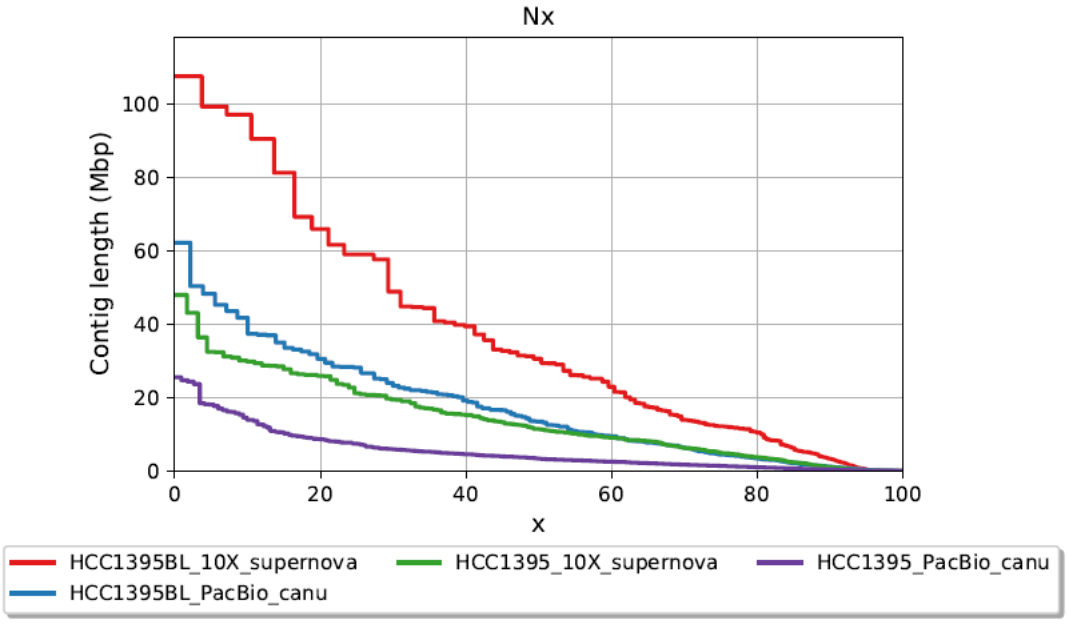
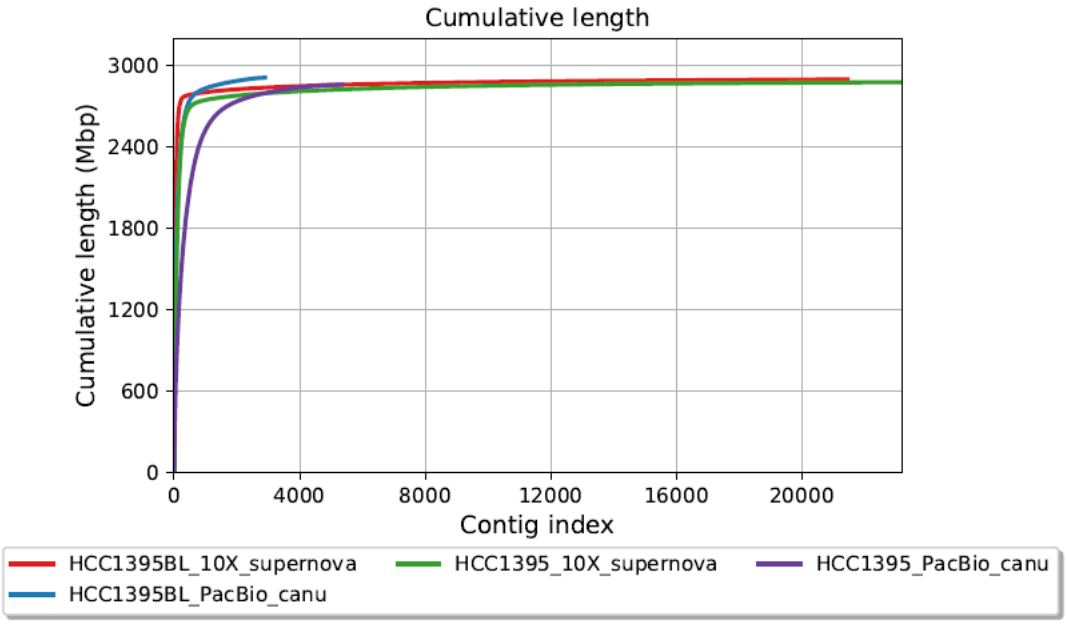
Supplementary materials

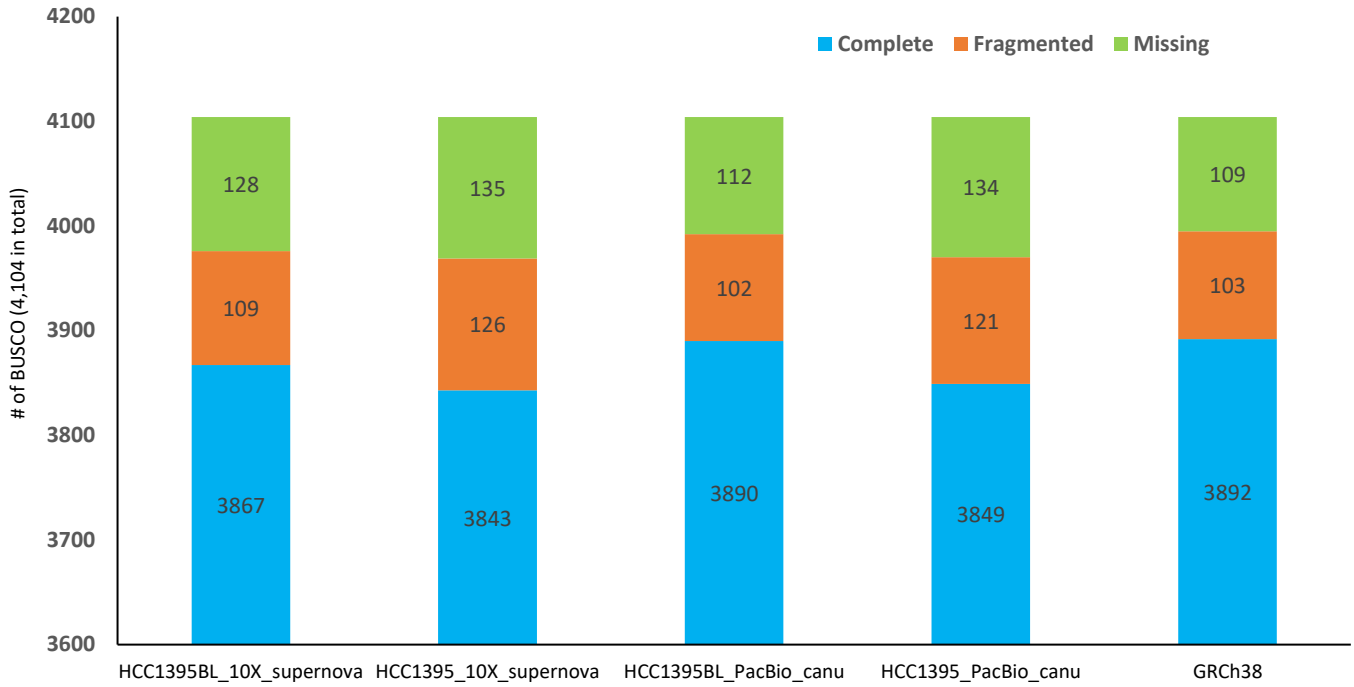
Supplementary Figures



Supplementary Figure 1. Nx plot of the assemblies from PacBio long reads using canu and 10X Genomics linked reads using supernova.

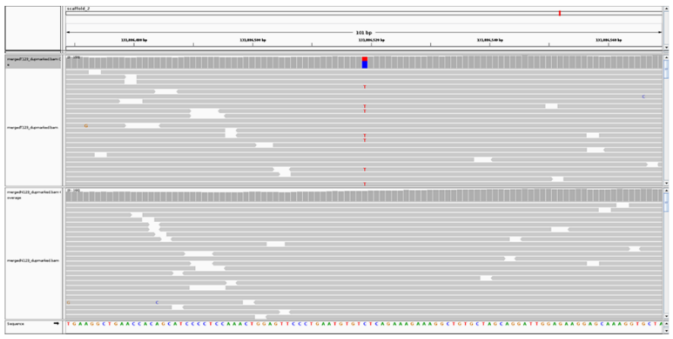


Supplementary Figure 2. Cumulative length plot of the assemblies from PacBio long reads using canu and 10X Genomics linked reads using supernova.

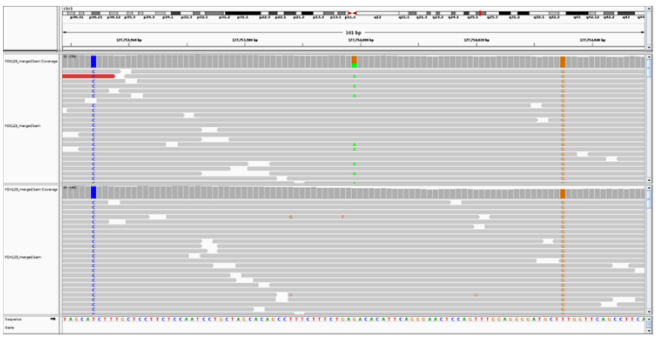


Supplementary Figure 3. BUSCO assessment of the assemblies from PacBio long reads using canu and 10X Genomics linked reads using supernova. GRCh38 (primary assembly) was listed here for comparison purposes.

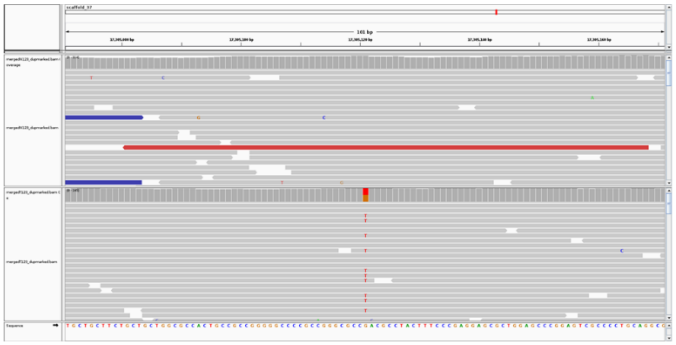
A. Intergenic SNV scaffod_2:131886519 on HCC1395BL_v1.0 assembly.



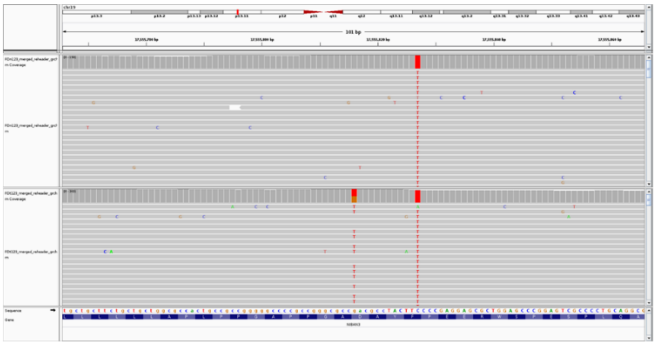
B. Intergenic SNV chr1:177753999 on GRCh38 with mismatches in flanking.



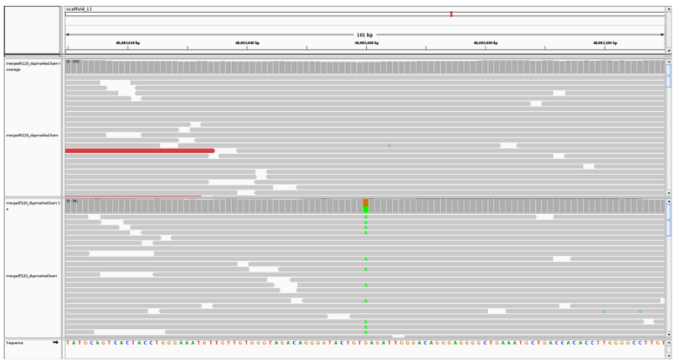
C. Exonic SNV scaffold_37: 17305121 on HCC1395BL_v1.0 assembly.



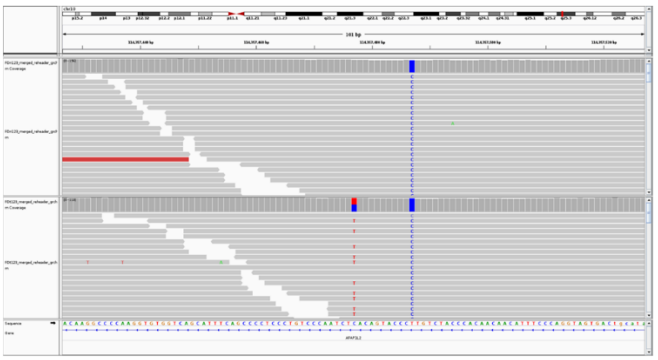
D. Exonic SNV chr19:17555816 on GRCh38 with mismatches in flanking.



E. Intronic SNV scaffold_12:48083060 on HCC1395BL_v1.0 assembly.



F. Intronic SNV chr10_114357477 on GRCh38 with mismatches in flanking.



G. BLAST alignment example of reference allele and alternate allele from SNV chr1:177753999 mapped onto de novo assembly.

```
Query= chr1_177753999_ref

Length=101

                                                                      Score     E
Sequences producing significant alignments:                          (Bits)  Value

scaffold_2                                                            176     2e-42


>scaffold_2
Length=159912780

 Score = 176 bits (95),  Expect = 2e-42
 Identities = 99/101 (98%), Gaps = 0/101 (0%)
 Strand=Plus/Minus

Query  1          TAGCATCTTTGCTCCTTCTCCAATCCTGCTAGCACAGCCTTTCTTTCTGAGACACATTCA  60
                  ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  131886569  TAGCACCTTTGCTCCTTCTCCAATCCTGCTAGCACAGCCTTTCTTTCTGAGACACATTCA  131886510

Query  61         GGGAACTCCAGTTTGGAGGGGATGCTTTGGTTCAGCCTTCA  101
                  |||||||||||||||||||||||| |||||||||||||
Sbjct  131886509  GGGAACTCCAGTTTGGAGGGGATGCTGTGGTTCAGCCTTCA  131886469


Query= chr1_177753999_alt

Length=101

                                                                      Score     E
Sequences producing significant alignments:                          (Bits)  Value

scaffold_2                                                            171     7e-41


>scaffold_2
Length=159912780

 Score = 171 bits (92),  Expect = 7e-41
 Identities = 98/101 (97%), Gaps = 0/101 (0%)
 Strand=Plus/Minus

Query  1          TAGCATCTTTGCTCCTTCTCCAATCCTGCTAGCACAGCCTTTCTTTCTGAAACACATTCA  60
                  ||||| ||||||||||||||||||||||||||||||||||||||||| |||||||||
Sbjct  131886569  TAGCACCTTTGCTCCTTCTCCAATCCTGCTAGCACAGCCTTTCTTTCTGAGACACATTCA  131886510

Query  61         GGGAACTCCAGTTTGGAGGGGATGCTTTGGTTCAGCCTTCA  101
                  |||||||||||||||||||||||| |||||||||||||
Sbjct  131886509  GGGAACTCCAGTTTGGAGGGGATGCTGTGGTTCAGCCTTCA  131886469
```

Supplementary Figure 4. Illustration of GRCh38 SNV mapping to *de novo* assembly with discrepancies.
(A) IGV snapshot for an intergenic SNV scaffold_2:131886519, the alleles were reverse-complement mapped on *de novo* assembly.
(B) IGV snapshot for SNV chr1:177753999 on GRCh38 with mismatches in flanking sequences. The same set of reads (266 reads from HCC1395BL sample, and 278 reads from HCC1395 sample) were found to align (with mapping quality 60) crossing the corresponding SNV regions (scaffold_2:131886469-131886569 for SNV scaffold_2:131886519 on HCC1395BL_v1.0; chr1:177753949-177754049 for SNV chr1:177753999 on GRCh38).
(C) IGV snapshot for an exonic SNV scaffold_37: 17305121 on HCC1395BL_v1.0 assembly.
(D) IGV snapshot for an exonic SNV chr19:17555816 on GRCh38 with mismatches in flanking. This somatic SNV causes amino acid change (Asp -> Tyr for GAC -> TAC) in gene COLGALT1 on chr19.
(E) IGV snapshot for an intronic SNV scaffold_12:48083060 on HCC1395BL_v1.0 assembly; (F) IGV snapshot for an intronic SNV chr10_114357477 on GRCh38 with mismatches in flanking. This somatic SNV is located in the intronic region of the gene AFAP1L2 on chr10.
(G) BLAST alignment example of reference allele and alternate allele from SNV chr1:177753999 mapped onto de novo assembly with reference allele 98% identity and 101 bps alignment for reference allele, and 97% identity and 101 bps alignment for alternate allele.

Supplementary Tables

Supplementary Table 1. Whole genome sequencing data of HCC1395 and HCC1395BL by multiple sequencing platforms used in this study.

Dataset	Sequencing center	Usage	HCC1395		HCC1395BL	
			# Reads	Total base pairs (Depths)	# Reads	Total base pairs (Depths)
Illumina HiSeq [ref 46-47]	Fudan (FD)	Polishing, unitig assembly, variant calling	3,403,236,080	510,485,412,000 (170.16x)	3,511,999,736	526,799,960,400 (175.59x)
10X Genomics Chromium [ref 46-47]	Fudan (FD)	Assembly; Scaffolding	3,206,110,814	480,916,622,100 (160.30x)	3,222,458,614	483,368,792,100 (161.12x)
PacBio Sequel [ref 46-47]	CSHL	Primary Contig assembly, phasing, variant calling	17,265,341	140,649,790,223 (46.88x)	17,671,082	160,626,774,754 (53.54x)
Hi-C	Dovetail	Scaffolding	NA	NA	1,432,923,164	214,938,474,600 (71.64x)
Oxford Nanopore	Loma Linda University	Phasing	NA	NA	11,585,856	47,006,385,877 (15.66x)

Supplementary Table 2: Summary of the *de novo* assemblies for the two cell lines using PacBio long reads and 10X Genomics linked reads.

	HCC1395BL (10X_supernova)	**HCC1395 (10X_supernova)**	**HCC1395BL (PacBio_canu)**	**HCC1395 (PacBio_canu)**
# contigs (>= 0 bp)	21,450	23,195	2,900	5,351
Total length (>= 0 bp)	2,893,054,450	2,871,887,885	2,905,219,353	2,853,495,799
# contigs (>= 10,000 bp)	3,416	4,666	2,828	4,949
Total length (>= 10,000 bp)	2,837,767,582	2,812,618,703	2,904,842,414	2,851,315,749
Largest contig	107,564,373	47,962,721	62,208,403	25,523,694
GC (%)	40.89	40.96	40.91	40.95
N50	30,593,498	11,455,133	13,480,407	3,338,913
L50	27	70	57	210
# contigs (>= 10,000 bp) matched GRCh38	3,176	4,449	2,526	4,627
# bps of contig (>= 10,000 bp) matched GRCh38	2,833,045,730	2,808,106,025	2,888,438,712	2,836,167,093
# cumulative bps matches on GRCh38	2,787,709,216	2,754,283,911	2,867,274,440	2,820,906,016
# novel contigs (>= 10,000 bp)	240	217	302	322

# bps of novel contigs (>= 10,000 bp)	4,721,852	4,512,678	16,403,702	15,148,656
RefSeq NMs mapped (95+% alignment covered)	48,061 (96.02%)	47,128 (94.15%)	49,287 (98.41%)	47,619 (95.13%)
RefSeq NRs mapped (95+% alignment covered)	14,423 (92.78%)	14,165 (91.12%)	15,115 (97.24%)	14,717 (94.67%)

Supplementary Table 3. The summary of genome phasing using WhatsHap with PacBio reads alone and reads from combinations of PacBio and Oxford Nanopore (ONT).

	PacBio reads	PacBio + ONT reads
# Blocks	6,368	3,204
Sum block length (bps)	2,421,024,137	2,543,961,355
Longest block (bps)	6,378,479	20,455,447
# Phased	3,134,631	3,135,816
# Hets in VCF	3,172,233	3,172,233

Supplementary Table 4. Summary of RefSeq genes and transcripts mapping onto HCC1395BL_v1.0 assembly. Genes from ChrY and all pseudogenes were excluded from this analysis. For comparison, the same set of RefSeq sequences were mapped onto GRCh38.

	# RefSeq NM	# genes for RefSeq NM	# RefSeq NR	# genes for RefSeq NR
# Input (excluding chrY, and all pseudo genes)	49,844	19,325	14,132	10,061
# Found on HCC1395BL_v1.0	49,838	19,322	14,131	10,060
# Not Found on HCC1395BL_v1.0	6	3	1	1
# Found 50%+ coverage & 95%+ identity on HCC1395BL_v1.0	49,798 (99.91%)	19,303 (99.89%)	14,120 (99.92%)	10,049 (99.88%)
# Found 95%+ coverage & 95%+ identity on HCC1395BL_v1.0	49,482 (99.27%)	19,164 (99.17%)	13,978 (98.91%)	9,958 (98.98%)
# Found 50%+ coverage & 95%+ identity on GRCh38	49,825 (99.96%)	19,318 (99.96%)	14,128 (99.97%)	10,058 (99.97%)
# Found 95%+ coverage & 95%+ identity on GRCh38	49,669 (99.65%)	19,232 (99.52%)	14,044 (99.37%)	10,002 (99.41%)

Supplementary Table 5. Summary of Illumina short read mappings on HCC1395BL_v1.0 assembly as opposed to GRCh38.

	HCC1395BL cell line		HCC1395 cell line	
	GRCh38 as reference	HCC1395BL_v1.0 as reference	GRCh38 as reference	HCC1395BL_v1.0 as reference
# Reads with proper-pairing	3,453,531,324	3,469,571,424	3,348,661,428	3,362,223,188
# Reads with improper-pairing	45,094,500	28,819,322	42,837,478	28,403,442
# Mismatches (from BAM NM tag)	3,287,384,223	2,736,108,247	3,034,142,098	2,545,029,100
# Reads with soft-clipping	134,583,157	116,428,804	129,114,705	113,160,104
# Reads with hard-clipping	14,076,941	9,704,136	13,634,238	9,820,328
# Reads mapped/paired	3,498,625,824	3,498,390,746	3,391,498,906	3,390,626,630

Supplementary Table 6. Summary of somatic SNVs/Indels detected by Strelka2 and MuTect2 on GRCh38 and HCC1395BL assembly, respectively.

	HCC1395BL_v1.0 as reference			**GRCh38 as reference**		
	Strelka2	MuTect2	Strelka2 / MuTect2 intersection	Strelka2	MuTect2	Strelka2 / MuTect2 intersection
# SNVs	57,314	52,137	43,285	46,742	49,676	41,669
# Indels (1~20 bps)	3,463	5,224	2,094	2,455	4,373	1,727
# Total	60,777	57,361	45,379	49,197	54,049	43,396

Supplementary Table 7. Summary of mapping 41,669 GRCh38-based SNVs (intersection of Strelka2/MuTect2 SNVs) to *de novo* assembly HCC1395BL_v1.0, and their function annotation using ANNOVAR.

	Mapped on de novo assembly WITH overlapping Strelka2/MuTect2 SNVs		Mapped on de novo assembly WITHOUT overlapping Strelka2/MuTect2 SNVs	Not-mapped on *de novo* assembly	Total
	No mismatch in flanking	Mismatch in flanking			
UTR3	363	28	2	1	394
UTR5	107	2	1	1	111
downstream	323	36	7	2	368
exonic	442	28	5	2	477
intergenic	17698	2112	480	135	20425
intronic	12726	1297	115	56	14194
ncRNA_exonic	264	24	3	2	293
ncRNA_intronic	4479	432	62	19	4992
upstream	360	34	7	1	402
splicing	11	2	0	0	13
Total # SNVs	**36,773 (88.25%)**	**3,995 (9.59%)**	**682 (1.64%)**	**219 (0.52%)**	**41,669 (100%)**