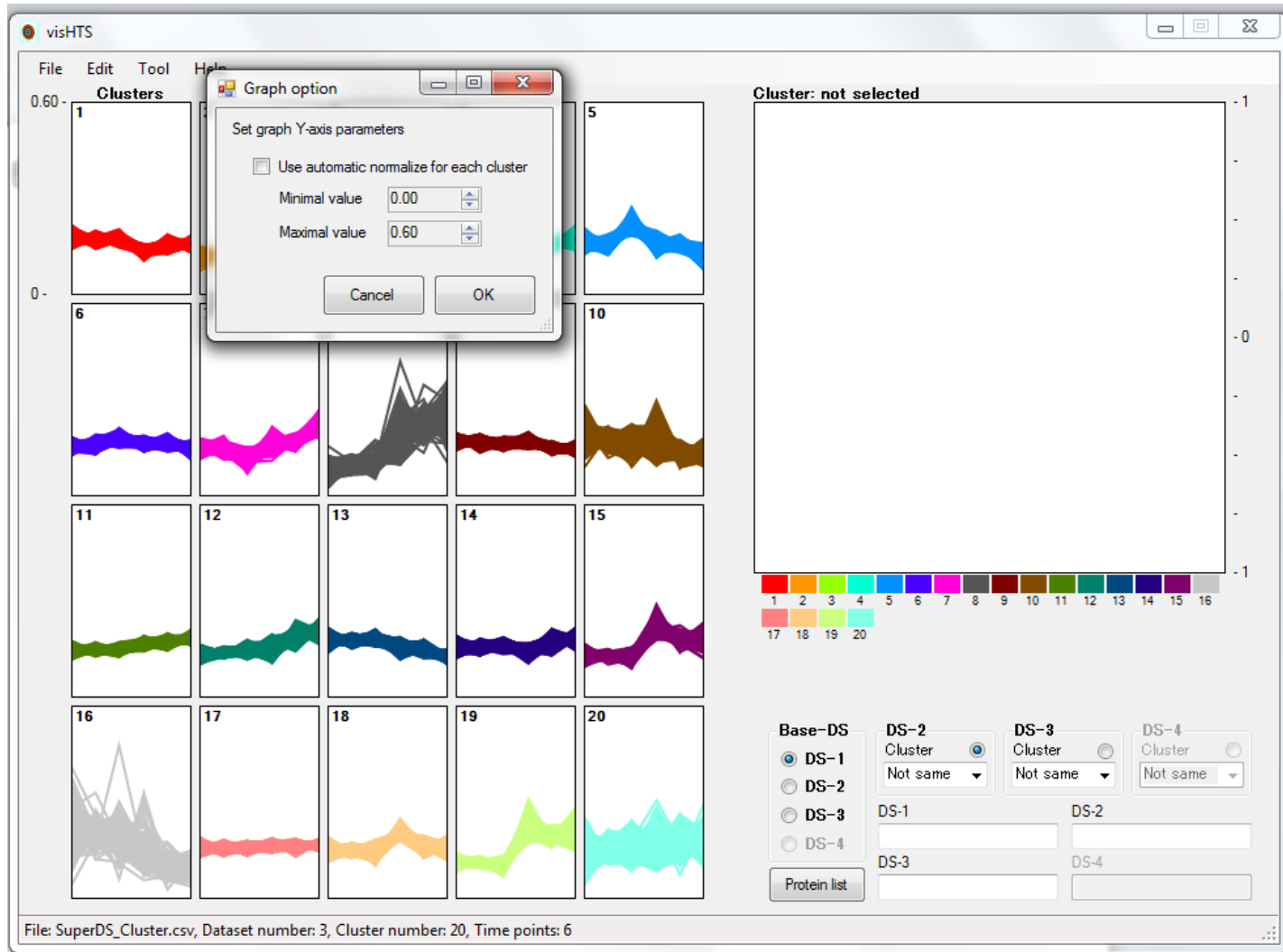
Importing a clustered super dataset



Importing a clustered super dataset



Change the Y-axis in the **Edit** dropdown



Important: use the arrows to change the values. Entering the values manually will crash the software

Example 1: proteins that behave the same in all three conditions – cluster 13

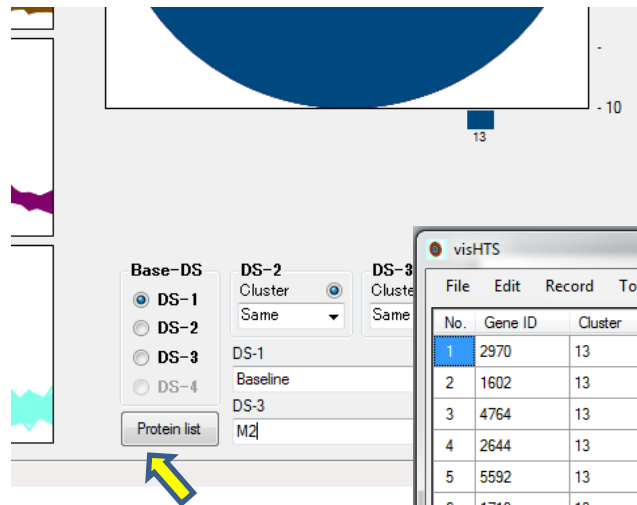


Rename datasets here

Example 1: proteins that behave the same in all three conditions



Example 1: Exporting the protein list



Retrieve the proteins

visHTS

File Edit Record Tool Help

No.	Gene ID	Cluster	Description	TMT1	TMT2	TMT3	TMT4	TMT5	TMT6
1	2970	13	GN=2970 Generic Protein Description	0.175284...	0.181419...	0.179316...	0.165819...	0.154776...	0.143382...
2	1602	13	GN=1602 Generic Protein Description	0.184263...	0.176156...	0.215957...	0.147411...	0.146858...	0.129353...
3	4764	13	GN=4764 Generic Protein Description	0.169434...	0.179769...	0.184005...	0.182988...	0.152321...	0.131480...
4	2644	13	GN=2644 Generic Protein Description	0.184842...	0.167282...	0.180961...	0.175970...	0.163585...	0.127356...
5	5592	13	GN=5592 Generic Protein Description	0.179888...	0.168555...	0.197877...	0.158841...	0.155423...	0.139413...
6	1719	13	GN=1719 Generic Protein Description	0.186636...	0.172265...	0.182157...	0.158081...	0.159574...	0.141284...
7	2722	13	GN=2722 Generic Protein Description	0.177367...	0.170273...	0.192089...	0.168144...	0.164774...	0.127350...
8	954	13	GN=954 Generic Protein Description	0.174641...	0.182675...	0.190010...	0.171323...	0.157527...	0.123821...
9	6137	13	GN=6137 Generic Protein Description	0.192752...	0.186006...	0.168658...	0.159984...	0.148419...	0.144178...
10	646	13	GN=646 Generic Protein Description	0.184229...	0.171518...	0.184045...	0.172070...	0.152358...	0.135777...

Edit dropdown – allows to copy to clip board

Record dropdown – allows to build a table and add more protein lists during a session

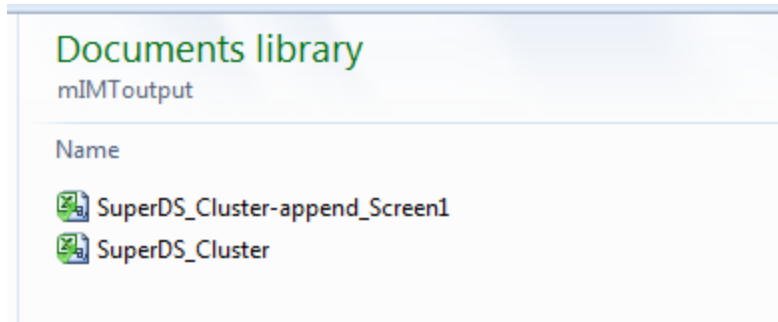
Exit this window here by clicking here!

Number of datasets: 3 Base-DS: 1 DS-1 Baseline Base-DS DS-2 M1 Same
Number of clusters: 20 DS-3 M2 Same

File: SuperDS_Cluster.csv, Dataset number: 3, Cluster number: 20, Time points: 6

Close

Example 1: Protein lists outputs – “Record” output

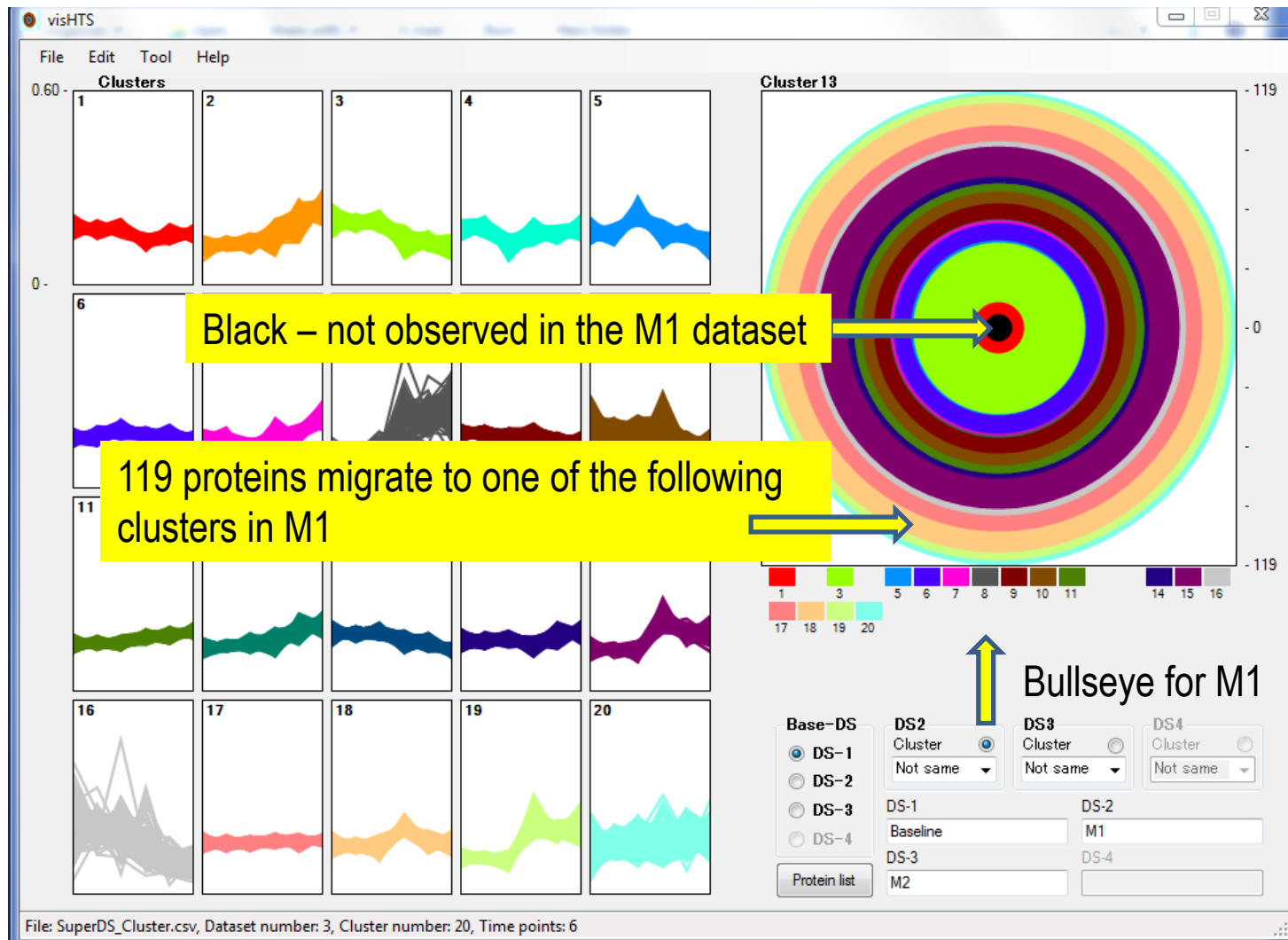


Record dropdown – allows to build a table (**Start** function) and **Add** more protein lists during a session.

“SuperDS_Cluster-append_Screen1” is the output file.

[illegible]

Example 2: Cluster 13 proteins from Baseline (dataset 1) that behave differently in **M1 (dataset 2)** and in M2 (dataset 3)



Example 2: Exporting cluster 13 proteins from Baseline that behave differently in M1

visHTS

File Edit Record Tool Help

No.	Gene ID	Cluster	Description	TMT1	TMT2	TMT3	TMT4	TMT5	TMT6
1	1914	3	GN=1914 Generic Protein Description	0.235460...	0.190016...	0.203202...	0.125029...	0.129503...	0.116788...
2	5273	8	GN=5273 Generic Protein Description	0.113199...	0.107765...	0.119198...	0.222322...	0.234661...	0.202852...
3	3424	10	GN=3424 Generic Protein Description	0.263435...	0.171496...	0.175974...	0.126975...	0.130663...	0.131454...
4	1588	15	GN=1588 Generic Protein Description	0.131717...	0.142123...	0.098919...	0.226159...	0.185063...	0.216016...
5	4805	18	GN=4805 Generic Protein Description	0.168265...	0.157327...	0.122497...	0.241460...	0.166077...	0.144371...
6	903	-1	GN=903 Generic Protein Description	-1	-1	-1	-1	-1	-1
7	715	3	GN=715 Generic Protein Description	0.236686...	0.193136...	0.216568...	0.145562...	0.115502...	0.092544...
8	4369	17	GN=4369 Generic Protein Description	0.163559...	0.158652...	0.152109...	0.187765...	0.166830...	0.171082...
9	1032	9	GN=1032 Generic Protein Description	0.175070...	0.159488...	0.168942...	0.188200...	0.150560...	0.157738...
10	5746	6	GN=5746 Generic Protein Description	0.152253...	0.162606...	0.184531...	0.175091...	0.163215...	0.162302...
11	1173	10	GN=1173 Generic Protein Description	0.193199...	0.176004...	0.168083...	0.187210...	0.141615...	0.133887...
12	5667	-1	GN=5667 Generic Protein Description	-1	-1	-1	-1	-1	-1
13	1053	9	GN=1053 Generic Protein Description	0.172235...	0.163107...	0.163279...	0.180502...	0.161212...	0.159662...
14	1735	-1	GN=1735 Generic Protein Description	-1	-1	-1	-1	-1	-1
15	3946	15	GN=3946 Generic Protein Description	0.135025...	0.145557...	0.095463...	0.251957...	0.177018...	0.194977...
16	5484	15	GN=5484 Generic Protein Description	0.140114...	0.124422...	0.106767...	0.240717...	0.183830...	0.204147...
17	5625	10	GN=5625 Generic Protein Description	0.160333...	0.185345...	0.204104...	0.188071...	0.143177...	0.118967...
18	5172	1	GN=5172 Generic Protein Description	0.190005...	0.186965...	0.196655...	0.133574...	0.137374...	0.155424...
19	1726	3	GN=1726 Generic Protein Description	0.190258...	0.199391...	0.200342...	0.154109...	0.136035...	0.119863...
20	6248	3	GN=6248 Generic Protein Description	0.198137...	0.190608...	0.223499...	0.127204...	0.155537...	0.105012...
21	6410	3	GN=6410 Generic Protein Description	0.205888...	0.188182...	0.217829...	0.138974...	0.141445...	0.107679...
22	4450	3	GN=4450 Generic Protein Description	0.195160...	0.205893...	0.189110...	0.147150...	0.143052...	0.119633...

Number of datasets: 3 Base-DS: 1 DS-1 Baseline Base-DS DS-2 M1 Not same

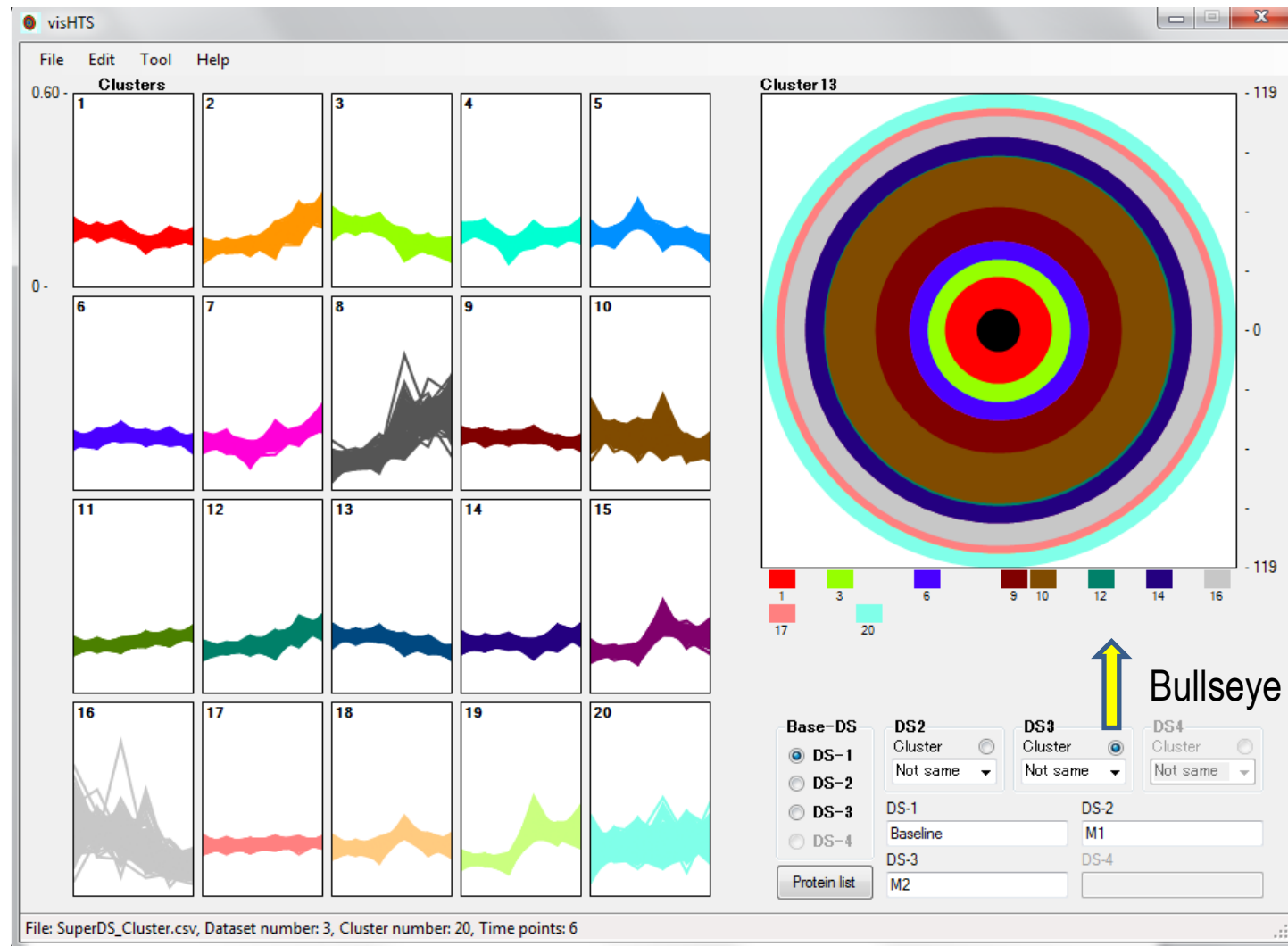
Number of clusters: 20 DS-3 M2 Not same

File: SuperDS_Cluster.csv, Dataset number: 3, Cluster number: 20, Time points: 6

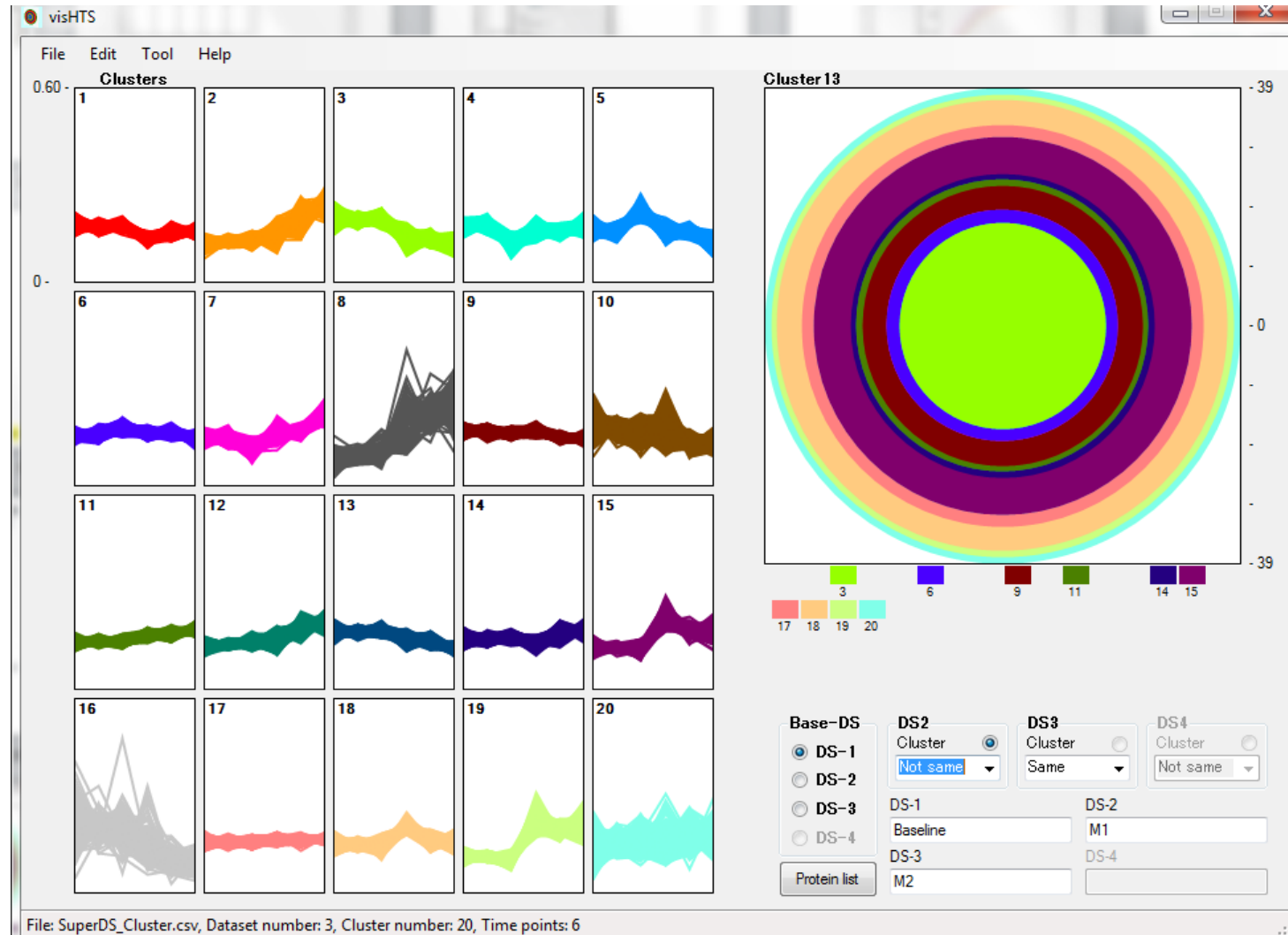
Close

“-1” in the table – not observed in the M1 dataset

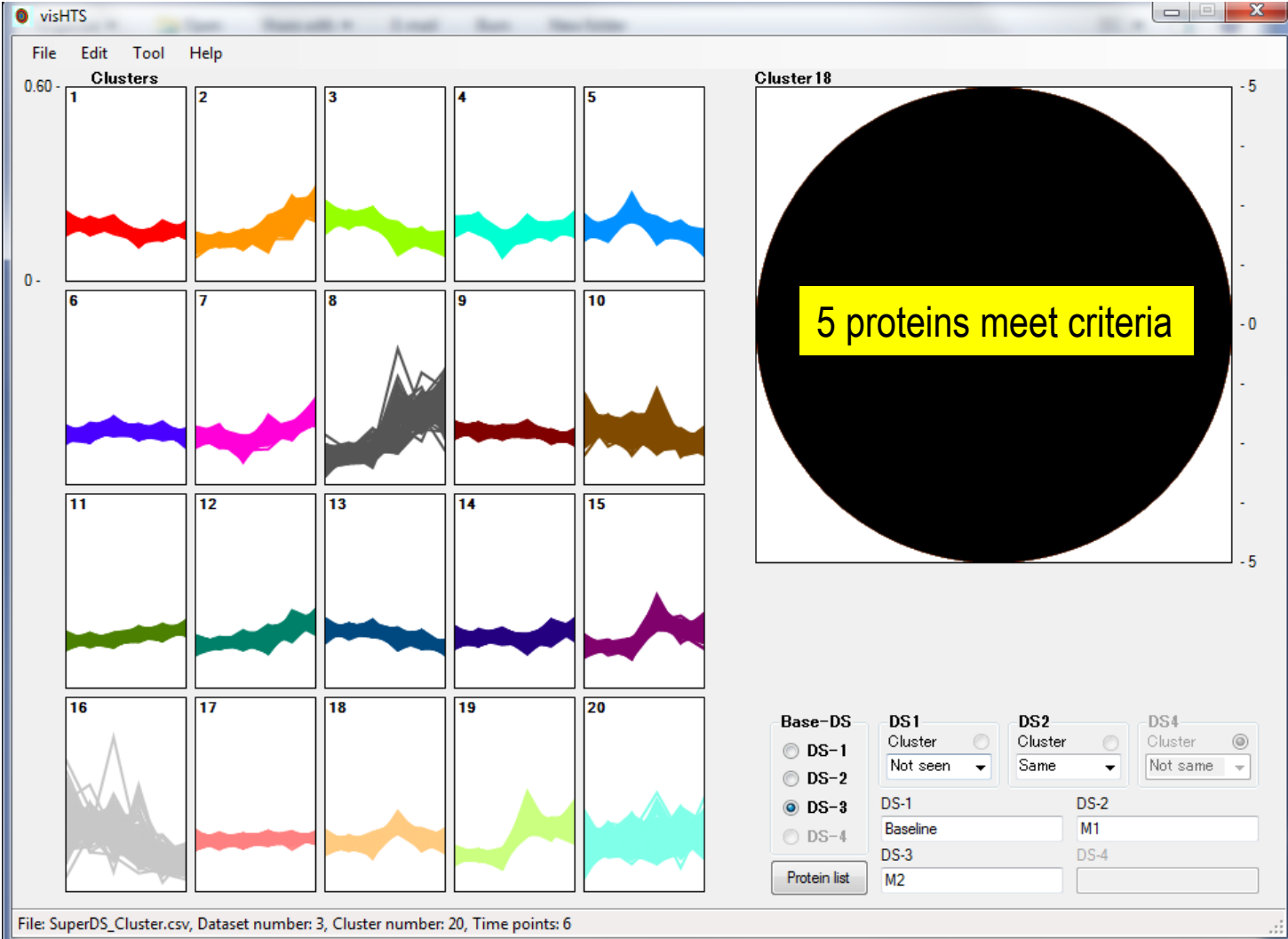
Example 2: Cluster 13 proteins from Baseline (dataset 1) that behave differently in M1 (dataset 2) and in **M2 (dataset 3)**



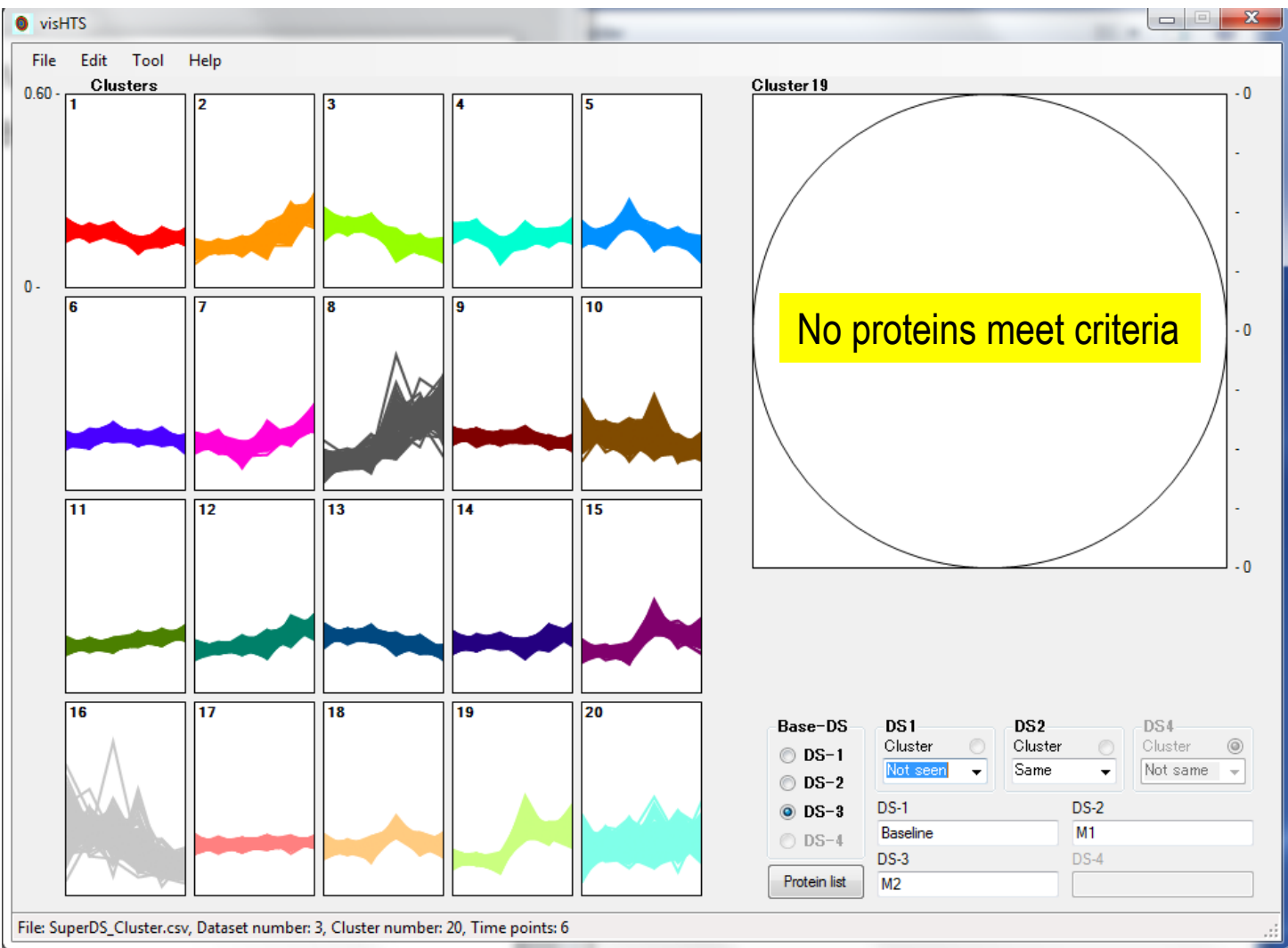
Example 3: Cluster 13 proteins that behave the same in Baseline and M1, but differently in M2



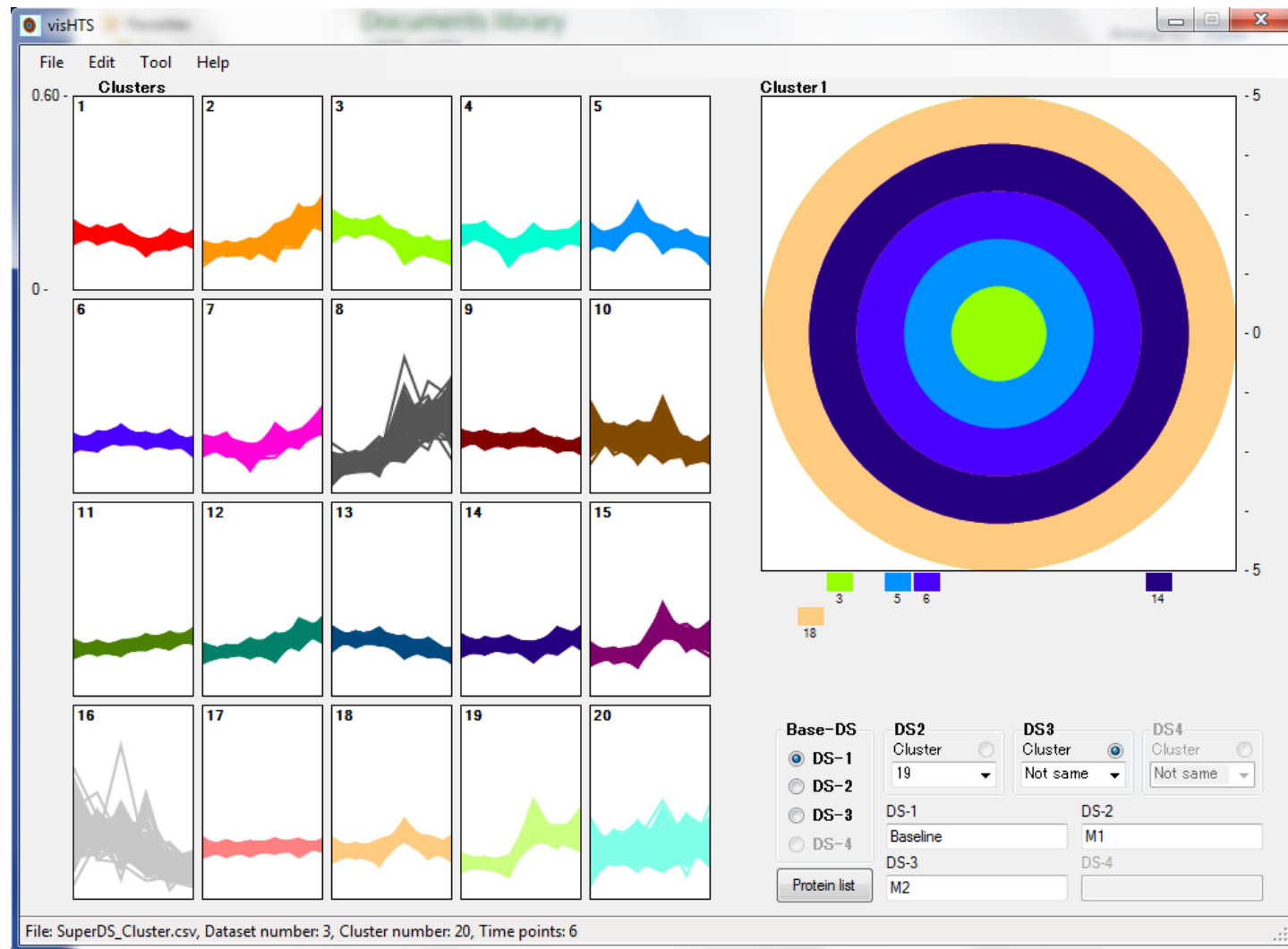
Example 4a: Cluster 18 proteins that behave the same in Baseline and in M2, and not detected in M1



Example 4b: Cluster 19 proteins that behave the same in Baseline and in M2, and not detected in M1



Example 5: Baseline proteins in cluster 1 that move to cluster 19 in M1, but do something different in M2



You can directly look for your favorite protein

In the Tools dropdown

