

Interactomics Basics and Applications
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Lecture – 17
Applications of protein microarrays in Malaria Research-II

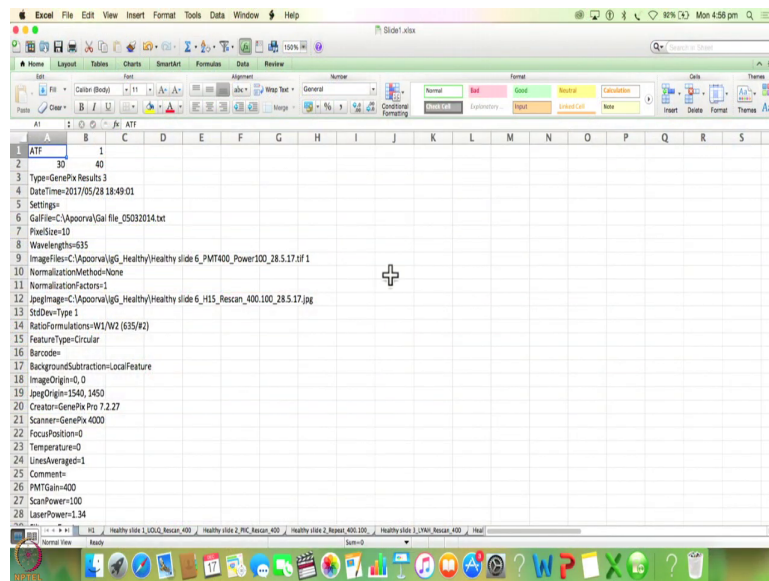
We are discussing about different microarray based platforms and how to perform some biological applications on these steps. In last lecture Ms. Apoorva Venkatesh showed you how to perform a microarray experiment using serum samples obtained from patients who had suffering from falciparum or vivax malaria.

Today we are going to continue the demonstration, and also show you the ways to do data normalization and how to do micro data analysis specifically if your goal was to look for a biological question of interest. In this case, we are going to talk about several ways of how to make meaningful data from the patients who are suffering from malaria using protein microarray based platforms. So, let us have that lecture and demonstration session today.

I am Apoorva Venkatesh your TA for this course. And today we are going to talk about microarray data normalization and analysis. In the last lecture we are trying to profile humeral responses of malaria positive patients using microarray technology. So, we are going to start from there.

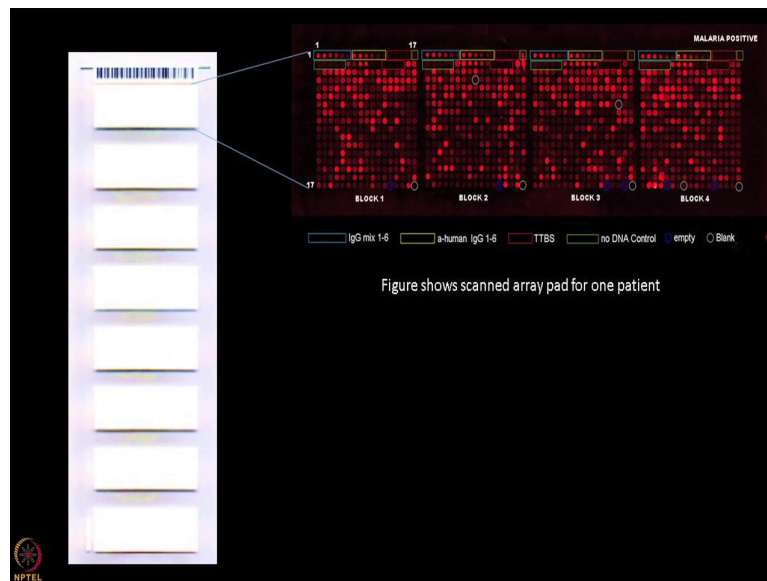
What we are going to do today is to see how to normalize microarray data using excel. So, what we will do is we will start with the raw file you get from the microarray scanner right. So, once you take your slide and you scan it in a scanner, you will extract the raw data.

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And here is the excel sheet you see. This is the type of data you get, I am showing you this one particular slide. So, I will just like to repeat that one slide can probe 8 patients serum.

(Refer Slide Time: 02:07)



So, here in this one particular excel which you see here, we actually have data for 8 patients. So, first what I am going to show you is how to reorganize this data ok. So, let us see first of all what kind of parameters are exported and you will see that all important parameters are provided in this excel, for example, start with pixel size is 10, the slide was scanned at a wavelength of 635 nanometer.

Then you go down normalization method this was not normalized yet, so it says none. Then if you scroll down further you can see the PMT gain which is 400, scan power 100, laser power 1.34. So, basically later on if you want to go back and check these slides again, if for example, differentiation if you forget the parameters we used, you can always go and open this excel to see what you had done right.

(Refer Slide Time: 02:59)

Excel File Edit View Insert Format Tools Data Window Help

File Home Layout Tables Charts SmartArt Formulas Data Review

Font Fill Color (Block) Font Styles Font Color Font Size Font

So, now let us scroll down further, you will see block column and row. So, this is very important again, let us go back to the slide layout one slide can probe 8 patients sera, and one particular pad that is one pad which probes one patient sera has 4 blocks, so which means that if I keep scrolling down. So, every 4 blocks represents 1 patient data right.

(Refer Slide Time: 03:37)

| | A | B | C | D | E | F | G | H | I | J | K | L |
|------|---|----|----|-------------------------|-------|-------|-----|-------|-------|-------|----|---|
| 1175 | 4 | 3 | 17 | hypothetic PVX_09773 | 16160 | 5830 | 170 | 5095 | 5083 | 924 | 18 | |
| 1176 | 4 | 4 | 17 | hypothetic PVX_08502 | 16410 | 5830 | 160 | 6124 | 6032 | 845 | 14 | |
| 1177 | 4 | 5 | 17 | Empty | 16850 | 5820 | 170 | 4961 | 4861 | 1125 | 23 | |
| 1178 | 4 | 6 | 17 | heat shock PFD075w4 | 16930 | 5830 | 170 | 4622 | 4646 | 978 | 21 | |
| 1179 | 4 | 7 | 17 | erythrocyte PFD1235w | 17170 | 5830 | 160 | 5698 | 5646 | 1129 | 19 | |
| 1180 | 4 | 8 | 17 | blank | 17410 | 5840 | 170 | 4863 | 4939 | 896 | 18 | |
| 1181 | 4 | 9 | 17 | amino acid PVX_11457 | 17670 | 5830 | 170 | 4985 | 4991 | 976 | 19 | |
| 1182 | 4 | 10 | 17 | Falstatin, pi PVX_09903 | 17910 | 5830 | 160 | 5273 | 5263 | 1045 | 19 | |
| 1183 | 4 | 11 | 17 | ubiquitin C PVX_09148 | 18160 | 5840 | 160 | 5365 | 5427 | 1203 | 22 | |
| 1184 | 4 | 12 | 17 | hypothetic PVX_00463 | 18410 | 5830 | 170 | 5430 | 5471 | 987 | 18 | |
| 1185 | 4 | 13 | 17 | Empty | 18650 | 5820 | 170 | 4803 | 4821 | 1124 | 23 | |
| 1186 | 4 | 14 | 17 | erythrocyte MAISP1.1 | 18910 | 5830 | 170 | 5219 | 5183 | 906 | 17 | |
| 1187 | 4 | 15 | 17 | erythrocyte PF11_0511 | 19160 | 5830 | 160 | 3966 | 4113 | 901 | 21 | |
| 1188 | 4 | 16 | 17 | Empty | 19400 | 5820 | 170 | 4903 | 4844 | 1218 | 25 | |
| 1189 | 4 | 17 | 17 | Blank | 19650 | 5830 | 170 | 982 | 985 | 51 | 5 | |
| 1190 | 5 | 1 | 1 | IgG mix 1 | 2090 | 10800 | 120 | 6535 | 56473 | 15582 | 27 | |
| 1191 | 5 | 2 | 1 | IgG mix 2 | 2340 | 10810 | 140 | 39073 | 37378 | 7767 | 20 | |
| 1192 | 5 | 3 | 1 | IgG mix 3 | 2590 | 10810 | 140 | 33724 | 31295 | 8292 | 26 | |
| 1193 | 5 | 4 | 1 | IgG mix 4 | 2840 | 10800 | 140 | 23712 | 22371 | 5342 | 23 | |
| 1194 | 5 | 5 | 1 | IgG mix 5 | 3090 | 10810 | 140 | 11551 | 11455 | 2602 | 22 | |
| 1195 | 5 | 6 | 1 | IgG mix 6 | 3340 | 10810 | 150 | 4586 | 4652 | 1013 | 21 | |
| 1196 | 5 | 7 | 1 | a-human Ig a-human Ig | 3590 | 10800 | 120 | 6535 | 61227 | 11580 | 18 | |
| 1197 | 5 | 8 | 1 | a-human Ig a-human Ig | 3840 | 10800 | 140 | 28638 | 26863 | 7737 | 28 | |
| 1198 | 5 | 9 | 1 | a-human Ig a-human Ig | 4090 | 10800 | 140 | 18224 | 17484 | 4939 | 28 | |
| 1199 | 5 | 10 | 1 | a-human Ig a-human Ig | 4340 | 10800 | 140 | 6985 | 6849 | 1758 | 25 | |
| 1200 | 5 | 11 | 1 | a-human Ig a-human Ig | 4590 | 10800 | 140 | 3102 | 3186 | 635 | 19 | |
| 1201 | 5 | 12 | 1 | a-human Ig a-human Ig | 4840 | 10810 | 130 | 1841 | 1833 | 240 | 13 | |
| 1202 | 5 | 13 | 1 | TTB5 | 5080 | 10800 | 170 | 1119 | 1125 | 102 | 9 | |

So, then when I keep scrolling down and I go to block 5 a new patient begins, so that is what I am going to talk to you about how to reorganize this. So, for example, if you see here four ends here right with blank and you will see that there is an IgG 1, IgG mix 1 which starts again. So, this is basically your new patient.

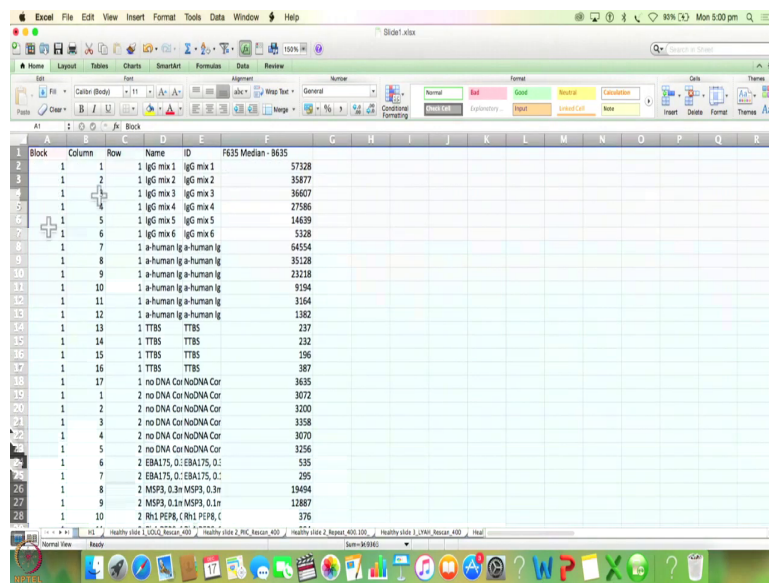
So, what we are going to do is, we are going to first reorganize this, but before this let me tell you which are the columns which are important for us. So, now I am going to scroll back up, and we are going to go through the columns which we have on this excel. So, now, apart from block column, row, the name, and the id basically this is your protein id, we do not need any of the other columns except for column.

So, you see column here. So, this is basically your F 635 median minus B 635 which is your basically your background signals right. So, this is our the column which we actually extract

and use for an analysis, and we do not need any other column here. So, what I am going to do, I am going to first delete all unwanted columns to make this excel less complicated.

So, let us delete all of this, and then we go and delete this as well. We also do not need these parameters for the analysis, I am also going to delete this. So, finally, this is what you get.

(Refer Slide Time: 04:55)



| Block | Column | Row | Name | ID | F635 Median - B635 |
|-------|--------|-----|---------------------------|-------|--------------------|
| 1 | 1 | 1 | 1 IgG mix 1 | 57328 | |
| 2 | 1 | 2 | 1 IgG mix 2 | 35877 | |
| 3 | 1 | 3 | 1 IgG mix 3 | 36807 | |
| 4 | 1 | 4 | 1 IgG mix 4 | 27586 | |
| 5 | 1 | 5 | 1 IgG mix 5 | 14639 | |
| 6 | 1 | 6 | 1 IgG mix 6 | 5328 | |
| 7 | 1 | 7 | 1 a-human Ig a-human Ig | 64554 | |
| 8 | 1 | 8 | 1 a-human Ig a-human Ig | 35128 | |
| 9 | 1 | 9 | 1 a-human Ig a-human Ig | 23218 | |
| 10 | 1 | 10 | 1 a-human Ig a-human Ig | 9354 | |
| 11 | 1 | 11 | 1 a-human Ig a-human Ig | 3164 | |
| 12 | 1 | 12 | 1 a-human Ig a-human Ig | 1382 | |
| 13 | 1 | 13 | 1 TTBS | 237 | |
| 14 | 1 | 14 | 1 TTBS | 232 | |
| 15 | 1 | 15 | 1 TTBS | 196 | |
| 16 | 1 | 16 | 1 TTBS | 387 | |
| 17 | 1 | 17 | 1 no DNA Cor NoDNA Cor | 3635 | |
| 18 | 1 | 1 | 2 no DNA Cor NoDNA Cor | 3072 | |
| 19 | 1 | 2 | 2 no DNA Cor NoDNA Cor | 3200 | |
| 20 | 1 | 3 | 2 no DNA Cor NoDNA Cor | 3358 | |
| 21 | 1 | 4 | 2 no DNA Cor NoDNA Cor | 3070 | |
| 22 | 1 | 5 | 2 no DNA Cor NoDNA Cor | 3256 | |
| 23 | 1 | 6 | 2 EBAL75, 0: EBAL75, 0: | 535 | |
| 24 | 1 | 7 | 2 EBAL75, 0: EBAL75, 0: | 295 | |
| 25 | 1 | 8 | 2 MSP3, 0.3n MSP3, 0.3n | 19494 | |
| 26 | 1 | 9 | 2 MSP3, 0.1n MSP3, 0.1n | 12887 | |
| 27 | 1 | 10 | 2 RH1 PEPR, C RH1 PEPR, C | 376 | |

So, now you keep scrolling down and then you arrange all patients side by side. So, when you do that this is what you get.

(Refer Slide Time: 05:07)

| Index | Row | Column | Spot | Spot Array | ADI spot ID (gal file) | ORF | PlasmidID | ORF_Fragment | Description |
|-------|-----|--------|------|------------|------------------------|-----|-----------|--------------|------------------|
| 1 | 1 | 1 | 1 | 1 | 1 IgG mix 1 | N/A | N/A | | IgG mix 1 |
| 2 | 1 | 1 | 2 | 2 | 2 IgG mix 2 | N/A | N/A | | IgG mix 2 |
| 3 | 1 | 1 | 3 | 3 | 3 IgG mix 3 | N/A | N/A | | IgG mix 3 |
| 4 | 1 | 1 | 4 | 4 | 4 IgG mix 4 | N/A | N/A | | IgG mix 4 |
| 5 | 1 | 1 | 5 | 5 | 5 IgG mix 5 | N/A | N/A | | IgG mix 5 |
| 6 | 1 | 1 | 6 | 6 | 6 IgG mix 6 | N/A | N/A | | IgG mix 6 |
| 7 | 1 | 1 | 7 | 7 | 7 a-human IgG 1 | N/A | N/A | | anti-human IgG 1 |
| 8 | 1 | 1 | 8 | 8 | 8 a-human IgG 2 | N/A | N/A | | anti-human IgG 2 |
| 9 | 1 | 1 | 9 | 9 | 9 a-human IgG 3 | N/A | N/A | | anti-human IgG 3 |
| 10 | 1 | 1 | 10 | 10 | 10 a-human IgG 4 | N/A | N/A | | anti-human IgG 4 |
| 11 | 1 | 1 | 11 | 11 | 11 a-human IgG 5 | N/A | N/A | | anti-human IgG 5 |
| 12 | 1 | 1 | 12 | 12 | 12 a-human IgG 6 | N/A | N/A | | anti-human IgG 6 |
| 13 | 1 | 1 | 13 | 13 | 13 TTBS | N/A | N/A | | TTBS |
| 14 | 1 | 1 | 14 | 14 | 14 TTBS | N/A | N/A | | TTBS |
| 15 | 1 | 1 | 15 | 15 | 15 TTBS | N/A | N/A | | TTBS |
| 16 | 1 | 1 | 16 | 16 | 16 TTBS | N/A | N/A | | TTBS |
| 17 | 1 | 1 | 17 | 17 | 17 noDNA | N/A | N/A | | noDNA Control |
| 18 | 1 | 1 | 18 | 18 | 18 noDNA | N/A | N/A | | noDNA Control |
| 19 | 1 | 1 | 19 | 19 | 19 noDNA | N/A | N/A | | noDNA Control |
| 20 | 1 | 1 | 20 | 20 | 20 noDNA | N/A | N/A | | noDNA Control |

So, you also see that there are additional columns here this is what you get from your gal file.

(Refer Slide Time: 05:13)

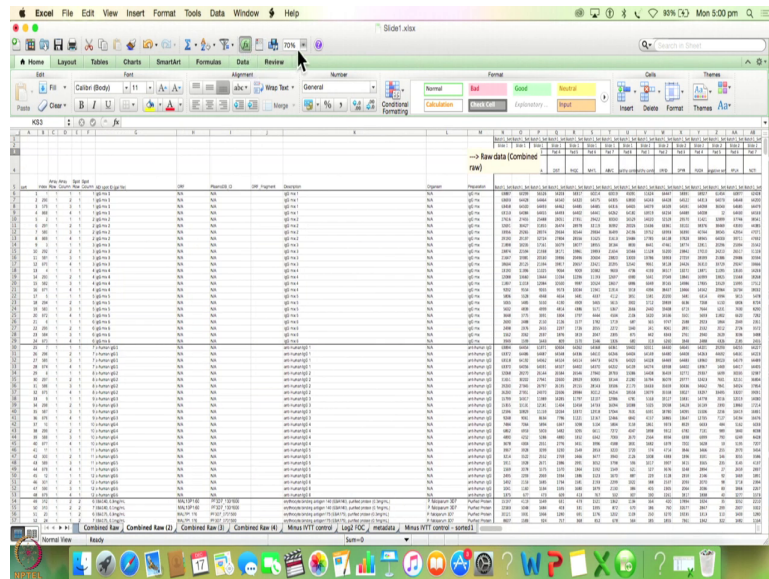
| | AB | AC | AD | AE | AF | AG | AH | AI | AJ | AK | AL | AM | AN | AO | AP | AQ | AR | AS |
|----|------------|------------|------------|------------|------------|------------|------------|-------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|--------------|
| 1 | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set |
| 2 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 |
| 3 | Pad 2 | Pad 3 | Pad 4 | Pad 5 | Pad 6 | Pad 7 | Pad 8 | Pad 1 | Pad 2 | Pad 3 | Pad 4 | Pad 5 | Pad 6 | Pad 7 | Pad 8 | Pad 1 | Pad 2 | Pad 3 |
| 4 | RICE | LMIA | DIST | FIQC | MHTL | ABVC | valthy | contwealthy | contri | ERFD | DPRR | FUOK | Vegetative | seri | RPLH | NCTI | positive | controactive |
| 5 | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set | Batch1_Set |
| 6 | 60299 | 56526 | 54203 | 58317 | 60214 | 60019 | 45091 | 51624 | 64447 | 58391 | 58927 | 61454 | 60877 | 62428 | 61427 | 64117 | 64425 | 64539 |
| 7 | 27455 | 25488 | 23051 | 27351 | 29422 | 30030 | 16529 | 14020 | 52529 | 29570 | 31421 | 32899 | 37746 | 38541 | 39029 | 32560 | 40244 | 29579 |
| 8 | 18235 | 17161 | 16079 | 18077 | 18955 | 18184 | 8833 | 8441 | 47461 | 18774 | 22811 | 20296 | 25394 | 25542 | 24584 | 22787 | 26667 | 19118 |
| 9 | 11396 | 11025 | 9064 | 9009 | 10382 | 9603 | 4736 | 4193 | 34517 | 13272 | 13871 | 11095 | 13535 | 14233 | 12192 | 13738 | 16799 | 10791 |
| 10 | 5528 | 4948 | 4654 | 3481 | 4337 | 4112 | 1851 | 1581 | 20200 | 5481 | 6314 | 4994 | 5815 | 5478 | 6116 | 6543 | 7063 | 5138 |
| 11 | 2488 | 2150 | 2126 | 1577 | 1782 | 1719 | 687 | 565 | 9747 | 2588 | 2929 | 1864 | 2349 | 2351 | 3673 | 2612 | 3393 | 2362 |
| 12 | 64454 | 63871 | 60604 | 64262 | 64568 | 64363 | 59402 | 50311 | 64303 | 64645 | 64201 | 29293 | 64255 | 64227 | 63924 | 64179 | 64158 | 64517 |
| 13 | 28270 | 26144 | 26584 | 26546 | 27840 | 28783 | 15086 | 14408 | 36459 | 32772 | 29337 | 6699 | 30035 | 32987 | 35097 | 33594 | 38812 | 33885 |
| 14 | 14317 | 11389 | 14285 | 11797 | 12107 | 12986 | 6781 | 5163 | 19127 | 15831 | 14778 | 2016 | 12019 | 14080 | 16662 | 14941 | 18089 | 14649 |
| 15 | 7064 | 5894 | 6347 | 5098 | 5104 | 5804 | 3159 | 1861 | 9373 | 8929 | 6633 | 484 | 5162 | 6033 | 7668 | 6813 | 8638 | 6753 |
| 16 | 3928 | 3299 | 3230 | 2549 | 2853 | 3220 | 1720 | 574 | 4714 | 3846 | 3406 | 255 | 2970 | 3454 | 4012 | 3304 | 4272 | 3448 |
| 17 | 2253 | 2069 | 1994 | 1386 | 1513 | 1670 | 887 | 229 | 3128 | 2359 | 2146 | 62 | 1469 | 1891 | 2049 | 1685 | 2731 | 1907 |
| 18 | 1115 | 974 | 832 | 188 | 210 | 241 | 151 | 40 | 1450 | 896 | 952 | 32 | 291 | 276 | 285 | 541 | 1158 | 838 |
| 19 | 562 | 551 | 625 | 141 | 145 | 170 | 82 | -8 | 1118 | 514 | 865 | 11 | 221 | 203 | 146 | 328 | 727 | 516 |
| 20 | 785 | 522 | 536 | 315 | 439 | 433 | 266 | -19 | 1054 | 524 | 502 | 15 | 398 | 475 | 540 | 280 | 1023 | 444 |
| 21 | 588 | 771 | 776 | 356 | 340 | 436 | 297 | -10 | 1252 | 589 | 691 | 96 | 388 | 443 | 494 | 295 | 359 | 546 |
| 22 | 3136 | 3439 | 4945 | 3126 | 5306 | 30843 | 1612 | 1549 | 6658 | 1031 | 5205 | 266 | 1479 | 2390 | 4061 | 5768 | 1357 | 2964 |
| 23 | 3389 | 3138 | 5739 | 4324 | 9238 | 10328 | 4864 | 1821 | 8522 | 2171 | 5526 | 1009 | 2644 | 4715 | 7035 | 6487 | 1425 | 3310 |
| 24 | 3026 | 2690 | 6000 | 3602 | 7584 | 14772 | 3029 | 1661 | 8278 | 2098 | 5782 | 449 | 1543 | 2682 | 4321 | 6769 | 1444 | 3268 |
| 25 | 1871 | 1634 | 6374 | 7590 | 6067 | 11348 | 3063 | 1713 | 7814 | 1771 | 6165 | 457 | 1006 | 474 | 1100 | 4432 | 6313 | 1301 |

And when you scroll right, you see that you all the patients are now placed next to each other right. So, this is that kind of excel you get first. Now, what I am going to do is, I am going to reorganize this excel to make it easier. This is combined data for all your patients and now we will reorganize the proteins. So, as you will see the proteins are present in the same order as they are present in your slide.

So, what we first do is now that this is common data for all patients we have already put them together, we are going to bring the IgG mix from all four blocks together. So, I will repeat this slide layout once more. Now, you have 6 IgG mix here this is present in your block 1. Similarly, you have the same spots present in all 4 blocks of the same pad.

So, what I am trying to do here, I am trying to get all the IgG mix together. So, you will have 24 such spots one after the other. We will also do the same thing for your anti human IgG mix. And similarly we are also going to do the same thing for your next spots.

(Refer Slide Time: 06:19)



So, when we rearrange our excel, this is how your excel will look.

(Refer Slide Time: 06:27)

---> Raw data (Cor raw)

| ORF | PlasmidOB_ID | ORF_Fragment | Description | Organism | Preparation | Batch1_Set Batc |
|-----|--------------|--------------|-------------|----------|-------------|-----------------|
| N/A | N/A | | IgG mix 1 | N/A | IgG mix | 63887 |
| N/A | N/A | | IgG mix 1 | N/A | IgG mix | 63693 |
| N/A | N/A | | IgG mix 1 | N/A | IgG mix | 63458 |
| N/A | N/A | | IgG mix 1 | N/A | IgG mix | 63153 |
| N/A | N/A | | IgG mix 2 | N/A | IgG mix | 27516 |
| N/A | N/A | | IgG mix 2 | N/A | IgG mix | 32591 |
| N/A | N/A | | IgG mix 2 | N/A | IgG mix | 33956 |
| N/A | N/A | | IgG mix 2 | N/A | IgG mix | 29190 |
| N/A | N/A | | IgG mix 3 | N/A | IgG mix | 21898 |
| N/A | N/A | | IgG mix 3 | N/A | IgG mix | 23874 |
| N/A | N/A | | IgG mix 3 | N/A | IgG mix | 21647 |
| N/A | N/A | | IgG mix 3 | N/A | IgG mix | 18524 |
| N/A | N/A | | IgG mix 4 | N/A | IgG mix | 13190 |
| N/A | N/A | | IgG mix 4 | N/A | IgG mix | 12088 |
| N/A | N/A | | IgG mix 4 | N/A | IgG mix | 11837 |
| N/A | N/A | | IgG mix 4 | N/A | IgG mix | 9292 |
| N/A | N/A | | IgG mix 5 | N/A | IgG mix | 5806 |
| N/A | N/A | | IgG mix 5 | N/A | IgG mix | 5065 |
| N/A | N/A | | IgG mix 5 | N/A | IgG mix | 5632 |
| N/A | N/A | | IgG mix 6 | N/A | IgG mix | 9648 |

What I have done here I have put all the 24 IgG mix of 1 pad together right.

(Refer Slide Time: 06:35)

| sort | Index | Array | Spot | Spot | ADI spot ID (gal file) | ORF | Plasmid_ID | ORF_Fragment | Description |
|------|-------|-------|------|------|------------------------|-----------|------------|--------------|-------------|
| 1 | 1 | 1 | 1 | 1 | 1 | IgG mix 1 | N/A | N/A | IgG mix 1 |
| 2 | 2 | 290 | 1 | 2 | 1 | IgG mix 1 | N/A | N/A | IgG mix 1 |
| 3 | 3 | 579 | 1 | 3 | 1 | IgG mix 1 | N/A | N/A | IgG mix 1 |
| 4 | 4 | 868 | 1 | 4 | 1 | IgG mix 1 | N/A | N/A | IgG mix 1 |
| 5 | 5 | 2 | 1 | 1 | 1 | IgG mix 2 | N/A | N/A | IgG mix 2 |
| 6 | 5 | 291 | 1 | 2 | 1 | IgG mix 2 | N/A | N/A | IgG mix 2 |
| 7 | 7 | 580 | 1 | 3 | 1 | IgG mix 2 | N/A | N/A | IgG mix 2 |
| 8 | 8 | 869 | 1 | 4 | 1 | IgG mix 2 | N/A | N/A | IgG mix 2 |
| 9 | 9 | 3 | 1 | 1 | 1 | IgG mix 3 | N/A | N/A | IgG mix 3 |
| 10 | 10 | 292 | 1 | 2 | 1 | IgG mix 3 | N/A | N/A | IgG mix 3 |
| 11 | 11 | 581 | 1 | 3 | 1 | IgG mix 3 | N/A | N/A | IgG mix 3 |
| 12 | 12 | 870 | 1 | 4 | 1 | IgG mix 3 | N/A | N/A | IgG mix 3 |
| 13 | 13 | 4 | 1 | 1 | 1 | IgG mix 4 | N/A | N/A | IgG mix 4 |
| 14 | 14 | 293 | 1 | 2 | 1 | IgG mix 4 | N/A | N/A | IgG mix 4 |
| 15 | 15 | 582 | 1 | 3 | 1 | IgG mix 4 | N/A | N/A | IgG mix 4 |
| 16 | 16 | 871 | 1 | 4 | 1 | IgG mix 4 | N/A | N/A | IgG mix 4 |
| 17 | 17 | 5 | 1 | 1 | 1 | IgG mix 5 | N/A | N/A | IgG mix 5 |
| 18 | 18 | 294 | 1 | 2 | 1 | IgG mix 5 | N/A | N/A | IgG mix 5 |
| 19 | 19 | 583 | 1 | 3 | 1 | IgG mix 5 | N/A | N/A | IgG mix 5 |
| 20 | 20 | 872 | 1 | 4 | 1 | IgG mix 5 | N/A | N/A | IgG mix 5 |
| 21 | 21 | 5 | 1 | 1 | 1 | IgG mix 6 | N/A | N/A | IgG mix 6 |
| 22 | 22 | 295 | 1 | 2 | 1 | IgG mix 6 | N/A | N/A | IgG mix 6 |
| 23 | 23 | 584 | 1 | 3 | 1 | IgG mix 6 | N/A | N/A | IgG mix 6 |
| 24 | 24 | 873 | 1 | 4 | 1 | IgG mix 6 | N/A | N/A | IgG mix 6 |

We will go through all the columns once more. For example, these are all just your spot details. Now, we will go to your ADI spot id which is your gal file. So, this is what you get from your gal file, I will come to this in a minute. Before that let us talk about ORF. So, this column here is basically your id.

(Refer Slide Time: 06:55)

| | | | | | | | | | | | |
|----|----|-----|---|---|----|----|---|---------------|---------------|--|---|
| 52 | 52 | 24 | 1 | 1 | 2 | 7 | EB175, 0.1mg/ml | MAU7P1.178 | PF3D7_0731500 | | erythrocyte binding antigen175 (EB175), purified p |
| 53 | 53 | 801 | 1 | 3 | 2 | 6 | MSP1, 0.3mg/ml | PF1478w | PF3D7_0930300 | | merozoite surface protein 1 (MSP1), purified protein |
| 54 | 54 | 802 | 1 | 3 | 2 | 7 | MSP1, 0.3mg/ml | PF1478w | PF3D7_0930300 | | merozoite surface protein 1 (MSP1), purified protein |
| 55 | 55 | 800 | 1 | 4 | 2 | 8 | MSP1, 0.3mg/ml | PF1478w | PF3D7_0930300 | | merozoite surface protein 2 (MSP2), purified protein |
| 56 | 56 | 891 | 1 | 4 | 2 | 7 | MSP1, 0.3mg/ml | PF1478w | PF3D7_0930300 | | merozoite surface protein 2 (MSP2), purified protein |
| 57 | 57 | 25 | 1 | 1 | 2 | 8 | MSP1, 0.3mg/ml | PF10_0345 | PF3D7_1035400 | | merozoite surface protein 3 (MSP3), purified protein |
| 58 | 58 | 26 | 1 | 1 | 2 | 9 | MSP1, 0.3mg/ml | PF10_0345 | PF3D7_1035400 | | merozoite surface protein 3 (MSP3), purified protein |
| 59 | 59 | 894 | 1 | 4 | 2 | 10 | PF CSP, 0.1mg/ml | MAU3P2.11 | PF3D7_0334400 | | circumsporozoite protein (CSP), purified protein (0.3 |
| 60 | 60 | 895 | 1 | 4 | 2 | 11 | PF CSP, 0.1mg/ml | MAU3P2.11 | PF3D7_0334400 | | circumsporozoite protein (CSP), purified protein (0.1 |
| 61 | 61 | 896 | 1 | 3 | 2 | 10 | PF LSA1, 0.1mg/ml | PF10_0356 | PF3D7_1038400 | | circumsporozoite protein (CSP), purified protein (0.3mg |
| 62 | 62 | 806 | 1 | 3 | 2 | 11 | PF LSA1, 0.1mg/ml | PF10_0356 | PF3D7_1038400 | | circumsporozoite protein (CSP), purified protein (0.1mg |
| 63 | 63 | 318 | 1 | 2 | 2 | 10 | Rh1 PEP1, 0.3mg/ml | PF3D7_0402300 | | reticulocyte binding protein homologue 1 (RH1) PEF | |
| 64 | 64 | 317 | 1 | 2 | 2 | 11 | Rh1 PEP1, 0.3mg/ml | PF3D7_0402300 | | reticulocyte binding protein homologue 1 (RH1) PEF | |
| 65 | 65 | 27 | 1 | 1 | 2 | 10 | Rh1 PEP1, 0.3mg/ml | PF3D7_0402300 | | reticulocyte binding protein homologue 1 (RH1) PEF | |
| 66 | 66 | 28 | 1 | 1 | 2 | 11 | Rh1 PEP1, 0.3mg/ml | PF3D7_0402300 | | reticulocyte binding protein homologue 1 (RH1) PEF | |
| 67 | 67 | 314 | 1 | 2 | 2 | 9 | Rh2, 0.3mg/ml | PF13_0198 | PF3D7_1335400 | | reticulocyte binding protein 2 homologue a (RH2a) |
| 68 | 68 | 315 | 1 | 2 | 2 | 9 | Rh2, 0.3mg/ml | PF13_0198 | PF3D7_1335400 | | reticulocyte binding protein 2 homologue a (RH2a) |
| 69 | 69 | 892 | 1 | 4 | 2 | 8 | Pv1ax AMA1 Ecto monomer prep2, 0.3mg/ml | EU395600.1 | NA | | apical membrane antigen 1 (AMA1) Ectodomain into |
| 70 | 70 | 893 | 1 | 4 | 2 | 9 | Pv1ax AMA1 Ecto monomer prep2, 0.3mg/ml | EU395600.1 | NA | | apical membrane antigen 1 (AMA1) Ectodomain into |
| 71 | 71 | 803 | 1 | 3 | 2 | 8 | Pv1ax AMA1, 0.3mg/ml | EU395600.1 | NA | | apical membrane antigen 1 (AMA1), purified protein |
| 72 | 72 | 804 | 1 | 3 | 2 | 9 | Pv1ax AMA1, 0.3mg/ml | EU395600.1 | NA | | apical membrane antigen 1 (AMA1), purified protein |
| 73 | 73 | 279 | 1 | 1 | 17 | 7 | PF1, 0.02g | PF1, 0.02g | NA | CDDR2 | erythrocyte membrane protein 1, PEMP1 (VAR) |
| 74 | 74 | 371 | 1 | 1 | 3 | 3 | PFAD10w2s2 | PFAD10w2s2 | PF3D7_0102200 | Exon 2 Segment 2 | erythrocyte membrane protein 1, PEMP1 (VAR) |
| 75 | 75 | 50 | 1 | 1 | 3 | 16 | PFAD12sc2 | PFAD12sc2 | PF3D7_0102200 | Exon 1 Segment 2 | erythrocyte binding antigen161 (EB161) |
| 76 | 76 | 991 | 1 | 4 | 8 | 5 | PFAD17sw_2c7 | PFAD17sw_2c7 | PF3D7_0103500 | Exon 2 of 7 | conserved Plasmodium protein, unknown function |
| 77 | 77 | 708 | 1 | 3 | 8 | 11 | PFAD36c_2a2 | PFAD36c_2a2 | PF3D7_0107300 | Exon 2 of 2 | probable protein, unknown function |
| 78 | 78 | 81 | 1 | 1 | 5 | 13 | PFAD41sw-s1 | PFAD41sw-s1 | PF3D7_0108300 | Segment 1 | conserved Plasmodium protein, unknown function |
| 79 | 79 | 370 | 1 | 2 | 5 | 13 | PFAD41sw-s2 | PFAD41sw-s2 | PF3D7_0108300 | Segment 2 | conserved Plasmodium protein, unknown function |

This is also going to give you details about the fragment which has been printed on the chip. So, let us go to a plasmid DB ID. Now, if you look at plasmid DB ID these are all basically each and every protein has a unique plasmid DB ID, so that is what is mentioned in this column here. If you go to the next column which is ORF fragment, so this will explain your ORF your column H better.

If you go here you will see that this specifies which exon segment is printed on the chip. So, basically as you know what is which are printed on the chip were not purified proteins, they were IVTT spots. And basically not, so what is IVTT in vitro transcription translation.

So, what was expressed the whole protein was not expressed here, only a certain segment of a particular exon of a protein was expressed right. So, basically it is not really right to say that

proteins were expression on the chip, instead you be better to say that polypeptides were expressed on the chip.

(Refer Slide Time: 07:55)

| | A | B | C | D | E | F | G | H | I | J | K |
|-----|----|-----|---|---|----|----|--|------------|-----------------|------------------|--|
| 75 | 70 | 893 | 1 | 4 | 2 | 9 | Pvixax AMA1 Ecto monomer prep2, 0.1mg/ml | EU395600.1 | N/A | | apical membrane antigen 1 (AMA1) Ectodomain mo |
| 76 | 71 | 903 | 1 | 3 | 2 | 8 | Pvixax AMA1, 0.3mg/ml | EU395600.1 | N/A | | apical membrane antigen 1 (AMA1), purified protein |
| 77 | 72 | 904 | 1 | 3 | 2 | 9 | Pvixax AMA1, 0.1mg/ml | EU395600.1 | N/A | | apical membrane antigen 1 (AMA1), purified protein |
| 78 | 73 | 279 | 1 | 1 | 17 | 7 | PFL 3002 C-0302 | PFL_3008 | N/A | | |
| 79 | 74 | 37 | 1 | 1 | 3 | 3 | PFAD115w2s2 | PFAD115w | PF3D7_0102200 | Exon 2 Segment 2 | erythrocyte membrane protein 1, P1EMPH (VAR) |
| 80 | 75 | 50 | 1 | 1 | 3 | 16 | PFAD115w2s1 | PFAD125c | PF3D7_0102500 | Exon 1 Segment 2 | erythrocyte binding antigen181 (EBA181) |
| 81 | 76 | 991 | 1 | 4 | 8 | 5 | PFAD175w_2o7 | PFAD175w | PF3D7_0103500 | Exon 2 of 7 | conserved Plasmodium protein, unknown function |
| 82 | 77 | 708 | 1 | 3 | 8 | 11 | PFAD360c_2o2 | PFAD360c | PF3D7_0107300 | Exon 2 of 2 | probable protein, unknown function |
| 83 | 78 | 81 | 1 | 1 | 5 | 13 | PFAD410w-s1 | PFAD410w | PF3D7_0108300 | Segment 1 | conserved Plasmodium protein, unknown function |
| 84 | 79 | 370 | 1 | 2 | 5 | 13 | PFAD410w-s2 | PFAD410w | PF3D7_0108300 | Segment 2 | conserved Plasmodium protein, unknown function |
| 85 | 80 | 947 | 1 | 4 | 5 | 12 | PFAD410w-s3 | PFAD410w | PF3D7_0108300 | Segment 3 | conserved Plasmodium protein, unknown function |
| 86 | 81 | 45 | 1 | 1 | 3 | 11 | PFAD430c1s1 | PFAD430c | PF3D7_0108700 | Exon 1 Segment 1 | secreted ookinete protein, putative (PSOP24) |
| 87 | 82 | 904 | 1 | 4 | 3 | 3 | PFAD430c1s2 | PFAD430c | PF3D7_0108700 | Exon 1 Segment 2 | secreted ookinete protein, putative (PSOP24) |
| 88 | 83 | 889 | 1 | 3 | 6 | 6 | PFAD490w_1o1 | PFAD490w | PF3D7_0110000 | Exon 1 of 1 | conserved Plasmodium protein, unknown function |
| 89 | 84 | 917 | 1 | 4 | 3 | 16 | PFAD510w-s2 | PFAD510w | PF3D7_0110500 | Exon 1 Segment 2 | brionodomain protein, putative |
| 90 | 85 | 628 | 1 | 3 | 3 | 16 | PFAD510w-s3 | PFAD510w | PF3D7_0110500 | Exon 1 Segment 3 | brionodomain protein, putative |
| 91 | 86 | 639 | 1 | 3 | 4 | 10 | PFBD210w (renamed) | PFBD210w | PF3D7_0200100 | Exon 1 of 1 | erythrocyte membrane protein 1, P1EMPH (VAR) |
| 92 | 87 | 713 | 1 | 3 | 8 | 16 | PFBD100c2s1 | PFBD100c | PF3D7_0200000 | Exon 2 Segment 1 | knob-associated histidine-rich protein (KAHRP) |
| 93 | 88 | 418 | 1 | 2 | 8 | 10 | PFBD106c_2o2 | PFBD106c | PF3D7_0202200 | Exon 2 of 2 | Plasmodium exported protein, unknown function |
| 94 | 89 | 617 | 1 | 3 | 3 | 5 | PFBD115w1s2 | PFBD115w | PF3D7_0202400 | Exon 1 Segment 2 | conserved Plasmodium protein, unknown function |
| 95 | 90 | 667 | 1 | 3 | 6 | 4 | PFBD120w_1o1 | PFBD120w | PF3D7_0202500 | Exon 1 of 1 | early transcribed membrane protein 2 (ETAMP2) |
| 96 | 91 | 53 | 1 | 1 | 4 | 2 | PFBD130c2s1 | PFBD130c | PF3D7_0203100 | Exon 2 Segment 3 | protein kinase, putative |
| 97 | 92 | 392 | 1 | 2 | 7 | 1 | PFBD179w_1o1 | PFBD179w | PF3D7_0203600 | Exon 1 of 1 | conserved Plasmodium protein, unknown function |
| 98 | 93 | 681 | 1 | 3 | 7 | 1 | PFBD250w_1o1 | PFBD250w | PF3D7_0205600 | Exon 1 of 1 | conserved Plasmodium protein, unknown function |
| 99 | 94 | 65 | 1 | 1 | 4 | 14 | PFBD300c | PFBD300c | PF3D7_0206800 | Exon 1 of 1 | merozoite surface protein 2 (MSP2) |
| 100 | 95 | 963 | 1 | 3 | 5 | 17 | PFBD305c_1o2 | PFBD305c | PF3D7_0206900.1 | Exon 1 of 2 | merozoite surface protein 5 (MSP5) |
| 101 | 96 | 90 | 1 | 1 | 8 | 5 | PFBD305c-e1 | PFBD305c | PF3D7_0206900.1 | Exon 1 | merozoite surface protein 5 (MSP5) |
| 102 | 97 | 374 | 1 | 2 | 5 | 17 | PFBD310c_1o2 | PFBD310c | PF3D7_0207000 | Exon 1 of 2 | merozoite surface protein 4 (MSP4) |

So, this particular column J gives us details about the polypeptide that was expressed in printed on the chip right. So, that is how you get this ADI spot spot id which is a unique id for each and every protein. What I mean here is that if you go to plasmo DB ID. And then if you try to look for duplicates will actually find duplicates here because it could be that for the same protein different exon fragments were printed on the chip.

So, you might get duplicates here. Whereas, if you go to your ad I sport id you will not find single do any single duplicate, because these are unique ids for each and every protein which takes into account the exon fragment which was printed on the chip, so that is what you see here.

(Refer Slide Time: 08:35)

| | | | | | | | | | | |
|-----|-----|------|---|---|---|-----------------------|----------|----------------|------------------|---|
| 91 | 86 | 639 | 1 | 3 | 4 | 10 PFB0010w (renamed) | PFB0010w | PF3D7_020100 | Exon 2 Segment 1 | erythrocyte membrane protein 1, PEEMP1 (VAR) |
| 92 | 87 | 713 | 1 | 3 | 8 | 16 PFB0100ce2i1 | PFB0100c | PF3D7_020200 | Exon 2 of 2 | knob-associated histidine-rich protein (KAHRP) |
| 93 | 88 | 418 | 1 | 2 | 8 | 10 PFB0104c_2a2 | PFB0104c | PF3D7_020200 | Exon 2 of 2 | Plasmodium exported protein, unknown function |
| 94 | 89 | 617 | 1 | 3 | 3 | 5 PFB0115w_1a2 | PFB0115w | PF3D7_020340 | Exon 1 Segment 2 | conserved Plasmodium protein, unknown function |
| 95 | 90 | 667 | 1 | 3 | 6 | 4 PFB0120w_1a1 | PFB0120w | PF3D7_020250 | Exon 1 of 1 | early transcribed membrane protein 2 (ETAMP2) |
| 96 | 91 | 53 | 1 | 1 | 4 | 2 PFB0150ce2i3 | PFB0150c | PF3D7_020310 | Exon 2 Segment 3 | protein kinase, putative |
| 97 | 92 | 392 | 1 | 2 | 7 | 1 PFB0170w_1a1 | PFB0170w | PF3D7_020360 | Exon 1 of 1 | conserved Plasmodium protein, unknown function |
| 98 | 93 | 681 | 1 | 3 | 7 | 1 PFB0210w_1a1 | PFB0210w | PF3D7_020560 | Exon 1 of 1 | conserved Plasmodium protein, unknown function |
| 99 | 94 | 65 | 1 | 1 | 4 | 14 PFB0300c | PFB0300c | PF3D7_020680 | Exon 1 of 1 | merozoite surface protein 2 (MSP2) |
| 100 | 95 | 663 | 1 | 3 | 5 | 17 PFB0305c_1a2 | PFB0305c | PF3D7_020690.1 | Exon 1 of 2 | merozoite surface protein 5 (MSP5) |
| 101 | 96 | 90 | 1 | 1 | 6 | 5 PFB0305c-e1 | PFB0305c | PF3D7_020690.1 | Exon 1 | merozoite surface protein 5 (MSP5) |
| 102 | 97 | 374 | 1 | 2 | 5 | 17 PFB0310c_1a2 | PFB0310c | PF3D7_020700 | Exon 1 of 2 | merozoite surface protein 4 (MSP4) |
| 103 | 98 | 987 | 1 | 4 | 8 | 1 PFB0310c_2a2 | PFB0310c | PF3D7_020700 | Exon 2 of 2 | merozoite surface protein 4 (MSP4) |
| 104 | 99 | 69 | 1 | 1 | 5 | 1 PFB0310c-e1 | PFB0310c | PF3D7_020700 | Exon 1 | merozoite surface protein 4 (MSP4) |
| 105 | 100 | 115 | 1 | 1 | 7 | 13 PFB0330c_2a4 | PFB0330c | PF3D7_020740 | Exon 2 of 4 | serine repeat antigen 7 (SERA7) |
| 106 | 101 | 1003 | 1 | 4 | 8 | 17 PFB0330ce2i1 | PFB0330c | PF3D7_020750 | Exon 3 Segment 1 | serine repeat antigen 8 (SERA8) |
| 107 | 102 | 901 | 1 | 4 | 2 | 17 PFB0340ce2i1 | PFB0340c | PF3D7_020760 | Exon 2 Segment 1 | serine repeat antigen 5 (SERAS) |
| 108 | 103 | 982 | 1 | 4 | 7 | 13 PFB0345c_2a4 | PFB0345c | PF3D7_020770 | Exon 2 of 4 | serine repeat antigen 4 (SERAA) |
| 109 | 104 | 428 | 1 | 2 | 9 | 3 PFB0345c_4a4 | PFB0345c | PF3D7_020770 | Exon 4 of 4 | serine repeat antigen 4 (SERAA) |
| 110 | 105 | 989 | 1 | 4 | 8 | 3 PFB0350c_2a4 | PFB0350c | PF3D7_020780 | Exon 2 of 4 | serine repeat antigen 3 (SERAS) |
| 111 | 106 | 116 | 1 | 1 | 7 | 14 PFB0755w_6a7 | PFB0755w | PF3D7_021670.1 | Exon 6 of 7 | conserved Plasmodium protein, unknown function |
| 112 | 107 | 996 | 1 | 4 | 8 | 10 PFB0900c_2a2 | PFB0900c | PF3D7_021870 | Exon 2 of 2 | Plasmodium exported protein (PHSTc), unknown function |
| 113 | 108 | 691 | 1 | 3 | 5 | 15 PFB0910w_2a2 | PFB0910w | PF3D7_021990 | Exon 2 of 2 | Plasmodium exported protein, unknown function |
| 114 | 109 | 948 | 1 | 4 | 5 | 13 PFB0915w-e2i1 | PFB0915w | PF3D7_022000 | Exon 2 Segment 1 | liver stage antigen 3 (LSA3) |
| 115 | 110 | 659 | 1 | 3 | 5 | 13 PFB0915w-e2i2 | PFB0915w | PF3D7_022000 | Exon 2 Segment 2 | liver stage antigen 3 (LSA3) |
| 116 | 111 | 698 | 1 | 3 | 8 | 1 PFB0926c_2a2 | PFB0926c | PF3D7_022050 | Exon 2 of 2 | Plasmodium exported protein (hyp2), unknown function |
| 117 | 112 | 408 | 1 | 2 | 7 | 17 PFB0930w_2a2 | PFB0930w | PF3D7_022060 | Exon 2 of 2 | Plasmodium exported protein (hyp6), unknown function |
| 118 | 113 | 705 | 1 | 3 | 8 | 8 PFB0931w_2a2 | PFB0931w | PF3D7_022070 | Exon 2 of 2 | Plasmodium exported protein (hyp6), unknown function |

If you say for example, let us look at this particular row if you say that this was the id and this is exon 1 of 2, you will actually see the id here and 1a2. So, this becomes your unique id for each and every protein. Why I am telling you all this is because this is very important for data analysis, for all sometimes you might just start with an analyzing your iref column, and then you will figure out later that there are a lot of duplicates and you do not know what you are actually doing.

So, what we need to do is if you want to shortlist any antigens, we need to consider the G column for analysis right. So, now let us move onto the next column which is your description. So, this we all know, this basically describe what was printed on the chip right these are just basically the names of them, basically the names of the antigens.

(Refer Slide Time: 09:29)

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Now, the next column is your organism. So, as you know you have two types of spots your plasmodium falciparum and plasmodium vivax.

(Refer Slide Time: 09:39)

| | K | L | M | N | O | P | Q | R | S | T |
|----|---|--------------------|------------------|-------|-------|-------|-------|------|-------|-------|
| 53 | anti-human IgG 6 | N/A | anti-human IgG | 1375 | 677 | 473 | 609 | 413 | 767 | 532 |
| 54 | erythrocyte binding antigen 140 (EBA140), purified protein (0.3mg/mL) | P. falciparum 3D7 | Purified Protein | 15197 | 4119 | 1549 | 631 | 473 | 1521 | 1362 |
| 55 | erythrocyte binding antigen 140 (EBA140), purified protein (0.1mg/mL) | P. falciparum 3D7 | Purified Protein | 22583 | 5048 | 1484 | 403 | 331 | 1395 | 872 |
| 56 | erythrocyte binding antigen 175 (EBA175), purified protein (0.3mg/mL) | P. falciparum 3D7 | Purified Protein | 20221 | 3331 | 1366 | 1260 | 691 | 1276 | 1202 |
| 57 | erythrocyte binding antigen 175 (EBA175), purified protein (0.1mg/mL) | P. falciparum 3D7 | Purified Protein | 8607 | 1583 | 924 | 757 | 368 | 852 | 678 |
| 58 | merozoite surface protein 1 (MSP1), purified protein (0.3mg/mL) | P. falciparum 3D7 | Purified Protein | 26420 | 16334 | 294 | 333 | 446 | 1048 | 925 |
| 59 | merozoite surface protein 1 (MSP1), purified protein (0.1mg/mL) | P. falciparum 3D7 | Purified Protein | 13938 | 8283 | 672 | 474 | 783 | 863 | 719 |
| 60 | merozoite surface protein 2 (MSP2), purified protein (0.3mg/mL) | P. falciparum 3D7 | Purified Protein | 17291 | 17732 | 903 | 8078 | 9174 | 2071 | 9867 |
| 61 | merozoite surface protein 2 (MSP2), purified protein (0.1mg/mL) | P. falciparum 3D7 | Purified Protein | 13110 | 18207 | 727 | 8151 | 7477 | 1980 | 10175 |
| 62 | merozoite surface protein 3 (MSP3), purified protein (0.3mg/mL) | P. falciparum 3D7 | Purified Protein | 10740 | 4711 | 4310 | 892 | 297 | 2952 | 1851 |
| 63 | merozoite surface protein 3 (MSP3), purified protein (0.1mg/mL) | P. falciparum 3D7 | Purified Protein | 7481 | 3326 | 2327 | 726 | 313 | 1995 | 1239 |
| 64 | circumsporozoite protein (CSP), purified protein (0.3mg/mL) | P. falciparum 3D7 | Purified Protein | 12416 | 7936 | 174 | 152 | 268 | 630 | 407 |
| 65 | circumsporozoite protein (CSP), purified protein (0.1mg/mL) | P. falciparum 3D7 | Purified Protein | 3792 | 6000 | 176 | 140 | 94 | 280 | 566 |
| 66 | liver stage antigen 1 (LSA1), purified protein (0.3mg/mL) | P. falciparum 3D7 | Purified Protein | 37614 | 34824 | 19824 | 17599 | 3078 | 13519 | 17585 |
| 67 | liver stage antigen 1 (LSA1), purified protein (0.1mg/mL) | P. falciparum 3D7 | Purified Protein | 18711 | 18898 | 9272 | 6790 | 1352 | 5206 | 9105 |
| 68 | reticulocyte binding protein homologue 1 (RH1) PEP1, purified protein (0.3mg/mL) | P. falciparum 3D7 | Purified Protein | 596 | 3086 | 189 | 197 | 210 | 242 | 270 |
| 69 | reticulocyte binding protein homologue 1 (RH1) PEP1, purified protein (0.1mg/mL) | P. falciparum 3D7 | Purified Protein | 198 | 626 | 163 | 116 | 175 | 188 | 271 |
| 70 | reticulocyte binding protein homologue 1 (RH1) PEP8, purified protein (0.3mg/mL) | P. falciparum 3D7 | Purified Protein | 302 | 359 | 284 | 236 | 187 | 417 | 673 |
| 71 | reticulocyte binding protein homologue 1 (RH1) PEP8, purified protein (0.1mg/mL) | P. falciparum 3D7 | Purified Protein | 92 | 180 | 175 | 172 | 158 | 183 | 273 |
| 72 | reticulocyte binding protein 2 homologue a (RH2a), purified protein (0.3mg/mL) | P. falciparum 3D7 | Purified Protein | 32348 | 4005 | 2439 | 3471 | 4877 | 14303 | 4903 |
| 73 | reticulocyte binding protein 2 homologue a (RH2a), purified protein (0.1mg/mL) | P. falciparum 3D7 | Purified Protein | 11257 | 2010 | 1367 | 1759 | 1887 | 5012 | 2196 |
| 74 | apical membrane antigen 1 (AMA1) Endocytosis monomer, purified protein (0.3mg/mL) | P. vivax Palo Alto | Purified Protein | 63088 | 43260 | 5127 | 1829 | 1468 | 49717 | 64027 |
| 75 | apical membrane antigen 1 (AMA1) Endocytosis monomer, purified protein (0.1mg/mL) | P. vivax Palo Alto | Purified Protein | 63048 | 38950 | 2709 | 1213 | 934 | 41403 | 63423 |
| 76 | apical membrane antigen 1 (AMA1), purified protein (0.3mg/mL) | P. vivax Palo Alto | Purified Protein | 63317 | 45303 | 5026 | 1817 | 1819 | 53167 | 63987 |
| 77 | apical membrane antigen 1 (AMA1), purified protein (0.1mg/mL) | P. vivax Palo Alto | Purified Protein | 63345 | 39267 | 3839 | 1716 | 1425 | 44904 | 64003 |
| 78 | erythrocyte membrane protein 1, PEEMP1 (VAR) | P. falciparum | IVTT | 6799 | 5126 | 2785 | 5373 | 4355 | 9072 | 20407 |
| 79 | ring-infected erythrocyte surface antigen (RESA) | P. falciparum 3D7 | IVTT | 13575 | 16656 | 2674 | 5852 | 3407 | 7356 | 13275 |
| 80 | erythrocyte binding antigen 181 (EBA181) | P. falciparum 3D7 | IVTT | 21760 | 4278 | 4717 | 7642 | 4723 | 8695 | 17838 |

So, basically this is going to tell you which organism does the antigen belong to. So, you have plasmodium falciparum 3D7 here for instance, and probably and if you scroll down further you will see plasmodium vivax sal 1 right. So, this is going to give you details of the organism.

(Refer Slide Time: 09:51)

| | K | L | M | N | O | P | Q | R | S | T |
|--|---------------|------|-------|-------|------|-------|------|-------|-------|---|
| 614 26S proteasome regulatory subunit, putative | P. vivax Sal1 | IVTT | 7146 | 2011 | 2689 | 4912 | 3071 | 7021 | 11803 | |
| 615 mercozole surface protein 5 | P. vivax Sal1 | IVTT | 8168 | 8845 | 2336 | 4246 | 3266 | 5864 | 961 | |
| 616 mercozole surface protein 4, putative | P. vivax Sal1 | IVTT | 4140 | 38796 | 2144 | 4495 | 4001 | 7769 | 23951 | |
| 617 mercozole surface protein 4, putative | P. vivax Sal1 | IVTT | 45412 | 38495 | 2588 | 5388 | 3925 | 7451 | 27106 | |
| 618 serine repeat antigen (SERA), putative | P. vivax Sal1 | IVTT | 8185 | 8314 | 2183 | 4253 | 3701 | 7907 | 14950 | |
| 619 serine repeat antigen 4 (SERA) | P. vivax Sal1 | IVTT | 8754 | 3513 | 3234 | 5284 | 4117 | 9971 | 18132 | |
| 620 serine repeat antigen 4 (SERA) | P. vivax Sal1 | IVTT | 6353 | 1905 | 1759 | 3148 | 2634 | 5224 | 10230 | |
| 621 serine repeat antigen 4 (SERA) | P. vivax Sal1 | IVTT | 11410 | 3687 | 3139 | 6952 | 5301 | 10654 | 14136 | |
| 622 serine repeat antigen 5 (SERA) | P. vivax Sal1 | IVTT | 7642 | 14822 | 2909 | 6110 | 4729 | 7608 | 16933 | |
| 623 serine repeat antigen 5 (SERA) | P. vivax Sal1 | IVTT | 23049 | 64174 | 3652 | 6957 | 4504 | 9734 | 15401 | |
| 624 serine repeat antigen 3 (SERA) | P. vivax Sal1 | IVTT | 5189 | 14020 | 1623 | 3090 | 3141 | 5698 | 14160 | |
| 625 serine repeat antigen 3 (SERA) | P. vivax Sal1 | IVTT | 5679 | 8712 | 2507 | 4570 | 4127 | 7090 | 14885 | |
| 626 eIF4Alike DEAD family RNA helicase, putative | P. vivax Sal1 | IVTT | 5471 | 2459 | 2056 | 6564 | 3376 | 5766 | 11607 | |
| 627 syntaxin, putative | P. vivax Sal1 | IVTT | 5159 | 3945 | 2872 | 3224 | 2967 | 5451 | 11431 | |
| 628 hypothetical protein, conserved | P. vivax Sal1 | IVTT | 7618 | 3624 | 2911 | 5777 | 4256 | 8904 | 16822 | |
| 629 hypothetical protein, conserved | P. vivax Sal1 | IVTT | 7493 | 2937 | 2742 | 5165 | 2986 | 8309 | 13107 | |
| 630 hypothetical protein, conserved | P. vivax Sal1 | IVTT | 4945 | 6028 | 2217 | 5162 | 4059 | 7021 | 15320 | |
| 631 cell cycle regulator with Zn finger domain, putative | P. vivax Sal1 | IVTT | 6747 | 5701 | 2440 | 5579 | 2981 | 8198 | 13233 | |
| 632 hap70 interacting protein, putative | P. vivax Sal1 | IVTT | 4263 | 2923 | 2316 | 3635 | 3368 | 7485 | 12594 | |
| 633 ubiquitin carboxyl-terminal hydrolase, putative | P. vivax Sal1 | IVTT | 6591 | 10200 | 2024 | 4410 | 3486 | 6364 | 11241 | |
| 634 hypothetical protein, conserved | P. vivax Sal1 | IVTT | 10331 | 2888 | 2612 | 35131 | 3686 | 7496 | 12567 | |
| 635 hypothetical protein, conserved | P. vivax Sal1 | IVTT | 5929 | 2353 | 2288 | 3975 | 3806 | 6708 | 14063 | |
| 636 hypothetical protein, conserved | P. vivax Sal1 | IVTT | 8092 | 13991 | 2576 | 4687 | 4418 | 7280 | 16114 | |
| 637 hypothetical protein, conserved | P. vivax Sal1 | IVTT | 6199 | 3151 | 2435 | 4280 | 3164 | 6634 | 10496 | |
| 638 hypothetical protein, conserved | P. vivax Sal1 | IVTT | 14621 | 11913 | 2444 | 5026 | 4467 | 7738 | 13909 | |
| 639 multidrug resistance protein (mdr1) | P. vivax Sal1 | IVTT | 6010 | 2052 | 1944 | 3951 | 3361 | 6726 | 11954 | |
| 640 hypothetical protein, conserved | P. vivax Sal1 | IVTT | 3779 | 1865 | 1796 | 3277 | 3225 | 5763 | 9709 | |
| 641 hypothetical protein, conserved | P. vivax Sal1 | IVTT | 7236 | 2088 | 1824 | 3691 | 2984 | 5350 | 11773 | |

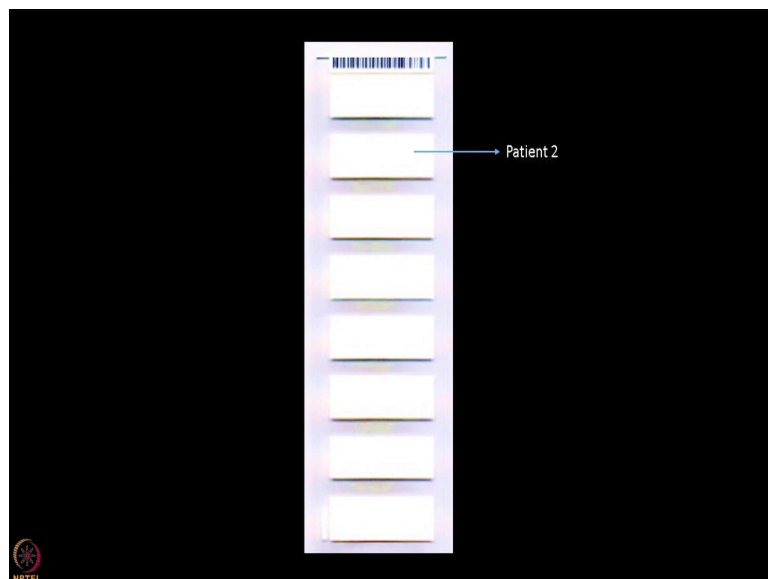
And then the next column which is m is going to talk to you about the preparation of the spot. Like for example, you have the first few spots are basically your IgG mix right. So, then the preparation is basically your IgG mix, it is not an IVTT spot. Now, if you scroll down further you will have similarly anti human IgG. Again you scroll down further you have certain purified proteins which are nothing but control proteins.

So, our control spots here were printed as purified proteins and not as IVTT spots. Now, if you scroll down further you will find all your other spots basically your antigens which you are trying to study had all printed as IVTT spots. So, basically this entire column m gives you details about the spot preparation. Now, the all other columns here are basically your patient samples.

So, if I just move this a little bit, what do you see here for example, let us consider the first sample this is basically a positive control which was part of batch 1, set 1, slide 1 and pad 1. So, let me again take you back to the experiment this experiment was performed in 4 sets of 2 batches or rather 2 batches of 4 sets. So, you have batch 1 set 1, batch 1 set 2, then you have batch 2 set 1 and batch 2 set 2.

So, basically what is this is telling me this is telling me that this particular positive control was probed on batch 1 set 1 on slide 1 and pad 1 right. Now, similarly, so let us go to the next one which is a real sample that was just a positive control. So, this is basically probed on batch 1, set 1, slide 1 and pad 2.

(Refer Slide Time: 11:41)



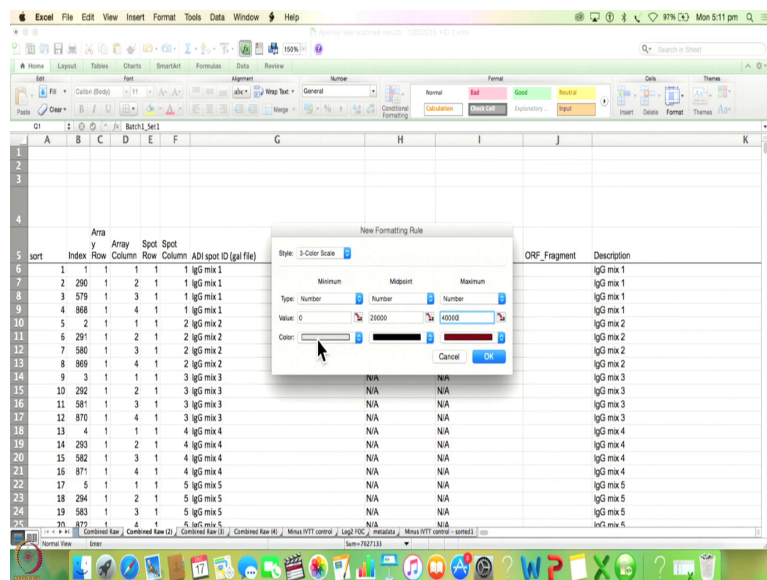
So, this is going to tell me my position of the sample. So, if ever I want to go back to the slides and to check the real spots right the images of the spots, then I will know exactly where

(Refer Slide Time: 12:07)

[illegible]

So, for which what I am going to do is I am going to go to conditional formatting, I am going to go to color scales more rules. And I am going to choose a three color scale and then I am going to choose number type ok.

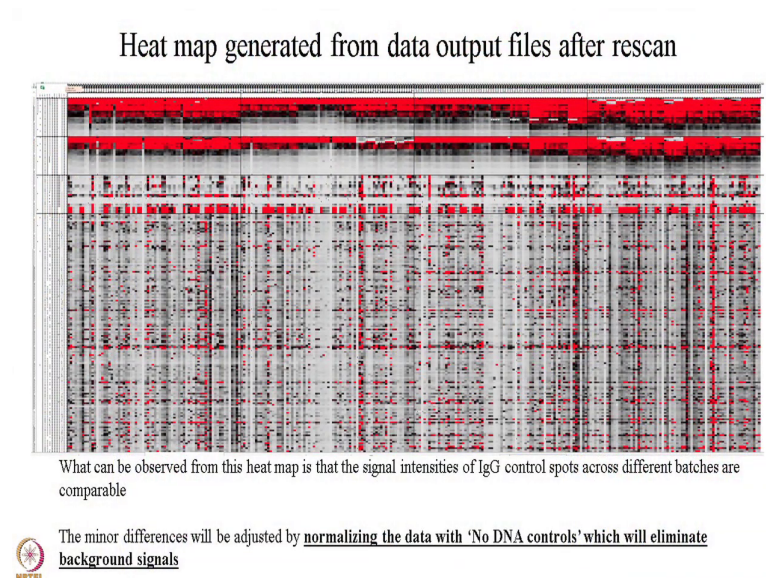
(Refer Slide Time: 12:53)



So, I am going to say 0. And I am going to assume that my entire data falls you know in between say certain negative values and maybe around 80000 is my maximum value. So, I am just going to assume that if my data falls in this range I am going to split my data be based on three numbers – 0, then my midpoint will be say 20000 and my highest will be 40000. And I am going to choose some colors here.

So, I say this is maybe gray, then I am going to keep this black, and I am going to keep this red. So, what this is going to do is all my values above 40000 are going to be in dark red, and then around 20000 will be black and the lowest or the least values will be gray and those which are in negatives will be almost white, so that is how I am going to apply a color gradient here.

(Refer Slide Time: 13:53)



So, you can see in this slide here basically what I have done is I have just minimized this excel a little bit. So, you can you will be able to see all four batches at once. So, I do not know if you can see a black line here. So, basically this is going to split your batches, so in fact, it is going to split your set, set 1 from set 2 of the first batch and set 1 and from set 2 of the second batch. So, basically this is batch 1 set 1 batch 1 set 2 batch 2 set 1 and batch 2 set 2.

So, when you minimize this excel a little bit and you apply this color gradient, what you can see is that the signal intensities particularly for this batch, in fact this whole batch, but batch 1, but batch 2 set 1 is really high compared to the rest of the batches. So, you will know that mainly from the IgG signals here. So, this particular line which you see here your IgG mix, and this particular line which you see here is your anti human igg.

So, basically this is your control which is going to tell you where you need to rescan your slide or not. So, if this is very high, then all your signals by default for this particular batch will also be high right, so that is going to screw up your results a little screw up your results later because all the patients in this batch are going to show high signals which will be which is not correct.

So, this IgG mix printed on the strip is going to basically help you in deciding whether you need to rescan your slides are different PMT settings and power settings right. So, what we will do here is we will rescan the slides once more bring these settings down a little bit, and bring these settings not as low as this, but a little lower because this is also a little high compared to this if you see right.

So, later on we realize if this is because of the membrane thickness of the slides, there could be other issues also which you might encounter later. So, to avoid this you need to first bring down the signals and then any changes after that will be corrected by normalization ok.


So, now having rescanned all the slides, as you can see in this slide the settings look pretty uniform, though it is still not very uniform and you still feel that batch 2 set 1 has higher signals, but overall it is because this will then be taken care of by normalization. So, now what we do is we will proceed with normalization using excel.


(Refer Slide Time: 16:21)

Data normalization

Two kinds of data normalization:

- 1. Sample specific median normalization:** Each raw value is subtracted from the median of its 'No DNA controls'
- 2. Log₂ transformed FOC:** Each raw value is divided by the median of its 'No DNA controls' and Log₂ transformed (Used for statistical analysis)- This is called fold-over-control (FOC) normalization which reduces the variation in signals that could potentially arise between probing operations performed at different times



 HPTEL

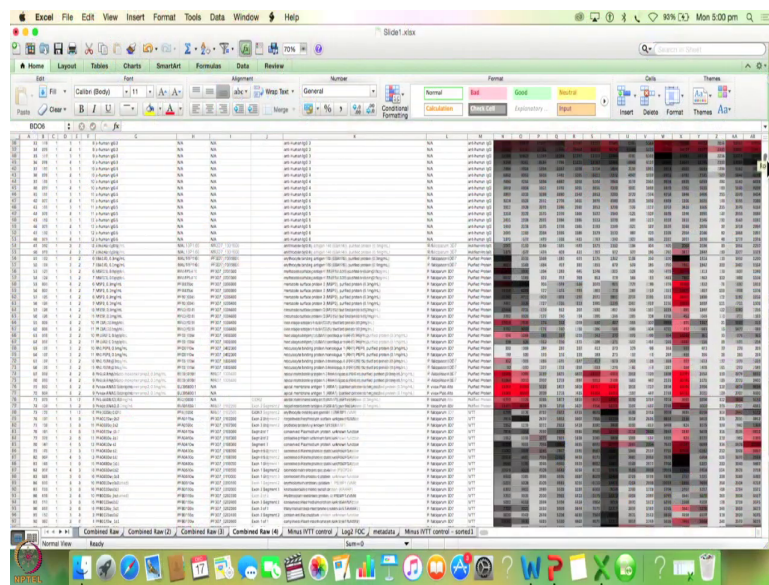
Now, there are two strategies I am going to talk to you about today. The first strategy is basically a very simple normalization method which we will use only for visualization. For example, if you want to prepare heat maps, then we will use this the first normalization method. However, if you want to perform statistical tests, then we will use the second kind of normalization which I will talk to you about.

So, let us first go through the first normalization method. So, what we are going to do in the first normalization is we are going to subtract the raw values for each of the IVTT spots from this sample specific median value of the no DNA controls. So, I am sure that this is a little confusing. So, what we will do is we will go step by step.

First I am going to show you what raw values are and then I am going to show you what the no DNA controls are, right. So, again we are going to come back to the same it is color coded

and you have view we have reached the stage. You also know that this now in this data, we have IgG makes, we have anti human IgG, we have purified proteins, we do not need any of those right now for our analysis, we are going to directly go down to the IVTT spots.

(Refer Slide Time: 17:19)



So, in fact, what we will do is we probably just delete those rows to avoid confusion. So, let us start from here. I am going to delete the first few maybe what I will do is I will just zoom this a little bit. So, I have just zoomed this a little bit. What we are going to do is we are going to delete unwanted rows right now. So, we do not want IgG mix, we do not want to anti human IgG, we do not want purified proteins right now.

Again let me tell you the purified proteins basically you do not require in the analysis, but it is important when for example, your slide is not worked at all. And or you have not got the

signals you required. You can always go back to the positive control spots to see what they signals were right. So, this is basically used for such you know analysis just as controls.

(Refer Slide Time: 18:13)

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So, right now we are going to delete those rows. And we are going to only keep rows which are IVTT mix right that is what this is. So, now we are going to have this wave 500 plasmodium falciparum IVTT spots, and 515 plasmodium falciparum vivax IVTT spots. So, we are going to go down, you have deleted unwanted rows. There are a few more rows below which we do not need.

(Refer Slide Time: 18:37)

| ID | Name | Exon | Protein | Value 1 | Value 2 | Value 3 | Value 4 |
|------|------------|---------------|---|---------------|---------|---------|---------|
| 1005 | PVX_122995 | Exon 1 of 1 | drug/metabolite exporter, drug/metabolite transporter | P. vivax SalI | IVTT | 7020 | 3633 |
| 1006 | PVX_123040 | Exon 1 of 1 | hypothetical protein, conserved | P. vivax SalI | IVTT | 4731 | 2726 |
| 1007 | PVX_123105 | Exon 2 of 6 | hypothetical protein, conserved | P. vivax SalI | IVTT | 6237 | 5189 |
| 1008 | PVX_123360 | Exon 1 of 1 | Segment bromodomain protein, putative | P. vivax SalI | IVTT | 16556 | 4177 |
| 1009 | PVX_123440 | Exon 1 of 2 | hypothetical protein, conserved | P. vivax SalI | IVTT | 3761 | 2658 |
| 1010 | PVX_123505 | Exon 1 of 1 | hypothetical protein, conserved | P. vivax SalI | IVTT | 7745 | 3635 |
| 1011 | PVX_123510 | Exon 1 of 1 | S4, putative | P. vivax SalI | IVTT | 8778 | 4332 |
| 1012 | PVX_123520 | | DNA-binding chaperone, putative | P. vivax SalI | IVTT | 8377 | 3982 |
| 1013 | PVX_123655 | Exon 1 of 3 | Segment hypothetical protein, conserved | P. vivax SalI | IVTT | 5320 | 1771 |
| 1014 | PVX_123705 | | hypothetical protein, conserved | P. vivax SalI | IVTT | 6164 | 3435 |
| 1015 | PVX_123745 | Exon 1 of 1 | endoplasmic precursor, putative | P. vivax SalI | IVTT | 11398 | 2863 |
| 1016 | PVX_123810 | Exon 2 of 2 | Segment hypothetical protein, conserved | P. vivax SalI | IVTT | 3732 | 2163 |
| 1017 | PVX_123845 | | polyadenylation-binding protein, putative | P. vivax SalI | IVTT | 7961 | 2907 |
| 1018 | PVX_123855 | Exon 2 of 2 | Chromatin assembly protein (ASF1), putative | P. vivax SalI | IVTT | 5567 | 15548 |
| 1019 | PVX_124015 | Exon 2 of 3 | hypothetical protein, conserved | P. vivax SalI | IVTT | 10785 | 2129 |
| 1020 | PVX_124140 | Exon 10 of 11 | Segment hypothetical protein, conserved | P. vivax SalI | IVTT | 4324 | 2604 |
| 1021 | NIA | | TTBS | NIA | TTBS | 1018 | 1115 |
| 1022 | NIA | | TTBS | NIA | TTBS | 635 | 562 |
| 1023 | NIA | | TTBS | NIA | TTBS | 572 | 785 |
| 1024 | NIA | | TTBS | NIA | TTBS | 605 | 588 |
| 1025 | NIA | | TTBS | NIA | TTBS | 56 | 122 |
| 1026 | NIA | | TTBS | NIA | TTBS | 33 | 97 |
| 1027 | NIA | | TTBS | NIA | TTBS | 14 | 212 |
| 1028 | NIA | | TTBS | NIA | TTBS | 113 | 154 |
| 1029 | NIA | | TTBS | NIA | TTBS | 782 | 1062 |
| 1030 | NIA | | TTBS | NIA | TTBS | 260 | 917 |
| 1031 | NIA | | TTBS | NIA | TTBS | 104 | 743 |
| 1032 | NIA | | TTBS | NIA | TTBS | 118 | 391 |

So, after these 15000 spots, there are few more like TTBS which is nothing but your buffer spot where only buffer is spotted and then you have some empty spots data then we have data for blank. So, this is also unwanted we are going to delete that as well.

(Refer Slide Time: 18:57)

| Sample ID | Sample Name | Value 1 | Value 2 | Value 3 |
|-----------|-------------|---------------|-----------|---------|
| 1021 | NIA | noDNA Control | N/A | noDNA |
| 1022 | NIA | noDNA Control | N/A | noDNA |
| 1023 | NIA | noDNA Control | N/A | noDNA |
| 1024 | NIA | noDNA Control | N/A | noDNA |
| 1025 | NIA | noDNA Control | N/A | noDNA |
| 1026 | NIA | noDNA Control | N/A | noDNA |
| 1027 | NIA | noDNA Control | N/A | noDNA |
| 1028 | NIA | noDNA Control | N/A | noDNA |
| 1029 | NIA | noDNA Control | N/A | noDNA |
| 1030 | NIA | noDNA Control | N/A | noDNA |
| 1031 | NIA | noDNA Control | N/A | noDNA |
| 1032 | NIA | noDNA Control | N/A | noDNA |
| 1033 | NIA | noDNA Control | N/A | noDNA |
| 1034 | NIA | noDNA Control | N/A | noDNA |
| 1035 | NIA | noDNA Control | N/A | noDNA |
| 1036 | NIA | noDNA Control | N/A | noDNA |
| 1037 | NIA | noDNA Control | N/A | noDNA |
| 1038 | NIA | noDNA Control | N/A | noDNA |
| 1039 | NIA | noDNA Control | N/A | noDNA |
| 1040 | NIA | noDNA Control | N/A | noDNA |
| 1041 | NIA | noDNA Control | N/A | noDNA |
| 1042 | NIA | noDNA Control | N/A | noDNA |
| 1043 | NIA | noDNA Control | N/A | noDNA |
| 1044 | NIA | noDNA Control | N/A | noDNA |
| 1045 | | | | |
| 1046 | | | | |
| 1047 | | | | |
| 1048 | | | | |
| | | | Median No | |
| | | | 7842 | 2726 |
| | | | | 2703.5 |

So, now what we have are 15000 IVTT spots and 24 no DNA control spots. So, now what are these no DNA control spots? So, basically these pots have the entire IVTT mix except the plasmid. So, basically what you expect here is no expression, because you do not even have the plasmid here, whereas the IVTT spots have the entire IVTT machinery just like no DNA, but they also have the plasmid, where you are going to express your gene of interest whereas, you do not have that here.

So, what is this going to provide is going to provide your background signal. So, what we are trying to do in the first type of normalization is, we are subtracting our raw signals from background. So, now there are 24 such spots which you remember we have rearranged and that is why it is come together group together like this. The first thing we are going to do is take a median of this which I have already provided you here.

So, each of these spots which you see here, I am going to subtract it and that is what is my median sample specific median normalization. So, let us scroll, so probably we will do this in the same excel ok. I have kept placed by that here.

So, this is called IVTT sports minus median of IVTT control, so that is exactly what we have to do we are going to say is equal to, then we are going to go to that particular spot. So, say let us take the first patient.

(Refer Slide Time: 20:45)

The screenshot displays an Excel spreadsheet with a complex data table. The table has columns labeled L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, AA, AB. The rows are numbered 1 to 25. The data is organized into a grid with multiple columns and rows. A red box highlights a specific cell in row 5, column M. A red arrow points to the cell in row 4, column M, which contains the text "Raw data (Combined raw)". The spreadsheet also shows standard Excel interface elements like the ribbon, status bar, and taskbar.

And then we see minus and we go to the median value, which is 7842.

(Refer Slide Time: 20:55)

| | L | M | N | O | P | Q | R | S | T | U | V | W | X | Y | Z | AA | AB |
|------|-----|---------------|-------|------|--------|------|--------|--------|-------|------|--------|------|--------|------|-----|------|--------|
| 1039 | NIA | ncDNA | 7913 | 2218 | 1533 | 3025 | 2509 | 3628 | 7305 | 1235 | 1186 | 5184 | 1371 | 3956 | 14 | 1437 | 2195 |
| 1040 | NIA | ncDNA | 8021 | 3684 | 3807 | 6333 | 4387 | 7425 | 15708 | 2596 | 1980 | 9836 | 2385 | 7062 | 89 | 2549 | 3277 |
| 1041 | NIA | ncDNA | 8162 | 2288 | 2129 | 4381 | 3924 | 7718 | 13473 | 2429 | 1198 | 5171 | 1151 | 9919 | 948 | 2252 | 2943 |
| 1042 | NIA | ncDNA | 8687 | 2397 | 1895 | 4550 | 4278 | 6056 | 14966 | 1595 | 1441 | 5628 | 1129 | 4052 | 274 | 2026 | 2511 |
| 1043 | NIA | ncDNA | 7771 | 2325 | 2268 | 4957 | 3867 | 4973 | 10740 | 1236 | 1127 | 5782 | 1184 | 3804 | 164 | 1311 | 2156 |
| 1044 | NIA | ncDNA | 10094 | 2593 | 2435 | 5442 | 4287 | 4734 | 9394 | 1321 | 1281 | 6452 | 1223 | 4091 | 11 | 1311 | 2256 |
| 1045 | | Median No DNA | 7842 | 2726 | 2703.5 | 5241 | 4295.5 | 7462.5 | 14586 | 2495 | 1492.5 | 6844 | 1554.5 | 4889 | 271 | 1832 | 2596.5 |
| 1046 | | | | | | | | | | | | | | | | | |
| 1047 | | | | | | | | | | | | | | | | | |
| 1048 | | | | | | | | | | | | | | | | | |
| 1049 | | | | | | | | | | | | | | | | | |
| 1050 | | | | | | | | | | | | | | | | | |
| 1051 | | | | | | | | | | | | | | | | | |
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| 1064 | | | | | | | | | | | | | | | | | |
| 1065 | | | | | | | | | | | | | | | | | |
| 1066 | | | | | | | | | | | | | | | | | |

So, now, because I want this row to remain constant throughout I am going to put a dollar sign in front of the row.

(Refer Slide Time: 21:15)

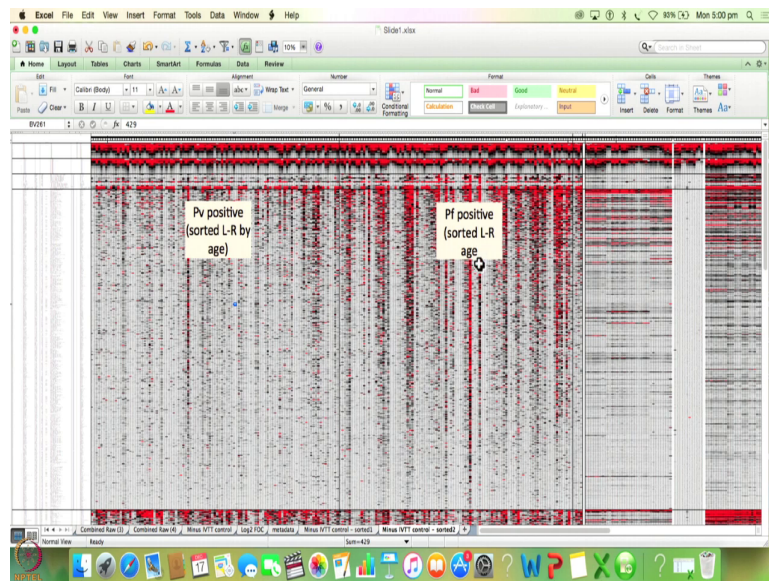
The screenshot shows an Excel spreadsheet with the following data table:

| | KJ | KK | KL | KM | KN | KO | KS |
|----|----------|------------|------------|----------|----------|----------|----------|
| 1 | Slide 8 | Slide 9 | Slide 9 | Slide 9 | Slide 9 | Slide 9 | Slide 9 |
| 2 | Pad 7 | Pad 8 | Pad 1 | Pad 2 | Pad 3 | Pad 4 | Pad 5 |
| 3 | div | Pos contro | Pos contro | mqv | xov | orm | wmic |
| 4 | | | | | | | |
| 5 | 0252 8 7 | 0252 8 8 | 0252 9 1 | 0252 9 2 | 0252 9 3 | 0252 9 4 | 0252 9 5 |
| 6 | 6705 | 7893 | 9128 | 8479 | 6018 | 6409 | 10411 |
| 7 | 7882 | 12075 | 11363 | 43142 | 5124 | 10085 | 10197 |
| 8 | 7138 | 11979 | 12568 | 11738 | 4818 | 6925 | 9718 |
| 9 | 6366 | 7101 | 6791 | 4814 | 4622 | 6940 | 8489 |
| 10 | 3689 | 5067 | 4579 | 2903 | 2545 | 4146 | 4678 |
| 11 | 6539 | 10618 | 36975 | 9112 | 11115 | 7537 | 7537 |
| 12 | 5535 | 7093 | 8356 | 20538 | 12274 | 5639 | 7402 |
| 13 | 6441 | | 11379 | 64543 | 13248 | 7161 | 7265 |
| 14 | 7457 | 8440 | 8109 | 4835 | 5333 | 7192 | 11126 |
| 15 | 6087 | 7441 | 6944 | 1116 | 5844 | 6398 | 36402 |
| 16 | 4111 | 6045 | 8948 | 7708 | 7859 | 8111 | 5329 |
| 17 | 5040 | 6394 | 6090 | 4814 | 5483 | 5519 | 9697 |
| 18 | 5571 | 7001 | 7561 | 5391 | 5226 | 6616 | 6448 |
| 19 | 7718 | 8079 | 9242 | 1116 | 6118 | 9655 | 9679 |
| 20 | 5834 | 9684 | 1578 | 6452 | 4595 | 7188 | 7533 |
| 21 | 5286 | 8419 | 10831 | 6862 | 4712 | 6191 | 8569 |
| 22 | 5340 | 6566 | 7039 | 4923 | 4138 | 5503 | 6521 |
| 23 | 4349 | 45961 | 65912 | 61881 | 13005 | 9934 | 10000 |
| 24 | 5521 | 5953 | 6326 | 6139 | 4996 | 8641 | 7372 |
| 25 | 6203 | 8431 | 9651 | 13251 | 6383 | 8440 | 11634 |

A yellow callout box with the text "---> IVTT spots minus median of IVTT control" is positioned over the data table.

So, this is what we get here. And now I am just going to drag this across as well as down.

(Refer Slide Time: 21:25)



So, once you drag and drop, this is the kind of excel you get. I have just minimized this, but if you apply your color gradient this is how it looks overall. So, this is what you can use now to make your heat maps and what I have also done is I have sorted this based on the antigens as well as the patients who were a falciparum positive and vivax positive. I have split them completely.

And I have also made another excel sheet based on age; you can also split them based on age. So, this is how I have sorted them. So, I have put all your P v positive patients together and P f positive together and I have also sorted based on age. So, you have P v positive, P f positive, as well as sorted by age. So, this way you can sort your excel in different ways.

You can also use other softwares to make a heat map, but basically this kind of not once you normalize it in this way, you do not perform any statistical analysis with this data. For

statistical analysis, I am going to now show you the next normalization method which is your log 2 transform fold over control normalization.

(Refer Slide Time: 22:33)

| | VW | VX | VY | VZ | WA | WB | WC | WD | WE | WF | WG | WH | WI | WJ | WK | W |
|----|----|----|-------------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | | | | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 | Slide 1 |
| | | | | Pad 1 | Pad 2 | Pad 3 | Pad 4 | Pad 5 | Pad 6 | Pad 7 | Pad 8 | Pad 1 | Pad 2 | Pad 3 | Pad 4 | Pad 5 |
| 4 | | | Set floor to 100 | | | | | | | | | | | | | |
| 5 | | | AD1 upper ID (gal file) | | | | | | | | | | | | | |
| 6 | | | PFL_000B_CIDR1 | | | | | | | | | | | | | |
| 7 | | | PFA0110we2s2 | | | | | | | | | | | | | |
| 8 | | | PFA01125ce1s2 | | | | | | | | | | | | | |
| 9 | | | PFA01175w_2s7 | | | | | | | | | | | | | |
| 10 | | | PFA0360c_2e2 | | | | | | | | | | | | | |
| 11 | | | PFA0410w-e1 | | | | | | | | | | | | | |
| 12 | | | PFA0410w-e2 | | | | | | | | | | | | | |
| 13 | | | PFA0410w-e3 | | | | | | | | | | | | | |
| 14 | | | PFA0430ce1s1 | | | | | | | | | | | | | |
| 15 | | | PFA0430ce1s2 | | | | | | | | | | | | | |
| 16 | | | PFA0490w_1s1 | | | | | | | | | | | | | |
| 17 | | | PFA0510we1s2 | | | | | | | | | | | | | |
| 18 | | | PFA0510we1s3 | | | | | | | | | | | | | |
| 19 | | | PFB0010w (renamed) | | | | | | | | | | | | | |
| 20 | | | PFB0100ce1s1 | | | | | | | | | | | | | |
| 21 | | | PFB0106c_2e2 | | | | | | | | | | | | | |
| 22 | | | PFB0115we1s2 | | | | | | | | | | | | | |
| 23 | | | PFB0120w_1s1 | | | | | | | | | | | | | |
| 24 | | | PFB0150ce1s3 | | | | | | | | | | | | | |
| 25 | | | PFB0170w_1s1 | | | | | | | | | | | | | |
| 26 | | | PFB0175w_1s1 | | | | | | | | | | | | | |

So, for this I am not going to show you the entire method again because now you know how to do it on excel, I am sure you all know. For this I am only going to show you the steps, the first thing what we do is we want to set a floor of 100. So, what with what we are trying to say here is that all the samples which are below 100 is going to have a value of 100, so this is going to remove all my negative values from my data. So, that is the first thing I have done it here for you and we are going to keep scrolling right.

(Refer Slide Time: 23:05)

The next step what we are going to do is to divide each and every raw value by the median of the IVTT controls control spots. So, just like we did previously, we subtracted raw values from the median of the IVTT control spots, this time we are going to divide it, so that is what is called fold over control.

(Refer Slide Time: 23:29)

The screenshot shows an Excel spreadsheet with a large table of data. The table has columns for various conditions (ASB, ASD, ASE, ASF, ASG, ASH, ASI, ASJ, ASK, ASL, ASM, ASN, ASO, ASP) and rows for different samples (Slide 9, Pad 3, Pad 4, Pad 5, Pad 6, Pad 7, Pad 8). A yellow box highlights the text "Log 2 Fold over control (Log2 FOC)".

So, once you set up flow of 100, then you divide it. And the next thing you are going to do is to convert this whole data into log values. So, you log to transform this entire data right and that is why it is called log 2 fold over control. So, once you do this, this data can be used for any statistical analysis, so this because this normalization is known to be more stringent ok.

So, now either you can use programming to do your statistical analysis or you can use different softwares which provide your statistical tests, but what you need to know is which type of test you need to use which is beyond the scope of this lecture. But you can always read about what you want to do and you can also decide which software you can you want to you want to use.

For example, graph pad prism is an excellent tool for preparing graphs, it also helps you in a lot of statistical analysis. But if your data is really huge like the one we have is not very huge,

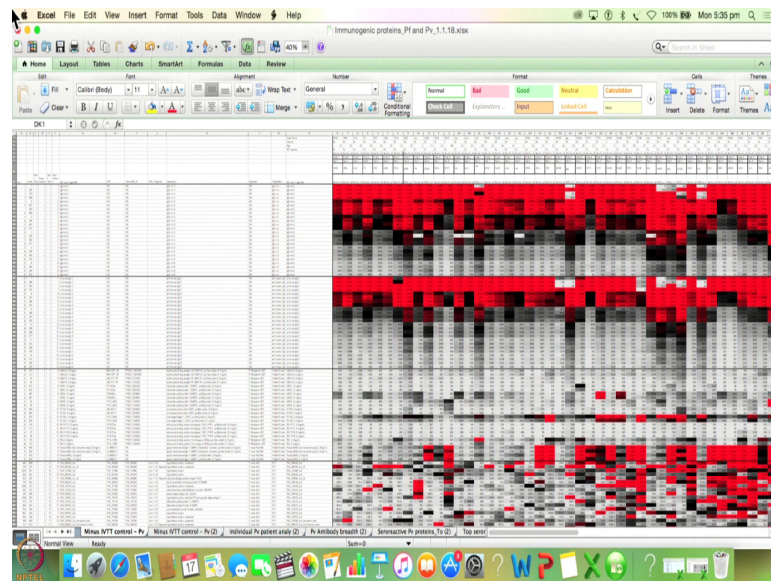
but still it is not very small for graph pad prism. So, for example, you can have graph pad prism can get stuck in the middle if you are using data even this size. So, of course, if you have bigger data sets, then it is very difficult to use software slipped off graph pad prism.

However, if you are going to have only 10 patients or 20 patients with 40 proteins or something graph pad prism does offer you a lot of statistical tests. Apart from that there are other softwares as well. You have metaboanalyst. Though it is for metabolomics data, you still have a module called significance analysis of microarrays in it which you can explore for your microarray data analysis.

But there are of course our programming and python and other things will definitely be much better for your analysis as you save a lot of time as well. So, what I am going to do towards this is we are coming to the end of the lecture, I am only going to show you very small analysis you do one I have done on excel.

So, basically what my aim here is to identify most zero reactive proteins in my from my chip which means that the proteins which elicit the maximum antibody response in malaria patients right, so that is my aim. So, now just to get this whole list of best zero reactive proteins, I you can also do this on excel using a particular formula which I will show you now.

(Refer Slide Time: 25:47)



So, let us go back to the excel. This is how our excel was right. Before we removed all of these rows which are I have IgG and anti human igg. So, I have written them for now and probably zoom this a little bit. So, now, if you see that I have retained all the rows. So, the first thing what we need to do of course, we do not need this, but I have still retained the entire sheet from the beginning.

(Refer Slide Time: 26:13)

| Row | Col | Value |
|-----|-----|-------|
| 10 | 10 | 10 |
| 11 | 11 | 11 |
| 12 | 12 | 12 |
| 13 | 13 | 13 |
| 14 | 14 | 14 |
| 15 | 15 | 15 |
| 16 | 16 | 16 |
| 17 | 17 | 17 |
| 18 | 18 | 18 |
| 19 | 19 | 19 |
| 20 | 20 | 20 |
| 21 | 21 | 21 |
| 22 | 22 | 22 |
| 23 | 23 | 23 |
| 24 | 24 | 24 |
| 25 | 25 | 25 |
| 26 | 26 | 26 |
| 27 | 27 | 27 |
| 28 | 28 | 28 |
| 29 | 29 | 29 |
| 30 | 30 | 30 |

You will see that there are these four patients which are deliberately kept out of the analysis. For example, there are so if you see here there is P f plus P v and everywhere right. So, basically these are my patients who were diagnosed with mixed infection. So, I do not want any such patients in my analysis.

So, I am going to purely have groups which are plasmodium falciparum and plasmodium vivax, and I am going to look at look for their response to plasmodium falciparum antigens and plasmodium vivax respectively. So, I am not going to have any of these mixed patients, I have going to have kept them out. So, if you want, we can also delete them right.

(Refer Slide Time: 26:51)

| | | | | | | | | | | | | |
|----|------|------|---|---|----|-------------------|-------------------------------------|--------------|--------------|---------------------|--|----------|
| 66 | 57 | 25 | 1 | 2 | 8 | MSPL 0.3mg/mL | PF10_0345 | PF027_036400 | | | membrane surface protein 3 (MSPL), purified protein (0.3mg/mL) | P. lalop |
| 67 | 58 | 26 | 1 | 2 | 9 | MSPL 0.3mg/mL | PF10_0345 | PF027_036400 | | | membrane surface protein 3 (MSPL), purified protein (0.3mg/mL) | P. lalop |
| 68 | 59 | 894 | 1 | 4 | 2 | 10 | PF CSP 0.3mg/mL | MAL3P2.11 | PF027_036400 | | circumsporozoite protein (CSP), purified protein (0.3mg/mL) | P. lalop |
| 69 | 60 | 895 | 1 | 4 | 2 | 11 | PF CSP 0.3mg/mL | MAL3P2.11 | PF027_036400 | | circumsporozoite protein (CSP), purified protein (0.3mg/mL) | P. lalop |
| 70 | 61 | 895 | 1 | 3 | 2 | 10 | PF SAL 0.3mg/mL | PF10_0366 | PF027_036400 | | liver stage antigen 1 (LSA1), purified protein (0.3mg/mL) | P. lalop |
| 71 | 62 | 895 | 1 | 3 | 2 | 11 | PF SAL 0.3mg/mL | PF10_0366 | PF027_036400 | | liver stage antigen 1 (LSA1), purified protein (0.3mg/mL) | P. lalop |
| 72 | 63 | 316 | 1 | 2 | 2 | 10 | RH1 PEP1 0.3mg/mL | PF027_040230 | | | reticulocyte binding protein homologue 1 (RH1) PEP1, purified protein (0.3mg/mL) | P. lalop |
| 73 | 64 | 317 | 1 | 2 | 2 | 11 | RH1 PEP1 0.3mg/mL | PF027_040230 | | | reticulocyte binding protein homologue 1 (RH1) PEP1, purified protein (0.3mg/mL) | P. lalop |
| 74 | 65 | 27 | 1 | 2 | 10 | RH1 PEP8 0.3mg/mL | PF027_040230 | | | | reticulocyte binding protein homologue 1 (RH1) PEP8, purified protein (0.3mg/mL) | P. lalop |
| 75 | 66 | 28 | 1 | 2 | 11 | RH1 PEP8 0.3mg/mL | PF027_040230 | | | | reticulocyte binding protein homologue 1 (RH1) PEP8, purified protein (0.3mg/mL) | P. lalop |
| 76 | 67 | 314 | 1 | 2 | 2 | 8 | RH2 0.3mg/mL | PF027_036400 | | | reticulocyte binding protein 2 homologue a (RH2a), purified protein (0.3mg/mL) | P. lalop |
| 77 | 68 | 315 | 1 | 2 | 2 | 9 | RH2 0.3mg/mL | PF027_036400 | | | reticulocyte binding protein 2 homologue a (RH2a), purified protein (0.3mg/mL) | P. lalop |
| 78 | 69 | 892 | 1 | 4 | 2 | 8 | PvAMA1 Ecto monomer prep2, 0.3mg/mL | EU089600.1 | NA | | apical membrane antigen 1 (AMA1) Ectodomain monomer, purified protein (0.3mg/mL) | P. vivax |
| 79 | 70 | 893 | 1 | 4 | 2 | 9 | PvAMA1 Ecto monomer prep2, 0.3mg/mL | EU089600.1 | NA | | apical membrane antigen 1 (AMA1) Ectodomain monomer, purified protein (0.3mg/mL) | P. vivax |
| 80 | 71 | 893 | 1 | 3 | 2 | 8 | PvAMA1 Ecto monomer prep2, 0.3mg/mL | EU089600.1 | NA | | apical membrane antigen 1 (AMA1), purified protein (0.3mg/mL) | P. vivax |
| 81 | 72 | 894 | 1 | 3 | 2 | 9 | PvAMA1 Ecto monomer prep2, 0.3mg/mL | EU089600.1 | NA | | apical membrane antigen 1 (AMA1), purified protein (0.3mg/mL) | P. vivax |
| 82 | 65 | 1028 | 1 | 4 | 10 | 8 | PvX_085560_342 | PvX_085560 | PvX_085560 | Exon 2 of 2 | hypothetical protein, conserved | P. vivax |
| 83 | 729 | 814 | 1 | 3 | 14 | 10 | PvX_085590_341_55 | PvX_085590 | PvX_085590 | Exon 1 of 1 Segment | hypothetical protein, conserved | P. vivax |
| 84 | 1016 | 837 | 1 | 3 | 16 | 4 | PvX_117880_342 | PvX_117880 | PvX_117880 | Exon 1 of 2 | hypothetical protein | P. vivax |
| 85 | 660 | 560 | 1 | 2 | 16 | 10 | PvX_081880_342 | PvX_081880 | PvX_081880 | Exon 2 of 2 | hypothetical protein | P. vivax |
| 86 | 103 | 504 | 1 | 2 | 13 | 11 | PvX_099880_341_52 | PvX_099880 | PvX_099880 | Exon 1 of 1 Segment | major bloodstage surface antigen Pv200 | P. vivax |
| 87 | 794 | 820 | 1 | 3 | 15 | 4 | PvX_096230_342 | PvX_096230 | PvX_096230 | Exon 1 of 2 | early transcribed membrane protein (ETRAMF) | P. vivax |
| 88 | 1046 | 537 | 1 | 2 | 15 | 10 | PvX_121930_342 | PvX_121930 | PvX_121930 | Exon 2 of 2 | hypothetical protein, conserved | P. vivax |
| 89 | 991 | 531 | 1 | 2 | 15 | 4 | PvX_115460_342 | PvX_115460 | PvX_115460 | Exon 1 of 2 | membrane associated holdenrich protein, MAHRP1 | P. vivax |
| 90 | 583 | 828 | 1 | 3 | 15 | 12 | PvX_000930_341 | PvX_000930 | PvX_000930 | Exon 1 of 1 | sexual stage antigen a16, putative | P. vivax |
| 91 | 1028 | 263 | 1 | 1 | 16 | 8 | PvX_118705_341 | PvX_118705 | PvX_118705 | Exon 1 of 1 | hypothetical protein, predicted Pf homolog liver stage antigen 3 | P. vivax |
| 92 | 373 | 272 | 1 | 1 | 16 | 17 | PvX_114145_341 | PvX_114145 | PvX_114145 | Exon 1 of 1 | Membrane surface protein 10, MSP10 | P. vivax |
| 93 | 883 | 850 | 1 | 3 | 16 | 17 | PvX_097825_341 | PvX_097825 | PvX_097825 | Exon 1 of 1 | Membrane Surface Protein 6, MSP6 | P. vivax |
| 94 | 954 | 244 | 1 | 1 | 15 | 6 | PvX_113245_342 | PvX_113245 | PvX_113245 | Exon 2 of 2 | cyclindependent protein kinase, predicted | P. vivax |
| 95 | 822 | 545 | 1 | 2 | 16 | 1 | PvX_091935_343 | PvX_091935 | PvX_091935 | Exon 2 of 3 | hypothetical protein | P. vivax |
| 96 | 136 | 508 | 1 | 2 | 16 | 14 | PvX_110935_341 | PvX_110935 | PvX_110935 | Exon 1 of 1 | hypothetical protein, conserved | P. vivax |
| 97 | 998 | 862 | 1 | 3 | 17 | 12 | PvX_116790_342 (tetraformed) | PvX_116790 | PvX_116790 | Exon 1 of 2 | hypothetical protein, conserved | P. vivax |
| 98 | 717 | 1143 | 1 | 4 | 17 | 4 | PvX_080025_341_52 (tetraformed) | PvX_080025 | PvX_080025 | Exon 1 of 1 Segment | hypothetical protein, conserved | P. vivax |

So, we need to basically start from row number 82 that is what we are interested in, because these are the IVTT spots. So, the first thing I am going to do is I am going to take an average for each and every spot. So, for example, let us write here average, and I am going to say is equal to.

(Refer Slide Time: 27:07)

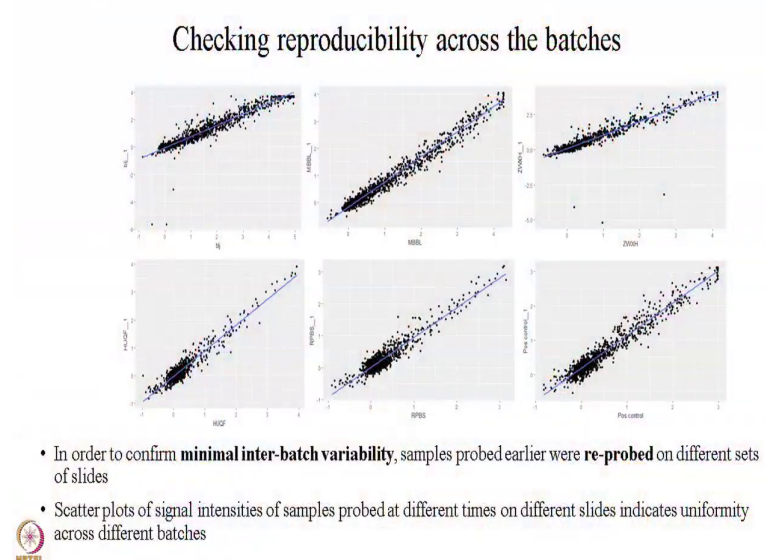
The screenshot shows an Excel spreadsheet with the following data structure:

| | DC | DD | DE | DF | DG | DH | DI | DJ | DK | DL | DM | DN | DO | DP | DQ | DR | DS | DT | DU | DV | DW | DX | DY |
|----|----|------|------|------|------|------|------|------|------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 65 | | 5245 | 5109 | 4575 | 5668 | 1062 | 1207 | | | | | | | | | | | | | | | | |
| 66 | | 5342 | 2322 | 2355 | 3907 | 4902 | 4440 | | | | | | | | | | | | | | | | |
| 67 | | 4543 | 1128 | 5406 | 5392 | 3433 | 9475 | | | | | | | | | | | | | | | | |
| 68 | | 5345 | 2953 | 3525 | 1824 | 3921 | 1027 | | | | | | | | | | | | | | | | |
| 69 | | 4277 | 8829 | 1252 | 1254 | 6824 | 1254 | 1252 | | | | | | | | | | | | | | | |
| 70 | | 4543 | 1128 | 5406 | 5392 | 3433 | 9475 | | | | | | | | | | | | | | | | |
| 71 | | 277 | 368 | 1182 | 261 | 1189 | 1847 | 3058 | 4808 | | | | | | | | | | | | | | |
| 72 | | 180 | 154 | 1090 | 142 | 1150 | 1346 | 651 | 3528 | | | | | | | | | | | | | | |
| 73 | | 878 | 376 | 1742 | 136 | 2444 | 1886 | 1411 | 672 | | | | | | | | | | | | | | |
| 74 | | 275 | 193 | 1173 | 103 | 1543 | 1257 | 561 | 308 | | | | | | | | | | | | | | |
| 75 | | 6443 | 8849 | 1252 | 3681 | 4543 | 9475 | 1252 | 1252 | | | | | | | | | | | | | | |
| 76 | | 1427 | 2813 | 8211 | 1713 | 1801 | 8211 | 1713 | 1801 | | | | | | | | | | | | | | |
| 77 | | 1446 | 1446 | 1446 | 1446 | 1446 | 1446 | 1446 | 1446 | | | | | | | | | | | | | | |
| 78 | | 7759 | 6524 | 4970 | 5246 | 1442 | 1442 | 1442 | 1442 | | | | | | | | | | | | | | |
| 79 | | 6184 | 4443 | 4182 | 4182 | 4182 | 4182 | 4182 | 4182 | | | | | | | | | | | | | | |
| 80 | | 8827 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | | | | | | | | | | | | | | |
| 81 | | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | | | | | | | | | | | | | | |
| 82 | | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | | | | | | | | | | | | | | |
| 83 | | 4452 | 4452 | 4452 | 4452 | 4452 | 4452 | 4452 | 4452 | | | | | | | | | | | | | | |
| 84 | | 1252 | 1252 | 1252 | 1252 | 1252 | 1252 | 1252 | 1252 | | | | | | | | | | | | | | |
| 85 | | 1442 | 1442 | 1442 | 1442 | 1442 | 1442 | 1442 | 1442 | | | | | | | | | | | | | | |
| 86 | | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | | | | | | | | | | | | | | |
| 87 | | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | | | | | | | | | | | | | | |
| 88 | | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | | | | | | | | | | | | | | |
| 89 | | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | | | | | | | | | | | | | | |
| 90 | | 4143 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | | | | | | | | | | | | | | |
| 91 | | 4143 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | | | | | | | | | | | | | | |
| 92 | | 4143 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | | | | | | | | | | | | | | |
| 93 | | 5105 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | | | | | | | | | | | | | | |
| 94 | | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | | | | | | | | | | | | | | |
| 95 | | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | | | | | | | | | | | | | | |
| 96 | | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | | | | | | | | | | | | | | |
| 97 | | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | | | | | | | | | | | | | | |
| 98 | | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | 1254 | | | | | | | | | | | | | | |

So, I get an average value, and I am going to just drag this down. So, you will have an average value for each and every spot for all the patients. What I am is telling you before is that in the previous sheet had many more columns here that is because they we had a lot more samples which have probed on the chips.

For example, we had positive controls which are nothing but samples from taken from a place which is a highly malaria endemic region. So, you know that those spots have to give you a signal right. So, those are my positive control samples. So, do not get confused between positive control samples and positive control spot, they are totally different.

(Refer Slide Time: 28:13)



So, these positive control samples I have excluded them from this from this particular analysis. We also had healthy controls which are basically malaria naive individuals means patients who were detected, were not detected with malaria at the time of admission. So, they were malaria negative, those patients were also taken and program the chip just to see there is a difference in response, such patients have also removed from the analysis.

There are also certain samples which I have probed repeatedly in probably in duplicates or four times in all you know once in all the sets just to check for reproducibility. So, here are some scatter plots you can see, where I am showing patient-patient reproducibility. So, basically I am showing a reproducible it between my badge runs. All of these patients also have removed from the analysis.

I have basically now in this excel 200 patients; 100 plasmodium vivax, and 96 plasmodium falciparum patients and 4 which a mixed infection also have removed. So, in this way you can choose to remove rows and columns based on what you want to study and you can make a excel less complicated right. So, that is what I had missed mentioning, but now that is done.

(Refer Slide Time: 29:33)

$$\text{Avg Raw value/antigen} > \text{Avg [Mean (No DNA controls)} \\ + 2\text{SD (Mean)}] = \text{Seroreactive antigens}$$



So, I have taken an average right now. Now, I am going to apply this particular formula which you see here. If the average value for a particular spot is more than twice the standard deviation of the mean of the no DNA controls, then that particular spot, then that particular antigen is zero reactive. So, what this means is that if this is the average, if this particular number is greater than this particular number which I am going to show you right now.

(Refer Slide Time: 30:01)

The screenshot displays an Excel spreadsheet with the following structure:

- Columns:** Labeled CQ, CR, CS, CT, CU, CV, CW, CX, CY, CZ, DA, DB, DC, DD, DE, DF, DG, DH, DI, DJ, DL, DM.
- Rows:** Numbered 606 to 638.
- Formula Bar:** Shows the formula =AVERAGE(D2:D3) .
- Data Table:** Contains numerical values for each cell. Some cells are highlighted in black, indicating specific data points or errors.
- Worksheet Name:** Immunogenic proteins_P1 and P2_1.1.18.xlsx.

If you take an average of the mean plus 2 times standard deviation of the no DNA spots, this is my number. So, if that raw value or if any raw value is greater than this value, then that spot is basically zero reactive, then that antigen is sorry then that antigen is basically zero reactive.

(Refer Slide Time: 30:41)

The screenshot shows an Excel spreadsheet with the following data structure:

| | CX | CY | CZ | DE | DF | DG | DH | DI | DJ | DK | DL | DM | DN | DO | DP | DQ | DR | DS | DT |
|----|---------|---------|-------|-------|-------|---------|---------|---------|---------|---------|---------|---------|----------|----|----|----|----|----|----|
| 61 | 362 | 689 | 108 | 1163 | 1072 | 1243 | 192 | 2208 | 1003 | 1687 | 1104 | 3487 | 1279 | | | | | | |
| 62 | 3442 | 1283 | 717 | 3142 | 1341 | 6156 | 1740 | 3792 | 3458 | 2472 | 2019 | 6476 | | | | | | | |
| 63 | 4272 | 493 | 206 | 1258 | 1258 | 474 | 2453 | 1159 | 2923 | 2809 | 2077 | 700 | 2065 | | | | | | |
| 64 | | | 8752 | 8710 | 9199 | 11803 | 11818 | 4692 | 8140 | 7687 | 8841 | | | | | | | | |
| 65 | | | 8261 | 8153 | 6642 | 11343 | 9818 | 3617 | 2676 | 5042 | 6037 | | | | | | | | |
| 66 | 4612 | 7402 | | | | 2645 | 5195 | 6175 | 3602 | 30432 | 10403 | | | | | | | | |
| 67 | 2169 | 4709 | 11367 | 35149 | 11626 | 5342 | 2912 | 2555 | 3907 | 4862 | 4848 | | | | | | | | |
| 68 | 1355 | 1340 | 8858 | 4122 | 12877 | 41614 | 3128 | 5496 | 5192 | 11021 | 5173 | | | | | | | | |
| 69 | 1792 | 1032 | 4925 | 2434 | 11146 | 1494 | 2915 | 325 | 1214 | 892 | 1037 | | | | | | | | |
| 70 | | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | 4444 | | | | | | | | |
| 71 | 8887 | 11341 | | 11401 | 11311 | 42114 | 3128 | 5496 | 5192 | 11021 | 5173 | | | | | | | | |
| 72 | 181 | 313 | 140 | 179 | 1487 | 277 | 360 | 1192 | 261 | 1389 | 1847 | 3054 | 4808 | | | | | | |
| 73 | 75 | 186 | 115 | 110 | 1150 | 180 | 156 | 1090 | 142 | 1150 | 1146 | 61 | 3528 | | | | | | |
| 74 | 179 | 1130 | 713 | 807 | 1792 | 873 | 176 | 1741 | 136 | 2444 | 6193 | 1411 | 672 | | | | | | |
| 75 | 152 | 2298 | 207 | 192 | 1154 | 271 | 193 | 1373 | 103 | 1543 | 1157 | 561 | 168 | | | | | | |
| 76 | 8416 | 3486 | 5412 | 2646 | 6463 | 8492 | 3411 | 8659 | 3679 | 8691 | 6173 | 2679 | 200 | | | | | | |
| 77 | 3588 | 1204 | 3104 | 1754 | 4185 | 5627 | 2813 | 6211 | 1733 | 8691 | 6173 | 2679 | 200 | | | | | | |
| 78 | 43424 | 17407 | 43024 | 40764 | 64430 | 12106 | 63123 | 11750 | 43063 | 64932 | 64810 | 80131 | 64904 | | | | | | |
| 79 | 12460 | 41301 | 17173 | 13887 | 13813 | 7219 | 43365 | 48970 | 42466 | 44942 | 44902 | 44902 | 44904 | | | | | | |
| 80 | 43139 | 44613 | 43796 | 12447 | 44444 | 44444 | 43044 | 44442 | 43122 | 43254 | 44796 | 42111 | 6206 | | | | | | |
| 81 | 17915 | 14414 | 34798 | 43045 | 43128 | 8037 | 43048 | 14441 | 43109 | 43021 | 44790 | 11107 | 45024 | | | | | | |
| 82 | 58075.5 | | 7493 | 48084 | 10740 | 1146 | 51957.5 | 63038.5 | 10272 | 34728.5 | 11522 | 86832.5 | 14799.04 | | | | | | |
| 83 | 44463.5 | 44463.5 | 51254 | 4054 | 44972 | 41462.5 | 47084.5 | 46297.5 | 46504 | 54175.5 | 1207 | 27483.5 | 41704.11 | | | | | | |
| 84 | 45492.5 | 45492.5 | 54444 | 41439 | 11115 | 10207 | 41462.5 | 48382.5 | 8915.5 | 46504 | 54175.5 | 1207 | 27483.5 | | | | | | |
| 85 | 12460.5 | 10213.5 | 10205 | 41472 | 38448 | 58471.5 | 10205 | 51921 | 54137.5 | 54073 | 62150.5 | 10205 | 10205 | | | | | | |
| 86 | 4205.5 | 10205 | 11461 | 43447 | 10461 | 63174.5 | 4925.5 | 11185.5 | 19104 | 54027.5 | 47506 | 48138.5 | 10205 | | | | | | |
| 87 | 12117.5 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | | | | | | |
| 88 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | | | | | | |
| 89 | 11121.5 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | | | | | | |
| 90 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | | | | | | |
| 91 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | | | | | | |
| 92 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | | | | | | |
| 93 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | | | | | | |

So, I am going to say if this is equal to if function, this spot is greater than this, then 1 else 0, so I get 1 here.

(Refer Slide Time: 31:11)

| | DF | DG | DH | DI | DJ | DK | DL | DM | DN | DO | DP | DQ | DR | DS | DT | DU | DV | DW | DX |
|----|-------|-------|-------|-------|-------|----------|----|----|----|----|------|-------|-------|-------|----|----|----|----|----|
| 48 | 3554 | | | 11435 | 9020 | | | | | | 6999 | 13764 | | 6545 | | | | | |
| 49 | 2654 | | | 14036 | 9352 | 7715 | | | | | 5628 | 13277 | | 6031 | | | | | |
| 50 | 1620 | 8801 | 7716 | 3531 | 2364 | | | | | | 3466 | 8446 | 13145 | 2221 | | | | | |
| 51 | 1722 | 11187 | 15446 | 4888 | 3123 | | | | | | 3591 | 8822 | 17688 | 2786 | | | | | |
| 52 | 1744 | 19638 | 10122 | 4587 | 3506 | | | | | | 3565 | 8236 | 12002 | 2612 | | | | | |
| 53 | 841 | 10552 | 7522 | 3651 | 2892 | | | | | | 2894 | 6226 | 11331 | 2368 | | | | | |
| 54 | 1024 | 5908 | 4893 | 1480 | 1138 | | | | | | 2146 | 4787 | 8871 | 947 | | | | | |
| 55 | 718 | 6742 | 7358 | 2288 | 1412 | | | | | | 2070 | 5261 | 10196 | 1247 | | | | | |
| 56 | 373 | 5265 | 5913 | 1696 | 1333 | | | | | | 2036 | 3930 | 6230 | 1176 | | | | | |
| 57 | 536 | 6685 | 3788 | 1442 | 1233 | | | | | | 1838 | 2345 | 6877 | 864 | | | | | |
| 58 | 1947 | 9010 | 10442 | 13261 | | 5187.65 | 1 | | | | 1924 | 8371 | 12639 | 11923 | | | | | |
| 59 | 3118 | 9867 | 11302 | 14114 | | 5812.56 | 1 | | | | 2847 | 7901 | | 1576 | | | | | |
| 60 | 2172 | 3920 | 2680 | 10617 | 4122 | 1933.34 | 0 | | | | 1313 | 1722 | 5557 | 6156 | | | | | |
| 61 | 1003 | 1687 | 1104 | 3487 | 1279 | 890.66 | 0 | | | | 1342 | 1504 | 2899 | 2968 | | | | | |
| 62 | 5791 | 5459 | 2872 | 2059 | 6875 | 1814.7 | 0 | | | | 312 | 4812 | 5405 | | | | | | |
| 63 | 2923 | 2829 | 2077 | 700 | 2065 | 911.25 | 0 | | | | 489 | 8698 | 2498 | 11677 | | | | | |
| 64 | 8243 | | 7687 | 8841 | | 7895.72 | 1 | | | | 1830 | 11341 | 17688 | 11964 | | | | | |
| 65 | 7676 | | 5042 | 6637 | | 6868.23 | 1 | | | | 1837 | 10249 | 12241 | 11321 | | | | | |
| 66 | 4573 | 5659 | 10652 | 10033 | | 7572.84 | 1 | | | | 1898 | 1504 | 7473 | 51261 | | | | | |
| 67 | 2555 | 3907 | 4862 | 4848 | 1981 | 4733.38 | 0 | | | | 1008 | 1100 | 4578 | 51942 | | | | | |
| 68 | 5382 | 18341 | 17176 | 9375 | 11842 | 4057.36 | 0 | | | | 1347 | 2748 | 10155 | 8662 | | | | | |
| 69 | 1524 | 3421 | | 3207 | | 3830.57 | 0 | | | | 645 | 2751 | 10196 | 8659 | | | | | |
| 70 | 5784 | 4582 | 12546 | 2145 | 10205 | 30797.21 | 1 | | | | | 8594 | 64119 | 51624 | | | | | |
| 71 | 11658 | 16291 | | 11181 | | 16407.79 | 1 | | | | | 8563 | 65250 | 39408 | | | | | |
| 72 | 261 | 1389 | 1847 | 3058 | 4808 | 599.83 | 0 | | | | 361 | 3788 | 1496 | 295 | | | | | |
| 73 | 142 | 1150 | 1346 | 651 | 3529 | 446.6 | 0 | | | | 156 | 2807 | 1644 | 225 | | | | | |
| 74 | 136 | 2444 | 6193 | 1411 | 672 | 941.82 | 0 | | | | 473 | 1063 | 2175 | 2087 | | | | | |
| 75 | 109 | 1543 | 5252 | 561 | 268 | 538.19 | 0 | | | | 305 | 1558 | 2042 | 482 | | | | | |

Then what I finally, get is an excel sheet like this where I have random ones and zeros right. So, all of these ones I am going to now say are my zero reactive proteins because they are greater than twice the standard deviation of my control spots. Now, a lot of people may also use healthy controls in their analysis right, but we do not have them.

So, what they do is they compare the signals in signal intensities in a malaria group versus a healthy population, but since we do not have all that I am going to simply say that this is mine, these are the list of my zero reactive proteins which I can now take forward for further analysis.

So, this is not a great start, this is not a statistical test this is only short listing my proteins, and here I am only short listing my proteins from 1500 to a handful which I can then take forward and study. So, this is what that sheet is. Now, what I have done here is I have taken

this for a group of patients, but now what if I want to check this for a particular patient, so that is what is my antibody breath which you see here. I have done this individually for every single patient, maybe I will zoom this a little bit.

(Refer Slide Time: 32:21)

| | DJ | DL | DM | DN | DO | DP | DQ | DR | DS | DT | DU | DV | DW | DX | DY | DZ | EA | EB | EC | ED | EE | EF | EG |
|-----|--------|---------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 559 | 65.5 | 224.13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 560 | -156.5 | 195.77 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 561 | 5076.5 | 195.61 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 562 | 481.5 | 362.72 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 563 | 555.5 | 355.73 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 564 | 22.5 | 132.47 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 565 | 1345.5 | 123.66 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 566 | 4.5 | 123.82 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 567 | 120.5 | 140.73 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 568 | -73.5 | 308.92 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 569 | 257.5 | 84.58 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 570 | -38.5 | 67.75 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 571 | 7768.5 | 51.16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 572 | 896.5 | -16.22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 573 | -41.5 | -8.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 574 | -21.5 | -8.8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 575 | 158.5 | -16.35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 576 | -45.5 | -25.57 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 577 | -109.5 | -139.01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 578 | -95.5 | -117 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 579 | -41.5 | -228.18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 580 | 2073.5 | -283.38 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 581 | 561.5 | -302.38 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 582 | 137.5 | -328.18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 583 | 34.5 | -372.15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 584 | -113.5 | -352.23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 585 | 4184.5 | -413.12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 586 | 486.5 | -508.01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 587 | -132.5 | -578.26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 588 | 195.5 | -752.18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 589 | 329.5 | -801.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 590 | 478.5 | -800.04 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 591 | 440.5 | -805.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

So, what you see here is that of zoom this for every single patient. So, basically the previous one which I showed you was it was the average for a single spot for a group of patients as well as the average for the no DNA control. Here I have done it for each patient which means it is a sample specific right.

(Refer Slide Time: 32:45)

The screenshot shows an Excel spreadsheet with the following data structure:

| | DJ | DK | DL | DM | DN | DO | DP | DQ | DR | DS | DT | DU | DV | DW | DX | DY | DZ | EA | EB | EC | ED | EE | EF |
|-----|--------|----------|----|-----------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 586 | 486.5 | -508.21 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 587 | -132.5 | -578.26 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 588 | 195.5 | -752.18 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 589 | 329.5 | -851.3 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 590 | 478.5 | -850.04 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 591 | 648.5 | -855.3 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 592 | -177.5 | -900.01 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 593 | 2041.5 | -1009.74 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 594 | 847.5 | -1211.7 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 595 | -534.5 | -1287.45 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 596 | 1088.5 | -1432.42 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 597 | -229.5 | -1483.71 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 598 | | | | | | | | | | | | | | | | | | | | | | | |
| 599 | 2126 | | | Ab breath | 12 | 11 | 13 | 38 | 15 | 31 | 34 | 24 | 77 | 20 | 23 | 48 | 39 | 75 | 43 | 12 | 36 | 53 | 94 |
| 600 | 2342 | | | | | | | | | | | | | | | | | | | | | | |
| 601 | 2093 | | | | | | | | | | | | | | | | | | | | | | |
| 602 | 1902 | | | | | | | | | | | | | | | | | | | | | | |
| 603 | 1858 | | | | | | | | | | | | | | | | | | | | | | |
| 604 | 1815 | | | | | | | | | | | | | | | | | | | | | | |
| 605 | 1832 | | | | | | | | | | | | | | | | | | | | | | |
| 606 | 2093 | | | | | | | | | | | | | | | | | | | | | | |
| 607 | 1912 | | | | | | | | | | | | | | | | | | | | | | |
| 608 | 1985 | | | | | | | | | | | | | | | | | | | | | | |
| 609 | 1826 | | | | | | | | | | | | | | | | | | | | | | |
| 610 | 1864 | | | | | | | | | | | | | | | | | | | | | | |
| 611 | 2315 | | | | | | | | | | | | | | | | | | | | | | |
| 612 | 2487 | | | | | | | | | | | | | | | | | | | | | | |
| 613 | 2634 | | | | | | | | | | | | | | | | | | | | | | |
| 614 | 2487 | | | | | | | | | | | | | | | | | | | | | | |
| 615 | 2358 | | | | | | | | | | | | | | | | | | | | | | |
| 616 | 2320 | | | | | | | | | | | | | | | | | | | | | | |
| 617 | 2129 | | | | | | | | | | | | | | | | | | | | | | |
| 618 | 1861 | | | | | | | | | | | | | | | | | | | | | | |

So, in this way if I if I scroll down what you will get here, this you will you will know the number of zero reactive antigens per patient which means that if one patient for example, here a zero reactive to only 12 antigens, whereas, there are some other patients which is zero reactive to 77 antigens. So, this is basically my antibody breath. So, these are the two basic kind of analysis which I can show you in excel for now.

Power of microarray technology is basically the fact that you can perform this experiment very fast probably in a day or two. And then using any kind of patient data, all you need to do is map this whole data which of microarray data to each and every patient clinical information that you have. And then you need to you can perform any kind of statistical analysis and you can generate several results from the same single experiment so that is the beauty of this.

I hope you have got a glimpse of how to perform data analysis and how and how this basic statistics can be done, and how this is not the only way to do statistics at all you can do use softwares and programming, and I would still recommend that people do programming because if you want to make even a single small change, you do not have to repeat the entire analysis.

Also tomorrow if somebody provides you some other clinical information of the same patient population, you do not have to repeat the analysis in excel, you can simply write a code for it and then in a few minutes you will get results for that as well. So, that is all for now. Thank you.

(Refer Slide Time: 34:07)

Points to Ponder

- Basic microarray data can be analysed using excel, however programming is highly recommended for larger data analysis
- R programming is a simple language and has been very useful for biologists
- Two types of normalization was used for the analysis: Sample specific median normalization is used for the purpose of visualization, while Log2 FOC is used for statistical analysis because it is more stringent



(Refer Slide Time: 34:23)

Points to Ponder

- The use of control samples and control spots are very important for any microarray data
- IgG positive controls are used for checking the overall experiment performance. In some cases, re-scanning at different PMT settings is recommended
- Multiple softwares could be explored for data analysis as well, in case programming is not an option



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After going through this demonstration session and the insights of doing microarray based data analysis, you must have realized that there are many ways of analyzing and representing microarray data. Of course, there is no single way, no correct way of telling you what is the best way of doing it analysis. There are many considerations you have to keep in mind when you are thinking about how to make meaningful information out of this high throughput data.

There are several questions that can be answered using microarray data provided your data passes the quality control checks and it is properly normalized. In such experiments your control features becomes very crucial. Both the positive controls and negative controls they guide you about how accurate and real a data is. They could distinguish between real signals and background noise after proper analysis methods.

In the next class, you will see another application of protein microarrays in a different application there we will shift the gears to the cancer research and also the platforms. So, far we have talked about cell free expression based protein microarray platform. We will not talk about how to take purified proteins printed on the chip using human proteome arrays, and then apply those to investigate a deadly disease cancer and try to talk to you about both extra mental demonstrations as well as the theoretical concepts involved in performing such biological experiments.

See you in next lecture.

Thank you.