NIPPON Genetics Co.,Ltd.

Customer feedback on products

Product Name : KAPA Hyper Prep Kit (KK8500, KK8502)
Manufacturer : KAPA BIOSYSTEMS
Application : Exome sequencing from small input-volume of FFPE genome DNA (50ng) using SureSelect XT (Agilent Technologies, Inc.)

All data here are available by courtesy of Dr. Hidewaki Nakagawa and Dr. Kazuhiro Maejima, Laboratory for Genome Sequencing Analysis, RIKEN Center for Integrative Medical Sciences, Japan. We are deeply grateful for their cooperation.

Introduction

Next-generation sequence analysis of genomic DNA extracted from formalin-fixed paraffin-embedded (FFPE) tissue samples is expected to play a very important role as an application, especially in the field of clinical research. However, it is also a well- known fact that this type of analysis is very difficult to perform due to such causes as:

- Degradation of DNA (e.g., fragmentation and/or substitution of cytosine to uracil due to hydrolysis)
- Protein-DNA cross-linking through formaldehyde
- Low efficiency of DNA extraction (only small amounts of DNA available)

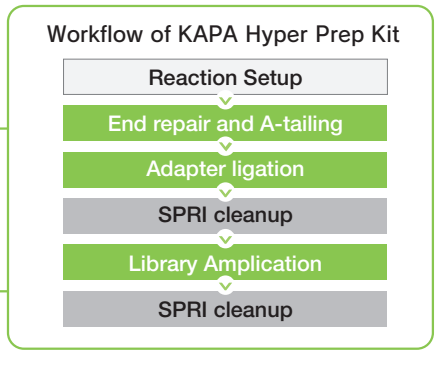
In general, it is difficult to prepare an enough volume of library for sequence analyses when using genomic DNA from FFPE. This will result in higher duplication rates and thus insufficient coverage and lower sequence depth.

In the study reported here, WES (Whole-exome sequencing) analysis of FFPE tissue samples were conducted by combining the SureSelect XT target enrichment system (Agilent Technologies) with KAPA Hyper Prep Kit, and very promising improvement was obtained.

Workflow of WES library preparation using KAPA Hyper Prep Kit and SureSelect XT target enrichment system (Agilent Technologies, Inc.)

① Extraction of genomic DNA (Pretreatment, including deparaffinization of FFPE samples, and DNA extract were conducted using QIAamp DNA FFPE Tissue Kit)
Size distribution check with Agilent TapeStation or Bioanalyzer
↓
② Fragmentation of genome DNA
↓
③ End-repair, dA-tailing
↓
④ Adapter-ligation

KAPABIOSYSTEMS KAPA Hyper Prep Kit



↓
⑤ Purification with AMPureXP
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⑥ Library amplification (pre-capture)

KAPABIOSYSTEMS KAPA Hyper Prep Kit

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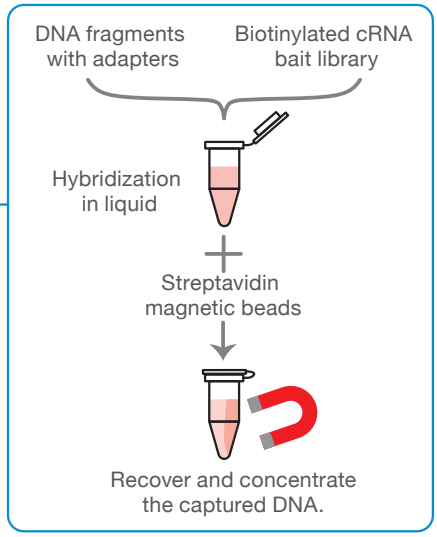
⑦ Purification with AMPureXP
↓
⑧ Size distribution check and validation of concentration with Agilent TapeStation or Bioanalyzer
↓
⑨ Concentration of pre-capture PCR product
↓
⑩ SureSelect Library Capture

Agilent Technologies
SureSelect XT
Human All Exon V5



↓
⑪ Library amplification (post-capture)
↓
⑫ Purification with AMPureXP
↓
⑬ Size check and concentration validation with Agilent TapeStation or Bioanalyzer
↓
⑭ Library pool
↓
⑮ Next-generation sequence (illumina HiSeq2500)

Production of Pre-capture PCR product from FFPE genomic DNA using KAPA Hyper Prep Kit and SureSelect Adapter

① Preparation of FFPE genomic DNA

Measurement of concentration by Picogreen
Quality evaluation of FFPE DNA by qPCR
Preparation of 50 ng to 200 ng of FFPE genomic DNA to make 50 µL (using 1x Low TE to increase the volume)

② Covaris fgragmentation

Fgragmentation of DNA using Covaris according to the protocol for SureSelect XT Kit
Cycle setting: 3 cycles (60sec x 3 cycles; Total 180sec)

Example: Setting for Covaris S2

Duty Cycle	10%
Intensity	5
Cycles per Burst	200
Time	60sec, **3cycles**
Setting mode	Frequency sweeping
Temperature	4℃ ～ 7℃

③ End Repair & A-Tailing

End repair and A-tailing according to the protocol for KAPA Hyper Prep Kit

Reaction composition

Fragmented DNA	50µL
End Repair & A-Tailing Buffer	7µL
End Repair & A-Tailing Enzyme	3µL
Total Volume per well	60µL

Thermal Cycler settings

20℃	30min	1 cycle
65℃	30min	
4℃	∞	

The HotTop of the thermal cycle should be set to OFF.

④ Adapter Ligation

Adapter ligation according to the protocol for KAPA Hyper Prep Kit

Reaction composition

End Repair & A-Tailing reaction Product	60µL
Nuclease-Free Water (*)	5µL
Ligation Buffer	30µL
DNA Ligase	10µL
SureSelect Adapter Oligo Mix (*)	5µL
Total Volume per well	110µL

* Adapter may be undiluted, diluted or increased depending on the quality of FFPE gDNA. When adapter is increased, Nuclease-free water should be decreased.

Thermal Cycler settings

20℃	15min	1 cycle
4℃	∞	

The HotTop of the thermal cycle should be set to OFF.

⑤ Post-Ligation Clean up

Cleanup according to the protocol for KAPA Hyper Prep Kit
For elution, 20 µL of nuclear-free water should be used.

⑥ Library Amplification (PreCapture PCR)

PCR conducted after changing the primer volume to 2.5 µL and the annealing temperature to 65°C

Reaction composition

Adapter-Ligated Library	20 µL
2x KAPA Hi-Fi HotStart Ready Mix	25 µL
SureSelect Primer	2.5µL
SureSelect ILM Indexing PreCapture PCR Reverse Primer	2.5µL
Total Volume per well	50 µL

Thermal Cycler settings

98℃	45sec	1 cycle
98℃	15sec	14 cycle (*)
65℃	30sec	
72℃	30sec	
72℃	1min	1 cycle
4℃	∞	1 cycle

* PCR cycle should be optimized according to the initial volume of DNA. SureSelect hybridization requires 750 ng of yield.

⑦ Post-Amplification Clean up

Cleanup of PCR product according to the protocol for KAPA Hyper Prep Kit
Elution conducted with 30µL of nuclease-free water

⑧ Evaluation by TapeStation or Bioanalyzer

Evaluation of pre-capture PCR products by TapeStation D1000 or Bioanalyzer DNA1000

⑨ Concentration of pre-capture PCR products

Concentration of 750 ng of pre-capture PCR product (recommended: 750 ng; minimum: 500 ng) using SpeedVac DNA 120 (Thermo Scientific)
Dissolution with 3.4 µL of nuclear-free water

⑩ From hybridization to preparation of capture library

Start with hybridization according to the protocol for SureSelect XT Kit using Agilent SureSelect XT Human All Exon V5 and hybridization reagent

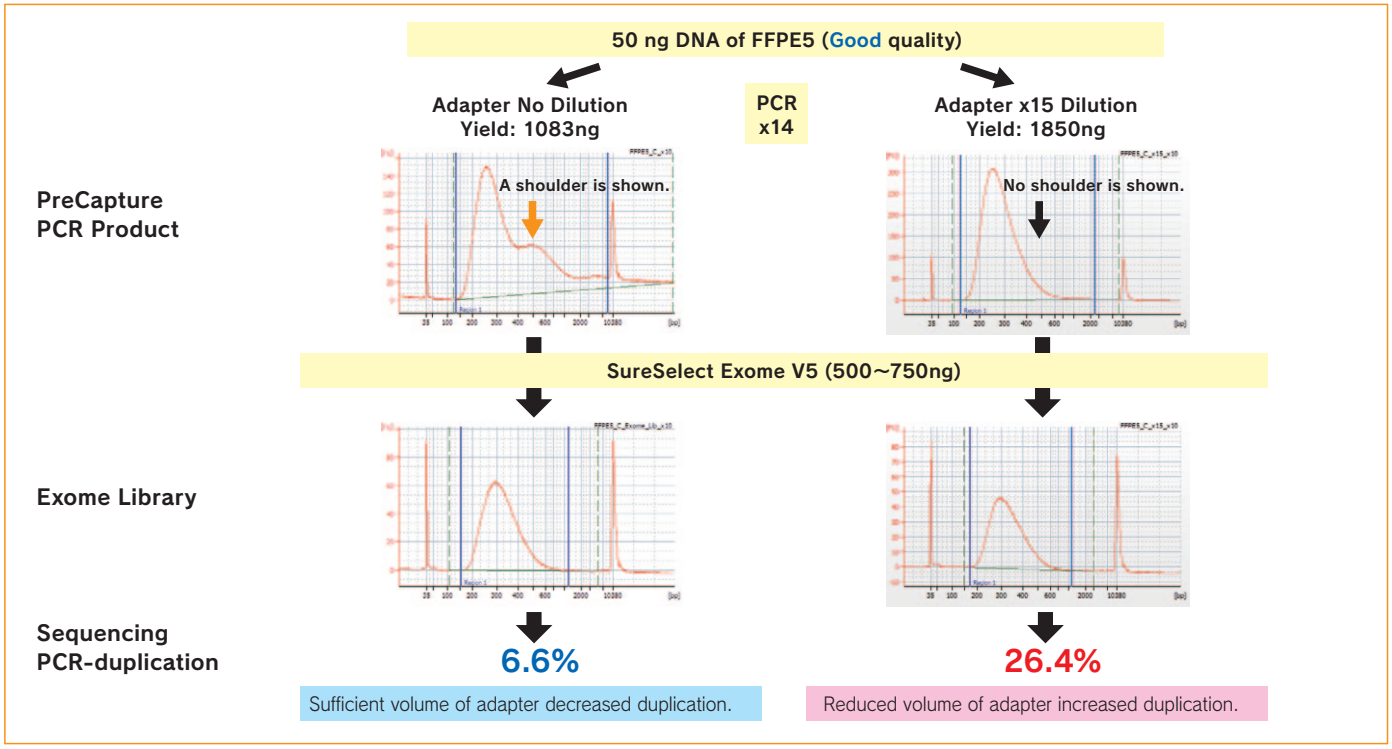
Process after ⑩ should be operated according to the protocol for Agilent SureSelect XT kit.

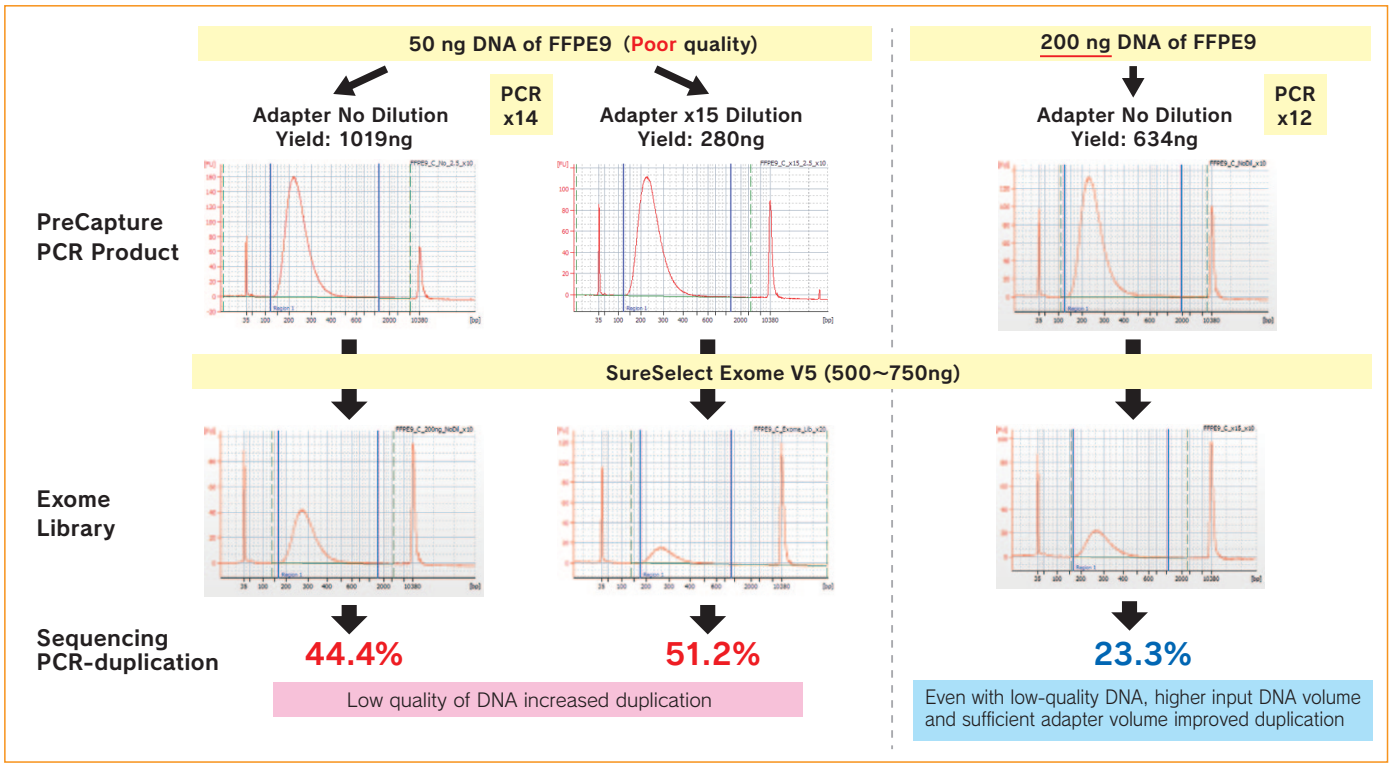
Evaluation of SureSelect XT library

Capture library reagent : SureSelect XT Human All Exon V5

Sample Name	DNA quality *	Input DNA [ng]	PCR Cycles	Adapter dilution	Pre-capture Yield [ng]	comment	Hybridization amount [ng]	Post-capture Yield [ng]
FFPE7_Cancer	Good	50	14	No	1179	2nd peak around 400-1000bp	750	264
FFPE5_Cancer	Good	50	14	No	1083	2nd peak around 400-1000bp	500	402
FFPE2_Cancer	Medium	50	14	No	1340	2nd peak around 400-1000bp	500	157
FFPE3_Cancer	Medium	50	14	No	1694	2nd peak around 400-1000bp	500	122
FFPE4_Cancer	Medium	50	14	No	1231	2nd peak around 400-1000bp	750	460
FFPE9_Cancer	Poor	50	14	No	1019		500	114
	Poor	200	12	No	634		634	187

* Use original qPCR method for FFPE DNA quality evaluation, not published.





Results of NGS (interim result)

Sample Name	DNA quality *	Input DNA [ng]	Initial fastq	Mapped	mapping rate	duplication rate	on target rate
FFPE7_Cancer	Good	50	67,960,522	62,814,748	92.4%	**8.2%**	66.8%
FFPE5_Cancer	Good	50	70,034,926	67,040,360	95.7%	**6.6%**	66.0%
FFPE2_Cancer	Medium	50	72,517,124	67,819,596	93.5%	**14.0%**	67.9%
FFPE3_Cancer	Medium	50	63,595,416	59,910,508	94.2%	**15.8%**	69.1%
FFPE4_Cancer	Medium	50	63,394,208	60,942,730	96.1%	**7.2%**	65.0%
FFPE9_Cancer	Poor	50	109,083,544	91,226,884	83.6%	**44.4%**	57.2%
	Poor	200	69,105,486	63,668,336	92.1%	**23.4%**	66.6%

* Use original qPCR method for FFPE DNA quality evaluation, not published.

Conclusion

- Exome sequencing is possible from 50 ng of FFPE DNA (biopsy samples).
- Performance may vary depending on the “quality” of FFPE evaluated by qPCR.
- Using extra volume of adapter may increase the yield and may be able to control the duplication.
- Existence of a “hump” during the library production may associate with the ratio of PCR duplication and other factors which decide feasibility of FFPE DNA sequencing.

<Customer's comments>

NGS analysis of FFPE samples is required for analyses of rare disorders (tumors), and for utilizing of a large amount of clinical data. For the development of the technique to clinical sequencing, methods suitable for NGS analysis of minute amounts of DNA taken from FFPE, such as biopsy samples taken in the past, must be established. We had hesitated to use DNA taken from FFPE samples because they tend to lead to a significantly high level of PCR duplication (more than 50% in some cases), and their data tend to be of low quality. The efficiency of KAPA Hyper Prep Kit in library construction was surprisingly high. There is no doubt that KAPA Hyper Prep Kit will make a great impact on the methods of clinical sequencing using FFPE. In the clinical sequencing, cancer tissue samples for example, are often available only in the form of biopsy FFPE. In such cases, KAPA Hyper Prep Kit is a very promising tool because it allows efficient construction of NGS library even from a several slices of biopsy FFPE (50–100 ng).

<Comment from Nippon Genetics Co., Ltd. >

We would like to show our deep gratitude to Dr. Nakagawa and Dr. Maejima for their performance to obtain good results in a short time.
In general, construction of NGS libraries from a minute amount of DNA samples, such as FFPE samples, tends to form dimer due to the extra amount of adaptor. Therefore, an appropriate amount of adaptor must be examined for the template. In addition, obtaining enough volume of library for sequencing is difficult, and as a result, the duplication rate will be high in the analysis of sequence data, causing a problem of insufficient levels of coverage and sequence depth.
With large improvement of reagents and protocol, KAPA Hyper Prep Kit has achieved very high efficiency for adapter ligation. In Dr. Nakagawa and Maejima’s study, this superior performance should have contributed to the effective construction of the library without dilution of DNA even with FFPE-derived genome DNA, thus low and favorable duplication rates.

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