

## Audit Level Report

Generated: Oct 09 2020 23:33

### Sample: CBA.G4F-426-L3W

#### Analysis Parameters

Analytes	Alpha1 AntiTrypsin, Apolipoprotein D, Ceruloplasmin, Fibrinogen, Haptoglobin, Immunoglobulin G, T
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.

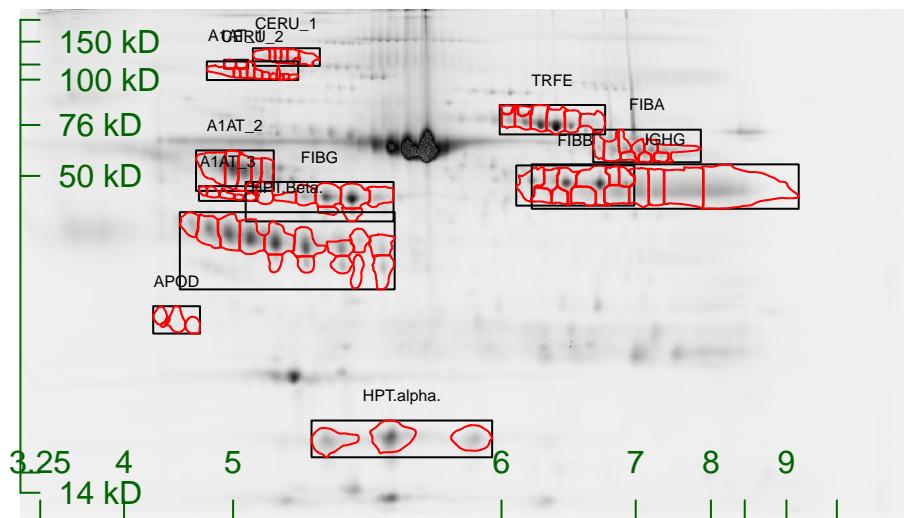
2µL Human Plasma is labelled with Cy5 (or equivalent) and a fixed standard labelled with Cy3 following protocols defined by biosignatures. Images of the channels are then uploaded to a cloud analysis platform. The Cy3 standard is then aligned into a fixed reference space and automated QC procedures assess 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.



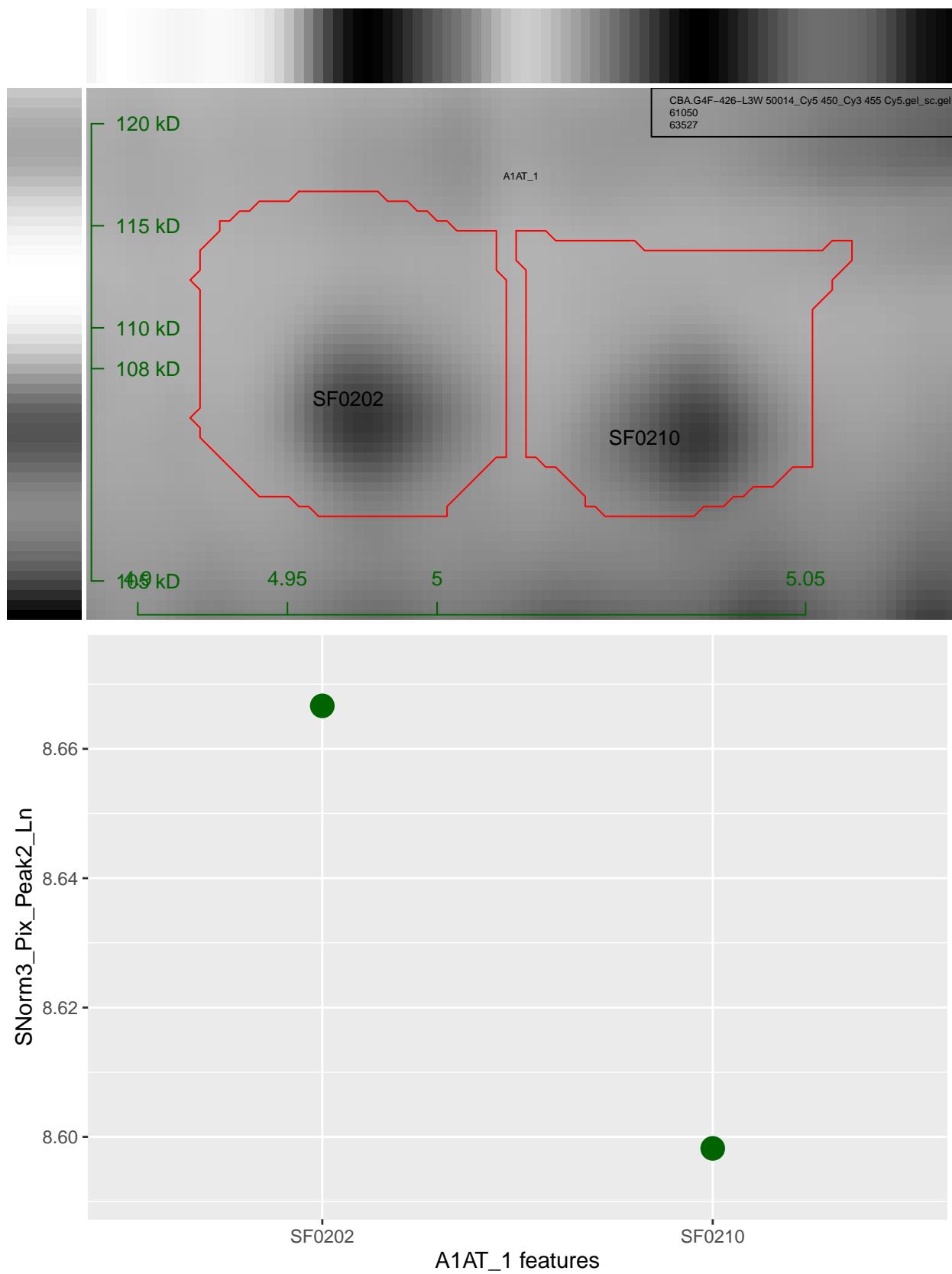
## Alpha-1-antitrypsin (P01009)

From: Human plasma protein N-glycosylation

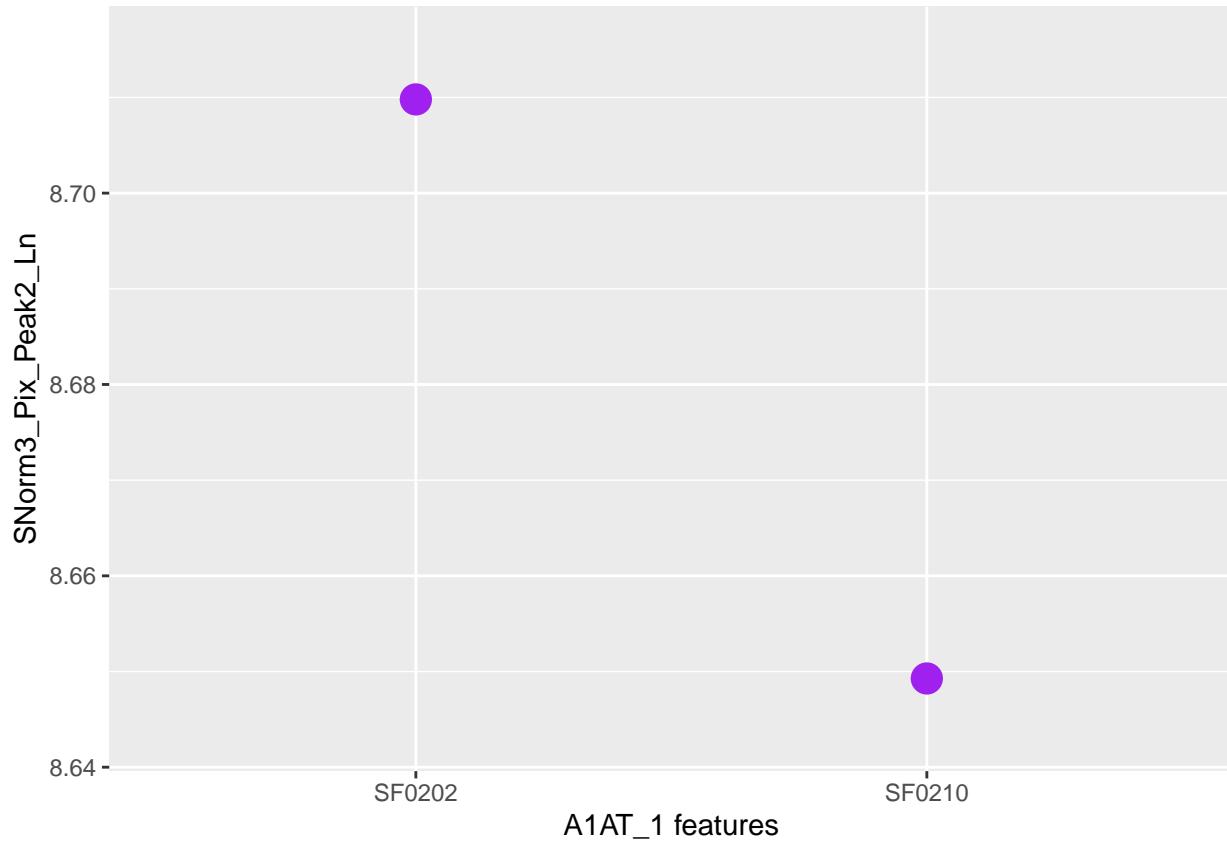
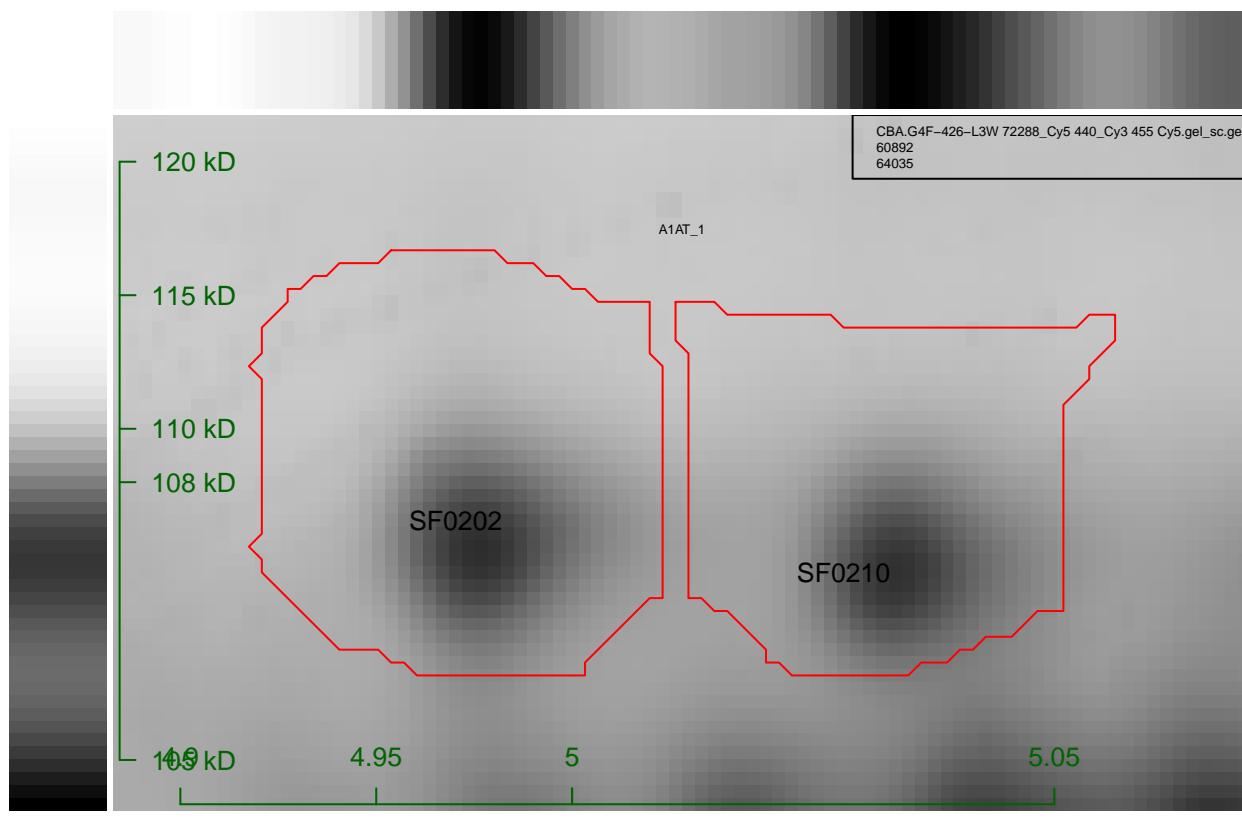
Alpha-1-antitrypsin (AAT), also known as alpha-1-protease inhibitor, alpha-1-antiproteinase or serpin A1, consists of 418 amino acids (including a 24 amino acid signal peptide) with an apparent mass of 51 kDa (including glycosylation). It is mainly produced in the liver by hepatocytes, but is also synthesized 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**

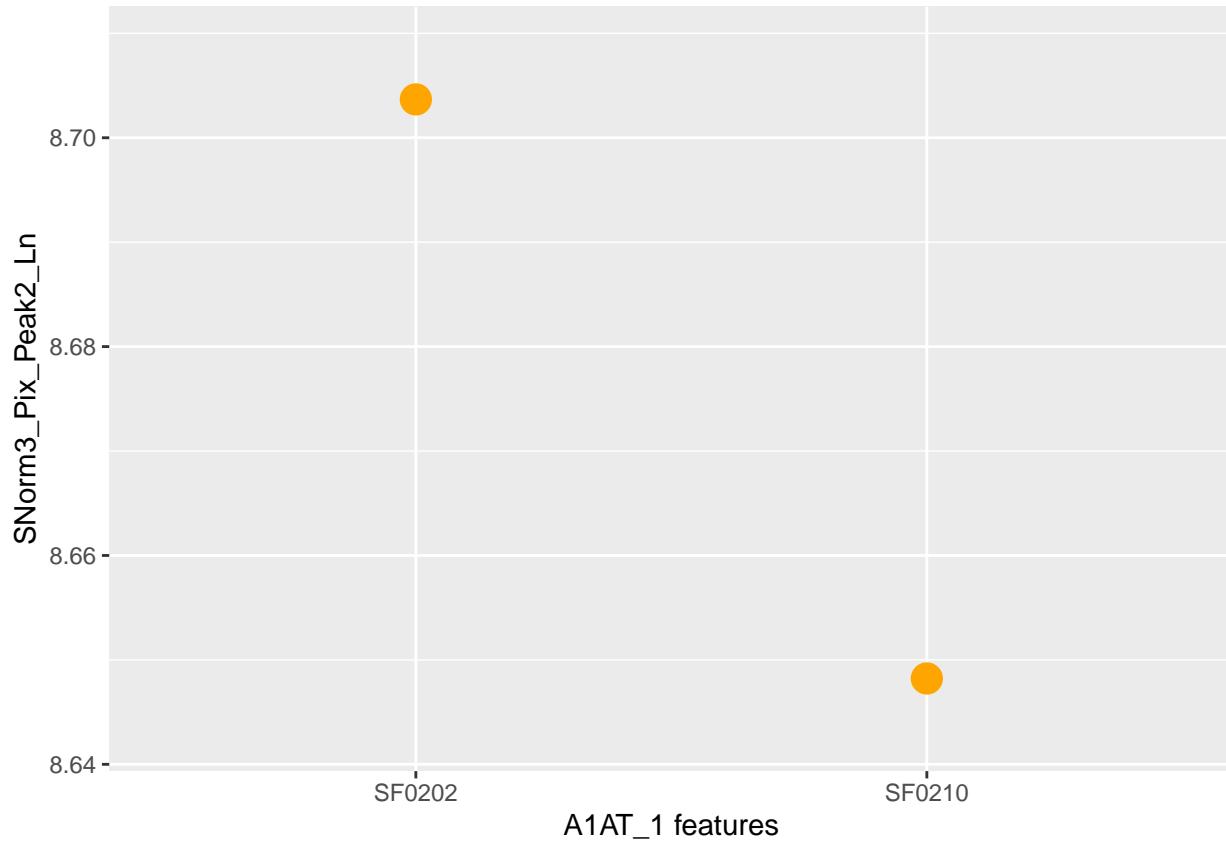
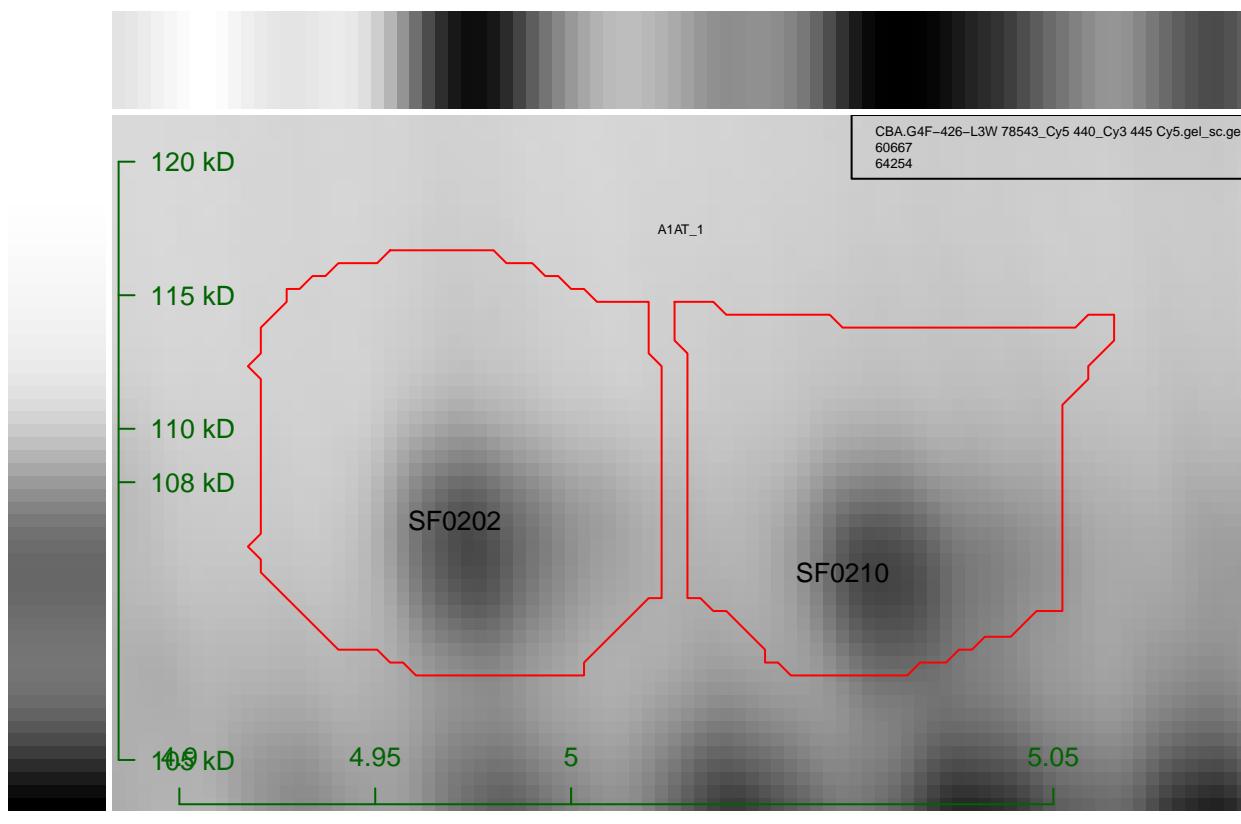
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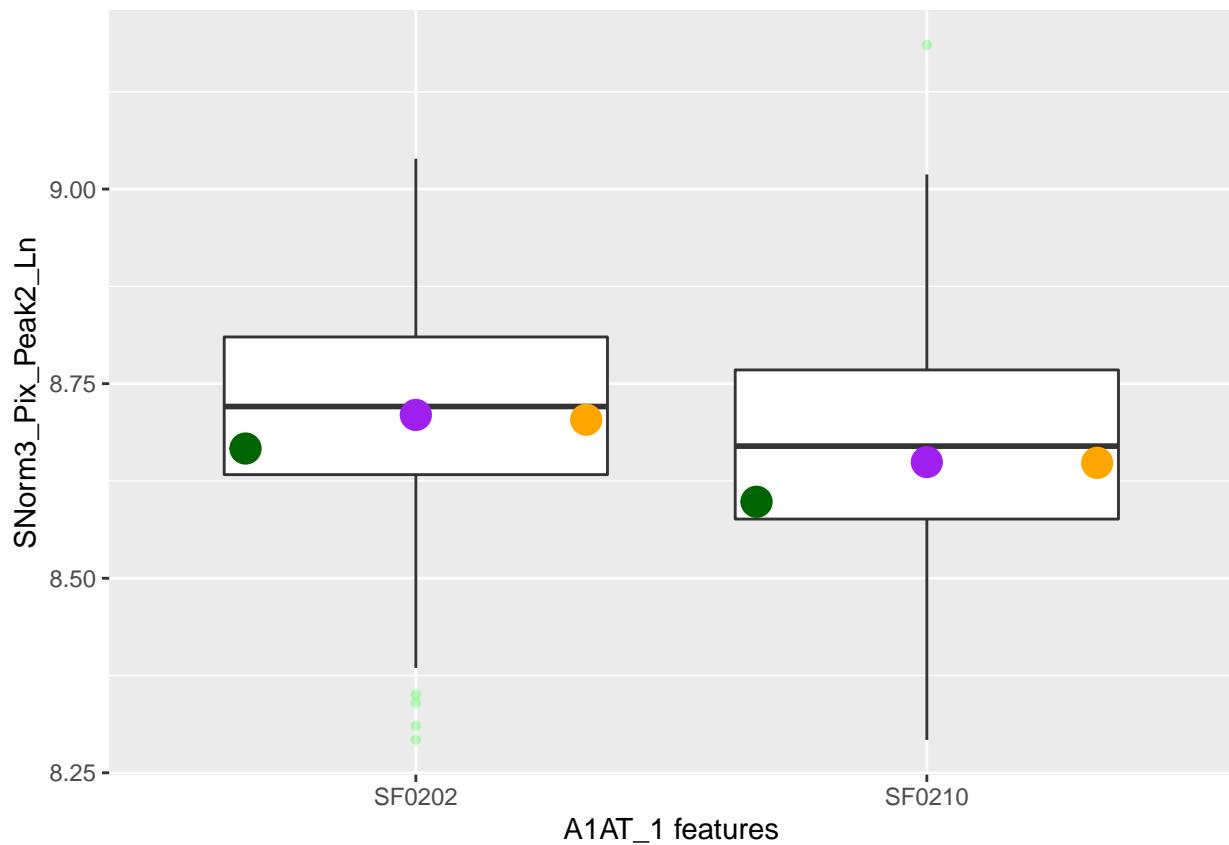


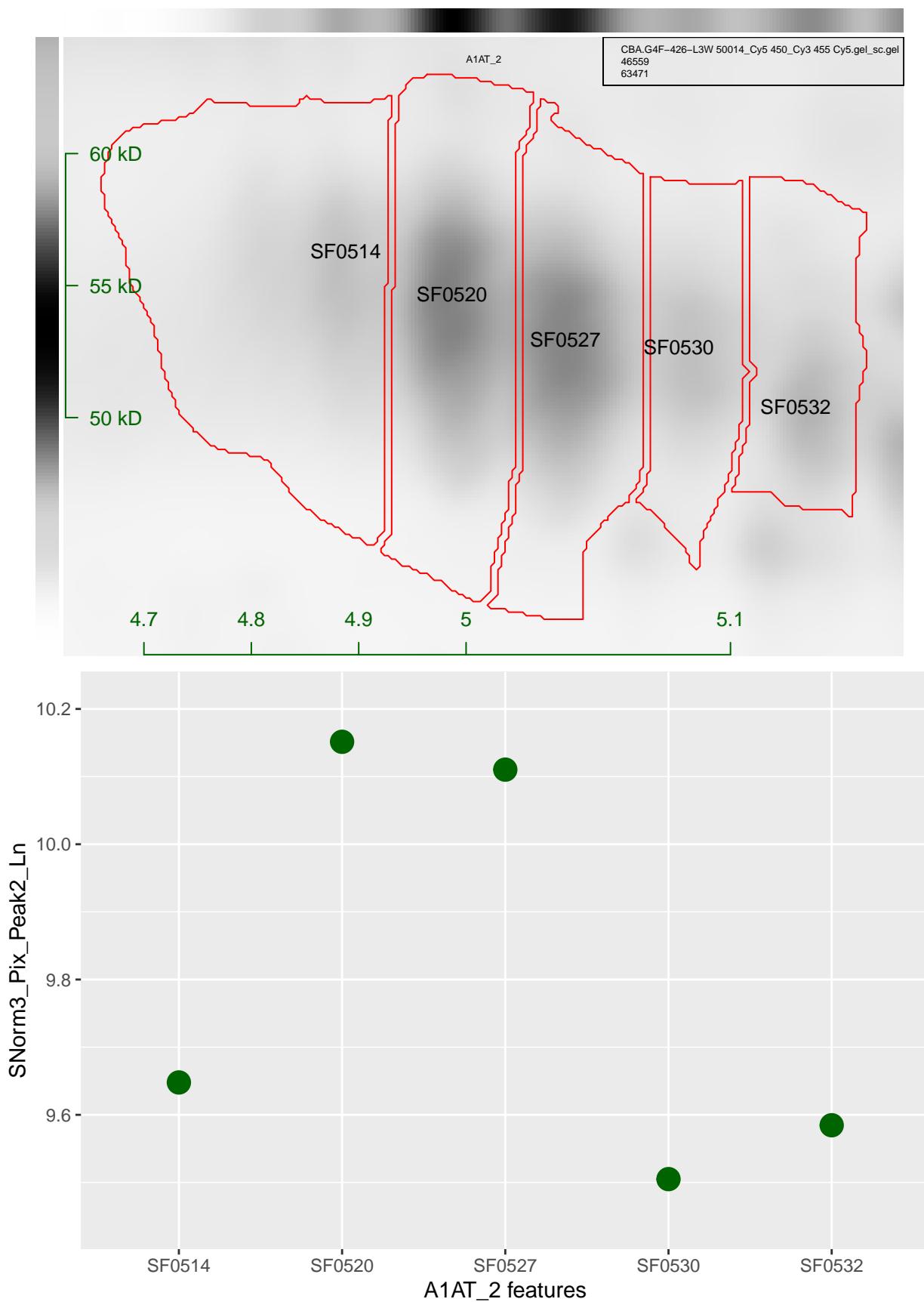
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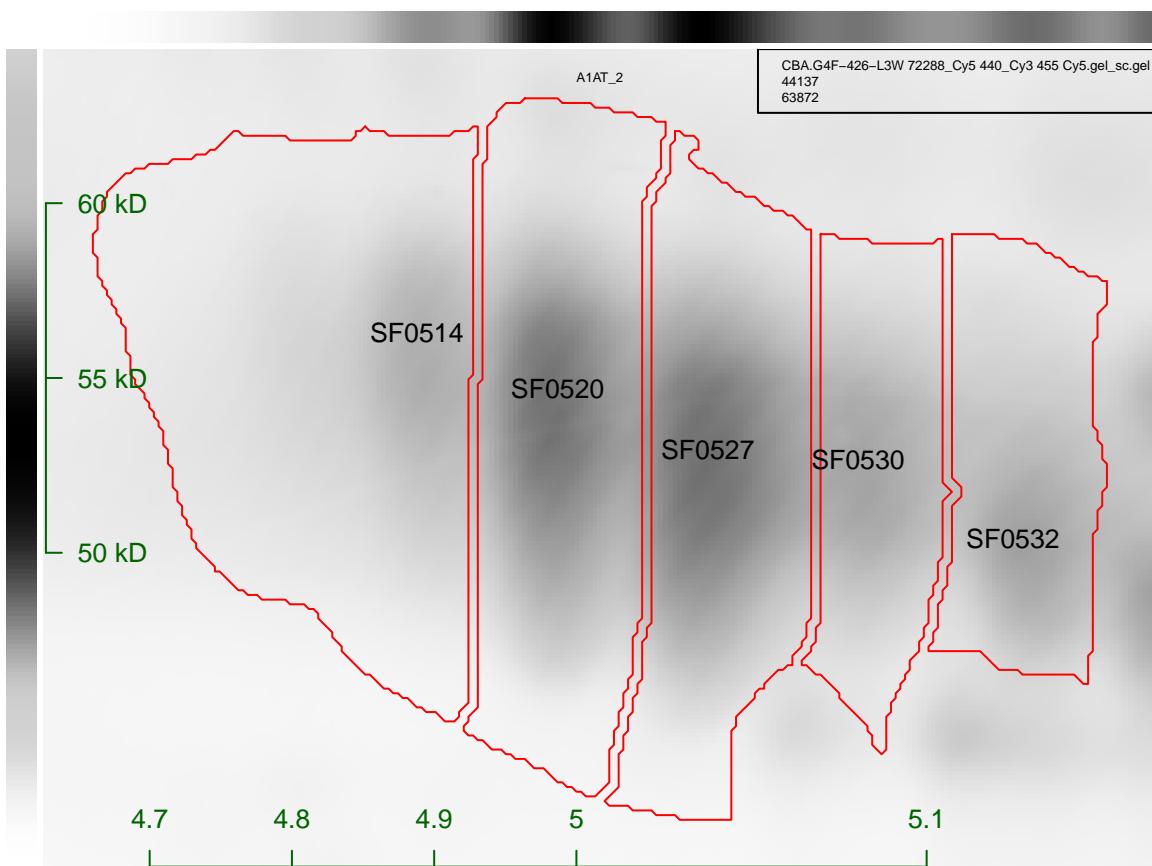
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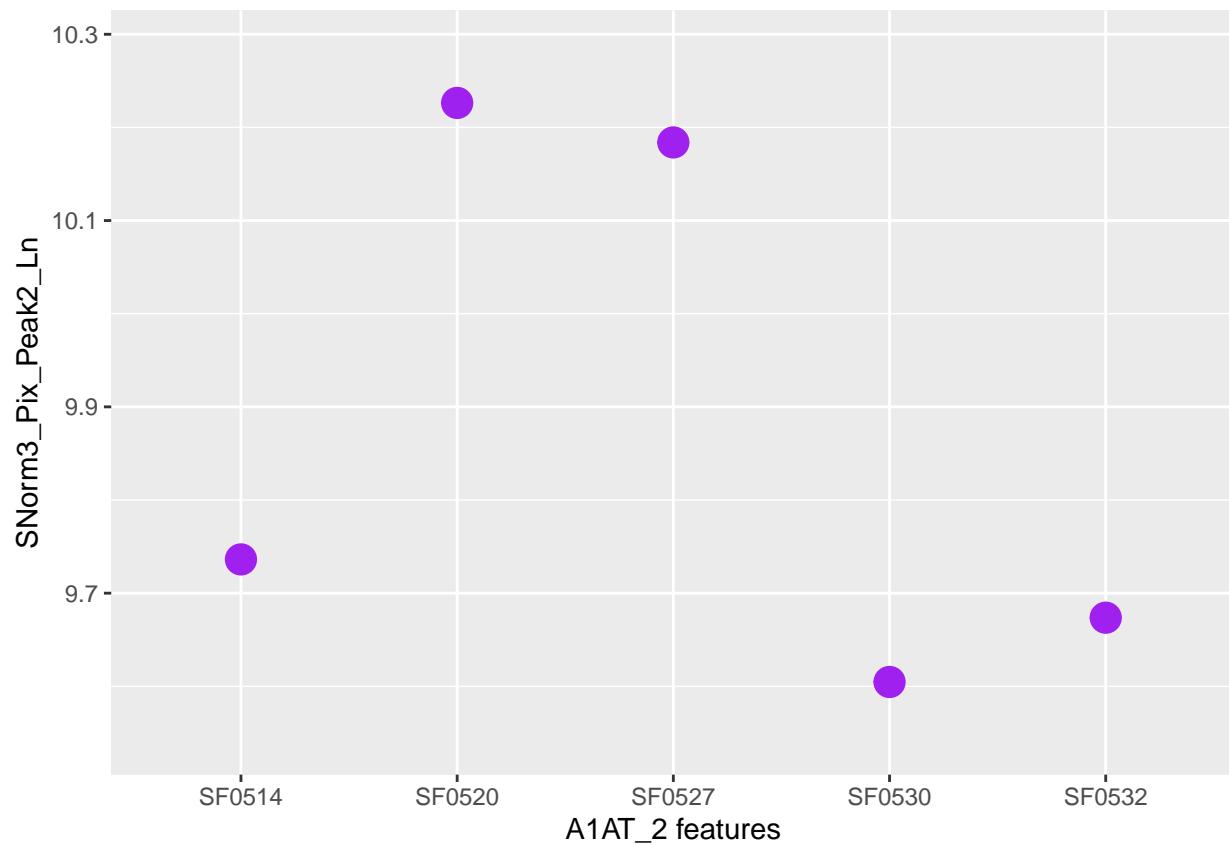


**CBA.G4F-426-L3W A1AT\_2****Replicate 1 : 50014\_Cy5 450\_Cy3 455 Cy5.gel\_sc.gel**

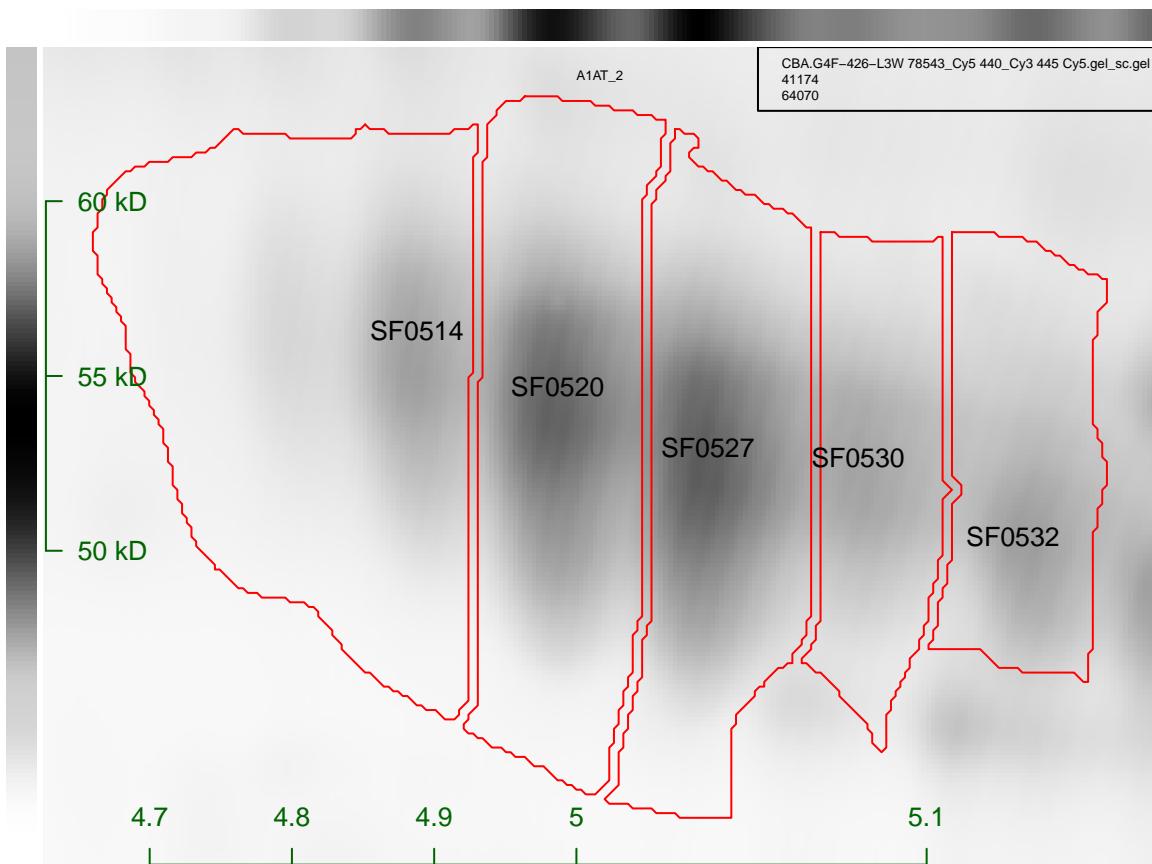


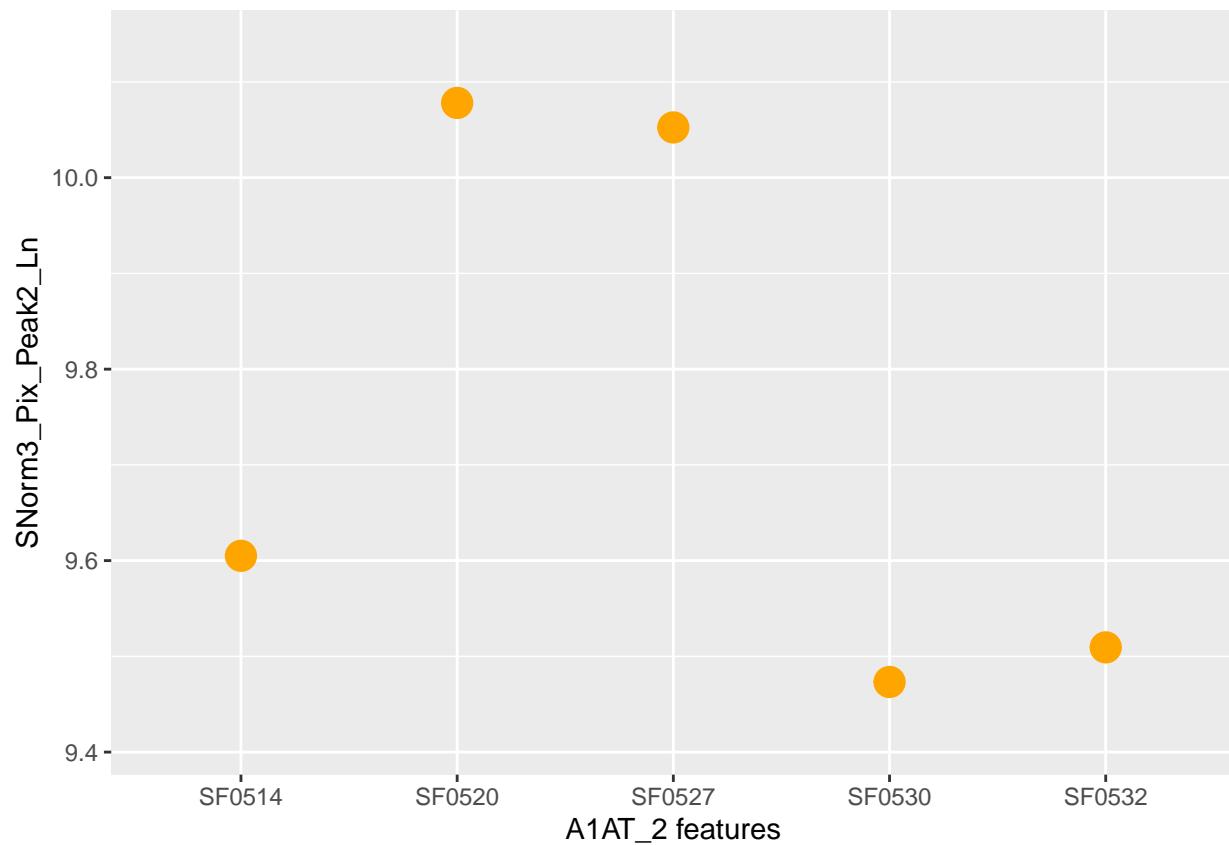
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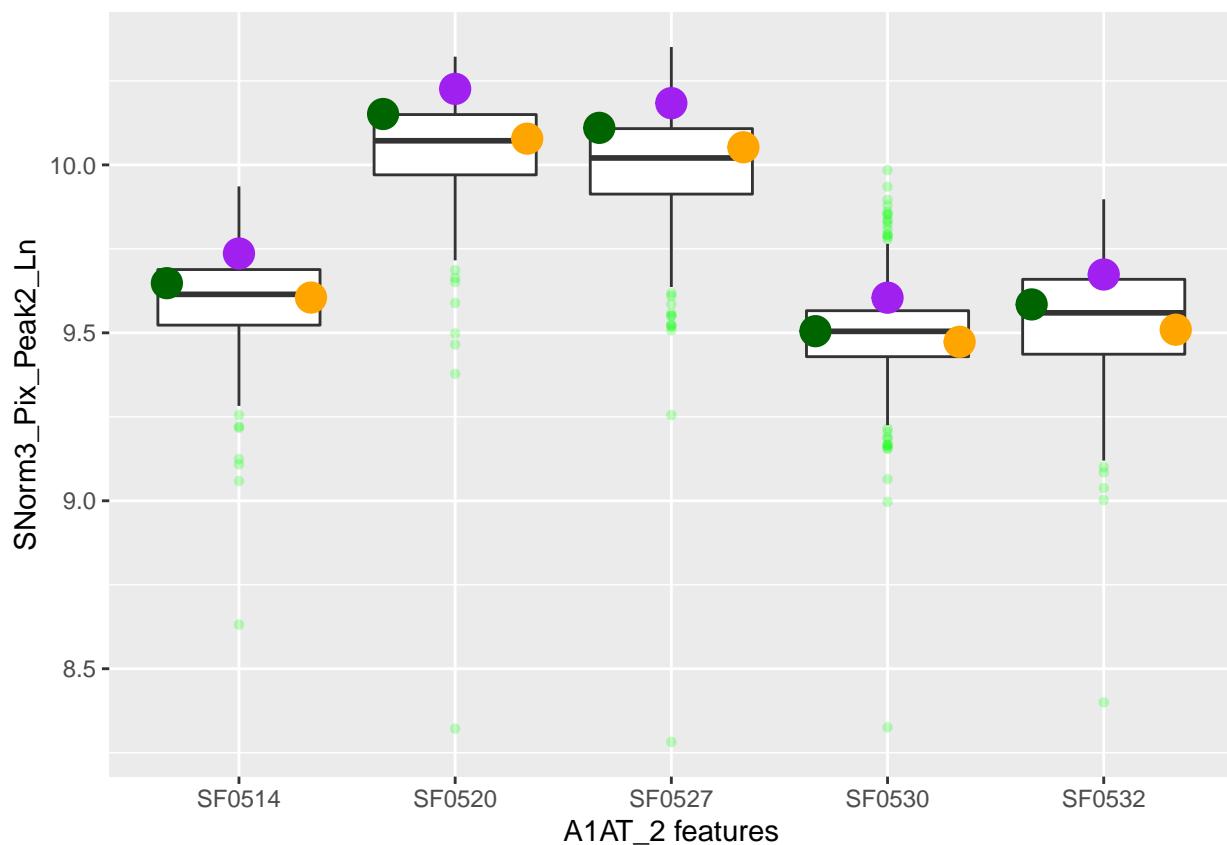




## Replicate 3 : 78543\_Cy5 440\_Cy3 445 Cy5.gel\_sc.gel

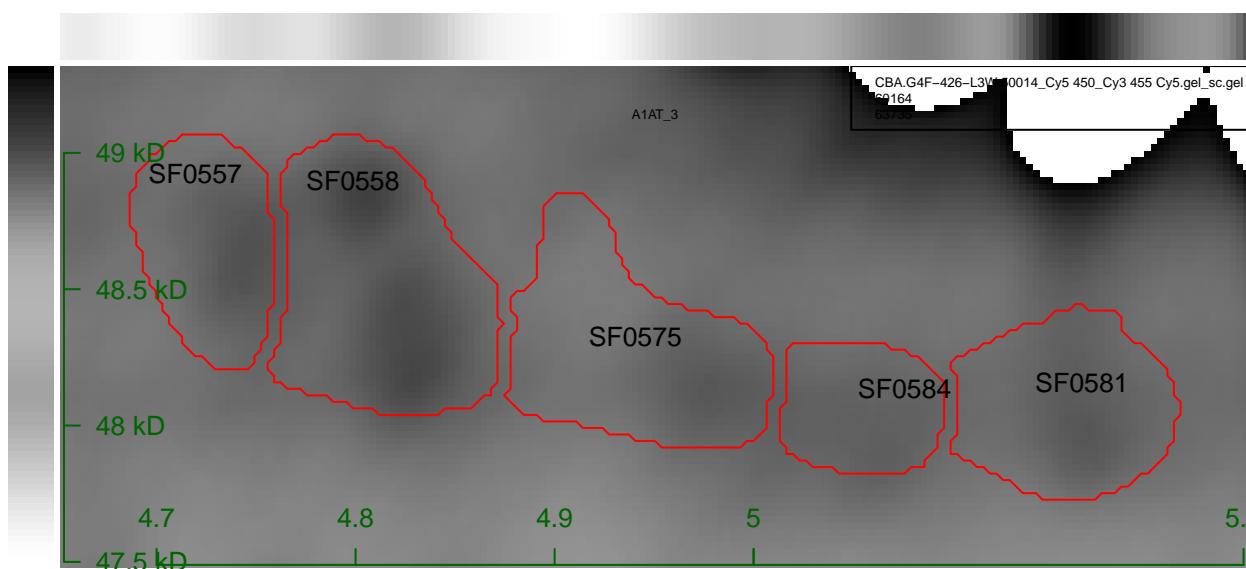


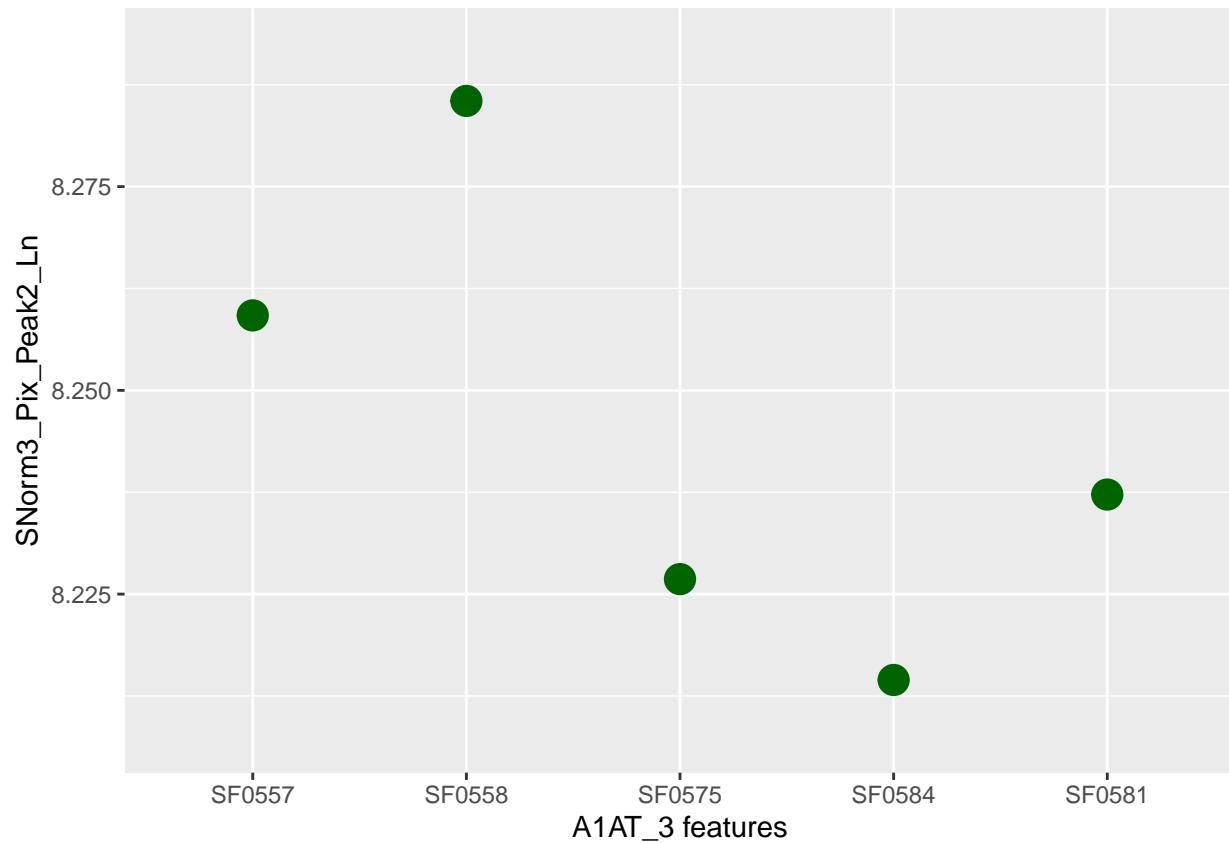




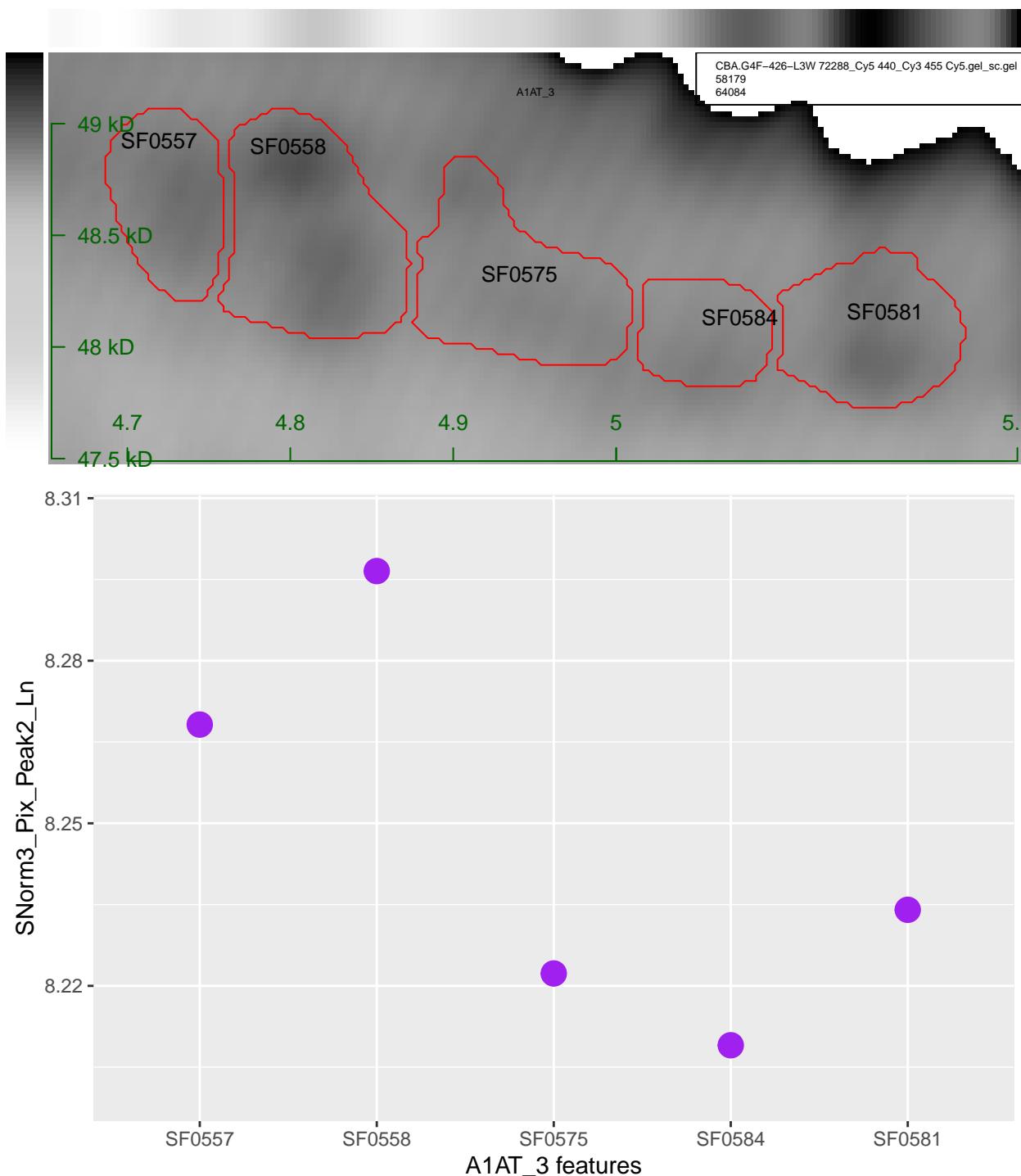
### CBA.G4F-426-L3W A1AT\_3

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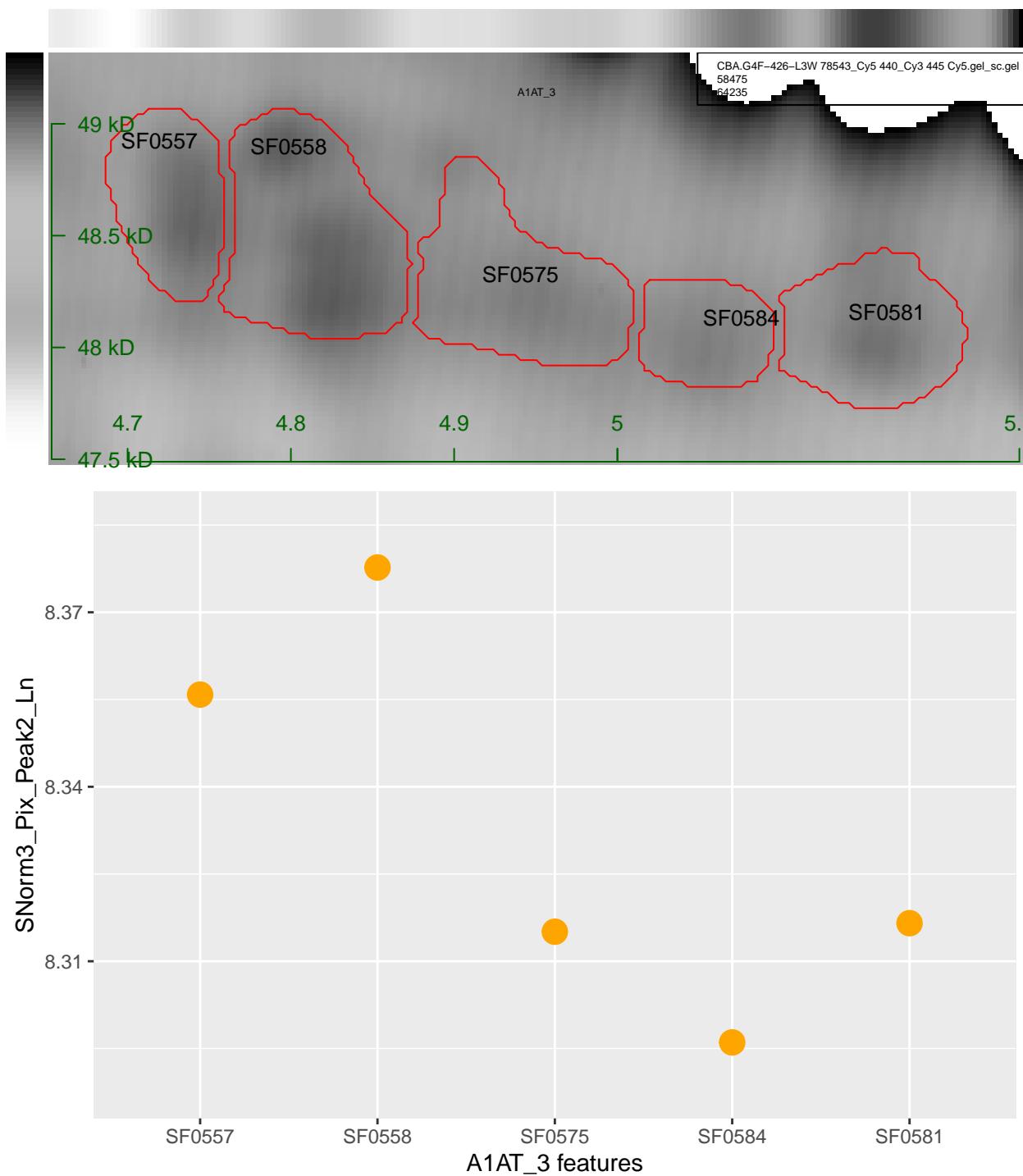


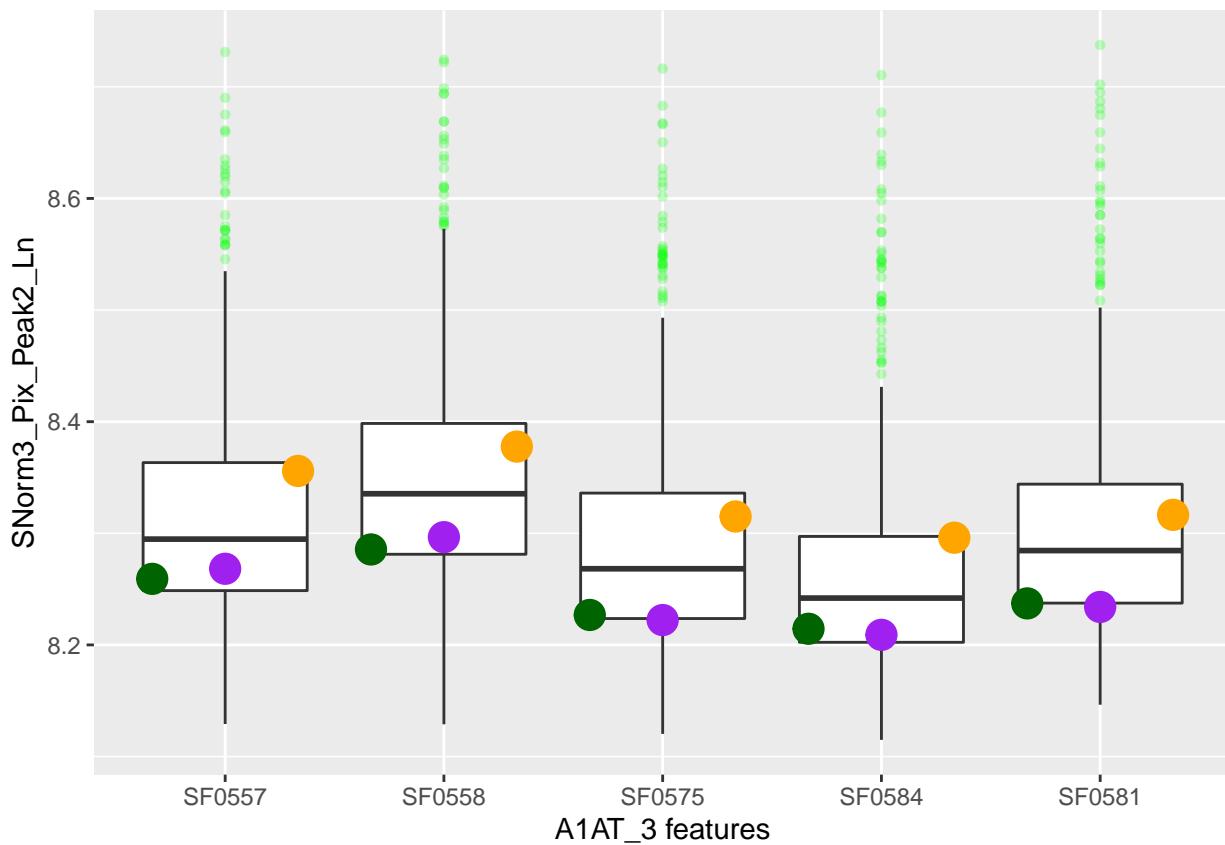


## Replicate 2 : 72288\_Cy5 440\_Cy3 455 Cy5.gel\_sc.gel



## 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

## Apolipoprotein D (P05090)

From: Human plasma protein N-glycosylation

Apolipoprotein D (Apo D), also referred to as thin line polypeptide, is a small glycoprotein of 189 amino acids (with a signal peptide of 20 amino acids), with a molecular weight varying between 19 and 32 kDa depending on its glycosylation [262, 263]. While it shares their name, it does in fact not resemble other apolipoproteins, and shares more homology with the lipocalin protein family [264]. It was originally assimilated to the apolipoprotein family due to its early association with lipid transport. Apo D is mainly synthesized in fibroblasts and to a lesser extent in the liver and intestine, where the other apolipoproteins are usually produced [265]. Its plasma levels are approximately 0.1 mg/mL [266, 267]. The common form of Apo D in plasma is a monomer, although it can also exist as a heterodimer linked to apolipoprotein A-2 via a disulfide bridge.

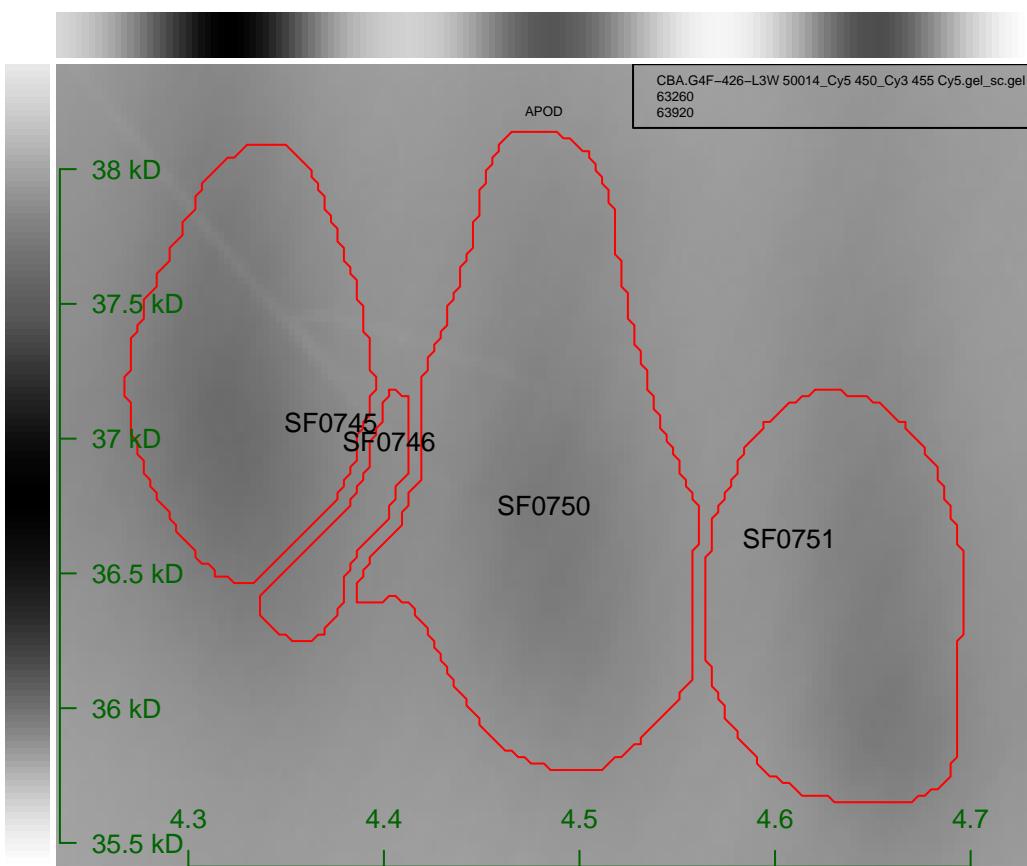
Apo D can form complexes with lecithin cholesterol acyltransferase and is implicated in the transport and transformation of lipids [264, 268–270]. It has been reported to have a potential role in colorectal cancer [265]. In addition, the protein is present at high concentrations in the cyst fluid where its concentration can be 500 times higher than in plasma, which can be associated with an increased risk of breast cancer [271–273]. Apo D has a tendency to accumulate in CSF and peripheral nerves of patients with Alzheimer's disease and other neurodegenerative conditions [274, 275]. A positive correlation between age and Apo D levels has been reported in females, but not in men [276, 277].

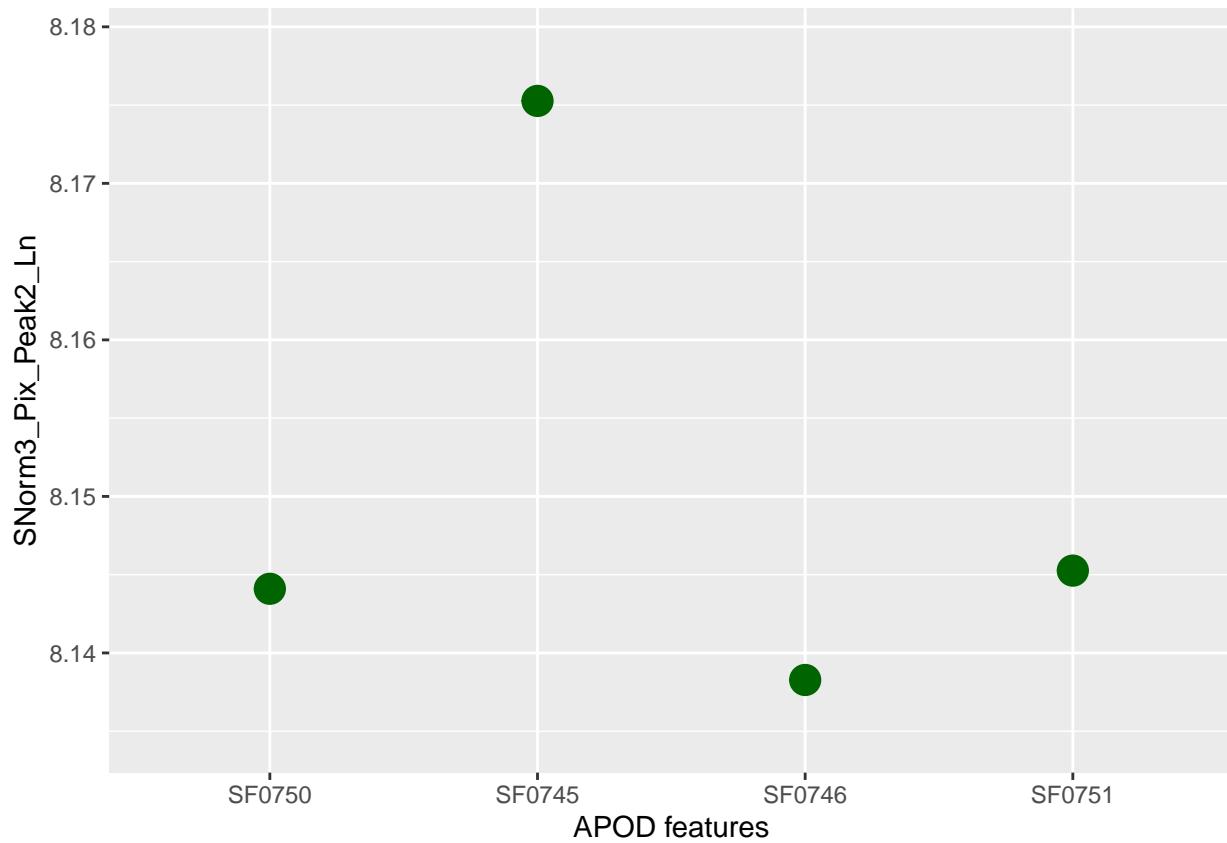
## ## Glycosylation

Two glycosylation sites have been reported and confirmed for Apo D, namely Asn65 and Asn98 [59, 60, 91]. These are mainly occupied by complex type N-glycans ranging from diantennary to tetraantennary structures, with potential elongation of the antennae in the form of N-acetylglucosamine (LacNAc) repeats [91]. LC-MS with exoglycosidase digestion has revealed the most abundant glycoforms per site as well. Asn65 mainly contains nonfucosylated triantennary structures with full sialylation (A3G3S3), less abundant signals including di- and tetraantennary species with high degrees of sialylation (A2G2S2; A4G4S4). Contrarily, Asn98 predominantly contains fucosylated species, also ranging from di- to tetraantennary, here the main signal being diantennary (A2FG2S2) [62, 67, 91, 92]. Treatment with  $\beta$ -galactosidase failed to trim one antenna of its galactosylation, strongly suggesting the presence of the antennary fucosylation, known to prevent this digestion [91, 93]. Studies have shown the implication of Apo D in conditions like Alzheimer's disease but no information about the role of glycosylation has been reported yet.

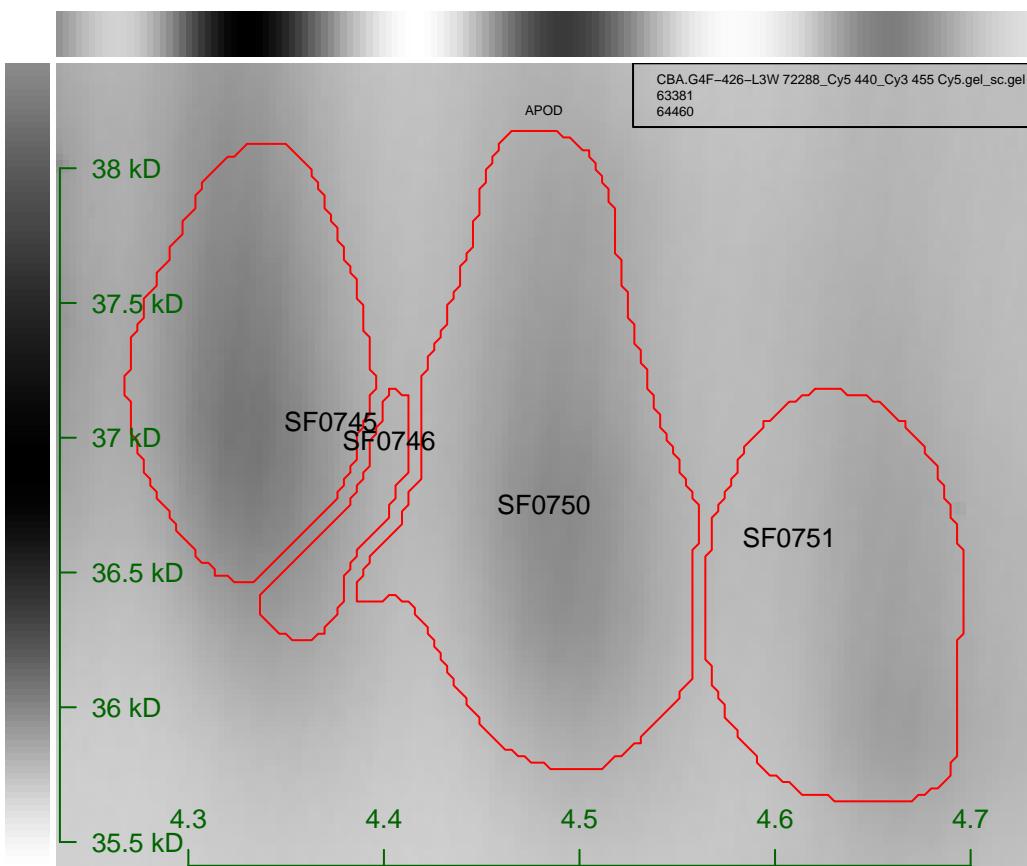
### CBA.G4F-426-L3W APOD

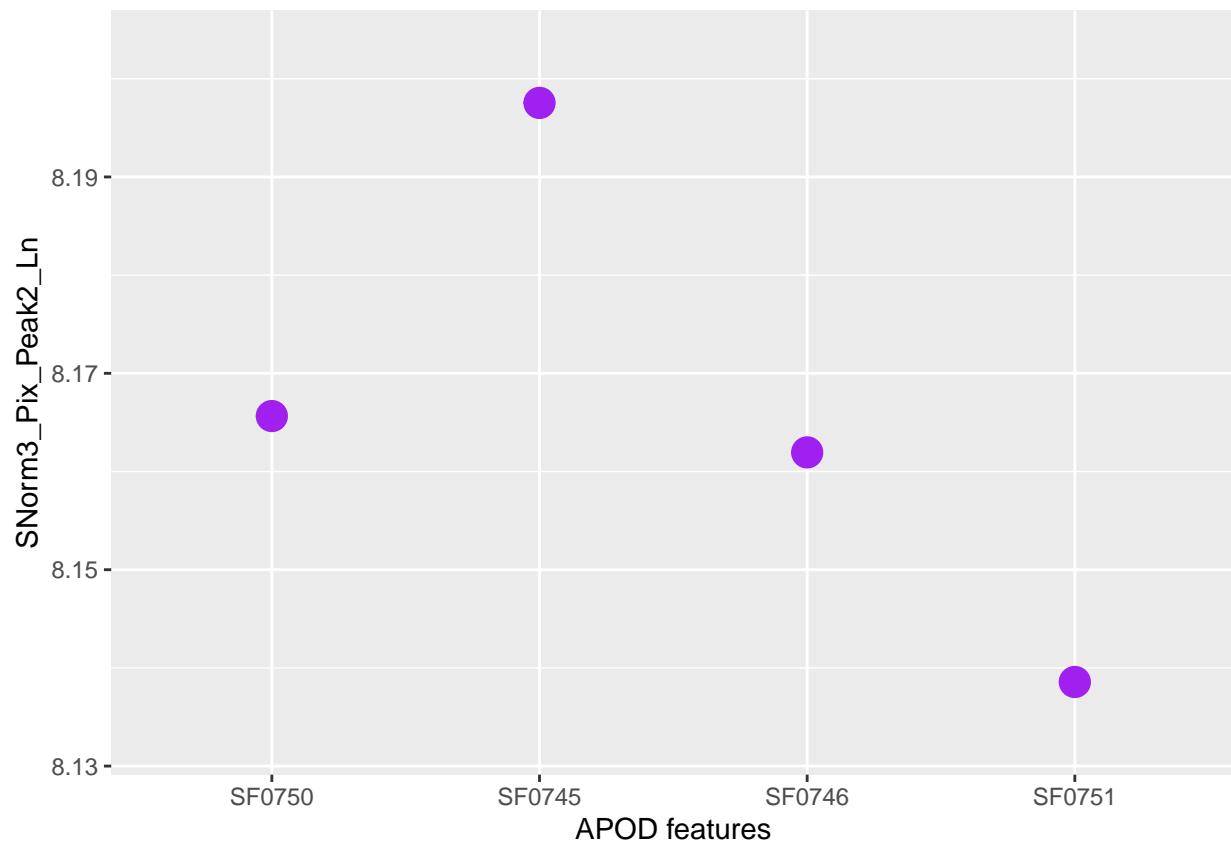
#### Replicate 1 : 50014\_Cy5 450\_Cy3 455 Cy5.gel\_sc.gel



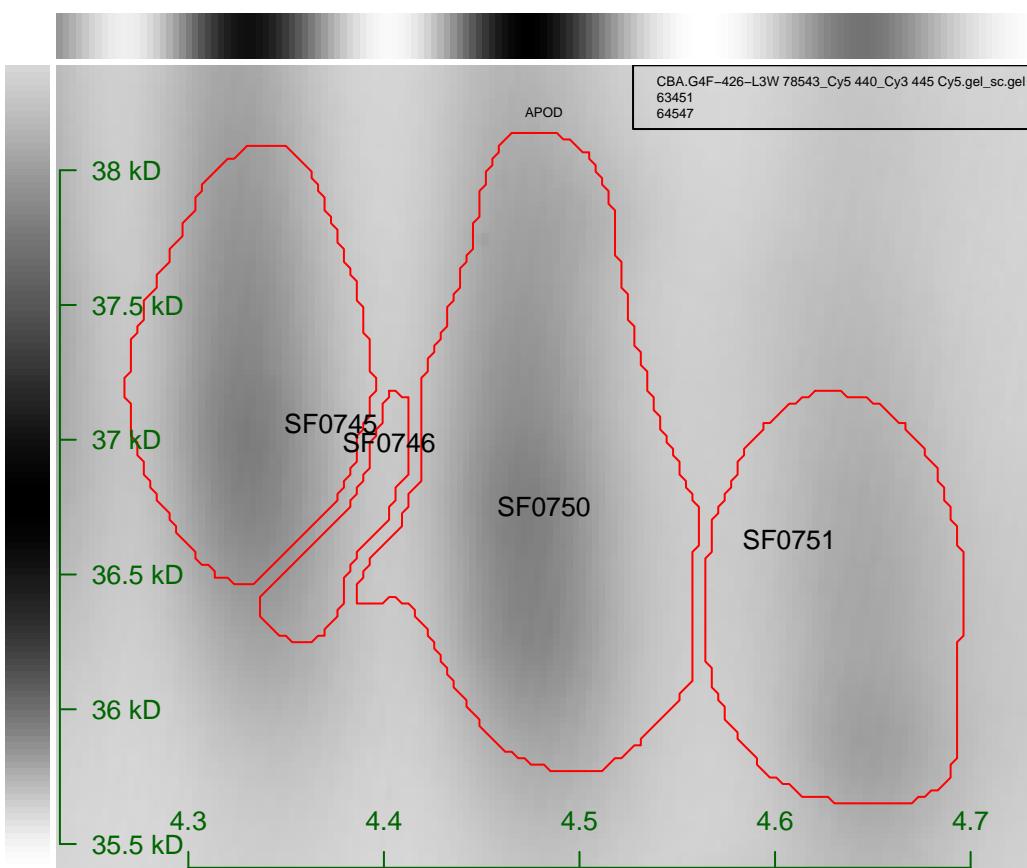


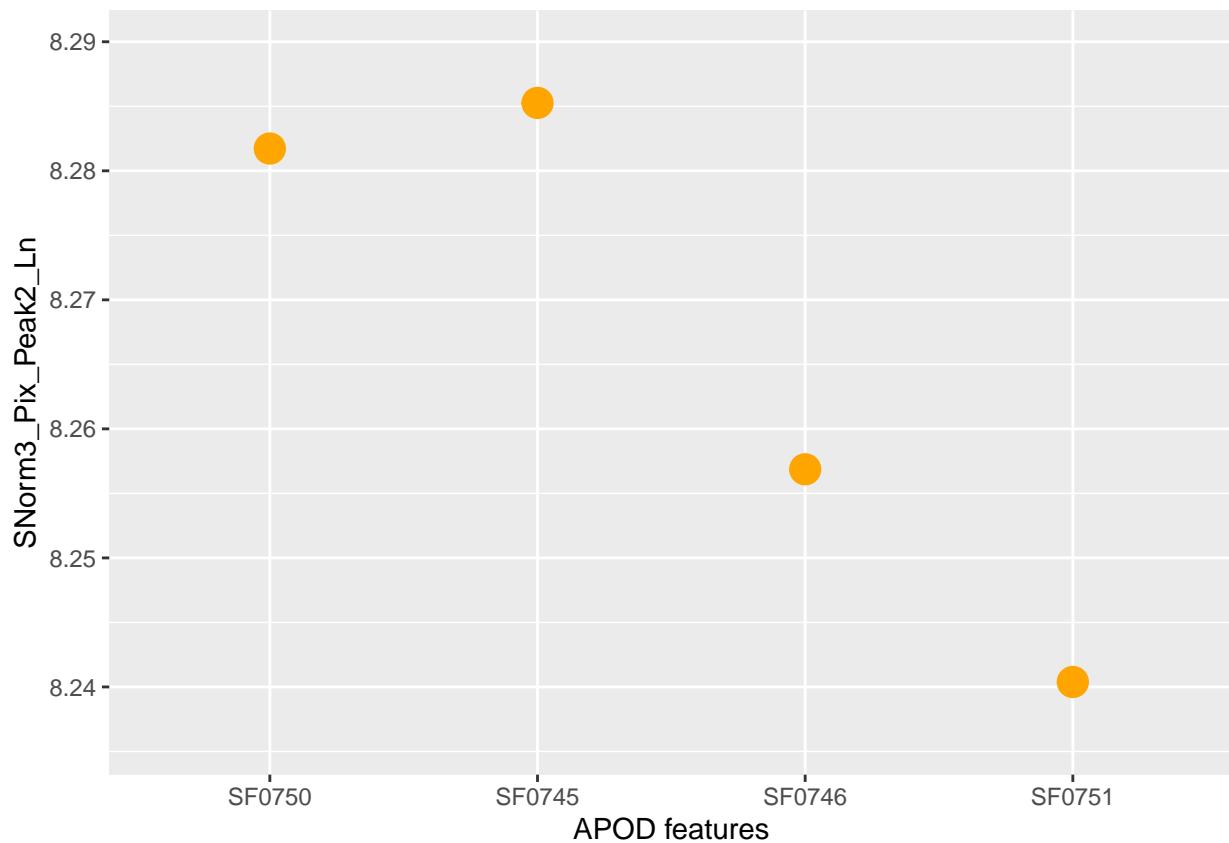
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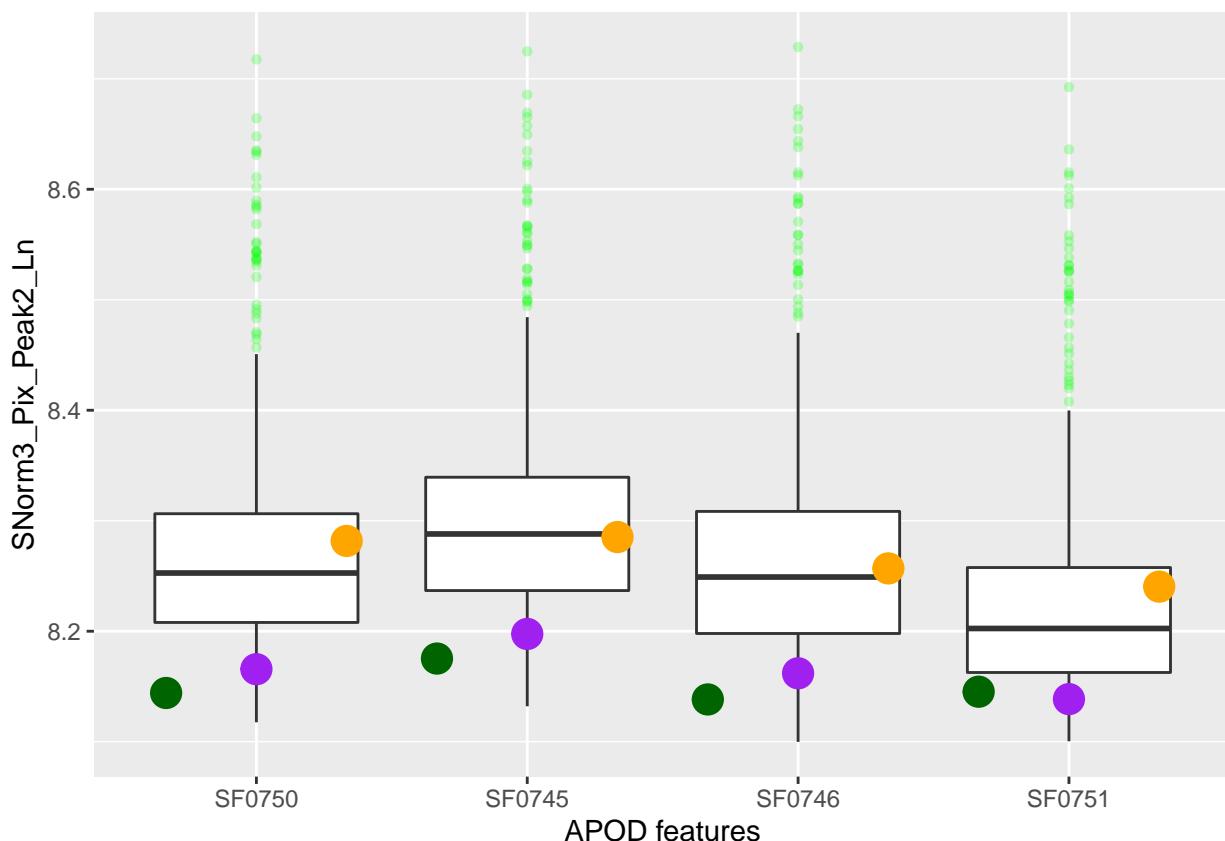




## Replicate 3 : 78543\_Cy5 440\_Cy3 445 Cy5.gel\_sc.gel







	SF0750	SF0745	SF0746	SF0751
50014_Cy5 450_Cy3 455 Cy5	8.144099	8.175266	8.138273	8.145260
72288_Cy5 440_Cy3 455 Cy5	8.165648	8.197539	8.161946	8.138565
78543_Cy5 440_Cy3 445 Cy5	8.281724	8.285261	8.256867	8.240385

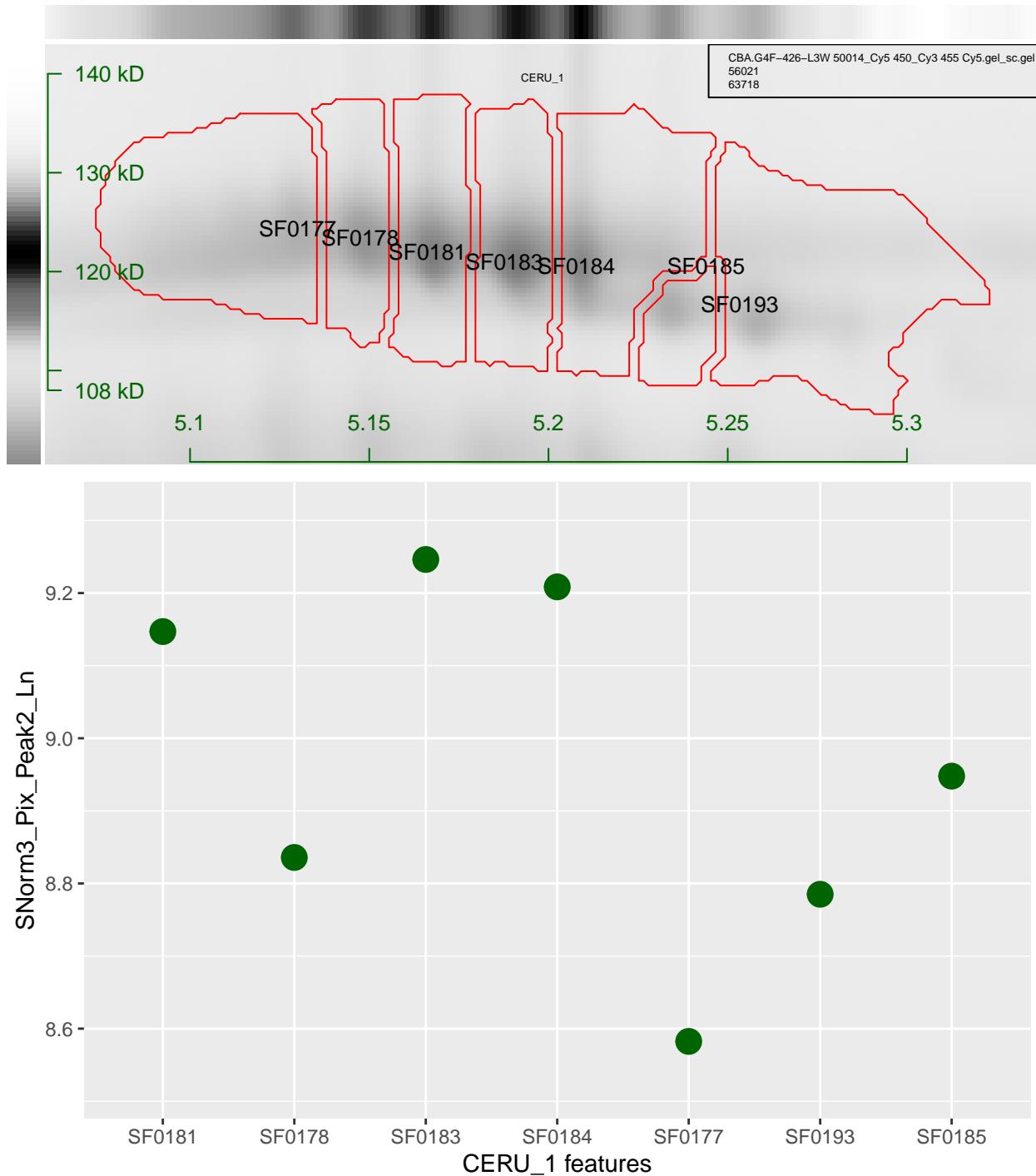
## Ceruloplasmin (P00450)

From: Human plasma protein N-glycosylation

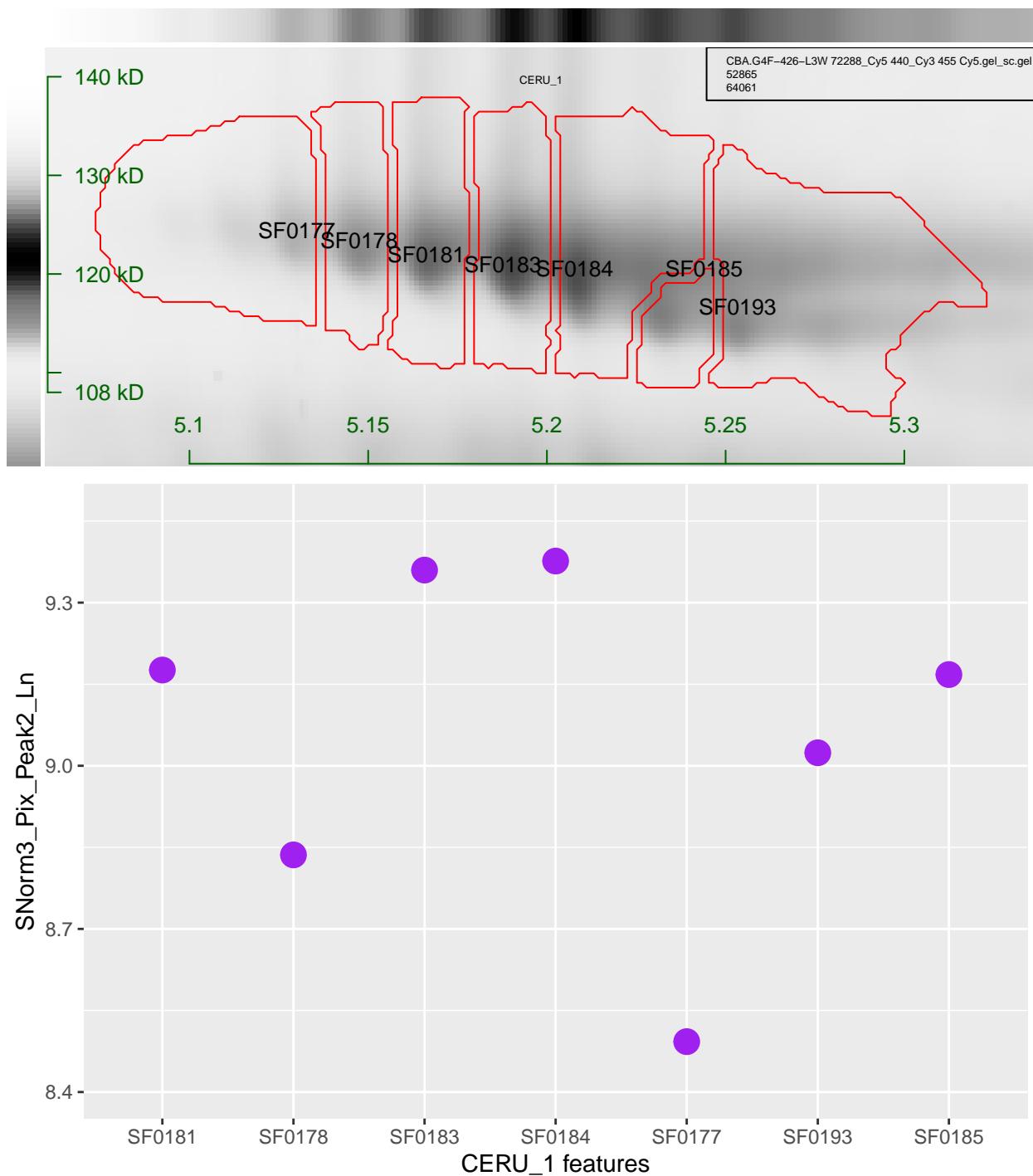
Ceruloplasmin (CP), also called ferroxidase, is a 132 kDa (120 kDa without glycosylation) 1065 amino acid (19 of which are signal peptide) glycoprotein synthesized by the liver [103]. It consists of a single polypeptide chain, and belongs to the multicopper oxidase family [103]. Concentrations for CP range from 0.15 to 0.96 mg/mL with a mean of 0.36 mg/mL, while elevated levels have been reported upon inflammatory stimulation [34, 288, 289]. CP can bind six to seven atoms of copper, in this manner containing and transporting 95 % of the copper found in plasma. The main function of the protein, however, is in iron metabolism. CP has ferroxidase activity oxidizing Fe2+ to Fe3+ without releasing radical oxygen species, while also facilitating iron transport across the cell membrane.

**CBA.G4F-426-L3W CERU\_1**

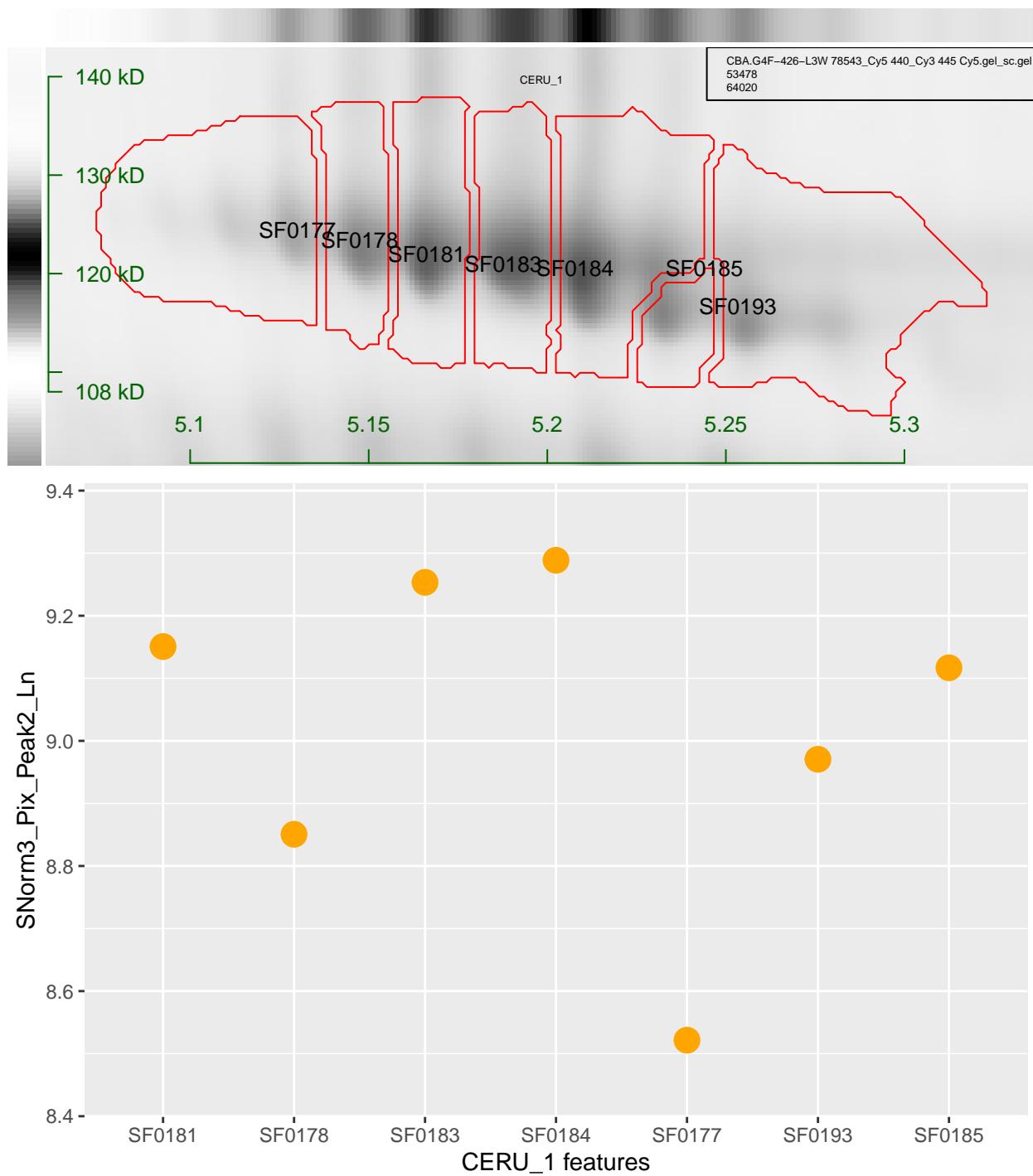
**Replicate 1 : 50014\_Cy5 450\_Cy3 455 Cy5.gel\_sc.gel**

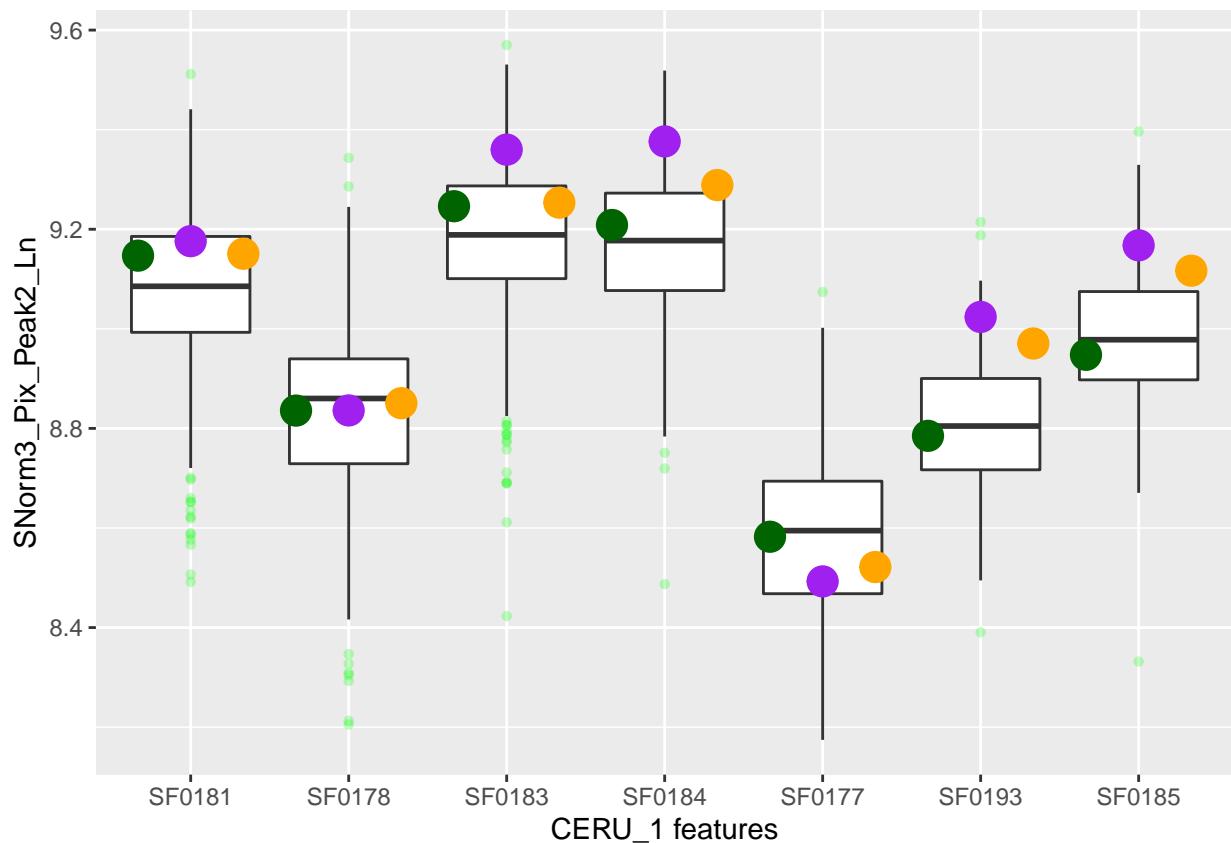


## Replicate 2 : 72288\_Cy5 440\_Cy3 455 Cy5.gel\_sc.gel



## Replicate 3 : 78543\_Cy5 440\_Cy3 445 Cy5.gel\_sc.gel



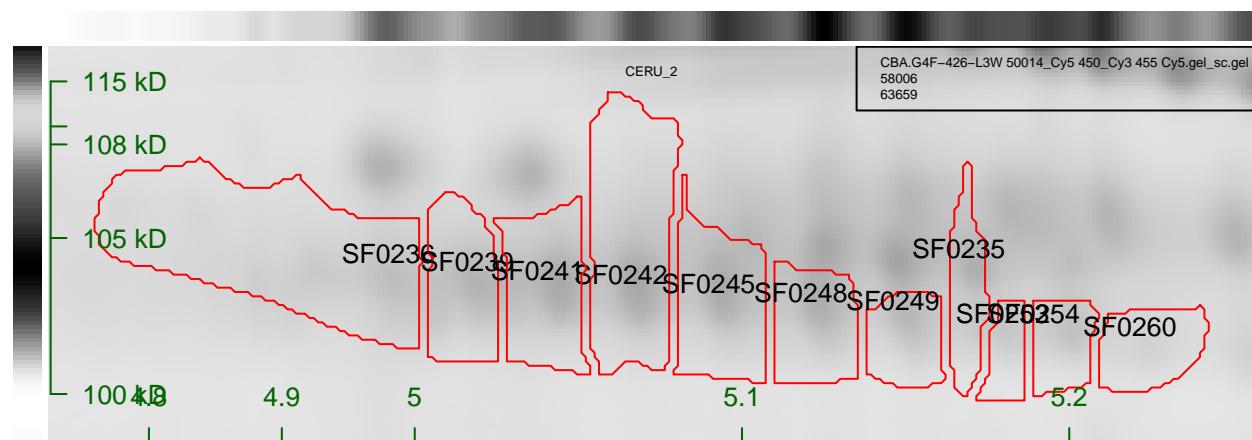


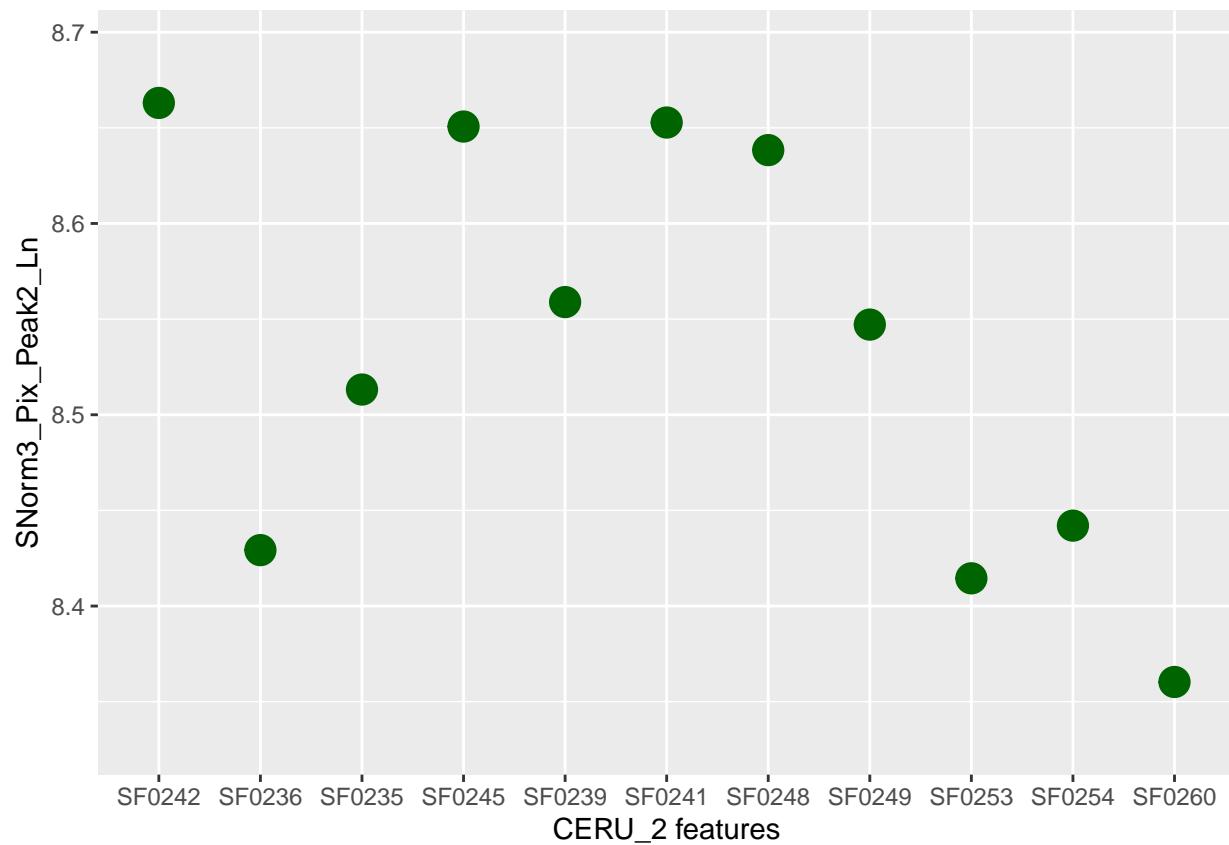
	SF0181	SF0178	SF0183	SF0184	SF0177	SF0193
50014_Cy5 450_Cy3 455 Cy5	9.146975	8.835792	9.246287	9.208539	8.582419	8.785081
72288_Cy5 440_Cy3 455 Cy5	9.176059	8.836083	9.359880	9.376448	8.492696	9.023890
78543_Cy5 440_Cy3 445 Cy5	9.150803	8.850661	9.253400	9.288689	8.521584	8.970559

	SF0185
50014_Cy5 450_Cy3 455 Cy5	8.947806
72288_Cy5 440_Cy3 455 Cy5	9.167746
78543_Cy5 440_Cy3 445 Cy5	9.116799

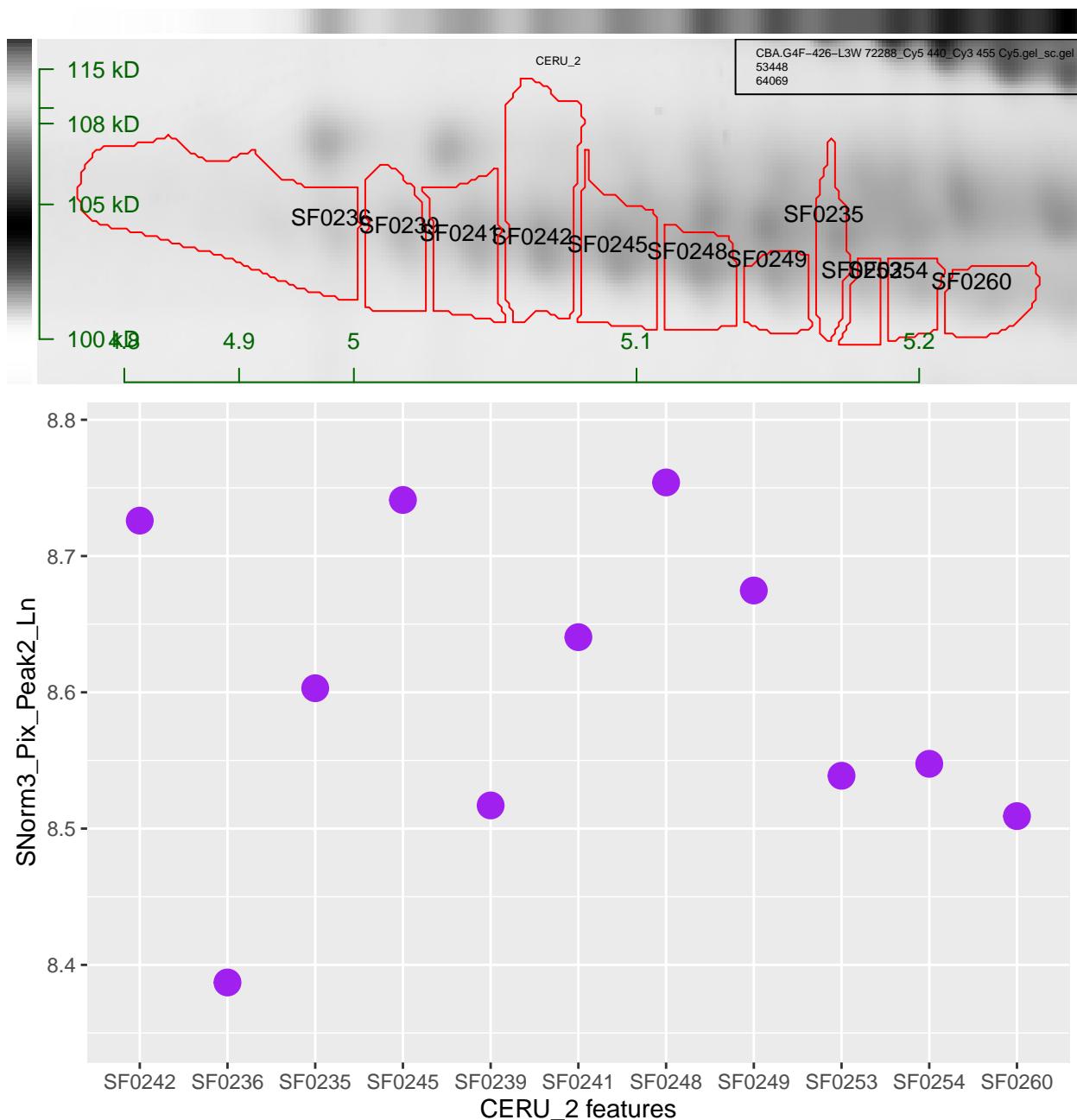
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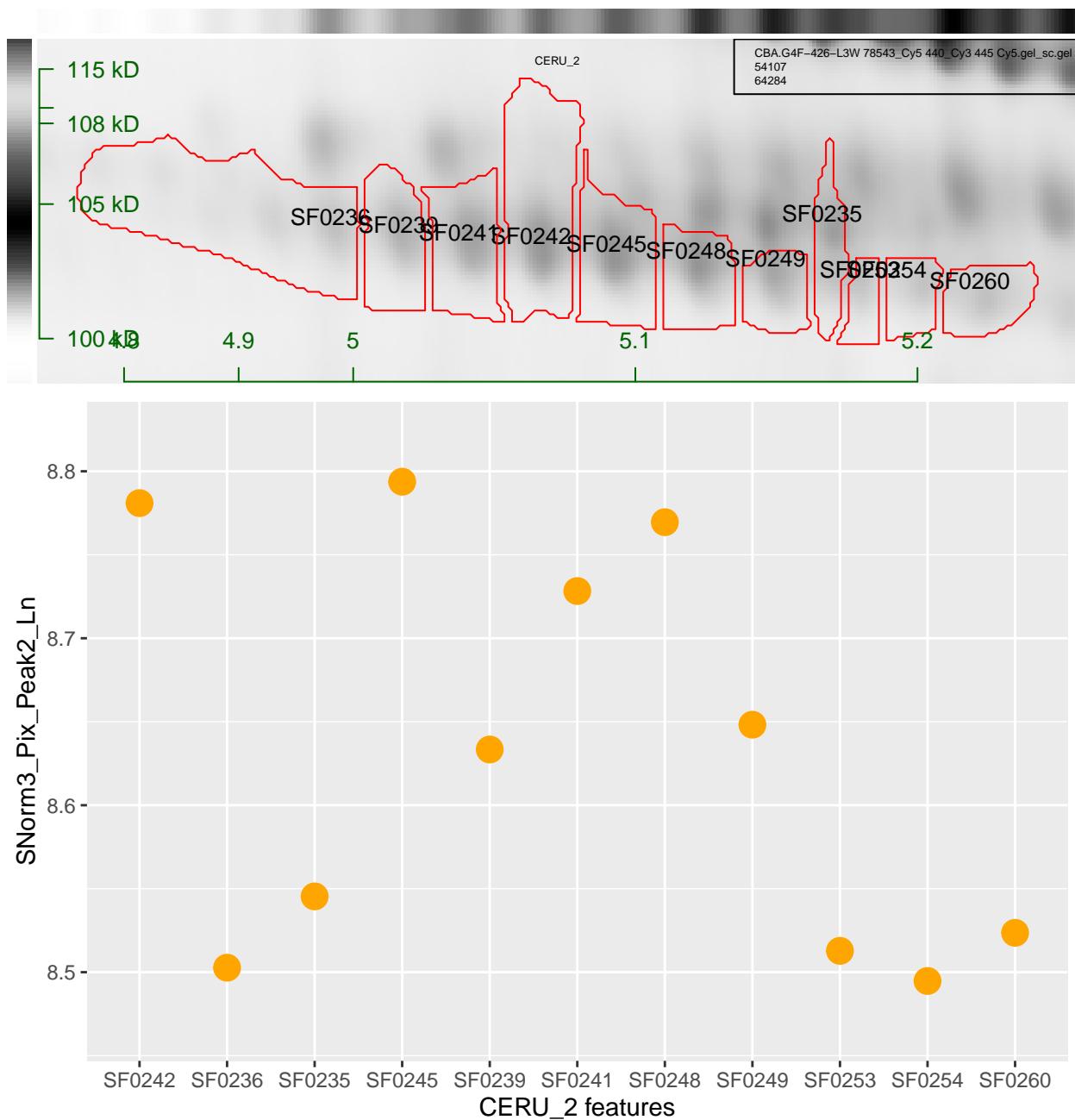


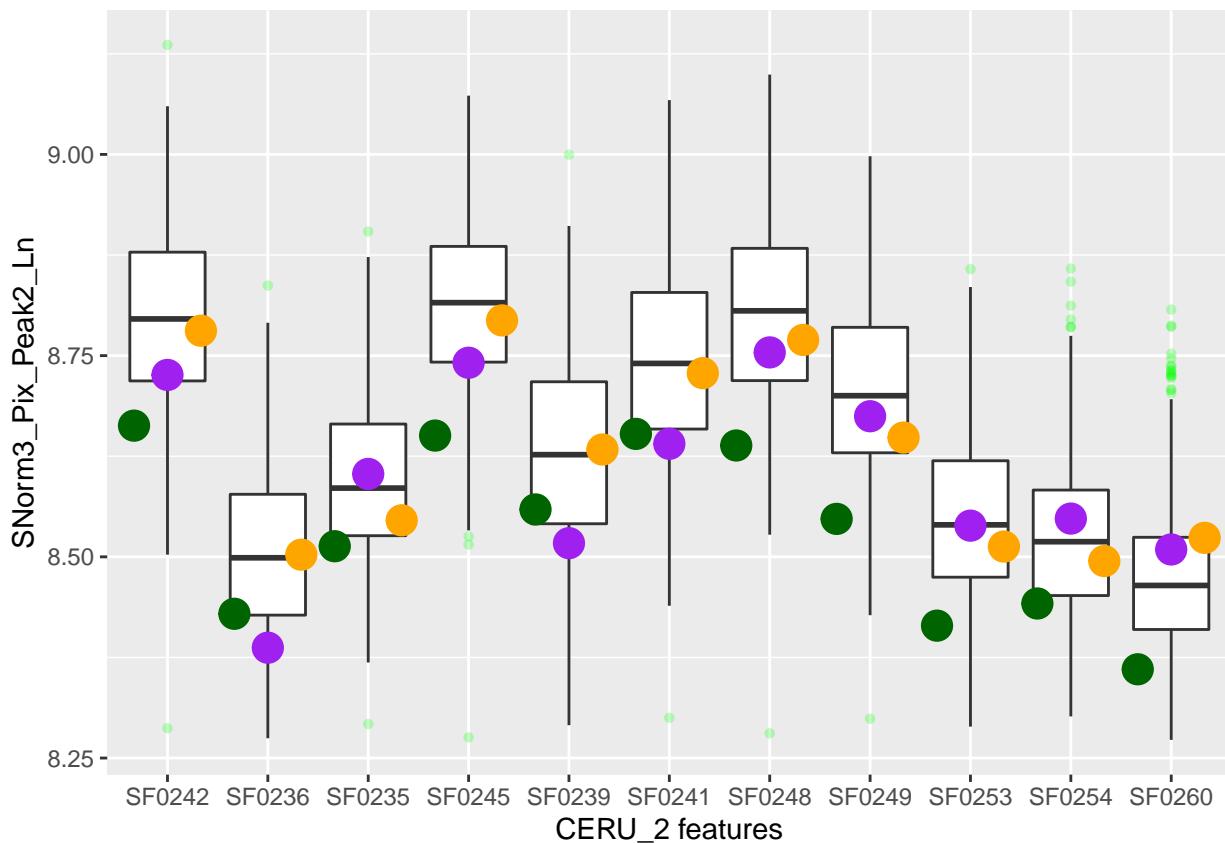


## Replicate 2 : 72288\_Cy5 440\_Cy3 455 Cy5.gel\_sc.gel



## Replicate 3 : 78543\_Cy5 440\_Cy3 445 Cy5.gel\_sc.gel





	SF0242	SF0236	SF0235	SF0245	SF0239	SF0241
50014_Cy5 450_Cy3 455 Cy5	8.663024	8.429236	8.513185	8.650675	8.558911	8.652772
72288_Cy5 440_Cy3 455 Cy5	8.725994	8.387085	8.603004	8.741136	8.516993	8.640472
78543_Cy5 440_Cy3 445 Cy5	8.780941	8.502688	8.545392	8.793764	8.633375	8.728264
	SF0248	SF0249	SF0253	SF0254	SF0260	
50014_Cy5 450_Cy3 455 Cy5	8.638348	8.547140	8.414496	8.442039	8.360305	
72288_Cy5 440_Cy3 455 Cy5	8.754003	8.674710	8.538759	8.547528	8.509161	
78543_Cy5 440_Cy3 445 Cy5	8.769507	8.648222	8.512783	8.494743	8.523573	

## Fibrinogen (P02671; P02675; P02679)

From: Human plasma protein N-glycosylation

Fibrinogen is a 340 kDa glycoprotein that is synthesized in the liver by hepatocytes, and plays a key role in blood clotting [290, 291]. The protein consists of two sets of three different polypeptide chains named the α-chain (610 amino acids), β-chain (461 amino acids), and <U+03B3>-chain (411 amino acids), arranged in a  $\alpha_2\beta_2<U+03B3>_2$  hexamer linked by disulfide bonds [106, 292, 293]. In plasma, fibrinogen is typically found at concentrations of 2–6 mg/mL with a mean of 3 mg/mL, with women having slightly higher levels, and it is also present in platelets, lymph nodes, and interstitial fluid [106, 293–296]. Fibrinogen is cleaved by thrombin into fibrin, one of the essential components of blood clots after injury [106, 291, 297]. Furthermore, it acts as a cofactor in platelet aggregation, assists rebuilding of epithelium, and can protect against infections in interferon <U+03B3> (IFN<U+03B3>)-mediated hemorrhage [106, 298, 299]. In addition, the protein can facilitate the immune response via the innate and T-cell pathways [300–303].

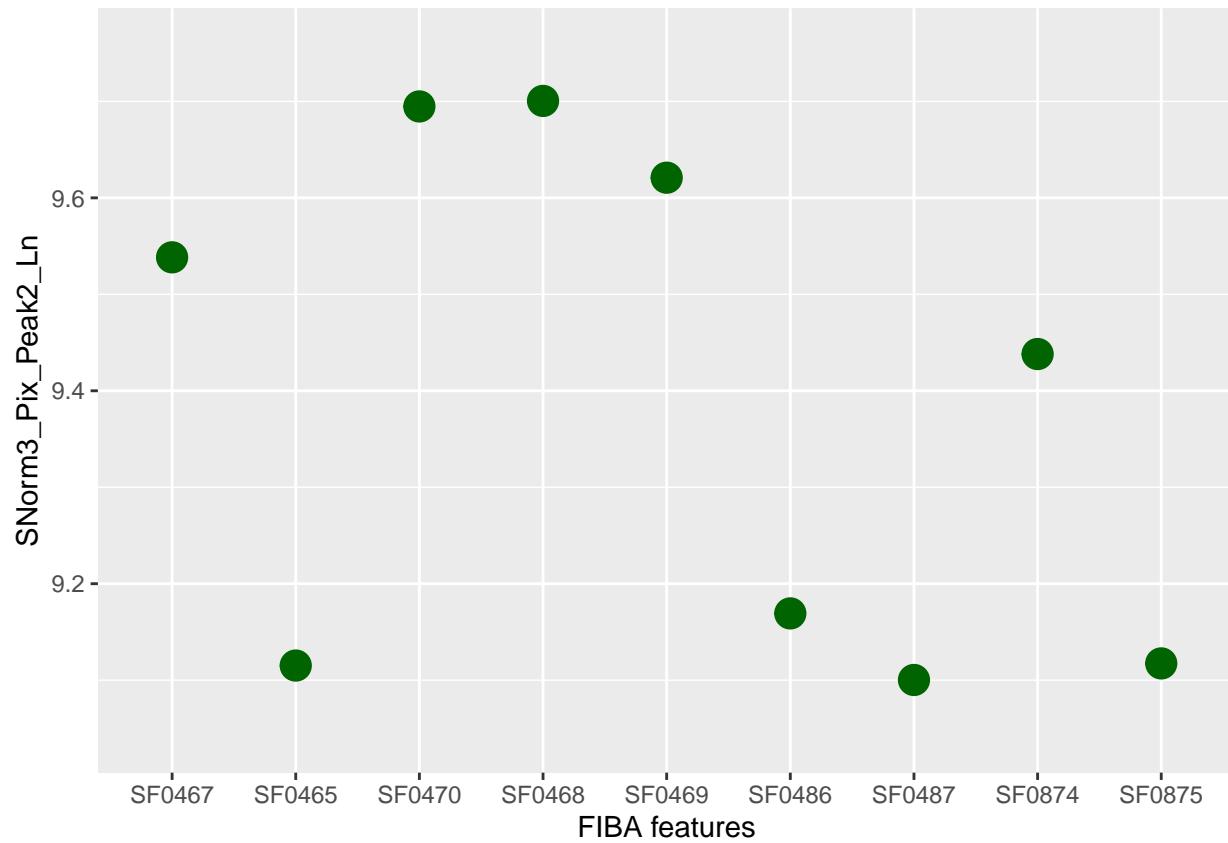
## Glycosylation

The  $\alpha$ -chain of fibrinogen is not N-glycosylated, even though it harbors two potential N-glycosylation sites at Asn453 and Asn686. The  $\beta$ - and <U+03B3>-chain are N-glycosylated at Asn394 and Asn78, respectively [106–108]. By MALDI-TOF-MS and HPLC with exoglycosidase digestion, the predominant glycan structures present on these chains were found to be A2G2S1 (53 %) and A2G2S2 (33 %). Sialic acids are mainly  $\alpha$ 2-6-linked, but a degree of  $\alpha$ 2-3-linkage has been reported as well depending on the source or analytical method [109, 110]. Bisecting N-acetylglucosamine and core fucosylation are found in minor quantities [110]. Comparisons between plasma and serum N-glycan profiles revealed that fibrinogen could contribute for 22 % to the total intensity of the diantennary monosialylated structures (A2G2S1) [110]. Site-specific analysis showed diantennary glycans with zero, one or two sialic acids on Asn394 ( $\beta$ -chain) and Asn78 (<U+03B3>-chain) [107]. The glycosylation sites have been confirmed in studies at the level of deglycosylated glycopeptides, showing occupancy of Asn394 of the  $\beta$ -chain and Asn78 of the <U+03B3>-chain, and surprisingly on the  $\alpha$ -chain Asn686 as well [59, 60, 70, 108]. The  $\beta$ -chain glycosylation site has furthermore been observed in a core-fucose targeted study [67]. In addition to N-glycosylation, all fibrinogen chains may carry O-glycans [107]. The general degree of sialylation may be influencing the solubility of fibrinogen, and thereby play a crucial role in blood clotting processes resulting in different fiber structures. [111–115]. In the Asahi mutant of the <U+03B3>-chain, Asn334 has been reported to contain an additional N-glycosylation site [116]. Patients exhibiting the Asahi variant of fibrinogen displayed abnormally long blood clotting time, suggesting that the effect induced by that extra glycosylation site disturbs the fibrin polymerization process [116, 117].

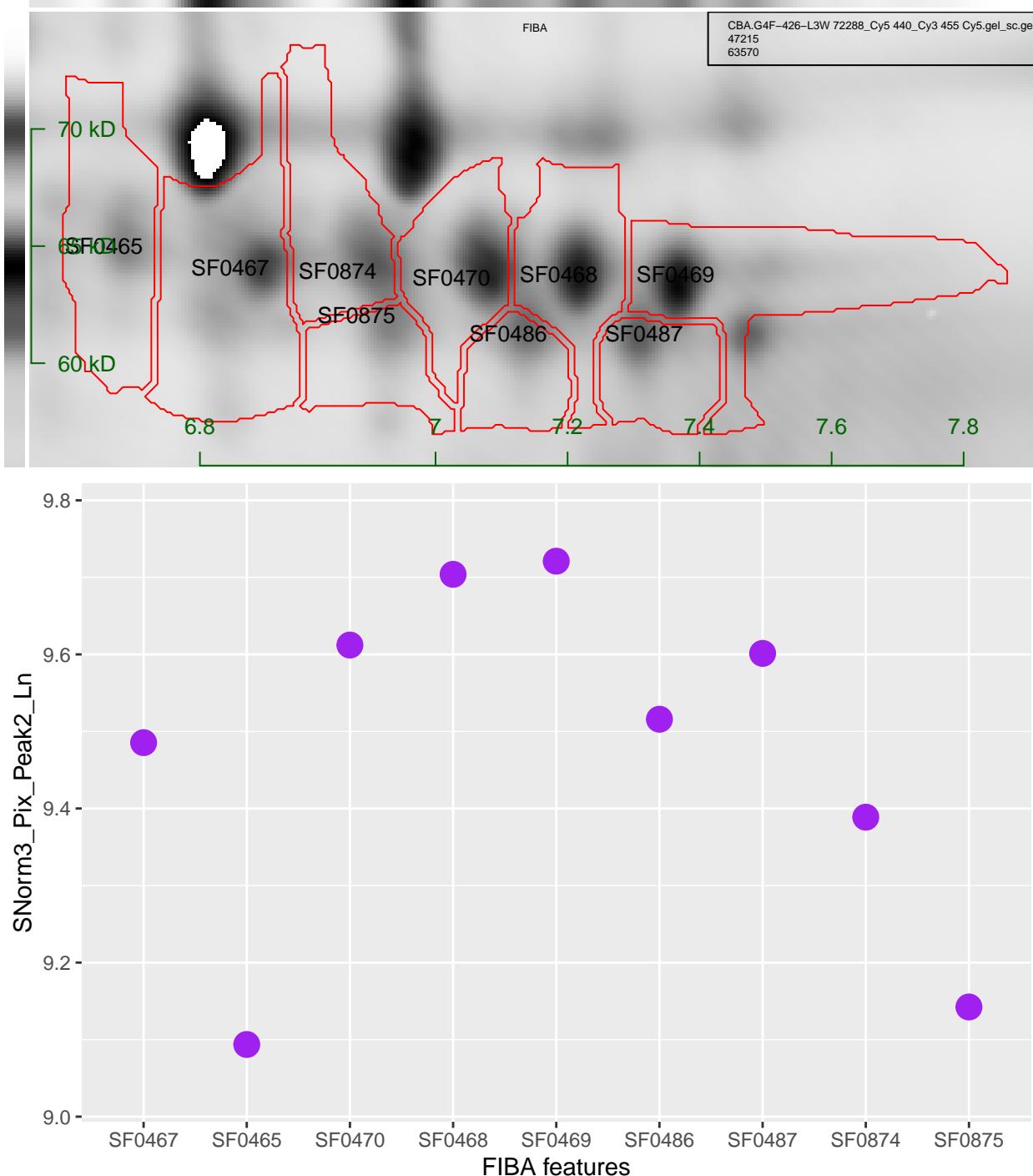
#### CBA.G4F-426-L3W FIBA

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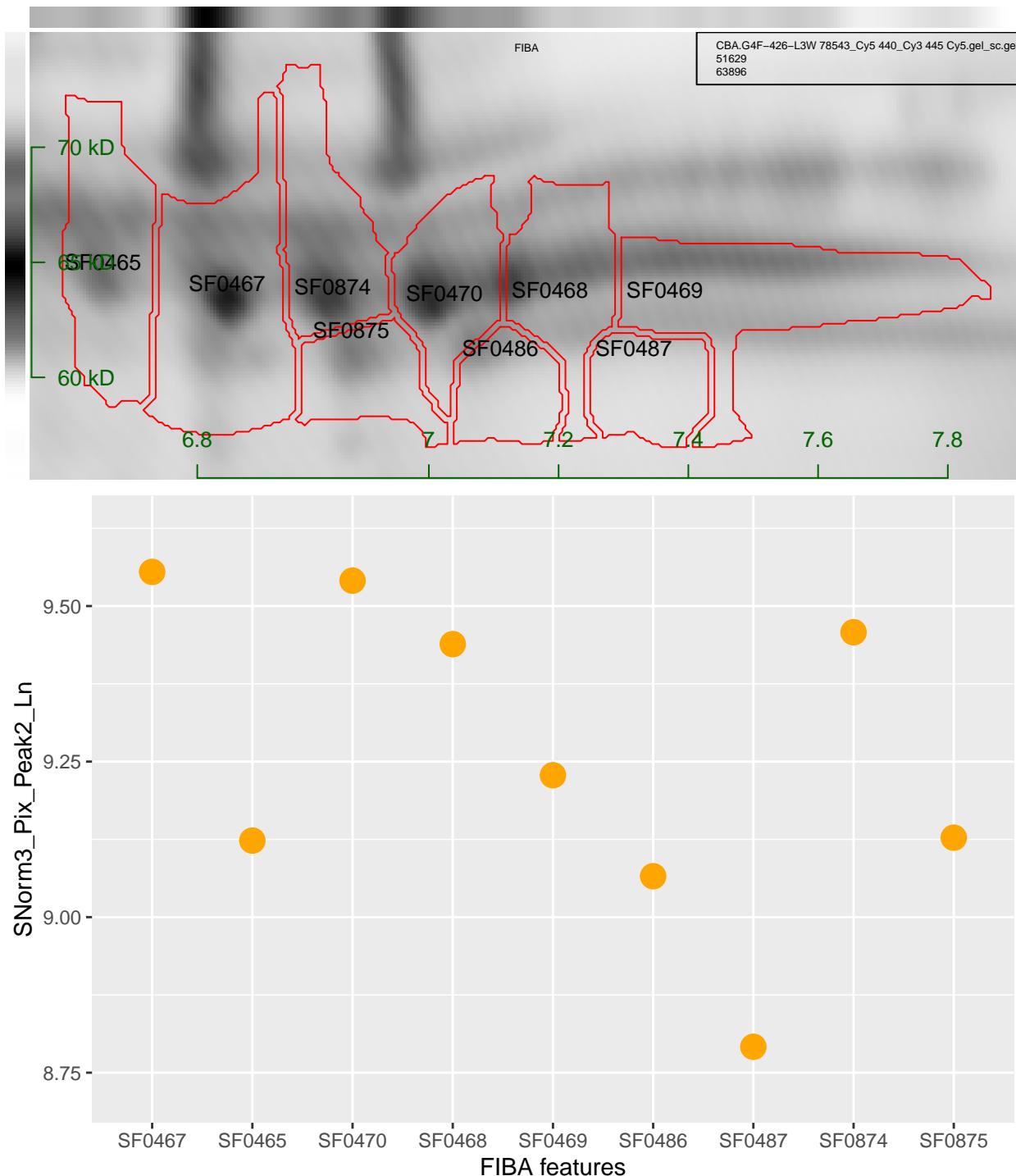


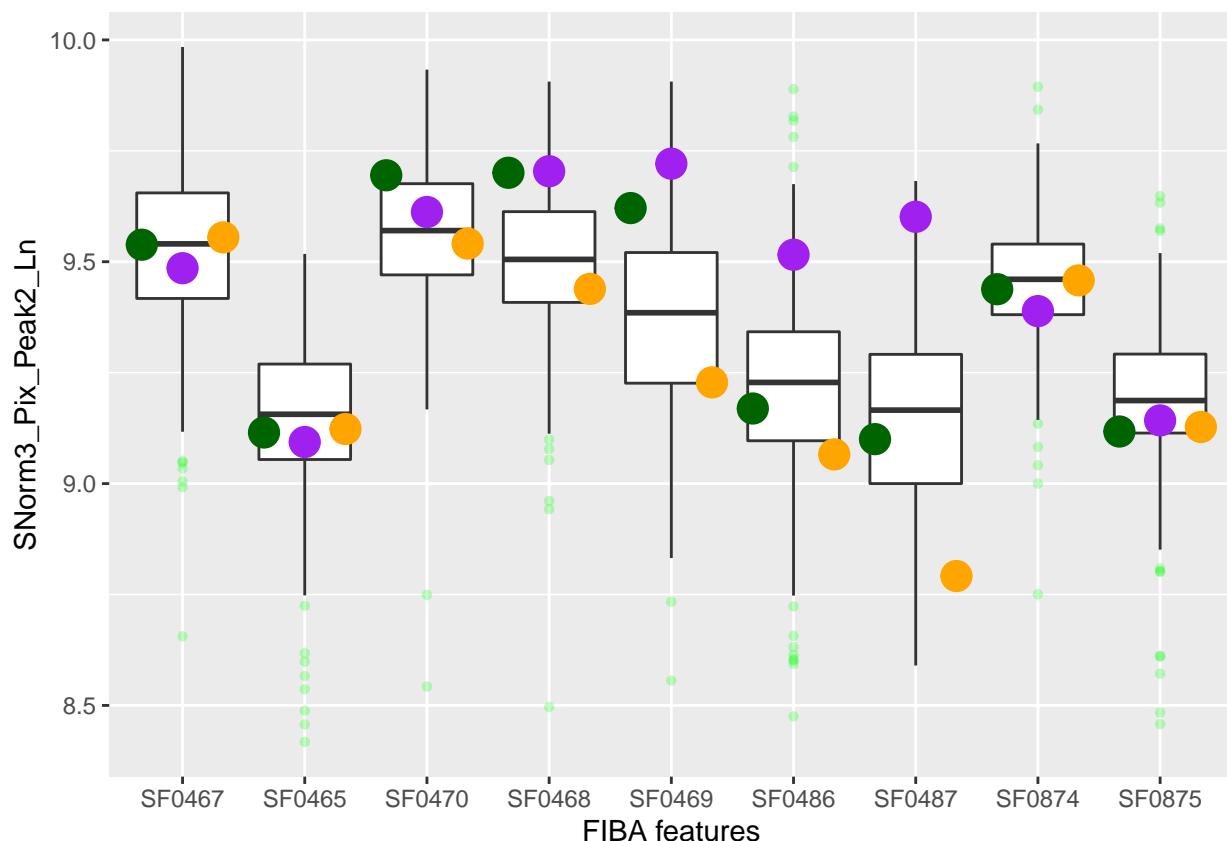


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## Replicate 3 : 78543\_Cy5 440\_Cy3 445 Cy5.gel\_sc.gel



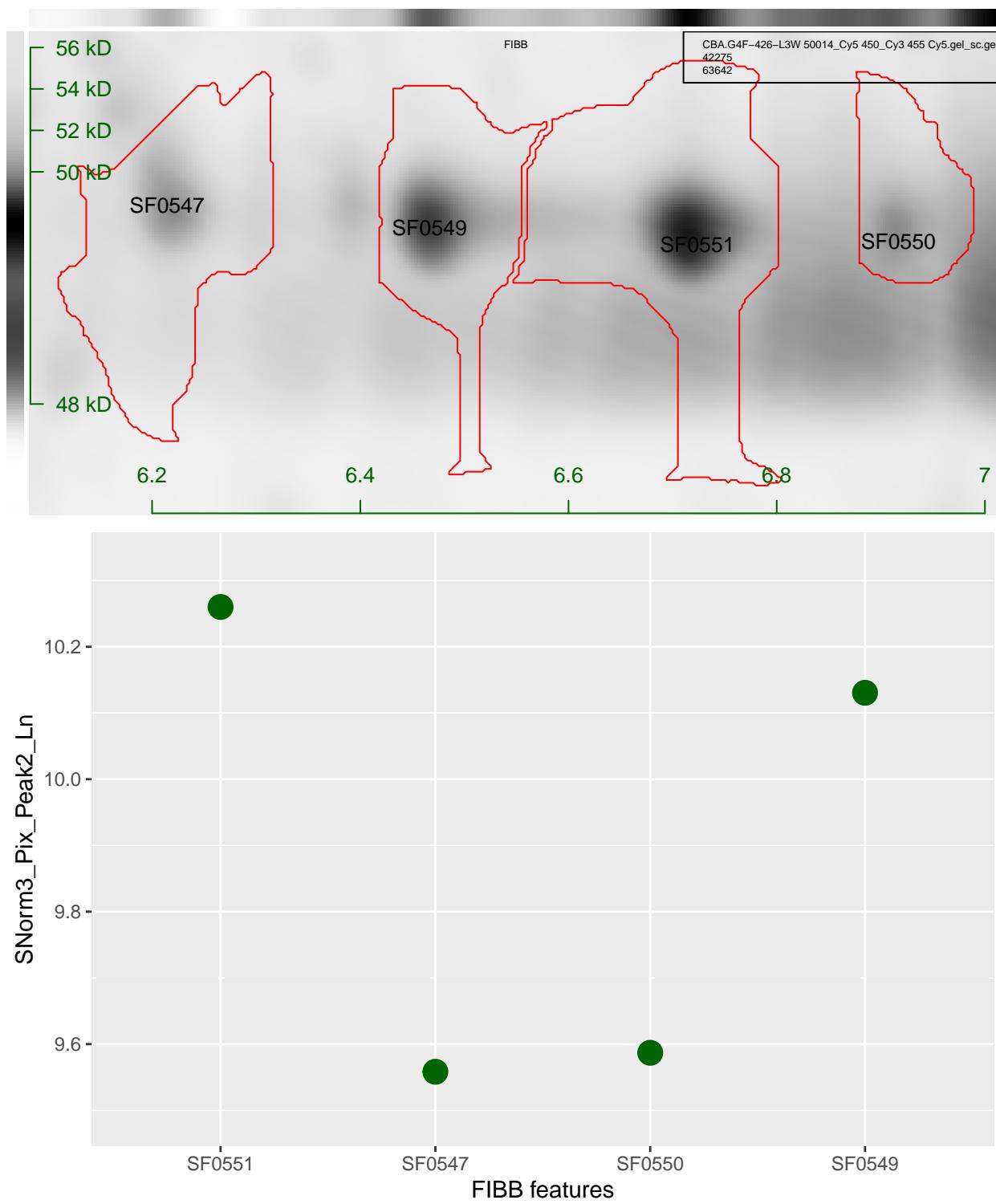


	SF0467	SF0465	SF0470	SF0468	SF0469	SF0486	SF0487	SF0875
50014_Cy5 450_Cy3 455 Cy5	9.538348	9.115150	9.694802	9.700514	9.620926	9.169206		
72288_Cy5 440_Cy3 455 Cy5	9.485469	9.093694	9.612132	9.703938	9.721006	9.515838		
78543_Cy5 440_Cy3 445 Cy5	9.554922	9.123038	9.540866	9.438830	9.228082	9.065777		

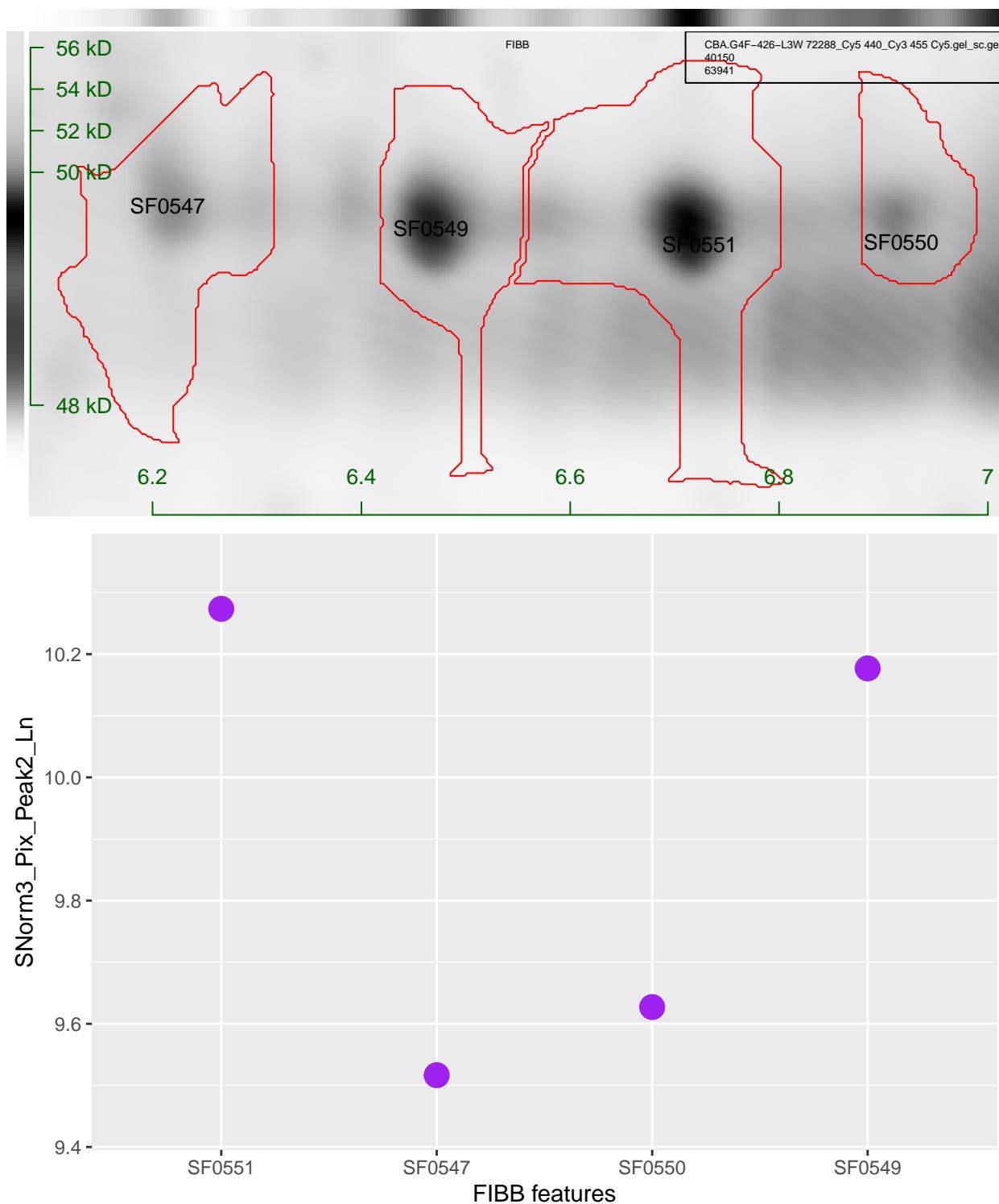
	SF0487	SF0874	SF0875
50014_Cy5 450_Cy3 455 Cy5	9.100191	9.438113	9.117347
72288_Cy5 440_Cy3 455 Cy5	9.601301	9.388738	9.142383
78543_Cy5 440_Cy3 445 Cy5	8.791638	9.457981	9.127719

### CBA.G4F-426-L3W FIBB

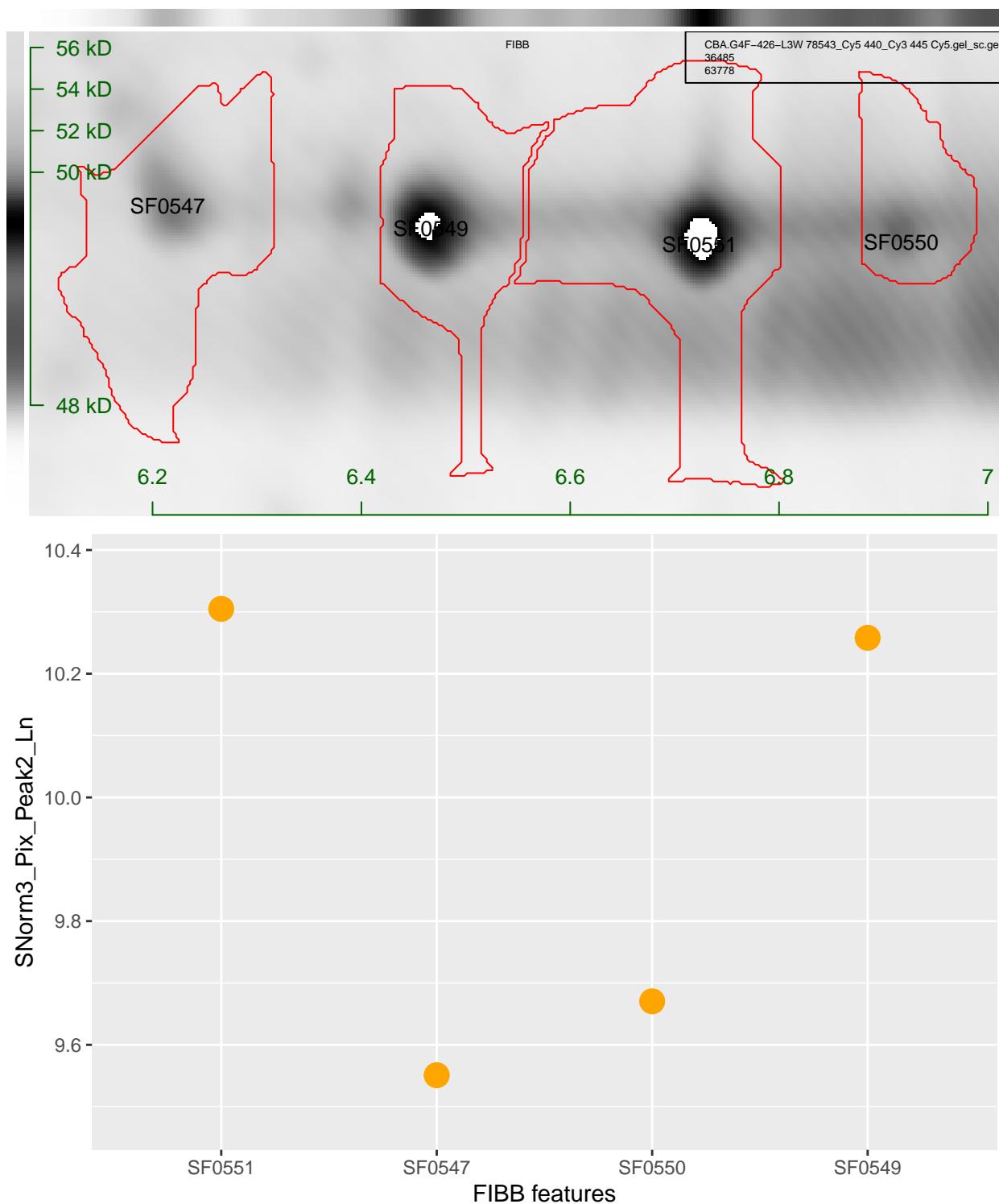
Replicate 1 : 50014\_Cy5 450\_Cy3 455 Cy5.gel\_sc.gel

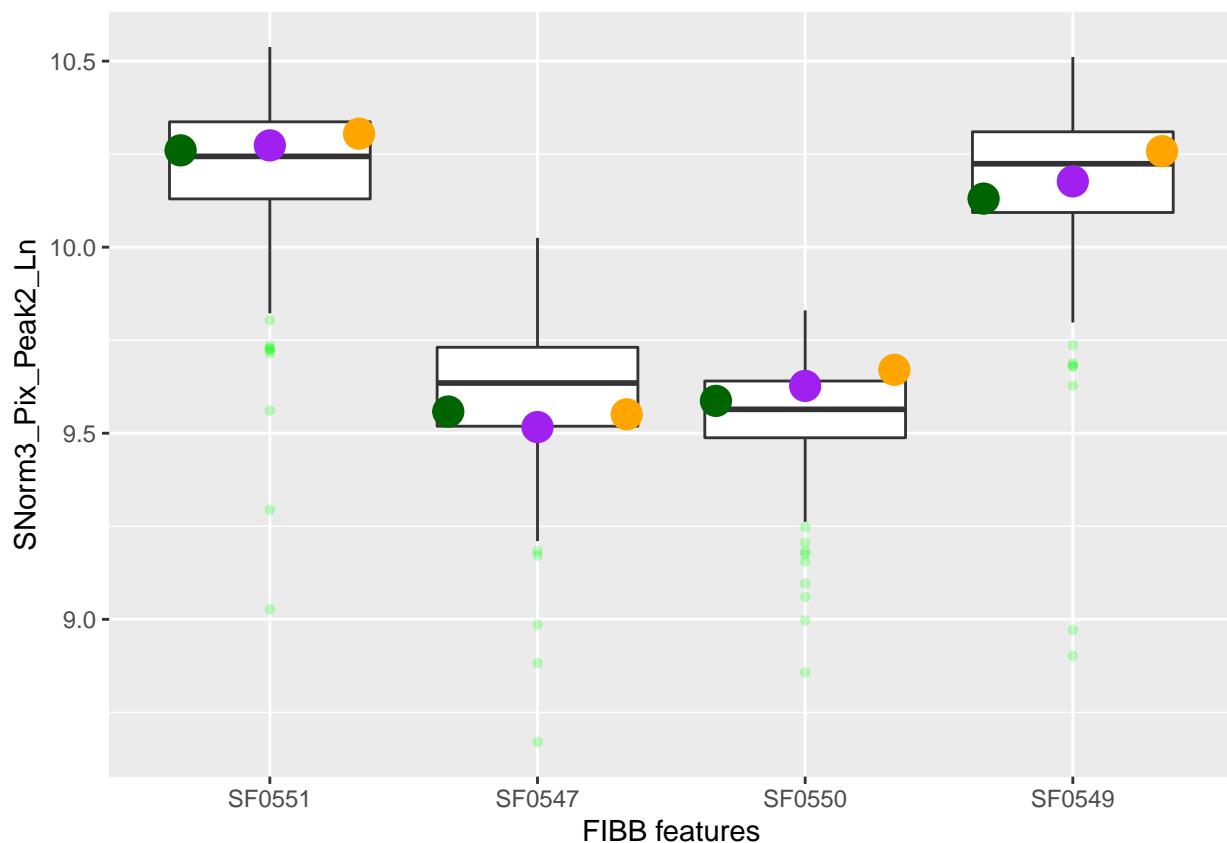


## Replicate 2 : 72288\_Cy5 440\_Cy3 455 Cy5.gel\_sc.gel



## Replicate 3 : 78543\_Cy5 440\_Cy3 445 Cy5.gel\_sc.gel

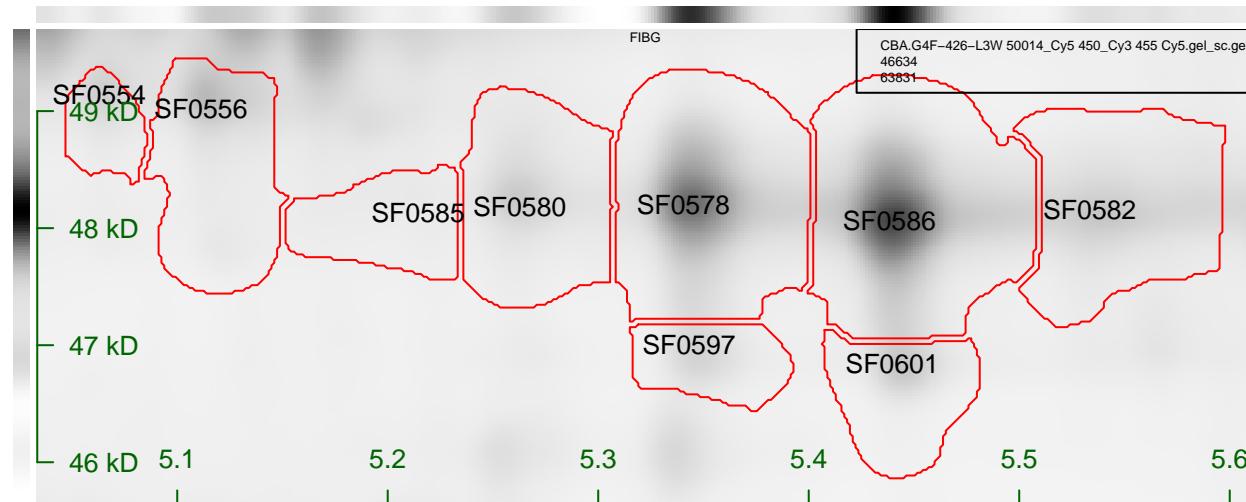


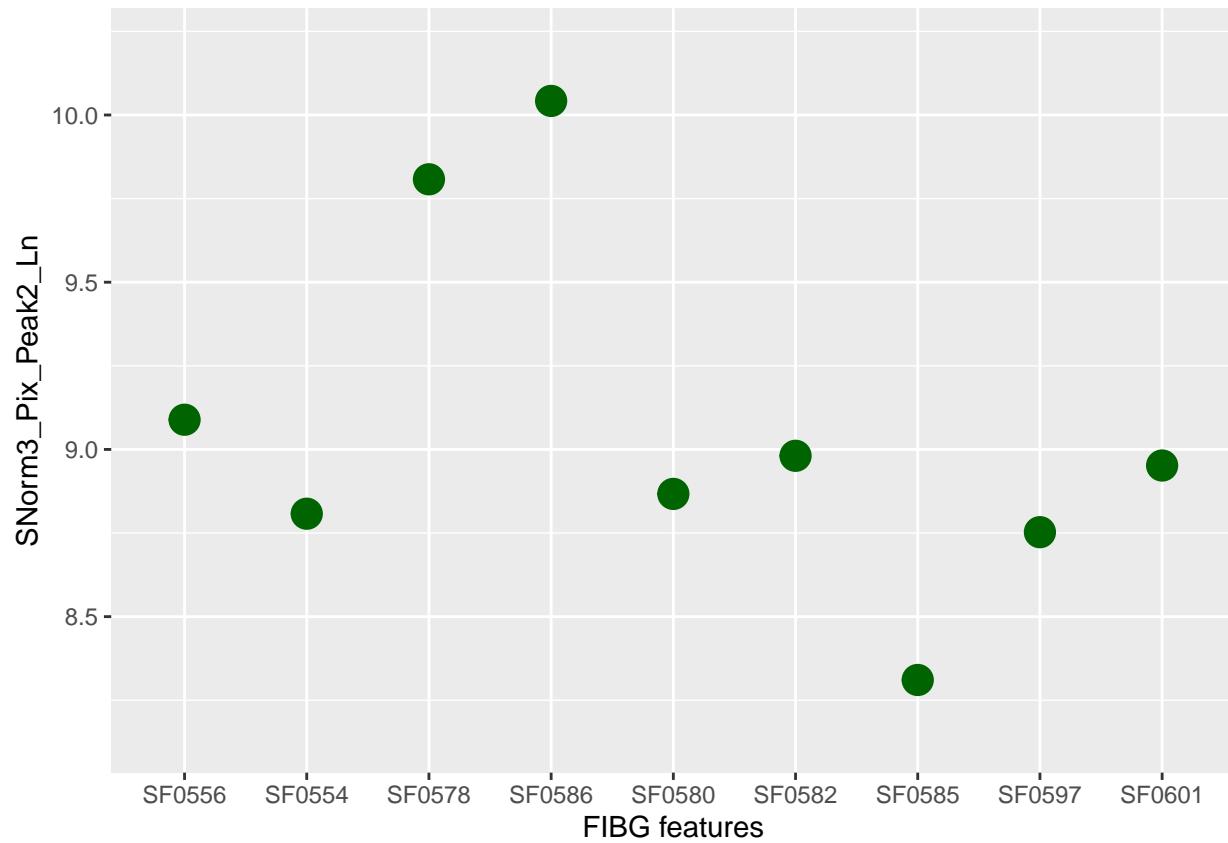


	SF0551	SF0547	SF0550	SF0549
50014_Cy5 450_Cy3 455 Cy5	10.26011	9.558318	9.586926	10.13050
72288_Cy5 440_Cy3 455 Cy5	10.27353	9.516574	9.627141	10.17687
78543_Cy5 440_Cy3 445 Cy5	10.30488	9.550876	9.670357	10.25805

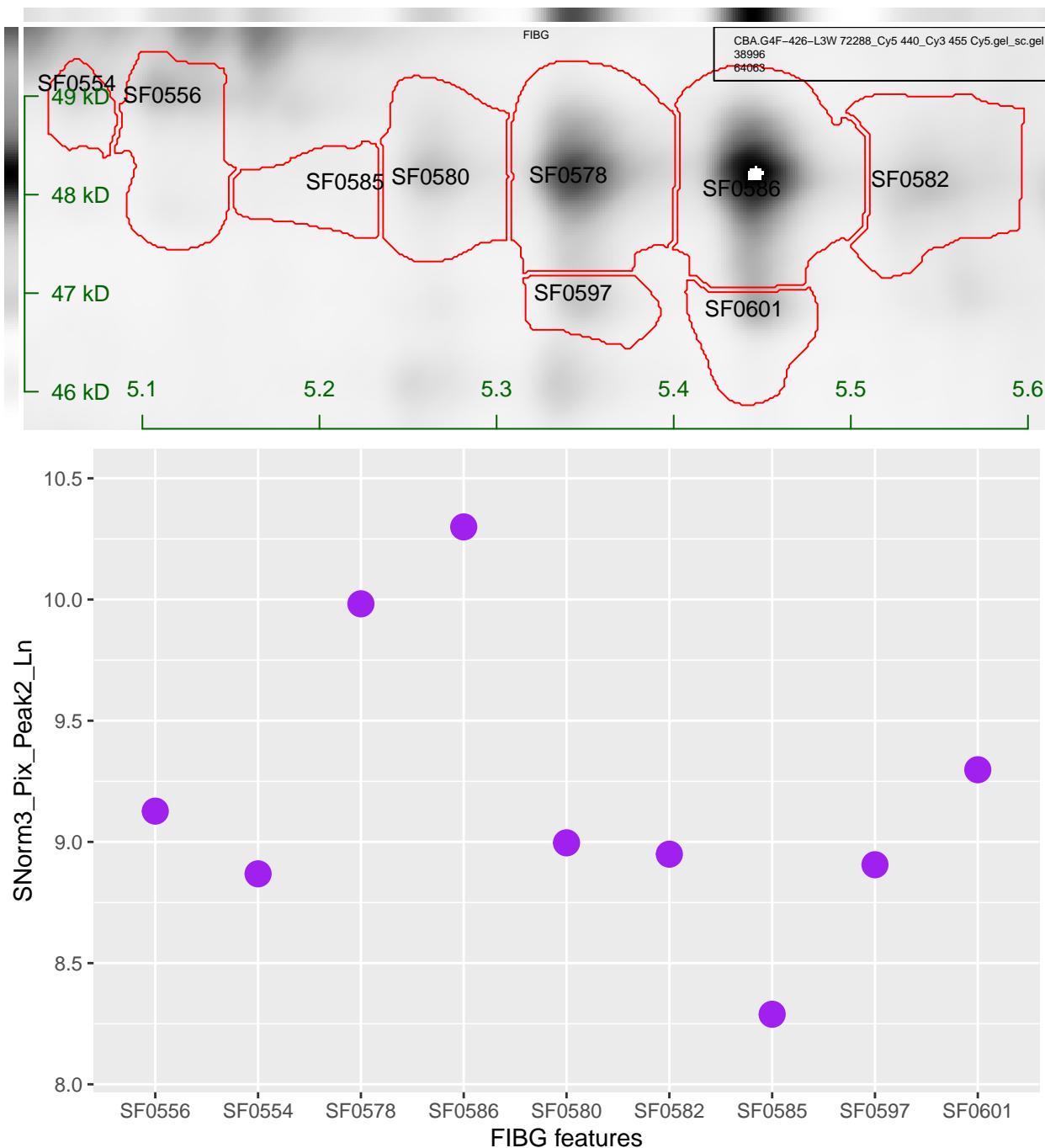
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Replicate 1 : 50014\_Cy5 450\_Cy3 455 Cy5.gel\_sc.gel

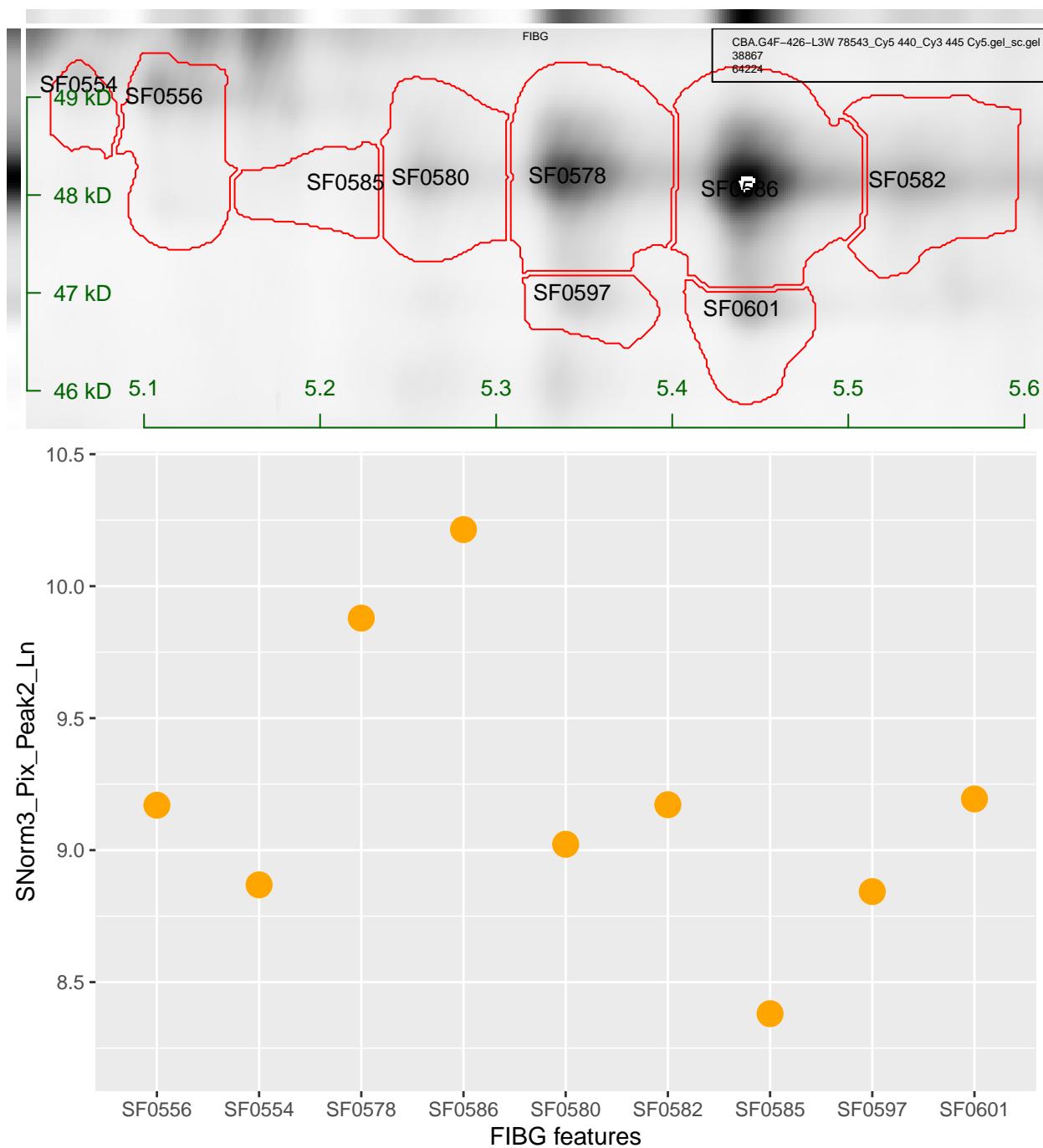


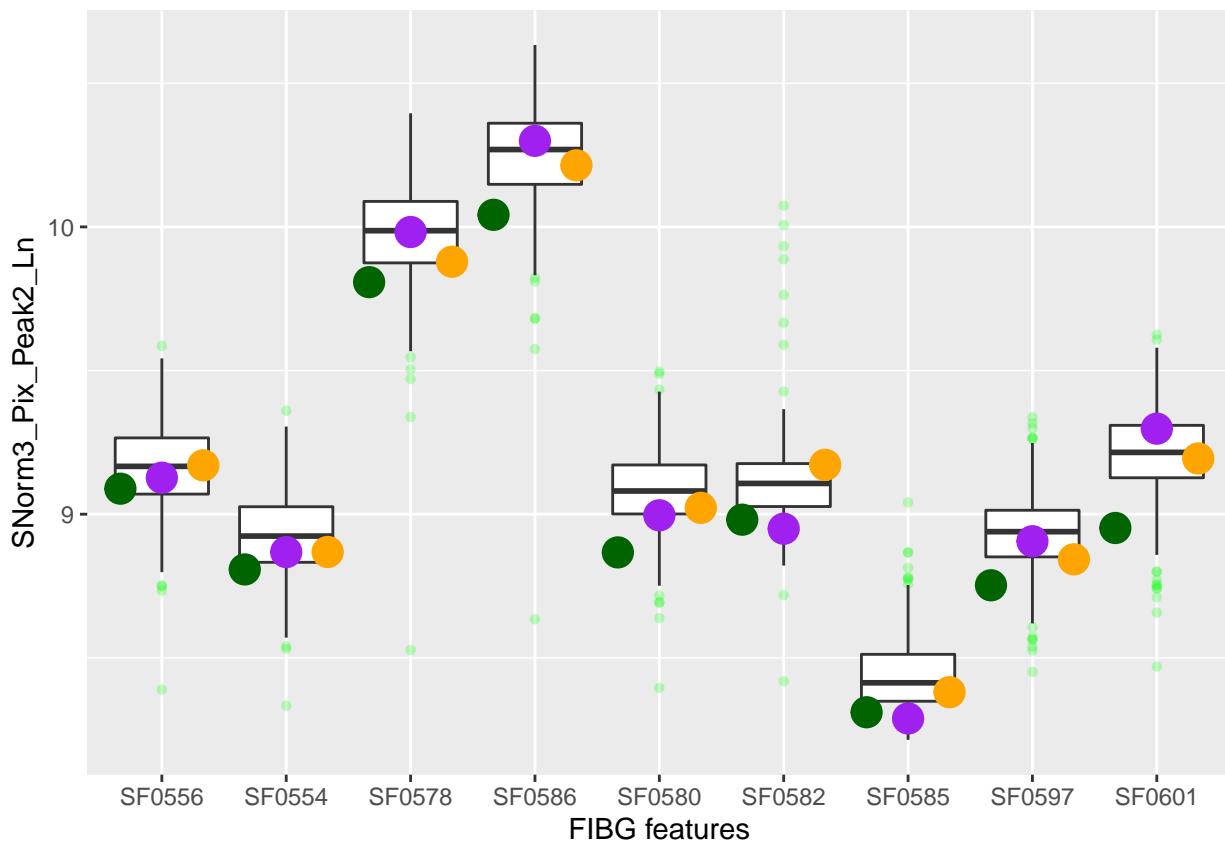


## Replicate 2 : 72288\_Cy5 440\_Cy3 455 Cy5.gel\_sc.gel



## Replicate 3 : 78543\_Cy5 440\_Cy3 445 Cy5.gel\_sc.gel





	SF0556	SF0554	SF0578	SF0586	SF0580	SF0582
50014_Cy5 450_Cy3 455 Cy5	9.088850	8.807771	9.807692	10.04207	8.867005	8.980927
72288_Cy5 440_Cy3 455 Cy5	9.126959	8.868272	9.982206	10.29954	8.996157	8.949495
78543_Cy5 440_Cy3 445 Cy5	9.170143	8.868554	9.878887	10.21439	9.022202	9.172015

	SF0585	SF0597	SF0601
50014_Cy5 450_Cy3 455 Cy5	8.310661	8.752581	8.951958
72288_Cy5 440_Cy3 455 Cy5	8.289037	8.905851	9.297710
78543_Cy5 440_Cy3 445 Cy5	8.380457	8.843038	9.193906

## Haptoglobin (P00738)

From: Human plasma protein N-glycosylation

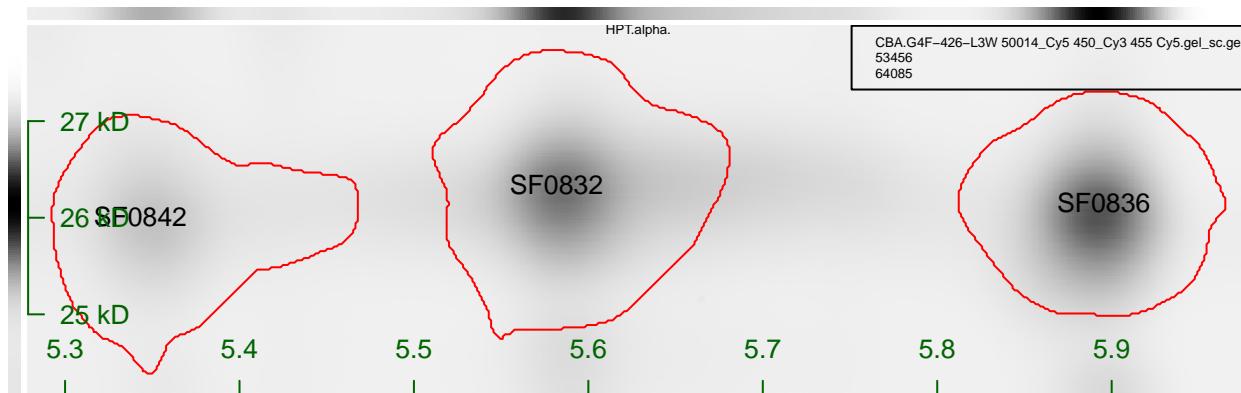
Haptoglobin (Hp) is a 406 amino acid (18 amino acid signal peptide) acute-phase glycoprotein with a peptide backbone of 45 kDa. It is synthesized in the liver by hepatocytes as a single polypeptide chain and is also found in skin [304, 305]. During its synthesis, Hp is cleaved into a light  $\alpha$  chain and a heavy  $\beta$  chain that are connected via disulfide bonds. Two variants of the  $\alpha$  chain originating from the sequence Val19-Gln160 and differing by the subsequence Glu38-Pro96 can exist,  $\alpha_1$  having this subsequence once while  $\alpha_2$  has it twice, resulting in chains of 83 or 142 amino acids with a respective molecular mass of 9 and 16 kDa. The 40 kDa  $\beta$  chain is made of 245 amino acids originating from the sequence Ile162-Asn406 [306, 307]. The combination of different allelic variants of the  $\alpha$  chain ( $\alpha_1$  and  $\alpha_2$ ) with  $\beta$  chain(s) creates the polymorphism observed in Hp. There are three major Hp phenotypes called Hp1-1, Hp2-1 and Hp2-2. They have a configuration of  $(\alpha_1\beta)_2$ ,  $(\alpha_1\beta)_2 + (\alpha_2\beta)_n = 0, 1, 2, \dots$  and  $(\alpha_2\beta)_n = 3, 4, 5, \dots$ , respectively, which are observed at different ratios among ethnicities [118, 308–310]. Caucasians have around 13 % of phenotype Hp1-1, 46 % of Hp2-1 and 41 % of Hp2-2. Hp is typically found at a plasma levels in the

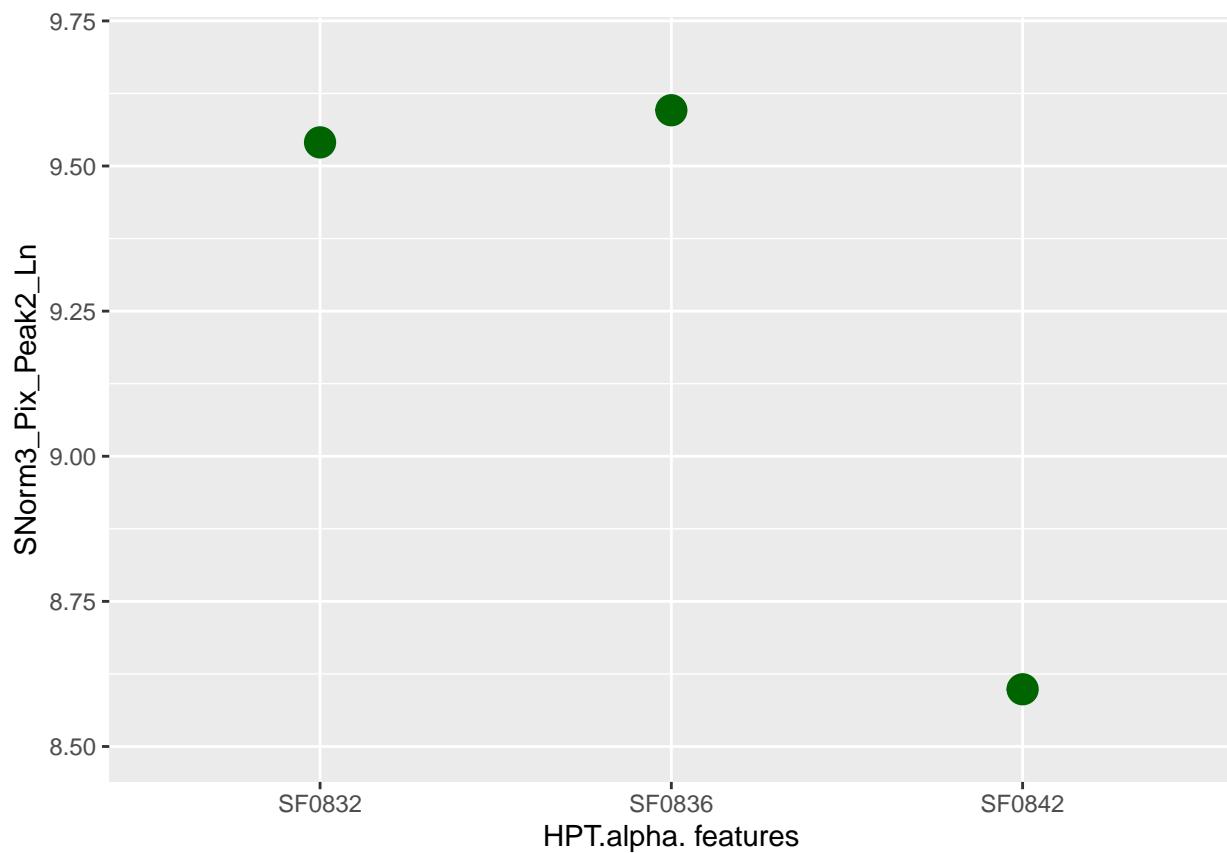
range of 0.6–2.3 mg/mL with a mean of 1.32 mg/mL [118]. Elevated Hp levels have been reported with inflammation and malignant diseases [308, 311, 312]. It should be taken into account that the concentration as well as the molecular mass including glycosylation may vary among phenotypes (86–900 kDa) [118]. The half-life of Hp is found to be on average four days.

The major function of Hp is to protect tissues from oxidative damage by capturing hemoglobin [307, 313]. It has been reported that Hp polymorphism has an effect on its physiological properties, for instance Hp1-1 binds hemoglobin stronger than Hp2-2 [314]. Certain diseases seem to be dependent on the polymorphism, as individuals with the Hp1-1 phenotype seem to have a higher concentration of induced antibodies in their plasma after vaccination, infections or liver diseases compared to the other phenotypes [118, 310].

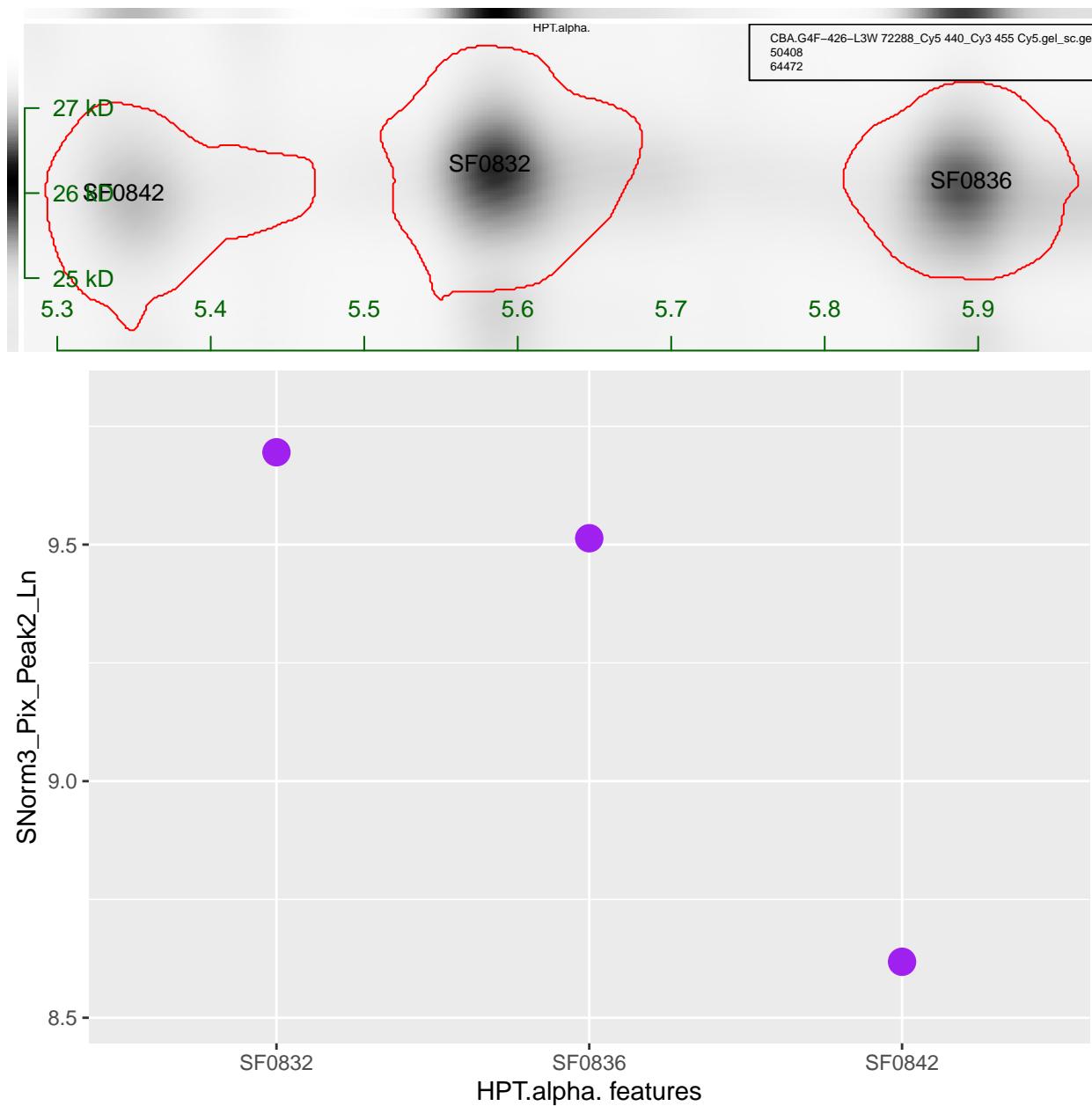
#### CBA.G4F-426-L3W HPT.alpha.

##### Replicate 1 : 50014\_Cy5 450\_Cy3 455 Cy5.gel\_sc.gel

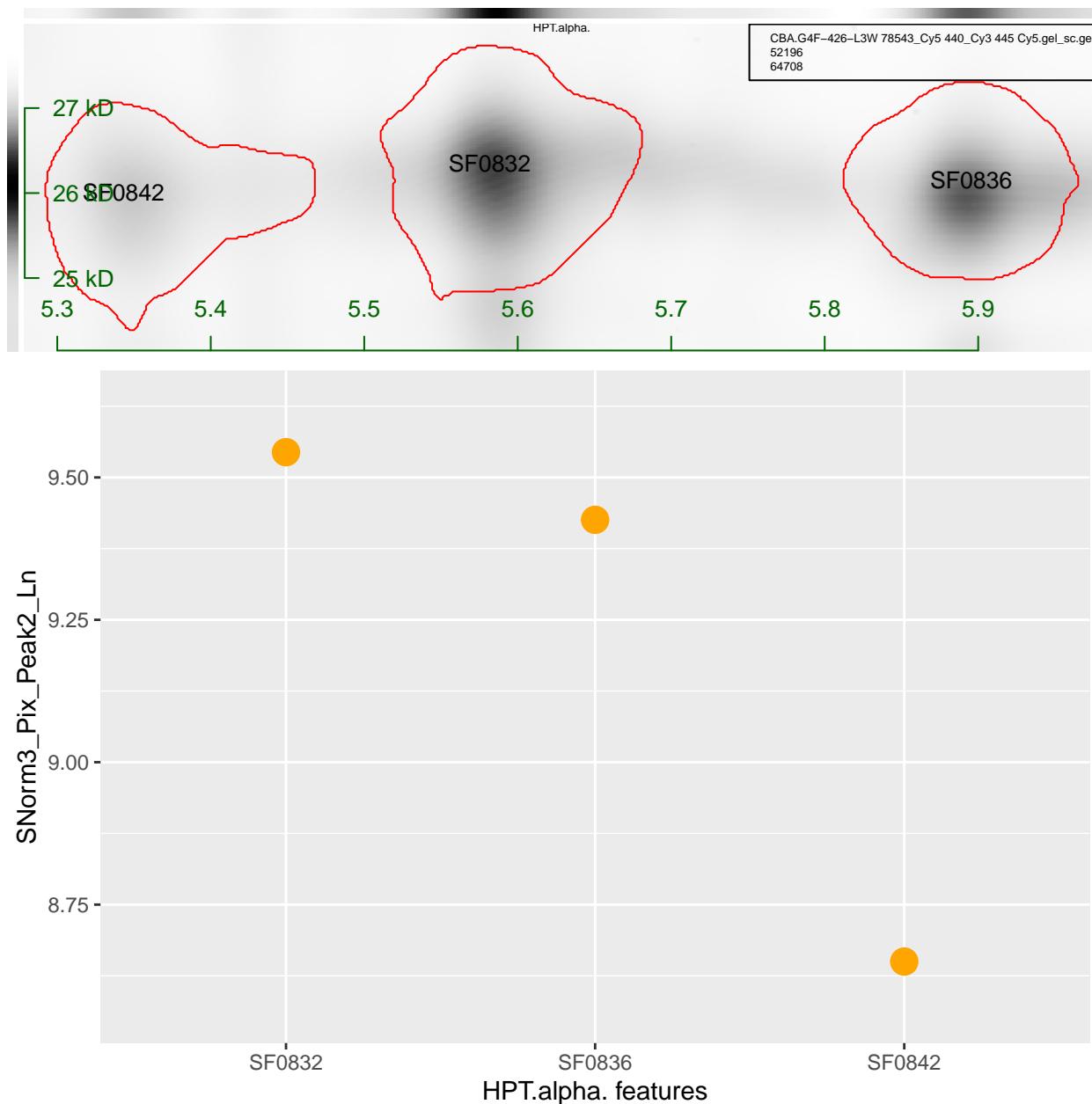


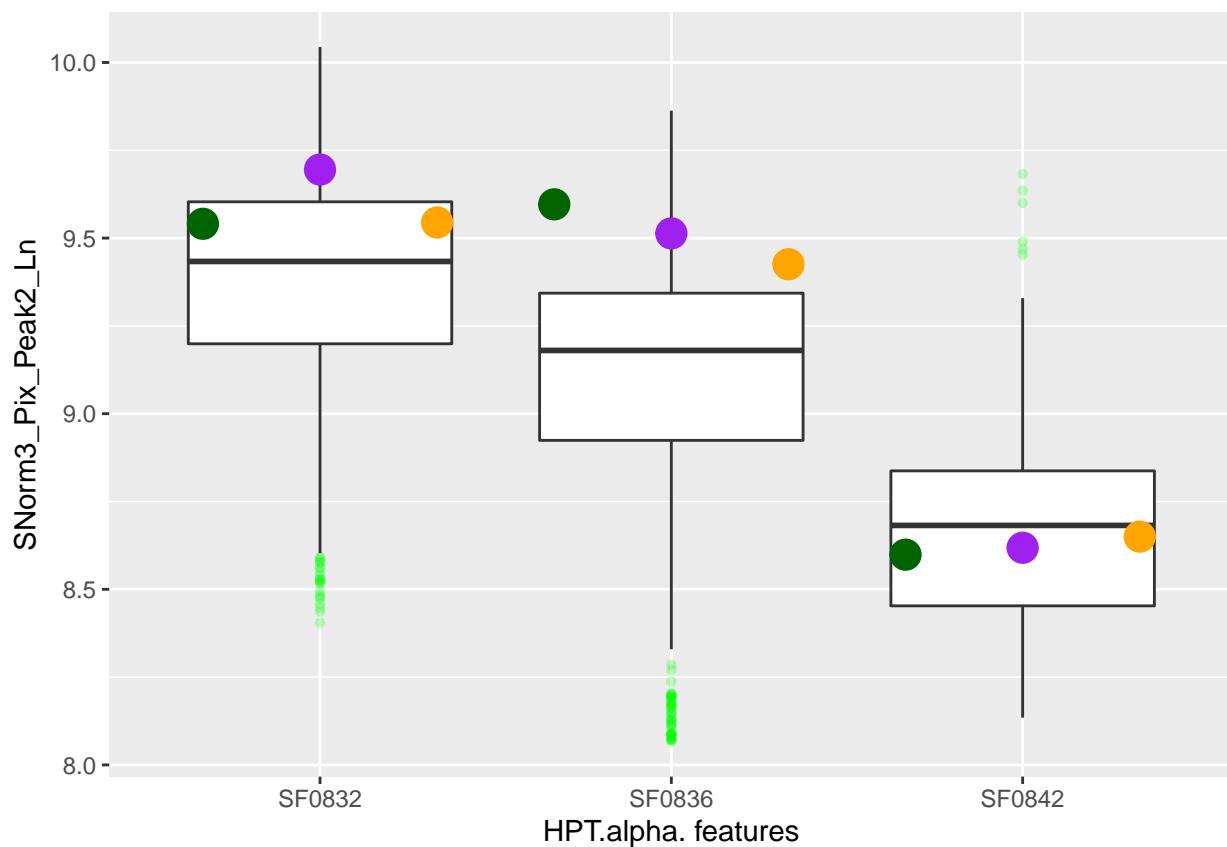


## Replicate 2 : 72288\_Cy5 440\_Cy3 455 Cy5.gel\_sc.gel



## Replicate 3 : 78543\_Cy5 440\_Cy3 445 Cy5.gel\_sc.gel

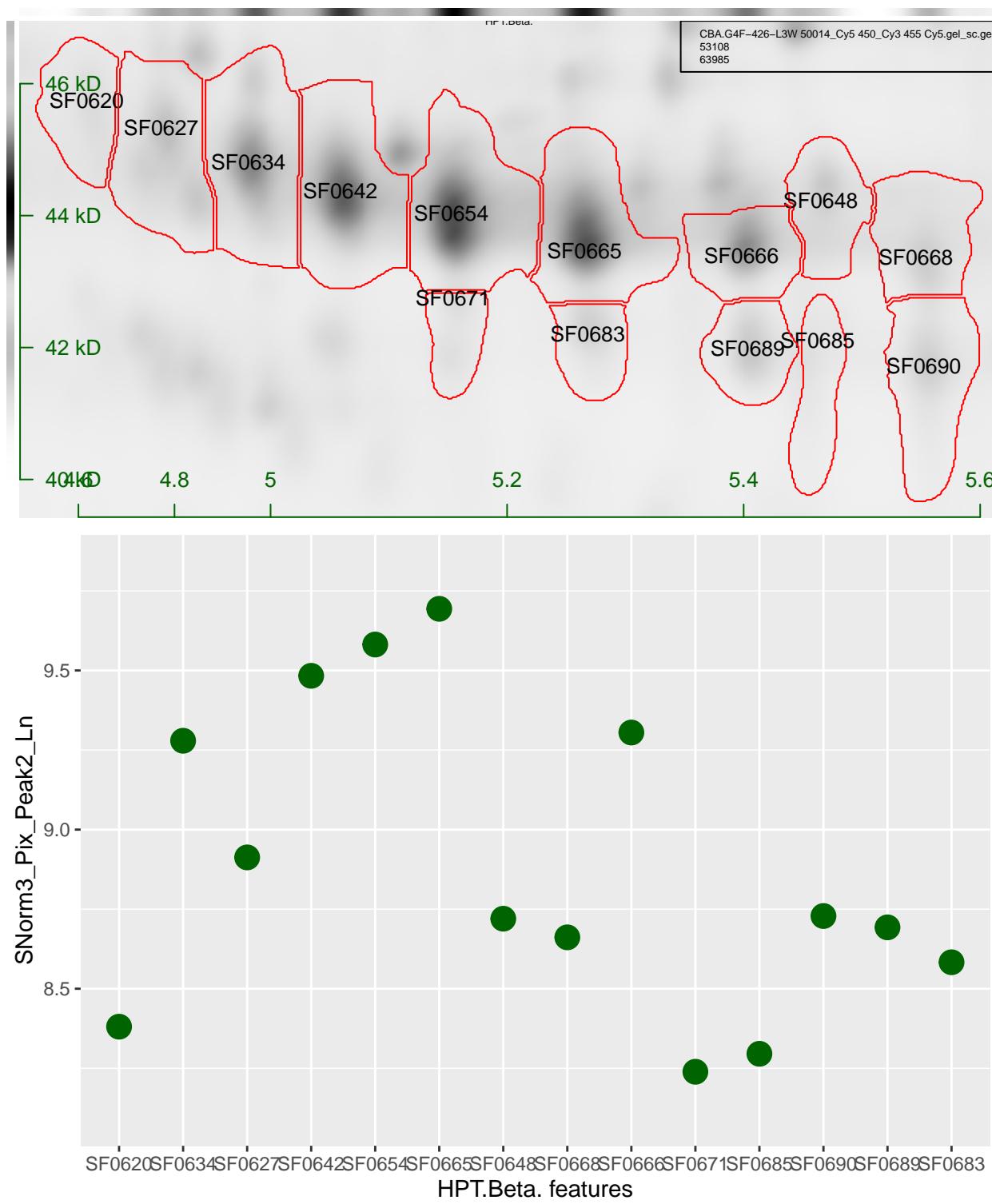




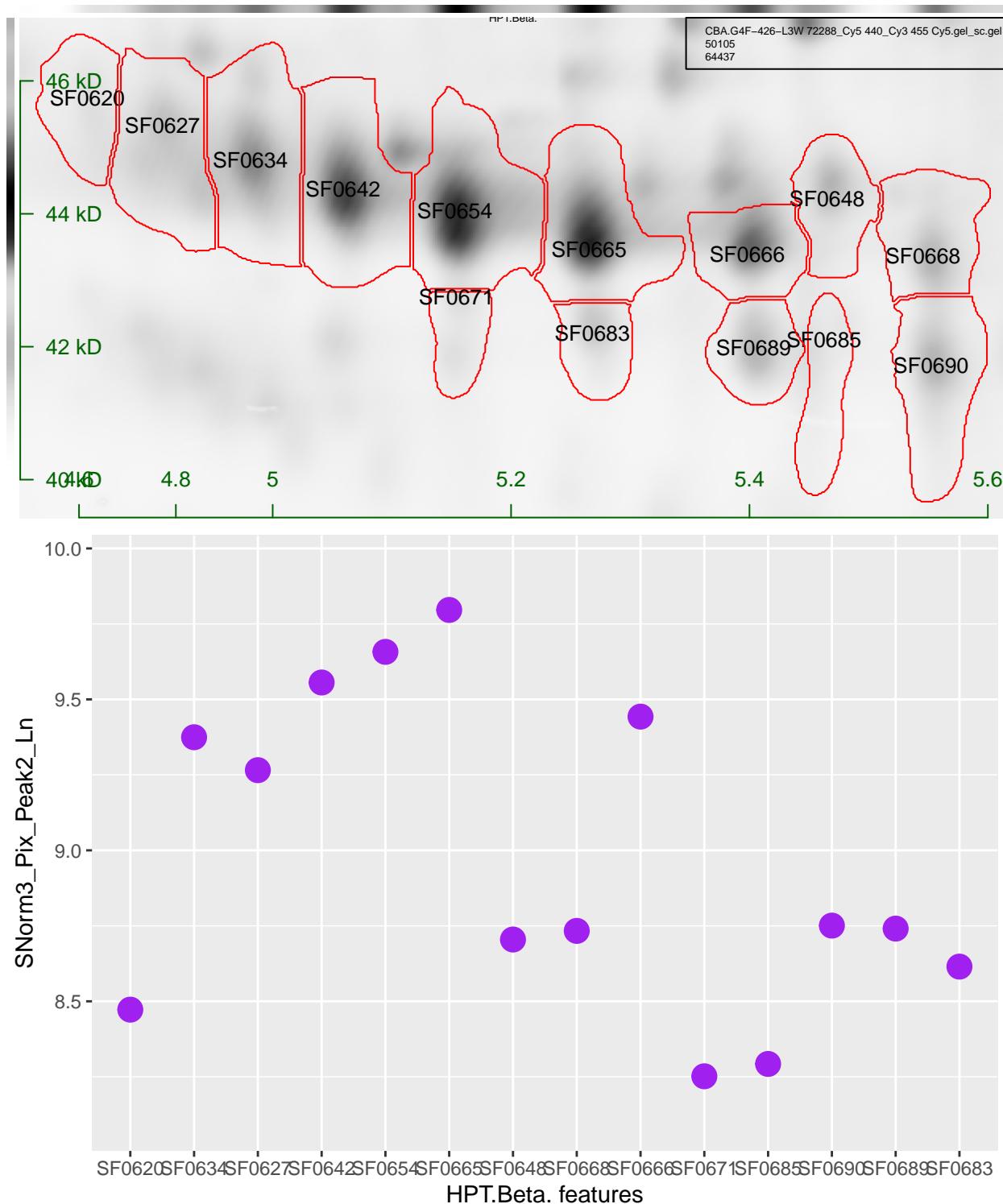
	SF0832	SF0836	SF0842
50014_Cy5 450_Cy3 455 Cy5	9.540723	9.596079	8.598589
72288_Cy5 440_Cy3 455 Cy5	9.695109	9.513404	8.618305
78543_Cy5 440_Cy3 445 Cy5	9.544381	9.425613	8.649974

#### CBA.G4F-426-L3W HPT.Beta.

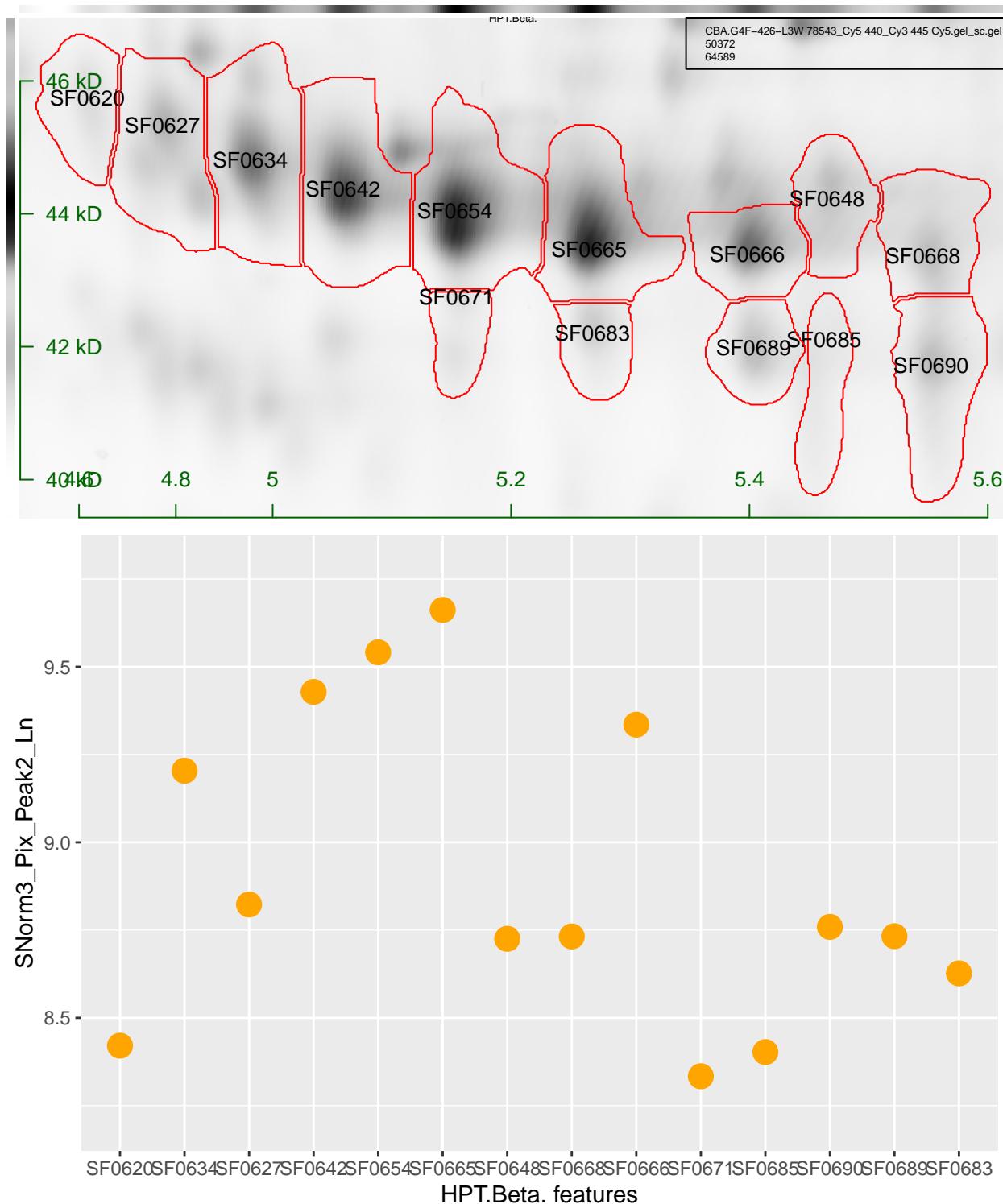
Replicate 1 : 50014\_Cy5 450\_Cy3 455 Cy5.gel\_sc.gel

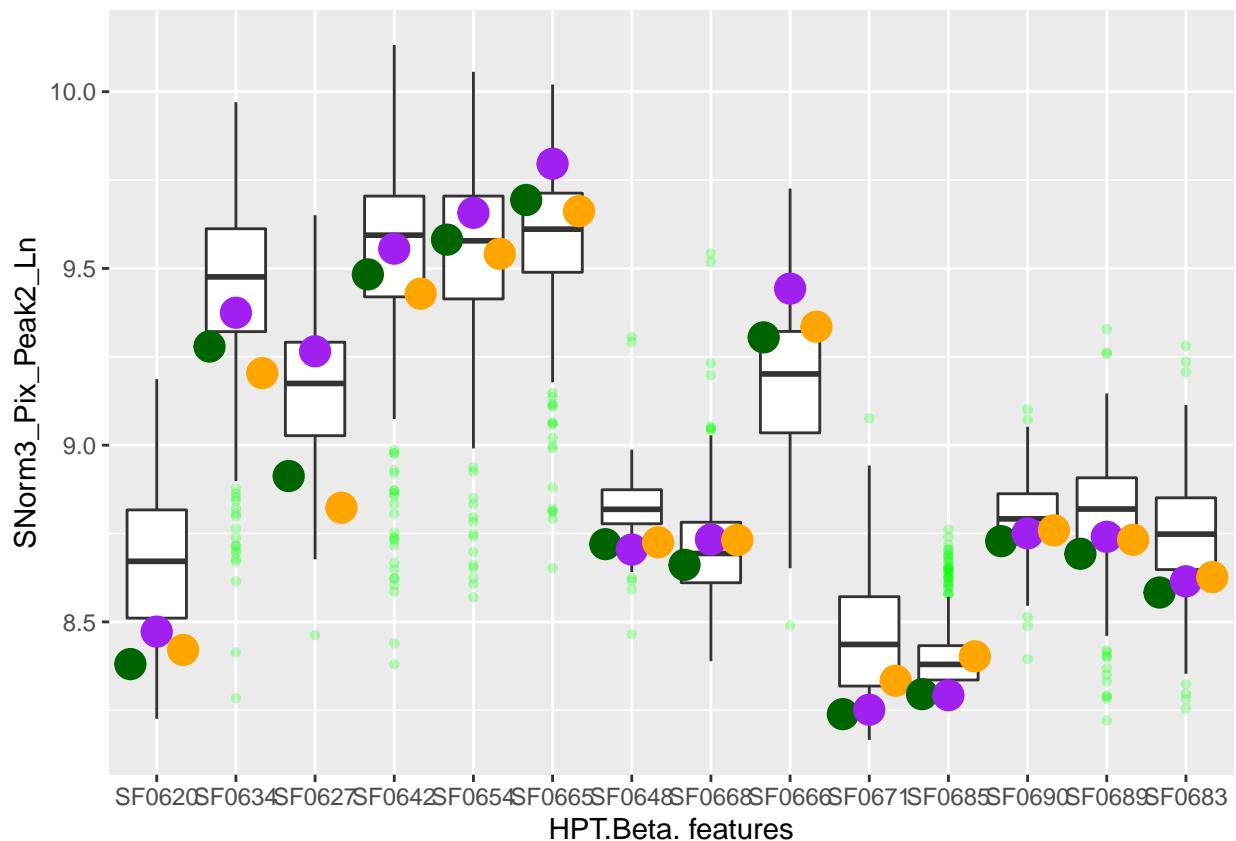


## Replicate 2 : 72288\_Cy5 440\_Cy3 455 Cy5.gel\_sc.gel



## Replicate 3 : 78543\_Cy5 440\_Cy3 445 Cy5.gel\_sc.gel





	SF0620	SF0634	SF0627	SF0642	SF0654	SF0665
50014_Cy5 450_Cy3 455 Cy5	8.380686	9.279120	8.912608	9.483188	9.581490	9.693692
72288_Cy5 440_Cy3 455 Cy5	8.472405	9.374668	9.265586	9.555985	9.657395	9.796292
78543_Cy5 440_Cy3 445 Cy5	8.420682	9.203819	8.822912	9.428512	9.541297	9.661989

	SF0648	SF0668	SF0666	SF0671	SF0685	SF0690
50014_Cy5 450_Cy3 455 Cy5	8.719971	8.661467	9.305014	8.239065	8.295549	8.728426
72288_Cy5 440_Cy3 455 Cy5	8.704668	8.733272	9.442959	8.251664	8.292549	8.750683
78543_Cy5 440_Cy3 445 Cy5	8.725183	8.731821	9.335121	8.333511	8.402456	8.759041

	SF0689	SF0683
50014_Cy5 450_Cy3 455 Cy5	8.692826	8.583168
72288_Cy5 440_Cy3 455 Cy5	8.740817	8.614864
78543_Cy5 440_Cy3 445 Cy5	8.732466	8.626765

## Immunoglobulin G (P01857; P01859; P01860; P01861)

From: Human plasma protein N-glycosylation

Immunoglobulin gamma (IgG) is a glycoprotein with a total molecular mass of approximately 150 kDa [159, 177]. The light chain consists of a domain covering the variable region (VL) as well as a constant (CL) domain. The heavy chain contains four domains with one domain which comprises the variable region (HL) followed by three constant domains: CH1, CH2 and CH3. Each light chain is paired with the HL and CH1 domain of the heavy chain to form a Fab portion, whilst CH2 and CH3 domains of the two heavy chains together form the Fc portion. Between the two Fab portions and the Fc portion a flexible hinge region is positioned, which makes it possible for the two Fab arms to move individually [159, 364]. The antibody is highly stable with a half-life of

approximately 12 days [369, 370]. Based on the amino acid sequence of the constant regions of the heavy chains, IgGs can be divided into four subclasses namely, IgG1 (P01857), IgG2 (P01859), IgG3 (P01860) and IgG4 (P01861) [371, 372]. Notably, IgG3 has a larger hinge region (62 amino acids) compared to the other subclasses (12 amino acids).

During a secondary immune response IgG is secreted in high amounts by B cells [373]. In healthy individuals the concentration of IgG in serum is between 7 and 18 mg/mL [374]. The average subclass-specific concentrations in plasma are reported as 5.03 mg/mL for IgG1, 3.42 mg/mL for IgG2, 0.58 mg/mL for IgG3 and 0.38 mg/mL for IgG4 [375]. IgG molecules are important for activating the complement system through the classical pathway (antibody-triggered) as well as binding to specific receptors on macrophages and neutrophils [364, 373]. The IgG subclasses differ in their ability to activate the complement system. The primary activators of the complement system are IgG1 and IgG3, whereas IgG2 can also activate it at a lower level. IgG4, on the other hand, is not capable of activating the complement system [364]. Furthermore, IgG molecules are the only antibodies that can pass from a mother to her child via the placenta, and maternal IgG has been shown to gradually decrease throughout pregnancy [364, 376, 377].

The majority of therapeutic antibodies is derived from IgG1, where the glycosylation plays an important role for their function [35, 364, 377–380].

## Glycosylation

Glycosylation can occur on both the Fc and Fab portions of the IgG molecules [178]. The Fc region has been extensively studied with a highly conserved N-glycosylation site in the CH2 domain at Asn297. Notably, this site may have a different number for different IgG subclasses and variants [178].

Another possible N-glycosylation site may be found at Asn322 of IgG3 although no occupation has yet been described [178]. The Fab portion is known to be N-glycosylated in 15–25 % of the cases [179].

The overall glycosylation of IgG has been the subject of many studies using a variety of different methods [24]. In a recent glycosylation MALDI-TOF-MS study on a released glycan level, the most abundant glycans were complex types, i.e. FA2G1 (31 %), FA2G2 (23 %), FA2G2S1(6) (13 %), FA2 (10 %) and FA2BG1 (5 %) [180]. Only a small portion of the glycans were found to be high mannose (0.21 %), of which Man8 (0.06 %) was the most abundant, followed by Man9 (0.05 %). Overall, 92 % of the total IgG pool was core-fucosylated, 13 % bisected, 18 % monosialylated and 3 % disialylated. Twelve percent of the glycans contained a2-6-linked sialic acids, against 0.2 % a2-3-sialylated species [180]. These findings are in agreement with previously reported sialylation values obtained by lectin interaction [181]. Next to the study of overall IgG glycosylation, differences between the Fab and Fc have also been studied by MALDI-TOF-MS after affinity capturing of the different regions [180]. The Fc region shows a similar profile as the total IgG profile, albeit with a lesser degree of sialylation. FA2G1 (32 %) was again the most pronounced glycan, followed by FA2G2 (27 %), FA2G2S1(6) (15 %), FA2 (9 %) and FA2BG1 (5 %), while the amount of high mannose type species was found to be very low (0.1 %). In contrast to Fc, the Fab region showed a significantly higher degree of sialylation, with 40 % of the species being monosialylated, and 52 % being disialylated. Also bisection and high mannose species were seen to be higher (45 and 4 % respectively). Specific compositions included FA2BG2S1(6) as most abundant with 21 %, followed by A2G2S2(6) (17 %), FA2BG2S2(6) (16 %), FA2G2S2(6) (16 %) and FA2G2S1(6) (10 %). For the high mannose types Man6 was the most abundant with 1.2 % followed by Man8 (1.0 %) and M5 (0.7 %) [180].

Affinity capturing followed by LC-MS with CID and electron-transfer dissociation (ETD) fragmentation of tryptic IgG glycopeptides revealed that the various IgG subclasses are similarly glycosylated, but with some notable differences [182, 183]. IgG1 tends to show higher galactosylation than the other subclasses, whereas IgG2 shows the highest degree of core-fucosylation and IgG3 the

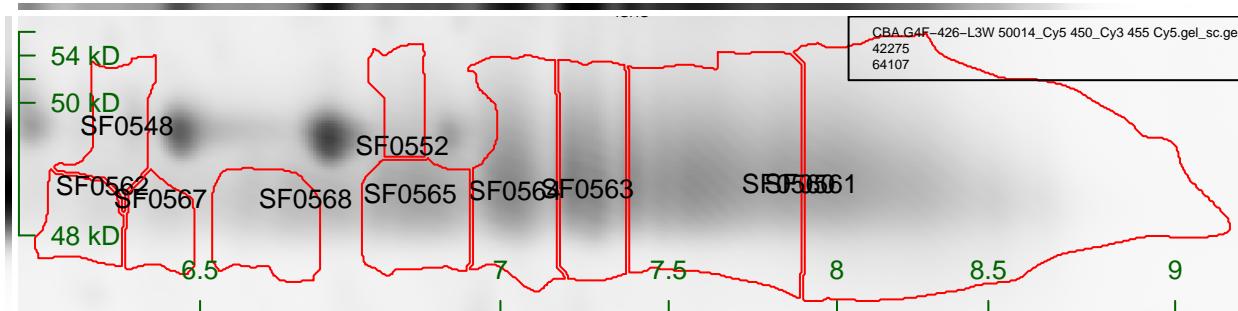
least. IgG4 was found more difficult to study due to its relatively low abundance [182]. Another study examined the O-glycosylation of IgG3 in the hinge region, revealing that the threonine sites (T) in the three repeated peptide sequences (CPRCPEPKSCDTPPPP) are partially occupied with core 1-type O-glycans [184].

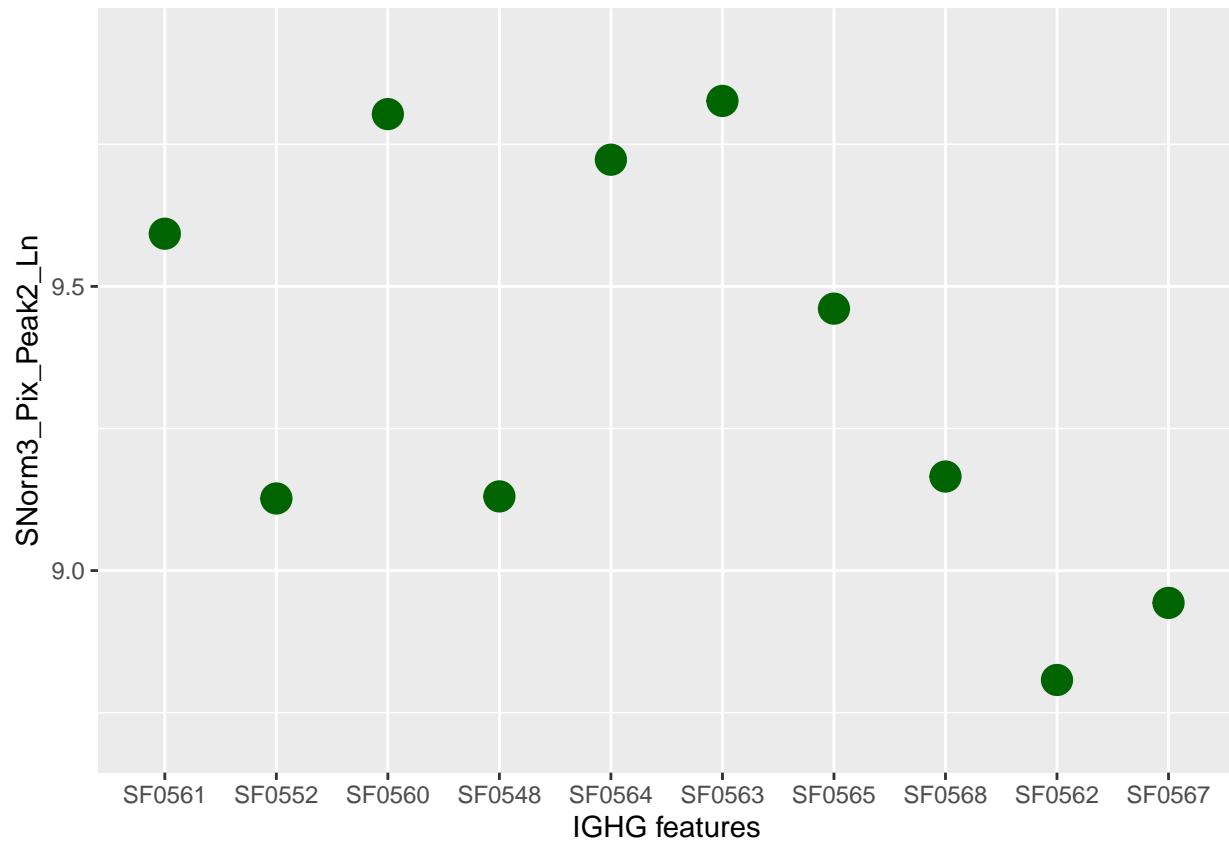
A vast body of literature exists describing disease-associated changes of IgG glycosylation, as well as the regulation and immunological effects of such glycosylation changes. In the following, only a very concise view of this field will be given, and we would like to refer the interested reader to more specialized reviews [177, 185, 186].

IgG glycosylation has been strongly associated with age, with a negative correlation between age and galactosylation [19, 20, 187, 188]. IgG FA2 seems to have a strong pro-inflammatory effect through various mechanisms, e.g. the lectin pathway of the complement system [19, 188, 189]. Increased levels of FA2 glycans and/or lowered levels of FA2G2 glycans is found in many diseases, including rheumatoid arthritis, Crohn's disease, granulomatosis with polyangiitis, tuberculosis, HIV and myositis [189–193]. Several studies revealed that core-fucosylation is an important factor in the binding capacity of the Fc region to the Fc<U+O3D2>RIIIa receptor [194, 195]. The lack of core-fucosylation suggests improvement in the binding extensively resulting in a higher degree of antibody-dependent cell mediated cytotoxicity (ADCC) receptor [194, 195]. The presence of sialic acids is able to reduce the binding capacity of the antibody to the Fc<U+O3D2>RIIIa receptor, as a consequence the activity of ADCC is decreased and anti-inflammatory effects are enhanced, although this only appears to be the case for a2-6-linked sialylation [33, 178, 196, 197]. Interestingly, during pregnancy the glycosylation also appears to change, especially in the Fc region where the levels of galactosylation and sialylation increase [28, 198–200]. This might be to suppress the immune response of the mother against her child [198]. Alterations in glycosylation have also been reported to occur in a subclass specific level, for example in patient suffering from hepatocellular carcinoma, cirrhosis, or myositis [190].

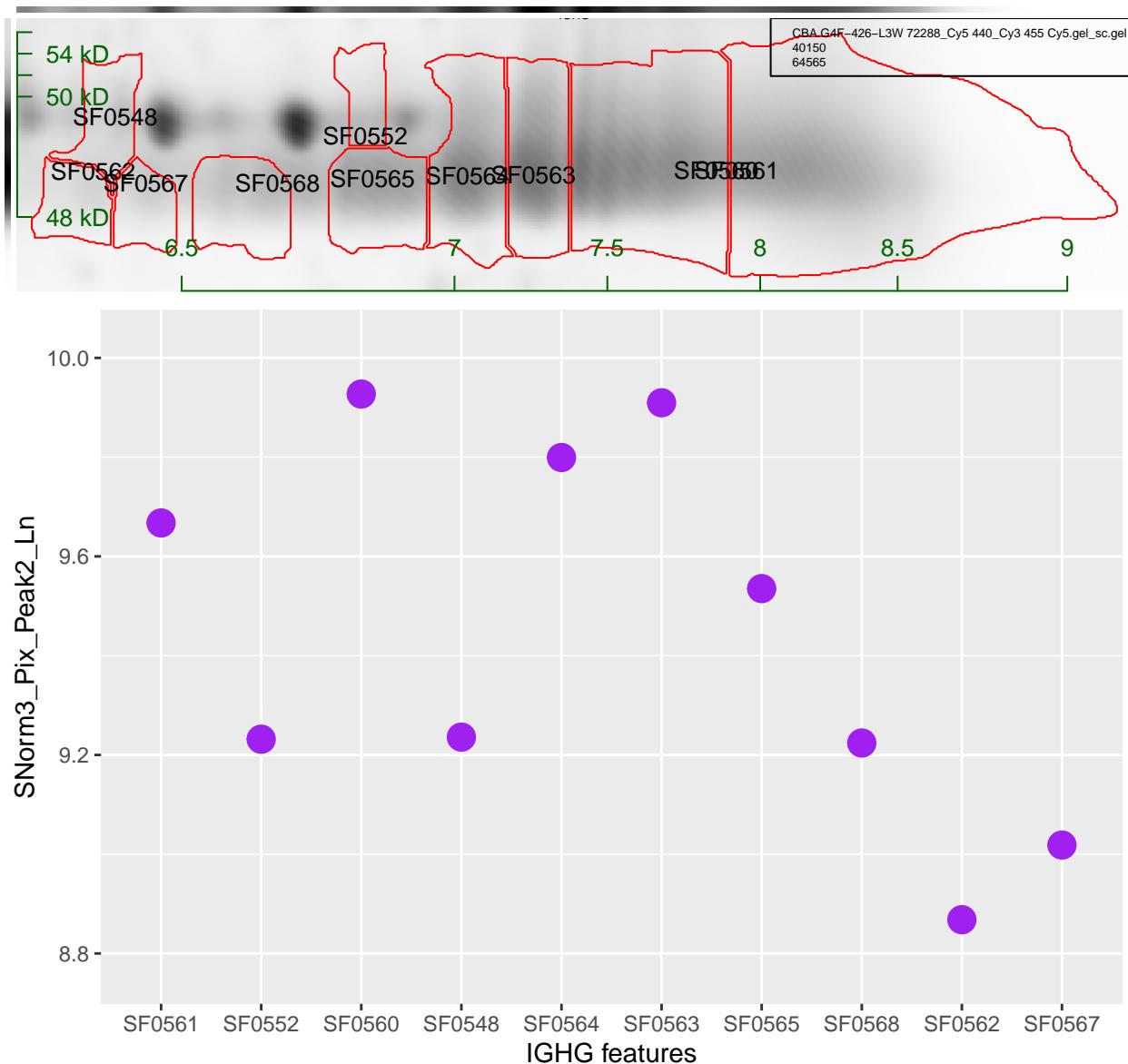
#### CBA.G4F-426-L3W IGHG

##### Replicate 1 : 50014\_Cy5 450\_Cy3 455 Cy5.gel\_sc.gel

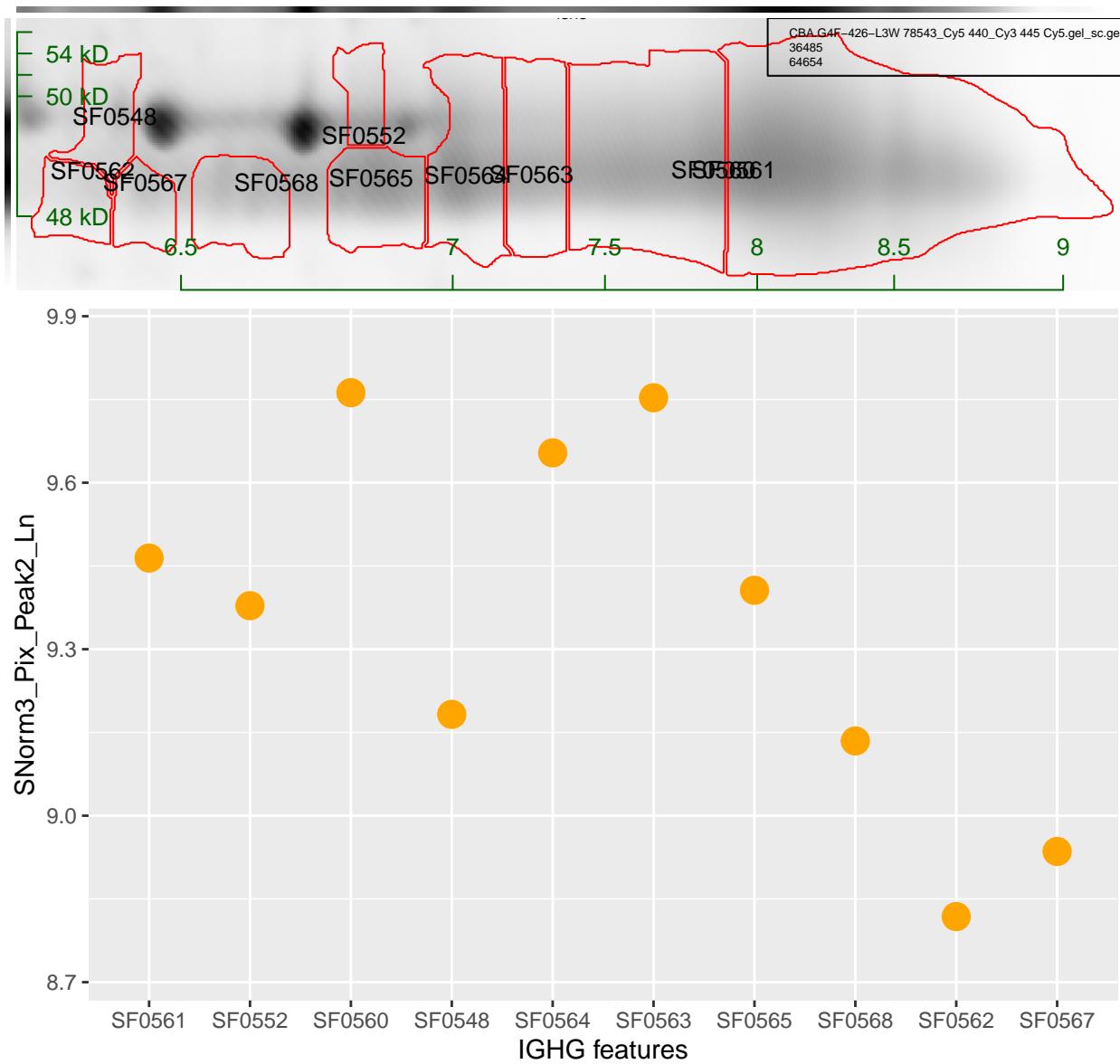


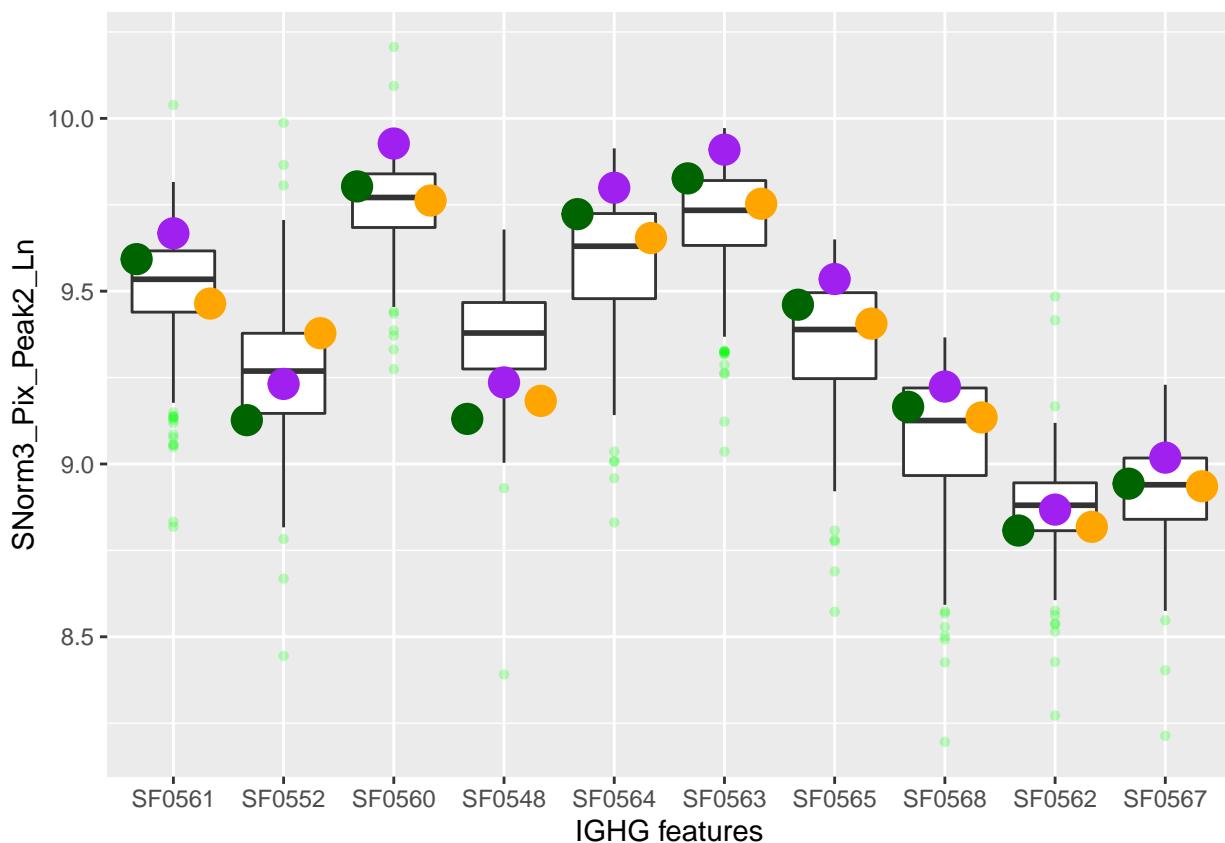


## Replicate 2 : 72288\_Cy5 440\_Cy3 455 Cy5.gel\_sc.gel



## Replicate 3 : 78543\_Cy5 440\_Cy3 445 Cy5.gel\_sc.gel





	SF0561	SF0552	SF0560	SF0548	SF0564	SF0563
50014_Cy5 450_Cy3 455 Cy5	9.592673	9.126850	9.803170	9.130431	9.722924	9.826337
72288_Cy5 440_Cy3 455 Cy5	9.667449	9.231710	9.927204	9.235911	9.799293	9.909569
78543_Cy5 440_Cy3 445 Cy5	9.464207	9.378394	9.762212	9.182558	9.653743	9.753072

	SF0565	SF0568	SF0562	SF0567
50014_Cy5 450_Cy3 455 Cy5	9.460866	9.165448	8.807472	8.943114
72288_Cy5 440_Cy3 455 Cy5	9.535174	9.224046	8.867991	9.018453
78543_Cy5 440_Cy3 445 Cy5	9.406072	9.134647	8.818186	8.935509

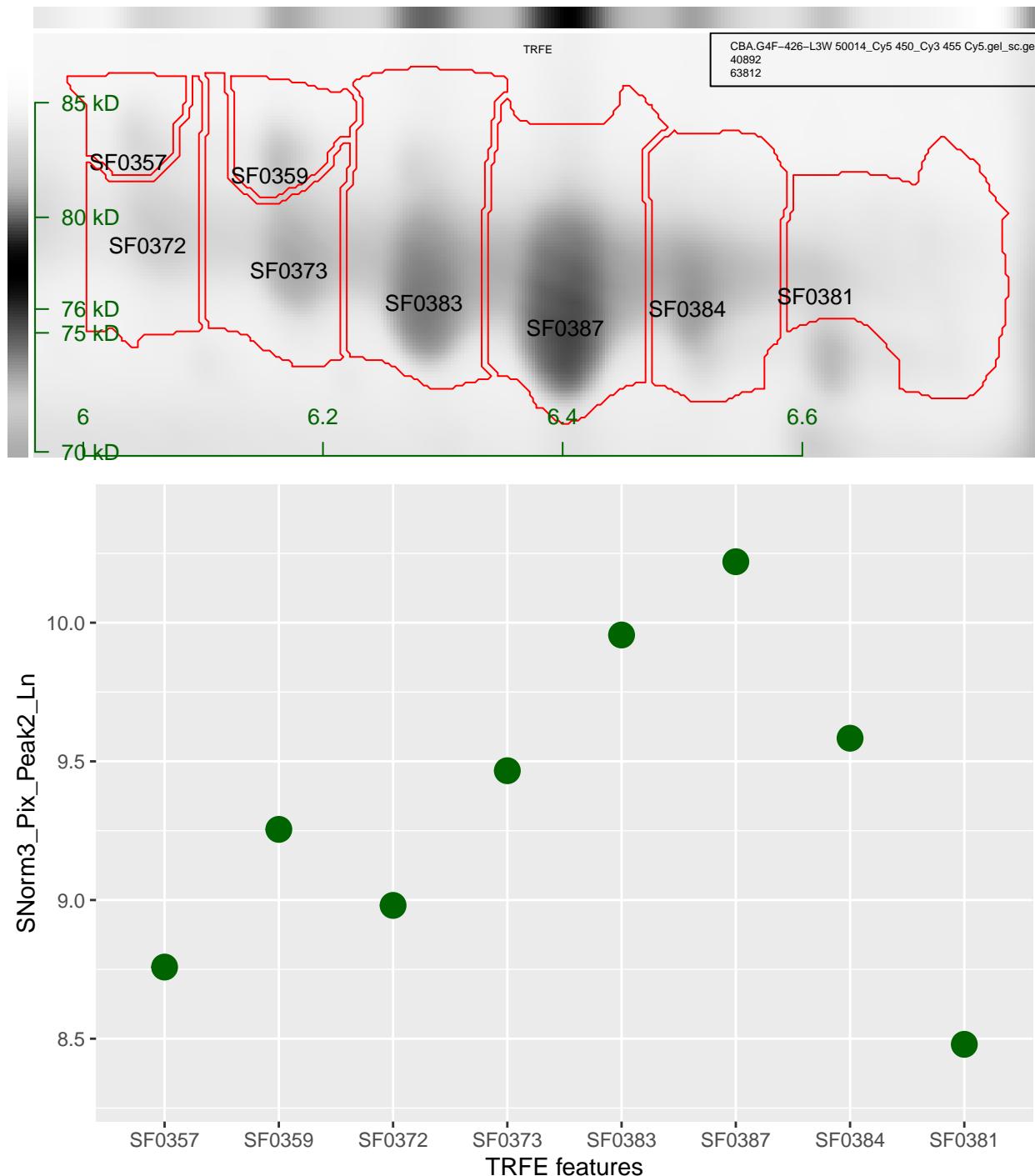
## Transferrin (P02787)

From: Human plasma protein N-glycosylation

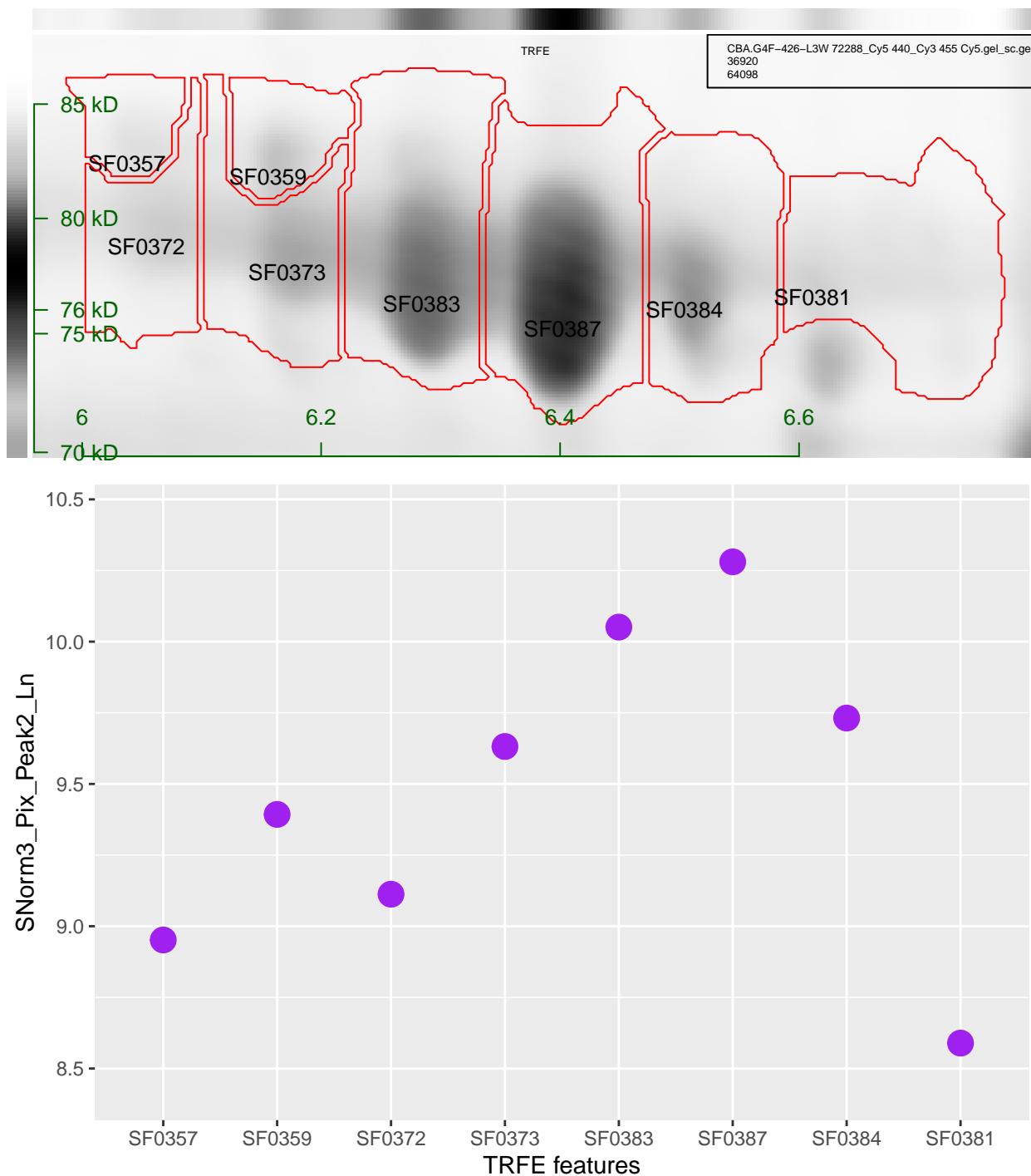
Serotransferrin (STF), also known as transferrin,  $\beta$ 1 metal binding globulin or siderophilin, is a 698 amino acid protein (19 amino acids of which are signal peptide) with a molecular mass of approximately 77 kDa (without glycosylation) [8, 337]. The protein consists of two globular domains, the 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].

**CBA.G4F-426-L3W TRFE**

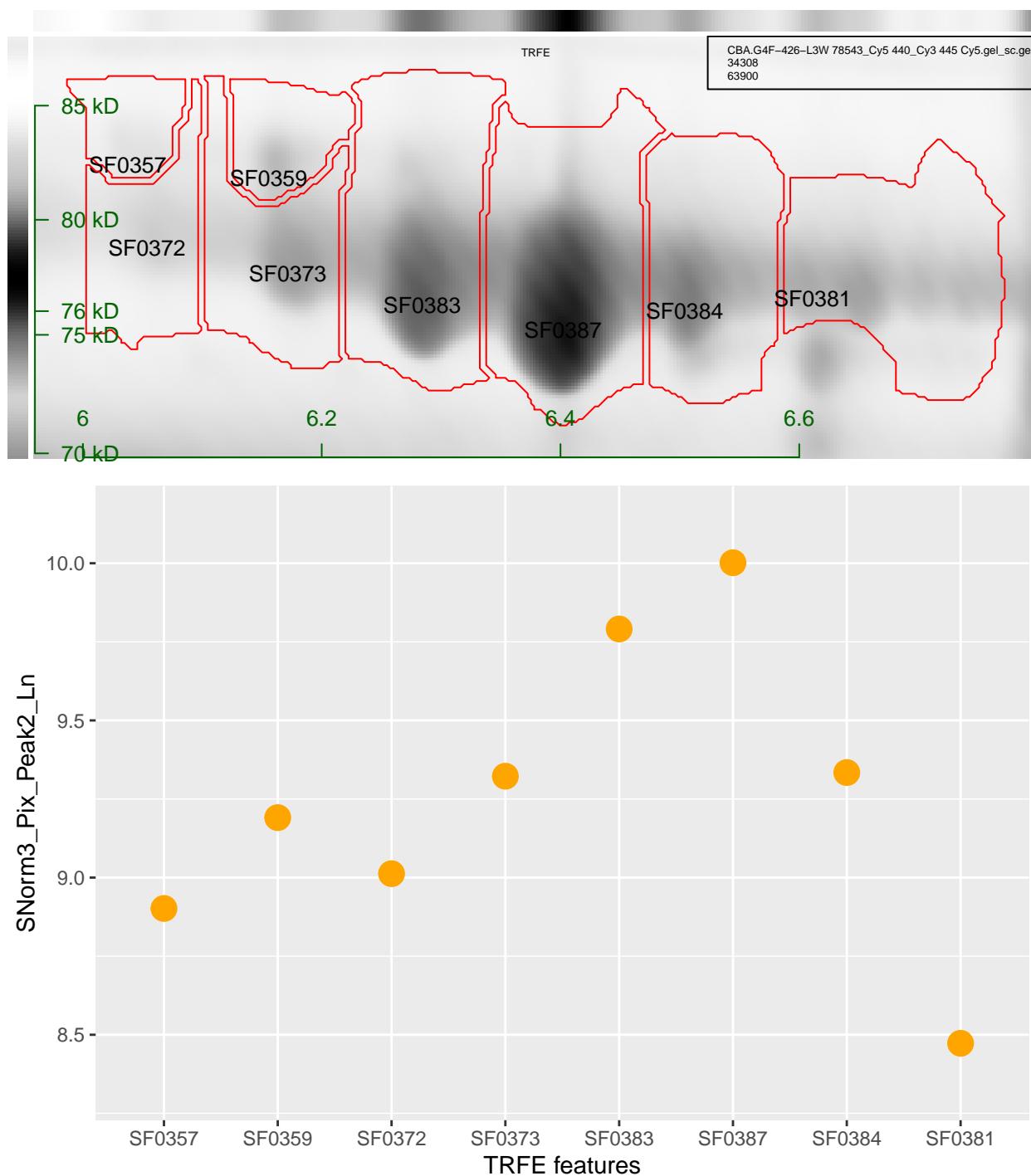
Replicate 1 : 50014\_Cy5 450\_Cy3 455 Cy5.gel\_sc.gel

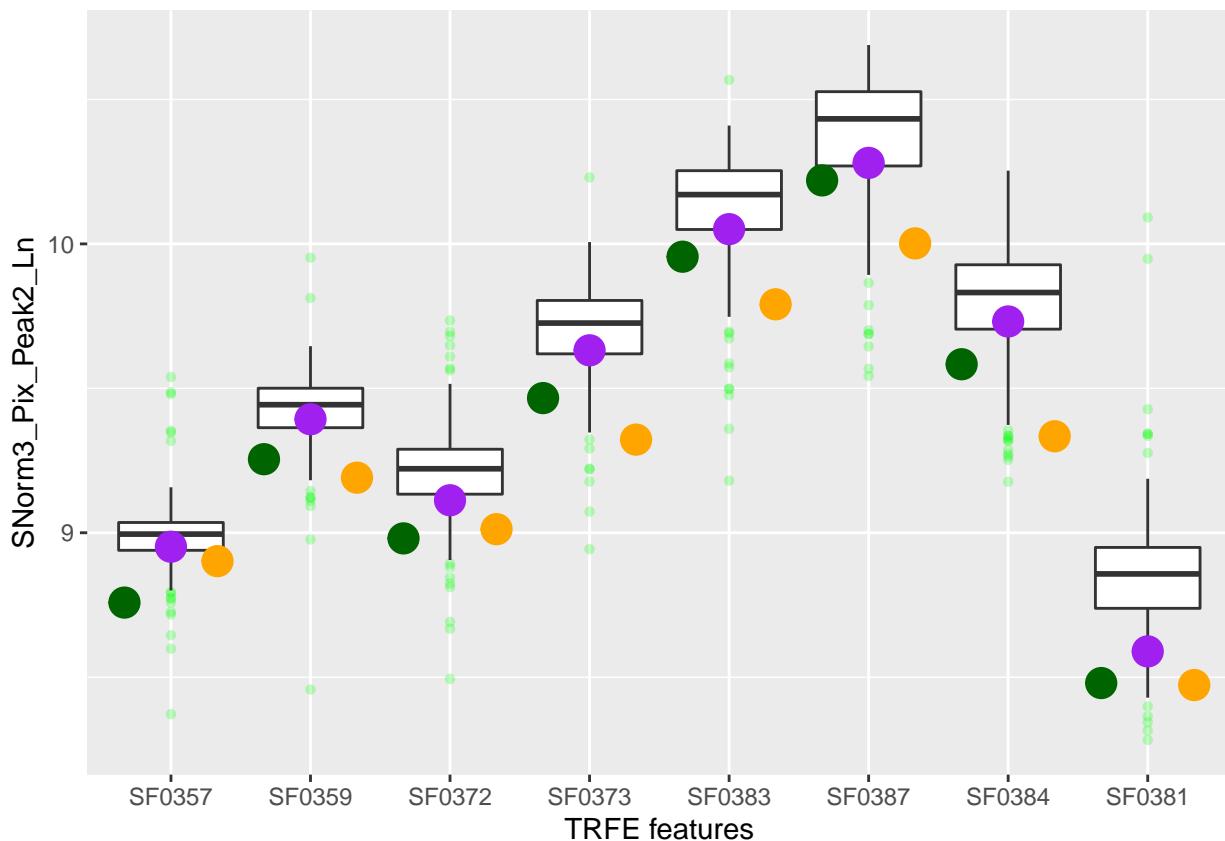


## Replicate 2 : 72288\_Cy5 440\_Cy3 455 Cy5.gel\_sc.gel



## 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

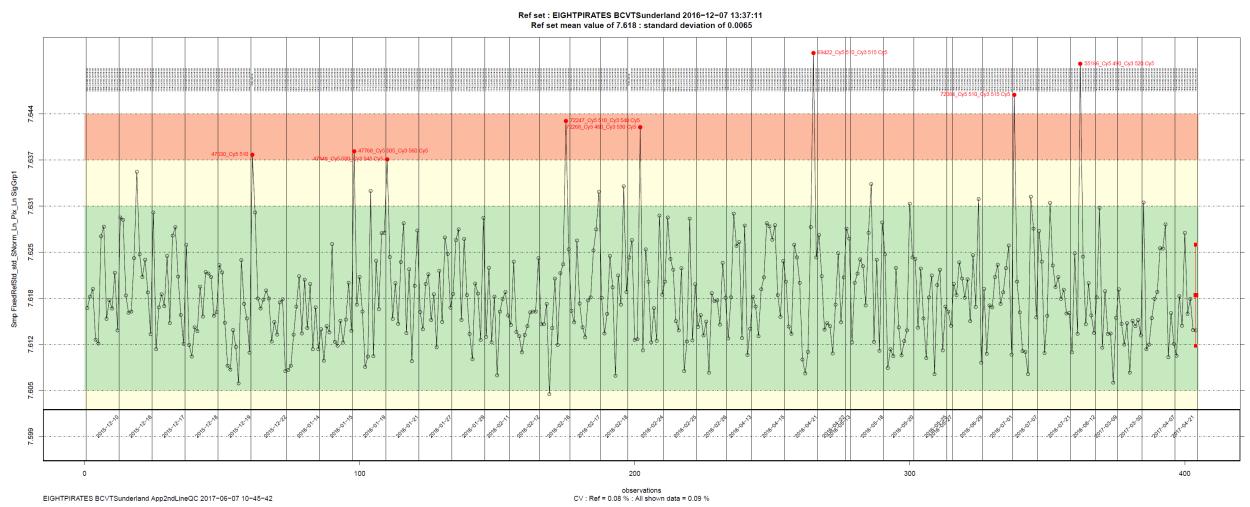
	SF0384	SF0381
50014_Cy5 450_Cy3 455 Cy5	9.583420	8.479699
72288_Cy5 440_Cy3 455 Cy5	9.731512	8.589328
78543_Cy5 440_Cy3 445 Cy5	9.334150	8.472823

## 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	ODD 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 #
<b>1x DIGE buffer</b>	14
<b>Control sample</b>	Human plasma/ SLCC1673
<b>IPG strips</b>	10277023
<b>Reswell Date</b>	22/09/2020
<b>Time strip 1</b>	16:10:00
<b>Time oil added</b>	16:40:00

Equipment	ID #
<b>Reswell tray</b>	3
<b>IPGphor unit</b>	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
<b>1</b>	Human plasma/ SLCC1673	NUBZ640100	49844	2	23.5
<b>2</b>	Human plasma/ SLCC1674	NUCX182416	49845	2	23.5
<b>3</b>	Human plasma/ SLCC1675	NUBZ648698	49846	2	23.5
<b>4</b>	Human plasma/ SLCC1676	NUAQ387360	49847	2	23.5
<b>5</b>	Human plasma/ SLCC1677	NUFH402412	49848	2	23.5
<b>6</b>	Human plasma/ SLCC1678	NUFT865086	49849	2	23.5
<b>7</b>	Human plasma/ SLCC1679	NUFR670624	49850	2	23.5
<b>8</b>	Human plasma/ SLCC1680	NUFT766262	49851	2	23.5
<b>9</b>	Human plasma/ SLCC1681	NUFU715690	49852	2	23.5
<b>10</b>	Human plasma/ SLCC1682	NUFT770122	49853	2	23.5
<b>11</b>	Human plasma/ SLCC1683	NUFT766280	49854	2	23.5
<b>12</b>	Human plasma/ SLCC1684	NUCX210223	49855	2	23.5

## 2D SDS

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

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

Equipment	Set
<b>Gel plates:</b>	1
<b>Casting box:</b>	1
<b>Optimiser:</b>	1

---

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

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