Target enrichment nanopore sequencing with adaptive sampling can determine the structure of the small supernumerary marker chromosomes

Tasuku Mariya[1,2], Takema Kato[1], Takeshi Sugimoto[1], Syunsuke Miyai[1], Hidehito Inagaki[1], Tamae Ohye[1], Eiji Sugihara[3], Yukako Muramatsu[4,5], Seiji Mizuno[6], Hiroki Kurahashi[1]

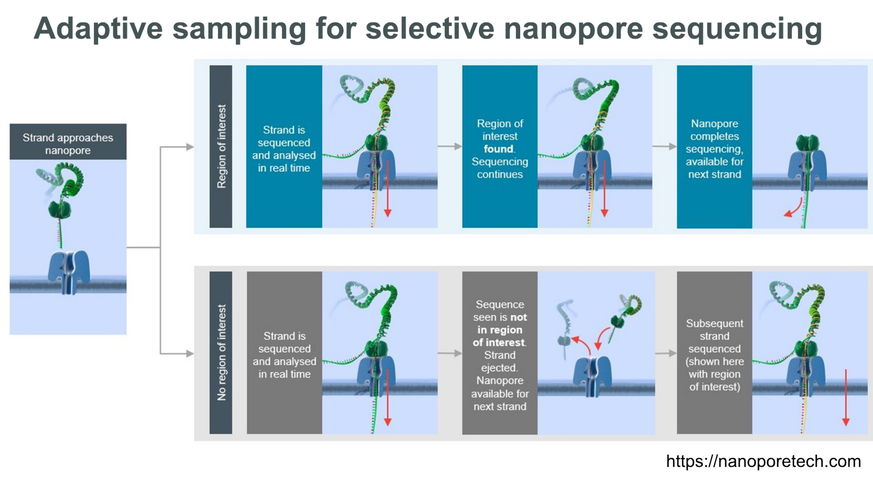
1. Division of Molecular Genetics, Institute for Comprehensive Medical Science, Fujita Health University, Aichi, Japan
2. Department of Obstetrics and Gynecology, School of Medicine, Sapporo Medical University, Sapporo, Japan
3. Center for Joint Research Facilities Support, Research Promotion and Support Headquarters, Fujita Health University, Aichi, Japan
4. Department of Pediatrics, Japanese Red Cross Aichi Medical Center Nagoya Daiichi Hospital, Nagoya, Japan.
5. Department of Pediatrics, Nagoya University Hospital, Nagoya, Japan.
6. Department of Clinical Genetics, Central Hospital, Aichi Developmental Disability Center, Aichi, Japan

Objective

Structural analysis of small supernumerary marker chromosomes (sSMCs) has revealed many sSMCs carrying complex chromosome structures with multiple fragments. However, structural analysis of sSMCs by short-read sequencing is inefficient because most sSMCs are mosaic and consist of a small region of the involved chromosome. In this study, we applied adaptive sampling using nanopore long-read sequencing technology to efficiently enrich the target region and determine the structure of sSMC.

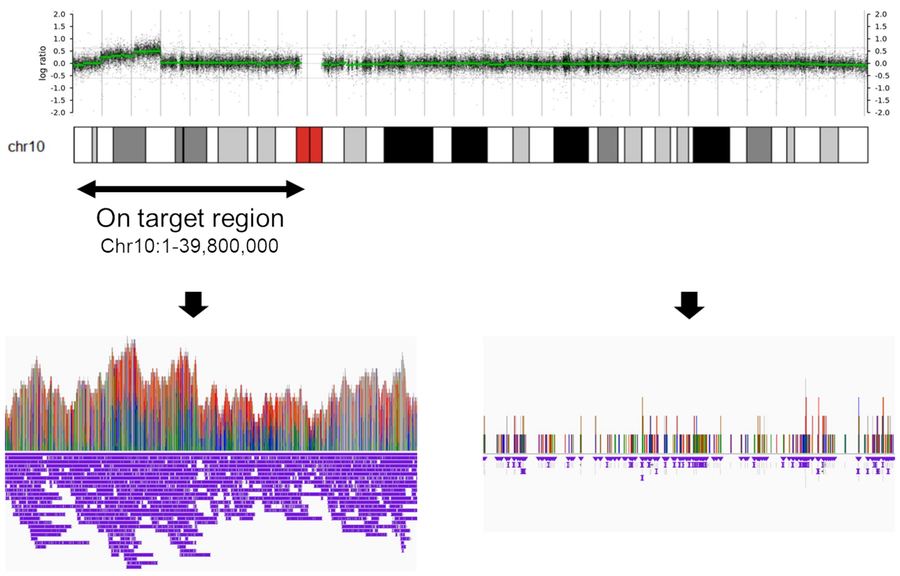
Methods

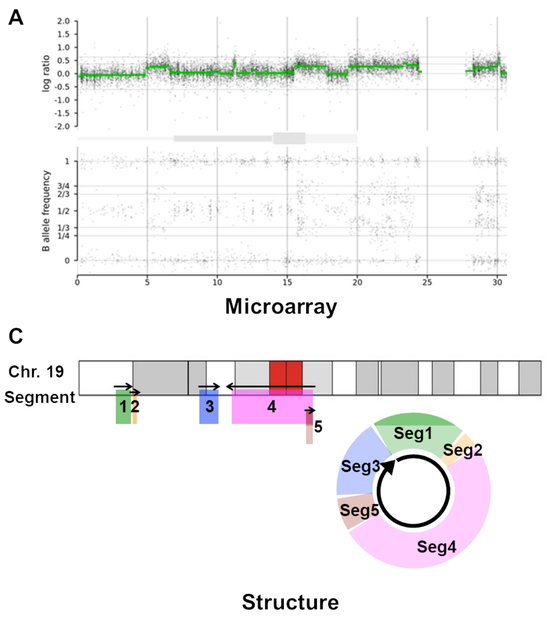
We applied adaptive sampling for two cases of sSMC with complex structural rearrangement in a single chromosome, which had already analyzed using chromosome cytogenetic microarray. To evaluate target enrichment, we performed both conventional pair-end short-read sequence, and nanopore sequencing with the adaptive sampling method. For enrichment reference, we used the FASTA file of the GRCh38 genome around a target region.

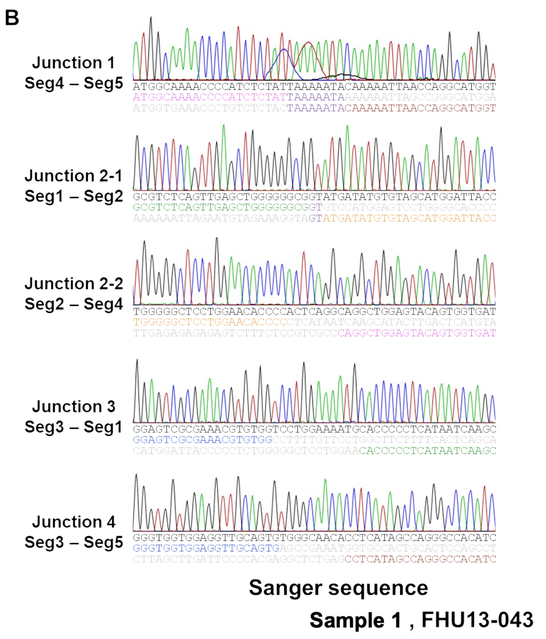


Results

Enrichment of on-target region (Case 2)







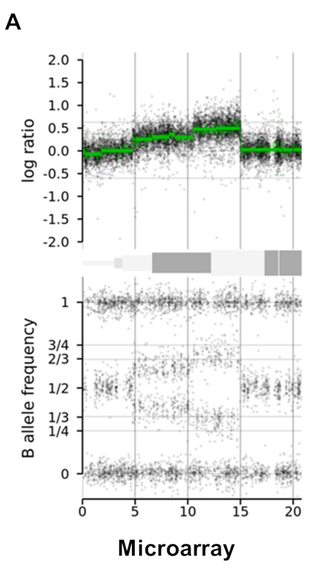
Short read, pair-end

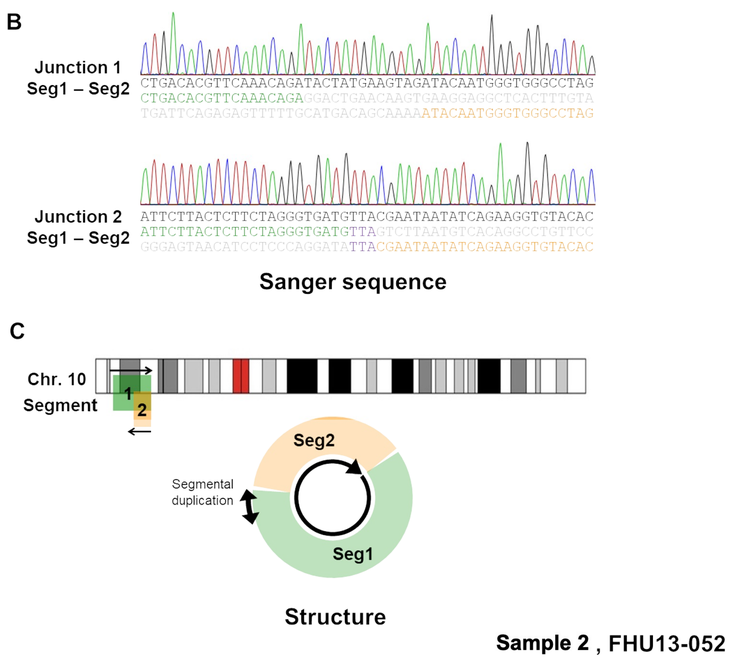
FHU13-43	Total	On-target (chr19:4,867,570-29,718,996)	Rate(%)
Read counts	31,098,474	353,017	1.14
Coverage	2,385,193,508	26,839,068	1.13
On-target coverage	-	1.08	-
FHU13-52	Total	On-target (chr10:4,709,424-14,979,491)	Rate(%)
Read counts	48,121,170	262,902	0.55
Coverage*	3,676,009,701	20,244,640	0.55
On-target coverage**	-	1.97	-

Nanopore, adaptive sampling

FHU13-43	Total	On-target (chr19:4,867,570-29,718,996)	Rate(%)
Read counts	5,432,286	42,282	0.78
Coverage	4,704,828,469	308,471,421	6.56
On-target coverage	-	12.41	-
FHU13-52	Total	On-target (chr10:4,709,424-14,979,491)	Rate(%)
Read counts	2,658,583	11,497	0.43
Coverage*	4,709,368,881	221,693,521	4.71
On-target coverage**	-	21.59	-

*Coverage: Total read bases of the region **On-target coverage: Total read bases on target / target size
Genomic positions of on-target region were result of microarray analysis based on GRCh38.





Conclusion

As a result of adaptive sampling, we could quickly determine the structure of sSMC. Adaptive sampling is a beneficial analysis method to determine the structure of complex chromosomal rearrangements.

CO I disclosure and statement

Tasuku Mariya
Affiliation: Department of Obstetrics and Gynecology, Sapporo Medical University of Medicine

There is no CO I to disclose. Oxford Nanopore Technologies products are not intended for use for health assessment or to diagnose, treat, mitigate, cure, or prevent any disease or condition.