

Customer feedback on products

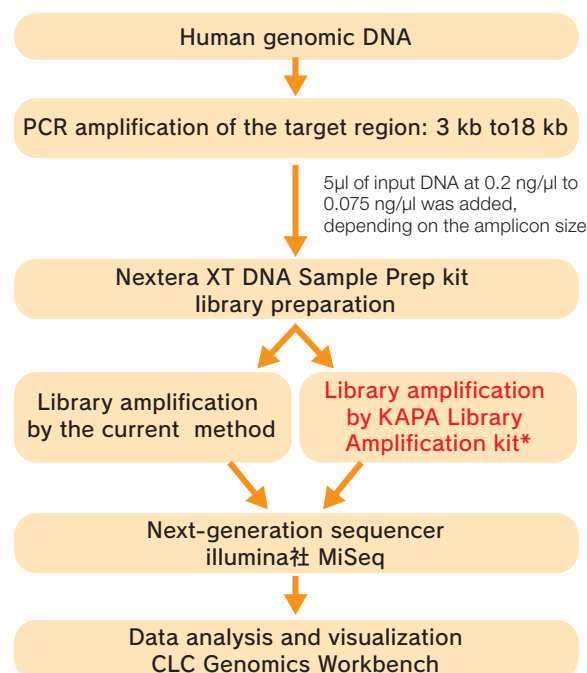
Product Name : KAPA Library Amplification Kit (KK2611, KK2612)
 Manufacturer : KAPA BIOSYSTEMS
 Application : Effective sequencing of long amplicons containing AT-rich regions targeting the PMS2 gene
 (one of the genes responsible for hereditary colorectal cancer)

The following data were provided by the courtesy of Dr. Gou Yamamoto, Dr. Miho Kakuta and Dr. Kiwamu Akagi at Division of Molecular Diagnosis and Cancer Prevention, Saitama Cancer Center, Japan.

Experimental method

The problem in sequencing long amplicons targeting the PMS2 gene (one of the genes responsible for hereditary colorectal cancer) was the low coverage resulting from fewer reads particularly in the AT-rich regions due to library amplification bias. Here, in the "tagmented library prepared from long amplicons by Nextera XT" prepared by the current method, we attempted to solve the above problem by amplifying the library using KAPA Library Amplification Kit (KAPA HiFi HotStart ReadyMix).

Workflow



* Library amplification by KAPA Library Amplification Kit

- ① After performing neutralization in the tagmentation step, 50µl (twice the volume of the reaction) of AMPureXP was added to 25µl of reaction, and cleanup was performed (washing twice with 80% ethanol)
- ② Elution was carried out with 15µl of 10 mM Tris-HCl (pH 8) or PCR-grade water
- ③ Library amplification was performed under the following conditions

Reaction composition

2xKAPA HiFi HS ReadyMix	25µL
Index 1 primer	5µL
Index 2 primer	5µL
Library DNA	15µL
	50µL RXN

PCR cycle

Initial Extension	72°C	3min	14cycles
Denaturation	98°C	30sec	
Denaturation	98°C	10sec	
Annealing	63°C	30sec	
Extension	72°C	3min	
Hold	10°C		

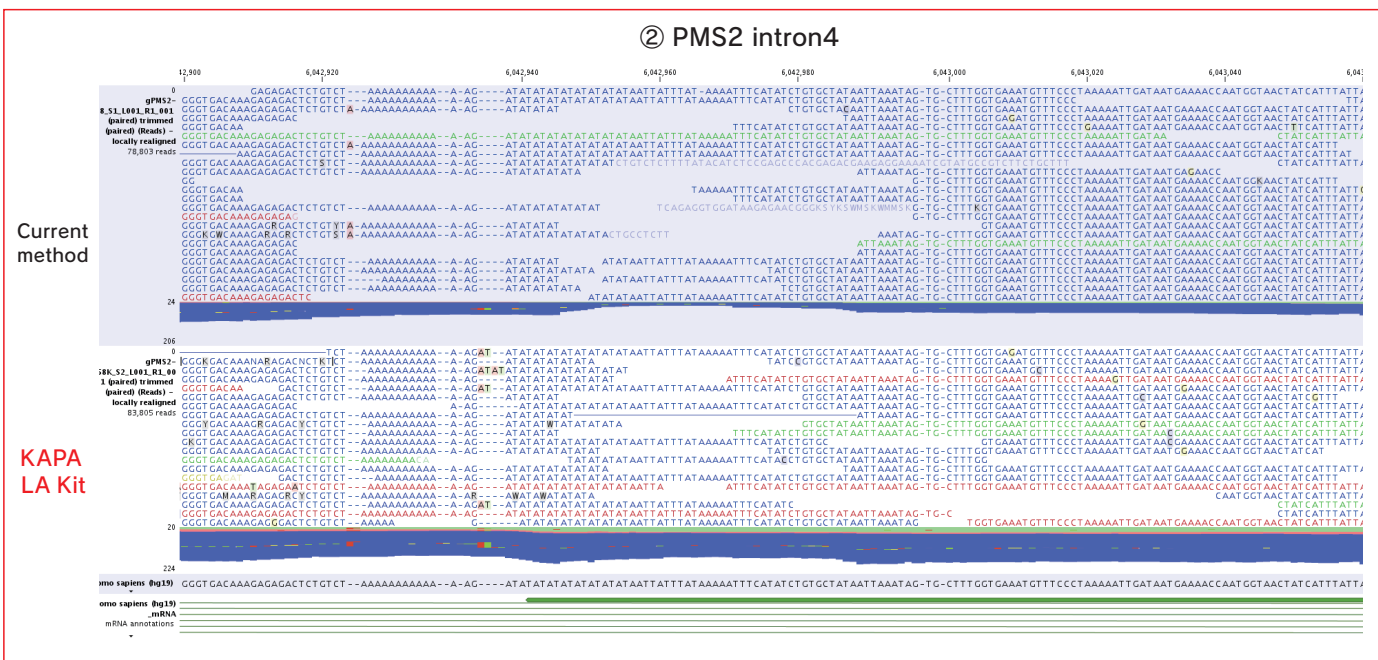
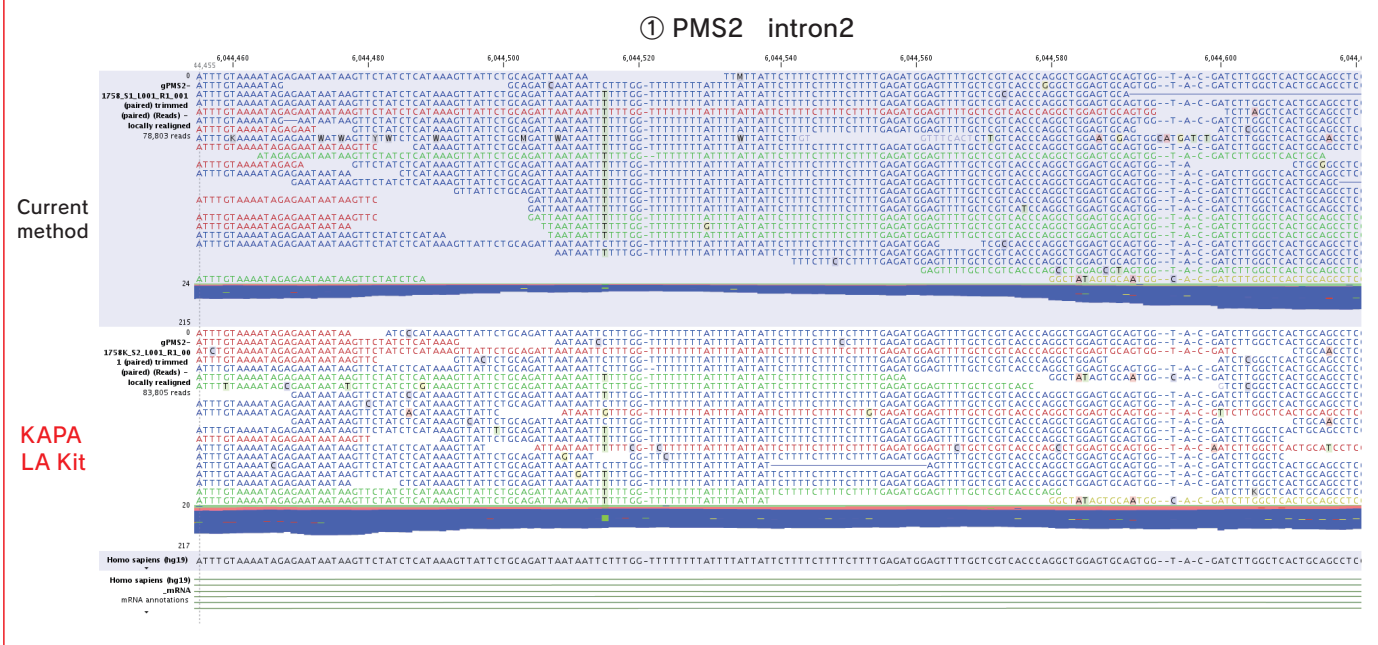
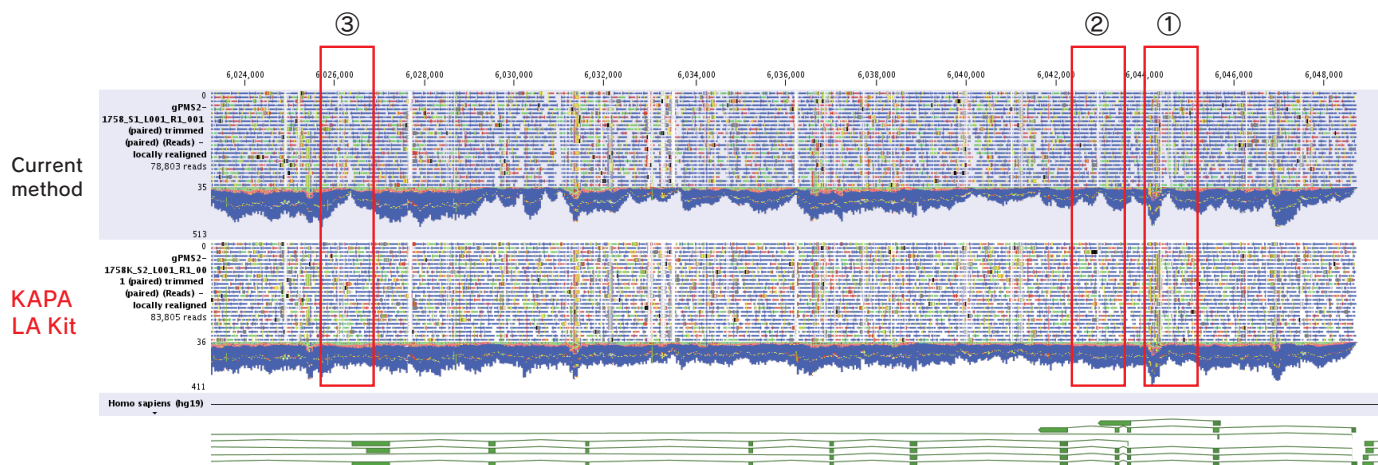
Existing method



Sequencing results of long amplicons targeting the PMS2 gene
 Fewer reads in AT-rich regions (e.g. regions indicated by arrows) resulted in low coverage.

Results

Libraries amplified using KAPA Library Amplification (LA) Kit showed improved coverage in AT-rich regions, resulting in more uniform coverage.

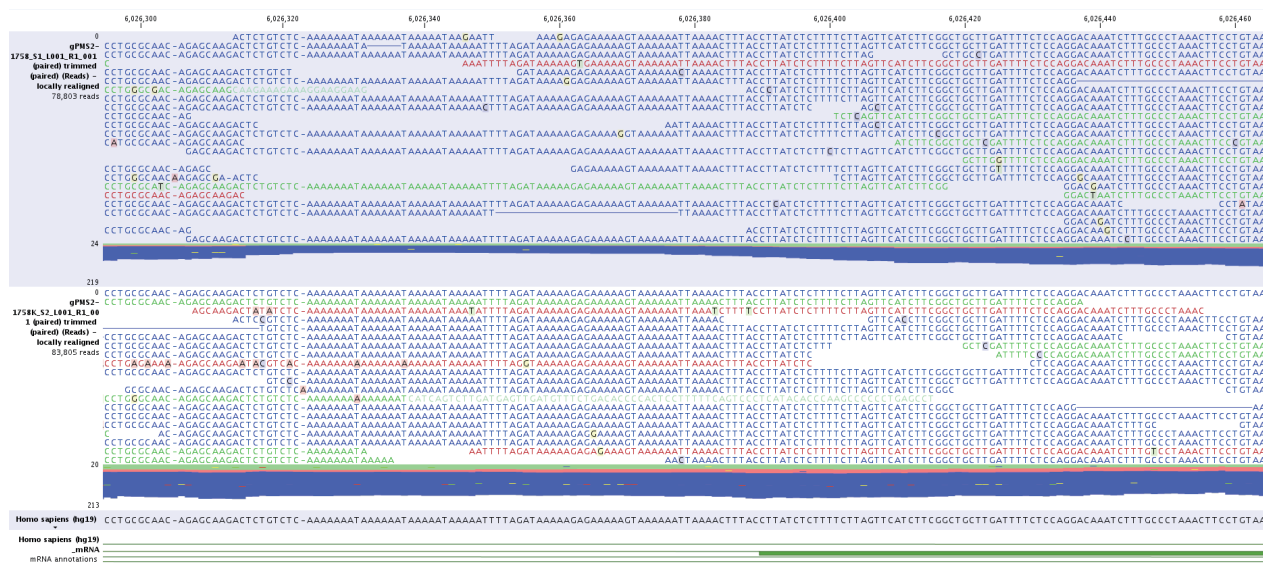


Results

③ PMS2 intron11_Exon11

Current method

KAPA LA Kit



The use of KAPA LA Kit also resulted in improvement of coverage in libraries amplified from a different sample (sample B at bottom).

PMS2 Exon1_Exon11

Sample A

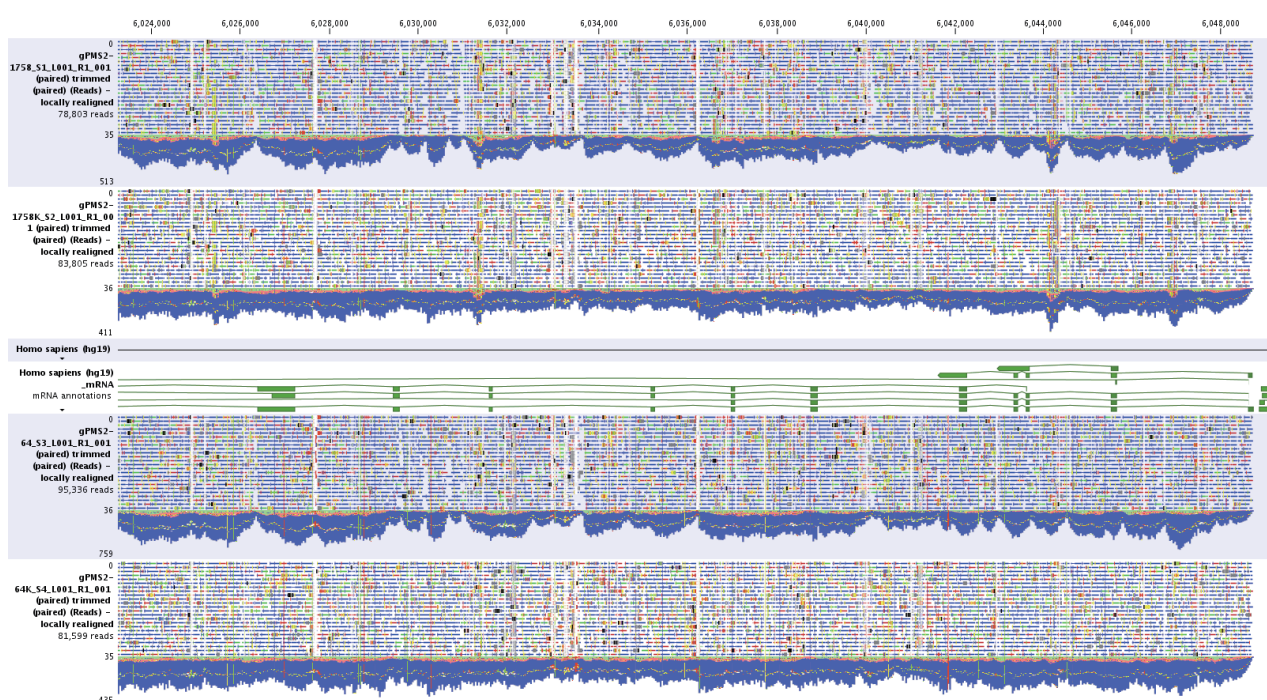
Current method

KAPA LA Kit

Sample B

Current method

KAPA LA Kit



<Customer's comments>

There was a substantial improvement in the results when using the KAPA Library Amplification Kit (KAPA HiFi HotStart ReadyMix) for amplification of AT-rich sequences.