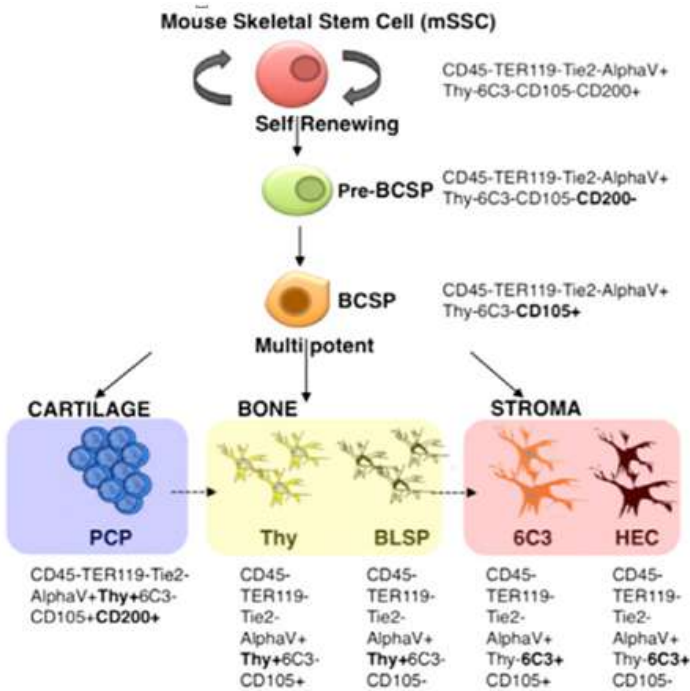


Transcriptome analysis of small synovial stromal populations in a murine model of rheumatoid arthritis

Nina Lukač, MD
Department of Anatomy
Laboratory for Molecular Immunology
Croatian Institute for Brain Research

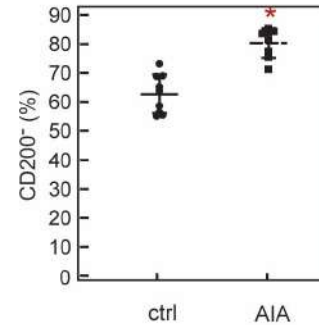
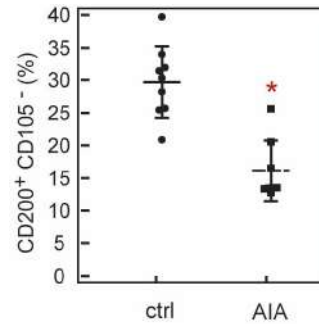


Altered synovial stromal populations rheumatoid arthritis

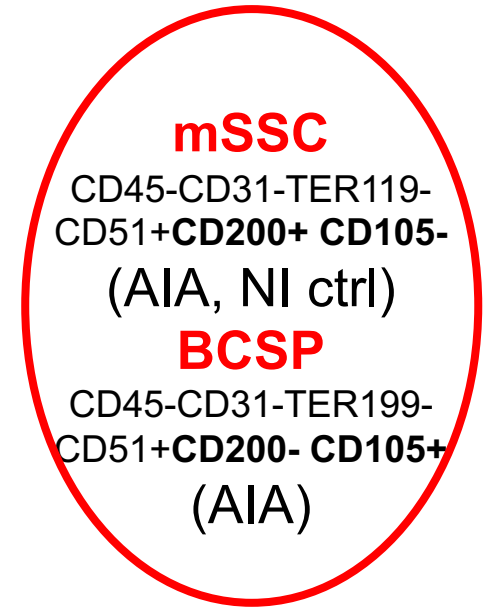
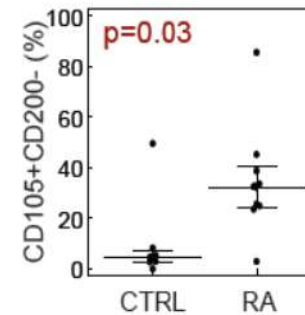
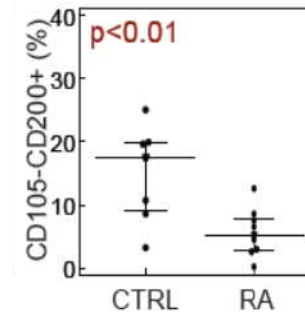


Chan et al. 2015, Cell

Murine model of RA



Human RA



Transcriptome analysis (RNA sequencing)

Amplicon QC Amplicon quantification

Library QC Library quantification

FACS

200-500 cells
+
ERCC Spike-Ins



SMART-Seq

(RT & product amplification)



Library Preparation

(tagmentation, indexing,
amplification)



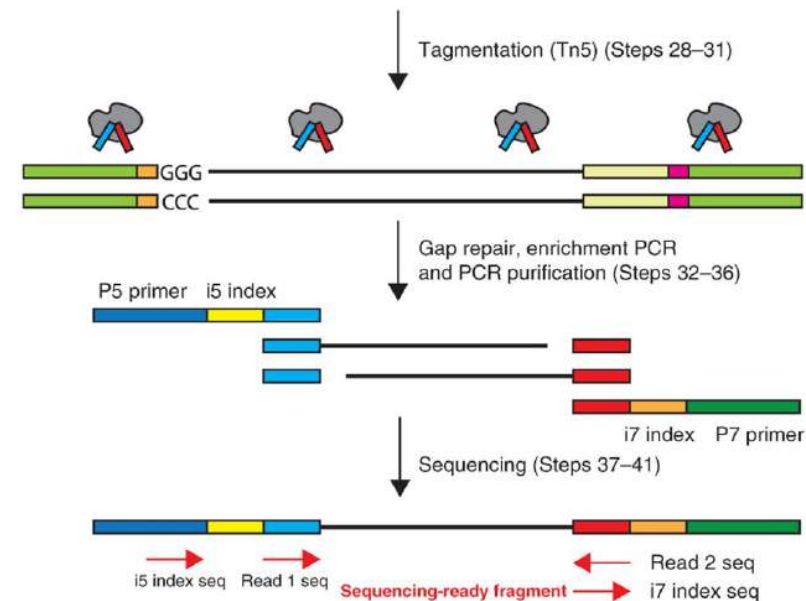
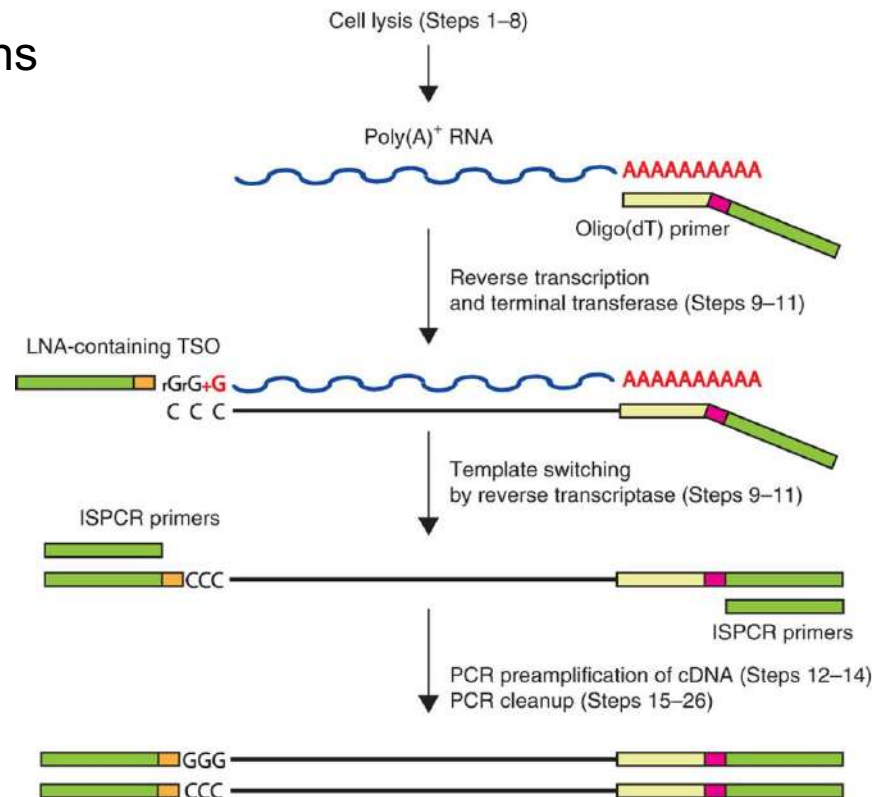
Sequencing

(50M reads/sample)



Bioinformatic analysis

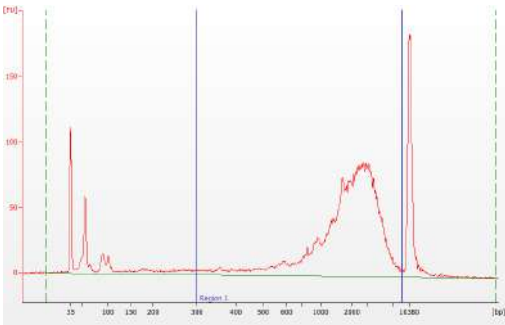
.FASTQ QC
alignment QC



Picelli et al. 2014, Nat Protoc.

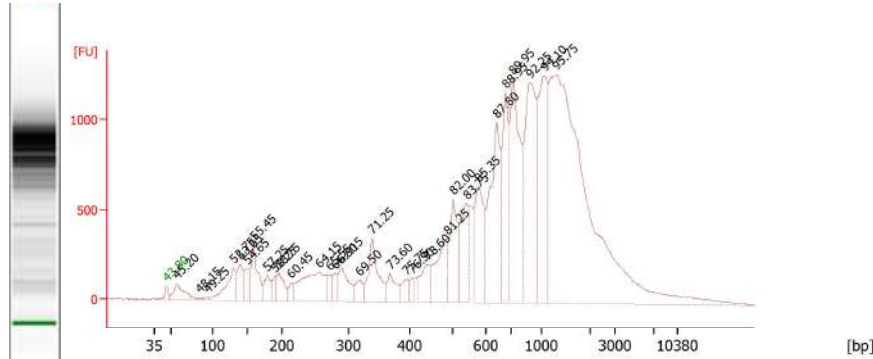
1. Amplicon QC & quantification

QC (Bioanalyzer)

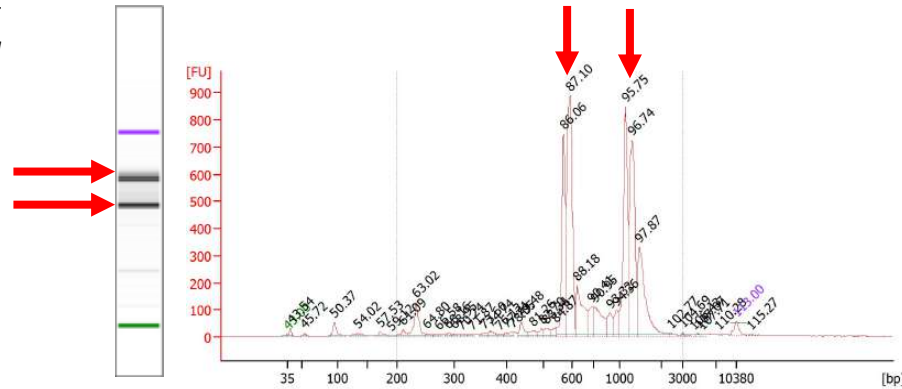


SMART-Seq® v4 Ultra® Low Input
RNA Kit for Sequencing User Manual

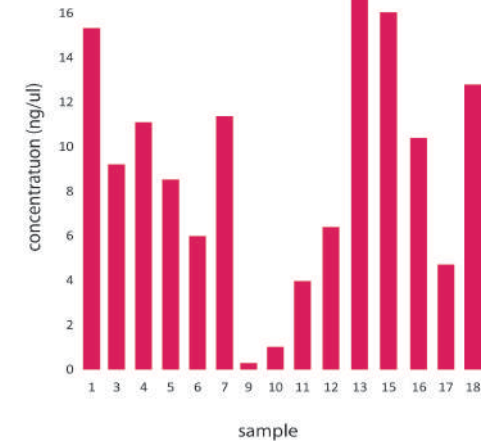
Ideal: peak spanning 400 - 10,000 bp



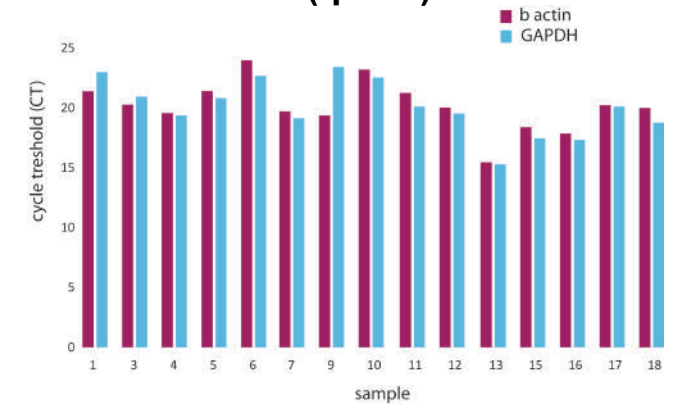
ERCC Spike-In overrepresentation



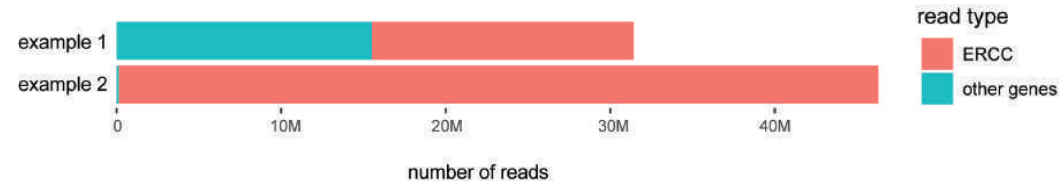
Concentration (Qubit)



Housekeeping genes (qPCR)

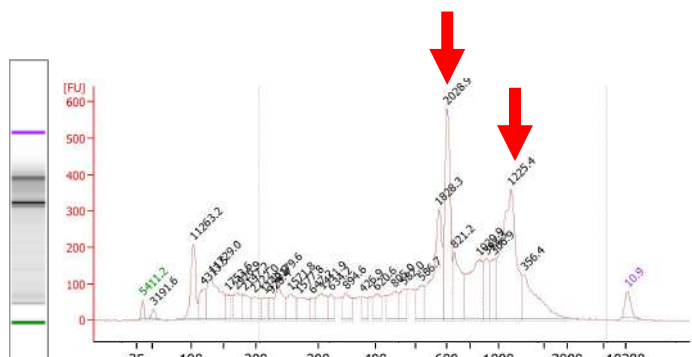


ERCC Spike-In overrepresentation

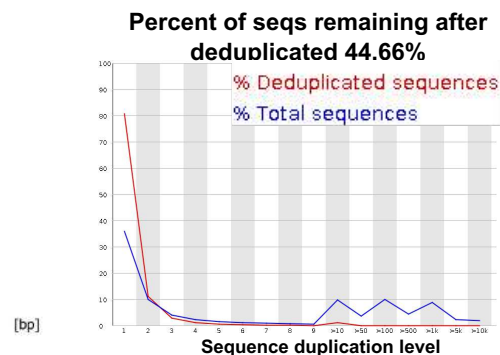


Example 1

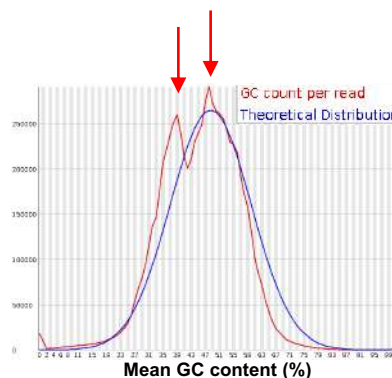
Bioanalyzer



Read duplication levels



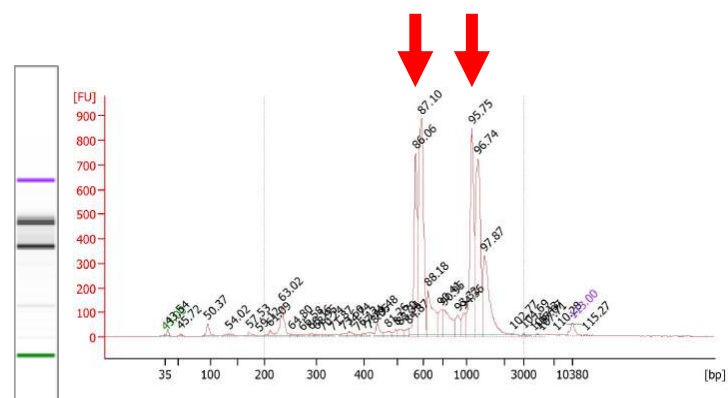
Per sequence GC content



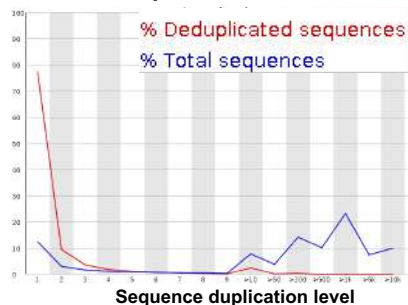
Read distribution

Total Reads	47551556		
Total Tags	48306380		
Total Assigned Tags	181202		
Group	Total_bases	Tag_count	Tags/Kb
CDS_Exons	36618602	100894	2.76
5'UTR_Exons	34250854	20935	0.61
3'UTR_Exons	59057538	37408	0.63
Introns	1087261145	18222	0.02
TSS_up_1kb	34344800	289	0.01
TSS_up_5kb	155604954	894	0.01
TSS_up_10kb	279616811	1430	0.01
TSS_down_1kb	36168639	1322	0.04
TSS_down_5kb	157745736	2055	0.01
TSS_down_10kb	279571842	2313	0.01

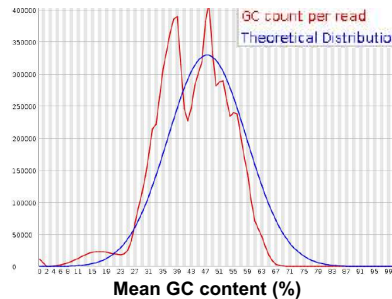
Example 2



Percent of seqs remaining after deduplicated 16.15%



Per sequence GC content



Total Reads	38358432		
Total Tags	42377054		
Total Assigned Tags	23129457		
Group	Total_bases	Tag_count	Tags/Kb
CDS_Exons	36618602	10898970	297.63
5'UTR_Exons	34250854	1339538	39.11
3'UTR_Exons	59057538	7365223	124.71
Introns	1087261145	3171879	2.92
TSS_up_1kb	34344800	28860	0.84
TSS_up_5kb	155604954	63389	0.41
TSS_up_10kb	279616811	109778	0.39
TSS_down_1kb	36168639	126815	3.51
TSS_down_5kb	157745736	206071	1.31
TSS_down_10kb	279571842	244069	0.87

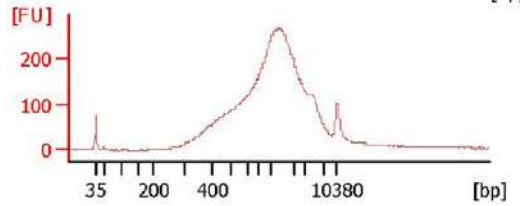
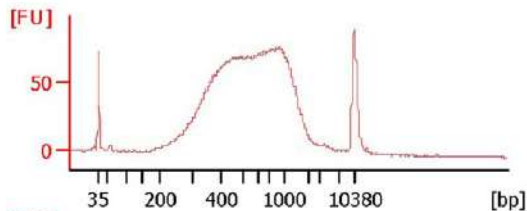
Amplicon QC

Raw reads QC

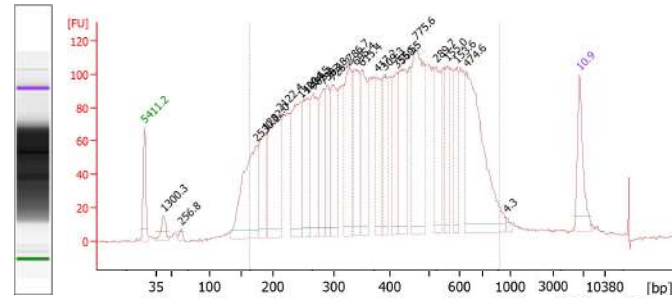
Alignment QC

3. Library QC & quantification

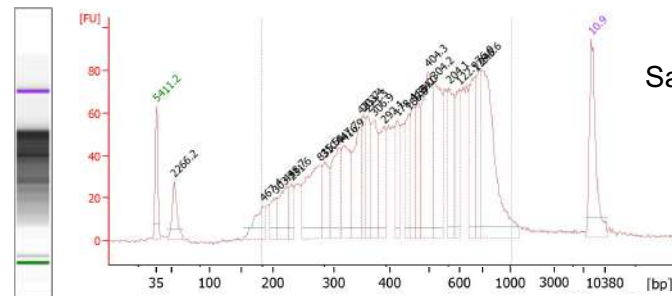
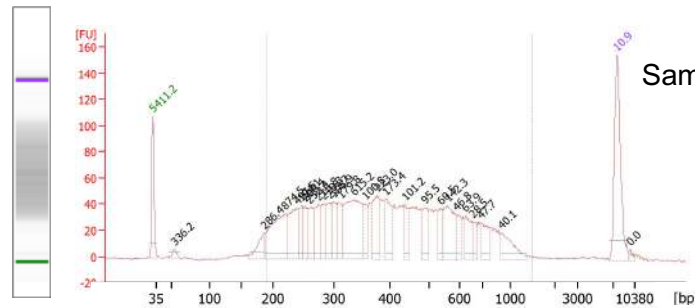
QC (Bioanalyzer)



Nextera XT DNA Library Prep Kit Reference Guide

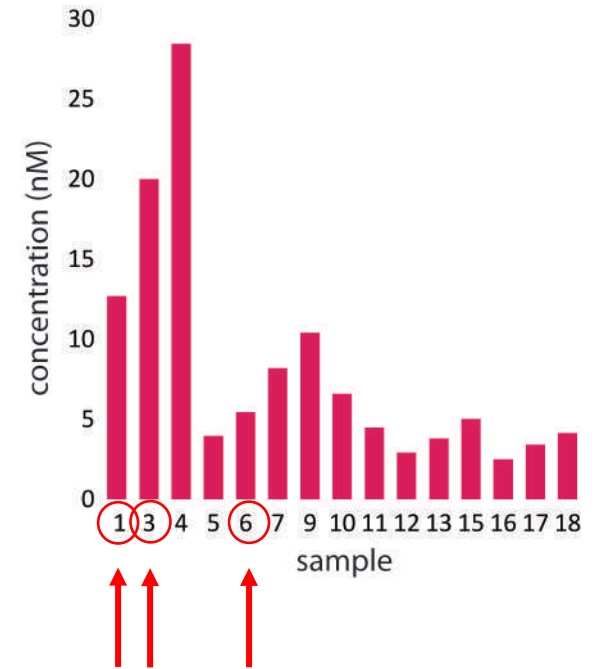


Sample 6



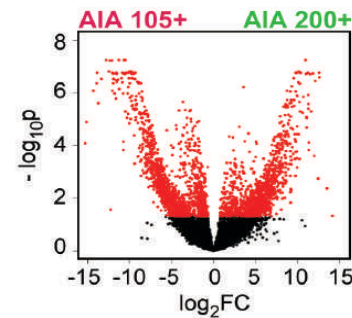
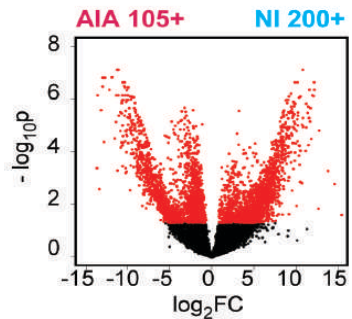
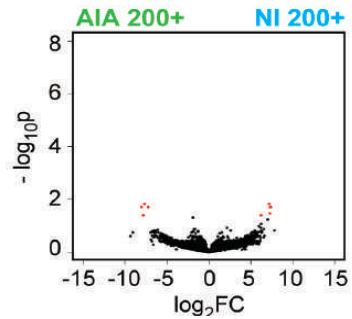
Sample 1

Concentration (Bioanalyzer)

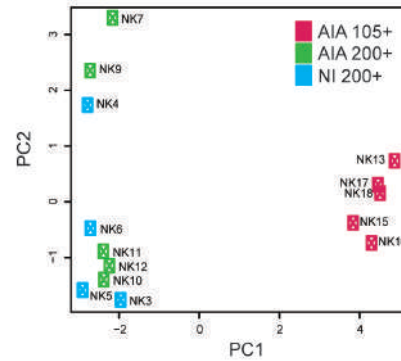


CD200+ cells have distinct transcriptional profile from CD105+ cells

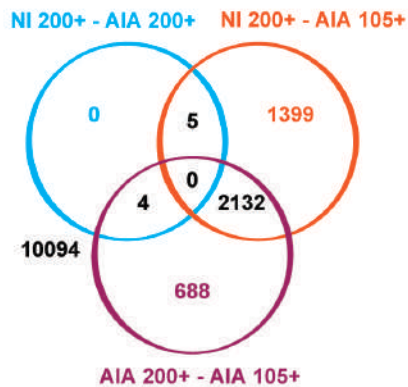
Differential expression



Principal component analysis



Venn diagram of DEGs



Enriched gene sets (GSEA)

