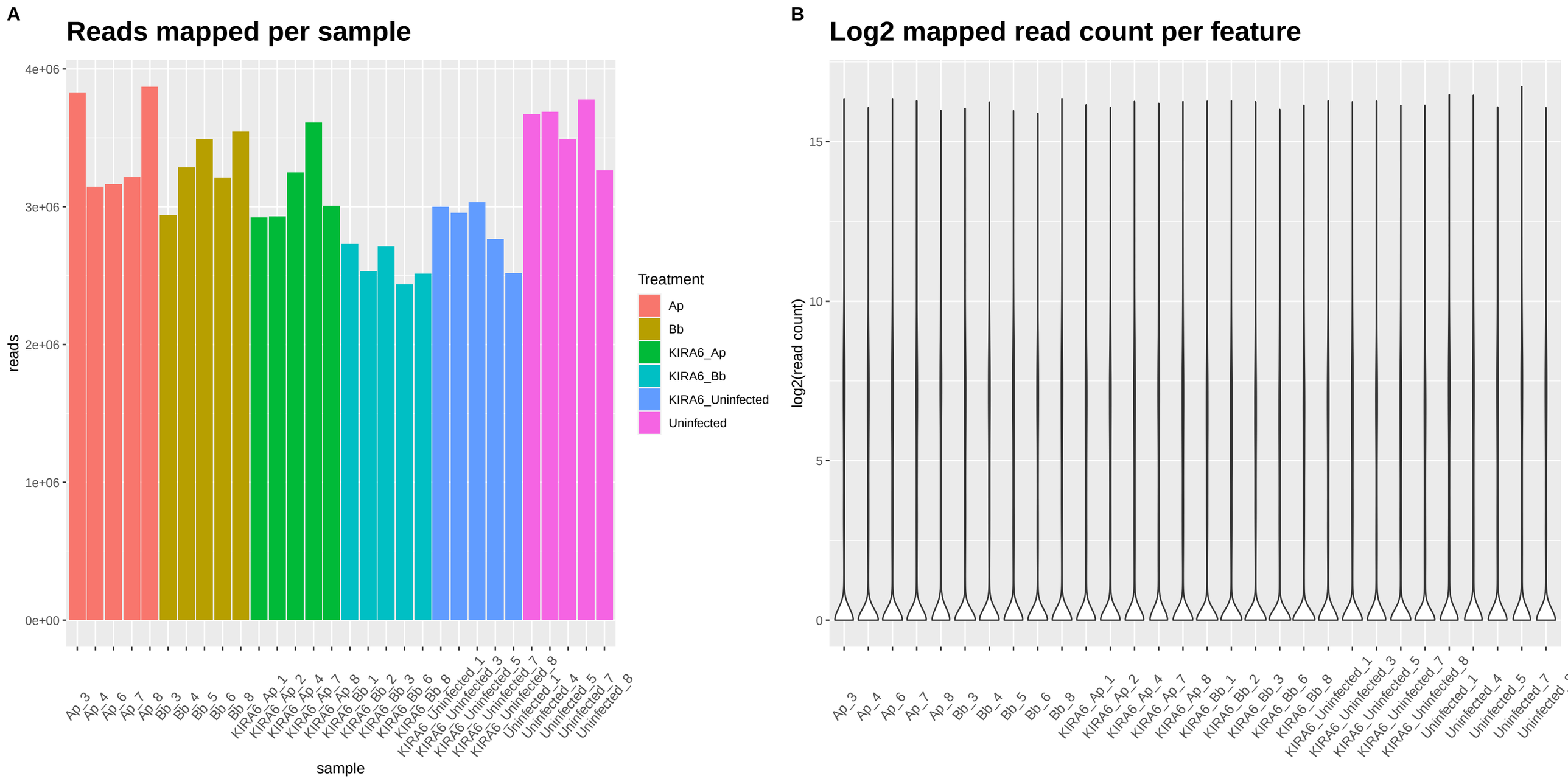
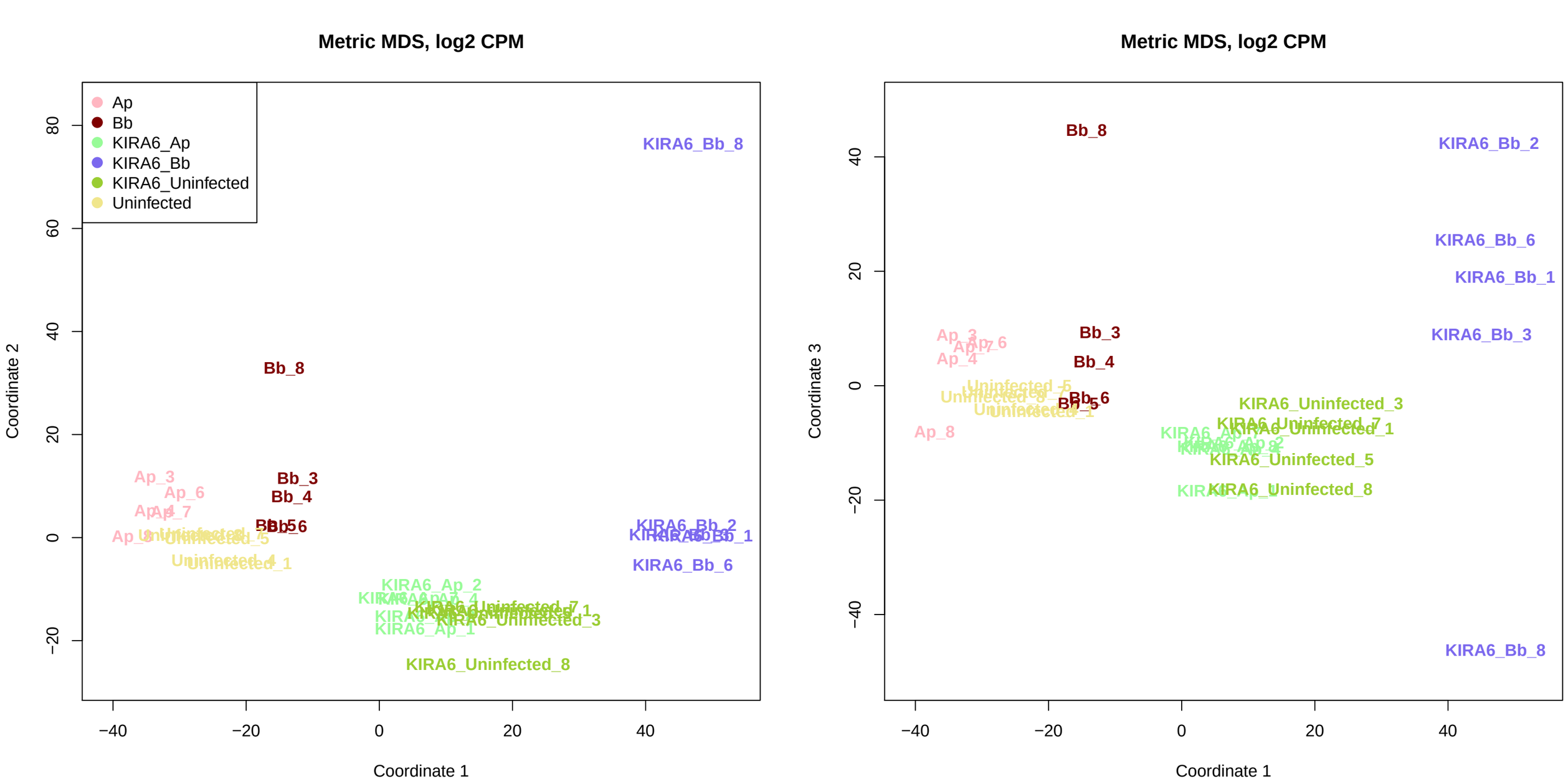
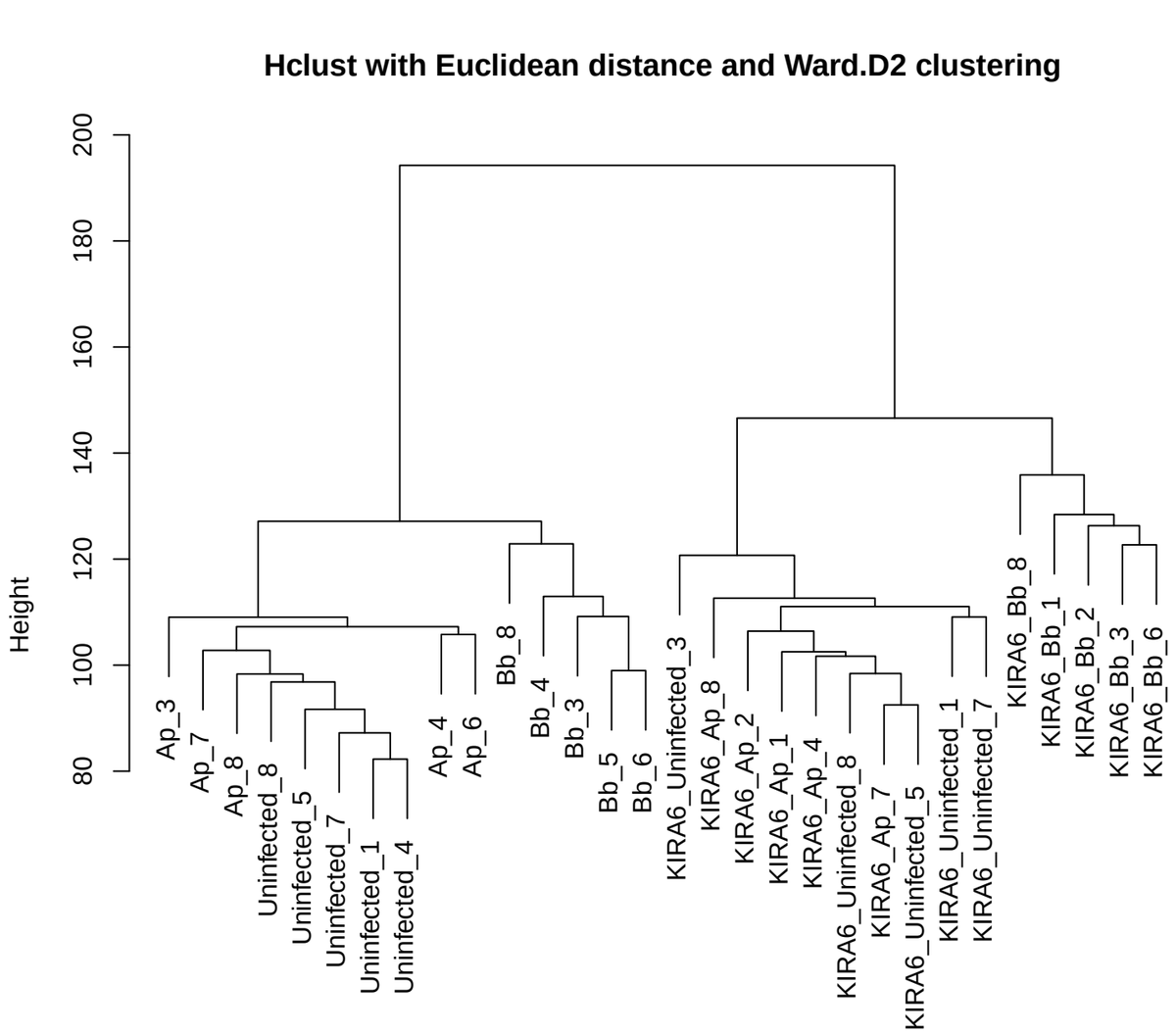
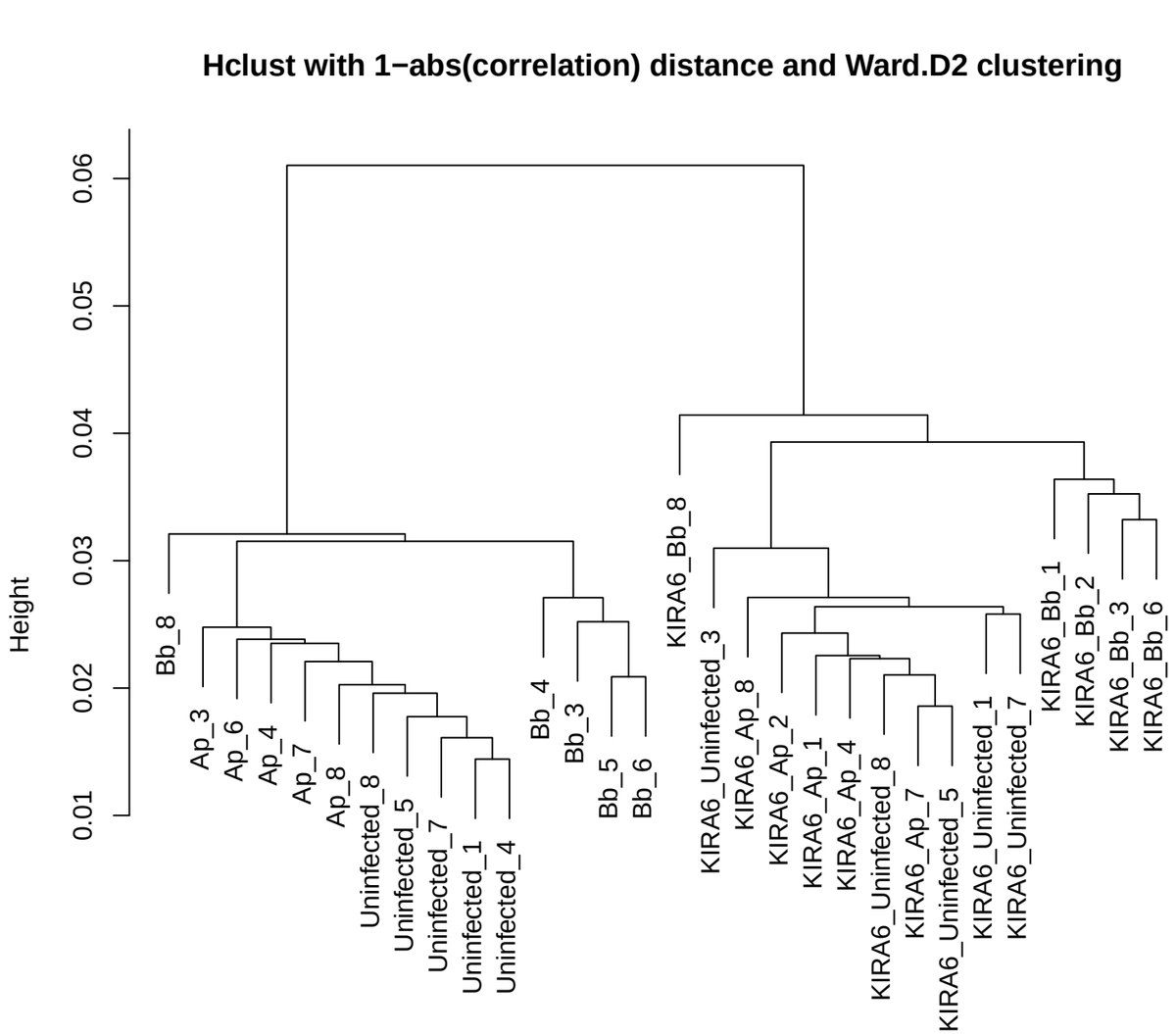
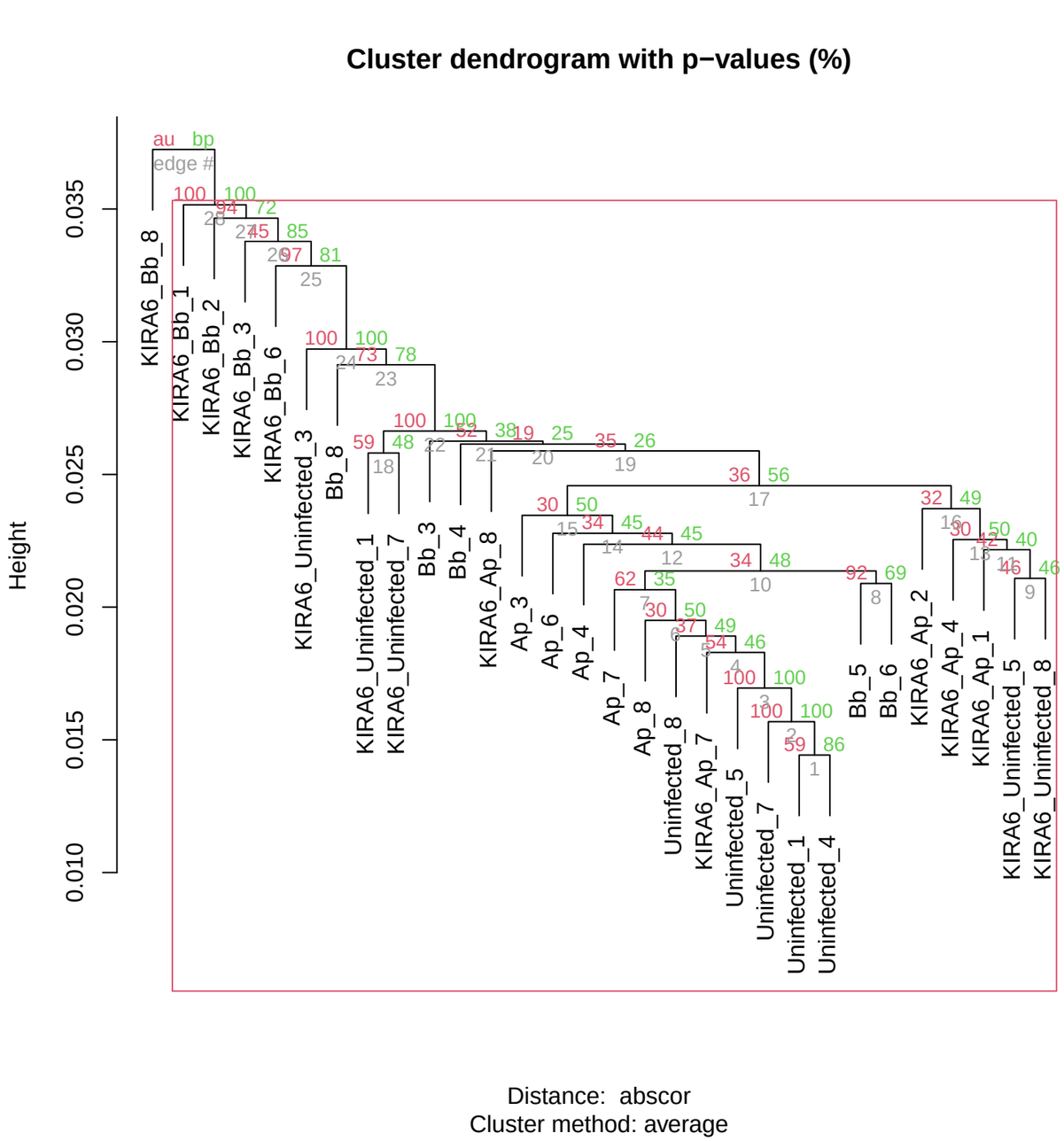
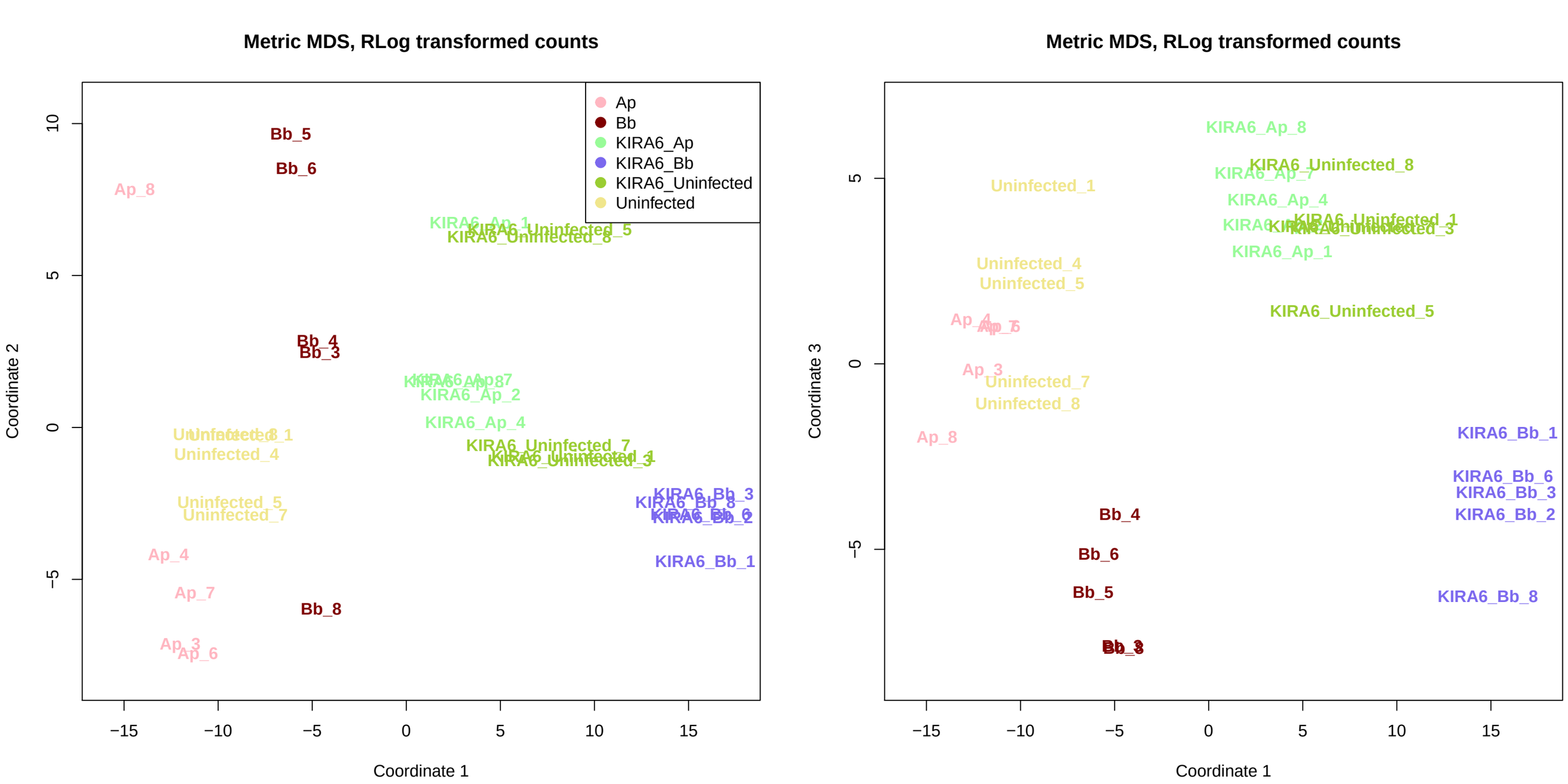

A
Reads mapped per sample
reads
4e+06
3e+06
2e+06
1e+06
0e+00
sample
Ap_3
Ap_4
Ap_6
Ap_7
Ap_8
Bb_3
Bb_4
Bb_5
Bb_6
Bb_8
KIRA6_Ap_1
KIRA6_Ap_2
KIRA6_Ap_4
KIRA6_Ap_7
KIRA6_Ap_8
KIRA6_Bb_1
KIRA6_Bb_2
KIRA6_Bb_3
KIRA6_Bb_6
KIRA6_Bb_8
KIRA6_Uninfected_1
KIRA6_Uninfected_3
KIRA6_Uninfected_5
KIRA6_Uninfected_7
KIRA6_Uninfected_8
Uninfected_1
Uninfected_4
Uninfected_5
Uninfected_7
Uninfected_8
Treatment
Ap
Bb
KIRA6_Ap
KIRA6_Bb
KIRA6_Uninfected
Uninfected
B
Log2 mapped read count per feature
log2(read count)
15
10
5
0

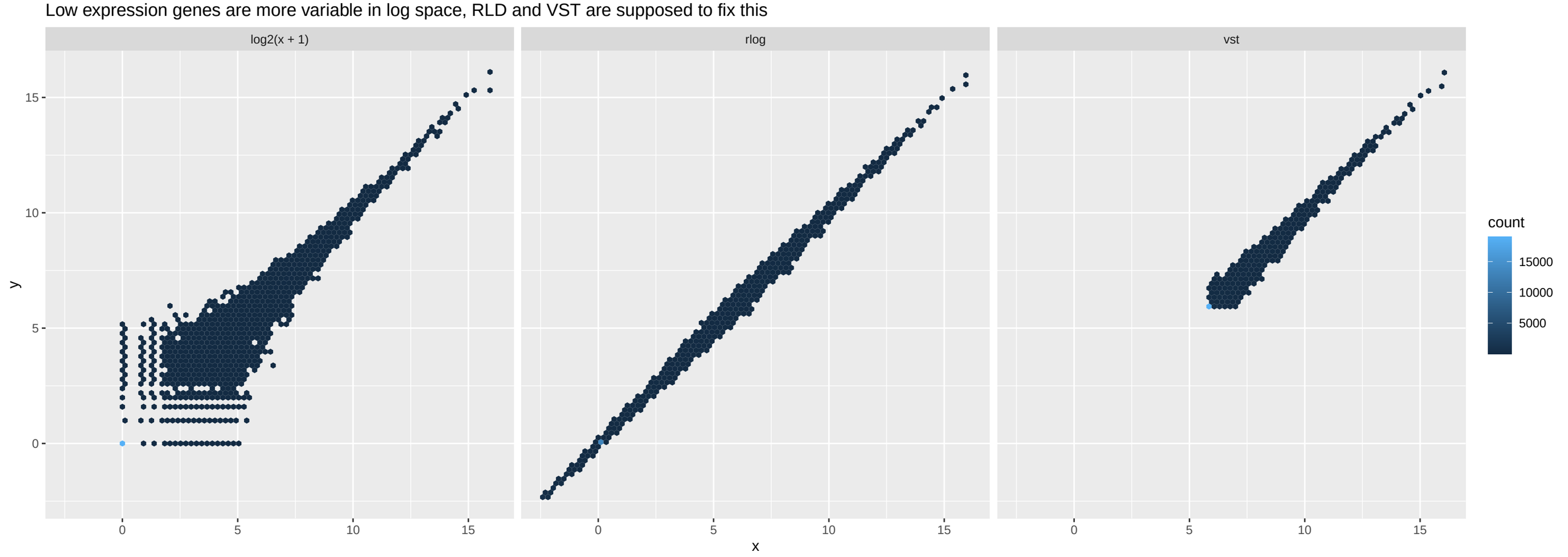

Metric MDS, log2 CPM
Ap
Bb
KIRA6_Ap
KIRA6_Bb
KIRA6_Uninfected
Uninfected
Coordinate 1
Coordinate 2
Metric MDS, log2 CPM
Coordinate 1
Coordinate 3

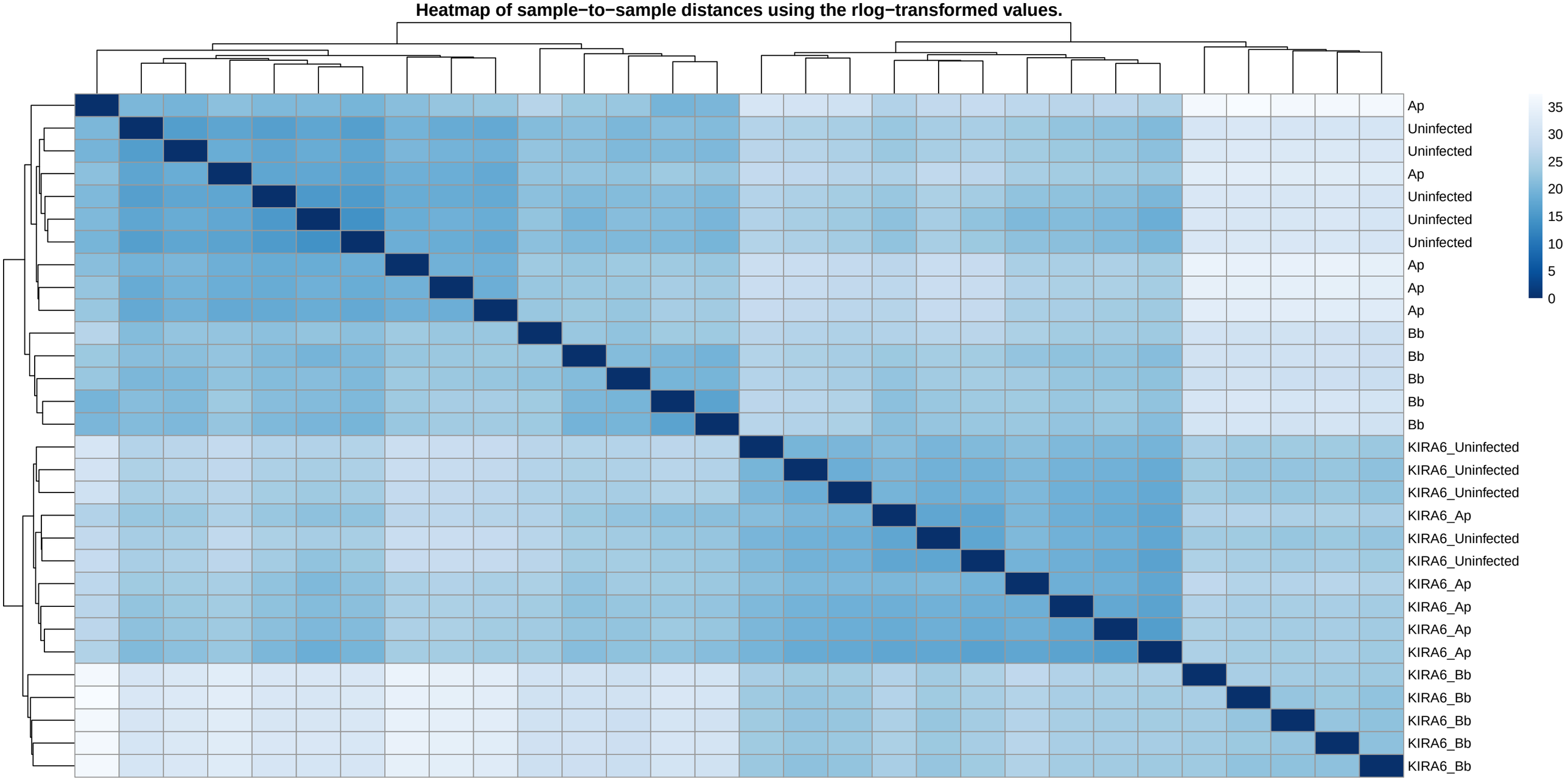

Hclust with Euclidean distance and Ward.D2 clustering
Height
200
180
160
140
120
100
80
Ap_3
Ap_7
Ap_8
Uninfected_8
Uninfected_5
Uninfected_7
Uninfected_1
Uninfected_4
Ap_4
Ap_6
Bb_8
Bb_4
Bb_3
Bb_5
Bb_6
KIRA6_Uninfected_3
KIRA6_Ap_8
KIRA6_Ap_2
KIRA6_Ap_1
KIRA6_Ap_4
KIRA6_Uninfected_8
KIRA6_Ap_7
KIRA6_Uninfected_5
KIRA6_Uninfected_1
KIRA6_Uninfected_7
KIRA6_Bb_8
KIRA6_Bb_1
KIRA6_Bb_2
KIRA6_Bb_3
KIRA6_Bb_6
dist(t(cpm(dds, log = T)))
hclust (*, "ward.D2")

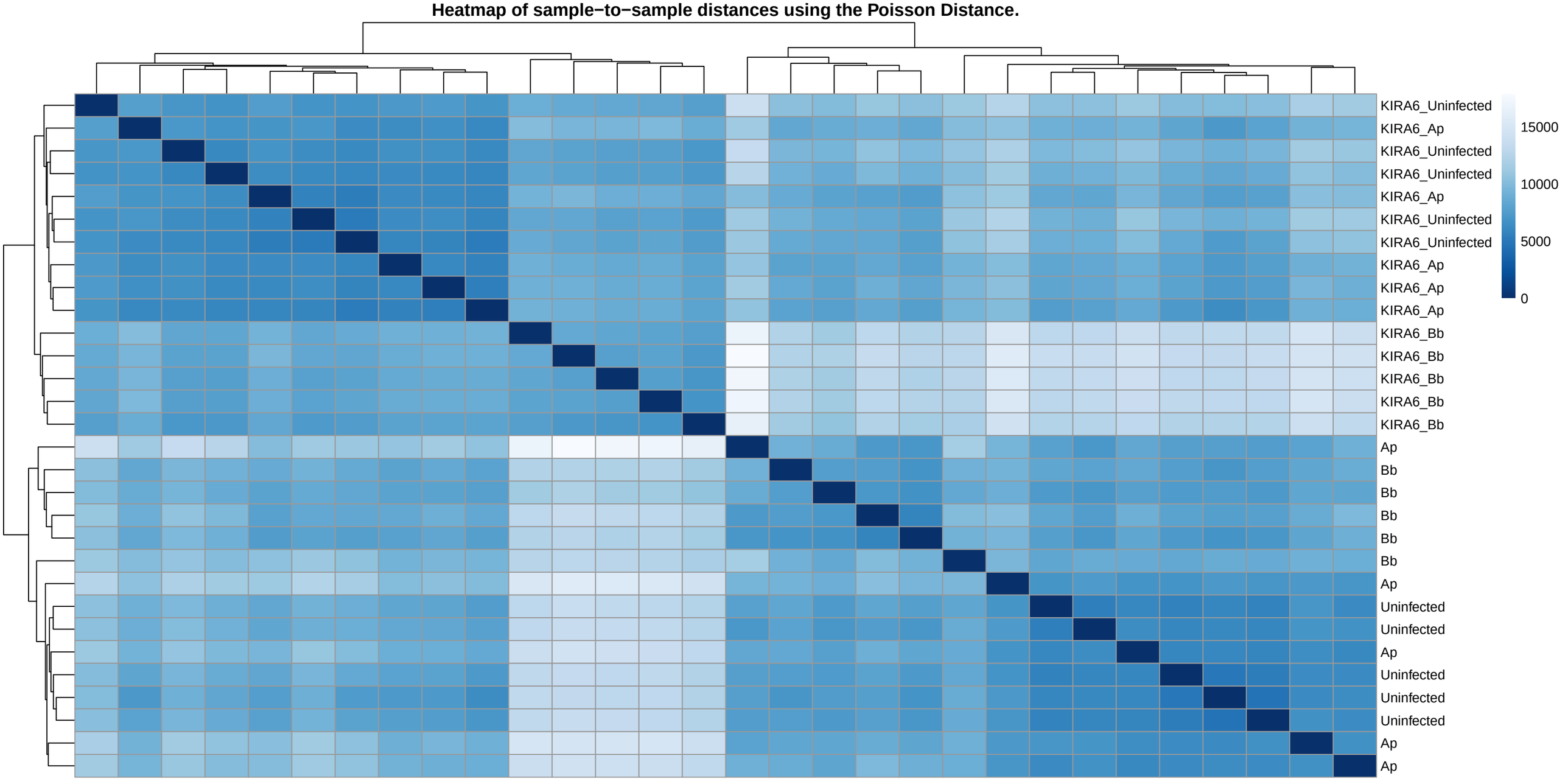

Hclust with 1−abs(correlation) distance and Ward.D2 clustering
Height
0.06
0.05
0.04
0.03
0.02
0.01
Bb_8
Ap_3
Ap_6
Ap_4
Ap_7
Ap_8
Uninfected_8
Uninfected_5
Uninfected_7
Uninfected_1
Uninfected_4
Bb_4
Bb_3
Bb_5
Bb_6
KIRA6_Bb_8
KIRA6_Uninfected_3
KIRA6_Ap_8
KIRA6_Ap_2
KIRA6_Ap_1
KIRA6_Ap_4
KIRA6_Uninfected_8
KIRA6_Ap_7
KIRA6_Uninfected_5
KIRA6_Uninfected_1
KIRA6_Uninfected_7
KIRA6_Bb_1
KIRA6_Bb_2
KIRA6_Bb_3
KIRA6_Bb_6
as.dist(1 − abs(cor(cpm(dds, log = T))))
hclust (*, "ward.D2")

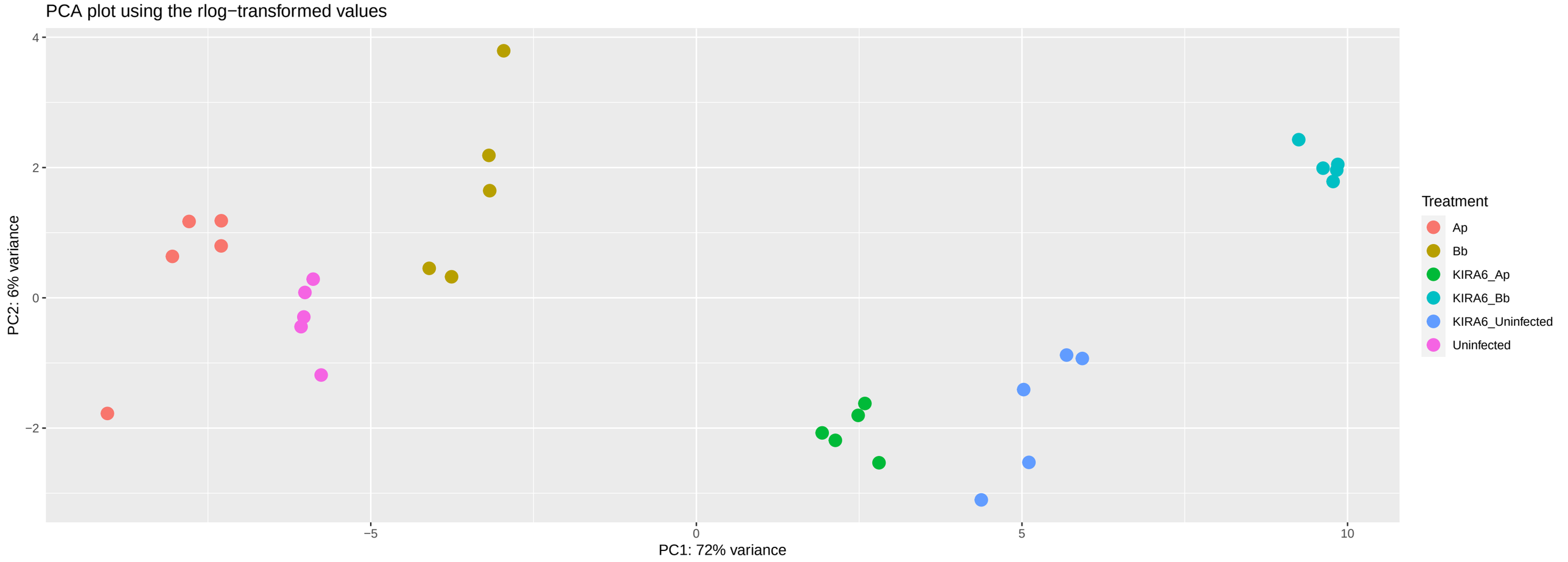

Cluster dendrogram with p−values (%)
au bp
edge #
Height
0.010
0.015
0.020
0.025
0.030
0.035
KIRA6_Bb_8
KIRA6_Bb_1
KIRA6_Bb_2
KIRA6_Bb_3
KIRA6_Bb_6
KIRA6_Uninfected_3
Bb_8
KIRA6_Uninfected_1
KIRA6_Uninfected_7
Bb_3
Bb_4
KIRA6_Ap_8
Ap_3
Ap_6
Ap_4
Ap_7
Ap_8
Uninfected_8
KIRA6_Ap_7
Uninfected_5
Uninfected_7
Uninfected_1
Uninfected_4
Bb_5
Bb_6
KIRA6_Ap_2
KIRA6_Ap_4
KIRA6_Ap_1
KIRA6_Uninfected_5
KIRA6_Uninfected_8
Distance: abscor
Cluster method: average

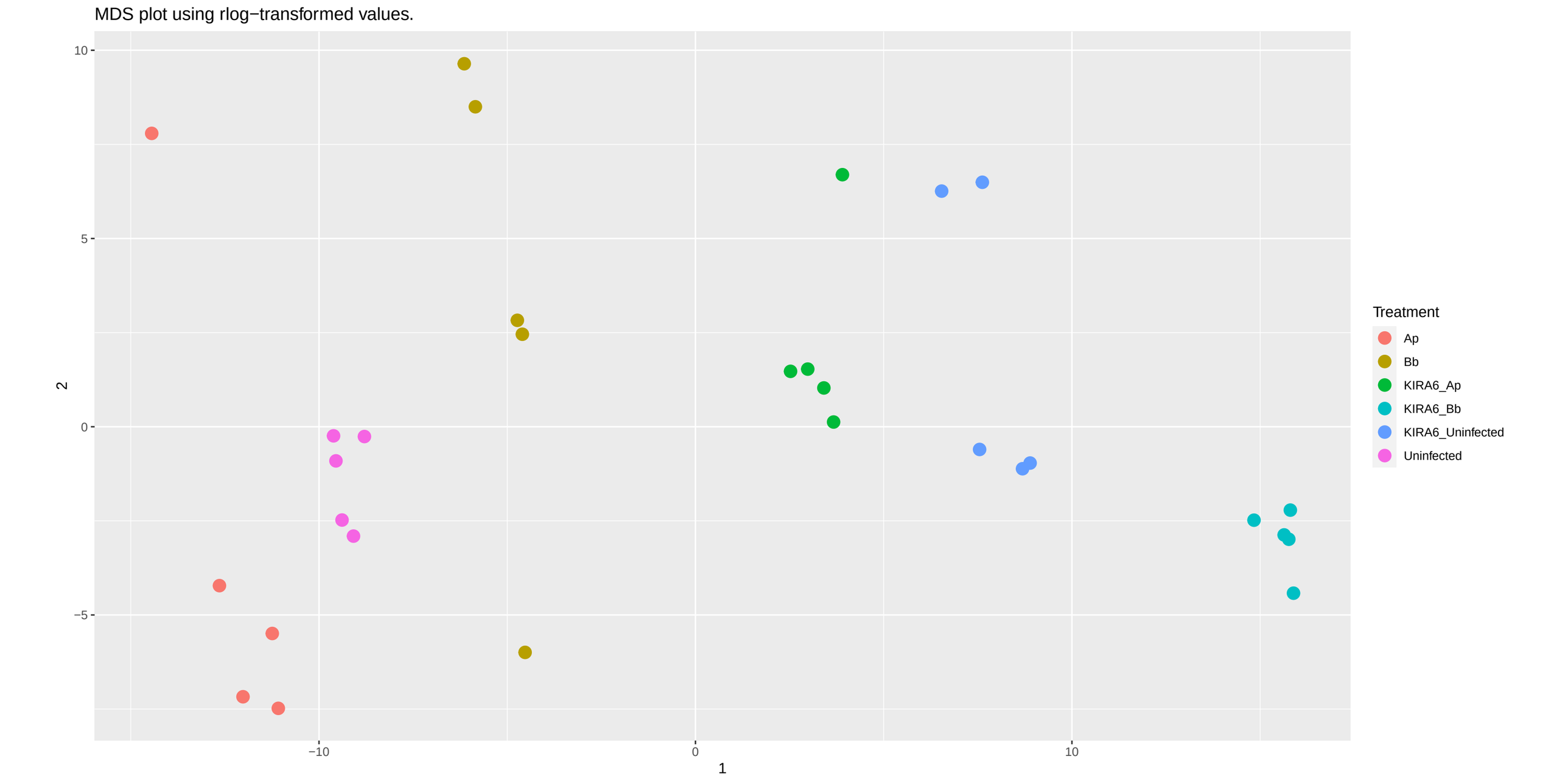

Metric MDS, RLog transformed counts
Ap
Bb
KIRA6_Ap
KIRA6_Bb
KIRA6_Uninfected
Uninfected
Coordinate 1
Coordinate 2
Metric MDS, RLog transformed counts
Coordinate 1
Coordinate 3

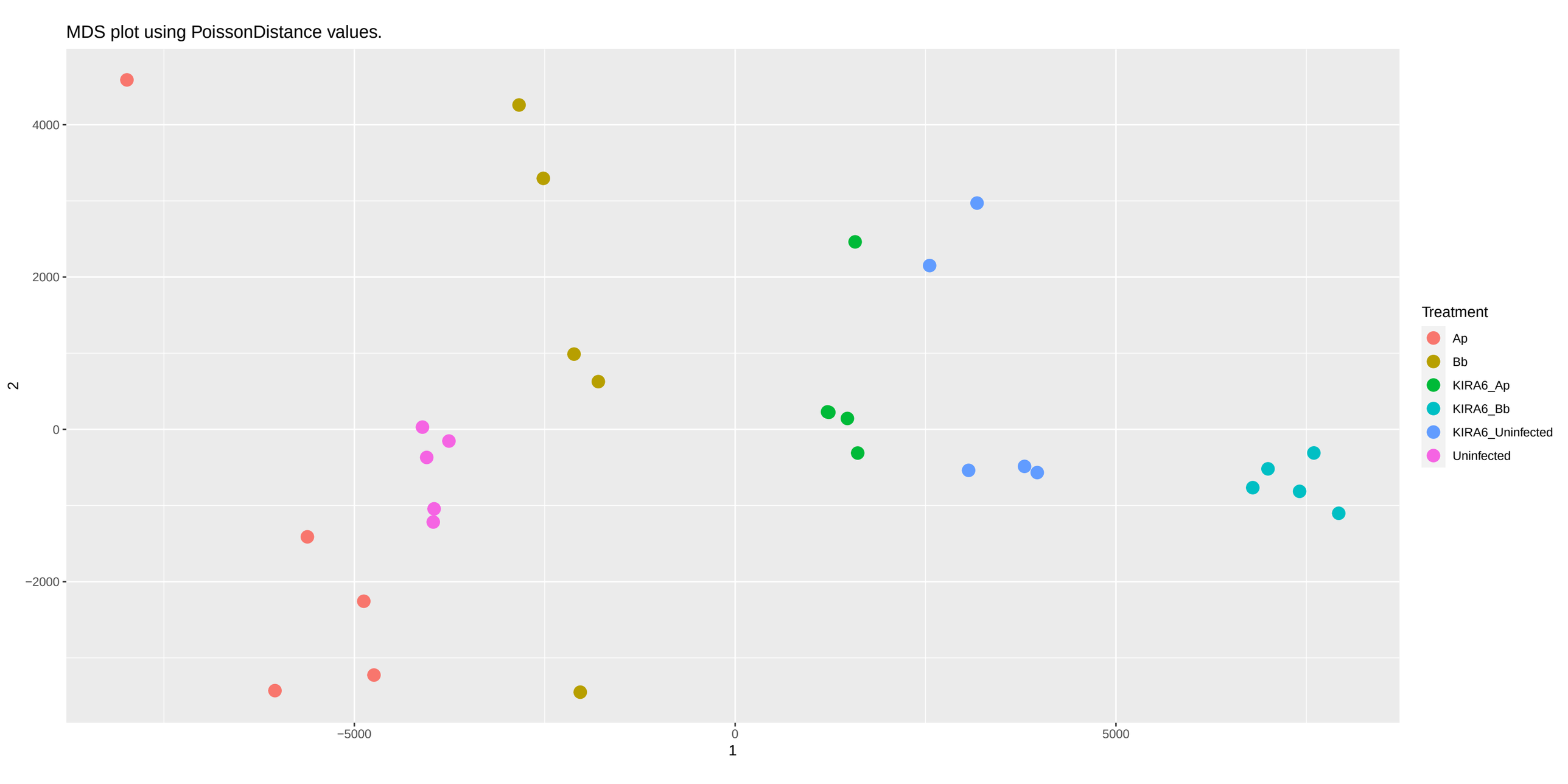

Low expression genes are more variable in log space, RLD and VST are supposed to fix this
log2(x + 1)
rlog
vst
y
x
0
5
10
15
count
15000
10000
5000


Heatmap of sample−to−sample distances using the rlog−transformed values.
Ap
Uninfected
Uninfected
Ap
Uninfected
Uninfected
Uninfected
Ap
Ap
Ap
Bb
Bb
Bb
Bb
Bb
KIRA6_Uninfected
KIRA6_Uninfected
KIRA6_Uninfected
KIRA6_Ap
KIRA6_Uninfected
KIRA6_Uninfected
KIRA6_Ap
KIRA6_Ap
KIRA6_Ap
KIRA6_Ap
KIRA6_Bb
KIRA6_Bb
KIRA6_Bb
KIRA6_Bb
KIRA6_Bb
35
30
25
20
15
10
5
0


Heatmap of sample−to−sample distances using the Poisson Distance.
KIRA6_Uninfected
KIRA6_Ap
KIRA6_Uninfected
KIRA6_Uninfected
KIRA6_Ap
KIRA6_Uninfected
KIRA6_Uninfected
KIRA6_Ap
KIRA6_Ap
KIRA6_Ap
KIRA6_Bb
KIRA6_Bb
KIRA6_Bb
KIRA6_Bb
KIRA6_Bb
Ap
Bb
Bb
Bb
Bb
Bb
Ap
Uninfected
Uninfected
Ap
Uninfected
Uninfected
Uninfected
Ap
Ap
15000
10000
5000
0


PCA plot using the rlog−transformed values
PC2: 6% variance
PC1: 72% variance
4
2
0
−2
−5
0
5
10
Treatment
Ap
Bb
KIRA6_Ap
KIRA6_Bb
KIRA6_Uninfected
Uninfected


MDS plot using rlog−transformed values.
10
5
0
−5
2
−10
0
10
1
Treatment
Ap
Bb
KIRA6_Ap
KIRA6_Bb
KIRA6_Uninfected
Uninfected


MDS plot using PoissonDistance values.
4000
2000
0
−2000
2
−5000
0
5000
1
Treatment
Ap
Bb
KIRA6_Ap
KIRA6_Bb
KIRA6_Uninfected
Uninfected