

Analysis of 40 small exRNA-Seq datasets from (offspring) FHS

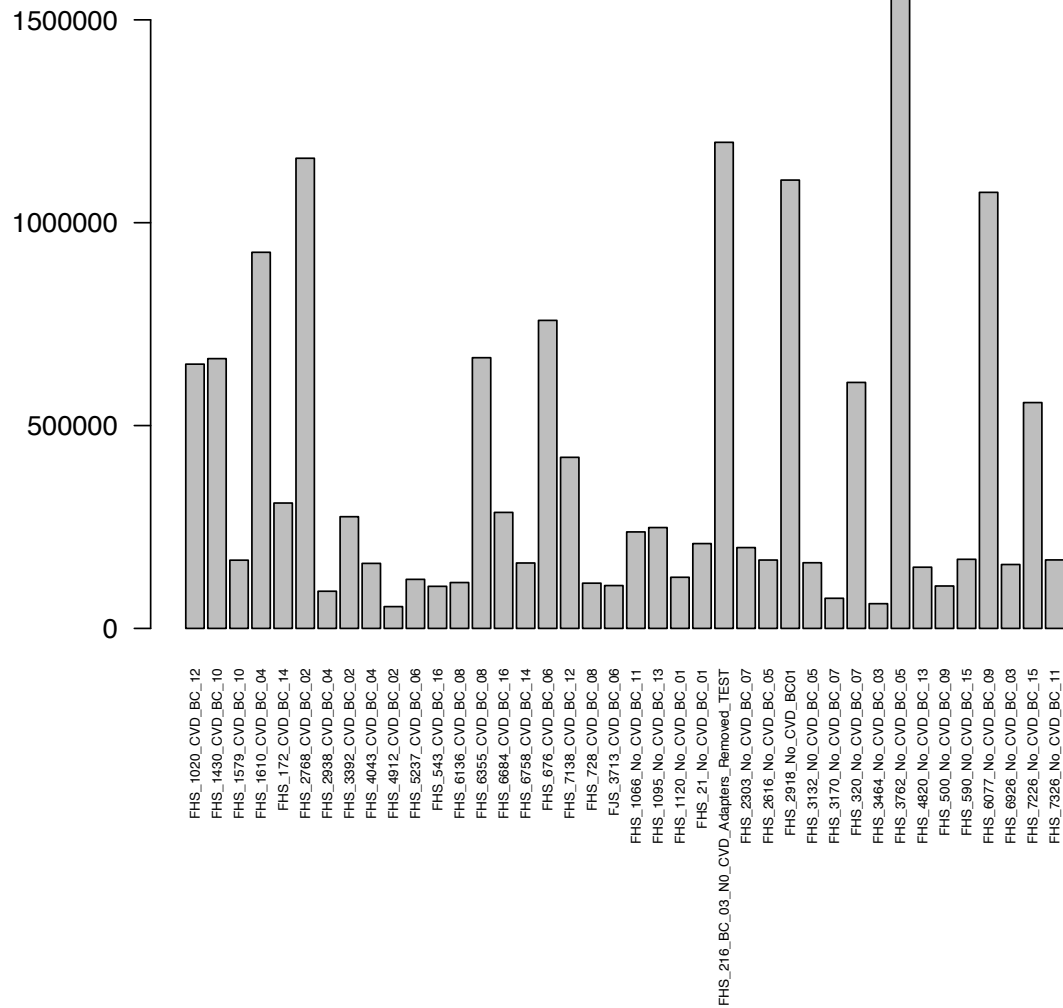
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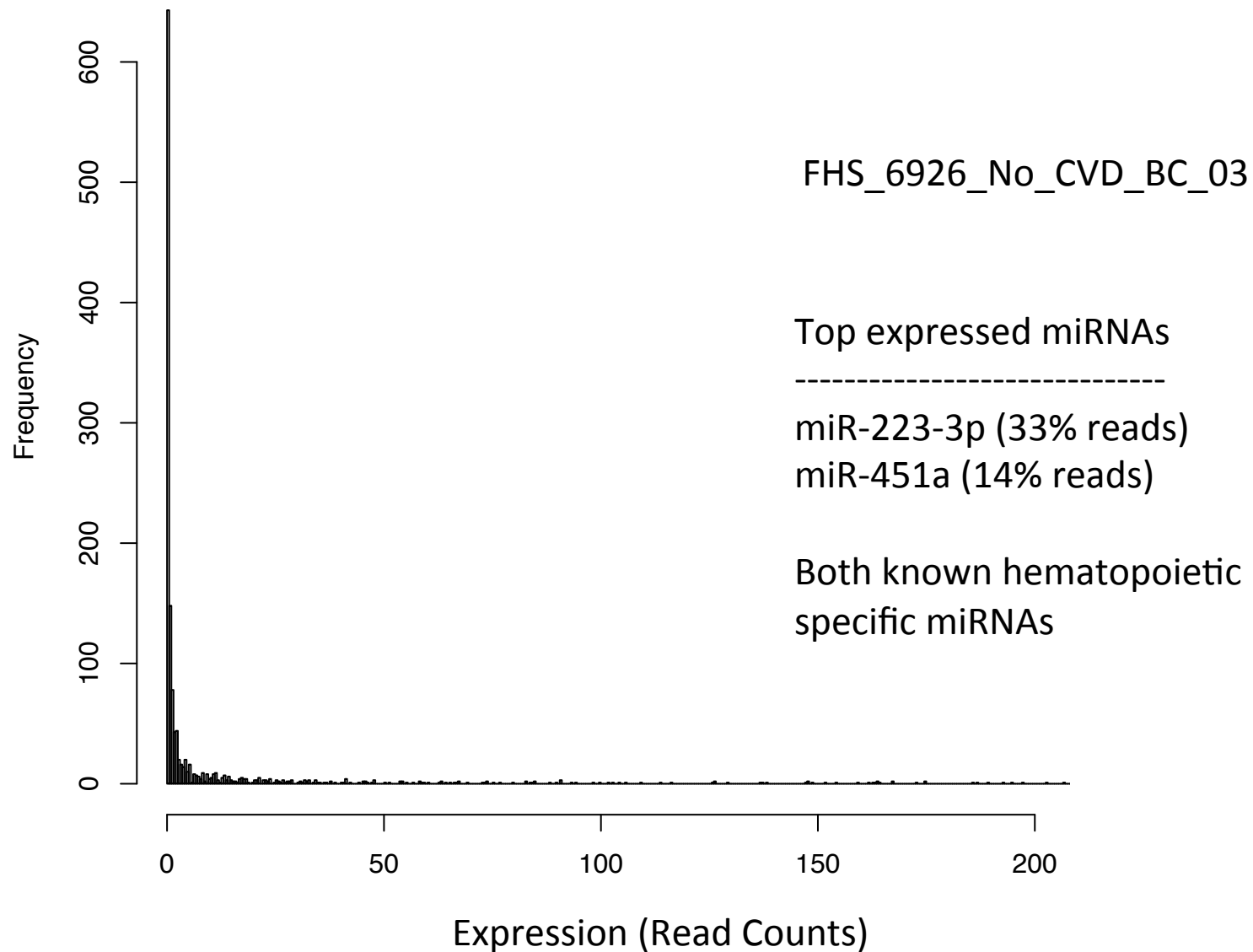
Datasets

- 40 small RNA-Seq datasets
 - 20 CVD & 20 No CVD
- 2 technical replicates with deeper sequencing (no multiplexing)
 - FHS_216_No_CVD_BC_03
 - FHS_6926_No_CVD_BC_03
- Ran all datasets through small exRNA-Seq pipeline
 - Quantified abundances of miRNAs, piRNAs, snoRNAs, tRNAs
- Number of mapped reads varies across the 40 samples:
 - 54k (FHS_4912_CVD_BC_02)
 - 1.76m (FHS_3762_No_CVD_BC_05)

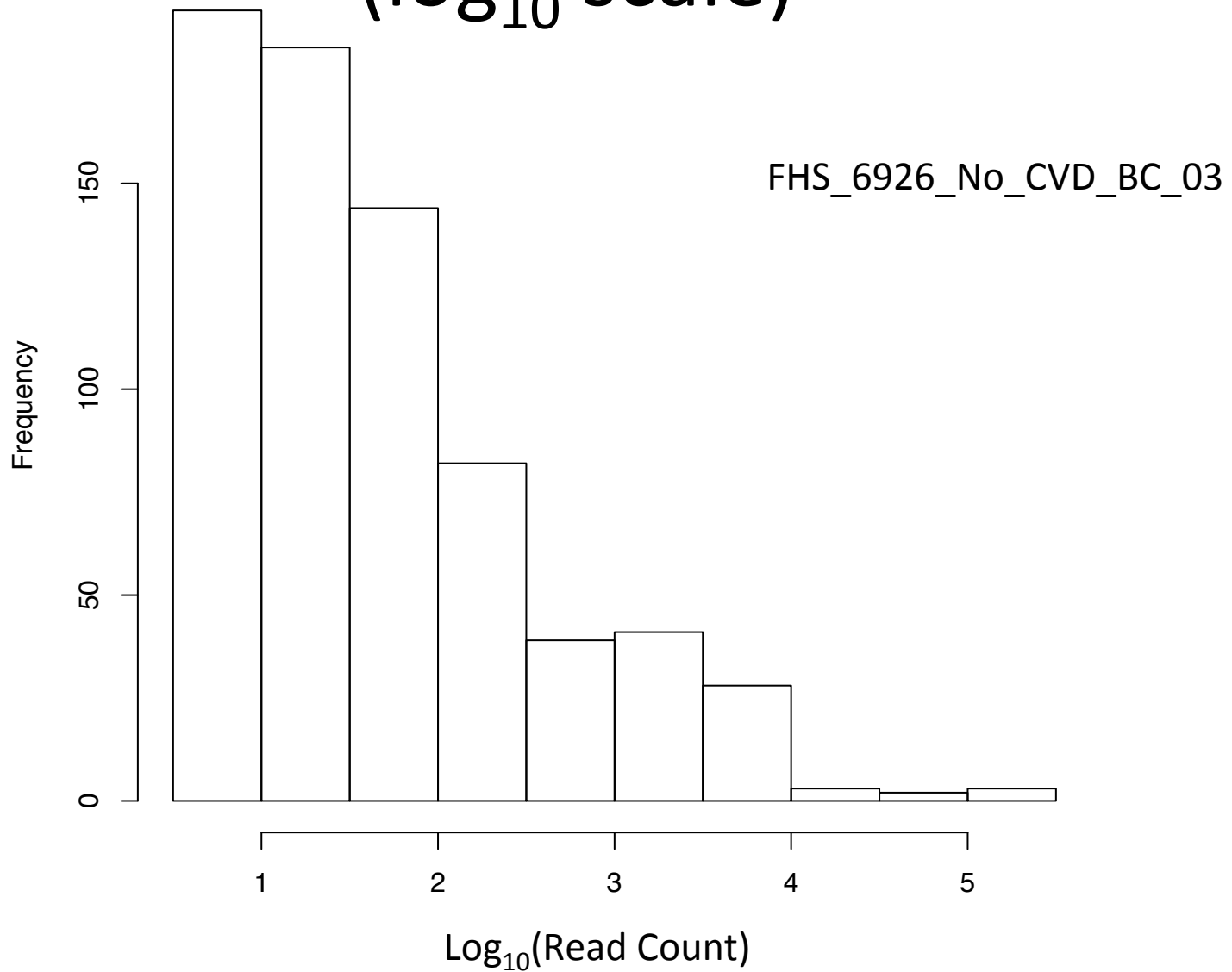
Number of mapped reads (40 samples)



Distribution of miRNA expression

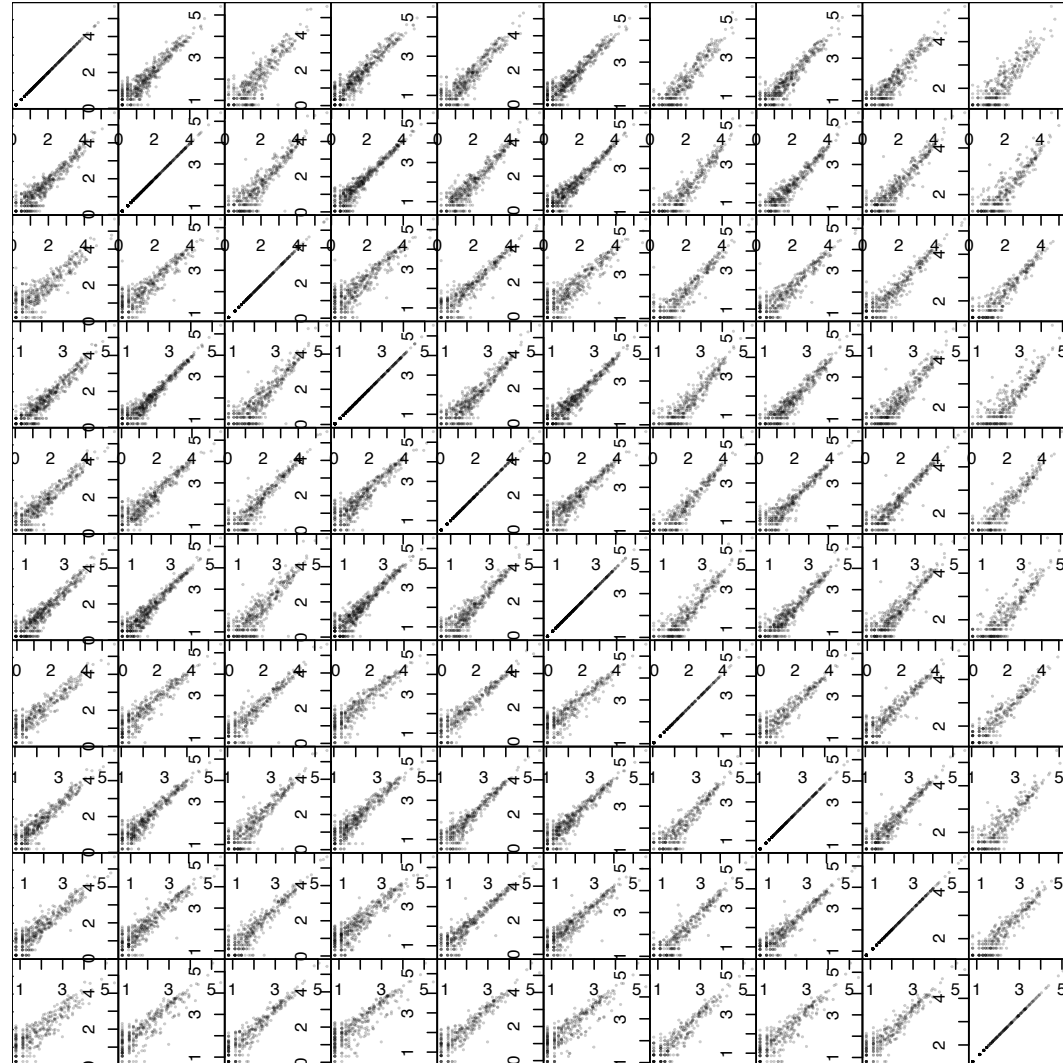


Distribution of miRNA expression (log₁₀ scale)

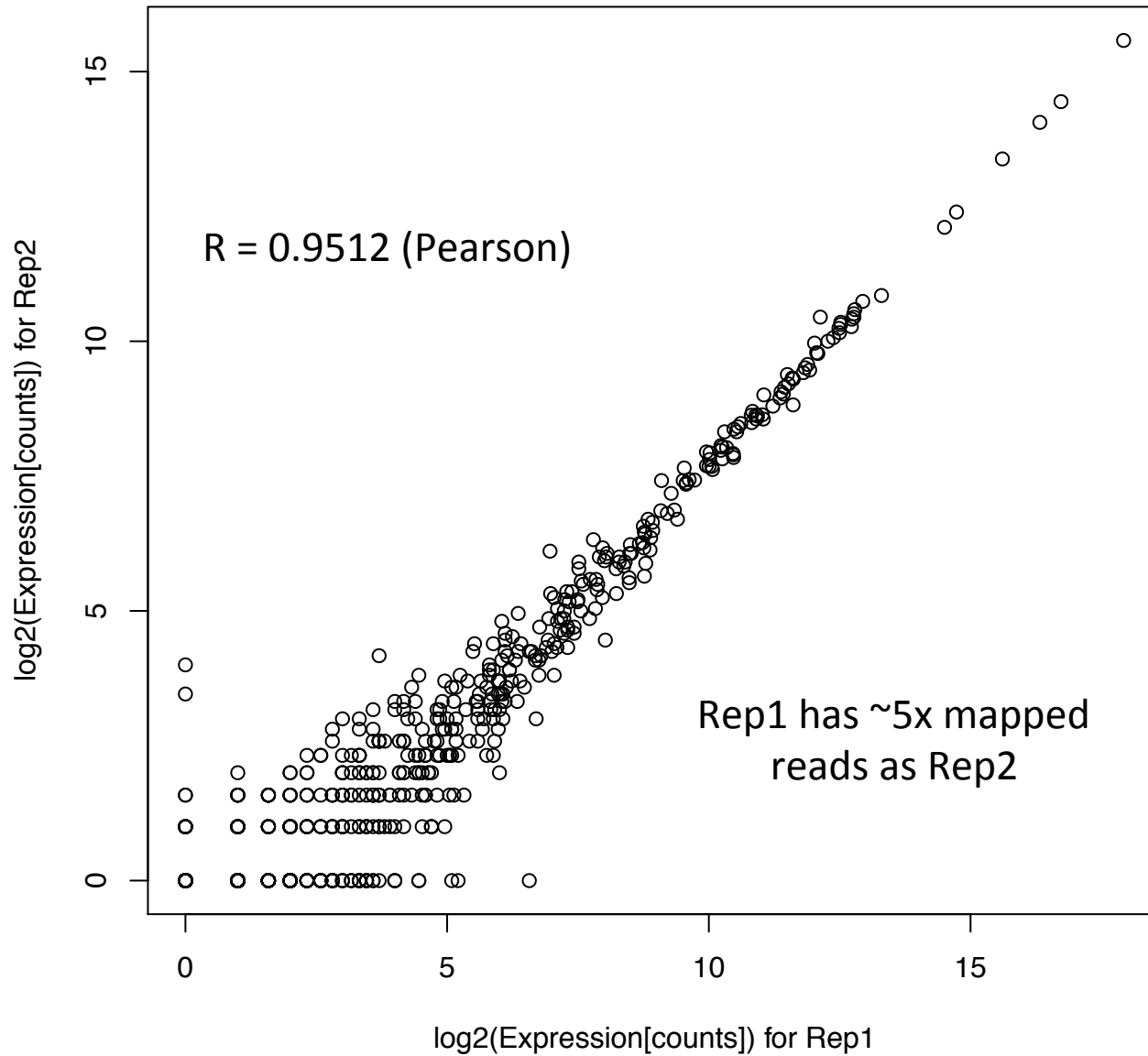


Correlations between 40 samples

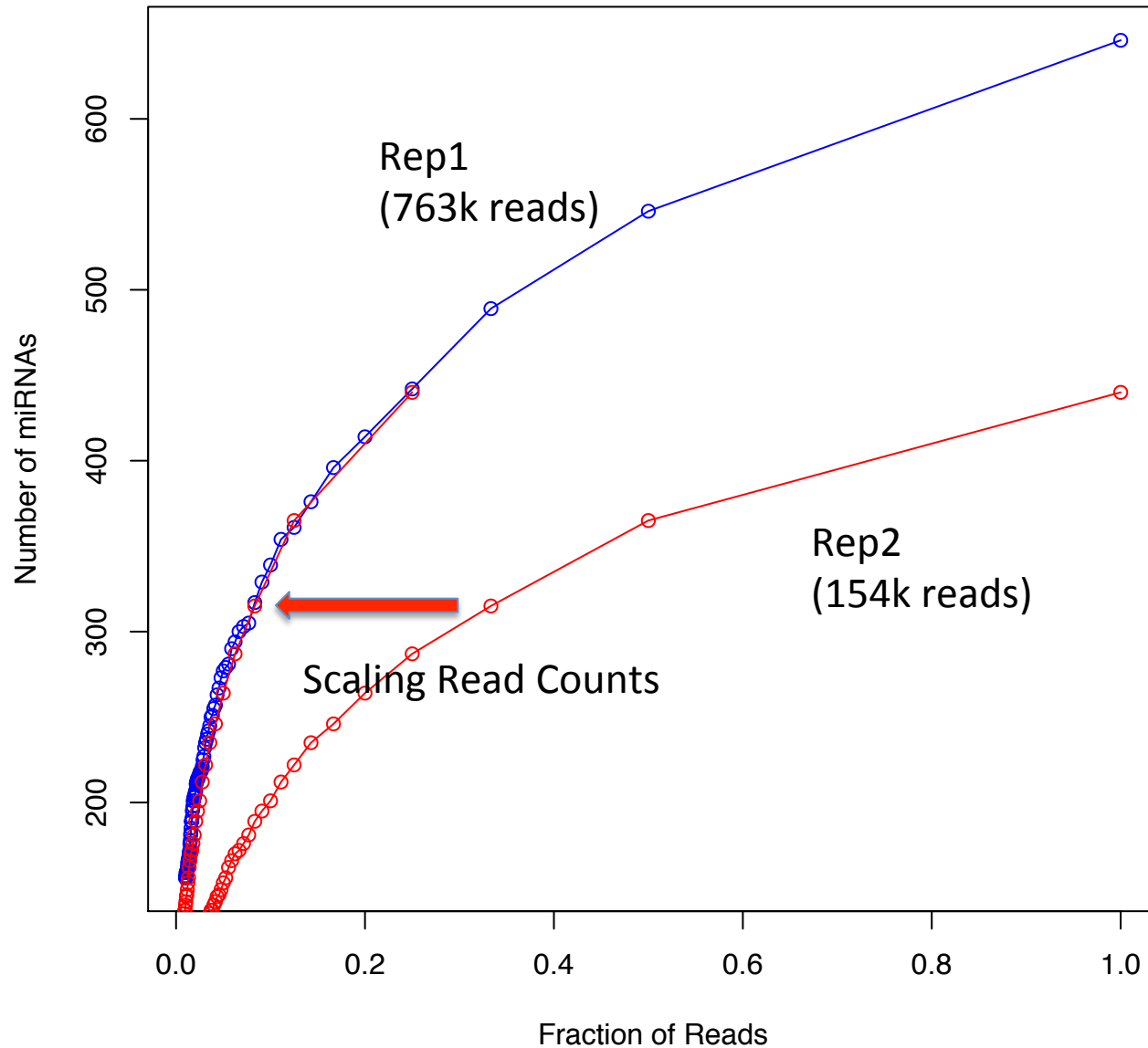
Pearson correlation
between pairs of samples
greater than 0.9



Comparison of Technical Replicates



Saturation Plot of Number of miRNAs Detected vs Sequencing Depth



Normalization & Differential Expression

- Normalized read counts per sample to 1,000,000 mapped reads (units are RPM)
- Performed differential expression (DE) analysis between CVD and no CVD samples
 - hsa-miR-589-3p (p-value = 0.01)
 - Nothing significant after correcting for multiple hypothesis testing

Small RNA selection for PCR analysis

- Selected all small RNA with a normalized read count of greater than 5 & 10 RPM averaged over the 40 samples
 - 545 small RNAs (10 RPM threshold)
 - 359 miRNAs, 25 piRNAs, 16 snoRNAs, 145 tRNAs
 - 688 small RNAs (5 RPM threshold)
 - 426 miRNAs, 36 piRNAs, 24 snoRNAs, 202 tRNAs
- Can easily adjust threshold if you want more