NIPPON Genetics Co.,Ltd.

Customer feedback on products

Product Name : KAPA Hyper Prep Kit (KK8500, KK8502, KK8504)
Manufacturer : KAPA BIOSYSTEMS
Application : *In situ* Hi-C Seq using 10^5 killifish embryo cells

The following data were provided by the courtesy of Dr. Masahiko Kumagaya and Professor Hiroyuki Takeda of the Laboratory of Embryology, Department of Biological Science, Graduate School of Science, The University of Tokyo, Japan.

Method

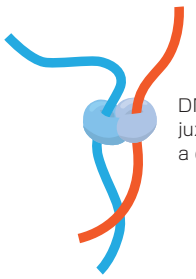
The improved performance and the expanded versatility of next-generation sequencers have led to the development of several new analytical application methods in various areas. The 3C (chromosome conformation capture) method, which has been developed for analyzing the three-dimensional conformation of chromosomes, enables detection of DNA regions closely juxtaposed in the chromosomes.[1)] The dilution Hi-C Seq method[2)], developed from the 3C method, and a further improved method, in situ Hi-C Seq method[3)] (the existing method), enable analysis with up to 10^7 and 10^6 killifish embryo cells, respectively.
In this study, we attempted an analysis with 10^5 cells using KAPA Hyper Prep Kit capable of preparing libraries from trace amounts of samples (1ng -).

1) Dekker J, *et al*, Science,295, 1306-1311, 2002
2) Lieberman-Aiden, E., *et al*. Science, 326, 289-293, 2009
3) Suhas S.P. Rao *et al*, Cell 159, 1665-1680, 2014

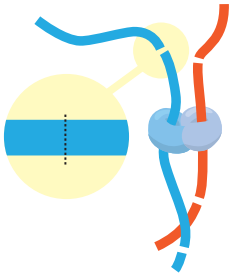
Species	: Killifish embryo cells
Initial sample volume	: 10^5 killifish embryo cells (Actually, we started from 2×10^5 cells and divided them into two groups in a particular step for comparison between the existing method and KAPA Hyper Prep Kit)
Library preparation kit	: KAPA Hyper Prep Kit
Sequencer	: HiSeq1500 (illumina)

in situ Hi-C Seq workflow

① **Cell preparation and DNA crosslinking**

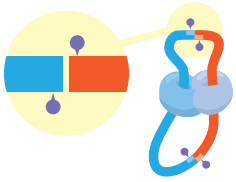


② **Cell lysis and digestion by restriction enzyme**

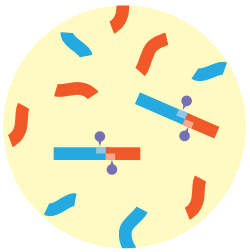


③ **Marking of DNA ends**

④ **Ligation**



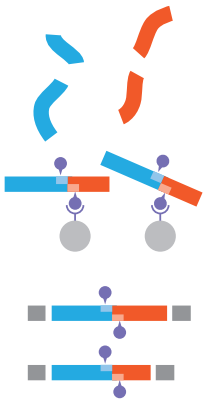
⑤ **DNA fragmentation and size selection**



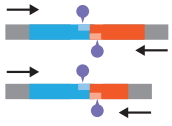
⑥ **Biotin pulldown and library preparation**

*In the biotin pulldown step using streptavidin beads, the sample cells were divided into two groups; in the subsequent library preparation steps, the two groups were treated separately using either the existing method or KAPA Hyper Prep Kit.
In the method using the KAPA Hyper Prep kit, after the biotin pulldown step, the sample cells were re-suspended in 50μl of distilled water, and library preparation was performed using the following reaction composition according to the protocol.

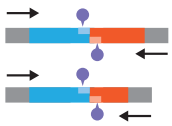
Sample	50μl
End Repair & A-Tailing Buffer	7μl
End Repair & A-Tailing Enzyme Mix	3μl



⑦ **Library amplification**



⑧ **Sequencing (Paired End)**



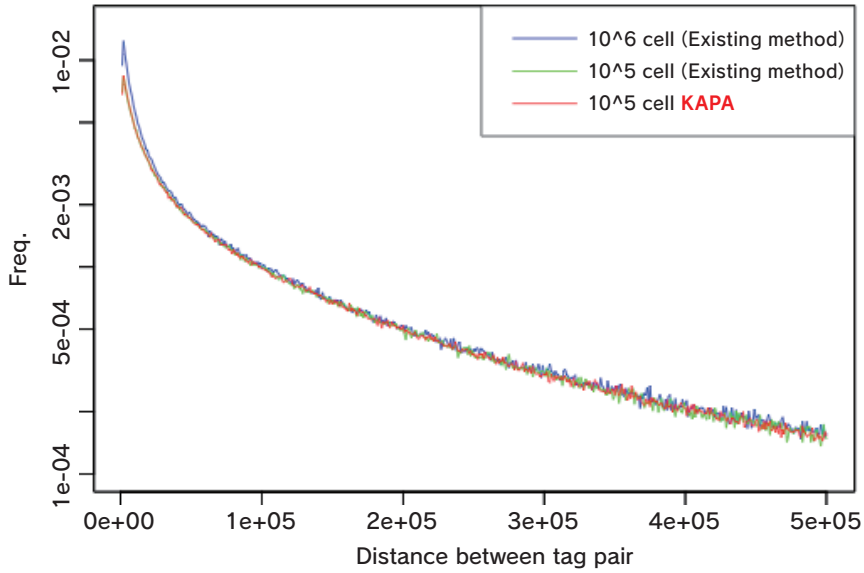
Results

Table 1 DNA amounts (amounts of DNA before and after the library preparation step)

	current method 10^6 cells	current method 10^5 cells	KAPA Hyper Prep 10^5 cells
Before library preparation (before step ⑥ in the workflow) total DNA amount (Qubit)	4,217ng	296ng	296ng
After library preparation (after step ⑥ in the workflow) total DNA amount (Qubit)	388ng (/50μl)	71.1ng (/50μl)	65.7ng (/50μl)
Number of library amplification cycles	7	8	8
Amount of library after library amplification (after step ⑦ in the workflow)	668ng	189ng	280ng

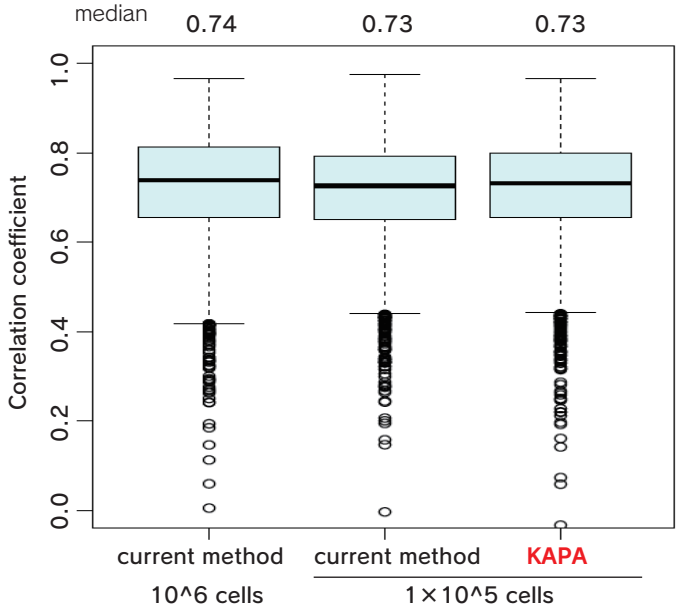
KAPA Hyper Prep kit could yield 1.5 times larger amount of library compared to the current method from 10^5 cells.

Fig.1 Frequency of paired end tag distance



The distribution patterns were nearly the same in two samples (10^5 cells and 10^6 cells).

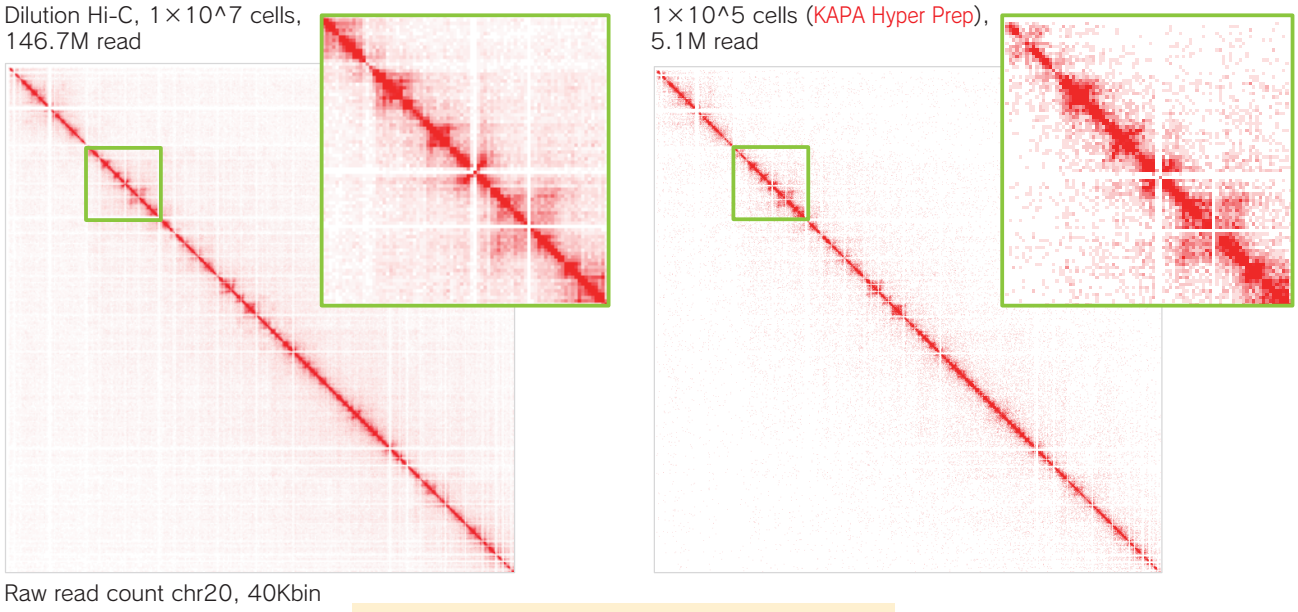
Fig.2 Distribution of correlation coefficient to dilution Hi-C with 10^7 cells
(Comparison of distribution of coefficients of correlation with the dilution Hi-C Seq data obtained with 10^7 cells)



The genome was divided into 100-kb windows, and the contact data for the individual windows were examined for their correlation with the dilution Hi-C Seq data obtained with 10^7 cells. Equivalent results could be obtained with smaller number of cells, i.e. 10^6 or 10^5.

Results

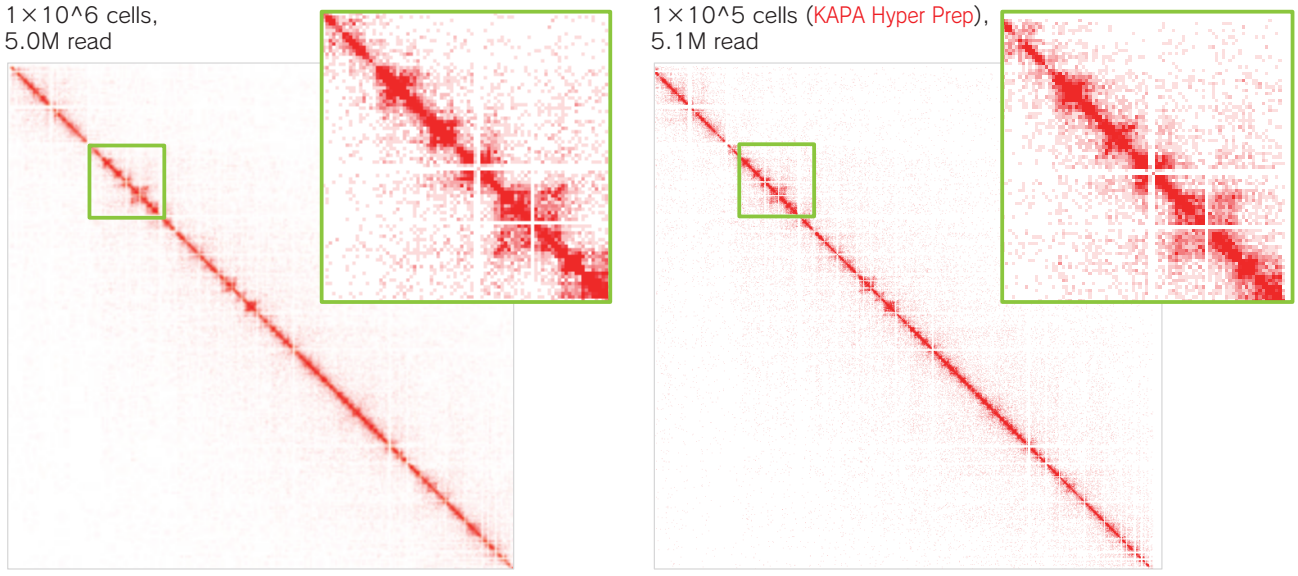
Fig.3 **Comparison between Dilution Hi-C Seq results obtained with 10^7 cells and *in situ* Hi-C Seq (KAPA Hyper Prep) results obtained with 10^5 cells**
Contact matrix of 40 kg bin (Contact matrix)



Raw read count chr20, 40Kbin

Both methods yielded favorable contact patterns.

Fig.4 **Comparison between *in situ* Hi-C Seq results obtained with 10^6 cells and *in situ* Hi-C Seq (KAPA Hyper Prep) results obtained with 10^5 cells**
Contact matrix of 40 kg bin (Contact matrix)



Raw read count chr20, 40Kbin

Both methods yielded favorable contact patterns.

In this study, the analysis results obtained with 10^5 cells using KAPA Hyper Prep Kit were similar to those obtained with 10^7 or 10^6 cells. It is expected that the future application of the method enables comprehensive analysis of the behavior of killifish chromosomes during initial development.

<Customer's comments>

KAPA Hyper Prep Kit requires few purification steps, so it could save time and labor and was easy to perform.
We have conventionally used the library amplification enzyme KAPA HiFi HotStart ReadyMix for library amplification because it can suppress amplification bias. The enzyme also yielded lowly-biased data in this study.
The kit ensures high amplification efficiency, so the number of library amplification cycles may be further reduced.
In the future, we expect that library preparation can be achieved from even smaller amounts of samples.

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