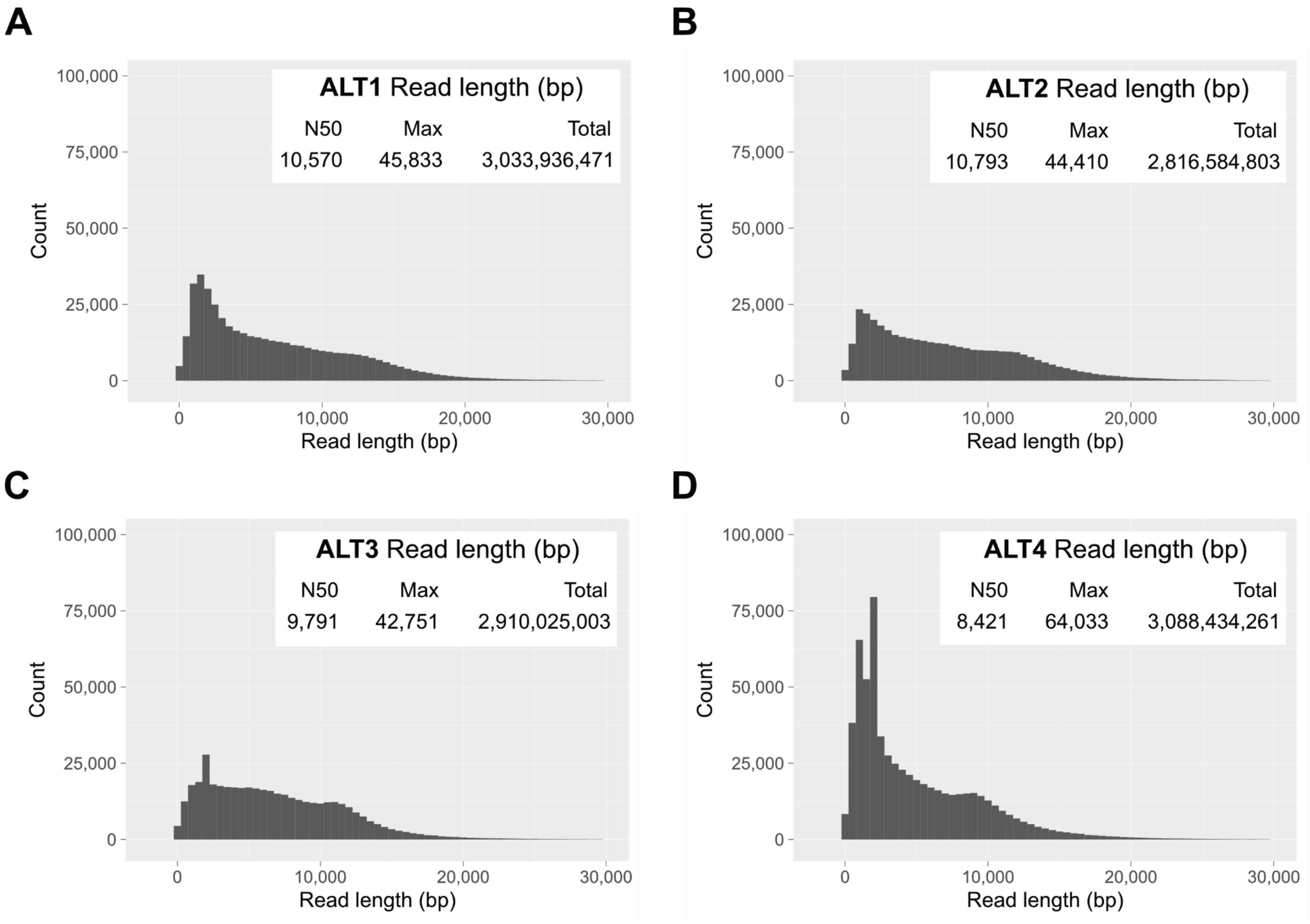
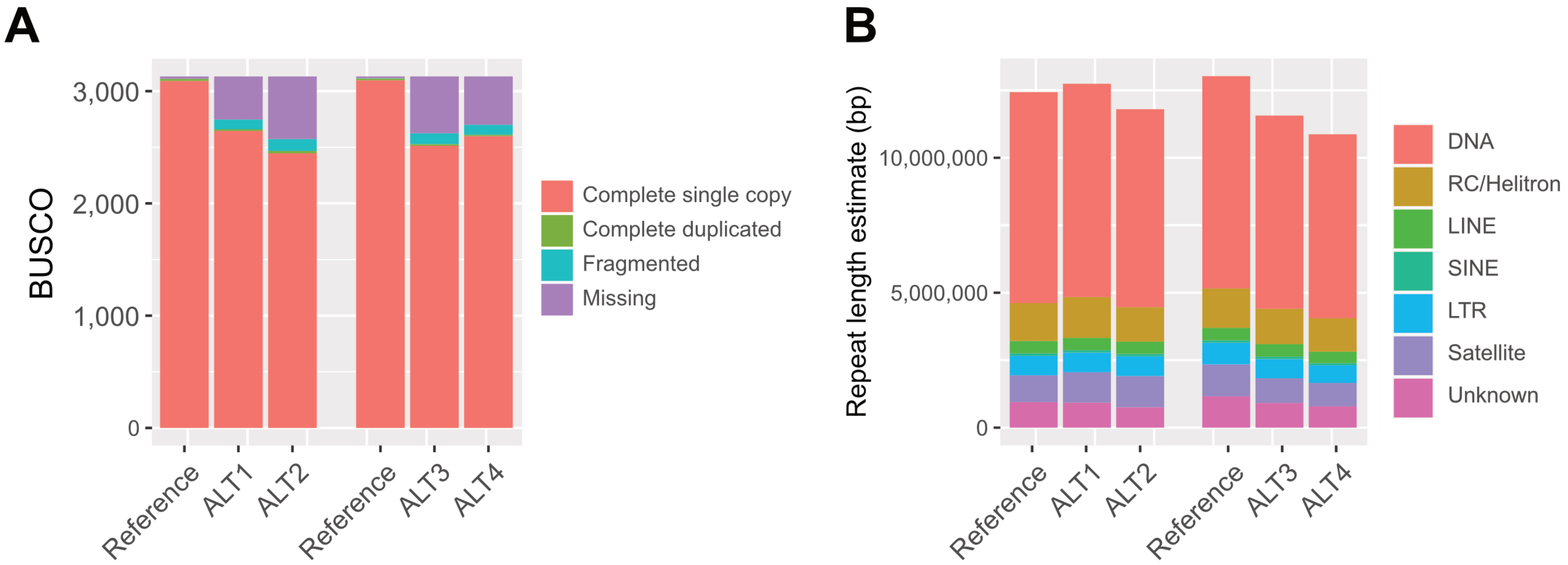
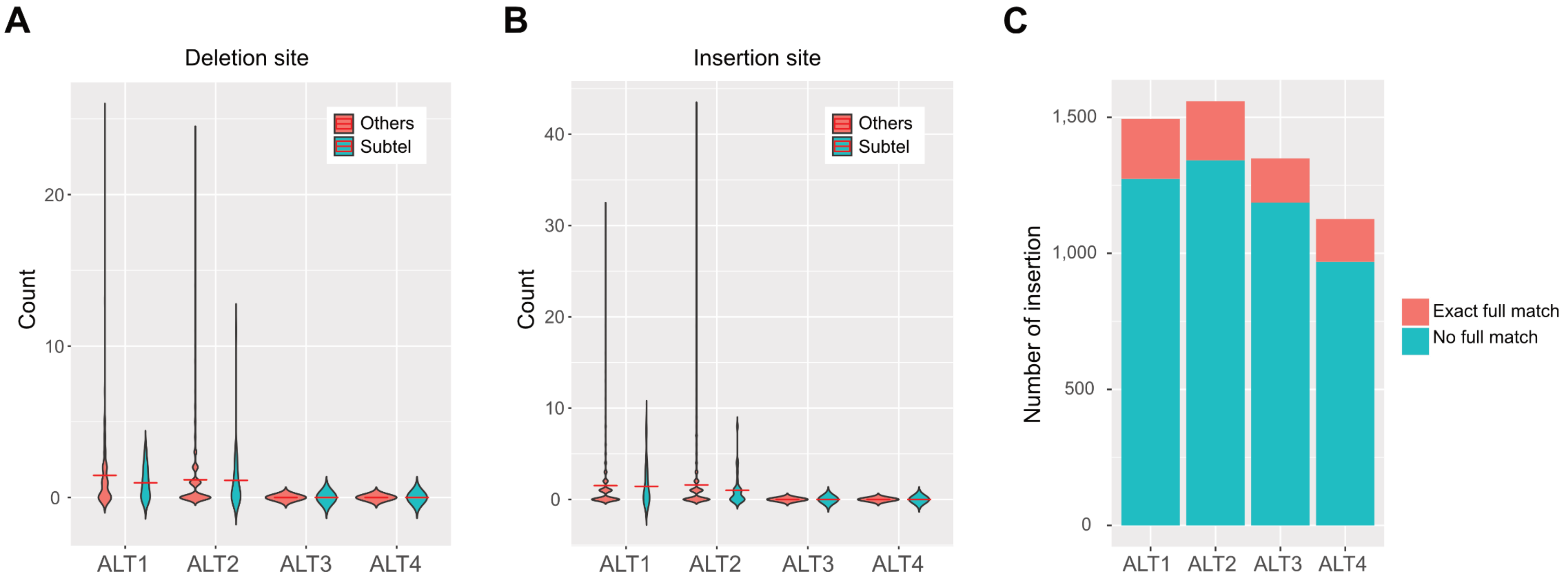


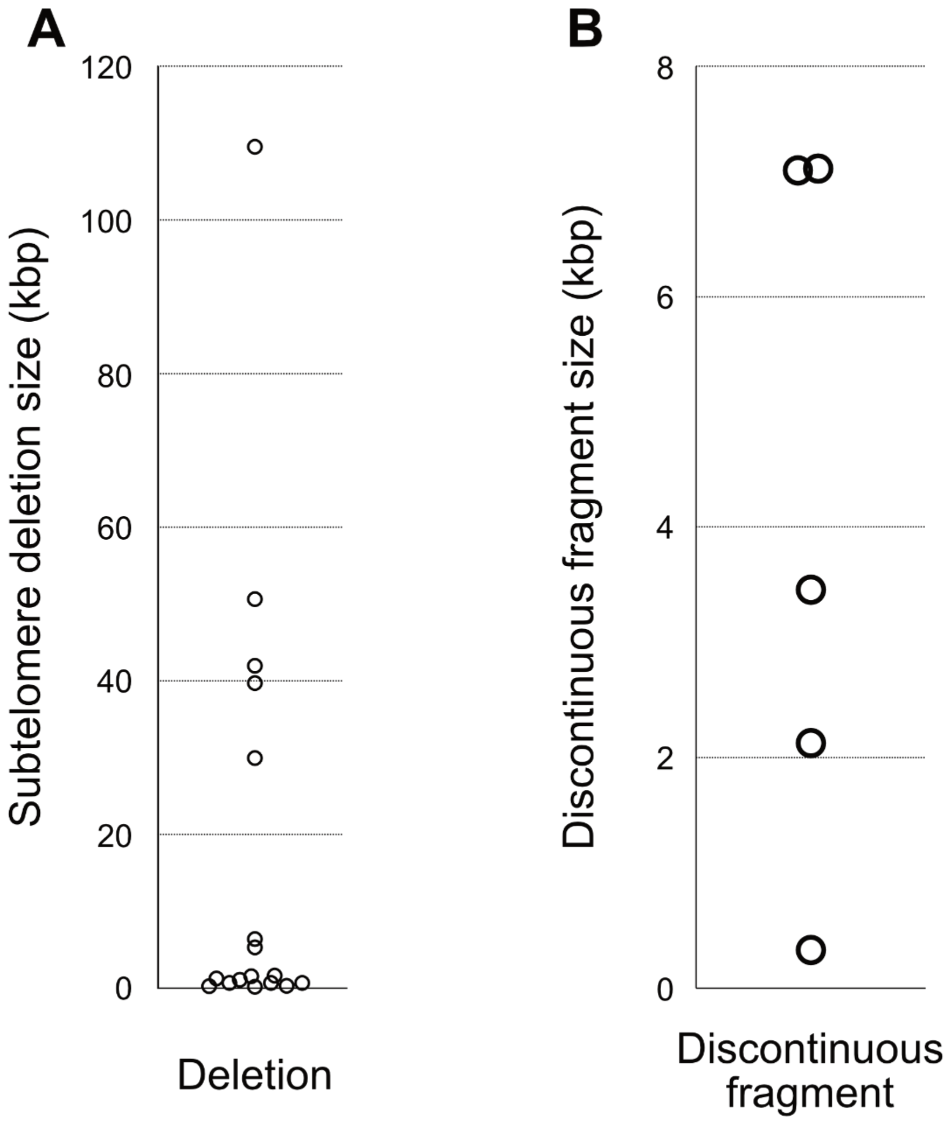
Supplementary Figure S1. PacBio read-length distributions of ALT1 (**A**), ALT2 (**B**), ALT3 (**C**) and ALT4 (**D**) survivor lines.



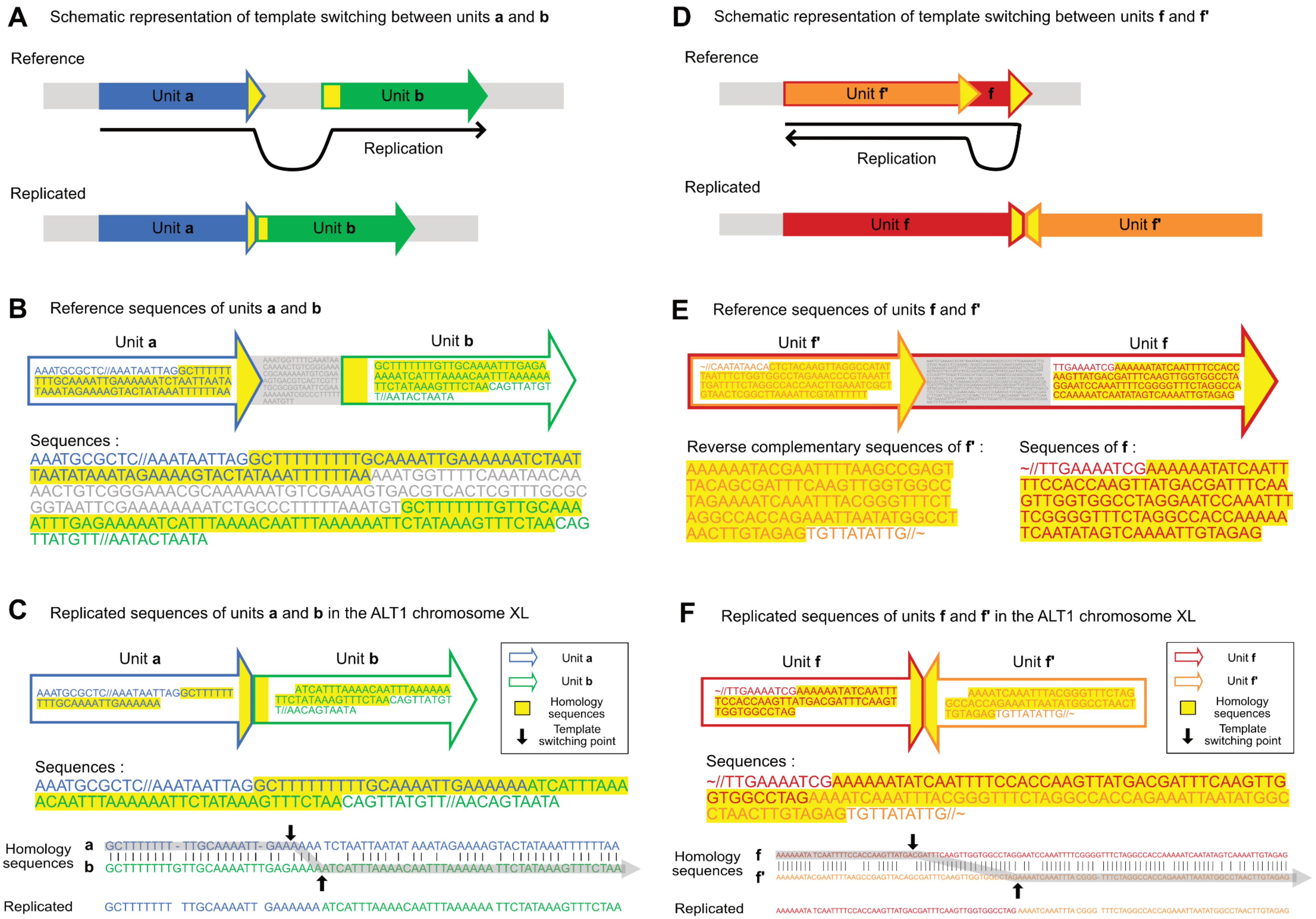
Supplementary Figure S2. Quality assessment for de novo genome assemblies of the four Caenorhabditis elegans ALT survivor lines. (**A**) The result of Benchmarking Universal Single-Copy Orthologs (BUSCO) analysis for the four genome assemblies. The first Reference corresponds to the scores of the CB4856 genome and the second Reference corresponds to those of the PD1074 genome, a long-read-based N2 genome assembly. (**B**) Estimated repetitive sequence lengths for each assembly based on the total read depths in annotated repeats. The first reference represents the CB4856 genome and the second represents the PD1074 genome.



Supplementary Figure S3. Insertions and deletions of the 4 ALT survivor lines (**A**) Deletion distributions in subtelomeric regions and other genomic regions. (**B**) Insertion distributions in subtelomeric regions and other genomic regions. Subtelomeric regions were defined as 200-kb regions from each chromosomal end. (**C**) Ratios of templated insertions to the insertions <50 bp are shown.



Supplementary Figure S4. Size of subtelomere changes in fusion chromosomes. (**A**) Distribution of subtelomere deletion size at each fused chromosome end. (**B**) Distribution of discontinuous fragment size between two nonhomologous chromosome ends.



A Schematic representation of template switching between units **a** and **b**

B Reference sequences of units **a** and **b**

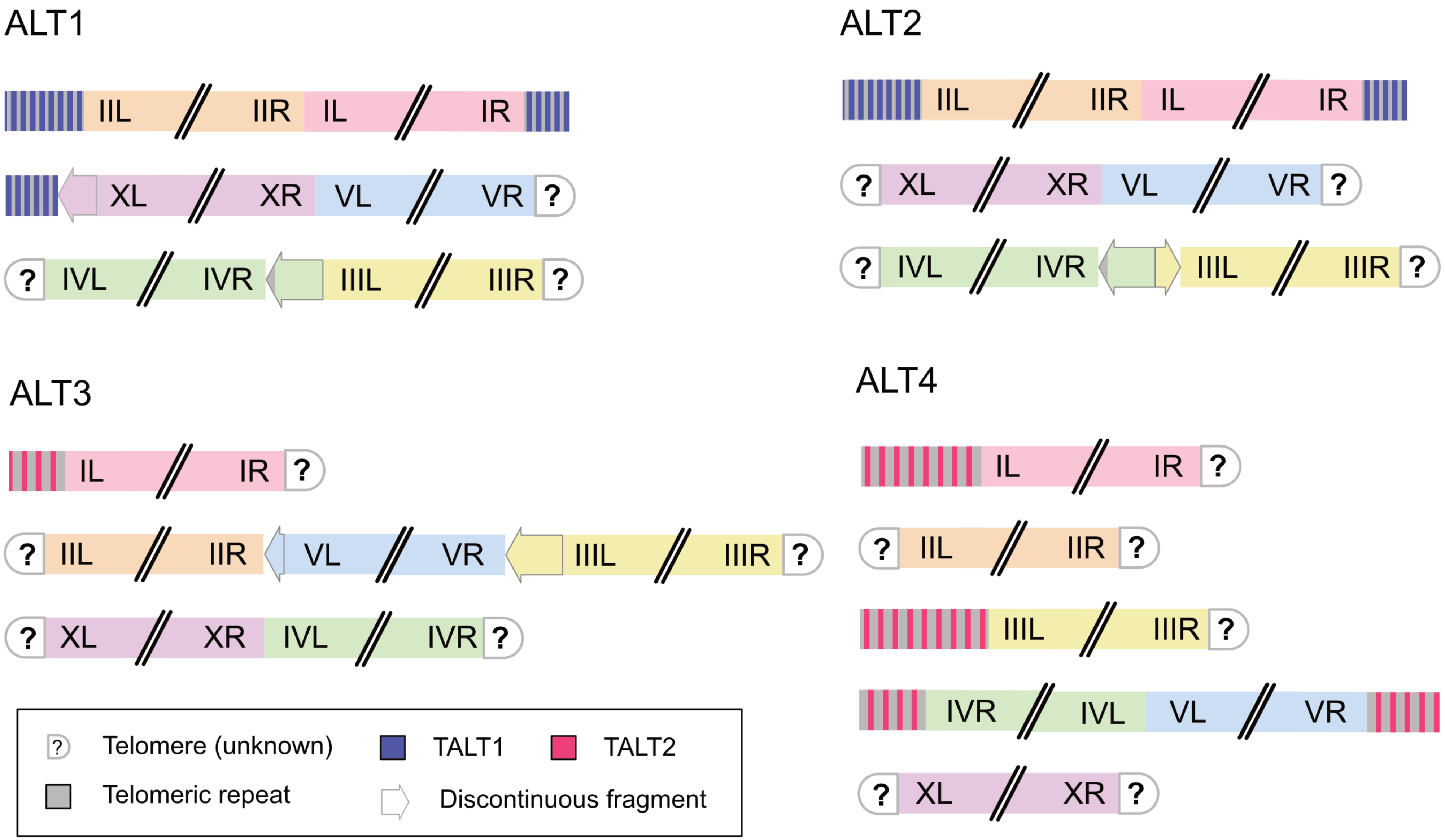
C Replicated sequences of units **a** and **b** in the ALT1 chromosome XL

D Schematic representation of template switching between units **f** and **f'**

E Reference sequences of units **f** and **f'**

F Replicated sequences of units **f** and **f'** in the ALT1 chromosome XL

Supplementary Figure S5. Sequence and homology information of units **a** and **b**, and **f** and **f′** in the reference CB4856 and ALT1 chromosome XL. (**A**) Schematic representation of template switching between units **a** (blue arrow) and **b** (green arrow). (**B**) Reference sequences of units **a** (blue arrow and sequences) and **b** (green arrow and sequences). Homologous regions between the two units are coloured in yellow background, and non-replicated sequences in the ALT1 survivor line are coloured in grey. (**C**) Replicated sequences of units **a** (blue arrow and sequences) and **b** (green arrow and sequences) in the ALT1 chromosome XL. Homology sequences are coloured in yellow background, and a possible template switching point is marked with black arrows. (**D**) Schematic representation of template switching between units **f** (red arrow) and **f′** (orange arrow). Unit **f′** is a part of unit **f** and 671-bp shorter than unit **f**. (**E**) Reference sequences of units **f** (red arrow and sequences) and **f′** (orange arrow and sequences). Homologous regions between the two units are coloured in yellow background, and non-replicated sequences in the ALT1 survivor line are coloured in grey. Unlike units **a** and **b**, units **f** and **f′** were connected in the opposite direction. Therefore, homologous sequences of unit **f′** are indicated as reverse complementary sequences for easy comparison with that of unit **f**. (**F**) Replicated sequences of units **f** (red arrow and sequences) and **f′** (orange arrow and sequences) in the ALT1 chromosome XL. Homology sequences are coloured in yellow background, and a possible template switching point is marked with black arrows. ‘//’ indicates that some sequences are omitted.



Supplementary Figure S6. Karyotypes of the four *Caenorhabditis elegans* ALT survivor lines. The fused chromosome ends identified are represented by their corresponding repair or reconstruction mechanisms. The other unknown chromosome ends are marked with question marks.

Supplementary Table S1. Genome assembly statistics of four ALT survivors.

	ALT1	**ALT2**	**ALT3**	**ALT4**
Sum (bp)	104,562,308	101,858,375	95,198,557	95,972,472
Contig number	763	754	891	764
Average length (bp)	137,041	135,090	106,844	125,618
Longest contig length (bp)	1,474,610	2,078,747	1,252,834	3,090,732
N50 length (bp)	388,786	389,851	241,979	386,771
Shortest contig length (bp)	1,092	1,706	1,497	1,081

Supplementary Table S2. Total lengths (bp) of assembled repetitive elements in the reference genomes and the four *Caenorhabditis elegans* ALT survivor lines.

	CB4856	**ALT1**	**ALT2**	**PD1074**	**ALT3**	**ALT4**
DNA	7,951,825	7,534,902	7,024,773	7,879,736	6,951,281	7,011,242
RC/Helitron	1,601,973	1,398,099	1,215,605	1,563,395	1,236,168	1,291,131
LINE	467,539	481,495	455,487	472,739	461,295	460,340
LTR	726,808	684,700	618,444	700,145	648,918	643,046
rRNA	34,349	37,259	45,133	13,916	33,828	35,741
Satellite	1,223,473	957,971	991,259	1,222,435	758,057	834,028
SINE	94,713	98,609	94,079	91,711	91,637	89,168
snRNA	8,426	8,187	7,843	8,754	8,600	8,151
tRNA	45,409	46,492	46,095	44,829	43,399	43,910
Unknown	1,038,780	834,964	721,372	1,009,842	770,126	774,647

Supplementary Table S3. Total estimated true lengths (bp) of repetitive elements in the reference genomes and the four *Caenorhabditis elegans* ALT survivor lines. The estimated true lengths were measured by raw read depth of repeat sequences normalised with average whole genome sequence depth.

	CB4856	**ALT1**	**ALT2**	**PD1074**	**ALT3**	**ALT4**
DNA	7,814,577	7,902,698	7,338,214	7,864,193	7,158,712	6,817,539
RC/Helitron	1,405,649	1,507,025	1,275,676	1,458,149	1,309,549	1,235,190
LINE	457,885	456,409	448,005	461,089	459,560	423,323
LTR	723,054	727,223	728,551	800,244	709,012	651,126
rRNA	342,214	193,129	398,146	250,824	303,545	1,147,672
Satellite	992,769	1,126,174	1,155,338	1,182,507	915,747	866,536
SINE	92,752	93,888	95,426	94,304	93,844	83,971
snRNA	8,353	8,074	8,263	8,115	8,222	8,258
tRNA	45,909	46,987	47,468	43,634	43,452	42,429
Unknown	946,520	927,411	758,480	1,163,335	916,396	79,2686

Supplementary Table S4. N2-type variants of ALT1 and ALT2 lines

	Deletion			**Insertion**		
	Shared	Specific	Ratio	Shared	Specific	Ratio
ALT1	420	202	0.675241	303	203	0.598814
ALT2	420	80	0.84	303	88	0.774936

Supplementary Table S5. Indels that ALT1 and ALT2 may or may not share

	Deletion			Insertion		
	≥ 5 bp and < 50 bp	≥ 50 bp	Total	≥ 5 bp and < 50 bp	≥ 50 bp	Total
Common in ALT1	66	24	90	74	17	91
Common in ALT2	76	23	99	74	18	92
ALT1-specific	769	633	1,402	1,420	54	1,474
ALT2-specific	751	357	1,108	1,486	46	1,532

* N2-type variants were not counted in this table.

Supplementary Table S6. Number of indels by size in 4 ALT survivors

	Deletion			Insertion		
	>1 bp and ≤200 bp	>200 bp	Total	>1 bp and ≤200 bp	>200 bp	Total
ALT1	1,136	356	1,492	1,527	38	1,565
ALT2	1,015	192	1,207	1,591	32	1,623
ALT3	797	149	946	1,366	9	1,375
ALT4	1,621	907	2,528	1,137	5	1,142

Supplementary Table S7. List of translocations in 4 ALT survivors

ALT1		ALT2		ALT3		ALT4	
Chromosome	Size (bp)	Chromosome	Size (bp)	Chromosome	Size (bp)	Chromosome	Size (bp)
III>IV	165,474	III>V	89,411	X>II	75,297	II>X	279,051
V>X	272,526	II>IV	467,213	X>IV	50,535	V>V	553,768
V>V	450,827	V>V	100,921	I>II	96,581	V>I	132,078
II>IV	168,931	X>III	105,744	I>V	127,558	X>IV	1,300,899
III>I	62,784	X>III	337,839	II>X	54,667	I>II	73,517
V>V	61,415			V>I	59,162	I>II	900,645
				V>V	249,995	I>I	397,594
				X>III	65,940	III>IV	159,619
						IV>V	94,146

Supplementary Table S8. Summary of categorising repair or reconstruction mechanisms at each chromosome end.

	ALT1	**ALT2**	**ALT3**	**ALT4**
IL	Simple fusion	Simple fusion	TALT end	TALT end
IR	TALT end	TALT end	.	.
IIL	TALT end	TALT end	.	.
IIR	Simple fusion	Simple fusion	BFB fusion	.
IIIL	BFB fusion	BFB fusion	BFB fusion	TALT end
IIIR	.	.	.	.
IVL	.	.	Simple fusion	Simple fusion
IVR	BFB fusion	BFB fusion	.	TALT end
VL	Simple fusion	Simple fusion	BFB fusion	Simple fusion
VR	.	.	BFB fusion	TALT end
XL	TALT end with FoSTeS	.	.	.
XR	Simple fusion	Simple fusion	Simple fusion	.

Supplementary Table S9. Sequence information of simple fusion breakpoints.

Line	Contig name	Fusion chromosome	Fusion sequence
ALT1	tig00000324	IIR-IL	GACTTGCTACAGTACTTTAGCTACAGTACCCCAGCTTGACTTGCTACAGTACTTTAGCTACAGTACCCCATCTTGACATGCTACAGTACACTATCTTGAC**CA**CGCCTGCTCGAATTTCGCAAAAAACGTGTCGTGTCGAGACCCCAATTACAGTATTTTTGACCCGAATATCGCGAAAATTCGAGTCTGGGTGAAAACAT
ALT1	tig00002922	XR-VL	ACGTCTTCTACACTTCTGGCTTCTGGGCGTACCCGATTCTCGGAGAACTTGCCGCACCATTCCGCCTTGTGTTCATTGCTGCCTGCATGTTCATTGTCA**TACC**ACTATCATTAAAACATTTGGAAATTATTTTTTGATTGTTTATATTCAAGTAGCAAGAAAGTCTCCTAGCCTTAAAAATAGGAAATTTTTTGCTCTTT
ALT2	tig00000558	IIR-IL	GACTTGCTACAGTACTTTAGCTACAGTACCCCAGCTTGACTTGCTACAGTACTTTAGCTACAGTACCCCATCTTGACATGCTACAGTACACTATCTTGAC**CA**CGCCTGCTCGAATTTCGCAAAAACGTGTCGTGTCGAGACCCCAATTACAGTATTTTTGACCCGAATATCGCGAAATTTCGAGTCTGGGTGAAAACATT
ALT2	tig00000501	XR-VL	ACGTCTTCTACACTTCTGGCTTCTGGGCGTACCCGATTCTCGGAGAACTTGCCGCACCATTCCGCCTTGTGTTCATTGCTGCCTGCATGTTCATTGTCA**TACC**ACTATCATTAAAACATTTGGAAATTATTTTTGATTGTTTATATTCAAGTAGCAAGAAAGTCTCCTAGCCTTAAAAATAGGAAATTTTTTGCTCTTTC
ALT3	tig00000627	XR-IVL	GACTTTATTAATGCAAAATAAACTCTAAAAACCGTCAGTTTCCAGCCTCCATTACGTCTTCTACACTTCTGGCTTCTGGGCGTACCCGATTCTCGG**AGAA**CT**TG**AAAAGGAATCCGAAAACAGTAGTTATTACAATGGTTACTTGAAATATGGACAGCTGATGAAGTGAGACATTTAAAAATTATTTTTGTATCACTCCC
ALT4	tig00001818	VL-IVL	AACGGCTCGAAAAGTTTGCACCGTCCGGATGGAAAAGTGTGGCACGCGTGGGCGTTAAGGTGCCATACGTGTTGCACTTACGATTCAAATTTTTATCCGCGTTAAGGCTCATGATGTTCTTGGAGAGCATCAGTGTTGTGCTCAGAAAAATGTTCGTAGTTTTGGAAGAATTACAAAAAGTTTACAAAACAAAATGATTA

* The colour of each sequence matches the colour of the fusion chromosome name. **Bold**: homologous sequences between two chromosomes

Supplementary Table S10. Sequence information of complex fusion breakpoints that contain discontinuous fragments.

Line	Contig name	Fusion chromosome	Fusion sequence
ALT1	tig00000145	IVR-IVR fragment 5'	CTTTTTTTCTGATACAAAAACACTAATTTCGTTACTTCAACTTTGCTGTTTTGCGTATTTTGCAATAAGTACACACGAAATTCAAAAATG*CCTATGCCTCAGCCTGA*GCCTAAGAAACAGCCATGAAATCATTTATTCAATCGAGTTTTCGTAGGAAATTTATCGAA
ALT1	tig00000145	IVR fragment 3'-IIIL	TTTTTTTTTTAAGTACAATCATCGACAAGACGTCGATTGGACTGACTTGGACGTCGGTCGGAGTCTATTTACCAGAATAACTTGACAATATTCATTGTT**CA**GTTATCGTTTTCTGTCCATTGTGAGTCACATTCGAATTACCATTTTTTTTCAATGACACTTTGGTGTGATTGGGCTGATCACAATTCATTGCAAATCCT
ALT2	tig00000192	IVR-IVR fragment 5'	CTTTTTTTCTGATACAAAAAACACTAATTTCGTTACTTCAACTTTGCTGTTTTGCGTATTTTGCAATAAGTACACACGAAATTCAAAAAATG*CCTATGCTCAGCCTGAGCCTGTTA*GCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCTCTAAGCCTAAACCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGCCTAAGAAACAGCCAATATGAAAATCATTTATTCAATCGGAGTTTCGTAGGATCCAA
ALT2	tig00000192	IVR fragment 3'-IIIL fragment 5'	TTTTTTTTTTAAGTACAATCATCGACAAGACGTCGATTGGACTGACTTGGACGTCGGTCGGAGTCTATTTACCAGAATAACTTGACAATATTCATTGTT**CA**GTTATCGTTTTCTGTCCATTGTGAGTCACATTCGAATTACCATTTTTTTTCAATGACACTTTGGTGTGATTGGGCTGATCACAATTCATTGCAAATCCT
ALT2	tig00000192	IIIL fragment 3'-IIIL	AGATGGATTGAAATGAAATGTATTCGATTTATGAGCGGGTAAGTAGTTATTACCAATTAAGATTATTTTTCAAATGAAATTAAAATGTATA**TGAT**A**TTT**T**T**C**TA**A**AATA**CATAACCTTCTTTGAGCTCTATGGCTTGTATATCATTAACTTTTTAACATTAAGGTATTTCTTACACTTTCAAATAATGCGACTAATTTTA

ALT3	tig00043120	IIR-VL fragment 5'	AATGGCCTATCCTTGTACCACGTGCAGGCCGAGAAAAGGAGGGGCGGGAAGACGCAGAACCTCTCGCTGTCTGCGTCTAACCCTTCTTGAGTC*TAACCCTTCCGTATA*AATTTTTCAGATTATCAGTGTTGATAACATTTTCAAGAAAAACATAAATAAACAAGAACAACGCATTTTGTATCAGGGAAAACAGCGAAAAA
ALT3	tig00043120	VL fragment 3'-VL	GAGCAAAAAATTTCCTATTTTTAGGCTAGGACACTTTCTTGCTACTTAAATATAAACAATCAAAAAATAATTTCCAAATGTTTTAATGGTAGTGGTATT*A*AATACCTAATAGGTATCCCCATTAATTTTTTTTTCAAGAAGAATGGCAACACAAACACCCCTCTCTGATGACTCGTGTGTCAAAAACGAGCTCACGGAACT
ALT3	tig00000971	VR-IIIL fragment 5'	ACCTTTTCCTTACTATTTGGTTGTCAAAATTACATTTATTTGGAAATTTTGTGAGTTTTAACTTGTATATAAACTTGTTTTTCCATAGTTGTTTGA**T**C**TT**C**AATT**CATTCACATCTACTTTTTGCTATCGGACATATTTTATTTAACTTCTGTGTTAAAGAAATTATATCTTTTGAAAATGGTAACAATCAGCCGAGAAG
ALT3	tig00000971	IIIL fragment 3'-IIIL	GTACTGTTATTCCCTATGCATCATCAGTCGCACCGCTCGTACTAGCTCCGTATCCTCCGCGCGTCATTAAGTAAGCCGCACGTTTGTTACCTATCGGGTCT**T**CGAAATAGGGCAGCGTAGATATAATATCATTGGGTGACACAAAAATAATCGATAATTCCTTCACATGAATGCATTGCTTAGATCTGAGGATGTTCAGAA

* The colour of each sequence matches the colour of the chromosome or fragment name. * **Bold**: homologous sequences between two chromosomes; *Red italicised*: randomly inserted sequences; Underlined: telomeric repeat sequences

Supplementary Table S11. Sequence identity between each discontinuous fragment and its origin.

	Aligned bases (%)	Average identity (%)	# of SNPs	# of Indels
ALT1 IVR fragment	99.97	99.49	2	30
ALT2 IIIL fragment	100	99.51	1	16
ALT2 IVR fragment	100	98.52	0	40
ALT3 VL fragment	100	98.2	2	4
ALT3 IIIL fragment	100	99.03	0	12

Supplementary Table S12. Estimated numbers of the units based on raw read-depth, and the identified numbers in the local re-assembled contigs.

Units	Estimated number	Identified number
a	8	3
b	10	4
c	1	1
d	4	1
e	4	2
f	2	2
g	1	1