

# virtualBlot program instruction

## System requirements

Java runtime version 1.7

## Input requirements

1. The MS file, two types of MS file applicable to use for generating the immunoblot depending on the experimental design.

### 1.1 Single or barcode experiment format

	A	B	C	D	E	F	G	H	I	J	K
1	Protein ID	Peptide	Slice Number	s1	s2	s3	s4	s5	s6	s7	s8
2	54114993 ras-related protein Rap-1A precursor [Rattus norvegicus]										
3		INVN EIFYDLVR	35	5.24E+06	5.24E+06	5.24E+06	5.24E+06	1.17E+07	1.17E+07	1.17E+07	1.17E+07
4		INVN EIFYDLVR	36	3.20E+06	3.20E+06	3.20E+06	3.20E+06	94891.9	94891.9	94891.9	94891.9
5		NGQG FALVYSIT AQSTFNDLQDLR	35	52268.7	52268.7	52268.7	52268.7	82671.2	82671.2	82671.2	82671.2
6		NGQG FALVYSIT AQSTFNDLQDLR	36	37216.4	37216.4	37216.4	37216.4	3353.88	3353.88	3353.88	3353.88
7		SALT VQFVQGIFVEK	35	824375	824375	824375	824375	762964	762964	762964	762964
8		SALT VQFVQGIFVEK	36	134095	134095	134095	134095	40773.5	40773.5	40773.5	40773.5
9		SALT VQFVQGIFVEK	34	79756.3	79756.3	79756.3	79756.3	88629.7	88629.7	88629.7	88629.7
10		SALT VQFVqGIFVEK	35	347530	347530	347530	347530	704568	704568	704568	704568
11		SKIN VNEIFYDLVR	35	378143	378143	378143	378143	118558	118558	118558	118558
12		SKIN VNEIFYDLVR	36	248002	248002	248002	248002	14909.9	14909.9	14909.9	14909.9
13		SKIN VNEIFYDLVR	34	524323	524323	524323	524323	139749	139749	139749	139749
14		SKIN VNEIFYDLVR	36	338111	338111	338111	338111	20427.7	20427.7	20427.7	20427.7
15		VK DTE DVP MILVGNK	35	1.31E+06	1.31E+06	1.31E+06	1.31E+06	693064	693064	693064	693064
16		VK DTE DVP MILVGNK	35	1.27E+06	1.27E+06	1.27E+06	1.27E+06	1.74E+06	1.74E+06	1.74E+06	1.74E+06
17		VK DTE DVP MILVGNK	35	455930	455930	455930	455930	210281	210281	210281	210281
18		nGQG FALVYSIT AQSTFNDLQDLR	36	43007.9	43007.9	43007.9	43007.9	1422.53	1422.53	1422.53	1422.53
19	10048460 basal cell adhesion molecule precursor [Mus musculus], Bcam										
20		LATQLTGPVMPPIR	32	851024	851024	851024	851024	5.36E+06	5.36E+06	5.36E+06	5.36E+06
21		LATQLTGPVMPPIR	33	1.29E+07	1.29E+07	1.29E+07	1.29E+07	1.16E+07	1.16E+07	1.16E+07	1.16E+07
22		LATQLTGPVMPPIR	33	5.61E+06	5.61E+06	5.61E+06	5.61E+06	4.52E+06	4.52E+06	4.52E+06	4.52E+06
23		STESLQANVQR	33	4.88E+06	4.88E+06	4.88E+06	4.88E+06	3.80E+06	3.80E+06	3.80E+06	3.80E+06
24	28461157 lambda-crystallin homolog [Rattus norvegicus]										
25		IVDDQVILSSSScLLPSK	30	198040	198040	198040	198040	140860	140860	140860	140860
26		LYDIEQQQITNALESIR	30	341736	341736	341736	341736	359936	359936	359936	359936
27		TFGPPVPEFSGDVTEK	30	1.01E+06	1.01E+06	1.01E+06	1.01E+06	1.23E+06	1.23E+06	1.23E+06	1.23E+06
28		VPDDPEHLAAR	30	380570	380570	380570	380570	355543	355543	355543	355543
29	6981110 inositol 1,4,5-trisphosphate receptor type 3 [Rattus norvegicus]										
30		FLQLLcENHNR	7	237280	237280	237280	237280	267698	267698	267698	267698
31		LVAVPHGNDIASLFELDPTTLQK	7	43861.7	43861.7	43861.7	43861.7	112920	112920	112920	112920
32	158187548 merlin [Rattus norvegicus]										
33		AKEADQLKQDLqEAR	32	574504	574504	574504	574504	1.67E+06	1.67E+06	1.67E+06	1.67E+06
34		AKEADQLKQDLqEAR	33	1.60E+06	1.60E+06	1.60E+06	1.60E+06	0	0	0	0
35	9625029 putative hydrolase RBBP9 [Rattus norvegicus]										
36		ASGYFSRPWQWEK	36	451385	451385	451385	451385	172549	172549	172549	172549
37	Gaps										
38		FLQLLcENHNR	7	237280	237280	237280	237280	267698	267698	267698	267698
39		LVAVPHGNDIASLFELDPTTLQK	7	43861.7	43861.7	43861.7	43861.7	112920	112920	112920	112920

### 1.2 Combined multiple experiments format

The data must be sorted by the protein name (peptides of the same protein will be place together)

	A	B	C	D	E	F	G	H	I	J	K	L
1	Protein	Peptide	slice number	S1	S2	S3	S4	S5	S6	S7	S8	
2	10048460 basal cell adhesion molecule precursor [Mus musculus]	AGAAGTSEATSSVR#	5	40048.7	0	0	0	0	52462.9	0	0	
3	10048460 basal cell adhesion molecule precursor [Mus musculus]	AGAAGTSEATSSVR#	6	84894.2	0	0	0	0	117328	0	0	
4	10048460 basal cell adhesion molecule precursor [Mus musculus]	AGAAGTSEATSSVR#	10	76659.9	0	0	0	0	102063	0	0	
5	10048460 basal cell adhesion molecule precursor [Mus musculus]	AGAAGTSEATSSVR	5	39846.3	0	0	0	0	51769.5	0	0	
6	10048460 basal cell adhesion molecule precursor [Mus musculus]	AGAAGTSEATSSVR	6	86993.4	0	0	0	0	121697	0	0	
7	11177922 replication factor C subunit 2 [Mus musculus]	EGNVNIIIAGPPGTGK@	17	128555	0	0	0	0	174537	0	0	
8	11177922 replication factor C subunit 2 [Mus musculus]	LNEIVGNEDTVSR#	17	221638	0	0	0	0	259893	0	0	
9	11177922 replication factor C subunit 2 [Mus musculus]	LNEIVGNEDTVSR	17	221638	0	0	0	0	254108	0	0	
10	11177922 replication factor C subunit 2 [Mus musculus]	VAEGVNSLLQMAGLLAR#	18	8443.28	0	0	0	0	9772.93	0	0	
11	11177922 replication factor C subunit 2 [Mus musculus]	VAEGVNSLLQMAGLLAR#	21	6421.43	0	0	0	0	7434.8	0	0	
12	9790219 dextrin [Mus musculus]	HEYQANGPEDINR#	31	39501.5	0	25106	0	7903.27	54391.5	0	24963.4	
13	9790219 dextrin [Mus musculus]	HEYQANGPEDINR	34	48243.4	0	334570	0	12951.3	63636	0	395816	
14	9845253 heterogeneous nuclear ribonucleoprotein H2 [Mus musculus]	VHIEIGPDGR	18	1.74E+06	0	4.10E+06	0	99870	2.06E+06	0	4.54E+06	
15	9845265 ubiquitin-60S ribosomal protein L40 [Mus musculus]	ESTLHLVLR#	39	261032	0	49379.7	0	23352.2	319606	0	53695.7	
16	9845265 ubiquitin-60S ribosomal protein L40 [Mus musculus]	TITLEVEPSTIENVK@	29	2.61E+06	0	104632	0	37968.3	2.95E+06	0	103146	
17	9845265 ubiquitin-60S ribosomal protein L40 [Mus musculus]	TITLEVEPSTIENVK@	5	1.45E+06	0	1.16E+06	0	18107	1.77E+06	0	1.25E+06	
18	9903607 protein canopy homolog 2 precursor [Mus musculus]	INPDGQSVVQVFPYAR	33	132850	0	183481	0	7058.49	157271	0	179048	
19	9910556 small ubiquitin-related modifier 3 precursor [Mus musculus]	VAGQDGSVVQVQFK@	33	105497	0	1.10E+06	0	70733.9	124143	0	1.20E+06	
20	9910556 small ubiquitin-related modifier 3 precursor [Mus musculus]	VAGQDGSVVQVQFK@	34	343749	0	1.53E+06	0	95472	461812	0	1.58E+06	
21	10048460 basal cell adhesion molecule precursor [Mus musculus]	QQGTQEEQLNVNLIK	13	0	7421.46	233003	0	37486.2	0	5214.24	222957	
22	10048460 basal cell adhesion molecule precursor [Mus musculus]	VEDYDADEEVQLVK@	13	0	20289.8	97112.5	0	38790	0	21032	93760.8	
23	10092608 glutathione S-transferase P 1 [Mus musculus]	PPYTIYFPPVR#	42	0	4510.45	7362.51	0	2818.38	0	5105.83	6314.07	
24	10863927 peptidyl-prolyl cis-trans isomerase A [Homo sapiens]	EGMNIVEAMER#	36	0	648038	111549	0	582389	0	711544	138826	
25	10863927 peptidyl-prolyl cis-trans isomerase A [Homo sapiens]	EGMNIVEAMER#	38	0	166640	137808	0	2678.68	0	172106	152234	
26	10863927 peptidyl-prolyl cis-trans isomerase A [Homo sapiens]	FEDENFILK@	36	0	3.08E+06	278620	0	2527.38	0	3.38E+06	329326	
27	10863927 peptidyl-prolyl cis-trans isomerase A [Homo sapiens]	SIYGKFEFENFILK	35	0	194386	163448	0	4206.25	0	242334	192145	
28	10863927 peptidyl-prolyl cis-trans isomerase A [Homo sapiens]	SIYGKFEFENFILK	36	0	9396.09	41187	0	3368.35	0	11414.2	47307.4	
29	10863927 peptidyl-prolyl cis-trans isomerase A [Homo sapiens]	VSFELFADK	41	0	74287.8	15928.4	0	5314.71	0	100260	21680.5	
30	10863927 peptidyl-prolyl cis-trans isomerase A [Homo sapiens]	VSFELFADK@VPK@	36	0	8287.42	35450.8	0	6064.85	0	15658.8	46082.6	

2. The Protein Marker file, which specify the molecular marker at the particular slice number

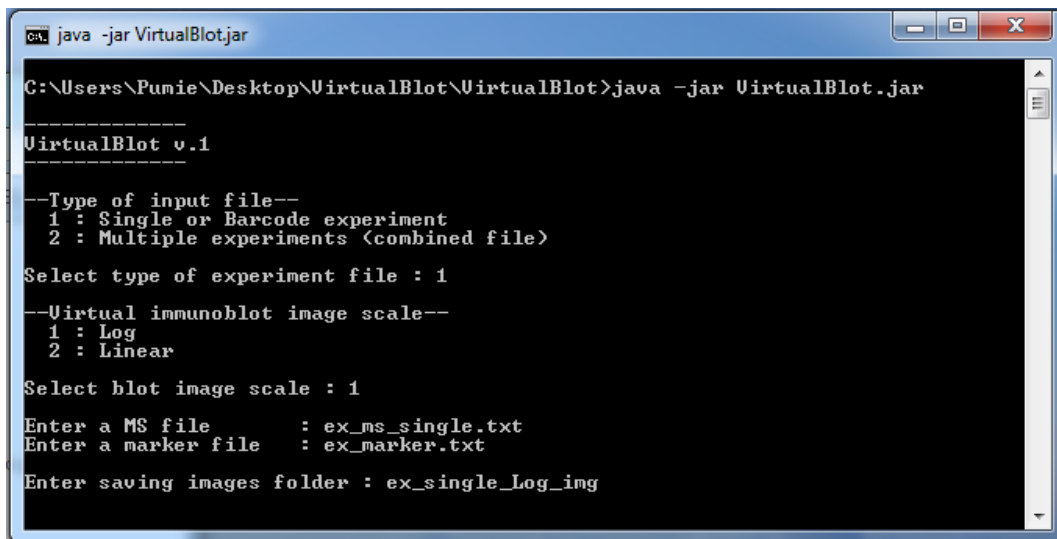
	A	B
1	1	
2	2	
3	3	
4	4	
5	5	
6	6	
7	7	250 kDa
8	8	
9	9	150 kDa
10	10	
11	11	100 kDa
12	12	
13	13	75 kDa
14	14	
15	15	
16	16	
17	17	
18	18	
19	19	
20	20	
21	21	
22	22	
23	23	37 kDa
24	24	
25	25	
26	26	
27	27	
28	28	25 kDa
29	29	
30	30	
31	31	20 kDa
32	32	
33	33	

## How to run the program

The program `virtualBlot.jar` is run under terminal in Max/Unix or `cmd` in Windows. As usual java command line program, user has to Java interpreter `java` to run it as

```
java -jar VirtualBlot.jar.
```

Before run the program, put the MS input and marker files in the same folder with jar file so user do not have to type the full path to the input files. Program will ask you to select type of MS file as mentioned above. Next, it will ask you to choose the image scale; 1 for Logarithm scale and 2 for linear scale. Then, it will ask you to specify the image output folder. Then it will generate all images for every protein in the input file.



```
cmd java -jar VirtualBlot.jar
C:\Users\Pumie\Desktop\VirtualBlot\VirtualBlot>java -jar VirtualBlot.jar
-----
VirtualBlot v.1
-----
--Type of input file--
 1 : Single or Barcode experiment
 2 : Multiple experiments <combined file>
Select type of experiment file : 1
--Virtual immunoblot image scale--
 1 : Log
 2 : Linear
Select blot image scale : 1
Enter a MS file      : ex_ms_single.txt
Enter a marker file  : ex_marker.txt
Enter saving images folder : ex_single_Log_img
```

The peptide intensities were converting into gray scale using linear or logarithm scaling function as user selected. Then all images were save as jpg file format in specified folder. Finally, the program will ask you if you would like to save the intensities matrix files or not.

```
ca. java -jar VirtualBlot.jar
-----
VirtualBlot v.1
-----
--Type of input file--
 1 : Single or Barcode experiment
 2 : Multiple experiments (combined file)
Select type of experiment file : 1
--Virtual immunoblot image scale--
 1 : Log
 2 : Linear
Select blot image scale : 1
Enter a MS file      : ex_ms_single.txt
Enter a marker file  : ex_marker.txt
Enter saving images folder : ex_single_Log_img
Reading file, please wait.
[[Ljava.lang.String;@41c17a8c
samNo: 8
Reading 1 of 1 : 54114993 ras-related protein Rap-1A precursor [Rattus norvegicus]
Reading 2 of 2 : 293341753
Reading 3 of 3 : 28461157 lambda-crystallin homolog [Rattus norvegicus]
Reading 4 of 5 : 6981110 inositol 1,4,5-trisphosphate receptor type 3 [Rattus norvegicus]
Reading 5 of 6 : 158187548 merlin [Rattus norvegicus]
Reading 6 of 7 : 9625029 putative hydrolase RBBP9 [Rattus norvegicus]
Reading 7 of 8 : Gaps
Reading 8 of 9 : 109484558 PREDICTED glyceraldehyde-3-phosphate dehydrogenase-like [Rattus norvegicus]
Reading 9 of 10 : 77539434 aquaporin-2 [Rattus norvegicus]
Generating image file, please wait.
Protein 1 of 9
Protein 2 of 9
Protein 3 of 9
Protein 4 of 9
Protein 5 of 9
Protein 6 of 9
Protein 7 of 9
Protein 8 of 9
Protein 9 of 9
Finished!
Would you like to delete intensities matrix files?
Y or N : Y
Intensities matrix deleted.
--- Press Anykey to Exit ---
```