biosignatures
Health information to live by!

Audit Level Report

Generated: Oct 08 2020 18:59

Sample: CBA.G4F-426-L3W

Analysis Parameters

Analytes	Transferrin, Alpha1 AntiTrypsin
Equipment Protocol	Vertical Tanks 1
Analysis Protocol	V1.01
Usage	Research Use Only

Summary

The analysis presented in this document resulted from following the biosignatures 'Gel-As-Assay' workflow. A hybrid 2D DiGE approach is used to increase automation, quality and reproducibility. The key difference is that a fixed standard is always used rather than a pooled standard.

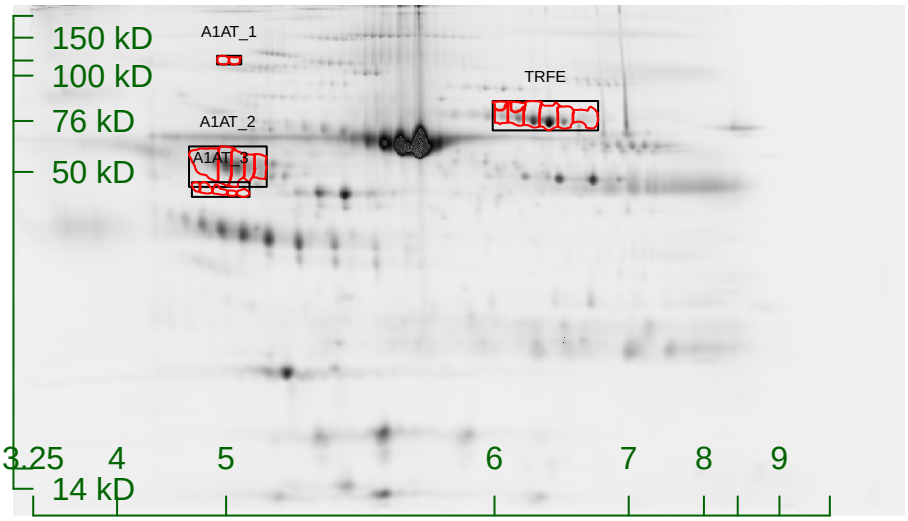
2µL Human Plasma is labelled with Cy5 (or equivalent) and a fixed standard labelled with Cy3 following protocols defined by biosignatures. Images of the channels are then uploaded to a cloud analysis platform. The Cy3 standard is then aligned into a fixed reference space and automated QC procedures assess the fixed standard. If the standard passes the automated procedures a fixed feature pattern is applied and feature measures obtained. This report is then automatically generated detailing the results for a selected subset of analytes.

summary table of results

Analytes:

Overview

The gel image below shows the locations of the analyte chains presented in this report.



Transferrin (P02787)

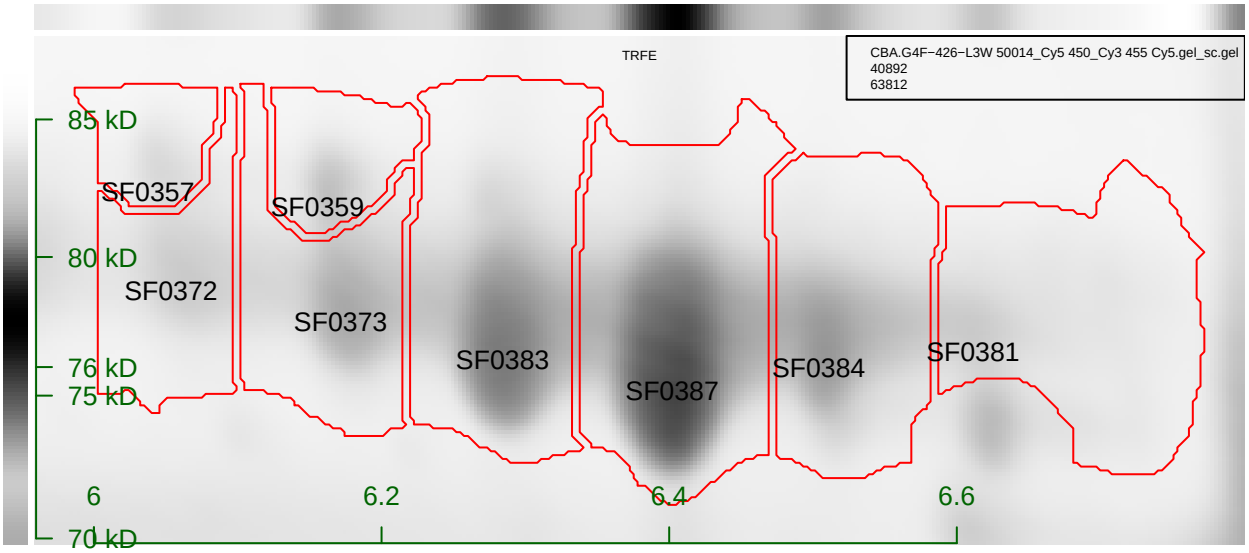
From: Human plasma protein N-glycosylation

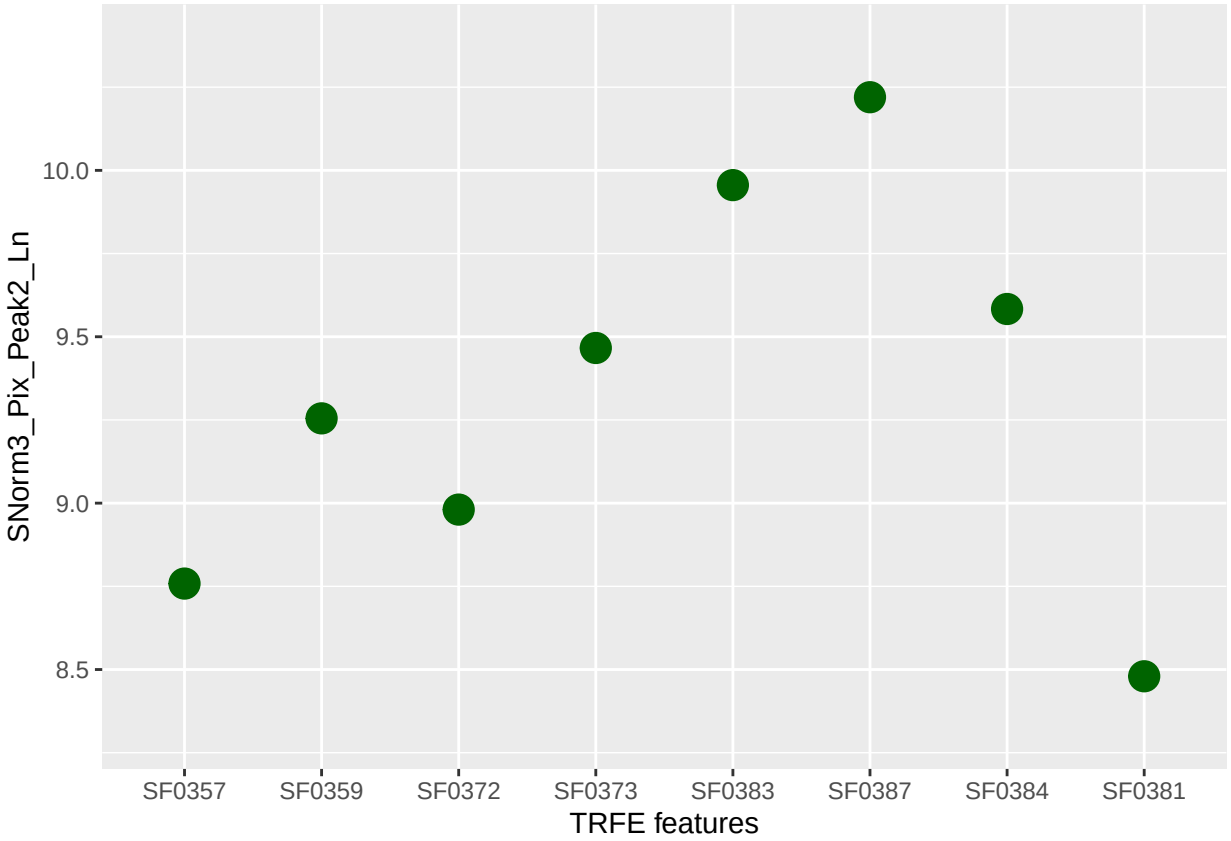
> Serotransferrin (STF), also known as transferrin, ß1 metal binding globulin or siderophilin, is a 698 amino acid protein (19 amino acids of which are signal peptide) with a molecular mass of approximately 77 kDa (without glycosylation) [8, 337]. The protein consists of two globular domains, the N-lobe and the C-lobe which divided into two subdomains each (N1, N2, C1 and C2). The two main domains are connected by a short linker peptide [337–339]. The N-lobe is 336 amino acids in size and spans from Val25 to Glu347, while the C-lobe is 343 amino acids long and ranges from Val361 to Lys683 [337]. The lobes can interact to form a hydrophilic metal ion binding site [337]. STF is mostly produced by hepatocytes, although other tissues have also shown expression, albeit at significantly lower amounts [337]. The plasma concentration is highly stable from the age of 2 years on, with a range between 2 and 3 mg/mL [337, 340]. Levels may increase during pregnancy up to 5 mg/mL [141].

A small change.

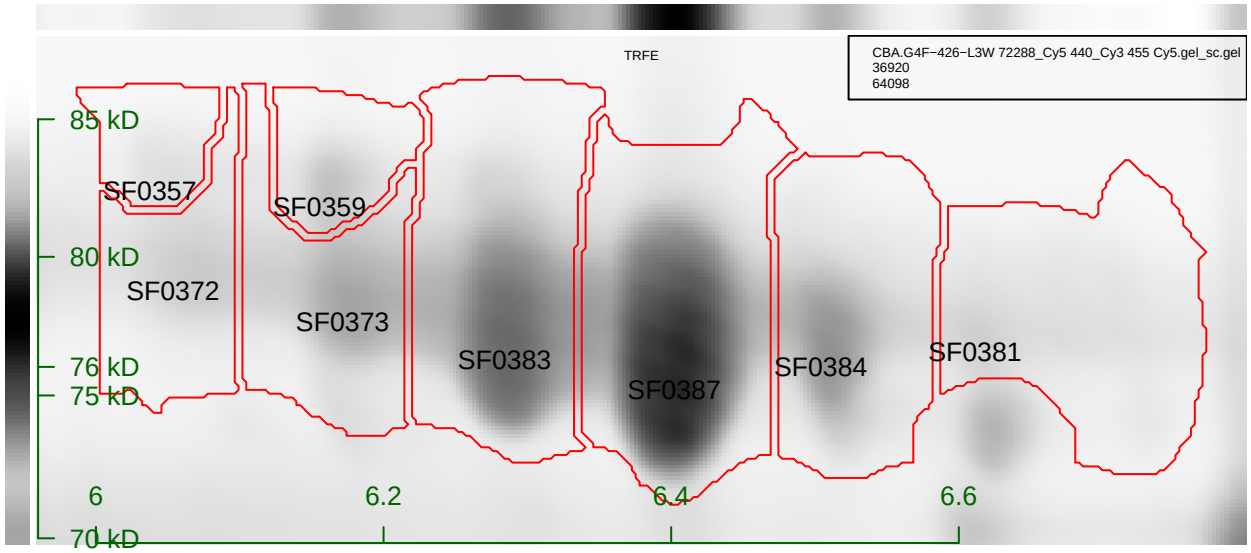
CBA.G4F-426-L3W TRFE

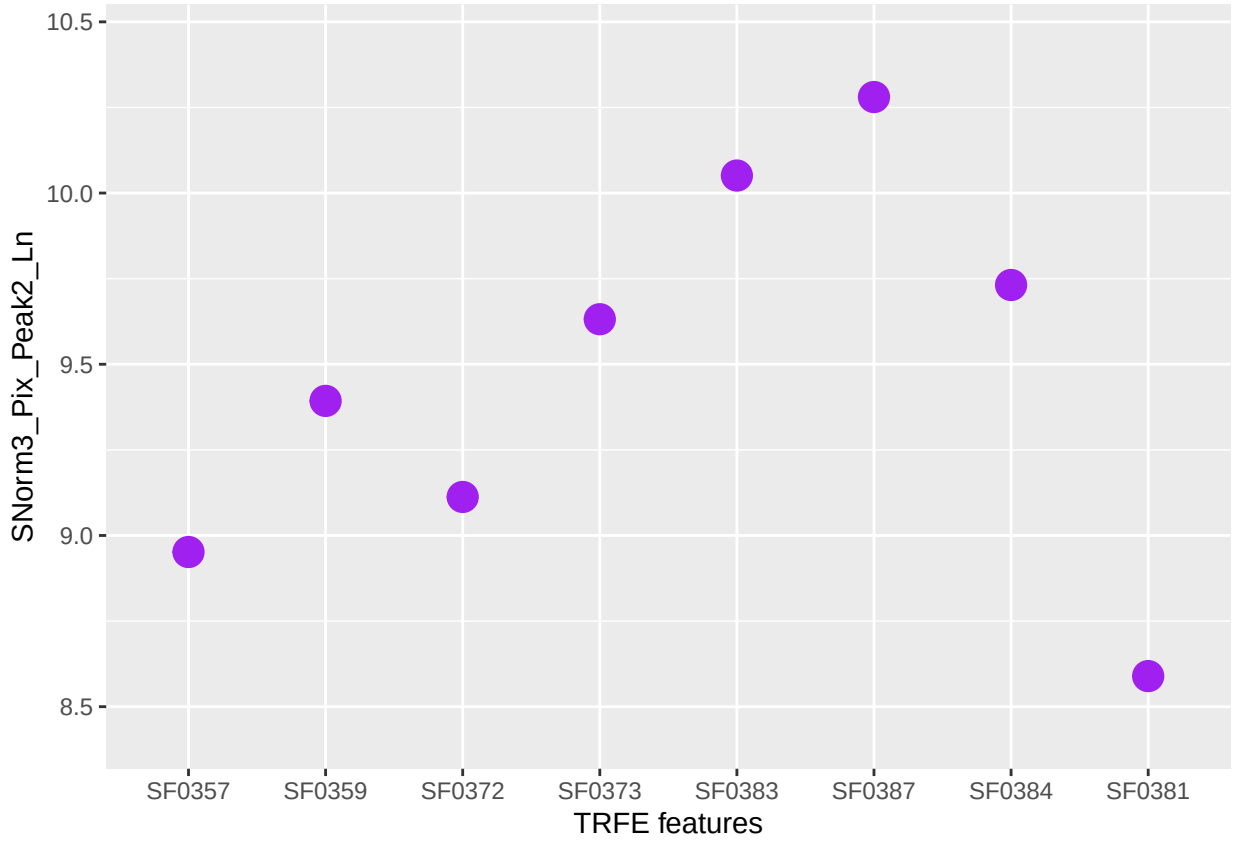
Replicate 1 : 50014_Cy5 450_Cy3 455 Cy5.gel_sc.gel


TRFE
CBA.G4F−426−L3W 50014_Cy5 450_Cy3 455 Cy5.gel_sc.gel
40892
63812
85 kD
80 kD
76 kD
75 kD
70 kD
SF0357
SF0359
SF0372
SF0373
SF0383
SF0387
SF0384
SF0381
6
6.2
6.4
6.6

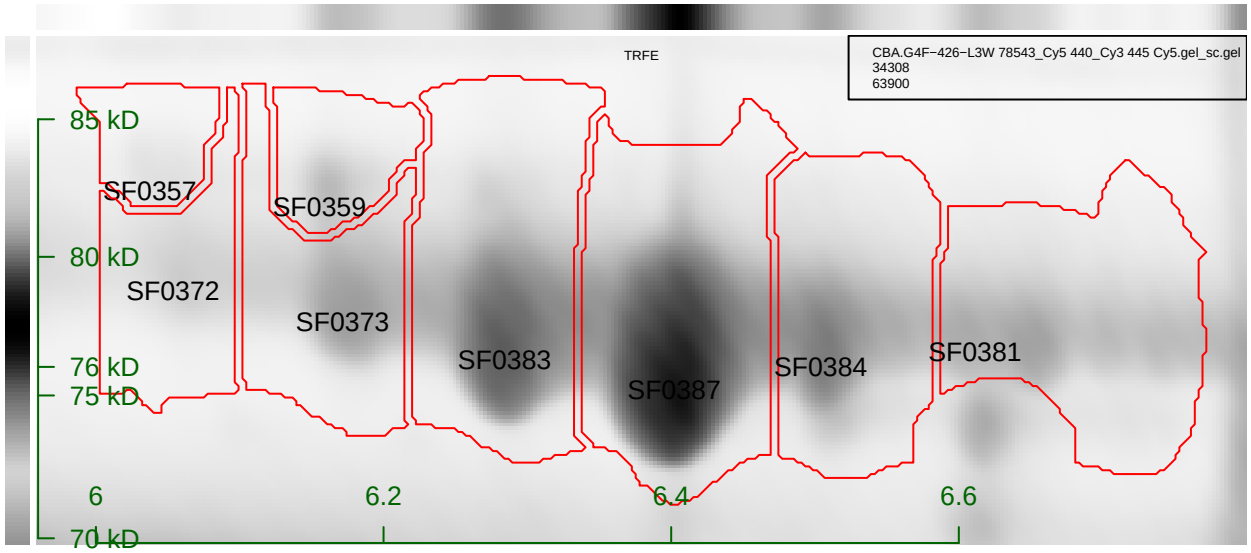

SNorm3_Pix_Peak2_Ln
10.0
9.5
9.0
8.5
SF0357
SF0359
SF0372
SF0373
SF0383
SF0387
SF0384
SF0381
TRFE features

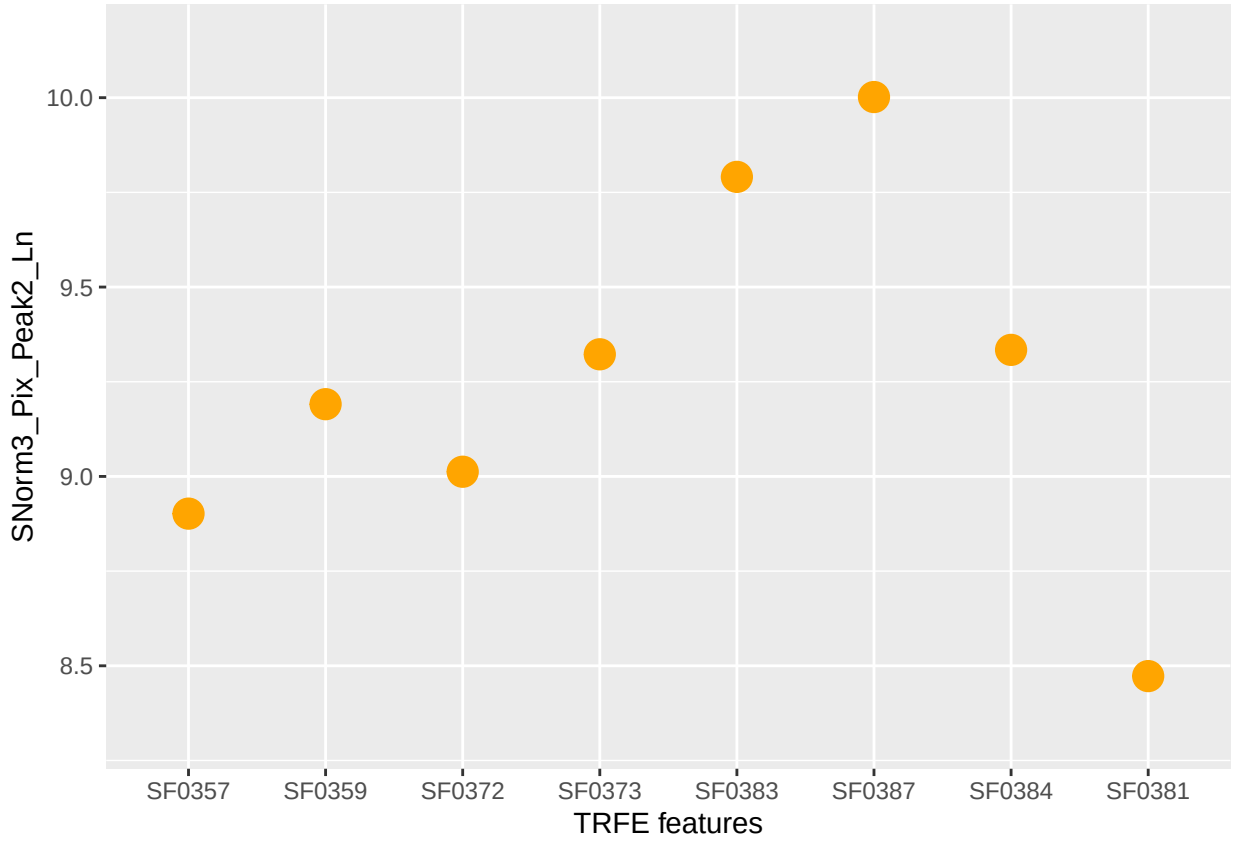
Replicate 2 : 72288_Cy5 440_Cy3 455 Cy5.gel_sc.gel

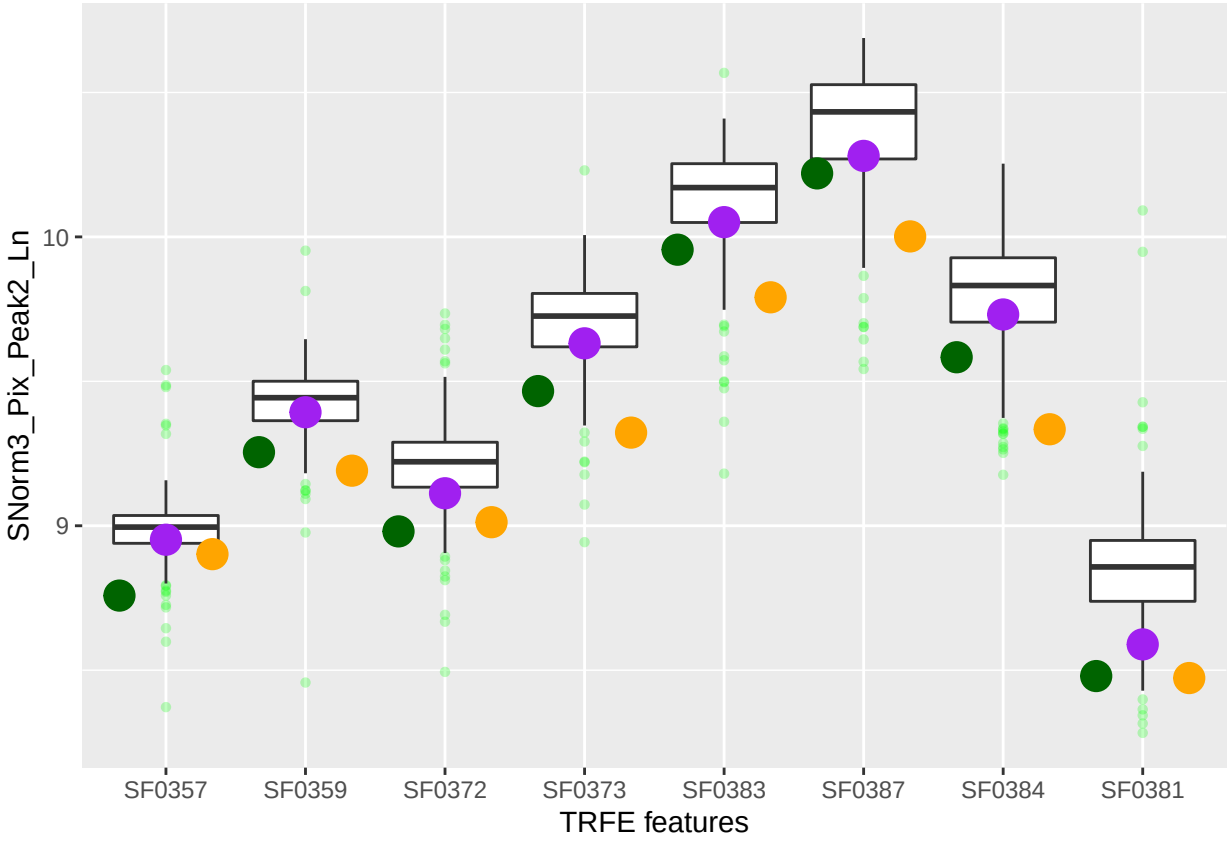




Replicate 3 : 78543_Cy5 440_Cy3 445 Cy5.gel_sc.gel







	SF0357	SF0359	SF0372	SF0373	SF0383	SF0387
50014_Cy5 450_Cy3 455 Cy5	8.758255	9.254740	8.980550	9.466222	9.955700	10.21983
72288_Cy5 440_Cy3 455 Cy5	8.951699	9.392995	9.112507	9.631548	10.051131	10.28042
78543_Cy5 440_Cy3 445 Cy5	8.901775	9.190750	9.012377	9.322508	9.790879	10.00170

	SF0384	SF0381
50014_Cy5 450_Cy3 455 Cy5	9.583420	8.479699
72288_Cy5 440_Cy3 455 Cy5	9.731512	8.589328
78543_Cy5 440_Cy3 445 Cy5	9.334150	8.472823

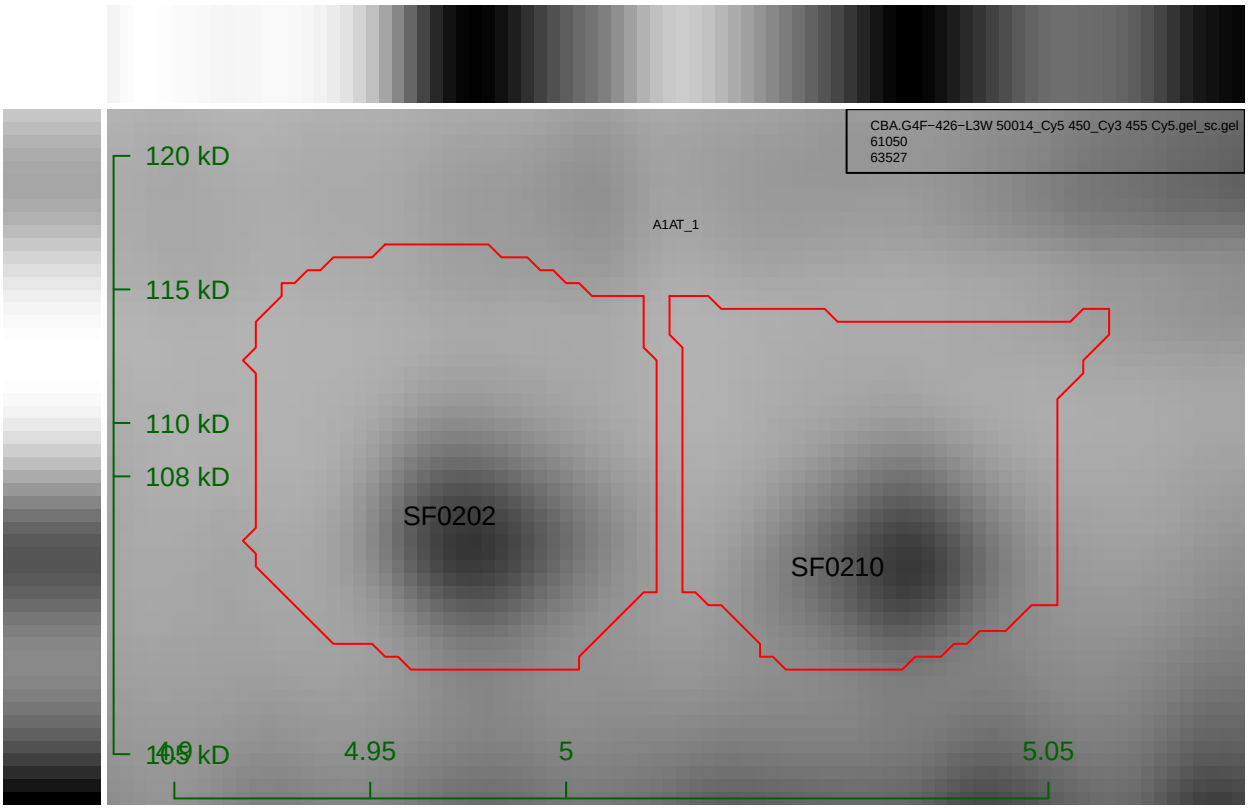
Alpha-1-antitrypsin (P01009)

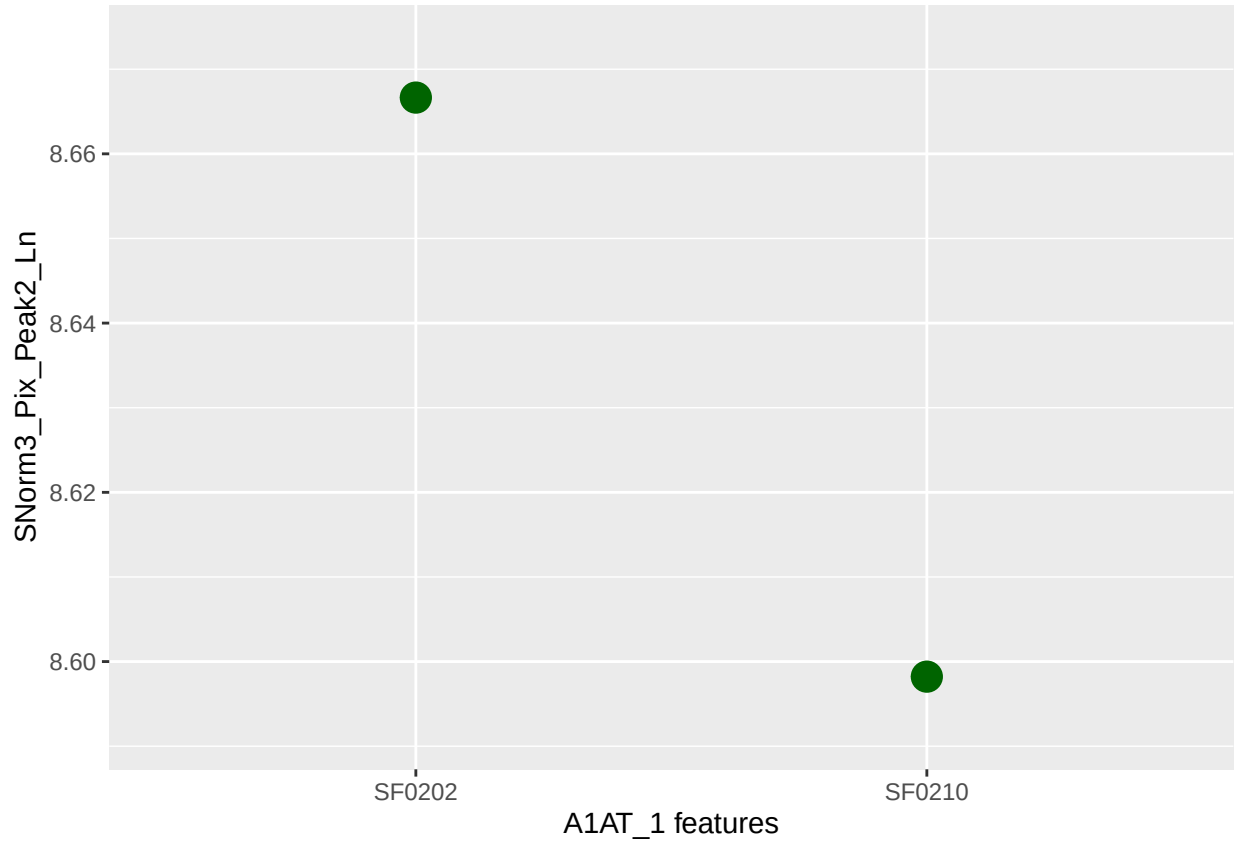
From: Human plasma protein N-glycosylation

> Alpha-1-antitrypsin (AAT), also known as alpha-1-protease inhibitor, alpha-1-antiproteinase or serpin A1, consists of 418 amino acids (including a 24 amino acid signal peptide) with an apparent mass of 51 kDa (including glycosylation). It is mainly produced in the liver by hepatocytes, but is also synthesized in monocytes, intestinal epithelial cells, and in the cornea [52, 208–211]. Due to its small size and polar properties, the glycoprotein can easily move into tissue fluids [52]. In healthy individuals, a plasma level of approximately 1.1 mg/mL is found, but the concentration can increase three- to four-fold during inflammation [212–215]. AAT occurs as three different amino acid sequences, of which the first is set as the standard sequence. Form 2 differs in the amino acid sequence 356–418 and form 3 lacks the amino acid sequence 307–418.

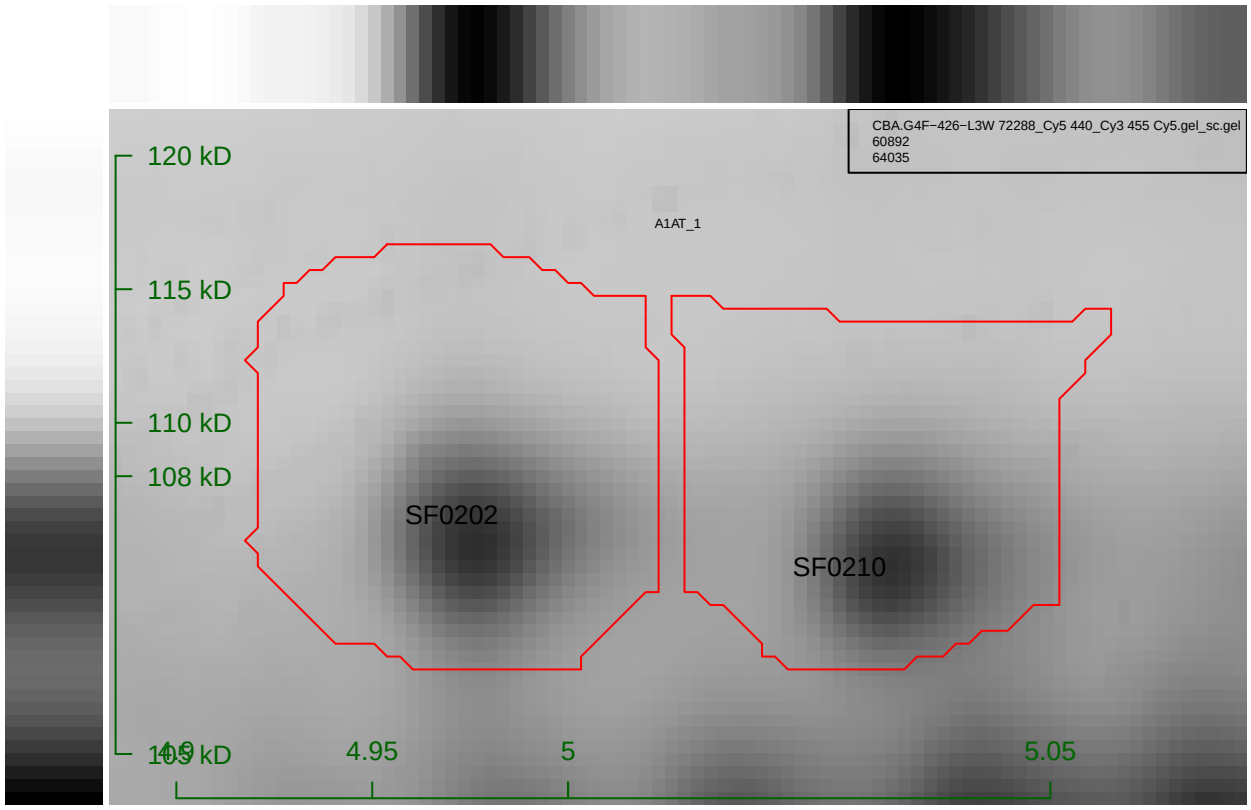
CBA.G4F-426-L3W A1AT_1

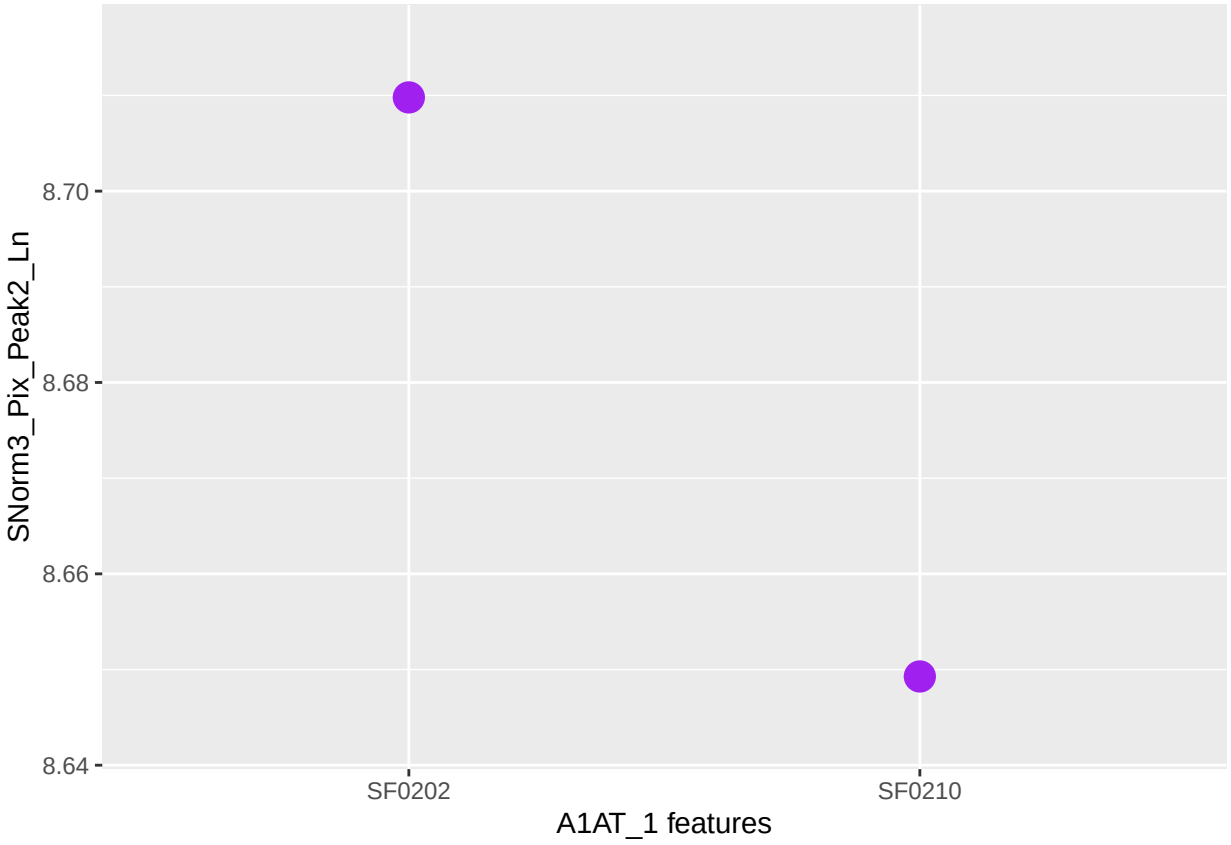
Replicate 1 : 50014_Cy5 450_Cy3 455 Cy5.gel_sc.gel


CBA.G4F−426−L3W 50014_Cy5 450_Cy3 455 Cy5.gel_sc.gel
61050
63527
A1AT_1
120 kD
115 kD
110 kD
108 kD
105 kD
SF0202
SF0210
4.9
4.95
5
5.05

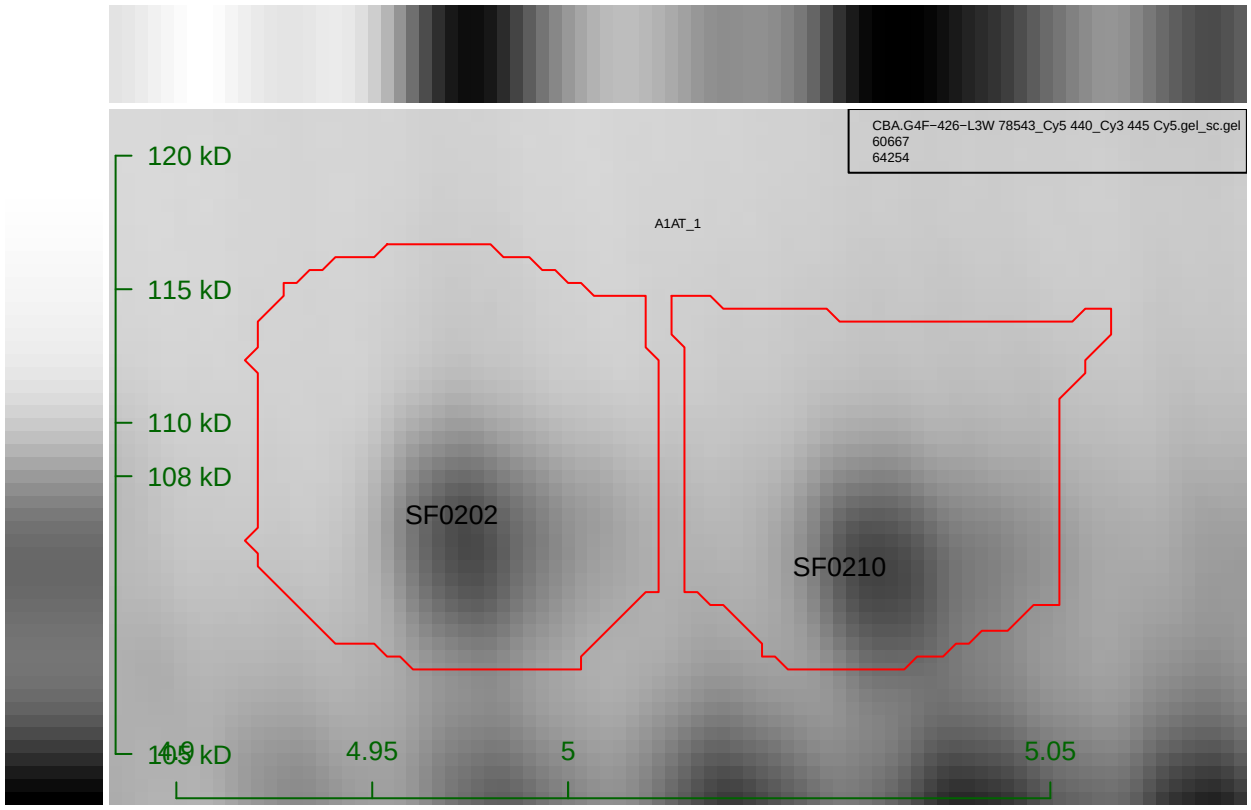

SNorm3_Pix_Peak2_Ln
8.66
8.64
8.62
8.60
SF0202
SF0210
A1AT_1 features

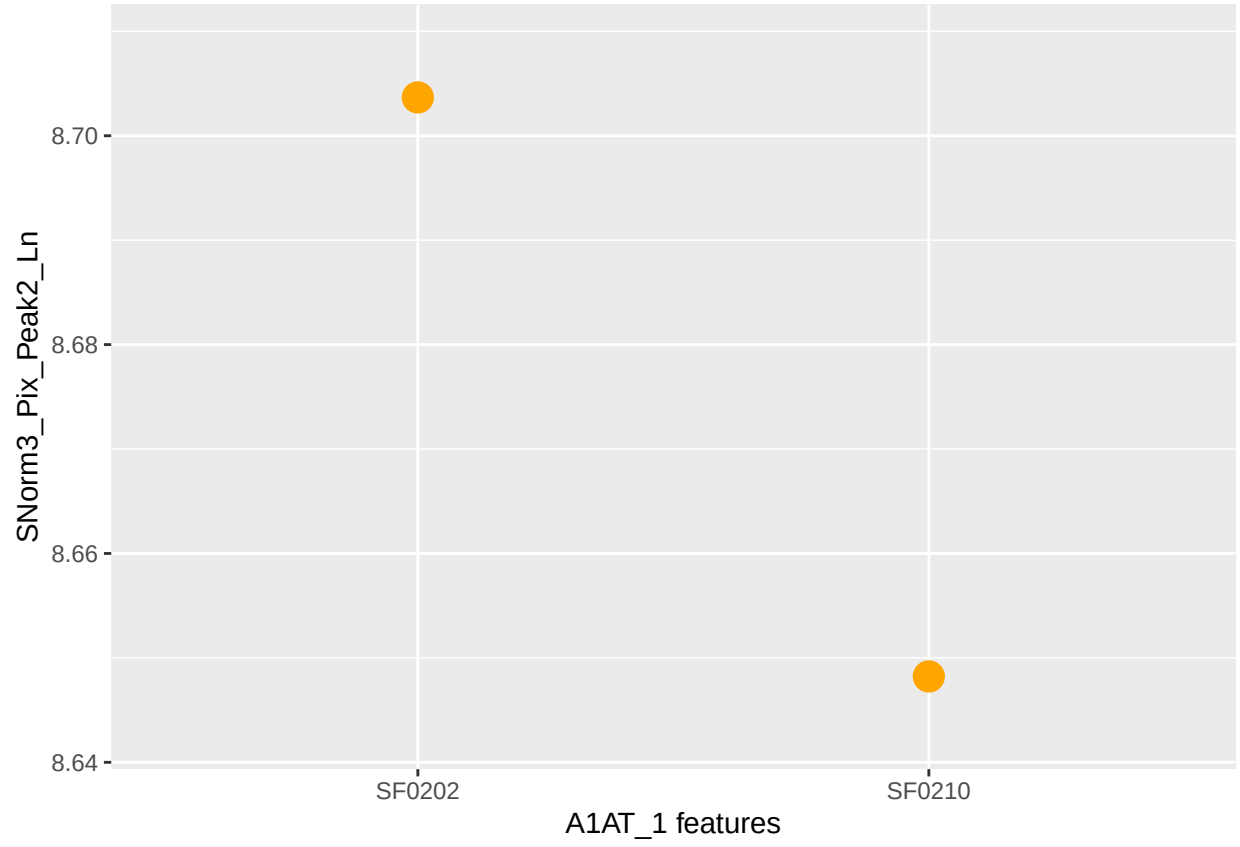
Replicate 2 : 72288_Cy5 440_Cy3 455 Cy5.gel_sc.gel

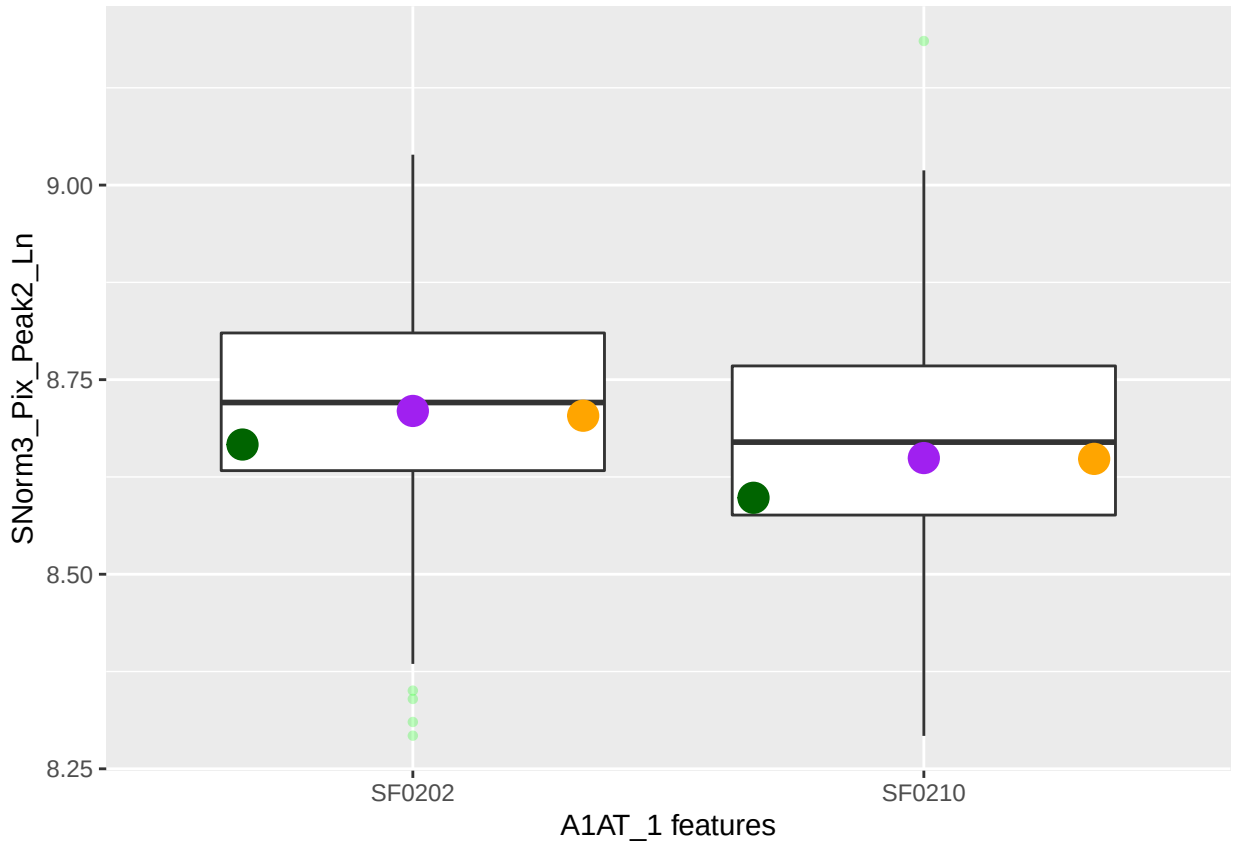




Replicate 3 : 78543_Cy5 440_Cy3 445 Cy5.gel_sc.gel



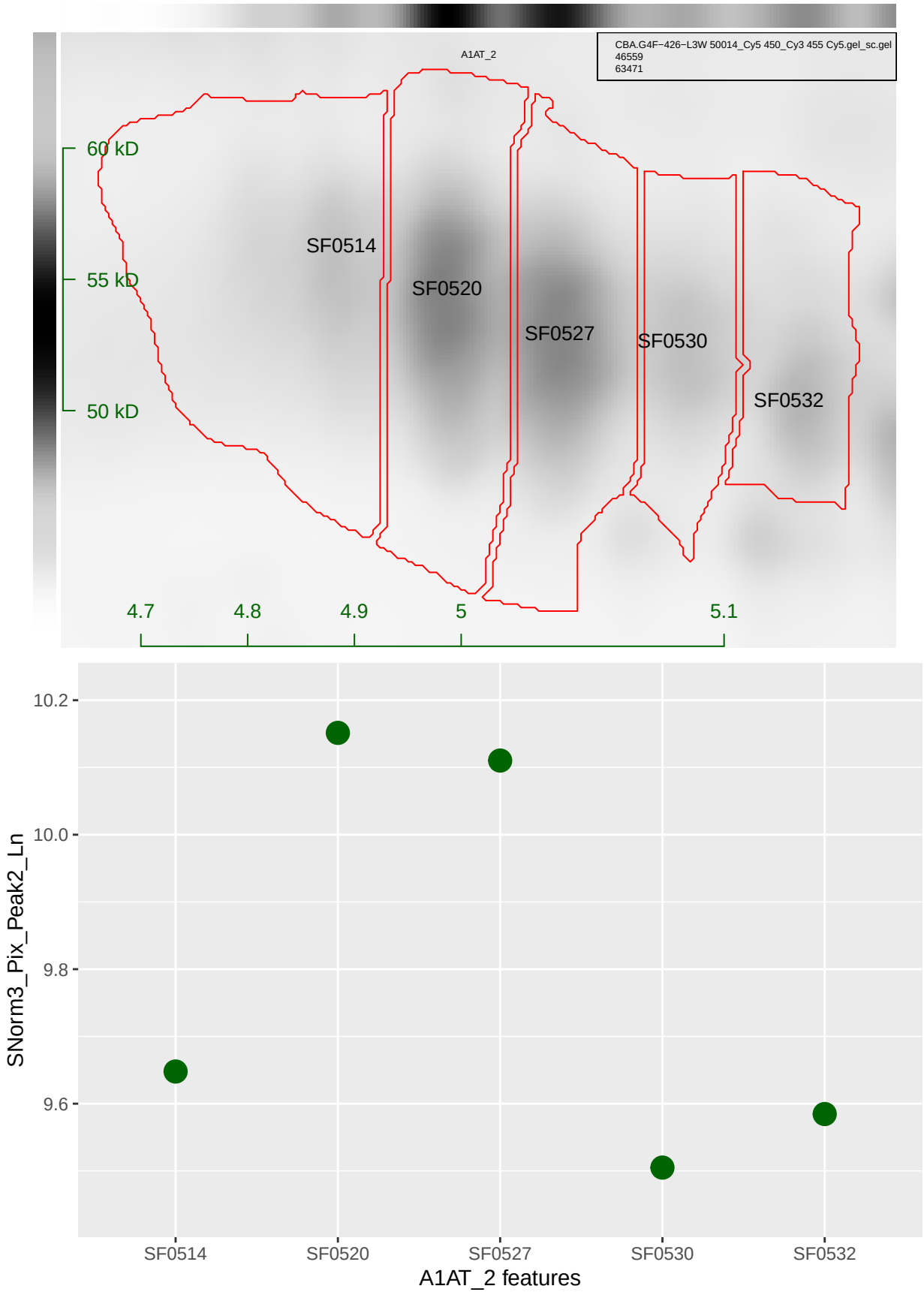




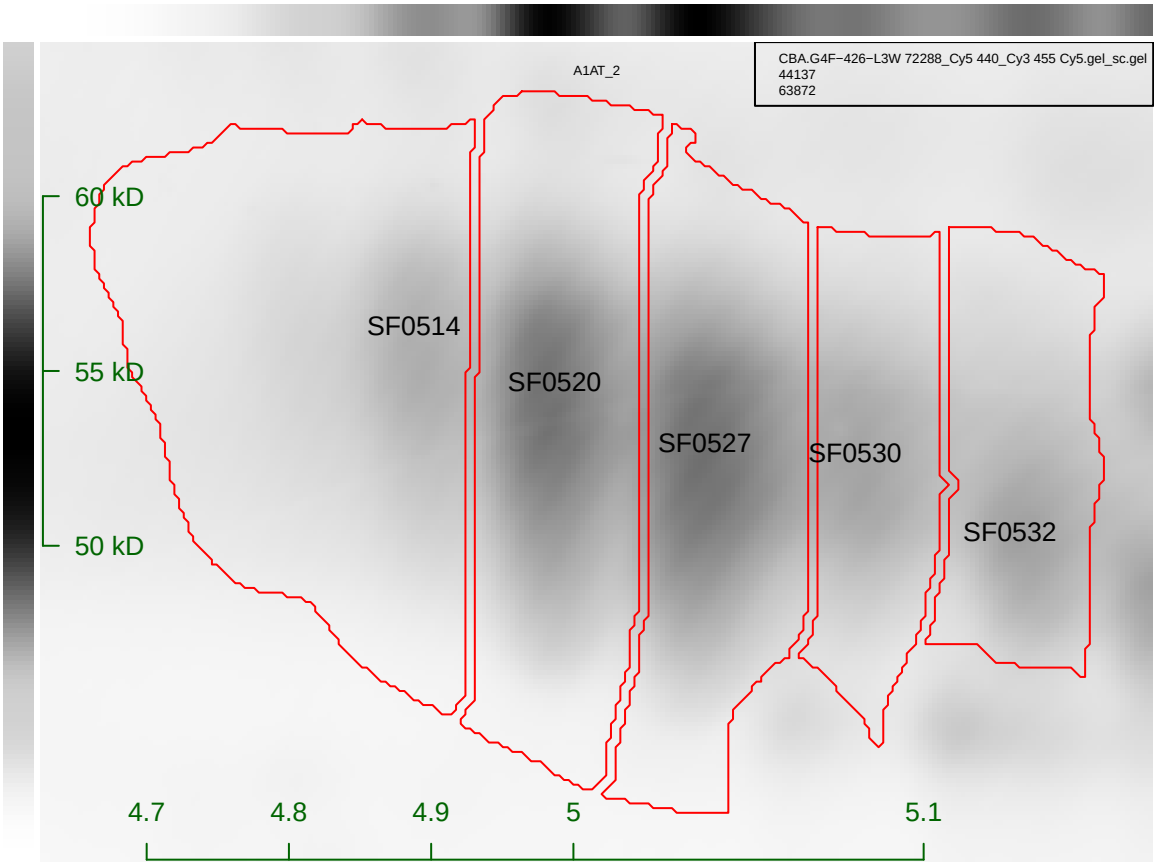
	SF0202	SF0210
50014_Cy5 450_Cy3 455 Cy5	8.666647	8.598220
72288_Cy5 440_Cy3 455 Cy5	8.709795	8.649273
78543_Cy5 440_Cy3 445 Cy5	8.703673	8.648222

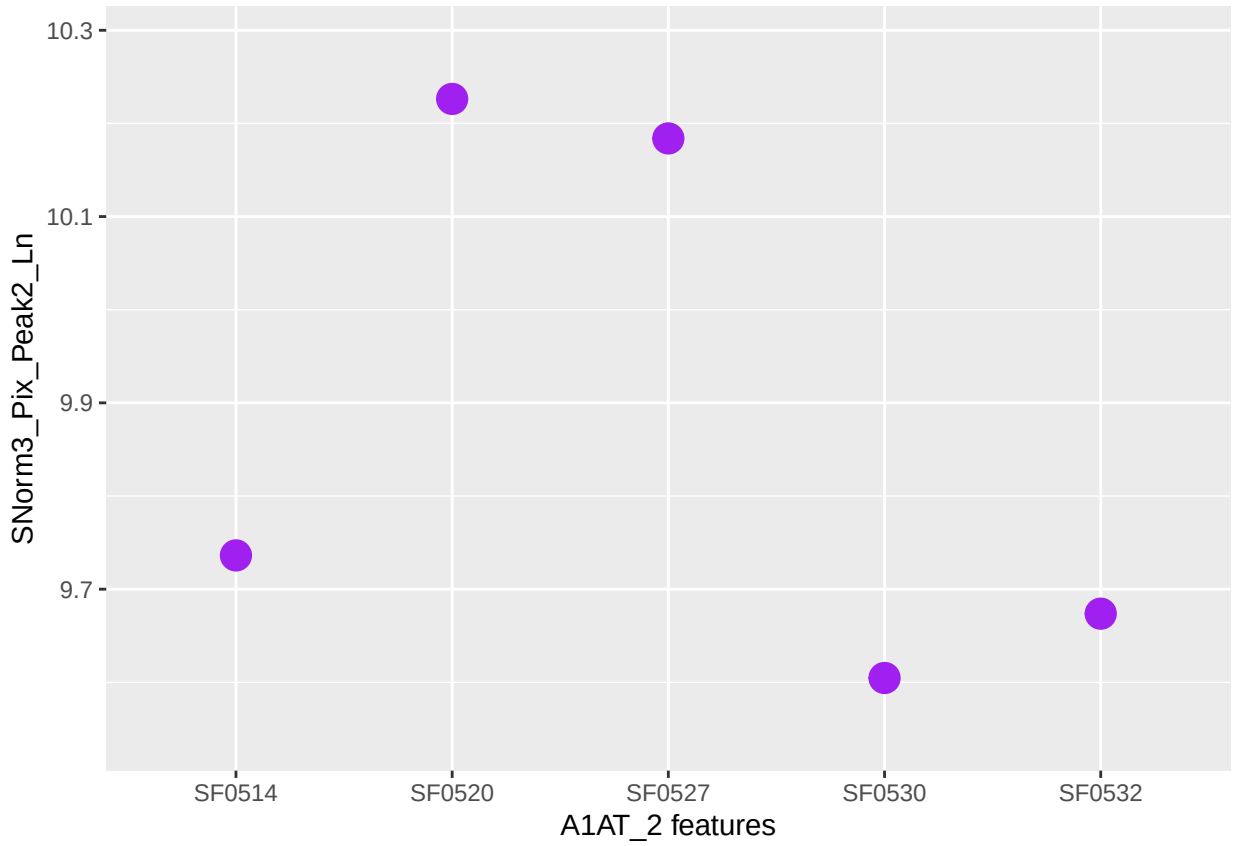
CBA.G4F-426-L3W A1AT_2

Replicate 1 : 50014_Cy5 450_Cy3 455 Cy5.gel_sc.gel

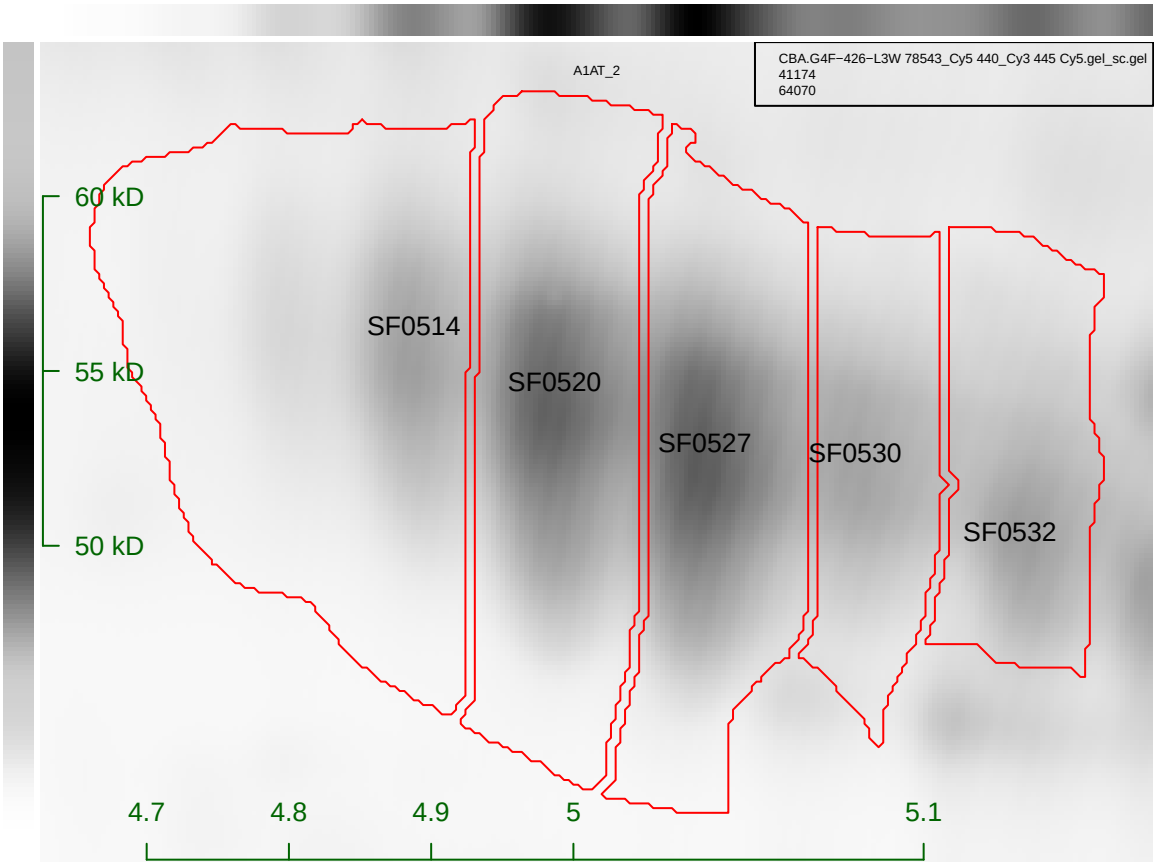

CBA.G4F−426−L3W 50014_Cy5 450_Cy3 455 Cy5.gel_sc.gel
46559
63471
A1AT_2
60 kD
55 kD
50 kD
SF0514
SF0520
SF0527
SF0530
SF0532
4.7
4.8
4.9
5
5.1
10.2
10.0
9.8
9.6
SNorm3_Pix_Peak2_Ln
SF0514
SF0520
SF0527
SF0530
SF0532
A1AT_2 features

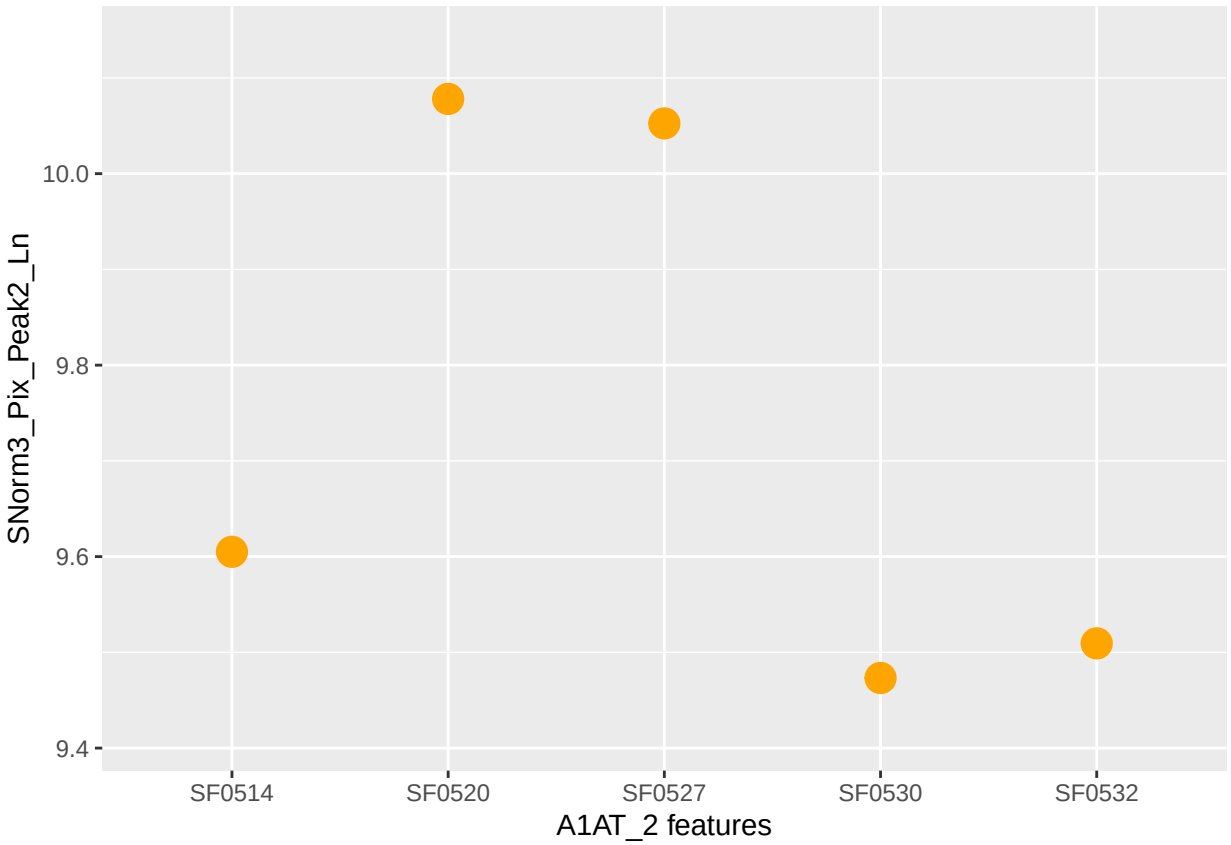
Replicate 2 : 72288_Cy5 440_Cy3 455 Cy5.gel_sc.gel

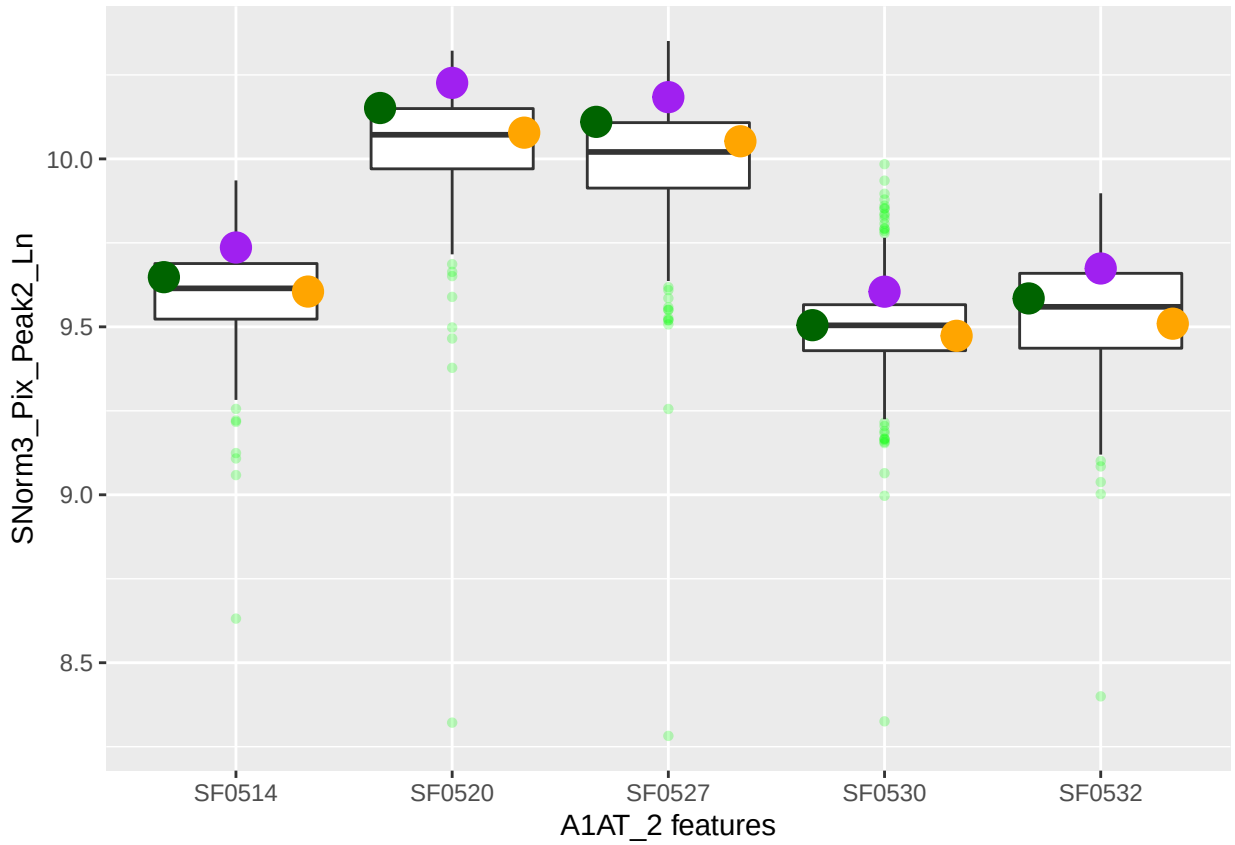



10.3
10.1
9.9
9.7
SNorm3_Pix_Peak2_Ln
SF0514
SF0520
SF0527
SF0530
SF0532
A1AT_2 features

Replicate 3 : 78543_Cy5 440_Cy3 445 Cy5.gel_sc.gel



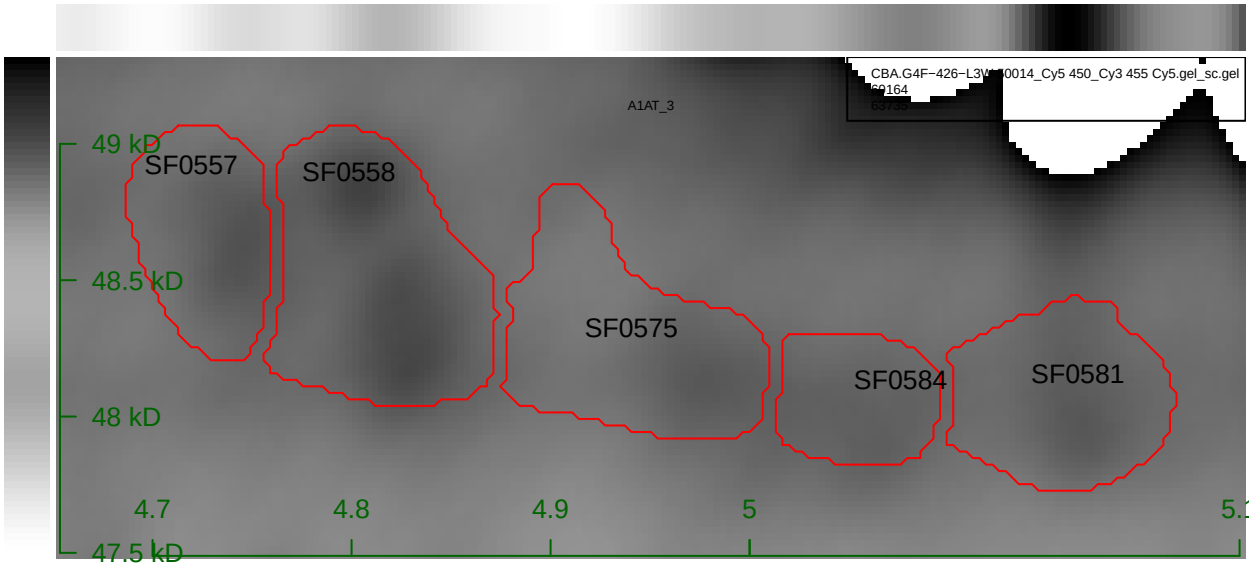

SNorm3_Pix_Peak2_Ln
10.0
9.8
9.6
9.4
SF0514
SF0520
SF0527
SF0530
SF0532
A1AT_2 features

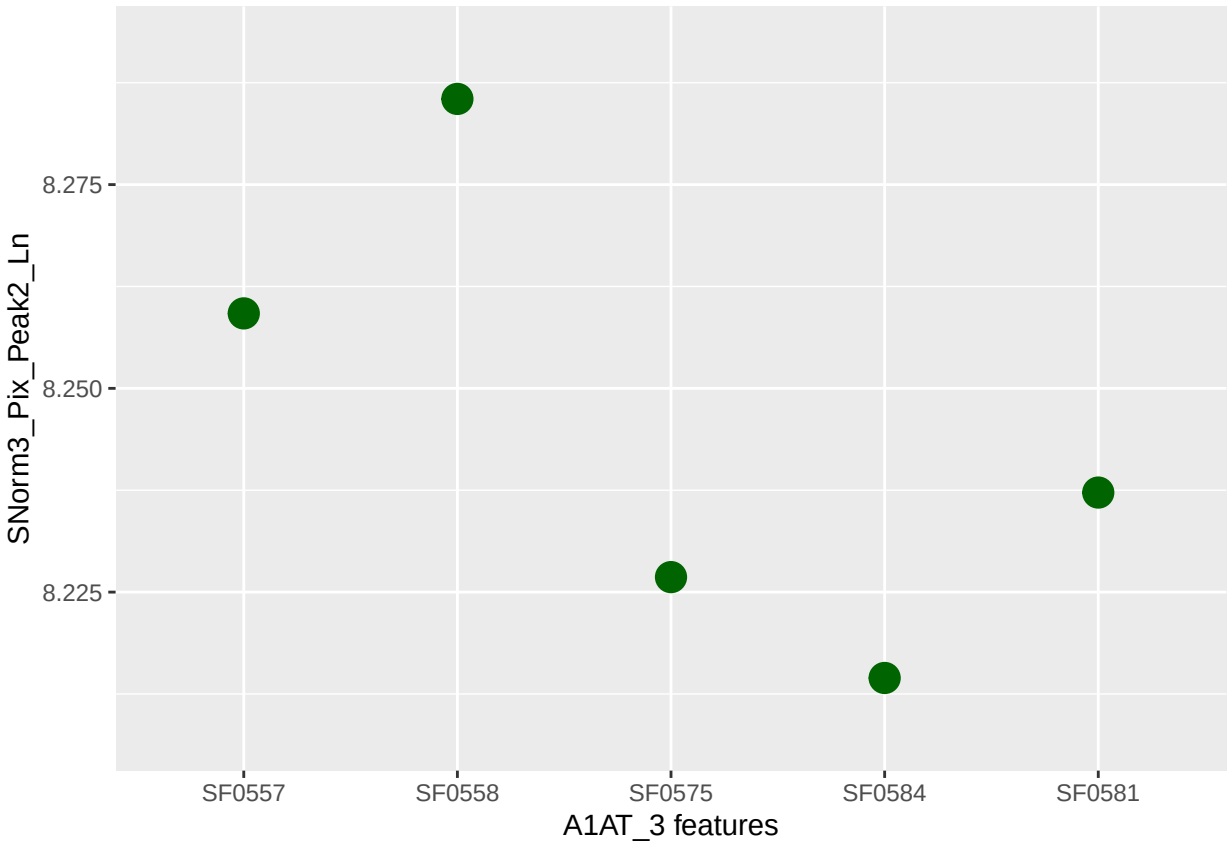


	SF0514	SF0520	SF0527	SF0530	SF0532
50014_Cy5 450_Cy3 455 Cy5	9.647821	10.15144	10.11026	9.504948	9.584590
72288_Cy5 440_Cy3 455 Cy5	9.736252	10.22630	10.18388	9.604677	9.673697
78543_Cy5 440_Cy3 445 Cy5	9.605014	10.07807	10.05247	9.473243	9.509407

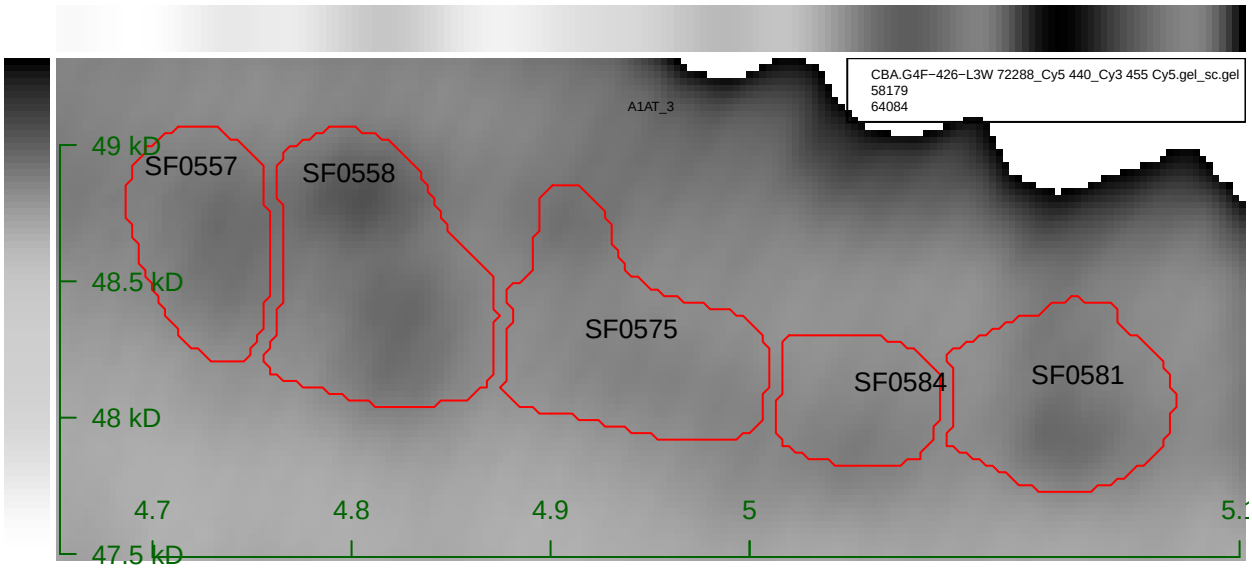
CBA.G4F-426-L3W A1AT_3

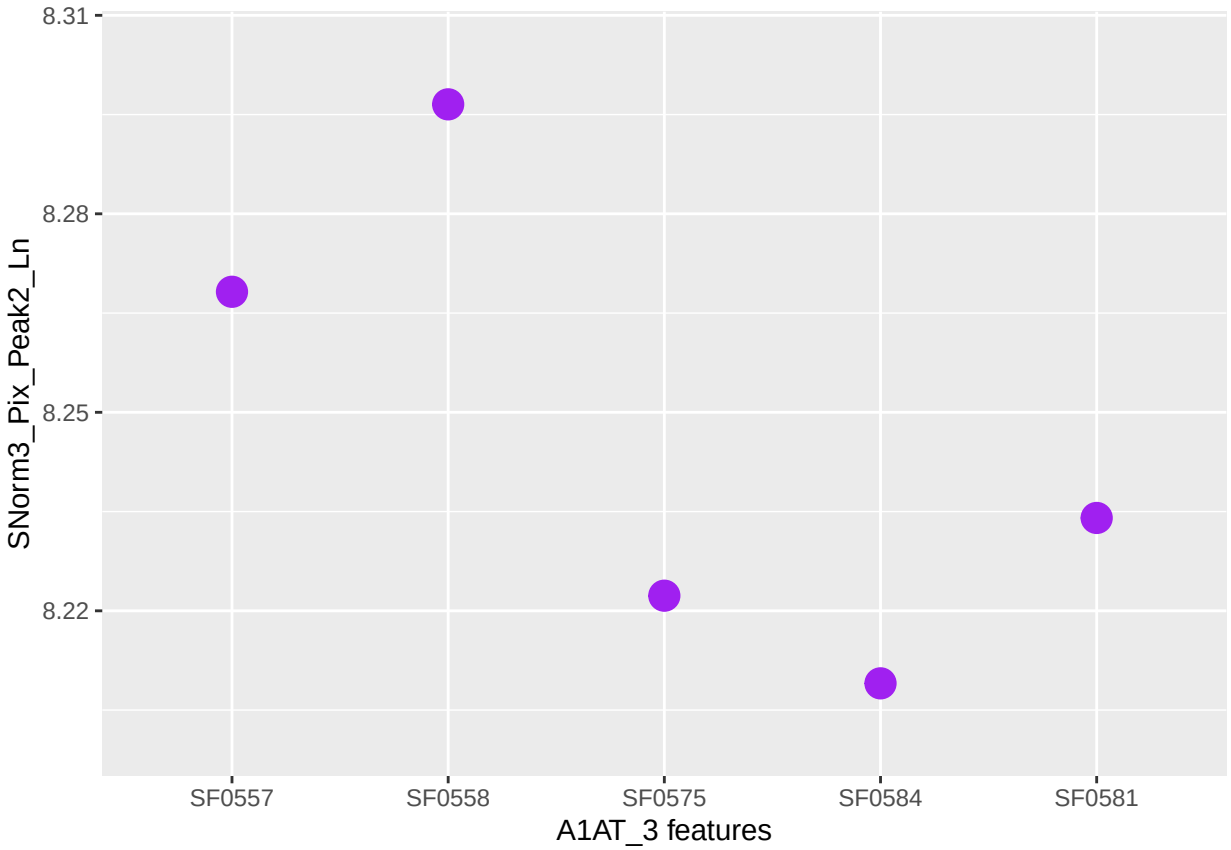
Replicate 1 : 50014_Cy5 450_Cy3 455 Cy5.gel_sc.gel



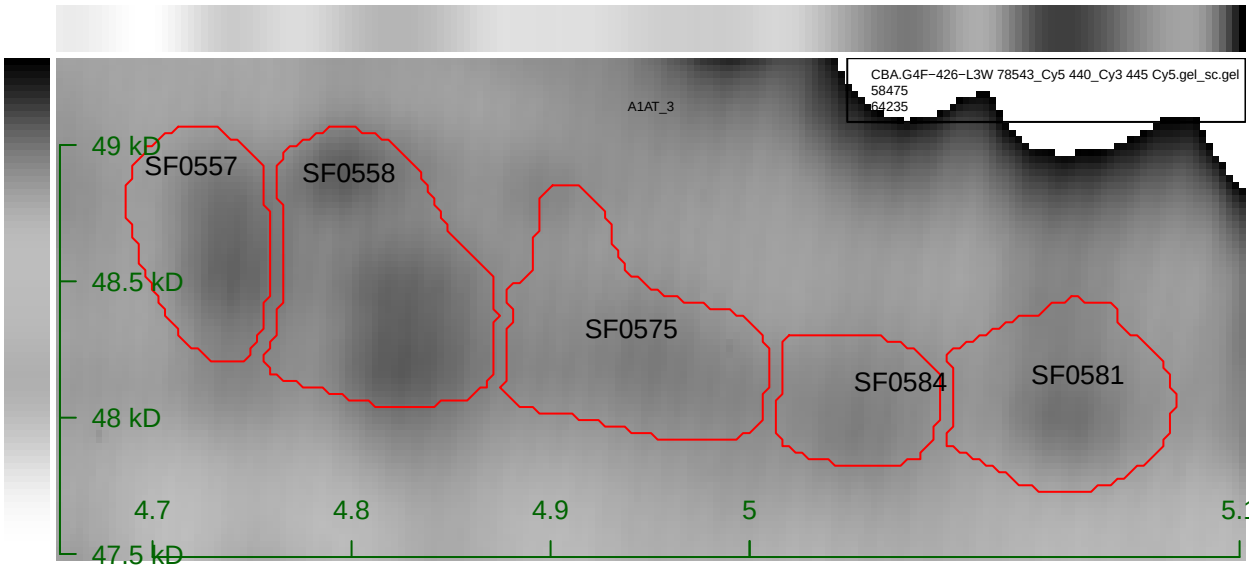

8.275
8.250
8.225
SNorm3_Pix_Peak2_Ln
SF0557
SF0558
SF0575
SF0584
SF0581
A1AT_3 features

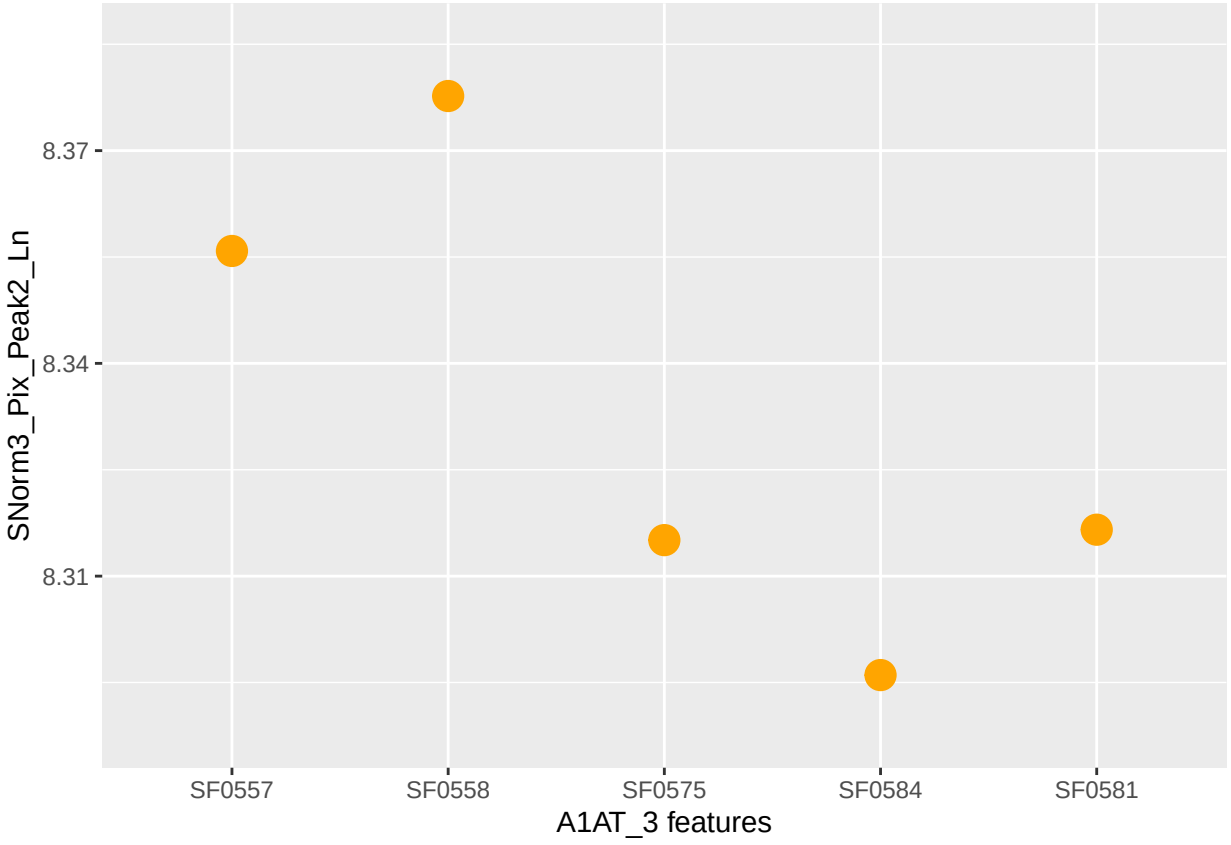
Replicate 2 : 72288_Cy5 440_Cy3 455 Cy5.gel_sc.gel

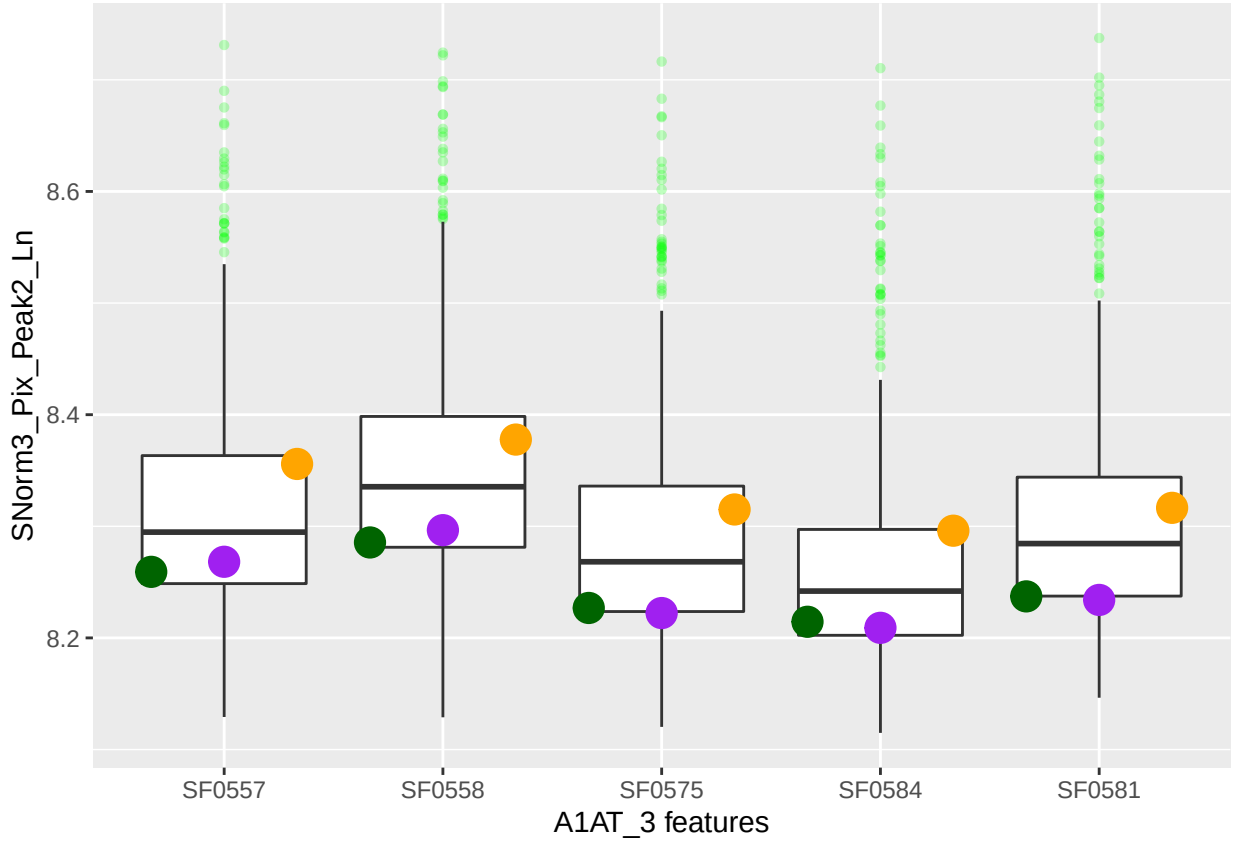




Replicate 3 : 78543_Cy5 440_Cy3 445 Cy5.gel_sc.gel







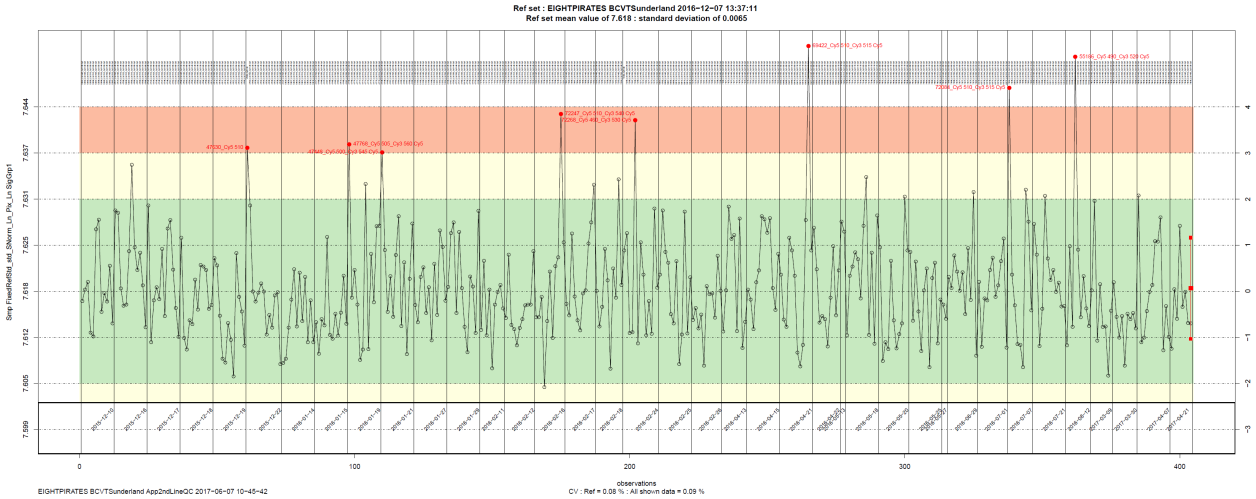
	SF0557	SF0558	SF0575	SF0584	SF0581
50014_Cy5 450_Cy3 455 Cy5	8.259199	8.285513	8.226841	8.214465	8.237215
72288_Cy5 440_Cy3 455 Cy5	8.268219	8.296546	8.222285	8.209036	8.234034
78543_Cy5 440_Cy3 445 Cy5	8.355850	8.377701	8.315077	8.296048	8.316545

Statistical Process Control

From: An introduction to statistical process control in research proteomics

> **Background:** Statistical process control is a well-established and respected method which provides a general purpose, and consistent framework for monitoring and improving the quality of a process. It is routinely used in many industries where the quality of final products is critical and is often required in clinical diagnostic laboratories [1,2]. To date, the methodology has been little utilised in research proteomics. It has been shown to be capable of delivering quantitative QC procedures for qualitative clinical assays [3] making it an ideal methodology to apply to this area of biological research.

Control charts and possibly image areas from the standard.



Audit Logs:

Run overview

Run #	208
Sample / Study	Human plasma (Lot: SLCC1673)/ NUBZ64100, NUCX182416, NUBZ648698, NUAQ387360, NUFH402412, NUFT865086, NUFR670624, NUFT766262, NUFU715690, NUFT770122, NUFT766280, NUCX210223
Completion (Scan) Date	25/09/2020
Comments	OOD GE strips, biotium dye, sample load 2uL. Pre-mix buffers. Standard IEF with cuploading. Standard 2D with type 2 water. 4% equilibration buffer.

Labelling

Technician:EA & NH

Sample: 2µL Human Plasma

Solution / reagent	Batch / Lot #	Reference
DIGE labelling buffer	6	LB-RS-06
Tris-HCl pH 8.5	2	LB-RS-02
DMF	STBH7014	
Cy3 (Biotium)	13C1212	
Cy5 (Biotium)	18C0626-1075	
Lysine	4	LB-RS-07
2x DIGE buffer	6	LB-RS-04

Iso Electric Focusing

Technician:EA & NH

Solution / reagent	Batch / Lot #
1x DIGE buffer	14
Control sample	Human plasma/ SLCC1673
IPG strips	10277023
Reswell Date	22/09/2020
Time strip 1	16:10:00
Time oil added	16:40:00

Equipment	ID #
Reswell tray	3
IPGphor unit	1

Transfer to IPGphor: 23/09/2020 10:54:00 **Technician**: EA & NH

#	Control ID (Cy3)	Sample ID (Cy5)	Strip ID	IEF Tray #	Run Time
1	Human plasma/ SLCC1673	NUBZ640100	49844	2	23.5
2	Human plasma/ SLCC1674	NUCX182416	49845	2	23.5
3	Human plasma/ SLCC1675	NUBZ648698	49846	2	23.5
4	Human plasma/ SLCC1676	NUAQ387360	49847	2	23.5
5	Human plasma/ SLCC1677	NUFH402412	49848	2	23.5
6	Human plasma/ SLCC1678	NUFT865086	49849	2	23.5
7	Human plasma/ SLCC1679	NUFR670624	49850	2	23.5
8	Human plasma/ SLCC1680	NUFT766262	49851	2	23.5
9	Human plasma/ SLCC1681	NUFU715690	49852	2	23.5
10	Human plasma/ SLCC1682	NUFT770122	49853	2	23.5
11	Human plasma/ SLCC1683	NUFT766280	49854	2	23.5
12	Human plasma/ SLCC1684	NUCX210223	49855	2	23.5

2D SDS

Casting (A2DE optimiser) **Technician**: NH

Solution / reagent	Batch / Lot #	Weight (g)	Reference
Tris-SDS	24		LB-RS-11
Glycerol	17		LB-RS-09
Acrylamide	07-19-22		WI-LB-001 5.2.1(1)
APS	MKCG5404		WI-LB-001 5.2.1(3a)
TEMED	STBH7073		WI-LB-001 5.2.1(3b)

Equipment	Set
Gel plates:	1
Casting box:	1
Optimiser:	1

Second dimension run properties (2D Tank1) **Technician**: EA & NH

Event	Time	W	mA	V
Start	15:32:00	8	170	46
Duration	17h30			
Power up	09:02:00			
Time off	09:18:00	25	190	130
Total time	17h 46m			

Imaging

Technician: EA & NH

Imager: Typhoon 9400

Calibration App Log File

The imager was calibrated on 25/09/2020 following protocol P-212 and Calibration App Version 1.01.

Dye channel	PMT
Cy5	450
Cy3	455

Scanner Driver App

The gels were imaged in pairs using Scanner Driver App version 1.01. The app automatically cropped and rotated the scans. Scans were performed at 100 and 200 micron resolutions. Cy3 and Cy5 channels were imaged simultaneously.

Whole gel visual review

The gels passed a visual review (under OP-28) before upload to the cloud for processing.

Scanning App Log File

Opening excel file: A:\Lab\GelRunning\Runs\0208\Run 208.xlsx

Loaded scans:

Strip1: 49844 Strip2: 49845

Strip1: 49846 Strip2: 49847

Strip1: 49848 Strip2: 49849

Strip1: 49850 Strip2: 49851

Strip1: 49852 Strip2: 49853

Strip1: 49854 Strip2: 49855

Starting scan of Strip1: 49844 Strip2: 49845

Finished scan of Strip1: 49844 Strip2: 49845

Scanned images in 00:31:26.9429270:

c:\Biosignatures data\2020-09-25-Run 208\9400\200\49844_Cy5 500_Cy3 530 Cy3.gel

c:\Biosignatures data\2020-09-25-Run 208\9400\200\49844_Cy5 500_Cy3 530 Cy5.gel

c:\Biosignatures data\2020-09-25-Run 208\9400\200\49845_Cy5 500_Cy3 530 Cy3.gel

c:\Biosignatures data\2020-09-25-Run 208\9400\200\49845_Cy5 500_Cy3 530 Cy5.gel

c:\Biosignatures data\2020-09-25-Run 208\9400\49844_Cy5 560_Cy3 590 Cy3.gel

c:\Biosignatures data\2020-09-25-Run 208\9400\49844_Cy5 560_Cy3 590 Cy5.gel

c:\Biosignatures data\2020-09-25-Run 208\9400\49845_Cy5 560_Cy3 590 Cy3.gel

c:\Biosignatures data\2020-09-25-Run 208\9400\49845_Cy5 560_Cy3 590 Cy5.gel

File 49844_Cy5 560_Cy3 590 Cy3.gel had 77362 saturated pixels of 5304000

File 49845_Cy5 560_Cy3 590 Cy3.gel had 107383 saturated pixels of 5304000

Starting scan of Strip1: 49846 Strip2: 49847

Finished scan of Strip1: 49846 Strip2: 49847

Scanned images in 00:31:16.6743397:

c:\Biosignatures data\2020-09-25-Run 208\9400\200\49846_Cy5 500_Cy3 530 Cy3.gel

c:\Biosignatures data\2020-09-25-Run 208\9400\200\49846_Cy5 500_Cy3 530 Cy5.gel

c:\Biosignatures data\2020-09-25-Run 208\9400\200\49847_Cy5 500_Cy3 530 Cy3.gel

c:\Biosignatures data\2020-09-25-Run 208\9400\200\49847_Cy5 500_Cy3 530 Cy5.gel

c:\Biosignatures data\2020-09-25-Run 208\9400\49846_Cy5 560_Cy3 590 Cy3.gel

c:\Biosignatures data\2020-09-25-Run 208\9400\49846_Cy5 560_Cy3 590 Cy5.gel

c:\Biosignatures data\2020-09-25-Run 208\9400\49847_Cy5 560_Cy3 590 Cy3.gel

c:\Biosignatures data\2020-09-25-Run 208\9400\49847_Cy5 560_Cy3 590 Cy5.gel

File 49846_Cy5 560_Cy3 590 Cy3.gel had 99403 saturated pixels of 5304000

File 49847_Cy5 560_Cy3 590 Cy3.gel had 85804 saturated pixels of 5304000

Starting scan of Strip1: 49848 Strip2: 49849
Finished scan of Strip1: 49848 Strip2: 49849
Scanned images in 00:31:19.2494869:
c:\Biosignatures data\2020-09-25-Run 208\9400\200\49848_Cy5 500_Cy3 530 Cy3.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\200\49848_Cy5 500_Cy3 530 Cy5.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\200\49849_Cy5 500_Cy3 530 Cy3.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\200\49849_Cy5 500_Cy3 530 Cy5.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\49848_Cy5 560_Cy3 590 Cy3.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\49848_Cy5 560_Cy3 590 Cy5.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\49849_Cy5 560_Cy3 590 Cy3.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\49849_Cy5 560_Cy3 590 Cy5.gel
File 49848_Cy5 560_Cy3 590 Cy3.gel had 97777 saturated pixels of 5304000
File 49849_Cy5 560_Cy3 590 Cy3.gel had 61913 saturated pixels of 5304000
Starting scan of Strip1: 49850 Strip2: 49851
Finished scan of Strip1: 49850 Strip2: 49851
Scanned images in 00:31:20.2025415:
c:\Biosignatures data\2020-09-25-Run 208\9400\200\49850_Cy5 500_Cy3 530 Cy3.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\200\49850_Cy5 500_Cy3 530 Cy5.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\200\49851_Cy5 500_Cy3 530 Cy3.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\200\49851_Cy5 500_Cy3 530 Cy5.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\49850_Cy5 560_Cy3 590 Cy3.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\49850_Cy5 560_Cy3 590 Cy5.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\49851_Cy5 560_Cy3 590 Cy3.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\49851_Cy5 560_Cy3 590 Cy5.gel
File 49850_Cy5 560_Cy3 590 Cy3.gel had 97818 saturated pixels of 5304000
File 49851_Cy5 560_Cy3 590 Cy3.gel had 69973 saturated pixels of 5304000
Starting scan of Strip1: 49852 Strip2: 49853
Finished scan of Strip1: 49852 Strip2: 49853
Scanned images in 00:31:18.1004212:
c:\Biosignatures data\2020-09-25-Run 208\9400\200\49852_Cy5 500_Cy3 530 Cy3.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\200\49852_Cy5 500_Cy3 530 Cy5.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\200\49853_Cy5 500_Cy3 530 Cy3.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\200\49853_Cy5 500_Cy3 530 Cy5.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\49852_Cy5 560_Cy3 590 Cy3.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\49852_Cy5 560_Cy3 590 Cy5.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\49853_Cy5 560_Cy3 590 Cy3.gel

c:\Biosignatures data\2020-09-25-Run 208\9400\49853_Cy5 560_Cy3 590 Cy5.gel
File 49852_Cy5 560_Cy3 590 Cy3.gel had 81429 saturated pixels of 5304000
File 49853_Cy5 560_Cy3 590 Cy3.gel had 82430 saturated pixels of 5304000
Starting scan of Strip1: 49854 Strip2: 49855
Finished scan of Strip1: 49854 Strip2: 49855
Scanned images in 00:31:19.8435209:
c:\Biosignatures data\2020-09-25-Run 208\9400\200\49854_Cy5 500_Cy3 530 Cy3.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\200\49854_Cy5 500_Cy3 530 Cy5.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\200\49855_Cy5 500_Cy3 530 Cy3.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\200\49855_Cy5 500_Cy3 530 Cy5.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\49854_Cy5 560_Cy3 590 Cy3.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\49854_Cy5 560_Cy3 590 Cy5.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\49855_Cy5 560_Cy3 590 Cy3.gel
c:\Biosignatures data\2020-09-25-Run 208\9400\49855_Cy5 560_Cy3 590 Cy5.gel
File 49854_Cy5 560_Cy3 590 Cy3.gel had 89075 saturated pixels of 5304000
File 49855_Cy5 560_Cy3 590 Cy3.gel had 71489 saturated pixels of 5304000