Whole Genome and Exome Sequencing Reference Datasets from A Multi-center and Cross-platform Benchmark Study

Yongmei Zhao[1#], Li Tai Fang[2], Tsai-wei Shen[1], Sulbha Choudhari[1], Keyur Talsania[1], Xiongfong Chen[1], Jyoti Shetty[3], Yuliya Kriga[3], Bao Tran[3], Bin Zhu[4], Zhong Chen[5], Wangqiu Chen[5], Charles Wang[5], Erich Jaeger[6], Daoud Meerzaman[7], Charles Lu[8], Kenneth Idler[8], Yuanting Zheng[9], Leming Shi[9], Virginie Petitjean[10], Marc Sultan[10], Tiffany Hung[11], Eric Peters[11] Jiri Drabek[12,13], Petr Vojta[12,13], Roberta Maestro[13,14], Daniela Gasparotto[13,14], Sulev Kõks[13,15,16], Ene Reimann[13,17], Andreas Scherer[13,18], Jessica Nordlund[13,19], Ulrika Liljedahl[13,19], Jonathan Foox[20], Christopher Mason[20], Chunlin Xiao[21], Wenming Xiao[22#]

[1] Advanced Biomedical and Computational Sciences, Biomedical Informatics and Data Science Directorate, Frederick National Laboratory for Cancer Research, Frederick, MD, USA

[2]Bioinformatics Research & Early Development, Roche Sequencing Solutions Inc., Belmont, CA, USA

[3]Sequencing Facility, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Frederick, MD, USA

[4]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

[5]Center for Genomics, School of Medicine, Loma Linda University, Loma Linda, CA, USA

[6]Core Applications Group, Product Development, Illumina Inc, Foster City, CA, USA

[7]Computational Genomics and Bioinformatics Branch, Center for Biomedical Informatics and Information Technology, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

[8]Computational Genomics, Genomics Research Center (GRC), North Chicago, IL, USA

[9]State Key Laboratory of Genetic Engineering, School of Life Sciences and Shanghai Cancer Center, Fudan University, Shanghai, China

[10]Biomarker Development, Novartis Institutes for Biomedical Research, Switzerland

[11]Companion Diagnostics Development, Oncology Biomarker Development, Genentech, South San Francisco, CA, USA

[12]IMTM, Faculty of Medicine and Dentistry, Palacky University, Olomouc, the Czech Republic

[13]Member of EATRIS ERIC - European Infrastructure for Translational Medicine

[14]Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, National Cancer Institute, Unit of Oncogenetics and Functional Oncogenomics, Italy

[15]Perron Institute for Neurological and Translational Science, Nedlands, Australia

[16]Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Murdoch, Australia

[17] Estonian Genome Centre, Institute of Genomics, University of Tartu, Tartu, Estonia

[18]Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Finland

[19]Department of Medical Sciences, Molecular Medicine and Science for Life Laboratory, Uppsala University, Uppsala, Sweden

[20]Department of Physiology and Biophysics, Weill Cornell Medicine, New York, NY, USA, 1305 York Ave., Y13-05, New York, NY, USA

[21]National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD, USA

[22]The Center for Devices and Radiological Health, U.S. Food and Drug Administration, FDA, Silver Spring, MD, USA

#Correspondence shall be addressed to: Yongmei Zhao at Yongmei.Zhao@nih.gov or Wenming Xiao at Wenming.Xiao@fda.hhs.gov

Abstract

With the rapid advancement of sequencing technologies in the past decade, next generation sequencing (NGS) analysis has been widely applied in cancer genomics research. More recently, NGS has been adopted in clinical oncology to advance personalized medicine. Clinical applications of precision oncology require accurate tests that can distinguish tumor-specific mutations from errors or artifacts introduced during NGS processes or data analysis. Therefore, there is an urgent need to develop best practices in cancer mutation detection using NGS and the need for standard reference data sets for systematically benchmarking sequencing platforms, library protocols, bioinformatics pipelines and for measuring accuracy and reproducibility across platforms and methods. Within the SEQC2 consortium context, we established paired tumor-normal reference samples, a human triple-negative breast cancer cell line and a matched normal cell line derived from B lymphocytes. We generated whole-genome (WGS) and whole-exome sequencing (WES) data using 16 NGS library preparation protocols, seven sequencing platforms at six different centers. We systematically interrogated somatic mutations in the paired reference samples to identify factors affecting detection reproducibility and accuracy in cancer genomes. These large cross-platform/site WGS and WES datasets using well-characterized reference samples will represent a powerful resource for benchmarking NGS technologies, bioinformatics pipelines, and for the cancer genomics studies.

Background & Summary

The NGS technology has become a powerful tool for precision medicine. More researchers and clinicians are utilizing NGS to identify clinically actionable mutations in cancer patients and to establish targeted therapies for patients based on the patient's genetic makeup or genetic variants of their tumor[1], there is a critical need to have a full understanding of the many different variables affecting the NGS analysis output. The rapid growing number of sample processing protocols, library preparation methods, sequencing platforms, and bioinformatics pipelines to detect mutations in cancer genome, presents great technical challenges for the accuracy and reproducibility of utilizing NGS for cancer genome mutation detections. To investigate how these experimental and analytical elements may affect mutation detection accuracy, recently we carried out a comprehensive benchmarking study using both whole-genome (WGS) and whole-exome sequencing (WES) data sets generated from two well-characterized reference samples: a human breast cancer cell line (HCC1395) and a B lymphocytes cell line (HCC1395BL) derived from the same donor (NBT-RS47789). We generated WGS and WES data using various NGS library preparation protocols, seven NGS platforms at six centers (NBT-A46164B).

Figure 1 shows our overall study design. Briefly, DNA was extracted from fresh cells or cell pellets mimicking the formalin-fixed paraffin-embedded (FFPE) process with fixation time of 1, 2, 6, or 24 hours. A small amount of DNA from fresh cells of HCC1395 and HCC1395BL was pooled at various ratios (3:1, 1:1, 1:4, 1:9 and 1:19) to create mixtures. Both fresh DNA and

FFPE DNA were profiled on NGS or microarray platforms following manufacturer recommended protocols. To assess the reproducibility of WGS and WES, six sequencing centers performed a total of 12 replicates (3x3 + 3) on each platform. In addition, 12 WGS libraries constructed using three different library preparation protocols (TruSeq PCR-free, TruSeq-Nano, and Nextera Flex) in four different quantities of DNA inputs (1, 10, 100, and 250 ng) were sequenced on an Illumina HiSeq 4000, and nine WGS libraries constructed using the TruSeq PCR-free protocol were sequenced on an Illumina NovaSeq. Finally, Cytoscan microarray and single-cell sequencing with 10X Genomics platform were performed to uncover the cytogenetics and heterogeneity of two cell lines. Table 1 contains the details of the platform, library protocols and read coverage information.

We first established reference call sets with evidence from 21 replicates of Illumina WGS runs with coverage ranging from 50X to 100X (1150X in total). We split mutation call confidence levels into four categories: HighConf, MedConf, LowConf, and Unclassified (NBT-RS47789). By combining all WGS runs, we were able to further confirm and improve our call set with tumor-normal pairs of 1500X data sets and identified mutations with VAF as low as 1.5%. A subset of reference mutation calls was validated by targeted exome sequencing (WES at 2,500X coverage) using HiSeq, and deep sequencing from AmpliSeq (at 2,000X coverage) using Miseq, and Ion Torrent (at 34X coverage), and long-read WGS by PacBio Sequel (at 40X coverage). In addition, we inferred subclones and heterogeneity of HCC1395 with bulk DNA sequencing. The results were confirmed by single-cell DNA sequencing analysis (NBT-RS47789B).

With defined reference call sets, we then systematically interrogated somatic mutations to identify factors affecting detection reproducibility and accuracy. By examining the interactions and effects of NGS platform, library preparation protocol, tumor content, read coverage, and bioinformatics process concomitantly, we observed that each component of the sequencing and analysis process can affect the final outcome. Overall WES and WGS results have high concordance and correlation. WES had a better coverage/cost ratio than WGS. However, sequencing coverage of the WES target regions was not even. In addition, WES showed more batch effects/artifacts due to laboratory processing and thus had larger variation between runs, laboratories, and likely between researchers preparing the libraries. As a result, WES had much larger inter-center variation and was less reproducible than WGS. Biological (library) replicates removed some artifacts due to random events ("Non-Repeatable" calls) and offered much better calling precision than did a single test. Analytical repeats (two bioinformatics pipelines) also increased calling precision at the cost of increased false negatives. We found that biological replicates are more important than bioinformatics replicates in cases where high specificity and sensitivity are needed (NBT-RS47789B).

Methods

Detailed methods were described in our two papers (NBT-A46164B and NBT-RS47789B, in press).

Cell line culture and DNA extraction

HCC1395; Breast Carcinoma; Human (*Homo sapiens*) cells (expanded from ATCC CRL-2324) were cultured in ATCC-formulated RPMI-1640 Medium, (ATCC 30-2001) supplemented with fetal bovine serum (ATCC 30-2020) to a final concentration of 10%. Cells were maintained at 37 °C with 5% carbon dioxide (CO_2) and were sub-cultured every 2 to 3 days, per ATCC recommended procedures using 0.25% (w/v) Trypsin-0.53 mM EDTA solution (ATCC 30-2101), until appropriate densities were reached. HCC1395BL; B lymphoblast; Epstein-Barr virus (EBV) transformed; Human (*Homo sapiens*) cells (expanded from ATCC CRL-2325) were cultured in ATCC-formulated Iscove's Modified Dulbecco's Medium, (ATCC Catalog No. 30-

150 2005) supplemented with fetal bovine serum (ATCC 30-2020) to a final concentration of 20%.
151 Cells were maintained at 37 °C with 5% CO_2 and were sub-cultured every 2 to 3 days, per
152 ATCC recommended procedures, using centrifugation with subsequent resuspension in fresh
153 medium until appropriate densities were reached. Final cell suspensions were spun down
154 and re-suspended in PBS for nucleic acid extraction.
155 All cellular genomic material was extracted using a modified Phenol- Chloroform-Iso-Amyl
156 alcohol extraction approach. Essentially, cell pellets were re-suspended in TE, subjected to
157 lysis in a 2% TritonX-100/0.1% SDS/0.1 M NaCl/10mM Tris/1mM EDTA solution and were
158 extracted with a mixture of glass beads and Phenol- Chloroform-Iso-Amyl alcohol. Following
159 multiple rounds of extraction, the aqueous layer was further treated with Chloroform-IAA
160 and finally underwent RNases treatment and DNA precipitation using sodium acetate (3 M,
161 pH 5.2) and ice-cold Ethanol. The final DNA preparation was re-suspended in TE and stored
162 at -80°C until use.
163
164 **FFPE processing and DNA extraction**
165 Please see Online methods in manuscript NBT-RA46164 for details.
166
167 **Illumina WGS Library Preparation**
168 The TruSeq DNA PCR-Free LT Kit (Illumina, FC-121-3001) was used to prepare samples for
169 whole genome sequencing. WGS libraries were prepared at six sites with the TruSeq DNA
170 PCR-Free LT Kit according to the manufacturers' protocol. The input DNA amount for WGS
171 library preparation with fresh DNA for TruSeq-PCR-free libraries was 1 ug unless otherwise
172 specified. All sites used the same fragmentation conditions for WGS by using Covaris with
173 targeted size of 350 bp. All replicated WGS were prepared on a different day.
174
175 The concentration of the TruSeq DNA PCR-Free libraries for WGS was measured by qPCR
176 with the KAPA Library Quantification Complete Kit (Universal) (Roche, KK4824). The
177 concentration of all the other libraries was measured by fluorometry either on the Qubit 1.0
178 fluorometer or on the GloMax Luminometer with the Quant-iT dsDNA HS Assay kit
179 (ThermoFisher Scientific, Q32854). The quality of all libraries was assessed by capillary
180 electrophoresis either on the 2100 Bioanalyzer or TapeStation instrument (Agilent) in
181 combination with the High Sensitivity DNA Kit (Agilent, 5067-4626) or the DNA 1000 Kit
182 (Agilent, 5067-1504) or on the 4200 TapeStation instrument (Agilent) with the D1000 assay
183 (Agilent, 5067-5582 and 5067-5583).
184
185 For the WGS library preparation from cross-site study, the sequencing was performed at six
186 sequencing sites using three different Illumina platforms including HiSeq 4000 instrument at
187 2 x 150 bases read length with HiSeq 3000/4000 SBS chemistry (cat# FC-410-1003), and on a
188 NovaSeq instrument at 2 x 150 bases read length using the S2 configuration (cat#PN
189 20012860), or on a HiSeq X Ten at 2x150bases read length using the X10 SBS chemistry (cat#
190 FC-501-2501). Sequencing was performed following the manufacturer's instructions.
191
192 For the comparison study of WGS library protocol using different input DNA amounts,
193 Illumina TruSeq DNA PCR-free protocol used 250ng input DNA, Illumina TruSeq Nano
194 protocol libraries were prepared with 1ng, 10ng, and 100ng input DNA amounts. Illumina
195 Nextera Flex libraries were prepared with 1ng, 10ng, and 100ng input DNA amounts. These
196 libraries sequenced at two sequencing sites using two different Illumina platforms including
197 HiSeq 4000 instrument (Illumina) at 2 x 150 bases read length with HiSeq 3000/4000 SBS
198 chemistry (Illumina, FC-410-1003) and NovaSeq instrument (Illumina) at 2 x 150 bases read
199 length using the S2 configuration (Illumina, PN 20012860). Sequencing was performed
200 following the manufacturer's instructions.
201

For the tumor purity study, 1µg tumor:normal dilutions were made in the following ratios using Resuspension Buffer (Illumina): 1:0, 3:1, 1:1, 1:4, 1:9, 1:19 and 0:1. Each ratio was diluted in triplicate. DNA was sheared using the Covaris S220 to target a 350 bp fragment size (Peak power 140w, Duty Factor 10%, 200 Cycles/Bursts, 55s, Temp 4 ^{o}C). NGS library preparation was performed using the Truseq DNA PCR-free protocol (Illumina) following the manufacturer's recommendations. The sample purity WGS libraries were sequenced on a HiSeq 4000 instrument (Illumina) at 2 x 150 bases read length with HiSeq 3000/4000 SBS chemistry (Illumina, FC-410-1003). Sequencing was performed following the manufacturer's instructions.

Whole Exome Library Construction and Sequencing

SureSelect Target Enrichment Reagent kit, PTN (Part No G9605A), SureSelect Human All Exon v6 + UTRs (Part No 5190-8881), Herculase II Fusion DNA Polymerase (Part No 600677) from Agilent Technologies and Ion Xpress Plus Fragment kit (Part No 4471269, Thermo Fischer Scientific Inc) were combined to prepare library according to the manufacturer's guidelines (User guide: SureSelect Target Enrichment System for Sequencing on Ion Proton, Version C0, December 2016, Agilent Technologies). Prior, during and after library preparation the quality and quantity of genomic DNA (gDNA) and/or libraries were evaluated applying QubitTM fluorometer 2.0 with dsDNA HS Assay Kit (Thermo Fischer Scientific Inc) and Agilent Bioanalyzer 2100 with High Sensitivity DNA Kit (Agilent Technologies).

WES libraries were sequenced at six sequencing sites with two different Illumina platforms, Hiseq4000 instrument (Illumina) at 2x150 bases read length with HiSeq 3000/4000 SBS chemistry (Illumina, FC-410-1003) and Hiseq2500 (Illumina) at 2x100 bases read length with HiSeq2500 chemistry (Illumina, FC-401-4003). Sequencing was performed following the manufacturer's instructions.

Whole Genome FFPE Sample Library Preparation and Sequencing

For the FFPE WGS study, NEBNext Ultra II (NEB) libraries were prepared according to the manufacturer's instructions. However, input adjustments were made according to the dCq obtained for each sample using the TruSeq FFPE DNA Library Prep QC Kit (Illumina) to account for differences in sample amplifiability. A total of 33 ng of amplifiable DNA was used as input for each sample.

FFPE WGS libraries were sequenced on two different sequencing canters on Hiseq4000 instrument (Illumina) at 2x150 bases read length with HiSeq 3000/4000 SBS chemistry (Illumina, FC-410-1003). Sequencing was performed following the manufacturer's instructions.

Whole Exome FFPE Sample Library Preparation and Sequencing

For the FFPE study, SureSelect (Agilent) WES libraries were prepared according to the manufacturer's instructions for 200 ng of DNA input, including reducing the shearing time to four minutes. Additionally, the adaptor-ligated libraries were split in half prior to amplification. One half was amplified for 10 cycles and the other half for 11 cycles to ensure adequate yields for probe hybridization. Both halves were combined after PCR for the subsequent purification step.

FFPE WES libraries were sequenced on at two sequencing sites with different Illumina platforms, Hiseq4000 instrument (Illumina) at 2x150 bases read length with HiSeq 3000/4000 SBS chemistry (Illumina, FC-410-1003) and Hiseq2500 (Illumina) at 2x100 bases

253 read length with HiSeq2500 chemistry (Illumina, FC-401-4003). Sequencing was performed
254 following the manufacturer's instructions.
255
256
257 **PacBio Library Preparation and Sequencing**
258 15 ug of material was sheared to 40 kbp with Megarupter (Diagenode). Per the Megarupter
259 protocol the samples were diluted to <50 ng/ul. A 1x AMPure XP bead cleanup was
260 performed. Samples were prepared as outlined on the PacBio protocol titled "Preparing >30
261 kbp SMRTbell Libraries Using Megarupter Shearing and Blue Pippin Size-Selection for PacBio
262 RS II and Sequel Systems." After library preparation, the library was run overnight for size
263 selection using the Blue Pippin (Sage). The Blue Pippin was set to select a size range of 15-50
264 kbp. After collection of the desired fraction, a 1x AMPure XP bead cleanup was performed.
265 The samples were loaded on the PacBio Sequel (Pacific Biosciences) following the protocol
266 titled "Protocol for loading the Sequel." The recipe for loading the instrument was generated
267 by the Pacbio SMRTlink software v5.0.0. Libraries were prepared using Sequel chemistry kits
268 v2.1, SMRTbell template kit 1.0 SPv3, magbead v2 kit for magbead loading, sequencing
269 primer v3, and SMRTbell clean-up columns v2. Libraries were loaded at between 4 pM and 8
270 pM.Sequencing was performed following the manufacturer's instructions.
271
272 **10X Genomics Chromium Genome Library Preparation and Sequencing**
273 Sequencing libraries were prepared from 1.25 ng DNA using the Chromium Genome Library
274 preparation v2 kit (10X Genomics, cat #120257/58/61/62) according to the manufacturer's
275 protocol (#CG00043 Chromium Genome Reagent Kit v2 User Guide). The quality of the
276 libraries was evaluated using the TapeStation D1000 Screen Tape (Agilent). The adapter-
277 ligated fragments were quantified by qPCR using the library quantification kit for Illumina
278 (KK4824, KAPA Biosystems) on a CFX384Touch instrument (BioRad) prior to cluster
279 generation and sequencing. Chromium libraries were sequenced on a HiSeq X Ten or a HiSeq
280 4000 instrument at 2 x 150 base pair (bp) read length and using sequencing chemistry v2.5 or
281 HiSeq 3000/4000 SBS chemistry (Illumina, cat# FC-410-1003) across five sequencing sites.
282 Sequencing was performed following the manufacturer's instructions.
283
284 **AmpliSeq library construction and sequencing**
285 AmpliSeq libraries were prepared in triplicate and prepared as specified in the Illumina
286 protocol (Document # 1000000036408 v04) following the two oligo pools workflow with 10
287 ng of input genomic DNA per pool. The number of amplicons per pool was 1517 and 1506
288 respectively. The libraries were quality-checked using an Agilent Tapestation 4200 with the
289 DNA HS 1000 kit and quantitated using a Qubit 3.0 and DNA high sensitivity assay kit. The
290 libraries were applied to a MiSeq v2.0 flowcell. They were then amplified and sequenced
291 with a MiSeq 300 cycle reagent cartridge with a read length of 2 × 150 bp. The MiSeq run
292 produced 7.3 Gbp (94.5%) at ≥Q30. The total number of reads passing filter was 47,126,128
293 reads.
294
295 **Whole Exome library Ion Platform Sequencing**
296 SureSelect Target Enrichment Reagent kit, PTN (Part No G9605A), SureSelect Human All Exon
297 v6 + UTRs (Part No 5190-8881), Herculase II Fusion DNA Polymerase (Part No 600677) from
298 Agilent Technologies and Ion Xpress Plus Fragment kit (Part No 4471269, Thermo Fisher
299 Scientific Inc) were combined to prepare libraries according to the manufacturer's guidelines
300 (User guide: SureSelect Target Enrichment System for Sequencing on Ion Proton, Version C0,
301 December 2016, Agilent Technologies). Prior, during, and after library preparation the quality
302 and quantity of genomic DNA (gDNA) and/or libraries were evaluated applying QubitTM
303 fluorometer 2.0 with dsDNA HS Assay Kit (Thermo Fisher Scientific Inc) and Agilent
304 Bioanalyzer 2100 with High Sensitivity DNA Kit (Agilent Technologies).

For sequencing the WES libraries, the Ion S5 XL Sequencing platform with Ion 540-Chef kit (Part No A30011, Thermo Fisher Scientific Inc) and the Ion 540 Chip kit (Part No A27766, Thermo Fisher Scientific Inc) were used. One sample per 540 chip was sequenced, generating up to 60 million reads with average length of 200 bp.

10X Genomics Single Cell CNV library construction, sequencing and analysis

HCC1395 and HCC1395 BL were cultured as described above. 500,000 cells of each culture were suspended in 1 mL suspension medium (10% DMSO in cell culture medium). Cells were harvested the next day for single-cell copy number variation (CNV) analysis via the 10X Genomics Chromium Single Cell CNV Solution (Protocol document CG000153) produces Single Cell DNA libraries ready for Illumina sequencing according to manufacturer's recommendations. Libraries were sequenced on a HiSeq 4000 instrument at 2 x 150 base pair (bp) read length and using sequencing chemistry v2.5 or HiSeq 3000/4000 SBS chemistry (Illumina, cat# FC-410-1003). Demultiplex BCL from sequencing run and Copy Number Variation analysis were performed using 10X Genomics Cell Ranger DNA version 1.1 software. CNV and heterogeneity visualization analysis was performed via 10X Genomics Loupe scDNA browser.

Affymetrix Cytoscan HD microarray

DNA concentration was measured spectrophotometrically using a Nanodrop (Life technology), and integrity was evaluated with a TapeStation 4200 (Agilent). Two hundred and fifty nanograms of gDNA were used to proceed with the Affymetrix CytoScan Assay kit (Affymetrix). The workflow consisted of restriction enzyme digestion with Nsp I, ligation, PCR, purification, fragmentation, and end labeling. DNA was then hybridized for 16 hr at 50 °C on a CytoScan array (Affymetrix), washed and stained in the Affymetrix Fluidics Station 450 (Affymetrix), and then scanned with the Affymetrix GeneChip Scanner 3000 G7 (Affymetrix). Data were processed with ChAS software (version 3.3). Array-specific annotation (NetAffx annotation release 36, built with human hg38 annotation) was used in the analysis workflow module of ChAS. Karyoview plot and segments data were generated with default parameters.

Reference genome

The reference genome we used was the decoy version of the GRCh38/hg38 human reference genome (https://gdc.cancer.gov/about-data/data-harmonization-and-generation/gdc-reference-files; GRCh38.d1.dv1.fa), which was utilized by the Genomic Data Commons (GDC). The gene annotation GTF file was downloaded from the 10X website as refdata-cellranger-GRCh38-1.2.0.tar.gz, which corresponds to the GRCh38 genome and Ensmebl v84 transcriptome.

All the following bioinformatics data analyses are based on the above reference genome and gene annotation.

Preprocessing and Alignment of WGS Illumina Data

For each of the paired-end read files (i.e., FASTQ 1 and 2 files) generated by Illumina sequencers (HiSeq, NovaSeq, X Ten platforms), we first trimmed low-quality bases and adapter sequences using Trimmomatic[2]. The trimmed reads were mapped to the human reference genome GRCh38 (see the read alignment section) using BWA MEM (v0.7.17)[3] in paired-end mode and bwa-mem was run with the –M flag for downstream Picard[5] compatibility.

Post alignment QC was performed based both FASTQ on BWA alignment BAM files, the read quality and adapter content were reported by FASTQC[4] software. The genome mapped percentages and mapped reads duplication rates calculated by BamTools (v2.2.3) and Picard (v1.84)[5]. The genome coverage and exome target region coverages as well as mapped reads insert sizes, and G/C contents were profiled using Qualimap(v2.2)[6] and custom scripts. Preprocessing QC reports were generated during each step of the process. MultiQC(v1.9)[7] was run to generate an aggregated report in html format. A standard QC metrics report was generated from a custom script. The preprocessing and alignment QC analysis pipeline is described in **Suppl. Figure 1a**.

Preprocessing and Alignment of WES Illumina Data

For each of the paired-end read files generated by Illumina sequencers (HiSeq2500, HiSeq4000 platforms), we first trimmed low-quality bases and adapter sequences using Trimmomatic[2]. The trimmed reads were mapped to the human reference genome GRCm38 (see the read alignment section) using BWA MEM (v0.7.17)[3] in paired-end mode. We calculated on-target rate based on the percentage of mapped reads that were overlap the target capture bait region file (target.bed). The post alignment QC methods are same as WGS Illumina data pre-processing.

DNA Damage Estimate for WGS, WES and FFPE Samples

The DNA Damage Estimator(v3)[8] was used to calculate the GIV score based on an imbalance between R1 and R2 variant frequency of the sequencing reads to estimate the level of DNA damage that was introduced in the sample/library preparation processes. GIV score above 1.5 is defined as damaged. At this GIV score, there are 1.5 times more variants on R1 than on R2. Undamaged DNA samples have a GIV score of 1.

Preprocessing and Alignment of PacBio Data

PacBio raw data were merged bam files using SMRTlink tool v6.0.1. which used minimap2[9] as default aligner. Duplicate reads were mark and removed from PBSV alignment bases on the reads coming from the same ZMW, the base pair tolerance was set to 100bp to remove the duplicated reads. The preprocessing and alignment QC analysis pipeline for PacBio data is described in **Suppl. Figure 1b**.

Genome coverage profiling

We used indexcov[10] to estimate coverage from the Illumina whole genome sequencing library cross-site comparison data set. The bam file for each library used as input to indexcov[10] to generate a linear index for each chromosome indicating the file (and virtual) offset for every 16,384 bases in that chromosome. This gives the scaled value for each 16,384-base chunk (16KB resolution) and provides a high-quality coverage estimate per genome. The output is scaled to around 1. A long stretch with values of 1.5 would be a heterozygous duplication; a long stretch with values of 0.5 would be a heterozygous deletion.

Preprocessing and Alignment of 10X Genomics WGS Data

The 10X Genomics Chromium fastq files were mapped and reads were phased using LongRanger to the hg38/GRCh38 reference genome using the LongRanger v2.2.2 pipeline [https://genome.cshlp.org/content/29/4/635.full]. The linked-reads were aligned using the Lariat aligner[11], which uses BWA MEM[3] [Li H. et al. 2010] to generate alignment candidates, and duplicate reads are marked after alignment. Linked-Read data quality was assessed using the 10X Genome browser Loupe. MultiQC(v1.9)[7] was run to generate an aggregated report in html format. A standard QC metrics report was generated from a custom script. The preprocessing and alignment QC analysis pipeline is described in **Suppl. Figure 1a**.

408
409 **Preprocessing and Alignment of Ion Torrent Data**
410 Raw reads were first filtered for low-quality reads and trimmed to remove adapter
411 sequences and low-quality bases. This step was performed using the BaseCaller module of
412 the Torrent SuitTM software package v5.8.0 (Thermo Fischer Scientific Inc). Low-quality
413 reads were retained from further analysis in the raw signal processing stage. Low-quality
414 bases were trimmed from the 5' end if the average quality score of the 16-base window fell
415 below 16 (Phred scale), cleaving 8 bases at once. Processed reads were mapped to the
416 GRCh38 reference genome by TMAP module of the Torrent Suite software package using the
417 default map4 algorithm with recommended settings. Picard (v1.84)[5] was then used to mark
418 PCR and optical duplicates on the BAM files.
419
420 **Preprocessing and alignment for AmpliSeq**
421 Low-quality bases and adapter sequences were trimmed with Trimmomatic[2]. The trimmed
422 reads were mapped to the human reference genome GRCh38 (see the read alignment
423 section) using BWA MEM (v0.7.17)[3] in paired-end mode. We calculated on-target rate based
424 on the percentage of mapped reads that were overlap the target capture bait region file
425 (target.bed). We counted the number of variant-supporting reads and total reads for each
426 variant position with MQ $\geq$ 40 and BQ $\geq$ 30 cutoffs. The preprocessing and alignment QC
427 analysis pipeline is described in **Suppl. Figure 1a**.
428
429 **Somatic Variant Analysis**
430 Four somatic variant callers, MuTect2 (GATK 3.8-0)[12], SomaticSniper (1.0.5.0)[13], Lancet
431 (1.0.7), and Strelka2 (2.8.4)[14], which are readily available on the NIH Biowulf cluster, were
432 run using the default parameters or parameters recommended by the user's manual.
433 Specifically, for MuTect2, we included flags for "-nct 1 -rf DuplicateRead -rf
434 FailsVendorQualityCheck -rf NotPrimaryAlignment -rf BadMate -rf
435 MappingQualityUnavailable -rf UnmappedRead -rf BadCigar", to avoid the running exception
436 for "Somehow the requested coordinate is not covered by the read". For MuTect2, we used
437 COSMIC v82 as required inputs. For SomaticSniper, we added a flag for "-Q 40 -G -L –F", as
438 suggested by its original author, to ensure quality scores and reduce likely false positives. For
439 TNscope (201711.03), we used the version implemented in Seven Bridges's CGC with the
440 following command, "sentieon driver -i $tumor_bam -i $normal_bam -r $ref --algo TNscope -
441 -tumor_sample $tumor_sample_name --normal_sample $normal_sample_name -d $dbsnp
442 $output_vcf". For Lancet, we ran with 24 threads on the following parameters "--num-
443 threads 24 --cov-thr 10 --cov-ratio 0.005 --max-indel-len 50 -e 0.005". Strelka2 was run with
444 24 threads with the default configuration. The rest of the software analyzed was run as a
445 single thread on each computer node. All mutation calling on WES data was performed with
446 the specified genome region in a BED file for exome-capture target sequences.
447
448 The high confidence outputs or SNVs flagged as "PASS" in the resulting VCF files were
449 applied to our comparison analysis. Results from each caller used for comparison were all
450 mutation candidates that users would otherwise consider as "real" mutations detected by
451 this caller.
452
453 **GATK indel realignment and quality score recalibration**
454 The GATK (3.8-0)-IndelRealigner was used to perform indel adjustment with reference indels
455 defined in the 1000Genome project
456 (https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=4&ved=0ahUKEwjlkc
457 fB5-
458 nbAhVOhq0KHXUWCKUQFgg7MAM&url=ftp%3A%2F%2Fftp.1000genomes.ebi.ac.uk%2Fvol1
459 %2Fftp%2Ftechnical%2Freference%2FGRCh38_reference_genome%2Fother_mapping_resou

rces%2FALL.wgs.1000G_phase3.GRCh38.ncbi_remapper.20150424.shapeit2_indels.vcf.gz&usg=AOvVaw0pLCj6zDgJg0A6zbFeMfQl). The resulting BAM files were then recalibrated for quality with "BaseRecalibrator" and dbSNP build 146 as the SNP reference. Finally, "PrintReads" was used to generate recalibrated BAM files.

Tumor Ploidy and clonality analysis from whole genome and exome data

To estimate the HCC1395 cell line ploidy, we used PURPLE[17] to determine the purity and copy number profile. To determine the clonality of HCC1395 and HCC1395 BL, we performed somatic SNV and CAN analysis using superFreq[16] on capture WES datasets. Mapped and markDuplicate bam files of a pair of HCC1395 and HCC1395BL were used as input and bam files of the remaining replicates of the HCC1395BL library were used to filter background. Analysis was run using the superFreq default parameters. The clonality of each somatic SNV was calculated based on the VAF, accounting for local copy number. The SNVs and CNAs undergo hierarchical clustering based on the clonality and uncertainty across replicates for the tumor sample.

Assessment of reproducibility and O_Score calculation

We created and used "tornado" plots to visualize the consistency of mutation calls derived from aligners, callers, or repeated NGS runs. The height of the "tornado" represents the number of overlapping calls in the VCF files in descending order. The top of each plot portrays SNVs called in every VCF file. The bottom of the plots contains SNVs present in only one VCF file. The width of the "tornado" represents the number of accumulated SNVs in that overlapping category, which is scaled by the total number of SNVs in the corresponding sub-group. In addition, we established following formula to measure reproducibility based on the overlapping SNVs:

$$O_{score} = \frac{\sum_{i=1}^{i \to n}((\frac{i}{n}) \times O_i)}{\sum_{i=1}^{i \to n} O_i}$$

where n is the total number of VCF results in the pool set, i is the number of overlaps, O_i is the number of accumulated SNVs in the set with i number of overlapping.

Data Records

All raw data (FASTQ files) are available on NCBI's SRA database (SRP162370). The truth set for somatic mutations in HCC1395, VCF files derived from individual WES and WGS runs, and source codes are available on NCBI's ftp site (ftp://ftp-trace.ncbi.nlm.nih.gov/seqc/ftp/release/Somatic_Mutation_WG/). Alignment files (BAM) are also available on Seven Bridges' s Cancer Genomics Cloud (CGC) platform.

Technical Validation

Assessment of whole genome and exome sequencing data quality

For whole genome sequencing, fresh DNA samples were prepared using standard TruSeq PCR-free libraries prepared from 1000 ng input DNA. A total of 24 data sets were generated from six sequencing centers. There were three different Illumina sequencing platforms in the cross-platform comparison including HiSeq4000, HiSeq X Ten, and NovaSeq 6000. The quality

510 assessment was based on the NGS preprocess pipeline produced quality metrics including
511 Percentage of Q30 bases, sequencing yields, percentage of adapter sequences, percentage
512 of mapped reads to reference genome, percentage of non-duplicate reads, GC content, DNA
513 fragment insert sizes, genome coverage, etc (**Suppl. Figure 1**).

514 All sequencing centers and platforms produced high quality data as base call Phred quality
515 scores above Q30, and greater than 99% of reads mapped to the reference genome (**Figure**
516 **2a**). The variation was observed in read coverage which was driven by sequencing platform
517 yield differences as well as sequencing library pooling variations. Most sequencing sites
518 produced genome coverage 50X (1,250 millions pair-end reads) per library, one sequencing
519 site targeted about 100X (2,500 millions pair-end reads) per genome sequencing depth
520 (**Figure 2b, Suppl. Figure 2a**). For whole exome sequencing, SureSelect Target Enrichment
521 Reagent kit, PTN (Part No G9605A), SureSelect Human All Exon v6 and SureSelect Human All
522 Exon v6 +UTRs were used, and sequencing was generated from 6 sequencing centers.
523 Illumina Hiseq4000, Illumina Hiseq3000/4000, and Illumina Hiseq2500 were used.
524 Sequencing quality from all sequences are high with greater than 99.1% of reads mapped to
525 reference genome across sites. The variation was also observed in read coverage, most
526 sequencing sites produced exome region on-target coverage 100X per library, and two
527 sequencing sites targeted about 300X and 550X per genome sequencing depth (**Figure 2c**).
528 When comparing WGS to WES libraries for the percentages of non-duplicated reads, all WGS
529 libraries have consistently high percentages of non-duplicate reads, which indicates higher
530 library complexity of WGS libraries than the targeted captures. In addition, there are much
531 high variations in targeted exome capture libraries.

532 To determine if the quality of sequencing data was substantially different between different
533 protocols, we also compared fresh DNA vs. FFPE DNA, different library protocols and input
534 DNA amount, as well as mixture tumor DNA and normal DNA for profiling the tumor purity
535 effect. Among the WGS libraries prepared using fresh cells, insert size distribution and G/C
536 content were uniform (40 – 43% G/C). WES libraries have higher GC content (47.2% for fresh
537 cells libraries, 51.1% for FFPE libraries) as well as higher variation (**Figure 2e**). All of the WGS
538 libraries had very low adapter contamination (<0.5%) (**Suppl. Figure 2b)**, while WES libraries
539 have higher adapter content due to smaller DNA fragment insert sizes (**Figure 2f**). WES
540 library sizes are between 150bps -280bps for fresh cells. FFPE WGS libraries all have much
541 shorter libraries sizes (225 - 300bps) than fresh DNA prepared WGS libraries (360 – 480bps).
542 The libraries with higher adapter contamination also had much higher G/C content
543 compared with the rest of the WES libraries (**Figure 2e**). When comparing library preparation
544 kits across different DNA inputs across TruSeq PCR-free (1000ng), TruSeq-Nano, and Nextera
545 Flex libraries prepared with 250, 100, 10, or 1 ng of DNA input, the percentage of non-
546 redundant reads was very low (<20%) for TruSeq-Nano with 1 ng input, due to PCR
547 amplification of a low input amount of DNA; higher input amount libraries have better
548 performance; for the same input amount, Nextera Flex libraries have less variation and
549 higher percentages of non-duplicated reads (**Suppl. Figure 2c**). We conclude the Nextera Flex
550 library protocol might be a better option for low input DNA library preparation.

551

552 **Assessment of reference sample sequencing coverage and genome heterogeneity**

553 We chose 26 replicates of HCC1395 and HCC1395BL data sets, which were libraries prepared
554 using the Ilumina TruSeq DNA PCR free (1000ng) protocol and sequenced on Illumina HiSeq
555 and NovaSeq. Each library was ranged from 50X to 100X genome coverage (**Figure 3a**). The
556 percentage of genome coverage with less than 5X is 0.9 – 7.7% (**Suppl. Figure 4a**). For 10X
557 Chromium libraries, each library has 45X - 120X genome coverage (**Figure 3b**), 6.4 – 7.3% of
558 genome regions have read coverage less than 5X (**Suppl. Figure 4b**). 10X Chromium linked

559 read technology produced input DNA molecule length in the range between 54 – 77kb. The
560 site-to-site variation was due to sequencing depth differences. For WES samples, the target
561 region has nearly 100% coverage by sequencing reads, however, we observed high variation
562 in the sequencing coverage within each replicate as well as among replicates (**Suppl. Figure**
563 **3c**).

564 In addition, we generated two PacBio libraries with 40X of genome coverage from subreads.
565 Long reads improve the map ability in repetitive genome regions where short-reads might
566 fail to map correctly. PacBio long-read sequencing may cover the genomic regions where
567 short reads cannot be mapped especially in the high GC/AT or low complexity genomic
568 regions (**Figure 3c**). However, its higher sequencing error rate than short-read sequencing
569 affects the accuracy for the low-frequency somatic mutation discovery. The variation in
570 genome coverage might be due to differences in sequencing technologies (**Figure 3d**). From
571 the study, short reads WGS has better uniform coverage compared to long reads. However,
572 there is better coverage for certain genomic regions in long-read technologies; most
573 noticeable are the highly repetitive regions, extreme GC regions, or around the centromere
574 regions.

575 The Indexcov[10] scaled read depth on reference genome for HCC1395 (**Suppl. Figure 4a**) and
576 HCC1395BL (**Suppl. Figure 4b**) showed HCC1395 harboring many Copy Number Variation
577 (gain or loss) events on every chromosome; HCC1395BL genome largely remains diploid
578 except for chr6 and chr16 and chrX. It showed loss of a chrX and a net loss of one copy of the
579 short-arm of chr6 and loss of one copy of the long-arm of chr16. Cytogenetic analysis with
580 Affymetrix Cytoscan HD microarray confirms the Cytogenetic view of HCC1395 which harbors
581 many copy numbers gains or losses; Cytogenetic view of HCC1395BL confirms the losses of
582 chr6p, chr16q, and chrX (NBT-RA46164).

583 For HCC1395 cell line, the tumor purity and ploidy estimated from Illumina WGS data set
584 (**Suppl. Figure 5a**) using PURPL[17] software showed the tumor purity is 99% and the ploidy is
585 around 2.85. Cell ploidy histogram from 10X Chromium single cell CNV data set (**Suppl.**
586 **Figure 5b**) displayed the vast majority of cells form a peak around ploidy 2.8. The analysis of
587 1270 cells for HCC1395 from 10X Single Cell CNV data set also revealed numerous
588 chromosome gains and losses events (**Suppl. Figure 5c**) consistently in sub-populations of
589 cells, which confirmed HCC1395 is a heterogeneous cell line.

590

591 **Assessment DNA Damage Artifacts**

592 A previous study has revealed that DNA damage accounts for the majority of the false calls
593 for the so-called low-frequency (1-5%) genetic variants in large public databases[8]. The DNA
594 damage directly confounds the determination of somatic variants in those data sets. The
595 Global Imbalance Value (GIV) score is commonly used to measure DNA damage based on an
596 imbalance between paired-end sequencing R1 and R2 variant frequency[8]. GIV scores to
597 capture the DNA damage due to the artifacts introduced during genomic library preparation,
598 the combination of heat, shearing, and contaminates can result in the 8-oxoguanine base
599 pairing with either cytosine or adenine, ultimately leading to G>T transversion mutations
600 during PCR amplification[18]. In addition, Formaldehyde also causes the deamination of
601 guanine. FFPE is known to cause G>T/C>A artifacts[19].

602 We calculated GIV score to monitor DNA damage in Illumina WGS and WES runs for both
603 fresh DNA libraries as well as FFPE libraries. We found lower GIV scores for the G>T/C>A
604 mutation pairs in fresh DNA WGS libraries (**Figure 4a)** than FFPE WGS libraries (**Figure 4b).** In
605 addition, both fresh cell DNA WES (**Figure 4c)** and FFPE WES Libraries (**Figure 4d)** all showed
606 increased GIV scores for the G>T/C>A mutation pairs relative to WGS libraries. The GIV for
607 G>T/C>A scores was inversely correlated with insert fragment sizes, and it is positively

608 correlated to DNA shearing time (**Suppl. Figure 5a/b/c**); WES libraries have consistently
609 shorter library insert sizes than all WGS library sizes (**Figure 2f)**. Thus, the GIV of G>T/C>A is a
610 good indicator of DNA damage introduced during genomic library preparation. We observe
611 the libraries have high G>T/C>A GIV scores also have a higher percentage of C/A mutation
612 called in WES from private mutation calls which are not shared among replicates as displayed
613 in **Suppl. Figure 5d**. Therefore, in order to improve cancer genomic variant call accuracy,
614 effective mitigation strategies to improve library preparation methods, or software tools to
615 detect and remove the DNA damage mutation calls are essential.

616
617
618 **Assessment reproducibility of somatic mutation calling from WES and WGS data sets**
619 To assess the concordance and reproducibility of the somatic variant detection with both
620 WES and WGS, we compared 12 replicates of WGS and WES for the matched tumor and
621 normal cell lines carried out at six sequencing centers. Using three mutation callers
622 (MuTect2[12], Strelka2[13], and SomaticSniper[14]) on alignments from three aligners (Bowtie2[15] ,
623 BWA MEM[3], and NovoAlign), we generated a total of 108 variant call files separately. We
624 were able to assess inter- and intra-centers reproducibility of the WES and WGS using the 12
625 repeat runs. The Venn diagram is widely used to display concordance of mutation calling
626 results from a small number of repeated analyses; however, this type of diagram is not
627 suitable for large data sets. To address this challenge, we applied the "tornado" plot to
628 visualize the consistency of mutation calls. The number of SNVs unique to one single VCF file
629 are represented by the width of the tornado at the bottom, and the number of SNVs called
630 in all VCF files are represented by the width at the top. Thus, like the actual meteorological
631 event, a tornado that is touching down is "bad" (many called variants are likely false
632 positives), and a tornado with the majority of the data at the top is "better" (many common
633 variants called across all conditions). As shown at the top of each plot (**Figure 5a**), we
634 observed relatively more library-specific variants at the bottom of the WES tornado plots
635 (bottom of tornado). In contrast, majority of called mutations (top of tornado) were shared
636 across all 12 WGS (**Figure 5b**). Therefore, calling results from WES tended to have more
637 inconsistent SNV calls (bottom of tornado) than those from WGS, indicating that WES results
638 were less consistent than WGS results (**Figure 5a/b**). Here we also introduced the O_Score, a
639 metric to measure reproducibility of repeated analyses (see Methods). O_Scores for WES
640 runs were not only significantly lower than WGS runs, but also more variable (**Suppl. Figure**
641 **7a**)**.** In addition, we measured reproducibility between replicates of WGS runs from both
642 NovaSeq and HiSeq platforms to assess cross-platform variation. Both platforms were
643 remarkably similar in terms of reproducibility, indicating that results from HiSeq and
644 NovaSeq are comparable (**Suppl. Figure 7b**). Overall, we observed the cross-center and
645 cross-platform variations for WGS were very small, indicating that all individual NGS runs,
646 regardless of sequencing centers or NGS platforms, detected most "true" mutations
647 consistently for WGS runs**.**
648
649 We also computed SNVs/indels calling concordance between WES and WGS from twelve
650 repeated runs. For direct comparison, SNVs/indels from WGS runs were limited to genomic
651 regions defined by an exome capturing protocol (SureSelect V6+UTR). WGS has a smaller
652 number of private calls for each sample than WES **(Figure 5c).** We observed the overlap
653 between the WES and WGS improved as sequencing depth increased. Moreover, the
654 correlation of MAF in overlapping WGS and WES SNVs/indels from repeated runs are
655 positively correlated with higher sequencing depth **(Figure 5d).** This indicates the benefit of
656 high read coverage not only improves the detection sensitivity of mutations with low MAF,
657 but also increases reproducibility of the calling sets. Overall, our results indicate the inter-
658 center variations for WES were larger than inter-center variations for WGS, whereas the
659 difference between intra-center variation between WES and WGS was not significant. As a

result, WGS had much less inter-center variation and thus provided better reproducibility than WES for cancer genomic variants detection.

Code Availability

All code used in processing the whole genome and exome-seq data are available on GitHub at the following link:
https://github.com/abcsFrederick/NGS_Preprocessing_Pipeline

Acknowledgements

The authors would like to thank Drs David Goldstein and Mariam Malik of the Office of Technology and Science at National Cancer Institute (NCI), National Institutes of Health (NIH), for the sponsorship and the usage of the NIH Biowulf cluster and support for this study; Dr. Jack Collins of the Advanced Biomedical and Computational Sciences, and Dr. Eric Stahlberg of Biomedical Informatics and Data Science Directorate at Frederick National Laboratory for Cancer Research for reviewing manuscript and providing suggestions; Seven Bridges Genomics for providing storage and computational support on the Cancer Genomic Cloud (CGC); this work also used the computational resources of Frederick Research Computing Environment (FRCE) at Frederick National Laboratory for Cancer Research. The authors sincerely thank members at NCI Sequencing Facility at Frederick National Laboratory for Cancer Research for their sequencing support for this study. This project has been funded in part with Federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261201800001I. The genomic work carried out at the Loma Linda University (LLU) Center for Genomics was funded in part by the NIH grant (S10OD019960), the Ardmore Institute of Health (AIH) grant (2150141), the American Heart Association (AHA) grant ((18IPA34170301), and Dr. Charles A. Sims' gift to LLU Center for Genomics. Drs. L. Shi and Y. Zheng were supported by the National Natural Science Foundation of China (31720103909), the National Key R&D Project of China (2018YFE0201600), and Shanghai Municipal Science and Technology Major Project (2017SHZDZX01). The work carried out at Uppsala University was supported by grants from the Swedish Research Council (2017-00630, 2019-01976) and the Knut and Alice Wallenberg Foundation. Ene Reimann was supported by the European Union through the European Regional Development Fund (Project No. 2014-2020.4.01.15-0012).

Disclaimer

This is a research study, not intended to guide clinical applications. The views presented in this article do not necessarily reflect current or future opinion or policy of the US Food and Drug Administration. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services. Any mention of commercial products is for clarification and not intended as endorsement.

Author contributions

WX and YZ conceived and designed the study. YZ and WX drafted the manuscript. YZ, WX, LTF, CW, JN, UL and DM edited the manuscript. BT, JS, YK, CW, EJ, CL, KI, YTZ, LS, VP, MS, TH, EP, JD, PV, RM, DG, SK, ER, AS, JN, UL, ZC, and WC performed library construction and

sequencing. YZ, XW, LTF, BZ, ZS, SC, KT and XFC performed bioinformatics data analyses. YZ and WX managed the project. All authors reviewed the manuscript. YZ finalized and submitted the manuscript.

Competing interests

Li Tai Fang is employee of Roche Sequencing Solutions Inc. Erich Jaeger is employee of Illumina Inc. Virginie Petitjean and Marc Sultan are employees of Novartis Institutes for Biomedical Research. Tiffany Hung and Eric Peters are employees of Genentech (a member of the Roche group). All other authors claim no conflicts of interest.

Figures

Figure 1. Study design for the experiment.
Figure 2. Overall data quality for WGS and WES data sets from Illumina platform
Figure 3. Genome coverage from WGS data from three technologies including Illumina, PacBio, and 10X Genomics.
Figure 4. Evaluation of DNA damage for WGS and WES libraries
Figure 5. Reproducibility of somatic mutation calling from WES and WGS data sets

Figure Legends

Figure 1. Study design for the experiment. DNA was extracted from either fresh cells or FFPE processed cells. Both fresh DNA and FFPE DNA were profiled on WGS and WES platforms for intra-center, inter-center and cross-platform reproducibility benchmarking. For fresh DNA, six centers performed WGS and WES in parallel following manufacture recommended protocols with limited deviation. Three library preparation protocols (TruSeq-Nano, Nextera Flex, and TruSeq PCR-free,) were used with four different quantities of DNA inputs (1, 10, 100, and 250 ng). DNA from HCC1395 and HCC1395BL was pooled at various ratios to create mixtures of 75%, 50%, 20%, 10%, and 5%. For FFPE samples, each fixation time point (1hm 2h, 6h, 24h) had six blocks that were sequenced at two different centers. All libraries from these experiments were sequenced on the HiSeq series. In addition, nine libraries using the TruSeq PCR-free preparation were run on a NovaSeq for WGS analysis.

Figure 2. Overall data quality for WGS and WES data sets from Illumina platform. **(a)** Percentage of total reads mapped to reference genome (hg38) for WGS (Green) and WES (Red) across 6 sequencing sites. **(b)** Mean coverage depth for WGS libraries across 6 sequencing sites. (**c)** Mean coverage depth in target capture regions for WES libraries across 6 sequencing sites. **(d)** Percentage of non-duplicated reads mapped to reference genome across 6 sequencing sites. WGS (Green) and WES (Red). (**e)** Percent GC content from different library prep protocols. WGS (Green) and WES (Red). (**f)** Mean insert size distribution from different library prep protocols. WGS (Green) and WES (Red).

Figure 3. Genome coverage from WGS data from three technologies including Illumina, PacBio, and 10X Genomics. Outer rainbow color track: chromosomes, red track: HCC1395, green track: HCC1395BL. **(a)** Genome coverage from WGS data by reads from Illumina platform. **(b)** Genome coverage from WGS data by reads from 10X Chromium linked-read technology **(c)** Genome coverage from WGS data by reads from PacBio platform. **(d)** Genome coverage from WGS data by reads from 3 platforms together. Inner track: PacBio. Middle track: 10X Genomics. Outer track: Illumina.

Figure 4. Evaluation of DNA damage for WGS and WES libraries. using GIV scores to capture the DNA damage due to the artifacts introduced during genomic library preparation. The estimation of damage is a global estimation based in an imbalance between R1 and R2 variant frequency. GIV score above 1.5 is defined as damaged. Undamaged DNA samples have a GIV score of 1. **(a)** DNA damage estimated for fresh cell prepared DNA for WGS Illumina libraries across different sites. **(b)** DNA damage estimated for FFPE WGS Illumina libraries. **(c)** DNA damage estimated for fresh cells prepared DNA for WES Illumina libraries across different sites **(d)** DNA damage estimated for FFPE WES Illumina libraries.

Figure 5. Reproducibility of somatic mutation calling from WES and WGS. The reproducibility "Tornado" plots for 12 repeated WES **(a)** and WGS runs **(b)**. The number in each plot represents the O_Score reproducibility measurement. **(c)** SNVs/indels calling concordance between WES and WGS from twelve repeated runs. For direct comparison, SNVs/indels from WGS runs were limited to genomic regions defined by an exome capturing kit (SureSelect V6+UTR). WES is shown on the left in the Venn diagram and WGS is on the right. Shown coverage depths for WES and WGS were effective mean sequence coverage on exome region, i.e. coverage by total number of mapped reads after trimming. **(d)** Correlation of MAF in overlapping WGS and WES SNVs/indels from repeated runs.

809
810 ## Tables
811 **Table 1**: Summary of all experiment data including WGS, WES discovery and validation data
812 sets used in the study.
813

Study Design	Technology	Platform	Sequecing	Number of Reads (coverage)	
				HCC1395	HCC1395BL
Discovery	WGS (Fresh DNA)	HiSeq	6 centers (24 libraries)	21 billion (750X)	21 billion (750X)
		NovaSeq	1 center (18 libraries)	13 billion (400X)	13 billion (400X)
	WES (Fresh DNA)	HiSeq	6 centers (24 libraries)	3 billion (2,500X)	3 billion (2,500X)
Validation	WGS Protocols	HiSeq	2 center (14 libraries)	9 billion (315X)	9 billion (315X)
	WGS Tumor Content	HiSeq	1 center (21 libraries)	64 billion (Mixture of samples, total 2300X)	
	WGS FFPE	HiSeq	2 center (26 libraries)	30 billion (980X)	27 billion (900X)
	WGS 10x Linked-Read	10X Genomics	5 centers (22 libraries)	20 billion (880X)	20 billion (880X)
	WGS PacBio	PacBio	1 center (2 libraries)	19 million (40X)	22 million (44X)
	WES FFPE	HiSeq	2 centers (17 libraries)	3 billion (2600X)	4 billion (3600X)
	Targeted Amplicon	Ion Torrent	1 centers (2 libraries)	67 million (34X)	82 million (47X)
	AmpliSeq	MiSeq	1 center (2 libraries)	25 million (2900x)	22 million (3300x)
	Microarray	AffyChip CytoScan HD	1 center (2 libraries)	2.1 million probes	
	Single Cell CNV	HiSeq	1 center (2 libraries)	1.5 billion (1465 cells)	1.3 billion (983 cells)

814
815
816 For the QC statistics for each data set, please reference the online-only supplementary
817 Table1 -10 for details.
818
819
820
821
822
823
824
825

References

1. Morash M, Mitchell H, Beltran H, Elemento O, Pathak J. The Role of Next-Generation Sequencing in Precision Medicine: A Review of Outcomes in Oncology. J Pers Med. 2018;8(3):30. Published 2018 Sep 17. doi:10.3390/jpm8030030

2. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics **30**, 2114–2120 (2014).

3. Li, H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997 [q-bio] (2013).

4. Babraham Bioinformatics - FastQC A Quality Control tool for High Throughput Sequence Data. Available at: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/. (Accessed: 4th September 2018)

5. Picard Tools - By Broad Institute. Available at: http://broadinstitute.github.io/picard/. (Accessed: 23rd December 2017)

6. Okonechnikov, K., Conesa, A. & García-Alcalde, F. Qualimap 2: advanced multi-sample quality control for high-throughput sequencing data. Bioinformatics **32**, 292–294 (2016).

7. Ewels, P. MultiQC: Aggregate results from bioinformatics analysis across many samples into a single report. Bioinformatics **32(19)**, 3047–8 (2016).

8. Chen, L., Liu, P., Evans, T. C. & Ettwiller, L. M. DNA damage is a pervasive cause of sequencing errors, directly confounding variant identification. Science **355**, 752–756 (2017).

9. Heng Li, Minimap2: pairwise alignment for nucleotide sequences, Bioinformatics, Volume 34, 18, 15 September 2018, Pages 3094-3100, https://doi.org/10.1093/bioinformatics/bty191

10. Pedersen, B. et al. Indexcov: fast coverage quality control control for whole-genome sequencing, GigaScience, 6, 2017, 1-6 doi: 10.1093/gigascience/gix090

11. Bishara, A., Liu, Y., Weng, Z., Kashef-Haghighi, D., Newburger, D. E., West, R., Sidow, A., & Batzoglou, S. (2015). Read clouds uncover variation in complex regions of the human genome. Genome research, 25(10), 1570–1580. https://doi.org/10.1101/gr.191189.115

12. Cibulskis, K., Lawrence, M., Carter, S. et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. Nat Biotechnol **31,** 213–219 (2013). https://doi.org/10.1038/nbt.2514

13. Saunders, C. T. et al. Strelka: accurate somatic small-variant calling from sequenced tumor–normal sample pairs. Bioinformatics **28**, 1811–1817 (2012).

14. Larson, D. E. et al. SomaticSniper: identification of somatic point mutations in whole genome sequencing data. Bioinformatics **28**, 311–317 (2012).

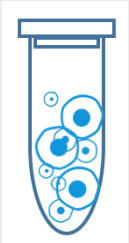
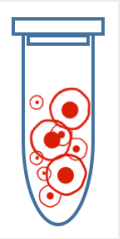
15. Langmead, B., Trapnell, C., Pop, M. & Salzberg, S. L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. **10**, R25 (2009).

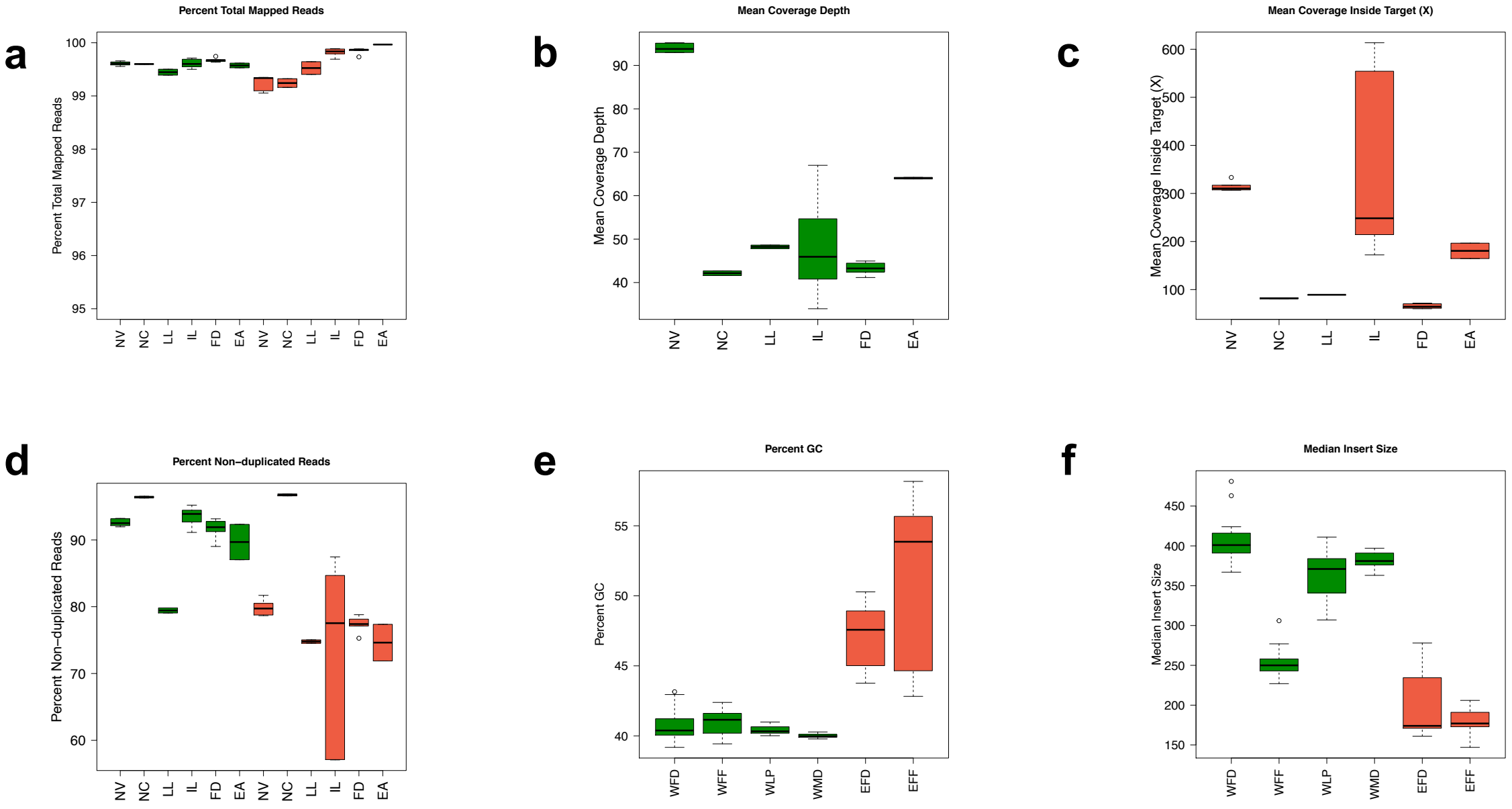
16. Flensburg, C, Sargeant, T, Oshlack, A, Majewski, IJ (2020) SuperFreq: Integrated mutation detection and clonal tracking in cancer. PLOS Computational Biology 16(2): e1007603. https://doi.org/10.1371/journal.pcbi.1007603

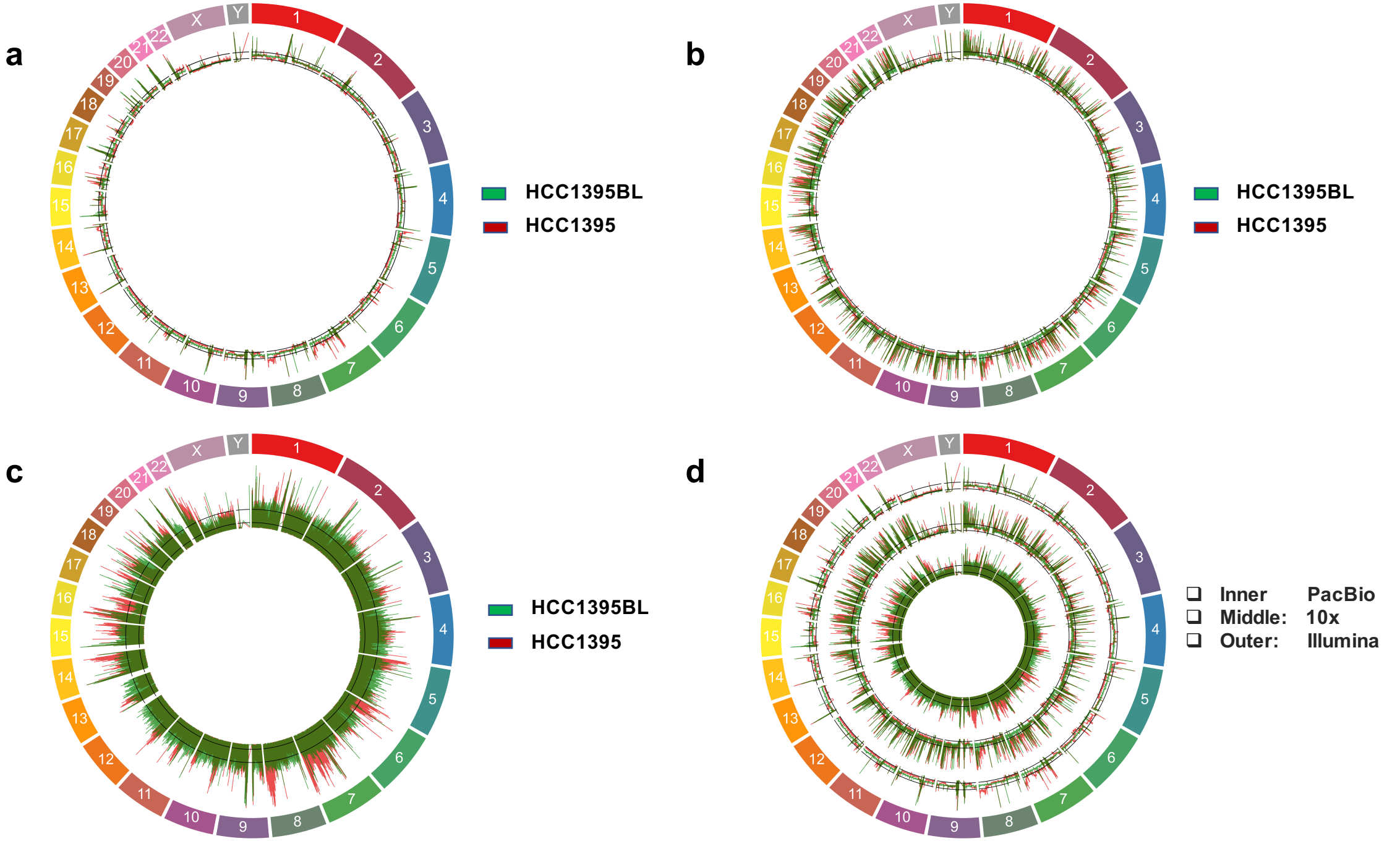
17. Cameron,D., Baber, J., Shale, C., Papenfuss, A., Valle-Inclan, J., Besselink, M., Priestley, C. GRIDSS, PURPLE, LINX: Unscrambling the tumor genome via integrated analysis of structural variation and copy number, bioRxiv 781013; doi: https://doi.org/10.1101/781013

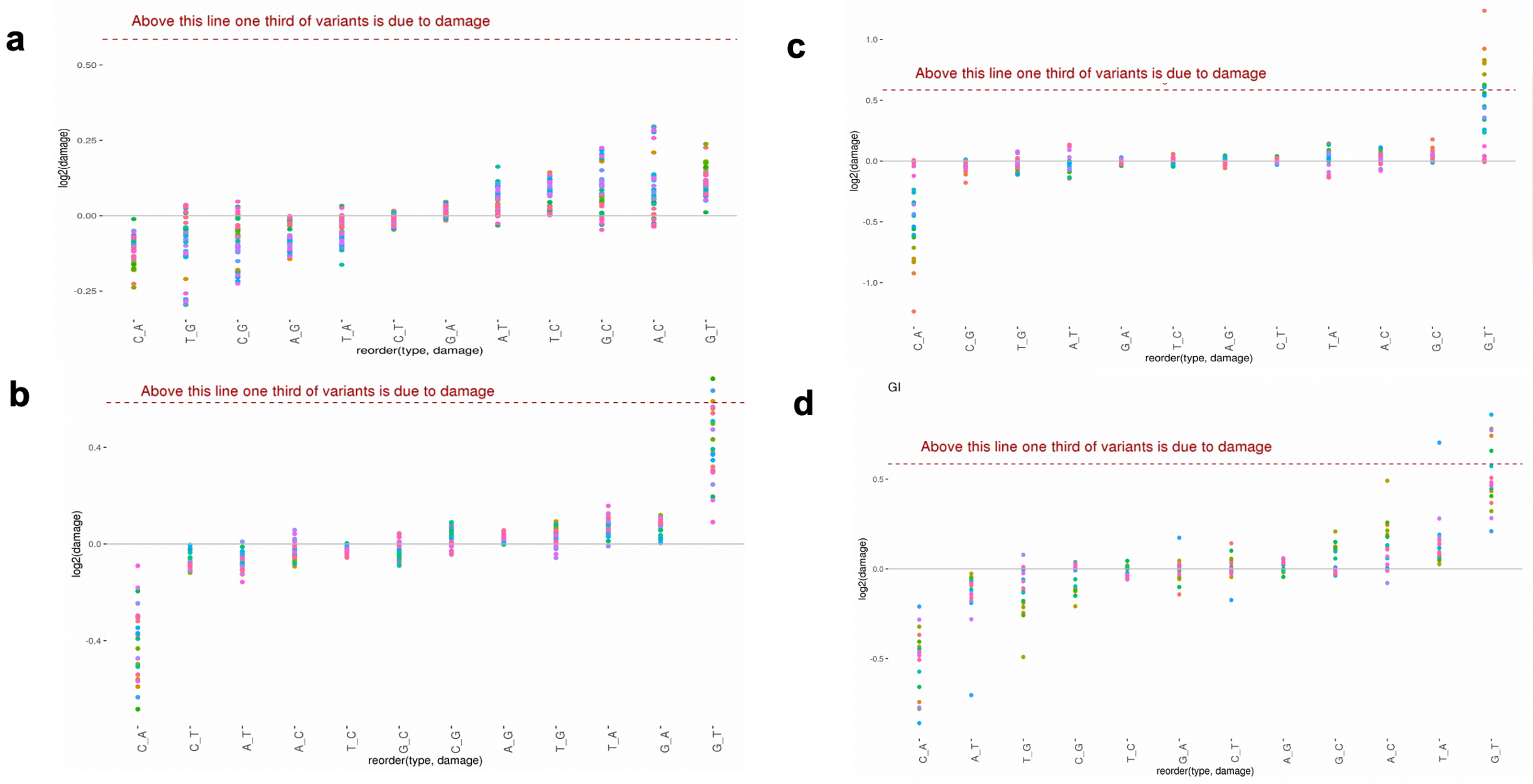
18. Costello, M. et al. Discovery and characterization of artifactual mutations in deep coverage targeted capture sequencing data due to oxidative DNA damage during sample preparation. Nucleic Acids Res **41**, e67 (2013).

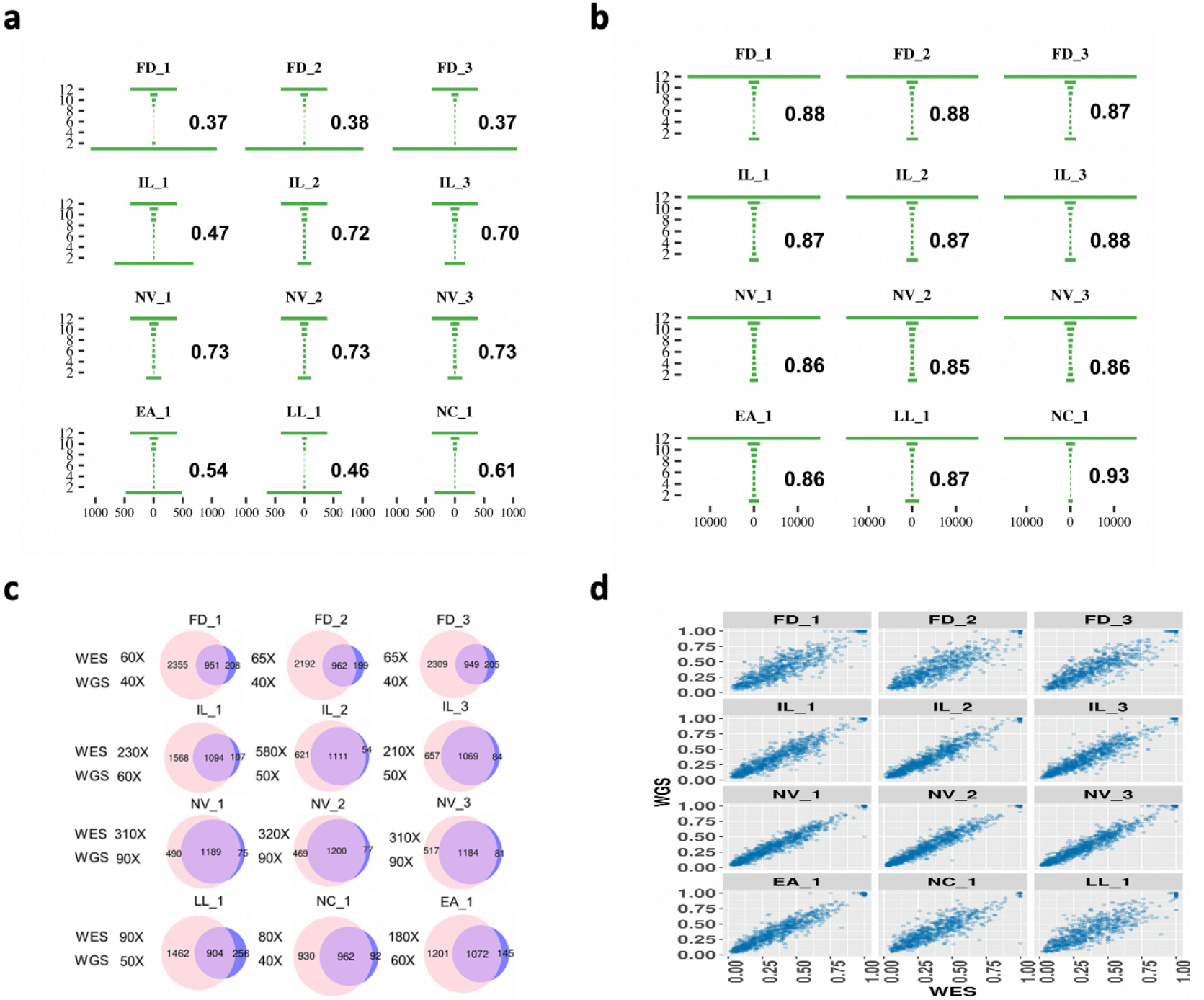
19. Do, H. & Dobrovic, A. Sequence Artifacts in DNA from Formalin-Fixed Tissues: Causes and Strategies for Minimization. Clinical Chemistry **61 (1)**, 64–71 (2015).

Reference sample	Sample types	NGS platform	Experiments
 Normal cell line HCC1395BL	Fresh DNA	WGS • Hi Seq • NovaSeq • PacBio • 10X Genomics WES • HiSeq • Ion Torrent AmpliSeq • MiSeq Microarray • AffyChip CytoScan HD	Reproducibility: • Intra-center • Inter-center • Cross-platform Library preparation: • Library kit • DNA input amount Validation • Confirmation • Specificity • Sensitivity
 Tumor cell line HCC1395	FFPE DNA Mixture DNA	WGS • HiSeq WES • HiSeq	FFPE process: • Fixing time: 1h, 2h, 6h, 24h • DNA damage Tumor purity: • 75%, 50%, 20%, 10%, 5%
	Fresh cells	scCNV • 10X Genomics	Number of cells: • HCC1395BL: 983 • HCC1395: 1465


a
Percent Total Mapped Reads
Percent Total Mapped Reads
100
99
98
97
96
95
NV NC LL IL FD EA NV NC LL IL FD EA
b
Mean Coverage Depth
Mean Coverage Depth
90
80
70
60
50
40
NV NC LL IL FD EA
c
Mean Coverage Inside Target (X)
Mean Coverage Inside Target (X)
600
500
400
300
200
100
NV NC LL IL FD EA
d
Percent Non−duplicated Reads
Percent Non−duplicated Reads
90
80
70
60
NV NC LL IL FD EA NV NC LL IL FD EA
e
Percent GC
Percent GC
55
50
45
40
WFD WFF WLP WMD EFD EFF
f
Median Insert Size
Median Insert Size
450
400
350
300
250
200
150
WFD WFF WLP WMD EFD EFF


a
b
c
d
HCC1395BL
HCC1395
Inner PacBio
Middle: 10x
Outer: Illumina


a
Above this line one third of variants is due to damage
log2(damage)
0.50
0.25
0.00
-0.25
C_A
T_G
C_G
A_G
T_A
C_T
G_A
A_T
T_C
G_C
A_C
G_T
reorder(type, damage)
b
Above this line one third of variants is due to damage
log2(damage)
0.4
0.0
-0.4
C_A
C_T
A_T
A_C
T_C
G_C
C_G
A_G
T_G
T_A
G_A
G_T
reorder(type, damage)
c
Above this line one third of variants is due to damage
log2(damage)
1.0
0.5
0.0
-0.5
-1.0
C_A
C_G
T_G
A_T
G_A
T_C
A_G
C_T
T_A
A_C
G_C
G_T
reorder(type, damage)
d
GI
Above this line one third of variants is due to damage
log2(damage)
0.5
0.0
-0.5
C_A
A_T
T_G
C_G
T_C
G_A
C_T
A_G
G_C
A_C
T_A
G_T
reorder(type, damage)


a
FD_1
FD_2
FD_3
0.37
0.38
0.37
IL_1
IL_2
IL_3
0.47
0.72
0.70
NV_1
NV_2
NV_3
0.73
0.73
0.73
EA_1
LL_1
NC_1
0.54
0.46
0.61
b
0.88
0.88
0.87
0.87
0.87
0.88
0.86
0.85
0.86
0.86
0.87
0.93
c
WES 60X
WGS 40X
2355 951 208
65X
40X
2192 962 199
65X
40X
2309 949 205
WES 230X
WGS 60X
1568 1094 107
580X
50X
621 1111 54
210X
50X
657 1069 84
WES 310X
WGS 90X
490 1189 75
320X
90X
469 1200 77
310X
90X
517 1184 81
WES 90X
WGS 50X
1462 904 256
80X
40X
930 962 92
180X
60X
1201 1072 145
d
WGS
WES