NIPPON Genetics Co.,Ltd.

Customer feedback on products

Product Name : **KAPA Hyper Prep Kit (KK8500, KK8502)**
Manufacturer : **KAPA BIOSYSTEMS**
Application : **Sequence determination of MtDNA from the ear specimen of *Tragulus kanchil* which was immersed in ethanol for long time**

All data in this paper was provided by the kindness of Dr. Taichi Ishige, The NODAI Genome Research Center, Tokyo University of Agriculture, Japan. We would like to express our deep appreciation for the contributions.

Introduction

DNA extracted from the sample which has been stored for a long time has generally less amount and poor quality. Here, the KAPA Hyper Prep Kit was used to be able to prepare a library from the sample having less amount and poor quality to obtain data without any problems and to report them.

Condition for experiment

Amount of the sample at the beginning: total DNA, see Table 2
Biological species: *Tragulus kanchil*
Sample material: the ear specimen of *Tragulus kanchil* in south-eastern Asia which was immersed in ethanol for 18 years
DNA extraction : QIAamp DNeasy Blood & Tissue Kit
Method for fragmentation of DNA : Covaris
Library preparation : KAPA Hyper Prep Kit for illumina
Adaptor* : KAPA Adapter Kit
Sequencer : HiSeq2500 (illumina)
*Supplementation by Nippon Genetics: At present, we recommend FastGene™ Adapter Kit (for illumina) (Cat. No. FG-NGSAD24).

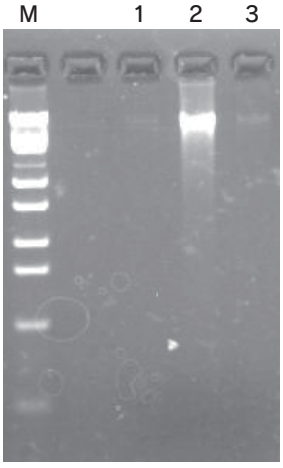
Working flow for preparing the library

① For the extraction of the genome DNA, QIAamp DNeasy Blood & Tissue Kit were used. Check of the property of DNA by agarose gel electrophoresis and measurement of the concentration by Qubit
↓
② Fragmentation of the genome DNA (covaris: 500bp)
↓
③ End-repair, dA-tailing
↓
④ Adapter-ligation
↓
⑤ Purify with AMPureXP
↓
⑥ Amplification of the library
↓
⑦ APurify with AMPureXP
↓
⑧ Size Selection by Pippin Prep
Gel cassette: 2.0%
↓
⑨ Check the distribution in the size by Agilent Bioanalyzer
↓
⑩ Measurement of the concentration by Kapa Library Quantification Kits
↓
⑪ Pool the library (added 1%PhiX)
↓
⑫ Next generation sequencing (illumina HiSeq 2500)

KAPABIOSYSTEMS
KAPA Hyper Prep Kit

Result

① For the extraction of the genome DNA (QIAamp DNeasy Blood & Tissue Kit) is used. Check of the property of DNA by agarose gel electrophoresis and measurement of the concentration by Qubit



M: 2.5kb ladder
1: Ear specimen 1 of chevrotain
2: Ear specimen 2 of chevrotain
3: Ear specimen 3 of chevrotain

Figure 1. Agarose gel electrophoresis of the extracted DNA

Table 1. Extracted amount of DNA measured by Qubit

name	Qubit (ng/μl)	Bio spec (ng/μl)
1	4.62	11.2
2	9.8	12.6
3	too low	1.8

Table 2. Amount of DNA used for preparing the library

name	input DNA (μl)	input DNA (ng)	
		Qubit	Bio spec nano
1	20	92.4	224
2	10.2	99.96	128.52
3	40	too low	72

Result

② Fragmentation of the genome DNA (covaris: 500 bp)

Table 3. Amount of DNA used for preparing the library

name	Qubit (ng)
1	too low
2	1.66
3	0.41

④ Adapter-ligation

Table 4. Amount of Adapter in the Adapter-ligation

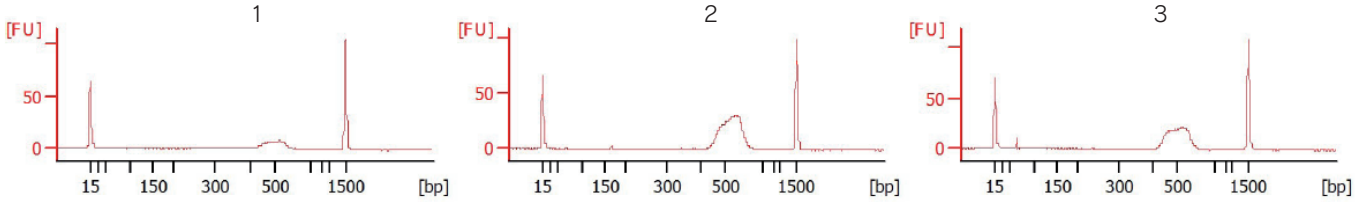
name	Concentration of the stock	Amount to be added or one reaction (total 110 μL)	Final concentration
1	750nM	5μL	34nM
2	15μM	5μL	680nM
3	7.5μM	5μL	340nM

⑥ Amplification of the library

Table 5. Number of PCR cycle

name	Number of PCR cycle
1	12
2	8
3	10

⑧ Size Selection by Pippin Prep



⑫ Next generation Sequencing (illumina HiSeq 2500)

Table 6. Result of sequencing

Sample Name	Sample Ref	Index	Control	Yield (Mbases)	% PF	# Reads	% of raw clusters per lane	% Perfect Index Reads	% One Mismatch Reads (Index)	% of >= Q30 Bases (PF)	Mean Quality Score (PF)
1	N	GCCAAT	N	10,367	100	103,666,038	35.02	98.99	1.01	93.73	36.58
2	N	ATCACG	N	9,594	100	95,938,014	32.41	99.19	0.81	93.39	36.48
3	N	CTTGTA	N	9,048	100	90,476,788	30.56	99.1	0.9	93.41	36.46

Table 7. Result of *de novo* assembly

Sample Name	N75	N50	N25	Minimum	Maximum	Average	Count	Total
1	1,119	1,268	1,661	651	175,450	1,455	33,956	49,418,356
2	1,136	1,283	1,540	712	31,211	1,333	81,957	109,238,541
3	1,132	1,273	1,522	644	48,192	1,334	73,867	98,512,409

Table 8. Result of *de novo* assembly (continued)

Sample Name	Consensus length (bp)	total reads	Average coverage
1	16,356	29,814	178.13
2	16,329	52,264	312.95
3	16,312	34,226	205.3

● Summary

Even in the sample 3 which amount at the time of the beginning is unknown, extremely high quality read information could be obtained to be 93% or more in read of Qscore greater than 30.
Almost full length of MtDNA could be determined in all samples by *De Novo* assembly in CLC Genomics Workbentch.

<Customer's comments>

Since a library could be prepared from DNA which has extremely low concentration and is poor in its condition, we believe that this method is useful for preparing a library of ancient DNA.
Since the loss of fragmented DNA is large due to the use of Covaris, we consider that this problem has to be solved.

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