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SUPPLEMENTARY INFORMATION

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Overcoming Wnt–β-catenin dependent anticancer therapy resistance in leukaemia stem cells

John M. Perry[1,2,3,4], Fang Tao[1,2], Anuradha Roy[5], Tara Lin[3], Xi C. He[1], Shiyuan Chen[1], Xiuling Lu[6], Jacqelyn Nemechek[2], Linhao Ruan[1,13], Xiazhen Yu[1,14], Debra Dukes[1], Andrea Moran[1], Jennifer Pace[2], Kealan Schroeder[2], Meng Zhao[1,15], Aparna Venkatraman[1], Pengxu Qian[1,16,17], Zhenrui Li[1,18], Mark Hembree[1], Ariel Paulson[1], Zhiquan He[7], Dong Xu[7], Thanh-Huyen Tran[6,19], Prashant Deshmukh[8], Chi Thanh Nguyen[9], Rajeswari M. Kasi[8,9], Robin Ryan[2], Melinda Broward[3], Sheng Ding[10], Erin Guest[2], Keith August[2], Alan S. Gamis[2], Andrew Godwin[3], G. Sitta Sittampalam[3,20], Scott J. Weir[11] and Linheng Li[1,12] ✉

[1]Stowers Institute for Medical Research, Kansas City, MO, USA. [2]Children's Mercy Kansas City, Kansas City, MO, USA. [3]University of Kansas Medical Center, Kansas City, KS, USA. [4]University of Missouri Kansas City School of Medicine, Kansas City, MO, USA. [5]High Throughput Screening Laboratory, University of Kansas, Lawrence, KS, USA. [6]Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT, USA. [7]Department of Electrical Engineering and Computer Science and C.S. Bond Life Sciences Center, University of Missouri, Columbia, MO, USA. [8]Polymer Program, Institute of Materials Science, University of Connecticut, Storrs, CT, USA. [9]Department of Chemistry, University of Connecticut, Storrs, CT, USA. [10]School of Pharmaceutical Science, Tsinghua University, Beijing, China. [11]Department of Cancer Biology, The Institute for Advancing Medical Innovation and University of Kansas Cancer Center, Kansas City, Kansas, USA. [12]Department of Pathology and Laboratory Medicine and Division of Medical Oncology, Internal Medicine, University of Kansas Medical Center, Kansas City, KS, USA. [13]Present address: Center for Cell Dynamics, Department of Cell Biology, School of Medicine, Johns Hopkins University, Baltimore, MD, USA. [14]Present address: Department of Hepatobiliary and Pancreatic Surgery, First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China. [15]Present address: Key Laboratory of Stem Cells and Tissue Engineering, Ministry of Education, Sun Yat-sen University, Guangzhou, Guangdong, China. [16]Present address: Center of Stem Cell and Regenerative Medicine and Bone Marrow Transplantation Center of the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China. [17]Present address: Institute of Hematology, Zhejiang University and Zhejiang Engineering Laboratory for Stem Cell and Immunotherapy, Hangzhou, China. [18]Present address: St. Jude, Memphis, TN, USA. [19]Present address: Department of Pharmaceutical Sciences, Northeastern University, Boston, MA, US. [20]Present address: Therapeutics for Rare and Neglected Diseases, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD, USA. ✉e-mail: lil@stowers.org

ATACseq Analysis QC Report

Analyst: Shiyuan (Cynthia) Chen

08 October, 2019

1. ATAC-seq Samples Details

ATAC-seq libraries were sequenced as 76bp paired-end reads on Illumina Nextseq 500 machine. Raw sequencing reads were demultiplexed into Fastq file format using Illumina's bcl2fastq v2.20.

Table 1: Table continues below

SampleRawFile	LibraryID	Group
Blast_Vehicle_Rep1_R1.fastq.gz	L36692	Blast Vehicle
Blast_Vehicle_Rep1_R2.fastq.gz	L36692	Blast Vehicle
Blast_Vehicle_Rep2_R1.fastq.gz	L36974	Blast Vehicle
Blast_Vehicle_Rep2_R2.fastq.gz	L36974	Blast Vehicle
Blast_Vehicle_Rep3_R1.fastq.gz	L36975	Blast Vehicle
Blast_Vehicle_Rep3_R2.fastq.gz	L36975	Blast Vehicle
Blast_LowDox_Rep1_R1.fastq.gz	L37176	Blast LowDoxTreated
Blast_LowDox_Rep1_R2.fastq.gz	L37176	Blast LowDoxTreated
Blast_LowDox_Rep2_R1.fastq.gz	L37177	Blast LowDoxTreated
Blast_LowDox_Rep2_R2.fastq.gz	L37177	Blast LowDoxTreated
LSC_Vehicle_Rep1_R1.fastq.gz	L37772	LSC Vehicle
LSC_Vehicle_Rep1_R2.fastq.gz	L37772	LSC Vehicle
LSC_Vehicle_Rep2_R1.fastq.gz	L37773	LSC Vehicle
LSC_Vehicle_Rep2_R2.fastq.gz	L37773	LSC Vehicle
LSC_LowDox_R1.fastq.gz	L37175	LSC LowDoxTreated
LSC_LowDox_R2.fastq.gz	L37175	LSC LowDoxTreated

IndexSequence	Read	Type	ReadLength
CGTACTAG	1	Paired Reads	76
CGTACTAG	2	Paired Reads	76
TAAGGCGA	1	Paired Reads	76
TAAGGCGA	2	Paired Reads	76
AGGCAGAA	1	Paired Reads	76
AGGCAGAA	2	Paired Reads	76
CGTACTAG	1	Paired Reads	76
CGTACTAG	2	Paired Reads	76
AGGCAGAA	1	Paired Reads	76
AGGCAGAA	2	Paired Reads	76
CGTACTAG	1	Paired Reads	76
CGTACTAG	2	Paired Reads	76
AGGCAGAA	1	Paired Reads	76
AGGCAGAA	2	Paired Reads	76
TAAGGCGA	1	Paired Reads	76
TAAGGCGA	2	Paired Reads	76

2. Analysis Method

Reference: ENCODE Guildlines (https://www.encodeproject.org/atac-seq/)

Step1: Adapter Trimming

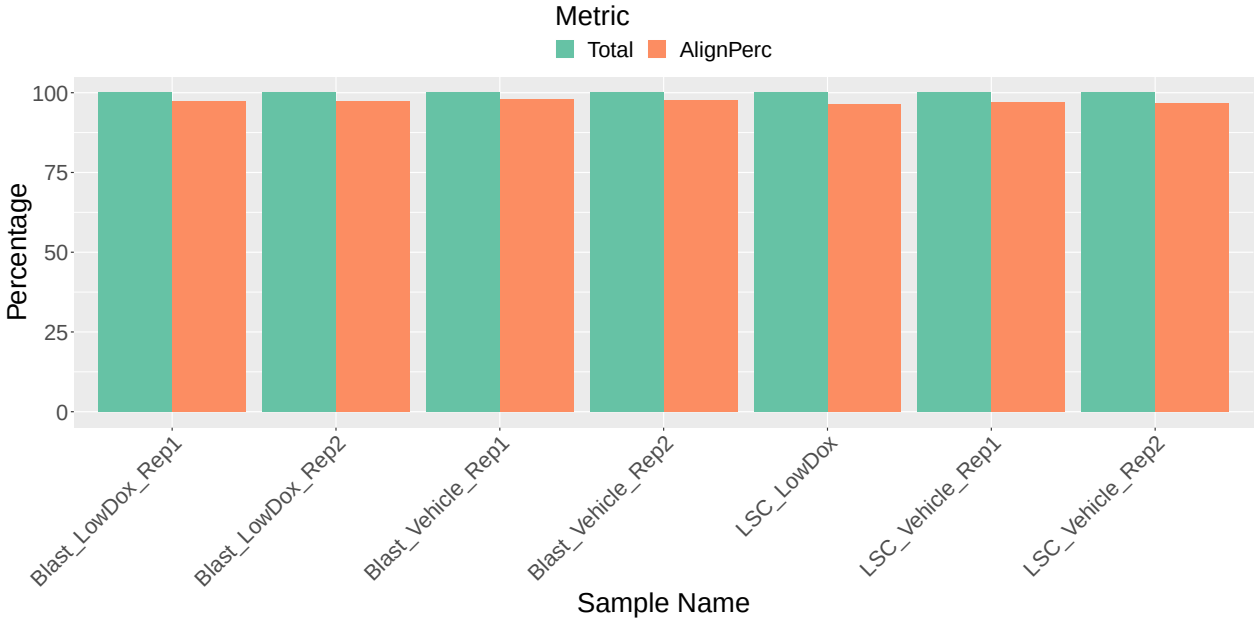
For data quality processing, 3' Nextera adapter CTGTCTCTTATA was first removed using cutadapt for both pairs. In addition, reads with at least 8bp overlap and at most 20% mismatches were kept for alignment. Pairs whose trimmed reads (R1 or R2) have length less than 50 bp will be discarded as well. After filtering, more than 94% of reads are kept for next step.

Sample	TotalReads	FilteredReads	FilterPercentage
Blast_Vehicle_Rep1	334884294	322612928	96.336%
Blast_Vehicle_Rep2	331761640	315160628	94.996%
Blast_LowDox_Rep1	346941242	335947632	96.831%
Blast_LowDox_Rep2	182890478	178860880	97.797%
LSC_Vehicle_Rep1	127426602	124461096	97.673%
LSC_Vehicle_Rep2	114023880	110513182	96.921%
LSC_LowDox	224299808	214672574	95.708%

Step2: Alignment using Bowtie2

Trimmed high quality reads were aligned to mm10 genome using bowtie2 with parameters "-X 2000 –very-sensitve". All samples have more than 95% overall alignment rate.

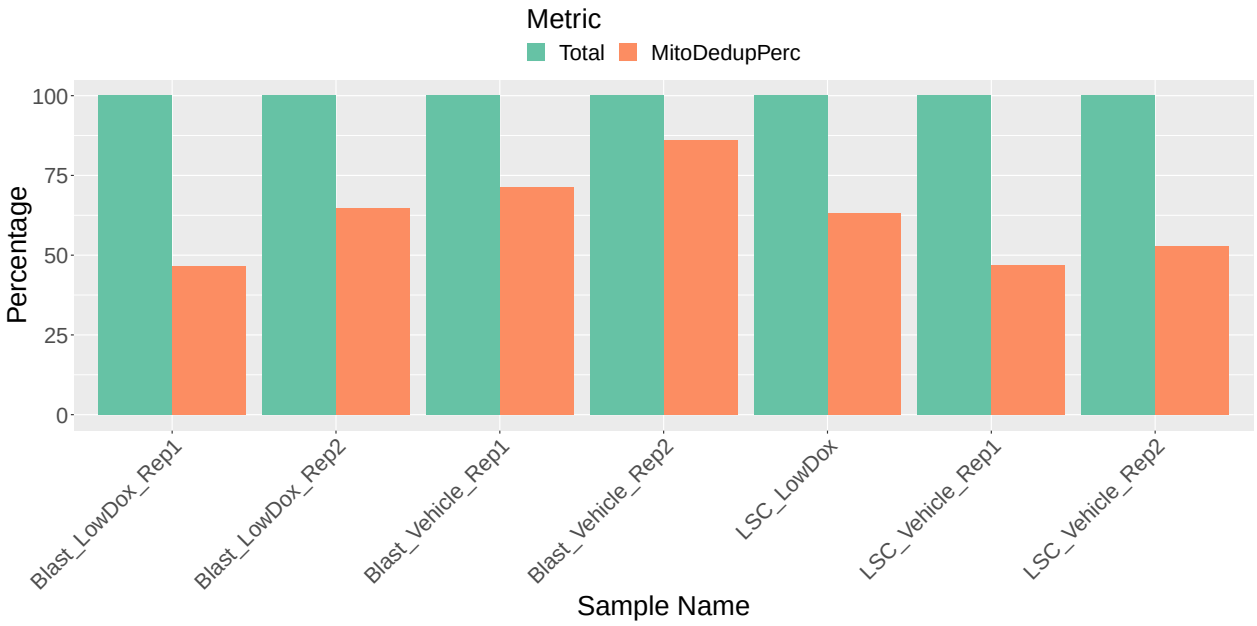
- Total - Number of read pairs after filterring
- AlignPerc - Number of pairs which aligned, including single and multiple mappings



Step3: Mitochondrial and Duplicated Reads Removal

After alignment, reads that mapped to mitochondrial chromosome were removed using samtools. Also, duplicated reads were evaluated and removed using Picard.

- Total - Number of read pairs after filterring
- MitoDedupPerc - Percentage of reads mapped to mitochondrail chromosome and are duplicated.



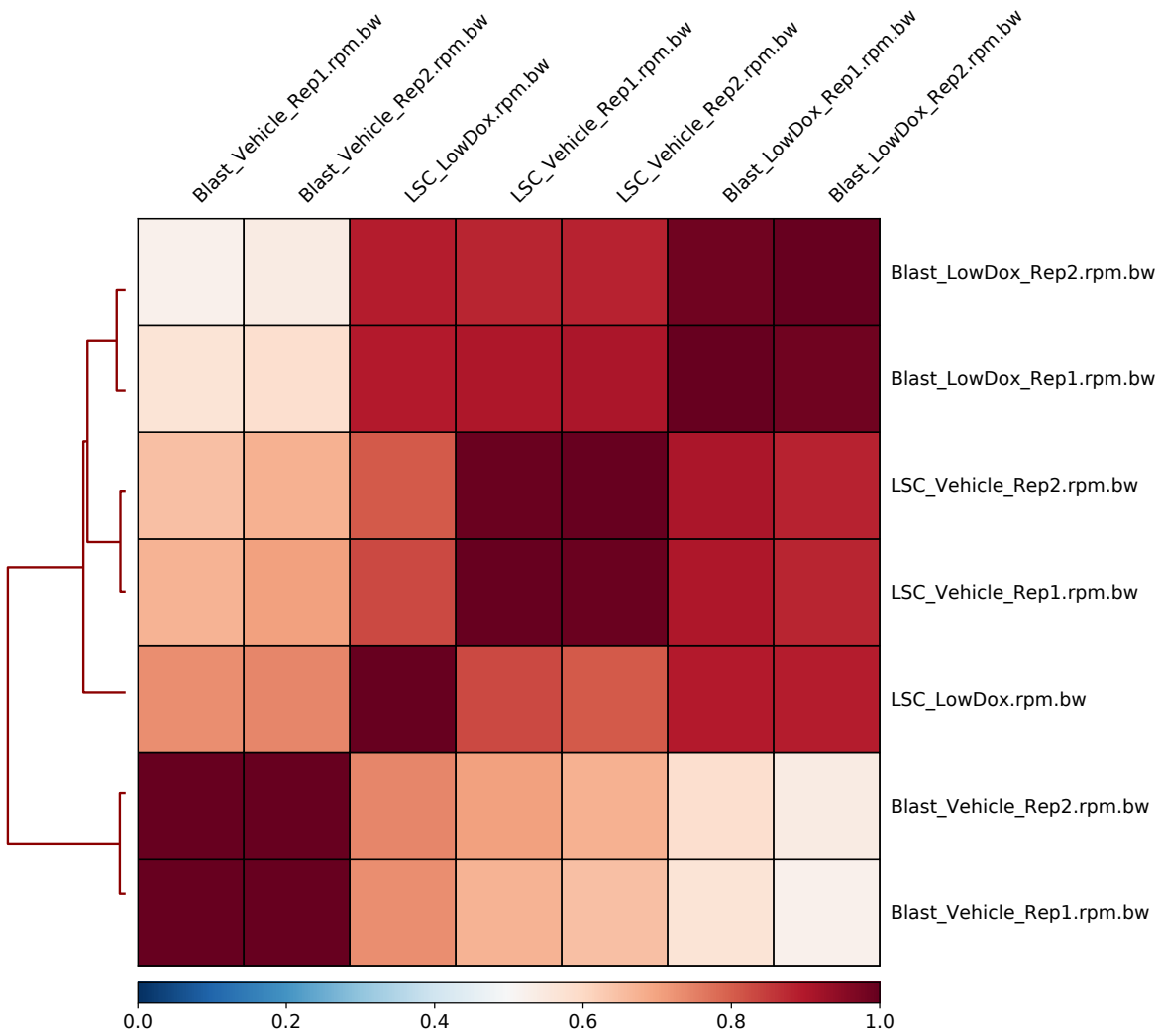
Summary

After all the data preprocessing, we have 46M ~ 174M reads left for samples, whose reads are primary alignments, proper-paired, and not duplicated.

Sample	FinalProcessedReads
Blast_Vehicle_Rep1	90408171
Blast_Vehicle_Rep2	42934911
Blast_LowDox_Rep1	174938157
Blast_LowDox_Rep2	61289871
LSC_Vehicle_Rep1	64000207
LSC_Vehicle_Rep2	50487364
LSC_LowDox	76352900

3. Sample Correlation

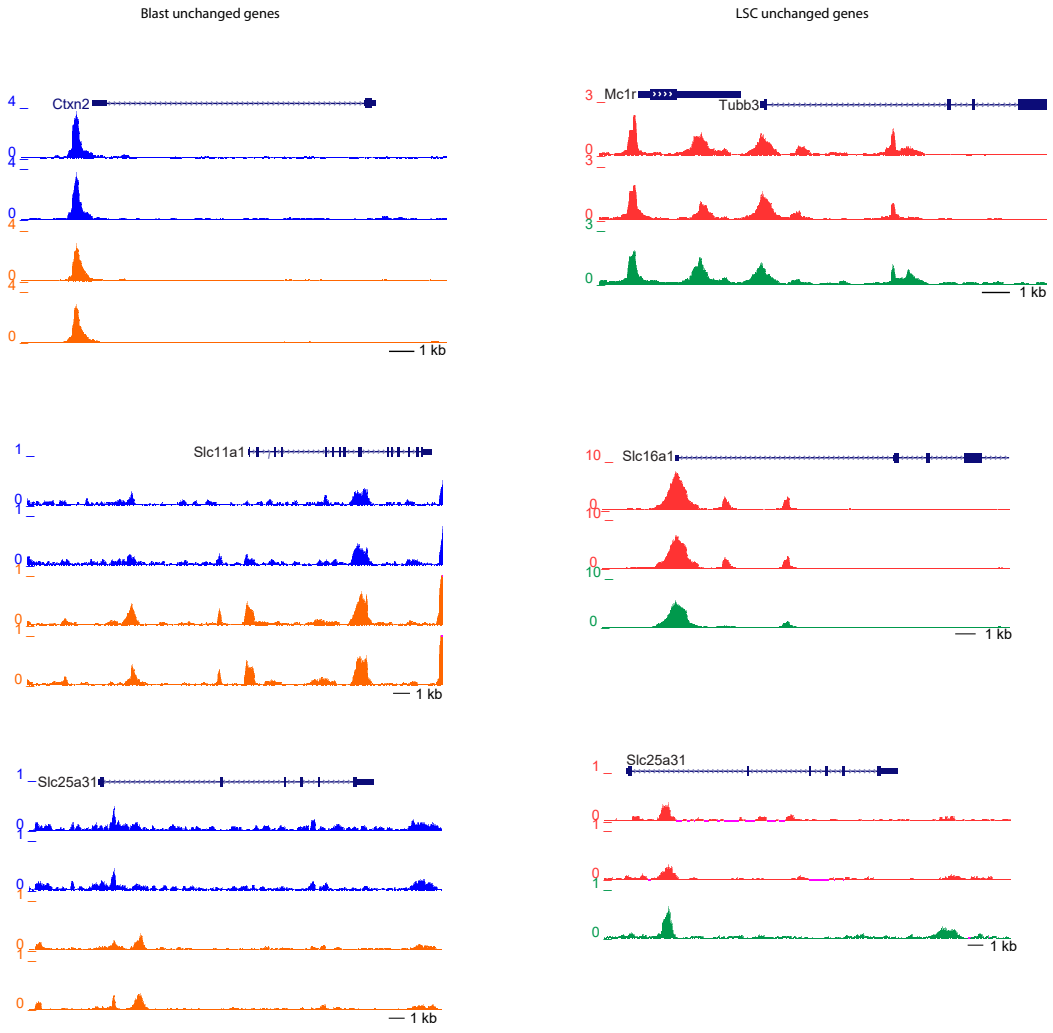
Pearson correlations between samples along with hierarchical clustering. We found high correlation between Blast replicates and LSCVehicle replicates.



4. Peak calling and differential accessibility analysis

- Peaks were called using MACS2 with parameters "-f BAMPE -q 0.05". Fragments per million normalized tracks in bigwig format were generated using fragments that are primary alignments, proper-paired, and not duplicated for downstream analysis.
- We take the filtered peaks (qvalue<0.05) and apply R package DiffBind to perform differential accessibility analysis. We compared BlastVehicle vs. BlastLowDox and LSCVehicle vs. LSCLowDox samples, respectively.
- **Due to the starting material restriction and high quality of LSC LowDoxTreated sample, reservoir random sampling (tools: seqtk) was used to create pseudo replicates and generate statistics for the LSC volcano plot.**

5. Gene tracks showing unaffected genes after treatment



6. Gene tracks showing affected gene in LSC cells