The DNA replication FoSTeS/MMBIR mechanism can generate human genomic, genic, and exonic complex rearrangements

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Supplementary Information

Supplementary Tables 1 to 4

Supplementary Figs. 1 to 10 with Legends

References

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Supplementary Table 1. Characteristics of the simple nonrecurrent rearrangements in 17p

Scale	Sample	Rearrangement	Size	Microhomology
Genomic	2661	dup	11.2 Mb	AT
	1861	dup	7.7 Mb	N.A.
	2488	dup	7.5 Mb	N.A.
	2362	dup	7.0 Mb	N.A.
	2440	dup	4.9 Mb	N.A.
Exonic	A10	del	16.8 kb	GATT
	A21	del	11.6 kb	C
	A11	del	7.2 kb	AC
	A14	del	212 bp	GC
	A12	del	9 bp	GACG

Abbreviation: del, deletion; dup, duplication; N.A., not available.

Supplementary Table 2. Five out of 23 (22%) CNV breakpoints sequenced in Perry *et al.*[1] show sequence complexities consistent with two or more FoSTeS/MMBIR events

Example	Genomic location	FoSTeS events	Microhomology
1	2q21.1	×2	GAGG, CA
2	2q32.3	×4	GAAGT, AATTC, ATTT, AAAACA
3	3q22.1	×2	CT, TG
4	4q26	×2	TTA, GAATA
5	5q33.1	×2	CAA, TCT

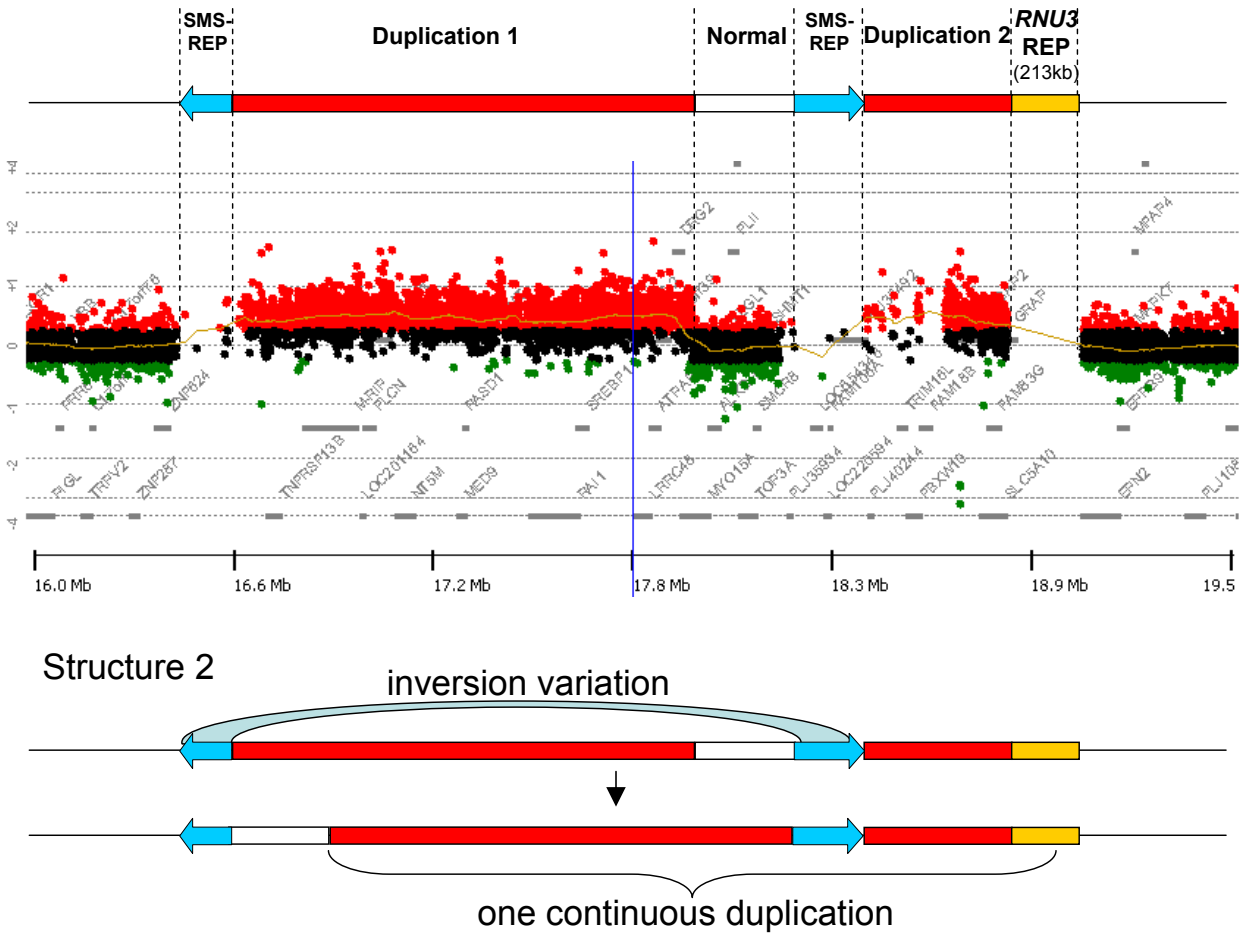
Supplementary Table 3. Reported complex gene rearrangements consistent with multiple FoSTeS/MMBIR events (http://www.hgmd.cf.ac.uk/ac/index.php)

Gene	Genomic location	Exon(s) involved	Events	Microhomology	Reference
*SLC3A1**	2p21	ex 2	×2	CTT, AAA	2
ATP2C1	3q22.1	ex 20	×2	AG, TGTGT	3
FOXL2	3q22.3	ex 1	×2	GC, GCAGCCGCGG	4
DSPP	4q22.1	ex 5	×2	AGCAG, AGCAGTGACAGCA	5
HPS1	10q24.2	ex 12-20	×2	GTTACTGGG, CCCCTCAGAGCC	6
HNF1B	17q12	ex 5	×2	TATT, AACA	7
BRCA1	17q21.31	ex 21, 22	×2	CCT, CACTGCACTCCA-GCCTGGGTGACAGAG	8
*SLC5A5**	19p13.11	ex 3-6	×2	CATC, AC	9
PRPF31	19q13.42	ex 1	×2	AAC, TCCTGGG	10
*OFD1**	Xp22.2	ex 7-9	×2	TCA, CTCAA	11
IL2RG	Xq13.1	ex 4	×2	TG, GTGA	12
*GLA**	Xq22.1	ex 5-7	×2	AC, AT	13
*F9**	Xq27.1	ex 4	×2	ATT, CAAAAA	14
*F8**	Xq28	ex 16	×2	AACT, TCA	15
*IDS**	Xq28	ex 5-7	×4	AATACATTT, GA, CAAGAG, CA	16
L1CAM	Xq28	ex 2	×2	GCT, GC	17
*MECP2**	Xq28	ex 4	×2	CC, AGCCC	18

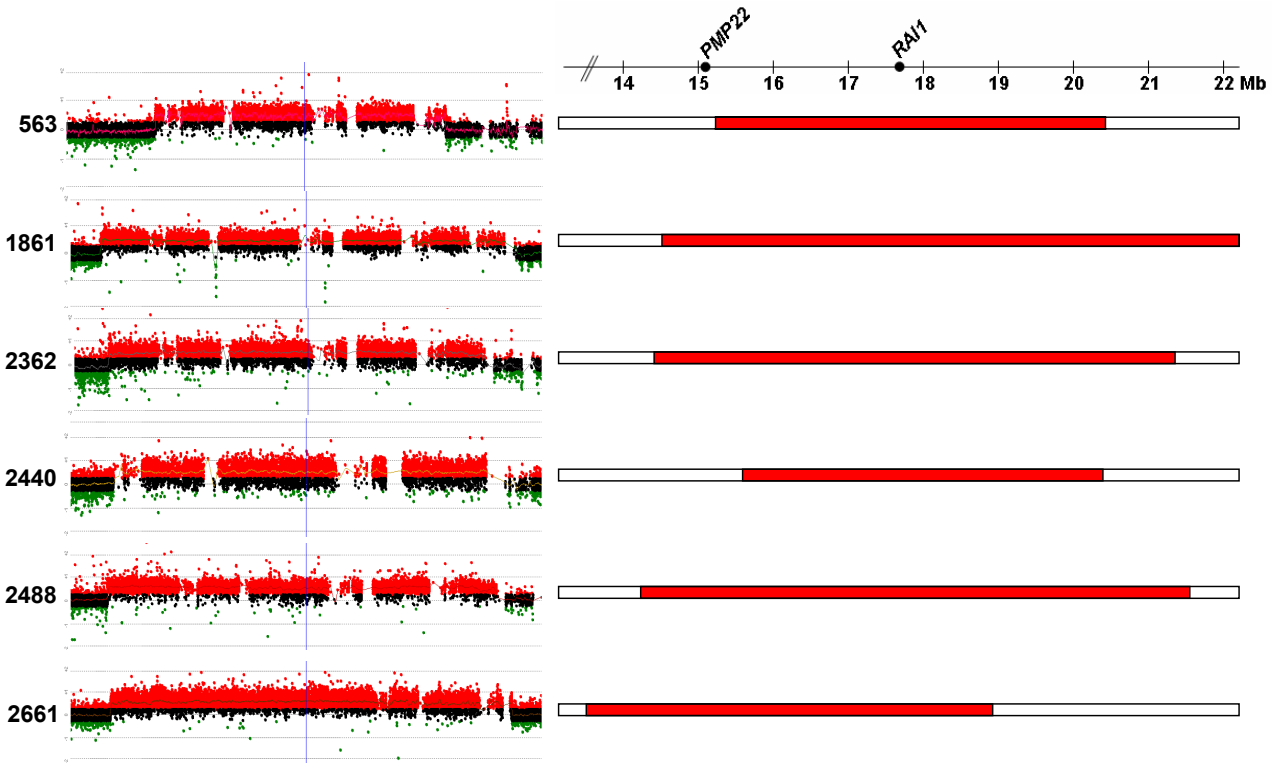
*Some complex gene rearrangements have been hypothesized to be caused by "serial replication slippage"[19,20], an alternative replication-based model proposed for small complex rearrangements.

Supplementary Table 4. Primers of achieved long-range PCR amplifications and the breakpoint junction products for sequencing in a proportion of nonrecurrent rearrangements on 17p

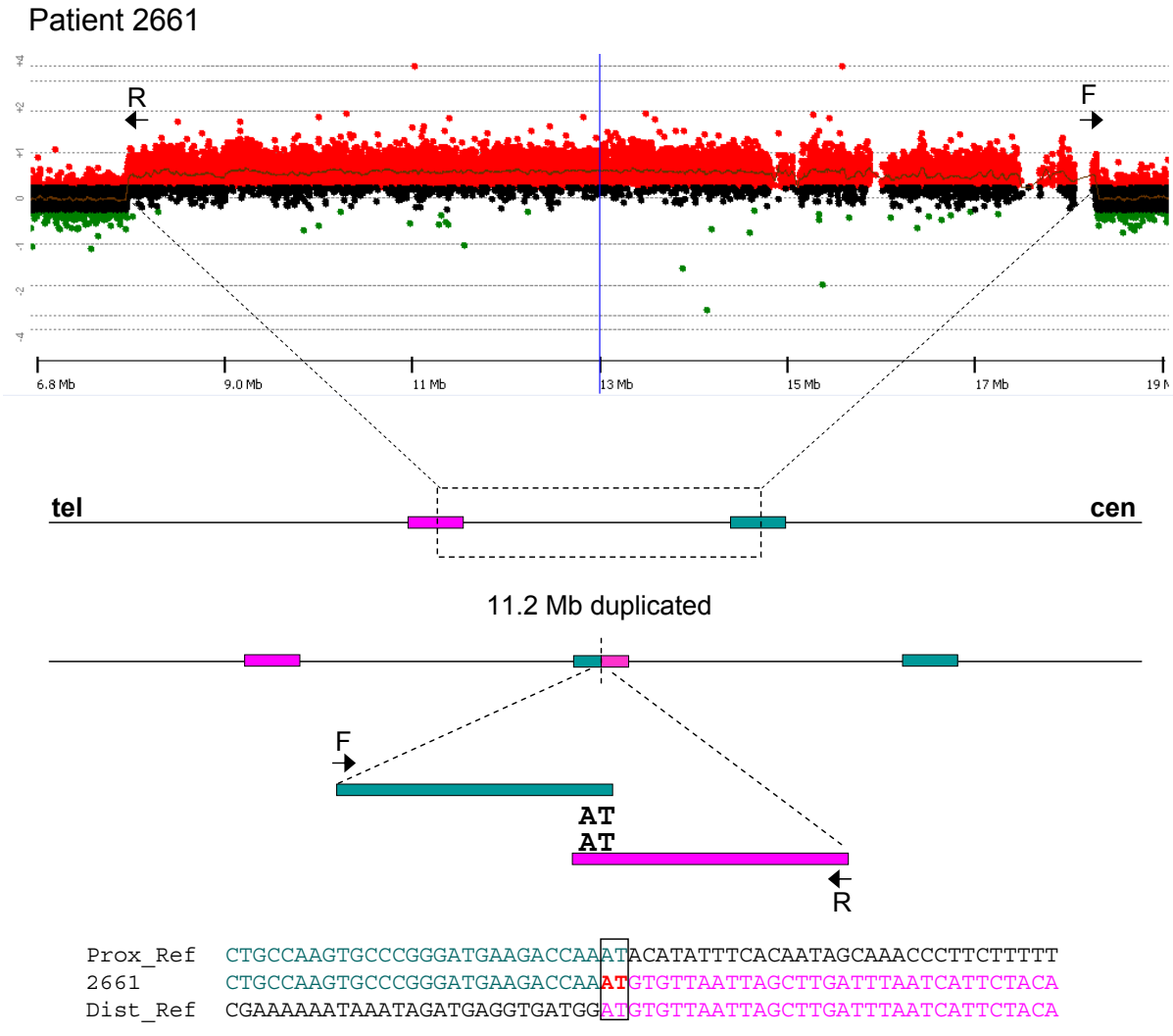
Sample	Primer coordinate (+/- strand), UCSC hg18		Size of product for Sequencing (kb)
	Forward	Reverse	
563	chr17:20775964-20775983 (+)	chr17:15225900-15225920 (-)	1.3
621	chr17:17034792-17034812 (+)	chr17:17021067-17021090 (-)	0.5
	chr17:17033028-17033053 (+)	Same with the forward primer	10
1229	chr17:15015801-15015820 (+)	chr17:15054771-15054791 (-)	0.5
2661	chr17:19124269-19124292 (+)	chr17:7977899-7977918 (-)	4.2
2695	chr17:16174939-16174959 (+)	chr17:16137968-16137993 (-)	3.9
2711	chr17:10870486-10870509 (+)	chr17:10955976-10955998 (-)	2.0
A10	chr17:15078686-15078705 (+)	chr17:15096288-15096307 (-)	0.8
A11	chr17:15096041-15096060 (+)	chr17:15104032-15104052 (-)	0.8
A12	chr17:15104214-15104233 (+)	chr17:15105252-15105272 (-)	1.1
A14	chr17:15104214-15104233 (+)	chr17:15105252-15105272 (-)	0.9
A15	chr17:15079113-15079132 (+)	chr17:15088798-15088817 (-)	1.1
A21	chr17:15093195-15093218 (+)	chr17:15105743-15105764 (-)	1.0



Supplementary Figure 1. Rearrangement for patient 2543. Oligonucleotide aCGH revealed a complex rearrangement with two separate duplications (structure 1) in patient 2543. Since NAHR between the SMS repeats (distal and middle SMS-REPs in reverse orientation) can lead to an inversion allele and such an inversion variation has been reported[21], the complexity in patient 2543 can alternatively be caused by a single duplication that occurred on an inversion allele (structure 2).

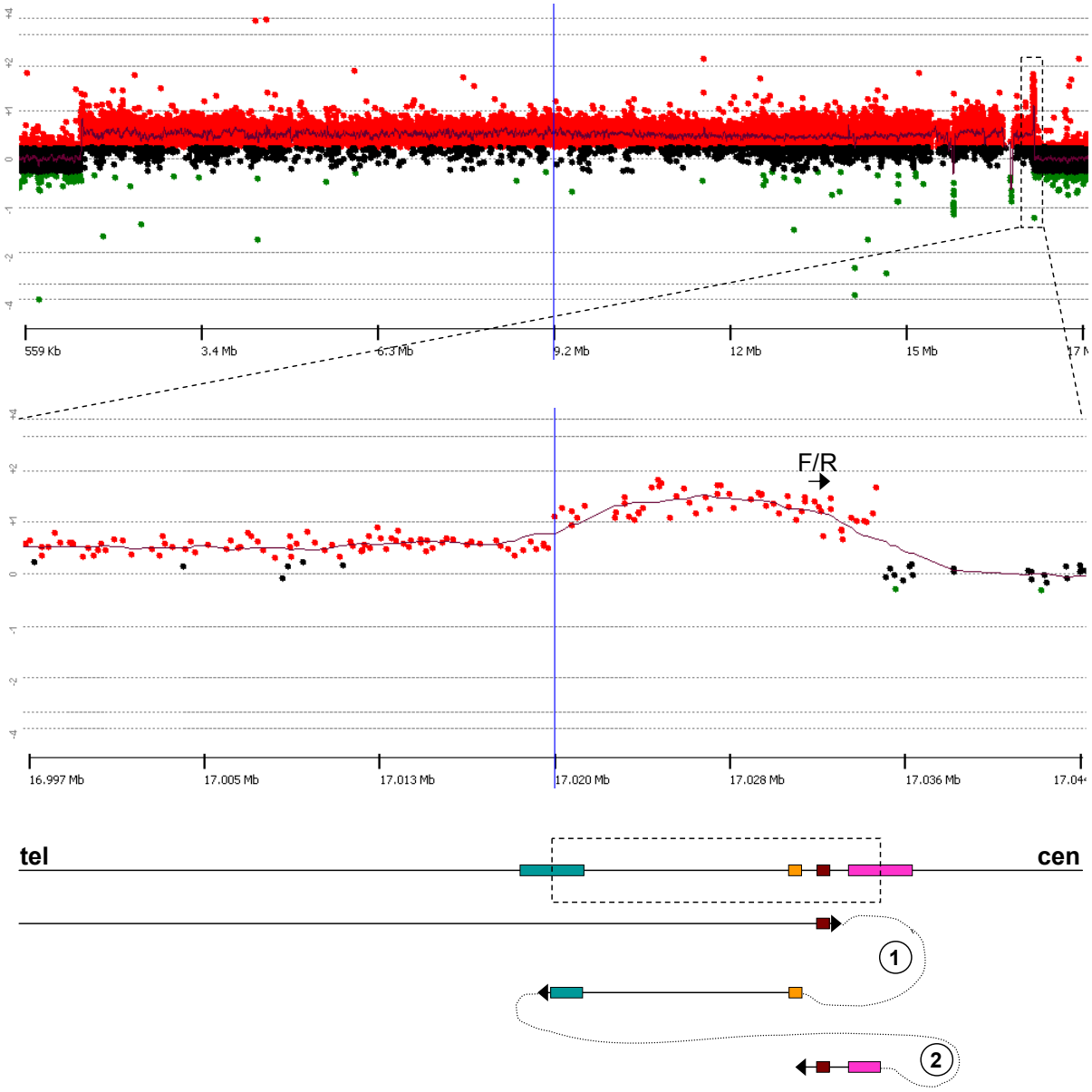


Supplementary Figure 2. No sequence complexity was found in 6 out of 14 nonrecurrent 17p11.2 duplications based on oligonucleotide aCGH. Further breakpoint sequence analyses revealed the FoSTeS/MMBIR-mediated complexity in patient 563.



Supplementary Figure 3. Junction sequence analysis of the duplication identified in 2661. Two base-pairs of microhomology (AT) were found at the breakpoint.

Patient 621



FoSTeS 1

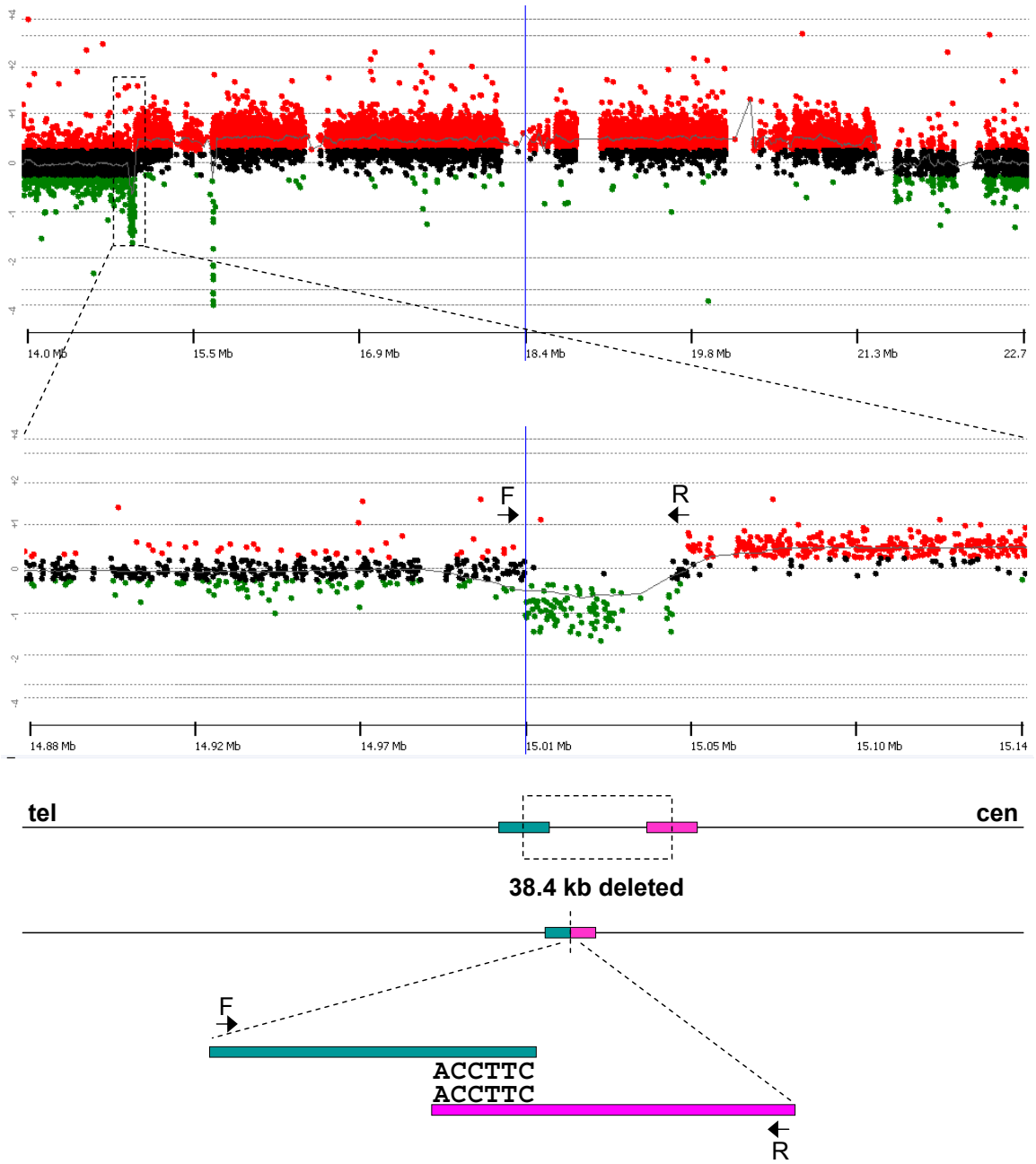
```
ProxRef1+ CAGGATGGTCTCGATTTCCTGACCTCGTGATCCGCCTGTCTCGGCCTCCCAAAGTGCTGGGATTACAGGC
621_1+    CAGGATGGTCTCGATTTCCTGACCTCGTGATCCGCCAGCCAGGGGTTTATACTCCCTATCTTAATGGGAT
DistRef2- CCACCTTGCTCCTAAAGTAGCAGAACCTGTTCCACCAGCCAGGGGTTTATACTCCCTATCTTAATGGGAT
```

FoSTeS 2

```
ProxRef2+ ATGTTTTTAATGACTGCTGCATCTTTGTAAAACGTTTGGTCATCTAACAGATGGTTTTAAAGTGTACAAT
621_2-    ATGTTTTTAATGACTGCTGCATCTTTGTAAAACGTTTGGTGGTCACTCAGTCACCTAGGGAATCTAGGAC
DistRef2+ AGGGACTCCTGGAGTCAGGGGAGATGACAGAATCATTGGTGGTCACTCAGTCACCTAGGGAATCTAGGAC
```

Supplementary Figure 4. Sequence analysis of one breakpoint junction of the complex rearrangement in patient 621. An about 10 kb junction product was amplified by only one primer at the proximal end (with respect to the reference sequence) of the complex rearrangement region. Sequencing analysis revealed two of the FoSTeS/MMBIR events causing complexity.

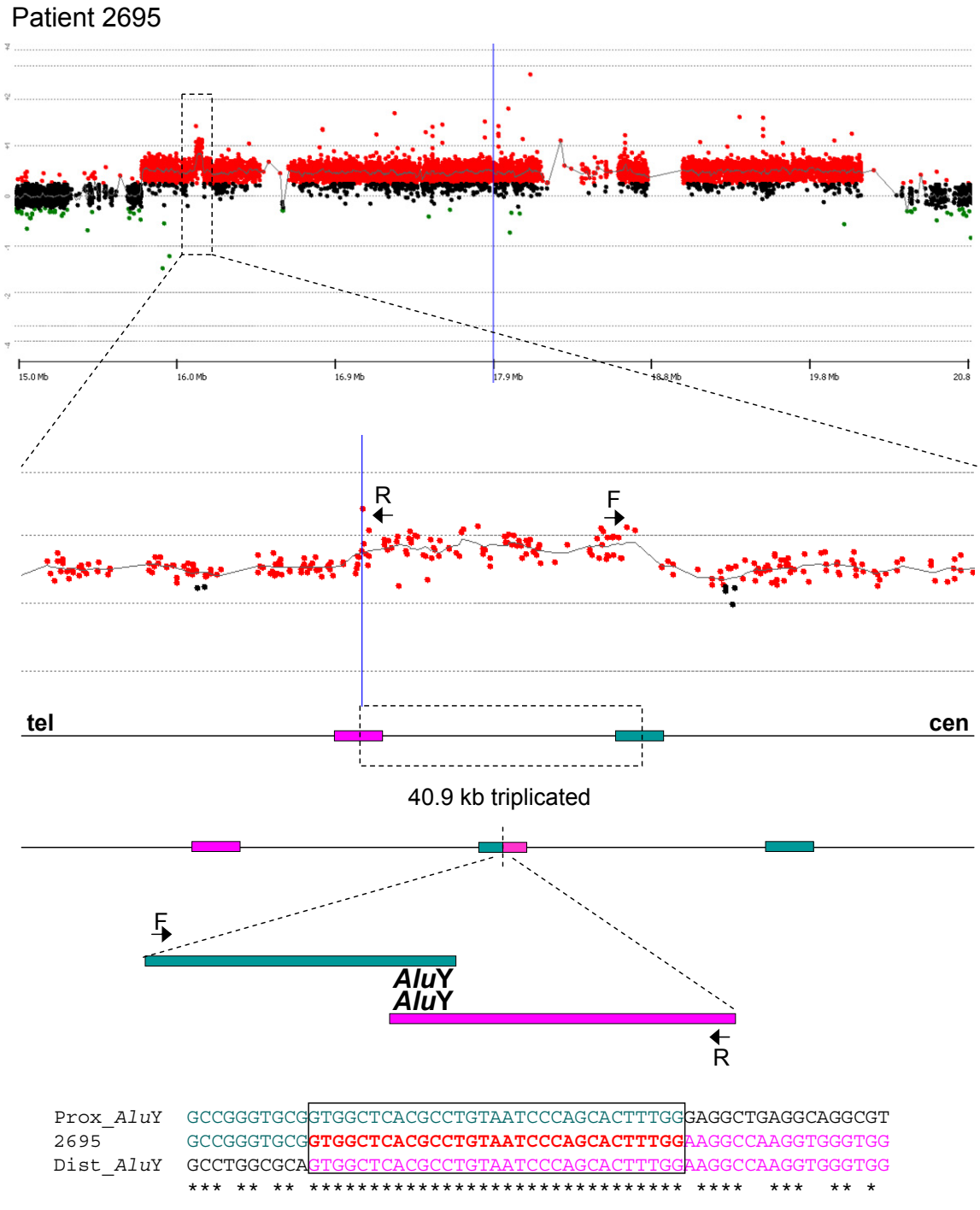
Patient 1229



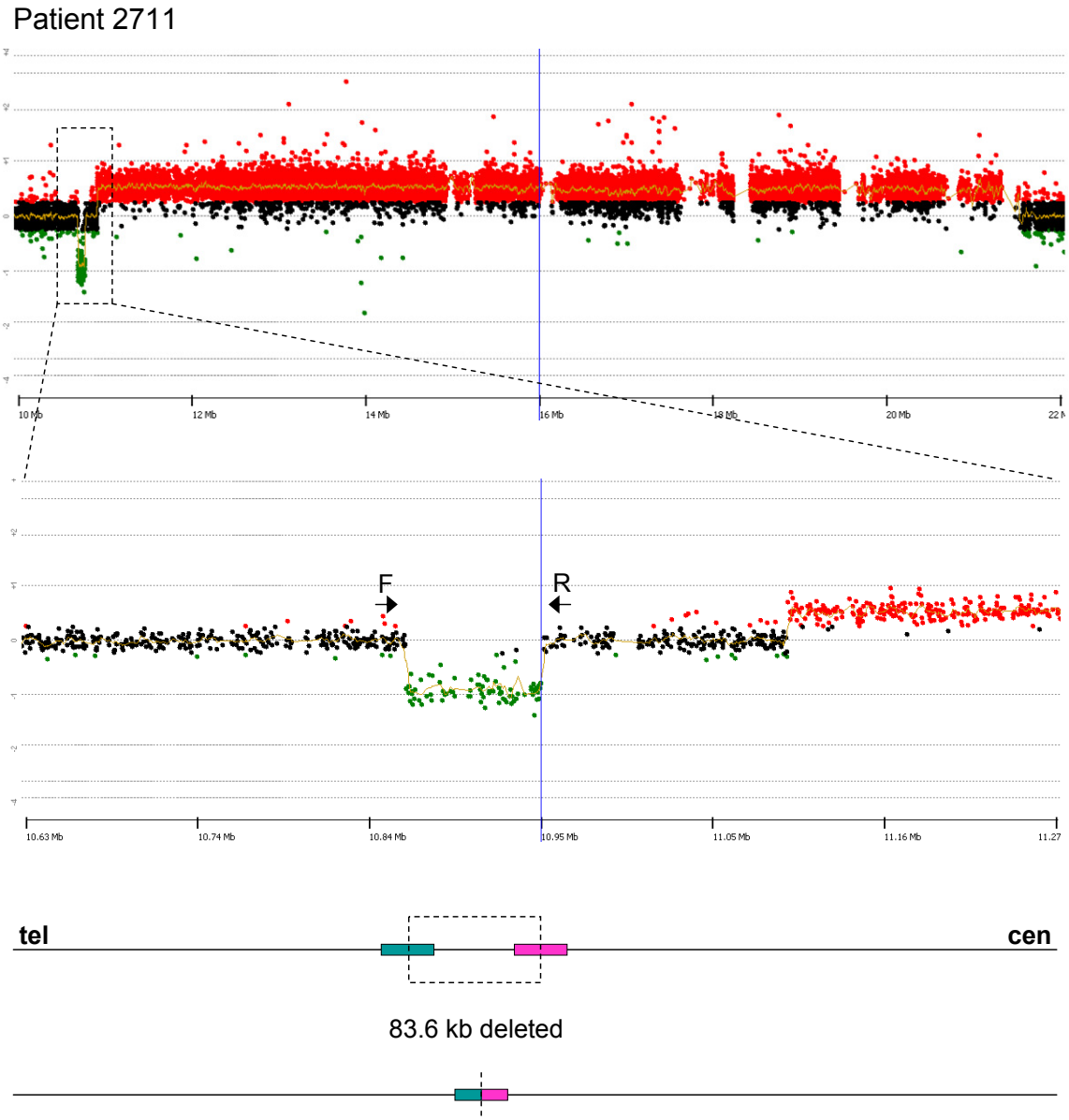
FoSTeS 1

```
Dist_Ref CTCCACATGTGGAATCTCCCCAGACCCCATGACCTTCATACACATTTCACCATTTCCCAGAGCCAGATTT
1229     CTCCACATGTGGAATCTCCCCAGACCCCATGACCTTCTGCTTCCTAACAACCACTGAGATAATTTGATAT
Prox_Ref TTACTGCAGGGGATTGGAGTCAACATAATTAACCTTCTGCTTCCTAACAACCACTGAGATAATTTGATAT
```

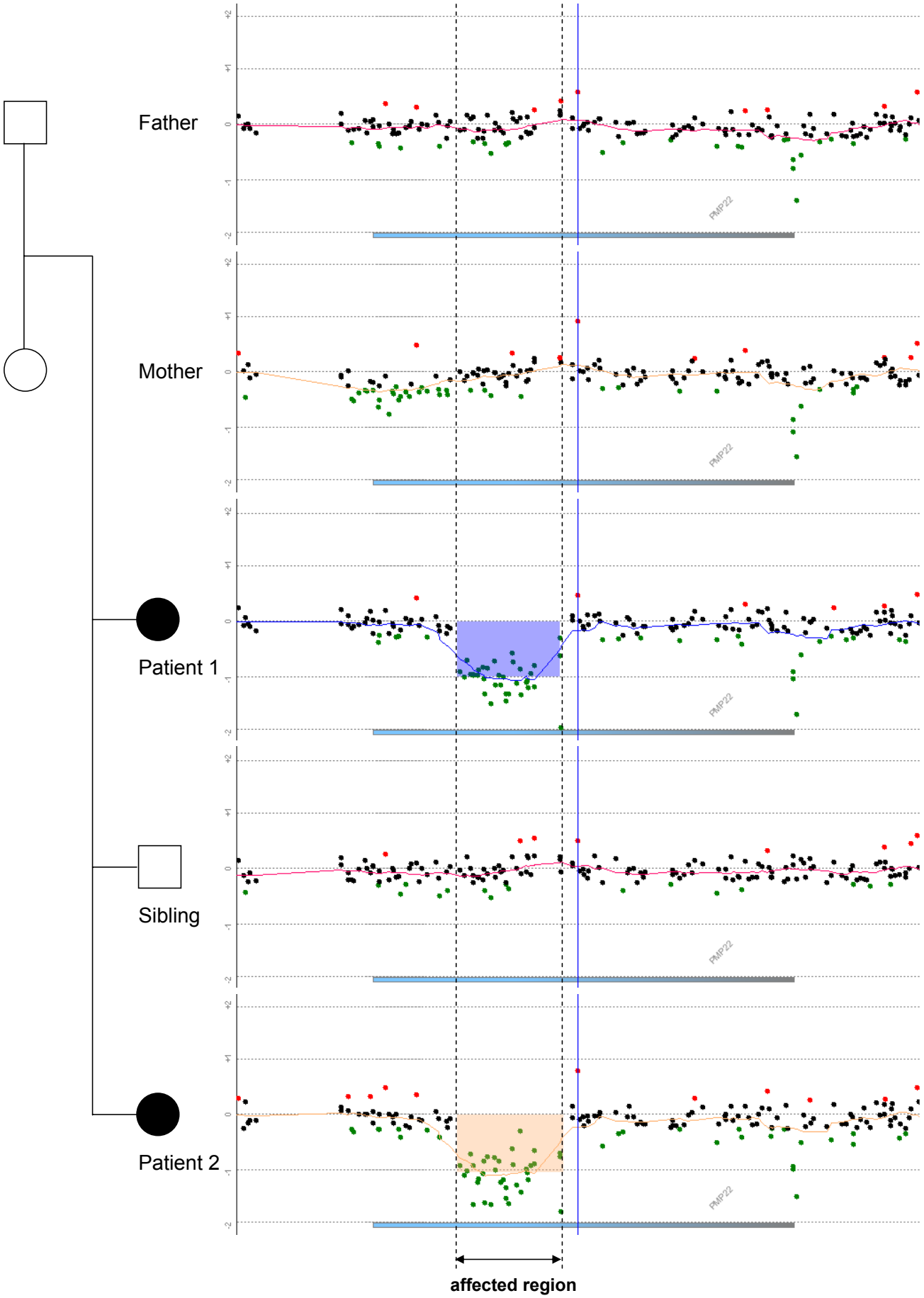
Supplementary Figure 5. Sequence analysis of one junction of a complex rearrangement identified in patient 1229. At this deletion breakpoint, a microhomology of 6 bp (ACCTTC) was found.



Supplementary Figure 6. Junction sequence analysis of the triplicated region in the complex rearrangement in patient 2695. At the breakpoint, there is 31 bp microhomology between two *Alu*Y elements.



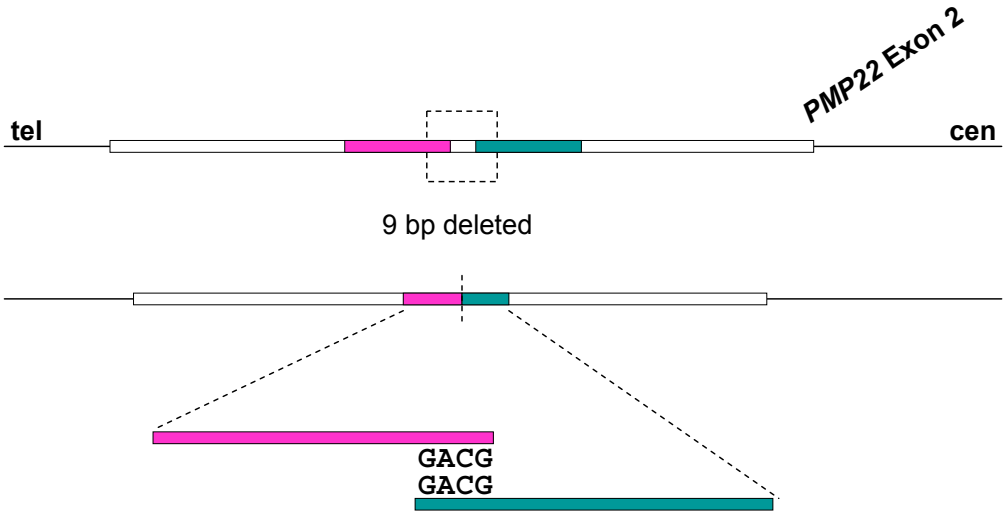
Supplementary Figure 7. Sequence analysis of one junction of the complex rearrangement in patient 2711. A “C” nucleotide was inserted at the deletion breakpoint.



Supplementary Figure 8. Oligonucleotide aCGH analysis of the *PMP22* gene region in one CMT1 family. The identical small deletions were identified in two affected siblings. No obvious copy number loss of the deletion region was found in healthy sibling and parents.

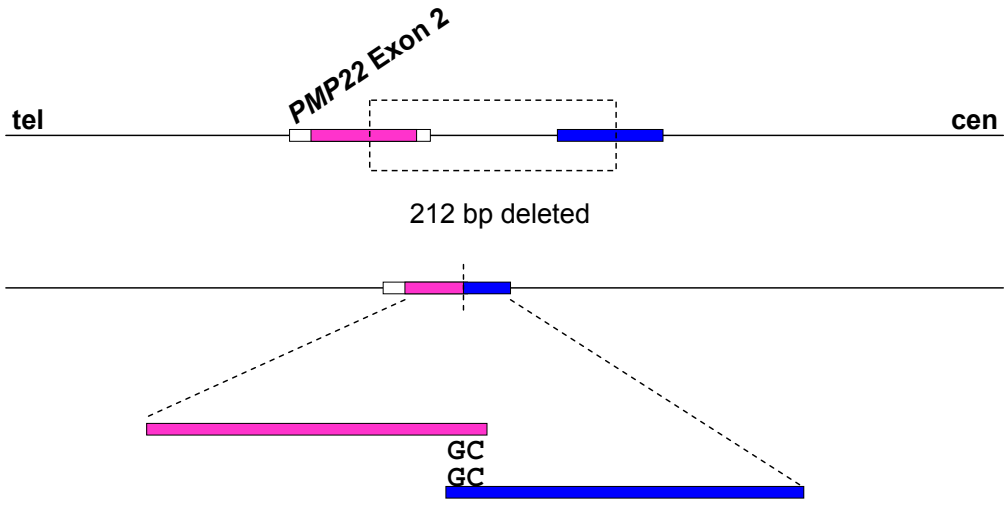
Patient A12

```
Ref    TCGTGGAGACGAACAGCAGCACCAGCACCGCGACGTGGAGGACGATGATACTCAGCAACAGGAGGAGCAT
A12    TCGTGGAGACGAACAGCAGCACCAGCACCGCGACG---------ATGATACTCAGCAACAGGAGGAGCAT
```



Patient A14

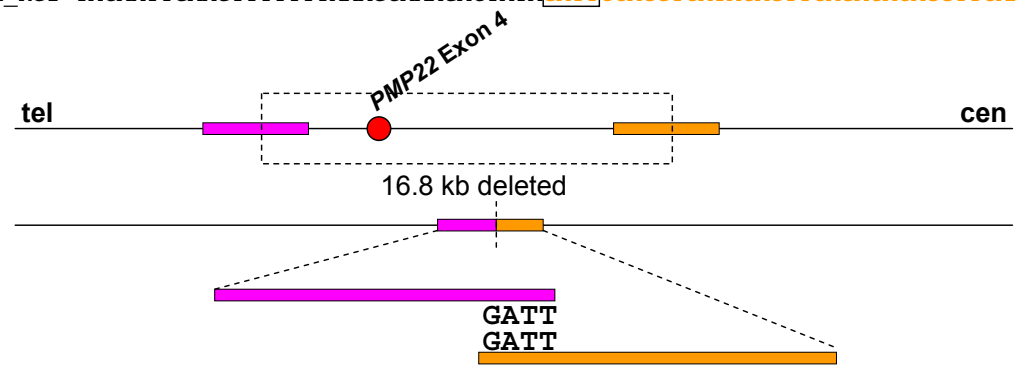
```
Dist_Ref GCACTCACGCTGACGATCGTGGAGACGAACAGCAGCACCAGCACCGCGACGTGGAGGACGATGATACTCA
A14      GCACTCACGCTGACGATCGTGGAGACGAACAGCAGCGCGGGTCAGGAGCCTTCGCGCCGCCTGCCGCCGA
Prox_Ref CCGGCCTGGCCCAGCGCCCGCAGCCCGACCGCCCGCGCGGGTCAGGAGCCTTCGCGCCGCCTGCCGCCGA
```



Supplementary Figure 9. Junction sequence analysis of small deletions within *PMP22* gene in patient A12 and A14. *PMP22* Exon 2 was represented by a white bar.

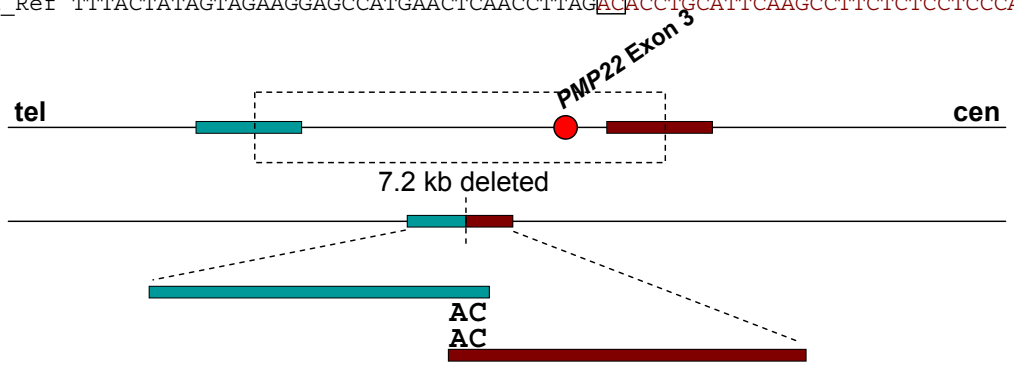
Patient A10

```
Dist_Ref CAATCCTCTCACCTCAGTCTTTCAAAGTGTTGGGATTACAGGCATTCGCCACCACACCTGGCCCAAATCC
A10_1    CAATCCTCTCACCTCAGTCTTTCAAAGTGTTGGGATTCCAGGTCATACAGTTCAGACACAGGTTCAAACA
Prox_Ref TACAATTGAAGTTTTTTTAAAGCAAAGACTATAGATTCCAGGTCATACAGTTCAGACACAGGTTCAAACA
```



Patient A11

```
Dist_Ref GGAAATTGTCTGGTGTCCTTGCCAAAATGACAGTGTCACCAAATTGATAAACAAGTCTGGGATCGTTTTG
A11_1    GGAAATTGTCTGGTGTCCTTGCCAAAATGACAGTGTCACACCTGCATTCAAGCCTTCTCTCCTCCCAGCA
Prox_Ref TTTACTATAGTAGAAGGAGCCATGAACTCAACCTTAGACACCTGCATTCAAGCCTTCTCTCCTCCCAGCA
```



Patient A21

```
Dist_Ref ACTTCAGGTCCTACCTCAATGCCTGCTCTGCTTTTAGGAAACCAGGGTACCTGCCCCAGGTTACCTAATT
A21_1    ACTTCAGGTCCTACCTCAATGCCTGCTCCGCCCGCGCGGGTCAGGAGCCTTCGCGCCGCCTGCCGCCGAG
Prox_Ref CGGCCTGGCCCAGCGCCCGCAGCCCGACCGCCCGCGCGGGTCAGGAGCCTTCGCGCCGCCTGCCGCCGAG
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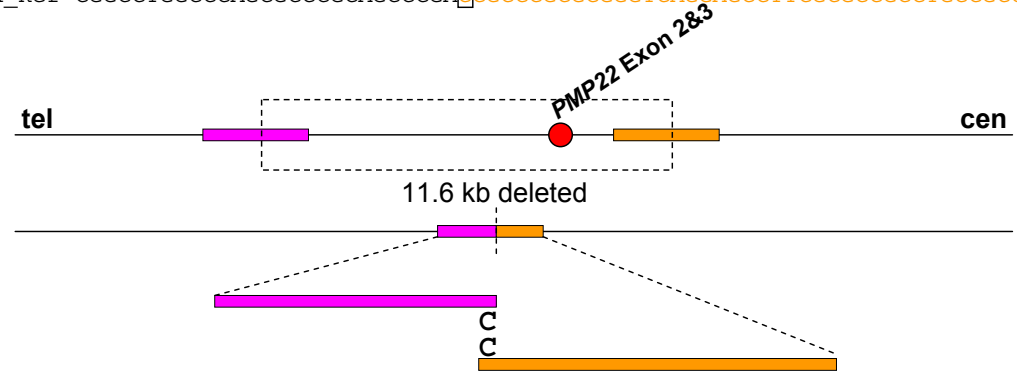

Supplementary Figure 10. Junction sequence analysis of small deletions within the *PMP22* gene in patients A10, A11, and A21.

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