<u>Multi⊕C</u> Sample MultiQC replort: Sample MultiQC report: NHD13\_GEO RNAseq NHD13\_GEO RNAseq Project Type RNA-seq **Library Preparation Sequencing Platform Data Formatting** bcltofastq-2.19.0 Data Cleaning fastp 0.20.1, --in1 ../\${SAMPLE}\_R1.fastq.gz --out1 clt\_\${SAMPLE}\_R1.fastq.gz --length\_required 35 --cut\_front\_mean\_quality 13 --cut\_tail\_window\_size 1 --cut\_tail\_window\_size 1 --cut\_tail\_mean\_quality 13 --cut\_tail\_mean\_quality 13 --cut\_tail\_mean\_quality 13 --cut\_tail\_window\_size 1 --cut\_tail\_mean\_quality 13 --cut\_tail\_window\_size 1 --cut\_tail\_window\_size Genome Alignment STAR\_2.7.6a, --twopassMode Basic --runMode alignReads --genomeDir \${GENOME} --readFilesIn \${SAMPLE} --outSAMtype BAM Unsorted --outSAMstrandField intronMotif --outFilterIntronMotifs RemoveNoncanonical Reference Genome Read Quantification1 subread-2.0.1, featurecounts, -s 2 -t exon -g gene\_name **Read Quantification3** salmon-1.3.0, --seqBias --gcBias --posBias Report generated on 2022-02-07, 13:31 based on data in: /gpfs/fs2/scratch/grc\_group/dalia\_ongoing/Project\_NHD13\_GE0 FeatureCounts (Unique Reads) **Welcome!** Not sure where to start? Watch a tutorial video (6:06) don't show again X **General Statistics** Copy table 

Configure Columns 

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Showing 6/6 rows and 10/12 columns. % PF GC content % Aligned M Aligned % Assigned M Assigned % Aligned M Aligned Sample Name % Duplication % Adapter 96.3% 31.6% 49.1% 80.6% 13.5 50.2% 11.5 88.7% 14.9 NHD13\_1 48.8% 96.4% 14.1 12.3 81.4% 15.8 NHD13\_2 32.9% 52.4% 91.3% 48.7% 2.6% 14.2 15.9 NHD13\_3 32.9% 96.5% 81.4% 52.1% 12.3 90.8% 10.8 13.9 31.7% 48.4% 96.5% 81.8% 12.5 52.7% 90.8% **WT\_1** 2.7% 11.7 48.8% 53.5% WT\_2 32.1% 82.2% 13.4 91.2% 96.4% 14.9 15.8 2.5% 48.2% 14.2 52.7% 12.3 32.9% **WT\_3** 96.4% 81.8% 91.0% **FastP** FastP An ultra-fast all-in-one FASTQ preprocessor (QC, adapters, trimming, filtering, splitting...) Filtered Reads Filtering statistics of sampled reads. Number of Reads Percentages Fastp: Filtered Reads Export Plot NHD13\_1 NHD13\_2 NHD13\_3  $WT_1$ WT\_2 WT\_3 500k 1000k 1500k 2000k 2500k 3000k 3500k 4000k 4500k 5000k 5500k 6000k 1500k 12000k 13500k 14000k 1500k 1500k 16000k 16500k 17000k 17500k 18000k 18500k 19000k19500k # Reads Passed Filter Low Quality Too Many N Too short Created with MultiQC **Duplication Rates** Duplication rates of sampled reads. Y-Limits: on Fastp: Duplication Rate Export Plot 80% 50% 40% 6 7 8 9 10 11 12 13 14 15 16 17 **Duplication** level Sequence Quality Average sequencing quality over each base of all reads. Y-Limits: on Read 1: Before filtering Read 1: After filtering Fastp: Sequence Quality Export Plot **Read Position** Created with MultiQC GC Content Average GC content over each base of all reads. Y-Limits: on Fastp: Read GC Content **Export Plot** 60% **Read Position** Created with MultiQC N content Y-Limits: on Average N content over each base of all reads. Read 1: Before filtering Read 1: After filtering Fastp: Read N Content **Export Plot** 2 4 6 8 10 12 14 16 18 20 22 24 26 STAR STAR is an ultrafast universal RNA-seq aligner. **Alignment Scores** Number of Reads Percentages STAR: Alignment Scores **Export Plot** NHD13\_2 NHD13\_3 WT\_1 WT\_3 9500k 10000k 10500k 11000k 11500k 12000k 12500k 13000k 13500k 14000k 14500k 15000k 15500k 16000k 16500k 17500k 17500k 18000k 18500k # Reads Uniquely mapped
 Mapped to multiple loci
 Mapped to too many loci
 Unmapped: too short
 Unmapped: other Created with MultiQC FeatureCounts (Unique Reads) Subread featureCounts is a highly efficient general-purpose read summarization program that counts mapped reads for genomic features such as genes, exons, promoter, gene bodies, genomic bins and chromosomal locations. Number of Reads Percentages featureCounts: Assignments Export Plot NHD13\_1 NHD13\_2 NHD13\_3 WT\_1 WT\_2 WT\_3 22M 11M 24M # Reads Assigned
 Unassigned\_MultiMapping
 Unassigned\_NoFeatures
 Unassigned\_Ambiguity Created with MultiQC Salmon Y-Limits: on Salmon is a tool for quantifying the expression of transcripts using RNA-seq data. Salmon: Fragment Length Distribution **Export Plot** 0.0175 0.015 0.0125 0.0025 150 200 250 300 350 400 450 500 650 700 750 800 100 600 850 950 Fragment Length (bp) Created with MultiQC

General Stats

Filtered Reads

**Duplication Rates** 

Sequence Quality

GC Content

N content

STAR

Salmon

FastP

MultiQC v1.9 - Written by Phil Ewels, available on GitHub. This report uses HighCharts, jQuery, jQuery UI, Bootstrap, FileSaver.js and clipboard.js.