

MEP-LINCs SS2 Pilot Analysis

2015-07-16

Summary

This experiment uses the MEP-LINCs SS2 staining set on PC3 cells in 8 well MEMAs.

Introduction

The plates were printed with 35 row by 20 column MEMAs using a 4x7 pin head that printed 5x5 blocks. Each sample spot contains one ECM protein paired with Collagen I. There are 46 different ECM proteins in the array arranged in a random fashion.

Images of each well were gathered on a Tecan LS Reloaded laser scanner and Olympus ScanR automated microscope. This staining set includes, DAPI, H3K9me3 (488nm), Fibrillarin (532 and 555nm) and EdU (635 and 647nm). Data from DAPI staining is only gathered by the ScanR.

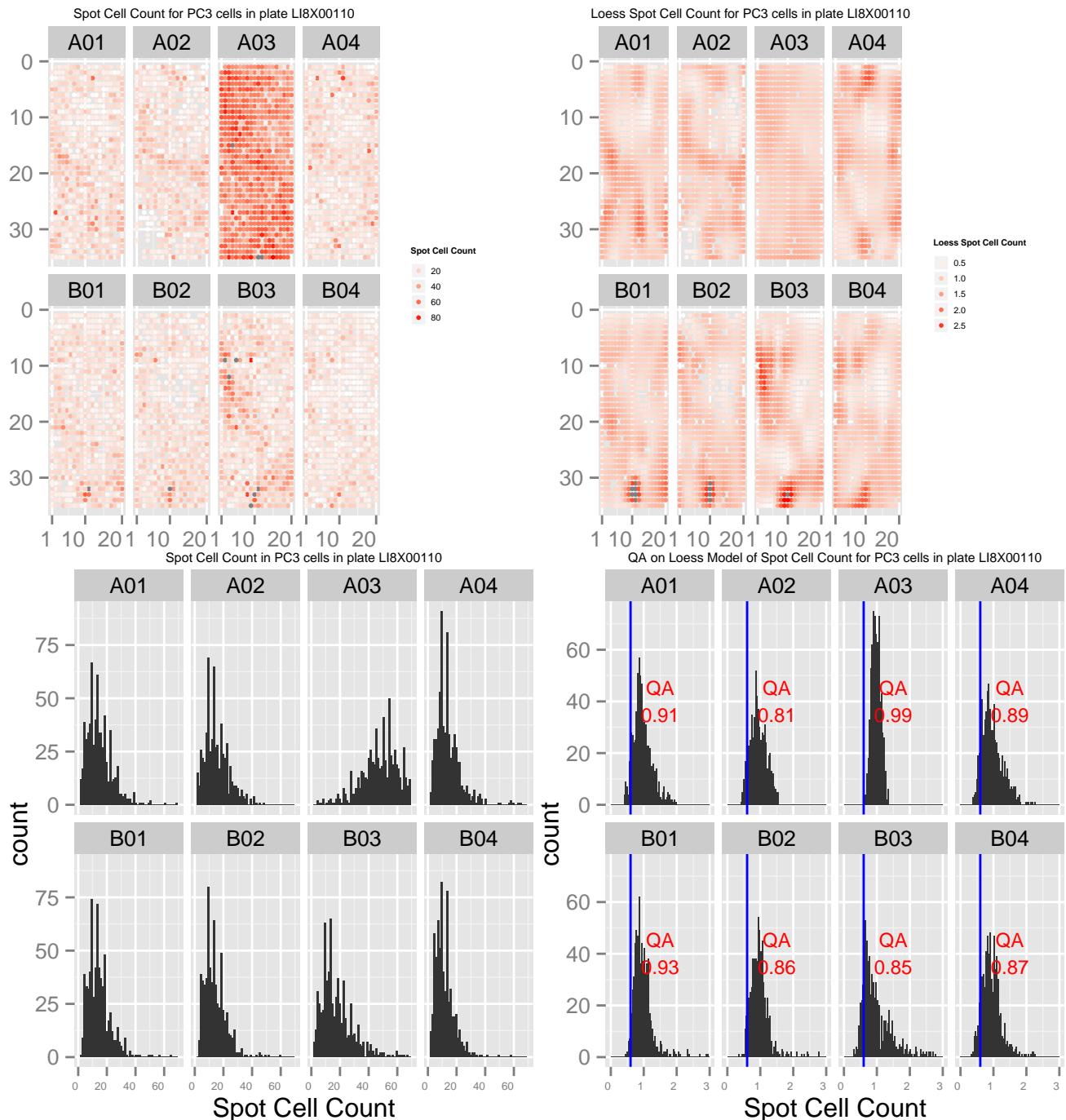
Tecan data is gathered at the spot population level by fitting round regions of interest (ROIs) to each spot. The Tecan data in this report uses the net values defined as the raw ROI value minus the mean of the local background.

The ScanR data comes from the nucleus as defined by the DAPI staining and a cytoplasmic annulus of 10 pixels that surrounds the nucleus.

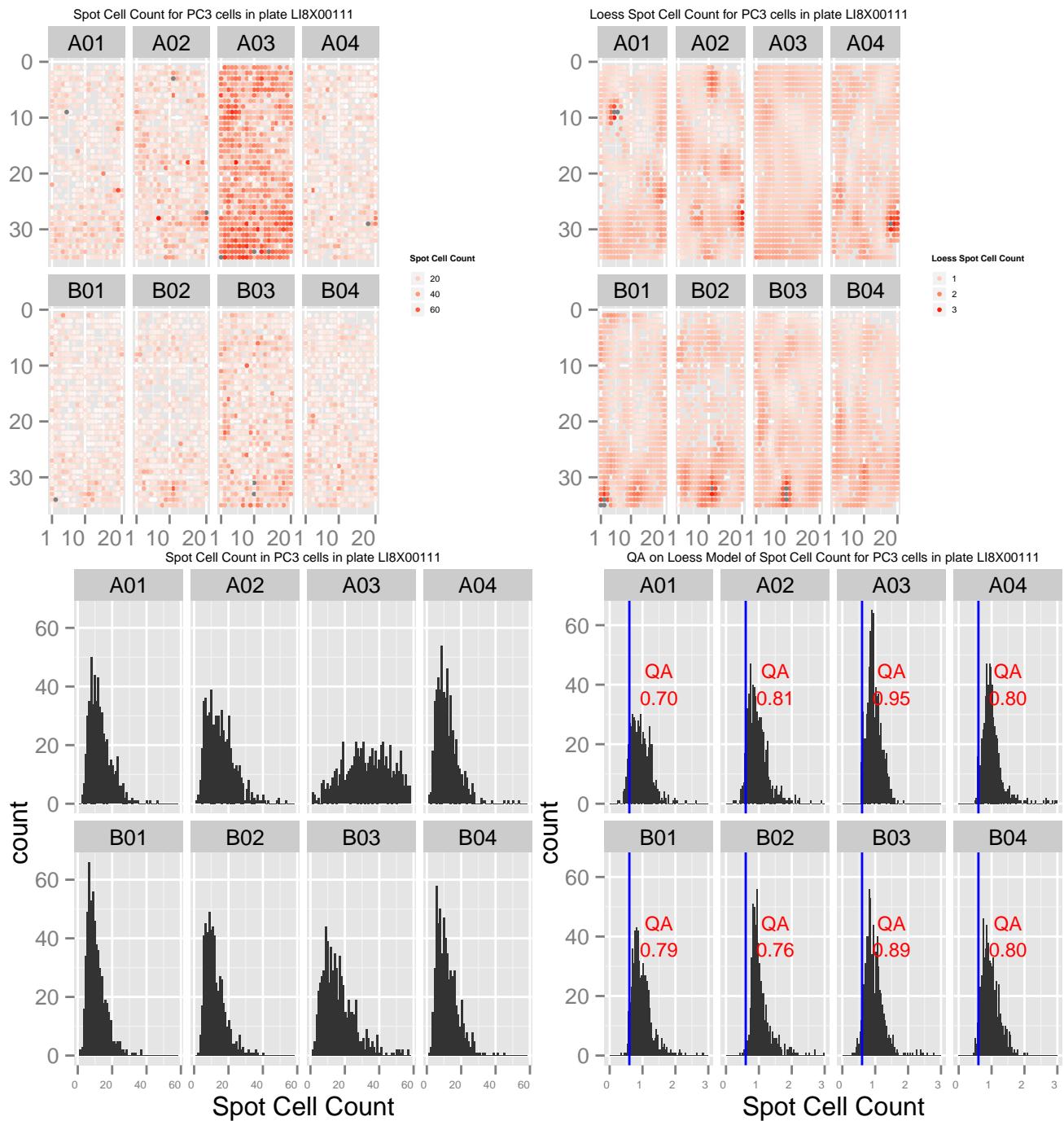
The spots that were not printed are labeled as blank.

QA Scoring of the dataset

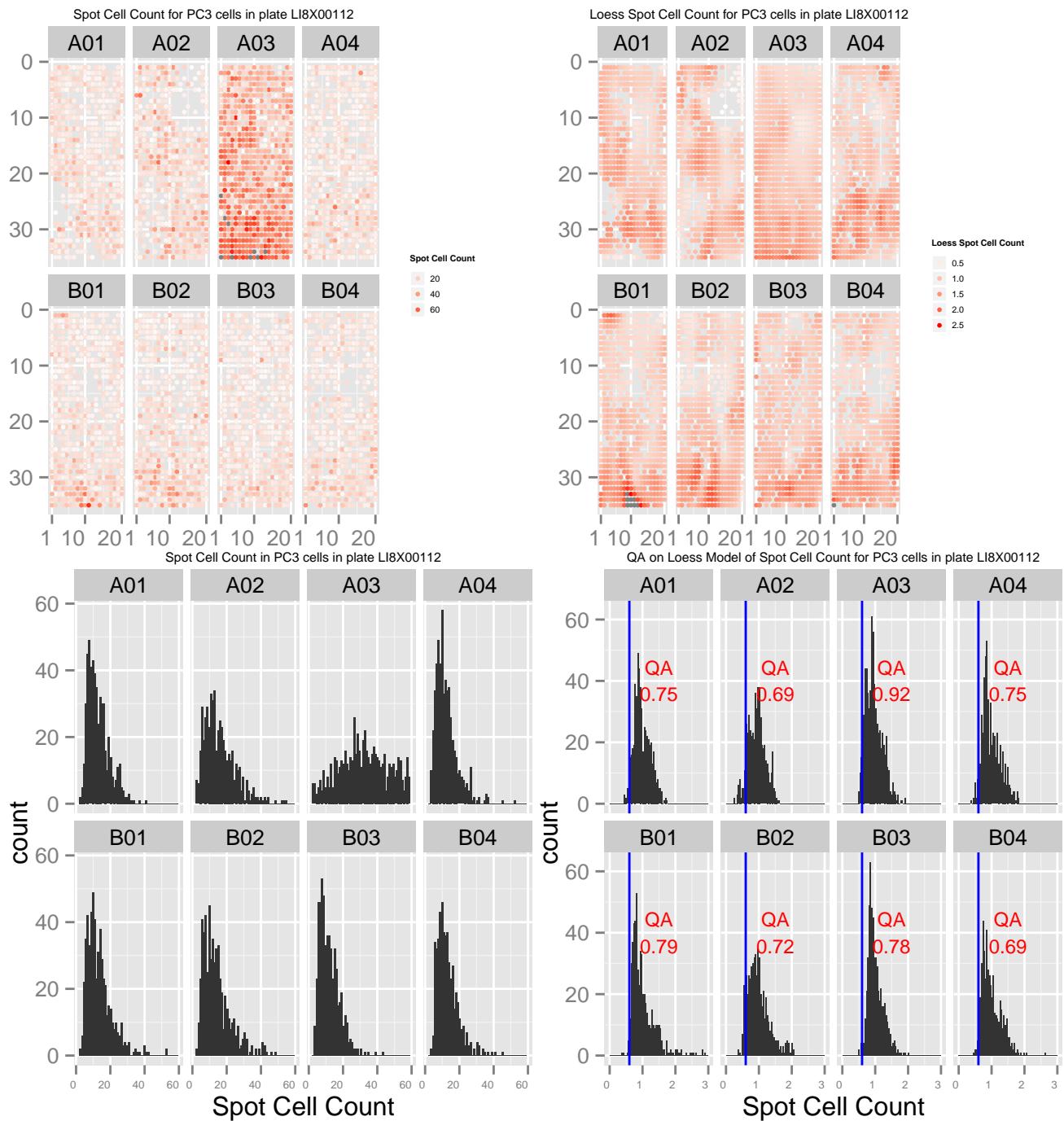
Each well is scored for even cell seeding according the count of the DAPI-stained nuclei.



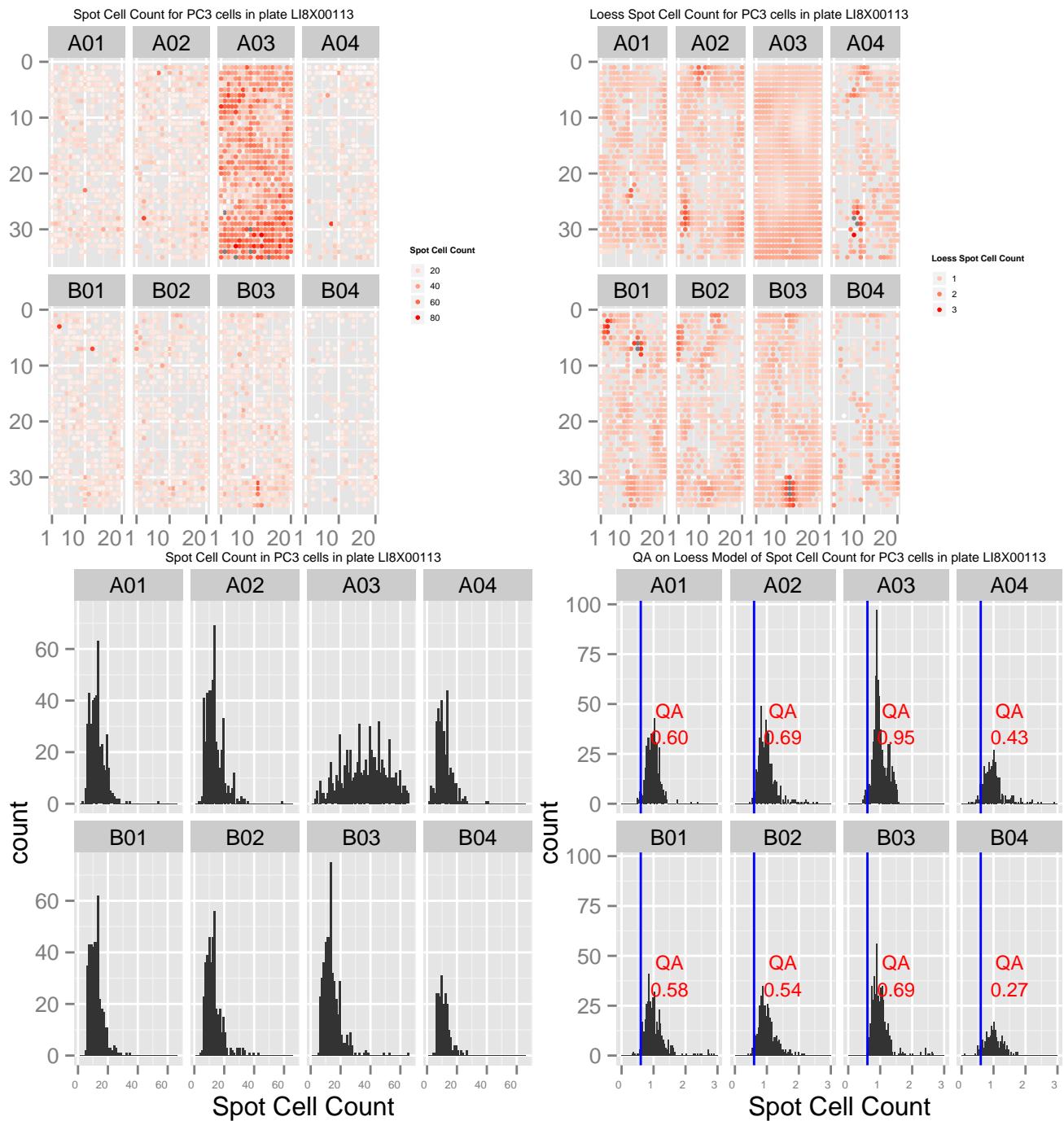
```
## Mean QA Score for LI8X00110 = 0.89
```



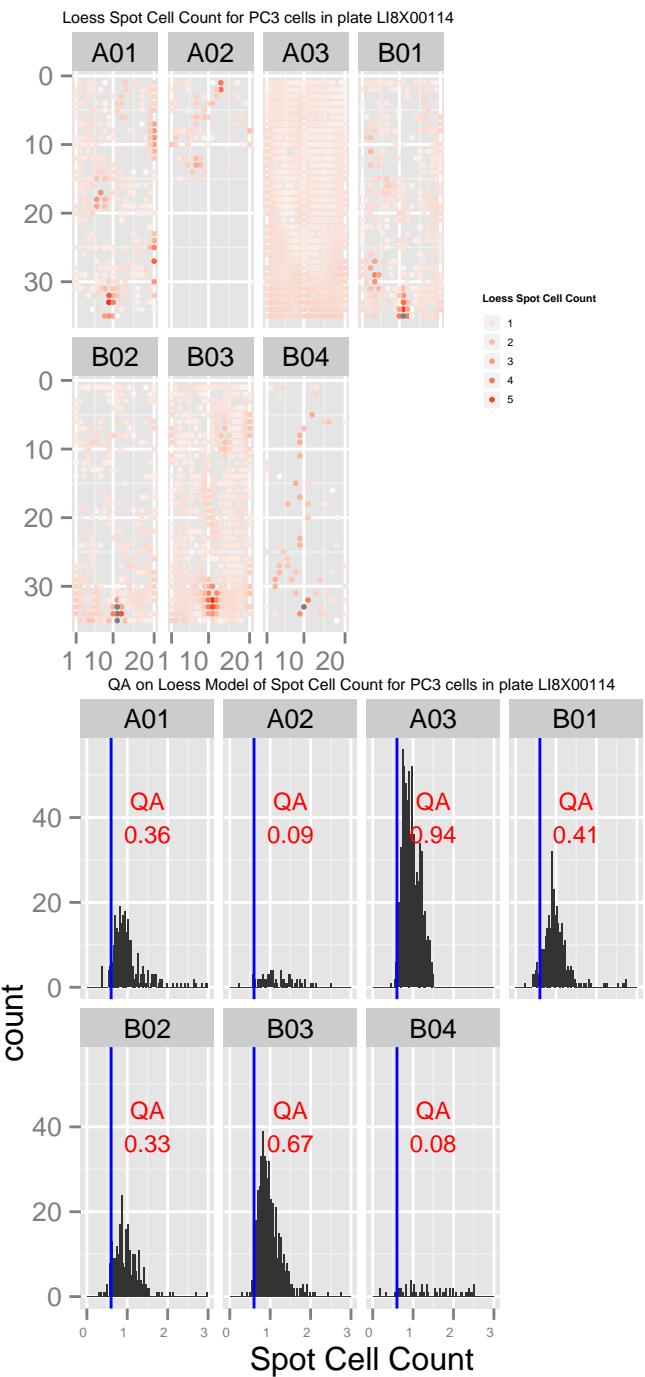
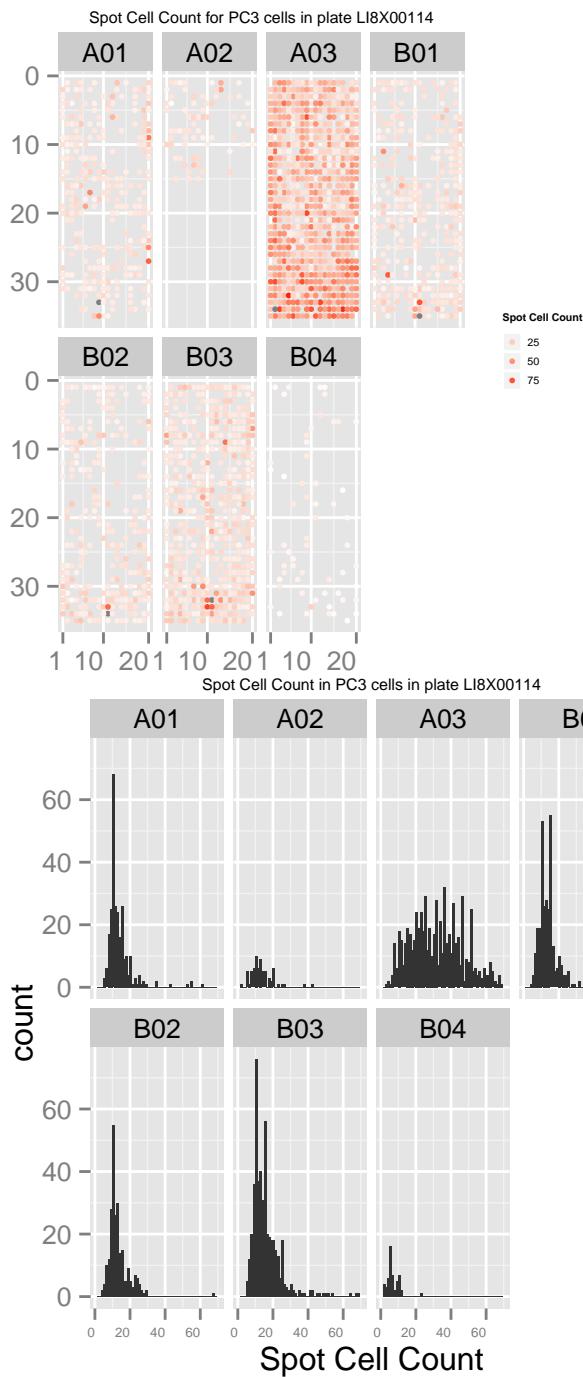
```
## Mean QA Score for LI8X00111 = 0.82
```



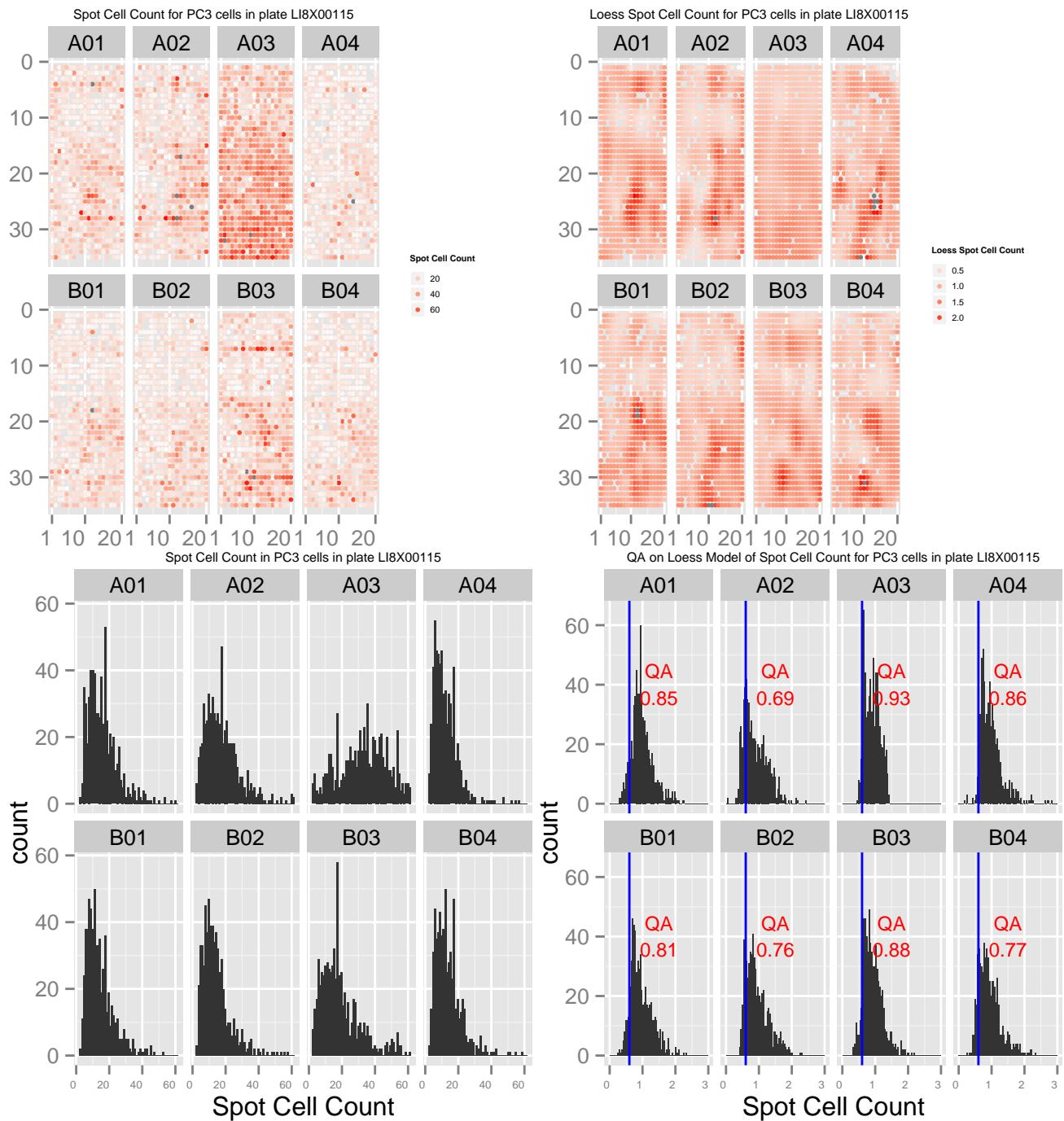
```
## Mean QA Score for LI8X00112 = 0.77
```



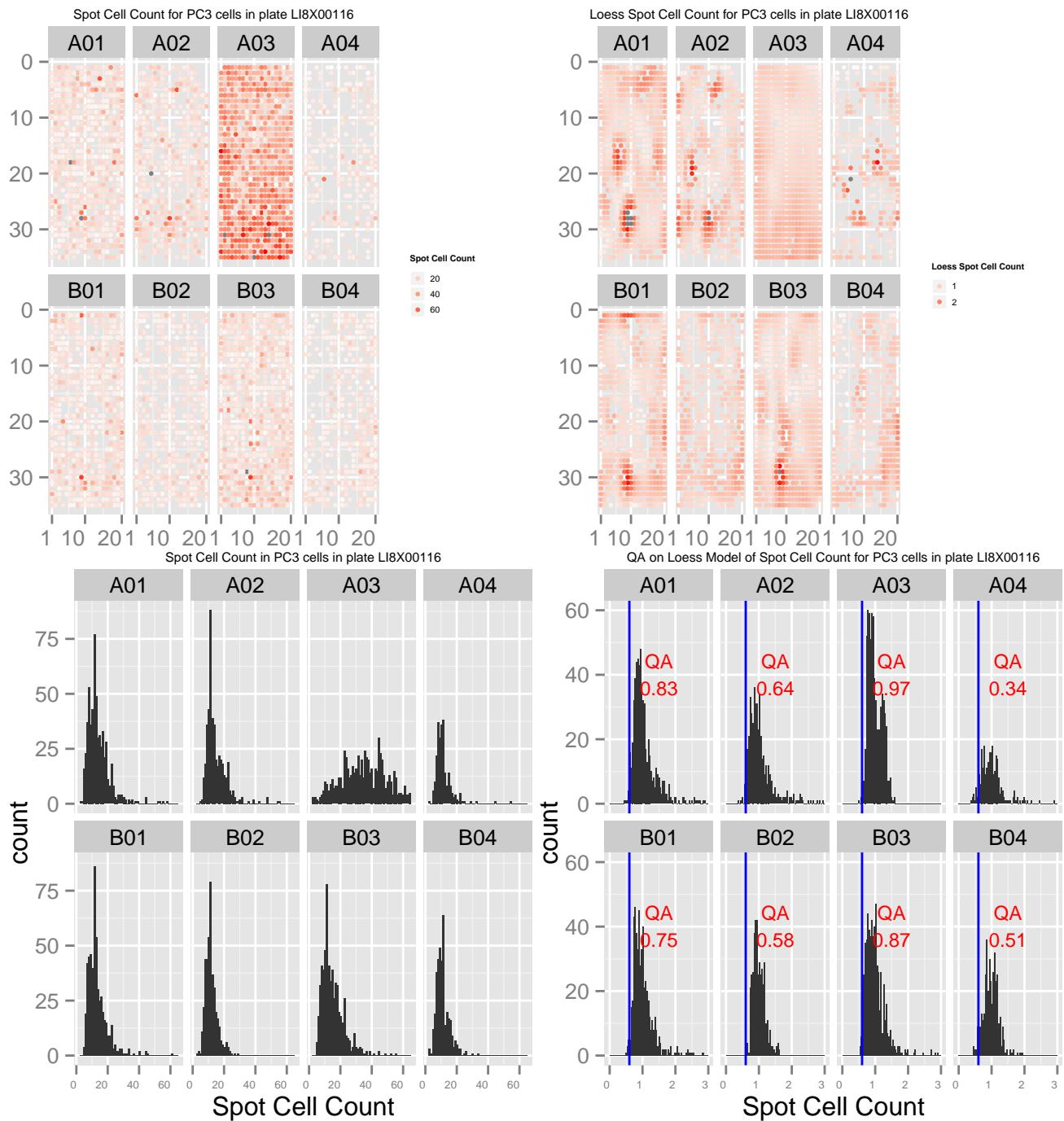
```
## Mean QA Score for LI8X00113 = 0.65
```



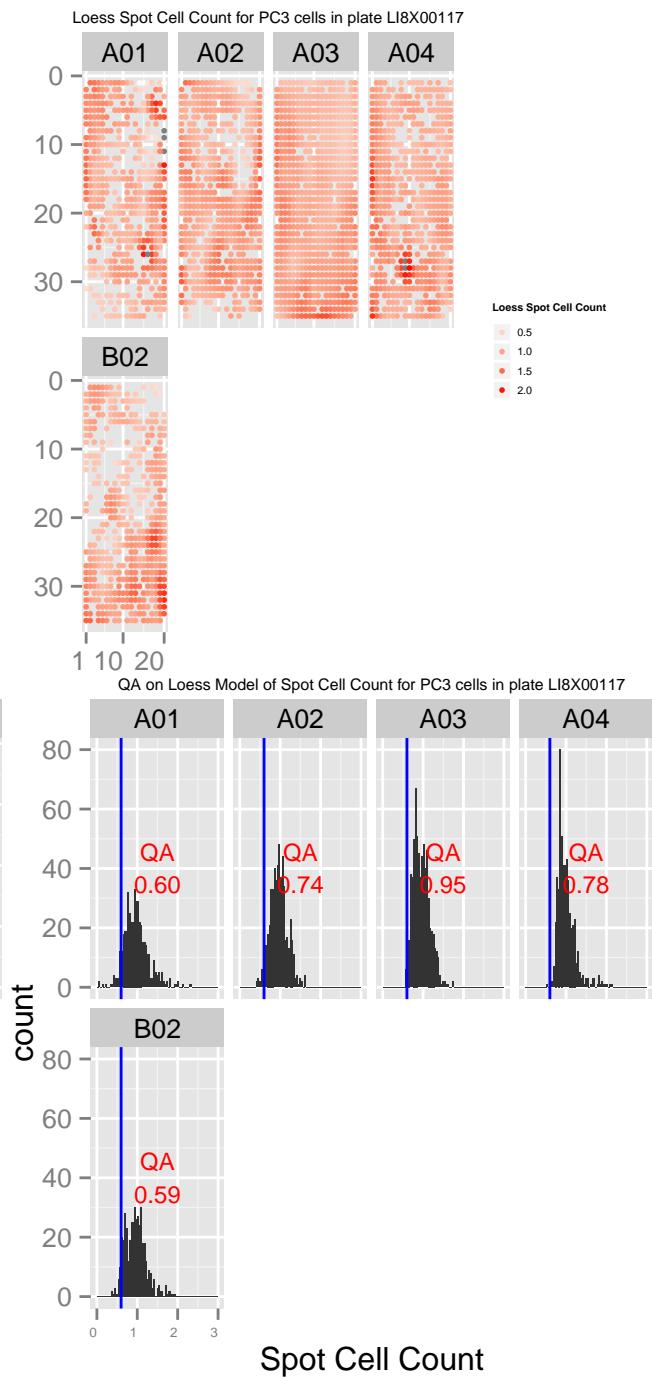
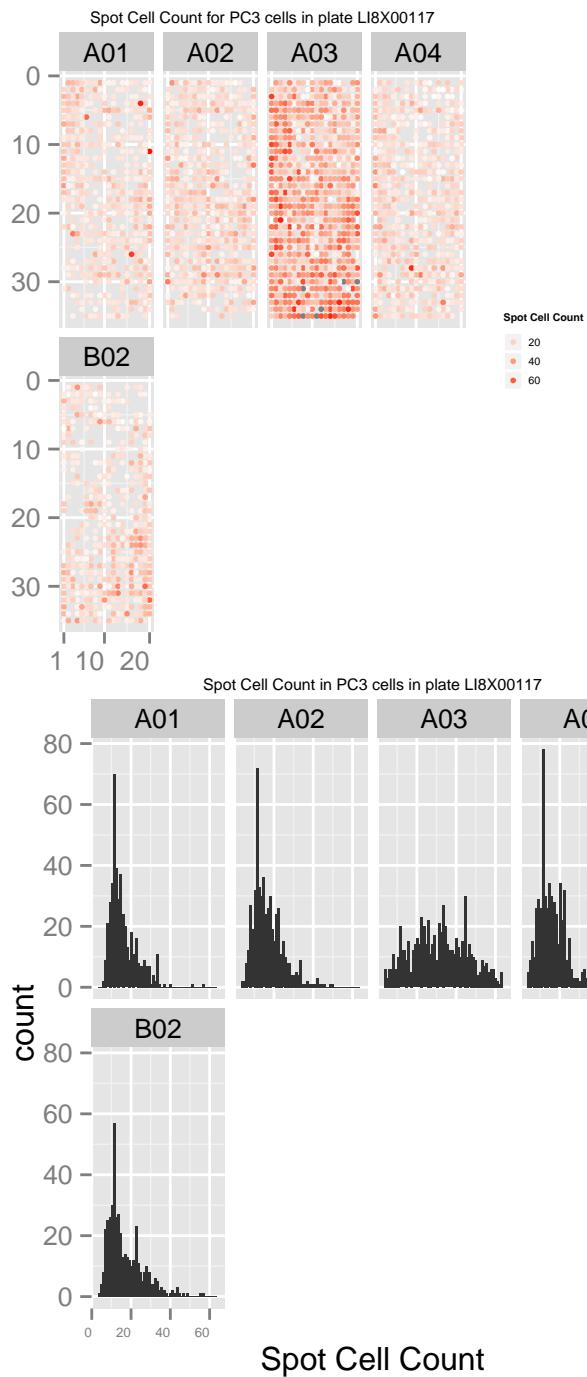
Mean QA Score for LI8X00114 = 0.60



```
## Mean QA Score for LI8X00115 = 0.82
```



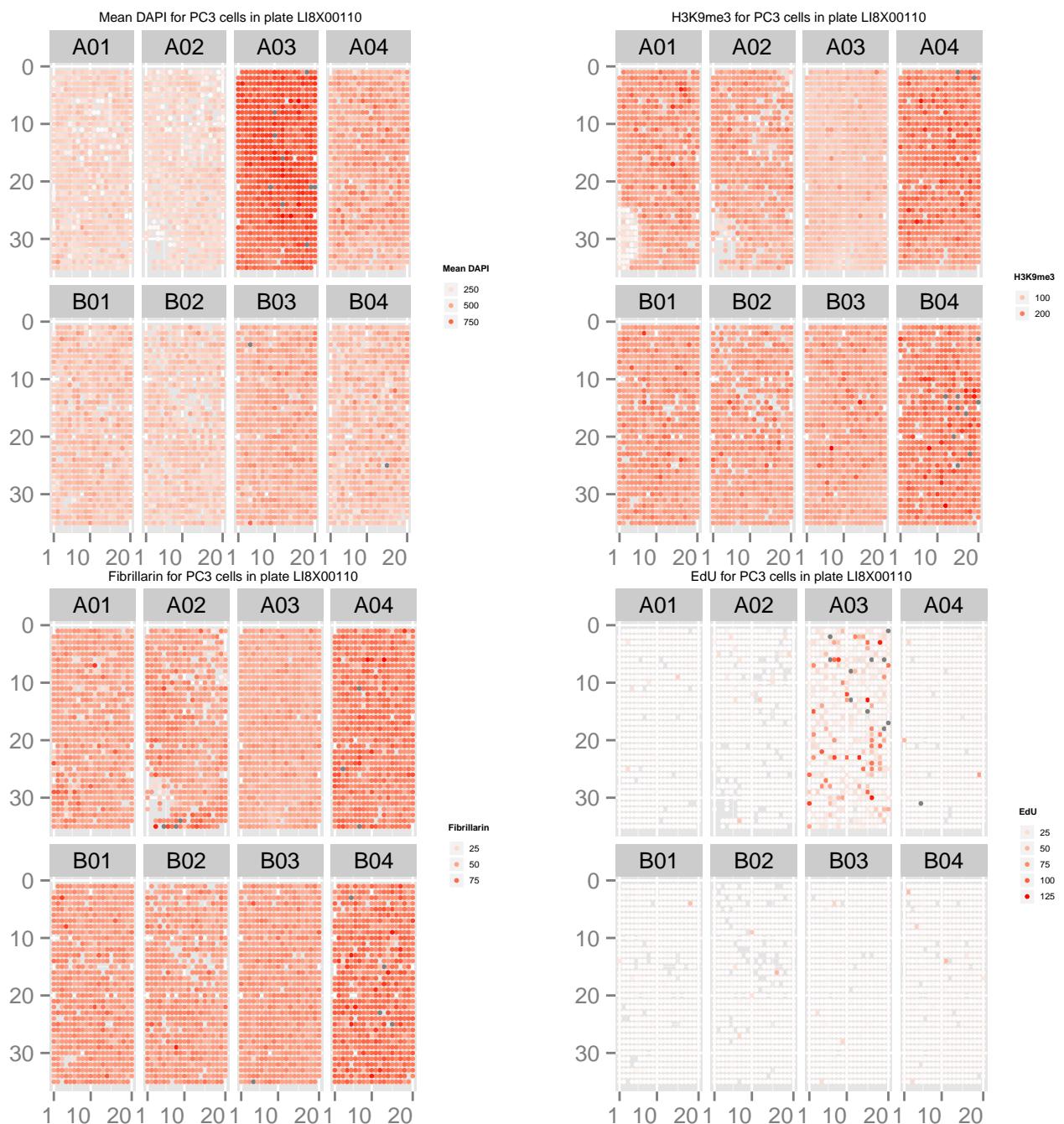
```
## Mean QA Score for LI8X00116 = 0.74
```

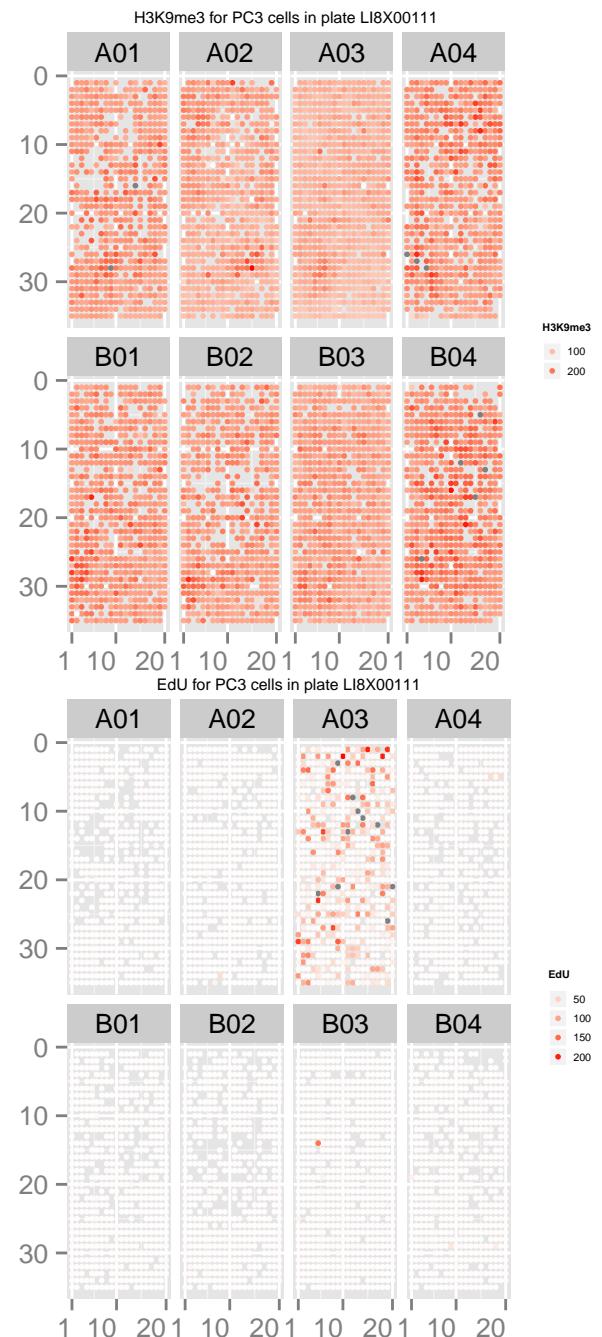
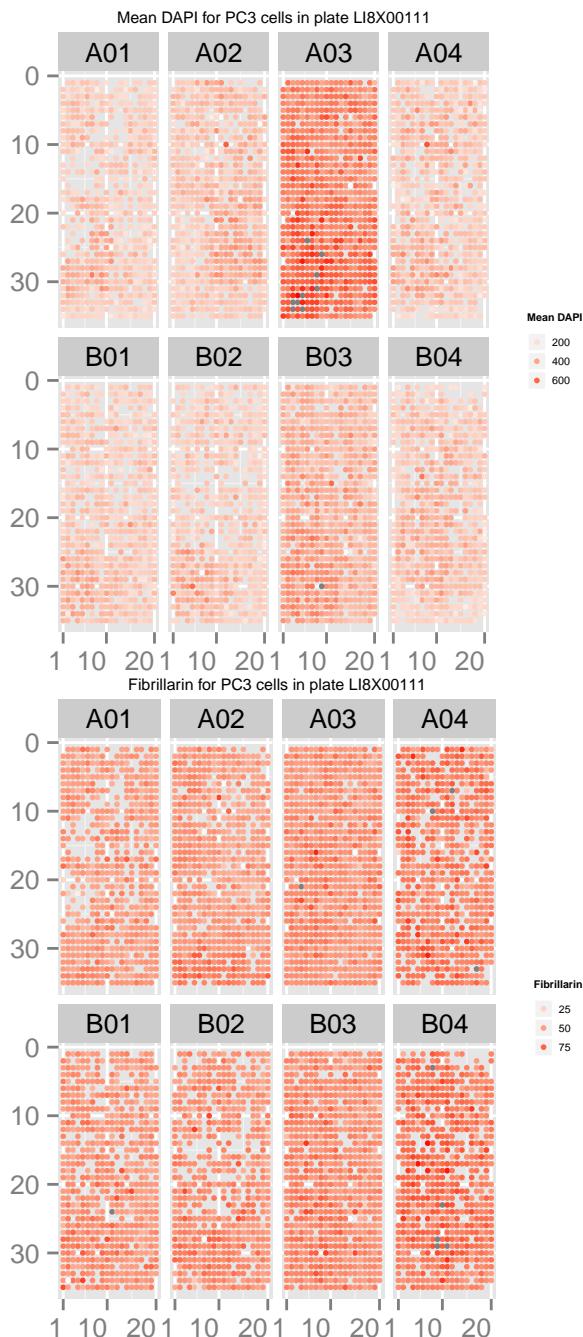


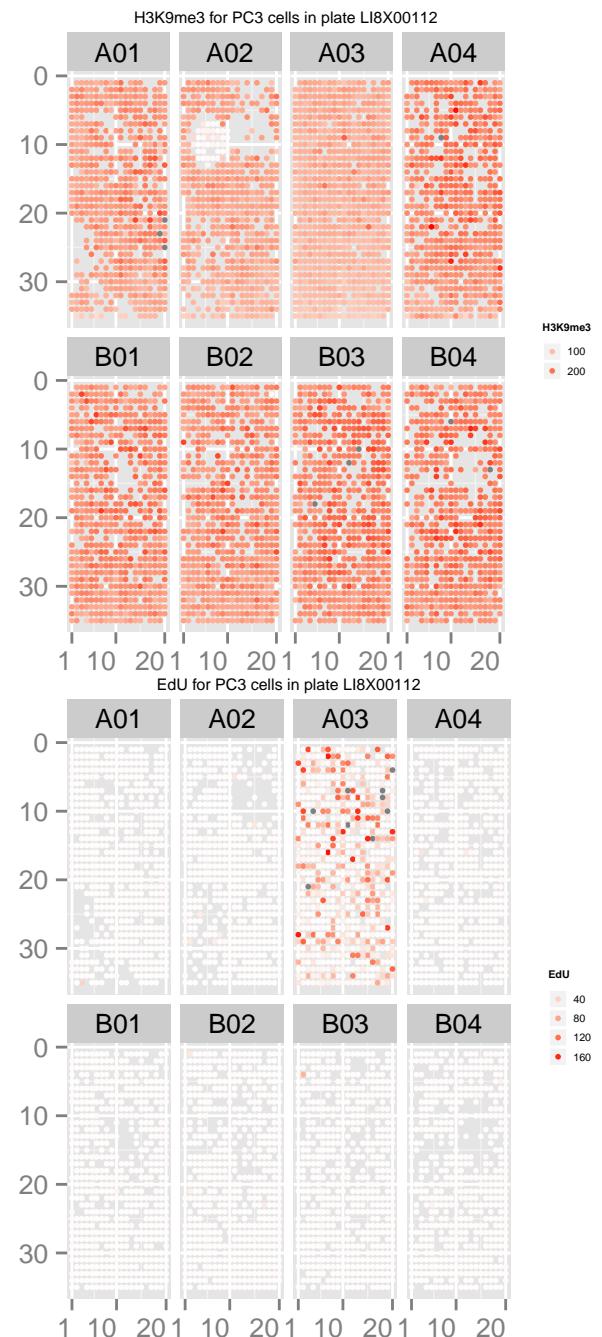
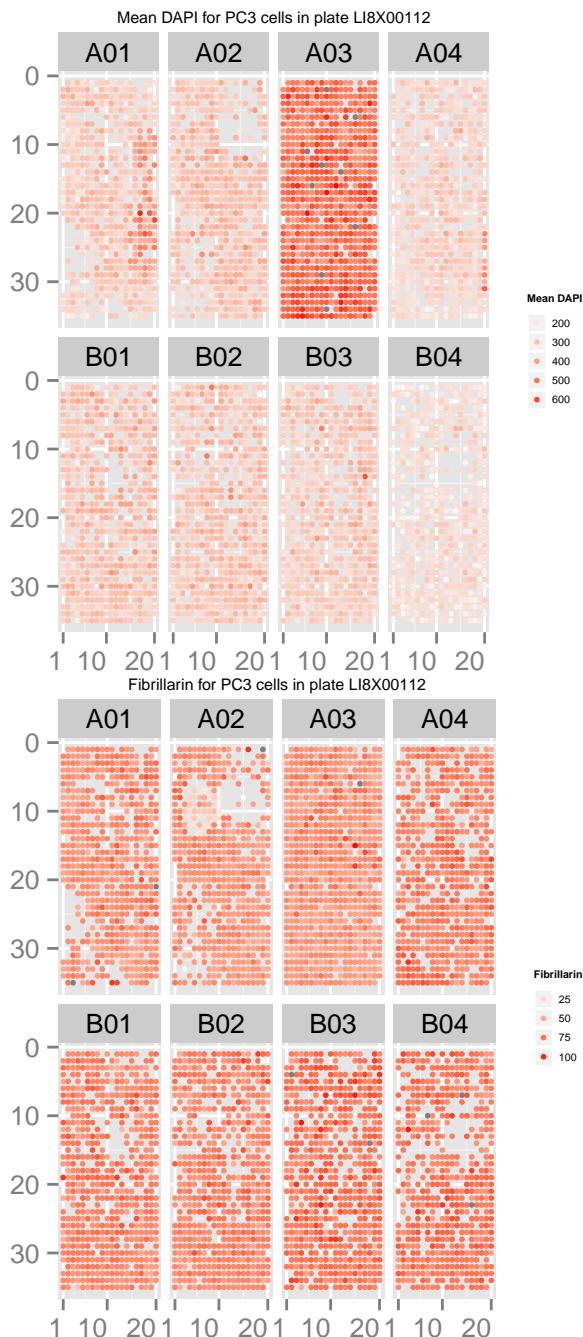
```
## Mean QA Score for LI8X00117 = 0.75
```

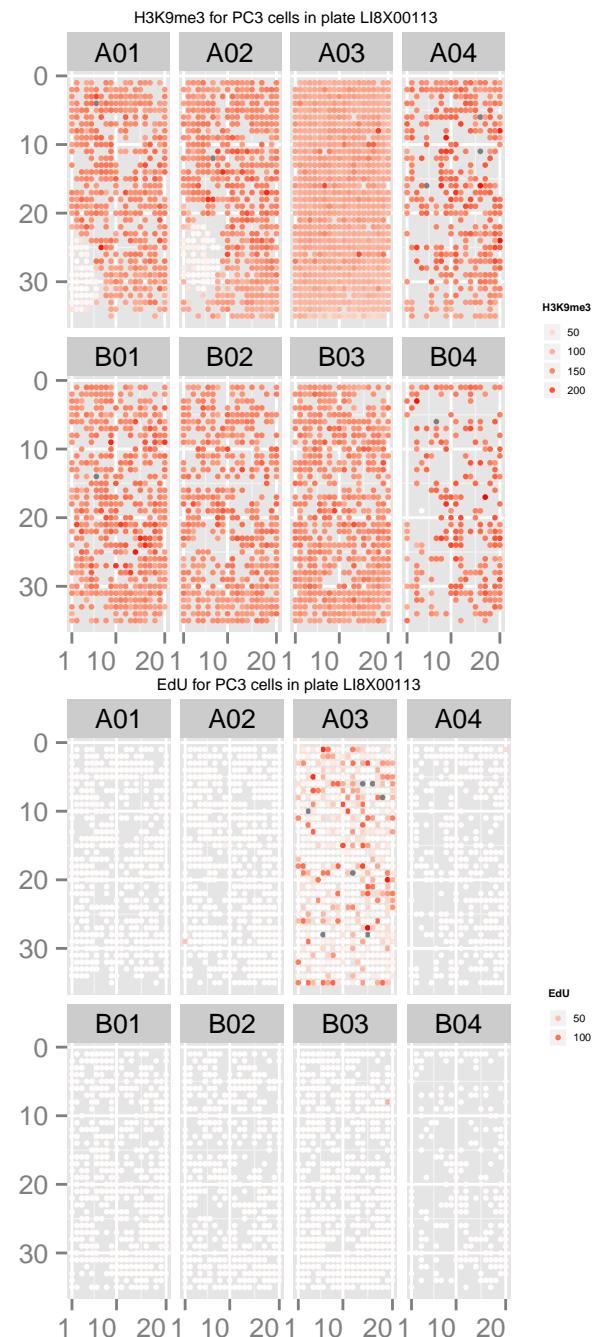
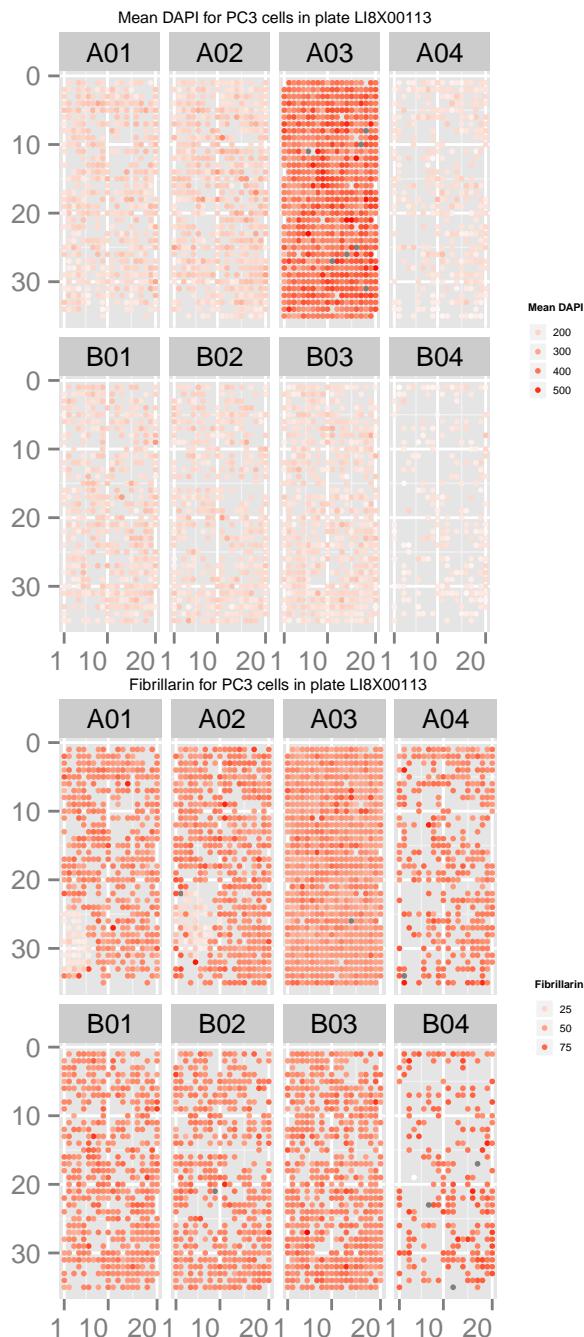
Stain Pseudoimages

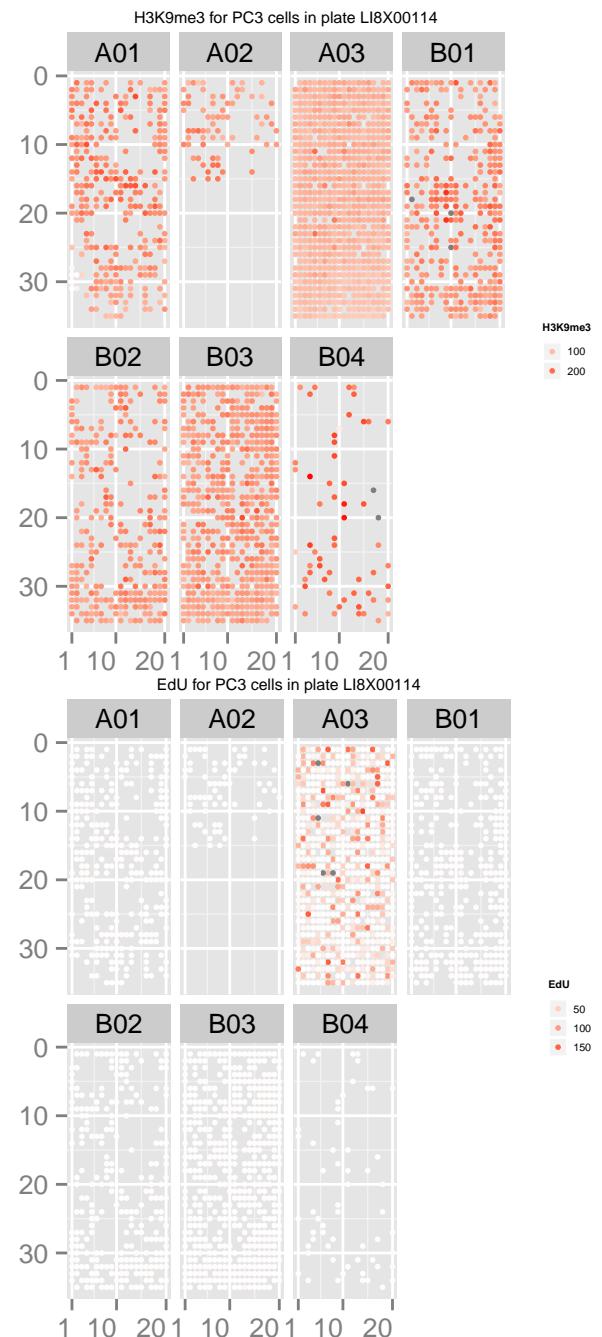
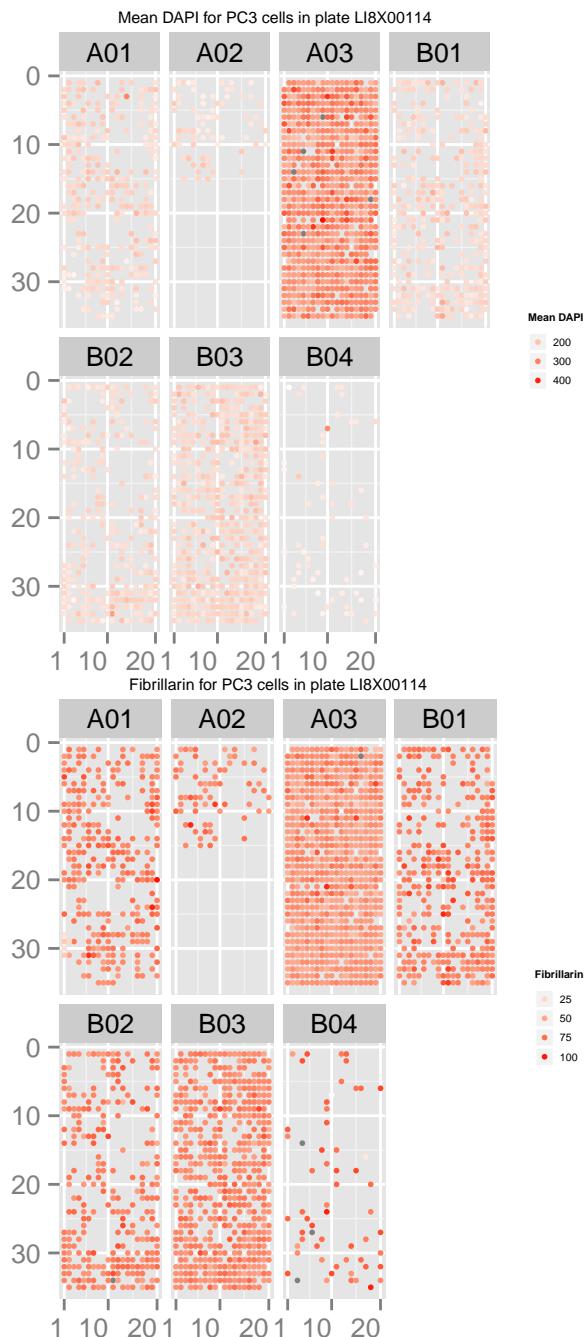
The pseudoimages of each well's raw signals are shown in the plots below.

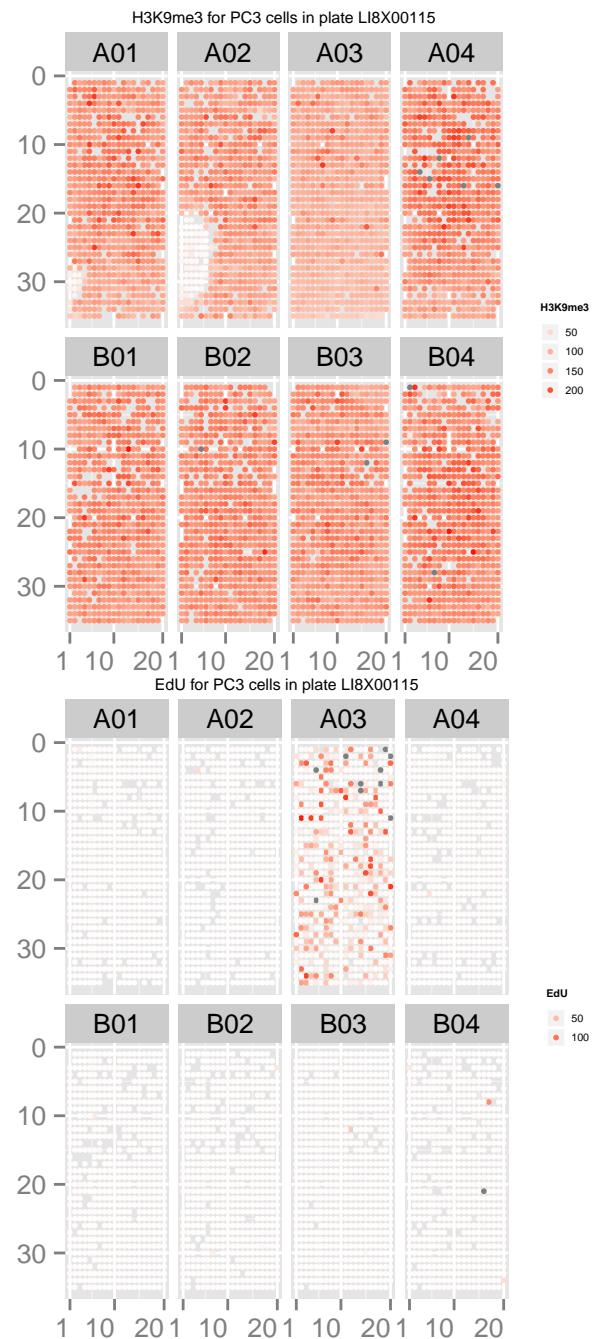
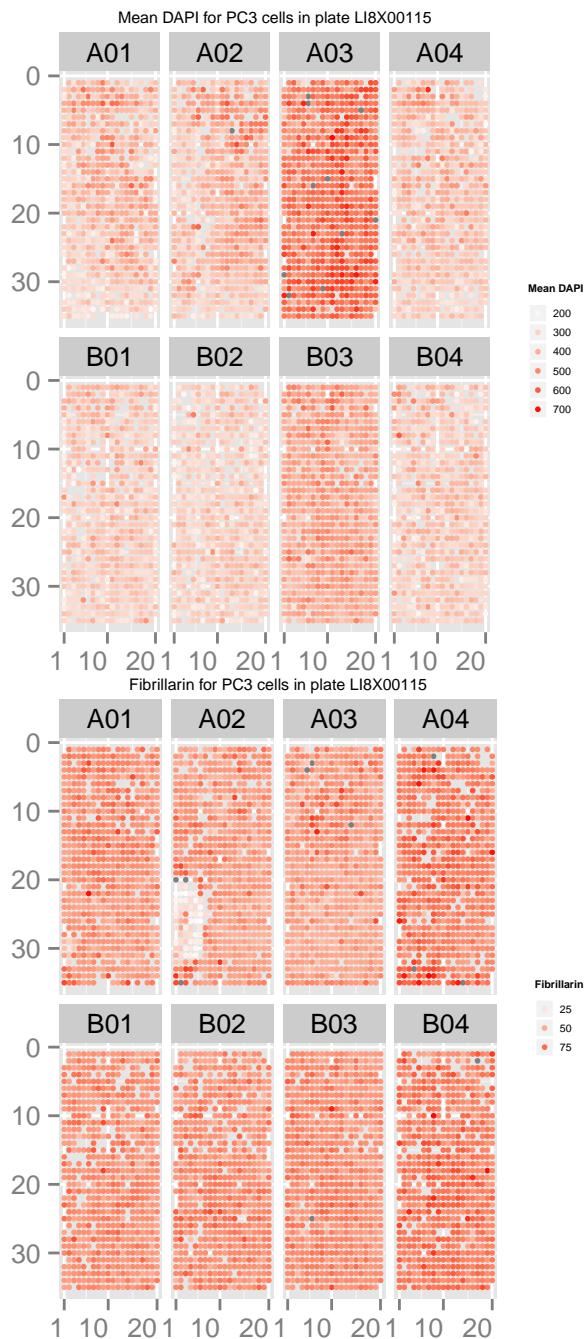


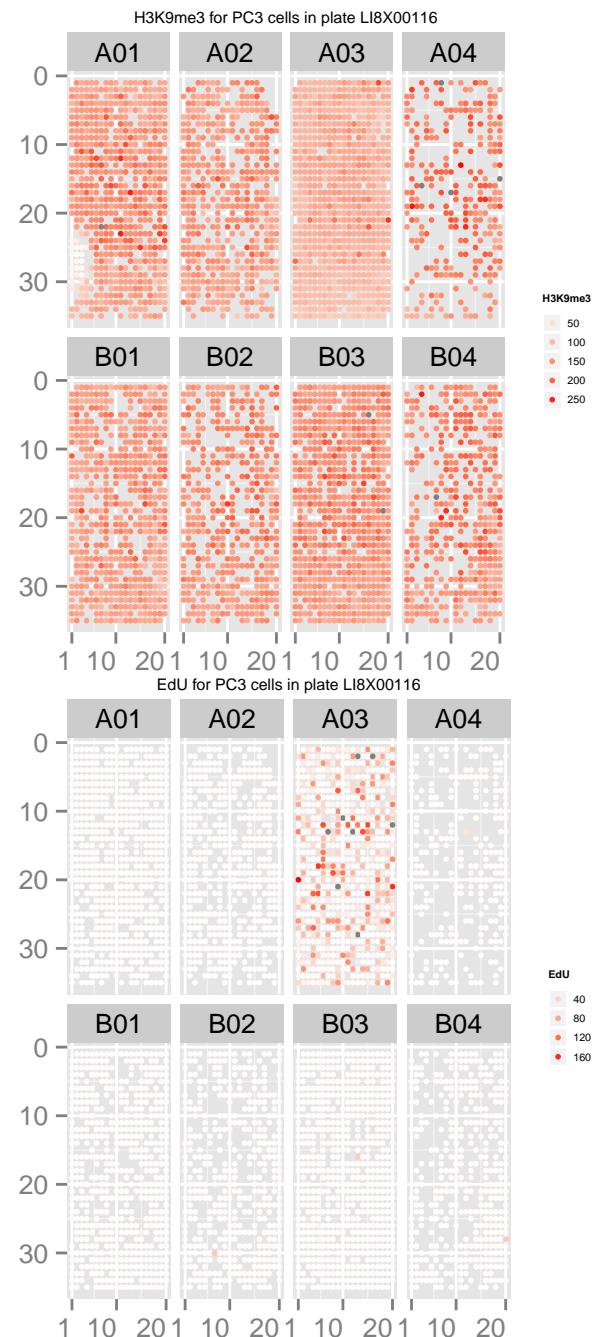
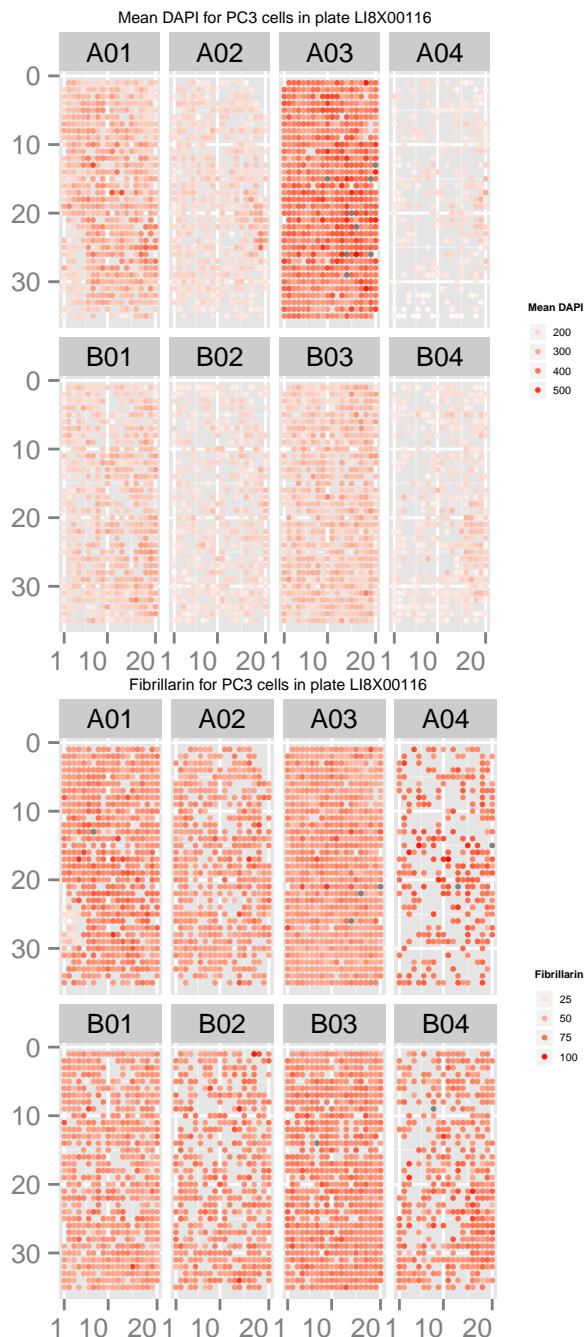


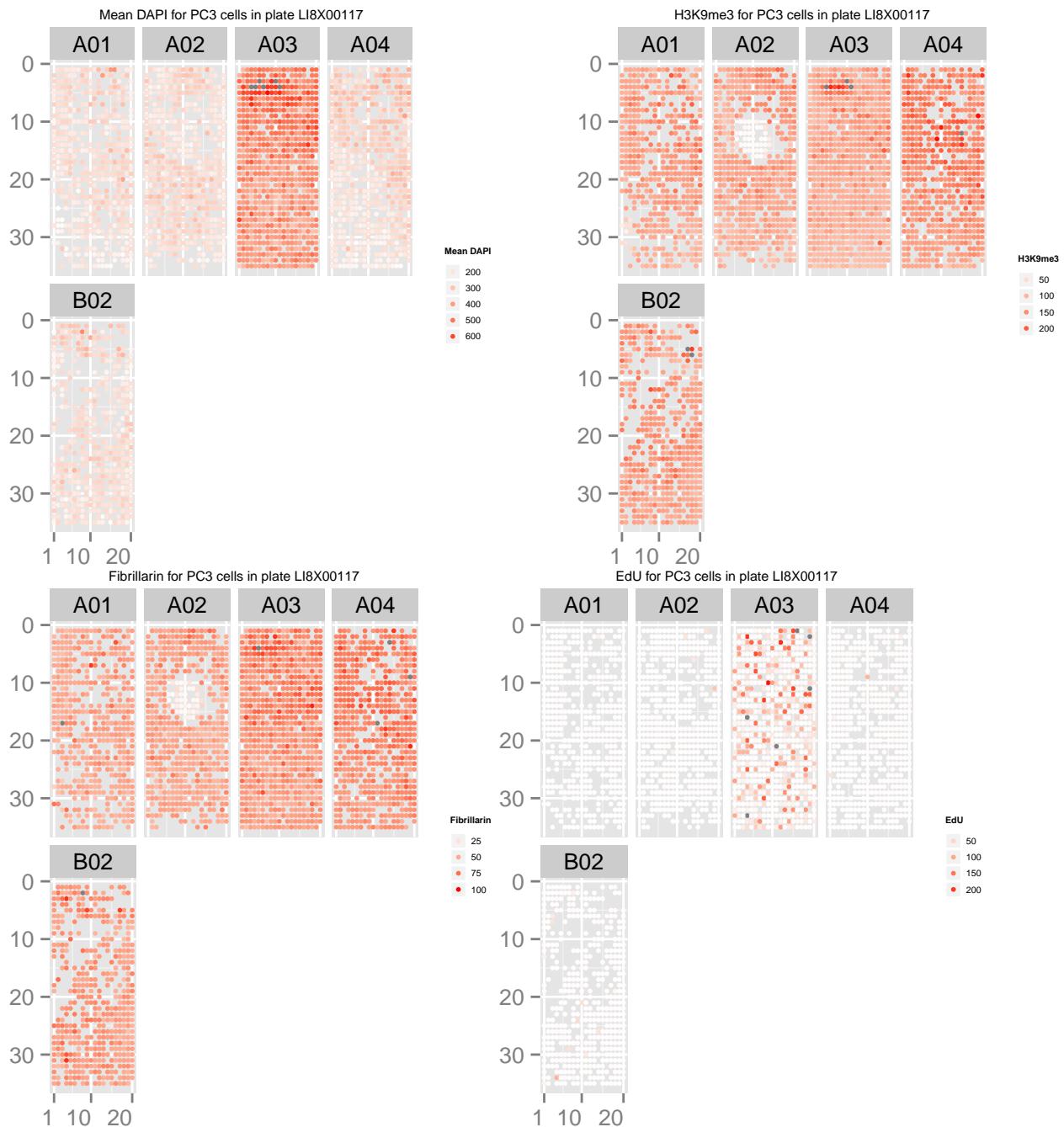












Z Height Pseudoimages

The pseudoimages of the focus Z heights are shown in the plots below.

Univariate Signal Distributions

The next step in the EDA is to look at the raw univariate cell-level signal responses.

Cell Cycle Plots

Filtering

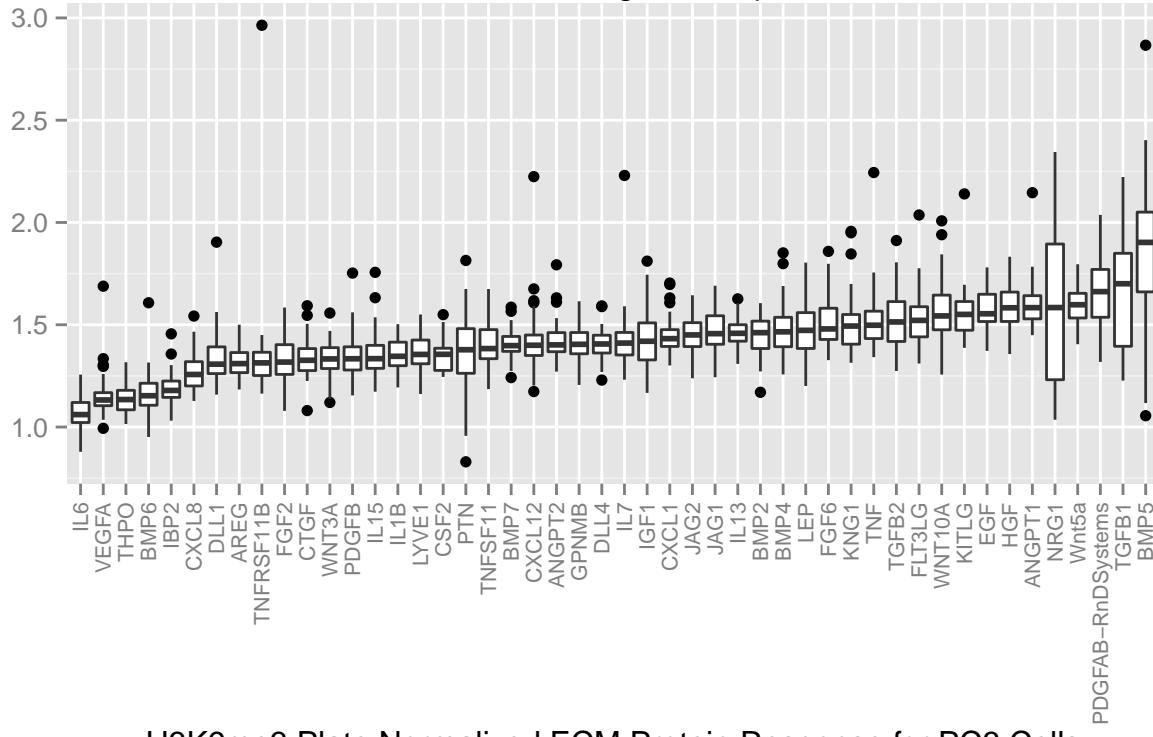
Wells with QA scores below 0 are removed from further analysis due to quality issues in the cell level data. The HighSerum control wells are also excluded from the plots.

MEP Perimeter Box Plots

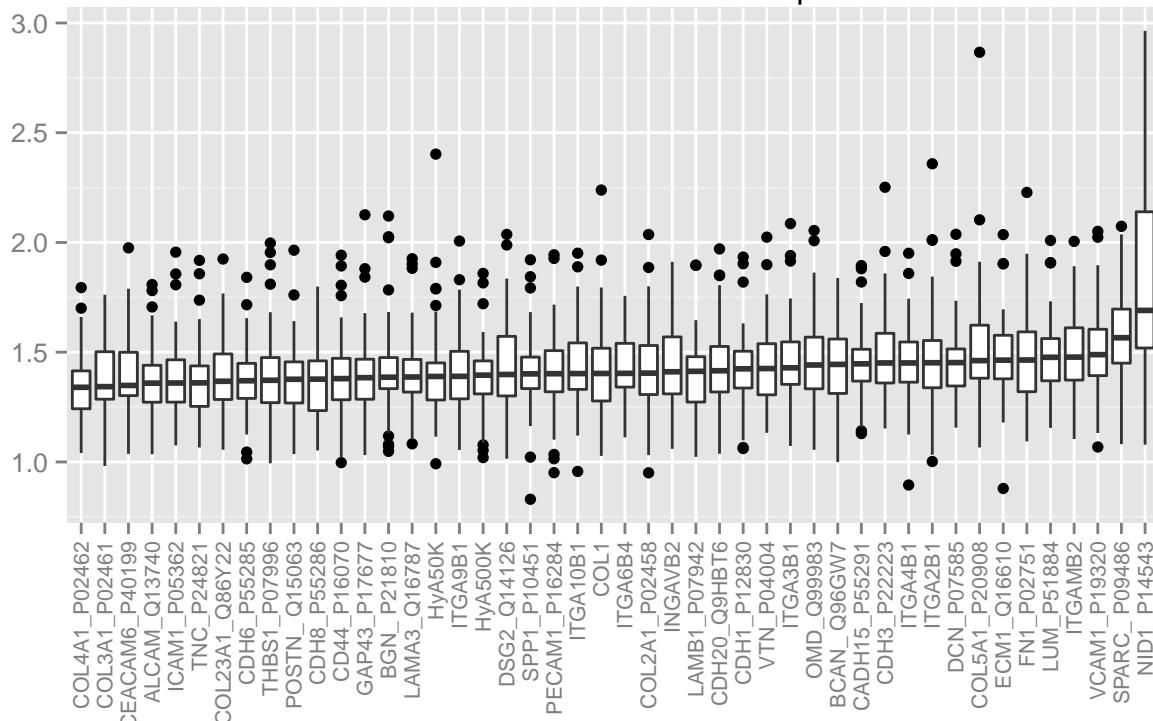
H3 Response

The following plots look at the plate normalized responses of the H3K9me3 signal stratified by ligand, ECM protein and MEP.

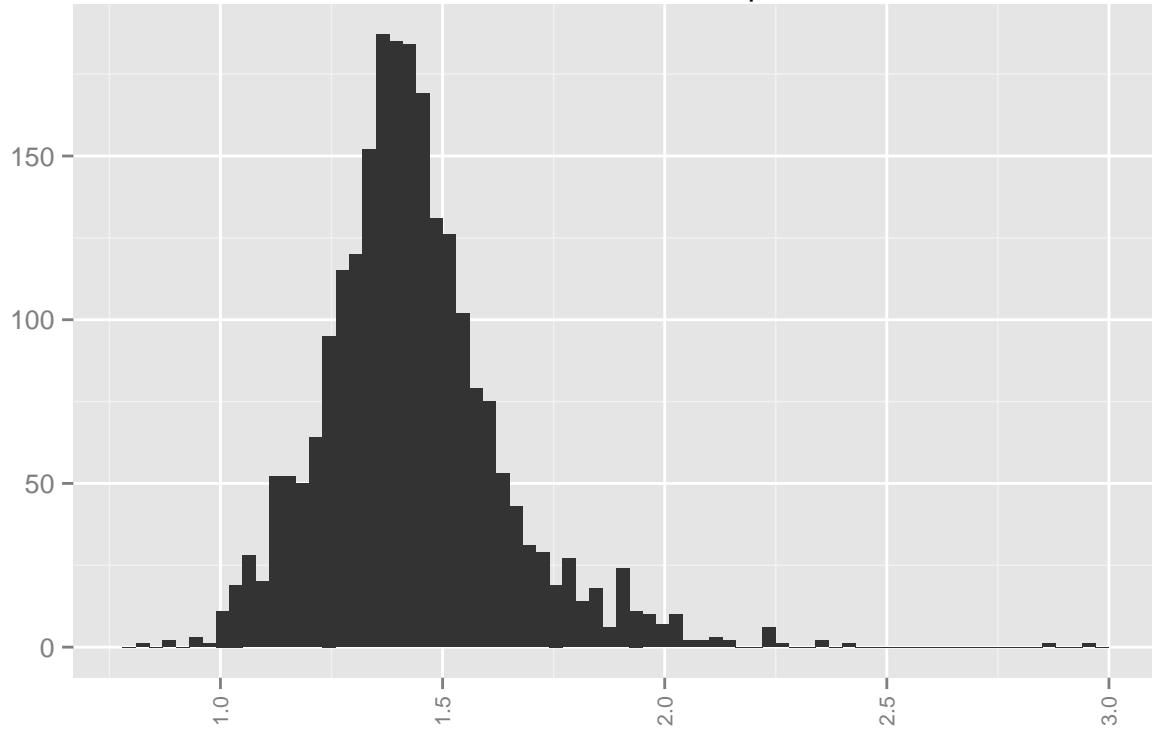
H3K9me3 Plate Normalized Ligand Response for PC3 Cells



H3K9me3 Plate Normalized ECM Protein Response for PC3 Cells



H3K9me3 Plate Normalized MEP Response for PC3 Cells



The following lists are the bottom and top 25 MEPS ordered by their normalized H3K9me3 values. These responses were divided by the high serum responses, then the high serum responses were filtered from the dataset.

##	Ligand	ECMp	MeanIntensityAlexa488MedNorm
## 1:	PTN	SPP1	0.8300528
## 2:	IL6	ECM1	0.8791469
## 3:	IL6	ITGA4B1	0.8949708
## 4:	IL6	COL2A1	0.9506287
## 5:	BMP6	PECAM1	0.9513076
## 6:	PTN	ITGA10B1	0.9569744
## 7:	BMP6	COL3A1	0.9815624
## 8:	IL6	HyA50K	0.9919050
## 9:	VEGFA	THBS1	0.9938397
## 10:	BMP6	CD44	0.9969691
## 11:	IL6	BCAN	0.9998555
## 12:	IL6	ITGA2B1	1.0019803
## 13:	IL6	CDH6	1.0140922
## 14:	IL6	DSG2	1.0145522
## 15:	THPO	PECAM1	1.0146753
## 16:	IL6	THBS1	1.0151283
## 17:	IL6	CD44	1.0192252
## 18:	IL6	HyA500K	1.0199619
## 19:	PTN	THBS1	1.0220802
## 20:	IL6	SPP1	1.0221054
## 21:	IL6	LAMB1	1.0231607
## 22:	IL6	COL1	1.0276652
## 23:	BMP6	THBS1	1.0290933
## 24:	IL6	GAP43	1.0309786
## 25:	IBP2	COL2A1	1.0310404

```

##      Ligand      ECMp MeanIntensityAlexa488MedNorm

##      Ligand      ECMp MeanIntensityAlexa488MedNorm
## 1:      NRG1      ECM1          2.035736
## 2:      BMP5    COL2A1          2.036276
## 3:    FLT3LG     SPARC          2.036613
## 4:      BMP5      DSG2          2.036747
## 5: PDGFAB-RnDSystems      DCN          2.037064
## 6:      BMP5    VCAM1          2.050696
## 7:      TGFB1      OMD          2.054413
## 8:      TGFB1     SPARC          2.073590
## 9:      TGFB1 ITGA3B1          2.085866
## 10:     NRG1    COL5A1          2.103215
## 11:     BMP5      BGN          2.120412
## 12:     BMP5     GAP43          2.126085
## 13:     KITLG     NID1          2.139743
## 14: ANGPT1     NID1          2.145584
## 15:      TGFB1     NID1          2.222275
## 16: CXCL12     NID1          2.224200
## 17:     BMP5      FN1          2.227898
## 18:      IL7     NID1          2.229837
## 19:     BMP5     COL1          2.238853
## 20:      TNF     NID1          2.244123
## 21:     BMP5     CDH3          2.251556
## 22:     NRG1     NID1          2.344717
## 23:     BMP5 ITGA2B1          2.358527
## 24:     BMP5   HyA50K          2.402504
## 25:     BMP5    COL5A1          2.866499
## 26: TNFRSF11B     NID1          2.964160
##      Ligand      ECMp MeanIntensityAlexa488MedNorm

```