NIPPON Genetics

Application

Quantitative analysis of gene expression in human bone marrow-derived mesenchymal stem cells (primer check)

Product Name: FastGene™ RNA Premium Kit（FG-81050, FG-81250）
Manufacturer: NIPPON Genetics EUROPE
Product Name: Transcriptor Universal cDNA Master（05893151001）
Manufacturer: Roche Diagnostics
Product Name: KAPA SYBR® FAST qPCR Kits（KK4610, KK4611）
Manufacturer: Kapa Biosystems, Inc.

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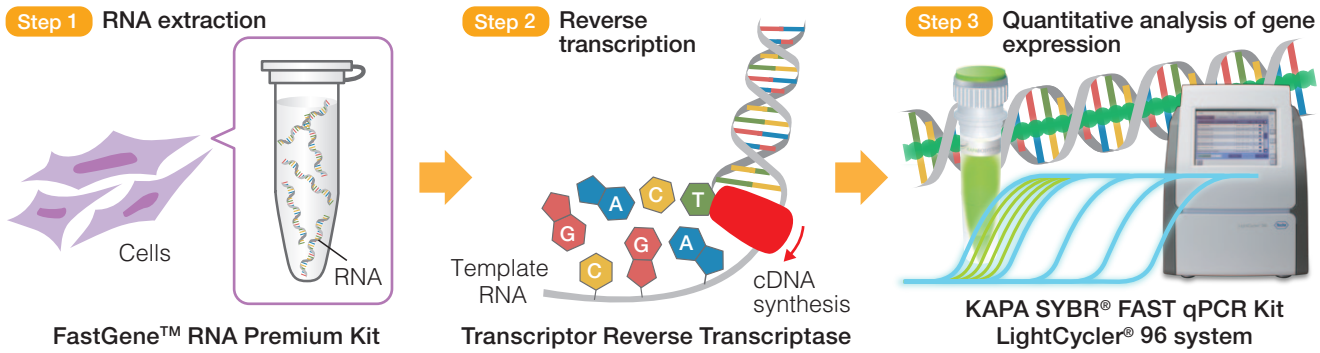
Total support with our products was possible!



FastGene™ RNA Premium Kit
Transcriptor Reverse Transcriptase
KAPA SYBR® FAST qPCR Kit
LightCycler® 96 system

Purposes of these experiments

1) In preparation for planned experiments in which hMSC (human mesenchymal stem cells) would be induced to differentiate into cartilage and bone, we checked primers targeting the following 10 genes using hMSC-BM (human bone marrow-derived mesenchymal stem cells).

   Genes: ACTB, GAPDH, RUNX2 (2 types), ALPL, Collagen Type I, SP7/Osterix, BGLAP (2 types), SOX9, Aggrecan, and Collagen Type II (11 types)

2) We verified whether 2.5 µg of RNA, which is a recommended maximum amount of input RNA for reverse transcription reaction, can be used for qPCR analysis (Sample A).

Experimental conditions

■ **RNA extraction：FastGene™ RNA Premium Kit**

- Starting sample: hMSC-BM (human bone marrow-derived mesenchymal stem cells) 1.8 × 10^6 cells
- The amount of lysis buffer RL: 600 µL
- Pretreatment of the sample: Lysis buffer RL (600 µL) was added onto the cells, and the cells were not homogenized but lysed by vortexing alone.
- Elution buffer RE: 50 µL
- Measurement of yield and purity: NanoDrop One (Thermo Fisher Scientific, Inc.)

■ **Reverse transcription：Transcriptor Universal cDNA Master**

- The amount of input RNA: Total RNA 1.0 µg (Sample B) or 2.5 µg (Sample A)
- The type of primers: Random hexamer primers
- The composition of the reaction mixture:

RNA	1.0 µg or 2.5 µg	
Transcriptor Universal Reaction Buffer	4	µL
Transcriptor Universal Reverse Transcriptase	1	µL
Distilled Water	up to 20	µL
total	20	µL

- Reaction program:

Annealing	25℃ · 5 min
↓	
Enzyme reaction	55℃ · 10 min
↓	
Denaturation and inactivation	85℃ · 5 min

■ Quantitative analysis of gene expression：KAPA SYBR® FAST qPCR Kit

- The amount of input cDNA: 2 µL (per 10-µL reaction) of the reverse transcription reaction solution diluted by 10-fold with DEPC-treated water
- Real-time machine: LightCycler® 96
- The composition of the reaction mixture:

cDNA	2	µL
KAPA SYBR® FAST qPCR Master Mix (2X)	5	µL
10 µM forward primer	0.2	µL
10 µM reverse primer	0.2	µL
PCR-grade water	Up to 10	µL
total	10	µL

- Reaction program:

Initial denaturation	95℃ · 3 min
↓	
Denaturation	95℃ · 10 sec
Annealing	60℃ · 20 sec
Extension reaction	72℃ · 1 sec
↓	
Melting	
↓	
Cooling	40℃ · 10 sec

Results

1. RNA extraction

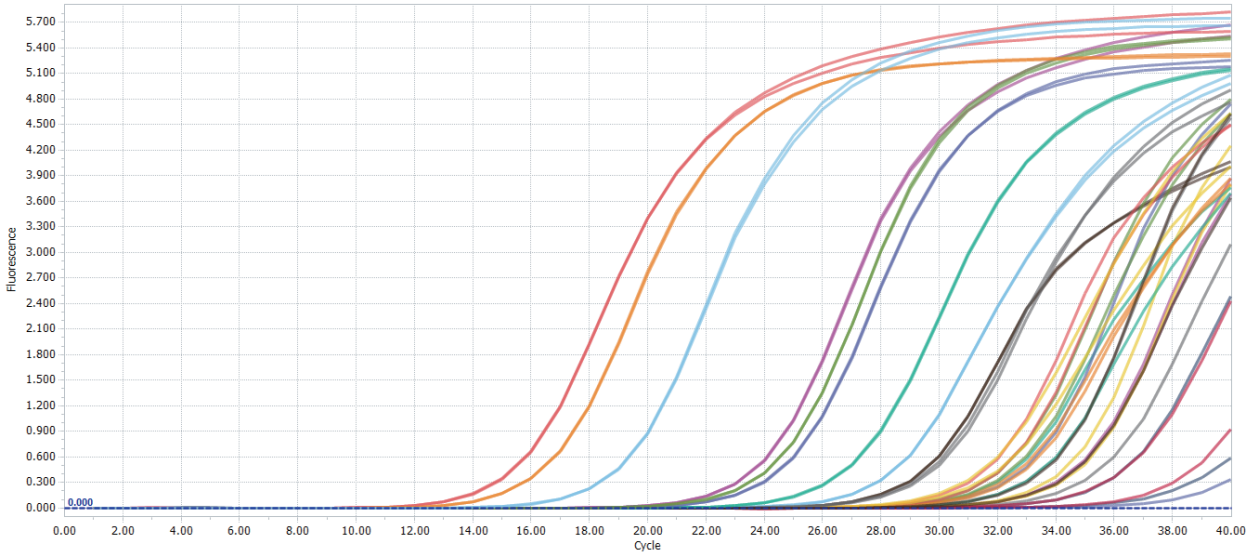
The volume of elution buffer (µL)	Concentration (ng/µL)	A260/A280	A260/A230	A260	A280
50	474.82	2.07	2.23	11.87	5.74

2. Quantitative analysis of gene expression

Sample A (RNA 2.5 µg)

As shown below, all the primers detected respective targets.

● **Amplification curve**



● **Cq values and positive/negative judgements**

Primers	No.	Cq value	Result
GAPDH	1	17.85	Positive
	2	17.89	Positive
ACTB	1	15.28	Positive
	2	15.27	Positive
RUNX2_1	1	22.98	Positive
	2	22.98	Positive
RUNX2_2	1	23.41	Positive
	2	23.39	Positive
ALPL	1	25.59	Positive
	2	25.63	Positive
Col1	1	14.26	Positive
	2	14.30	Positive

Primers	No.	Cq value	Result
SP7	1	33.48	Positive
	2	32.48	Positive
Bglap1	1	28.53	Positive
	2	28.66	Positive
Bglap3	1	30.24	Positive
	2	30.73	Positive
SOX9	1	22.53	Positive
	2	22.54	Positive
Aggrecan	1	37.41	Positive
	2	35.06	Positive
Col2 9/4_1	1	31.00	Positive
	2	30.48	Positive

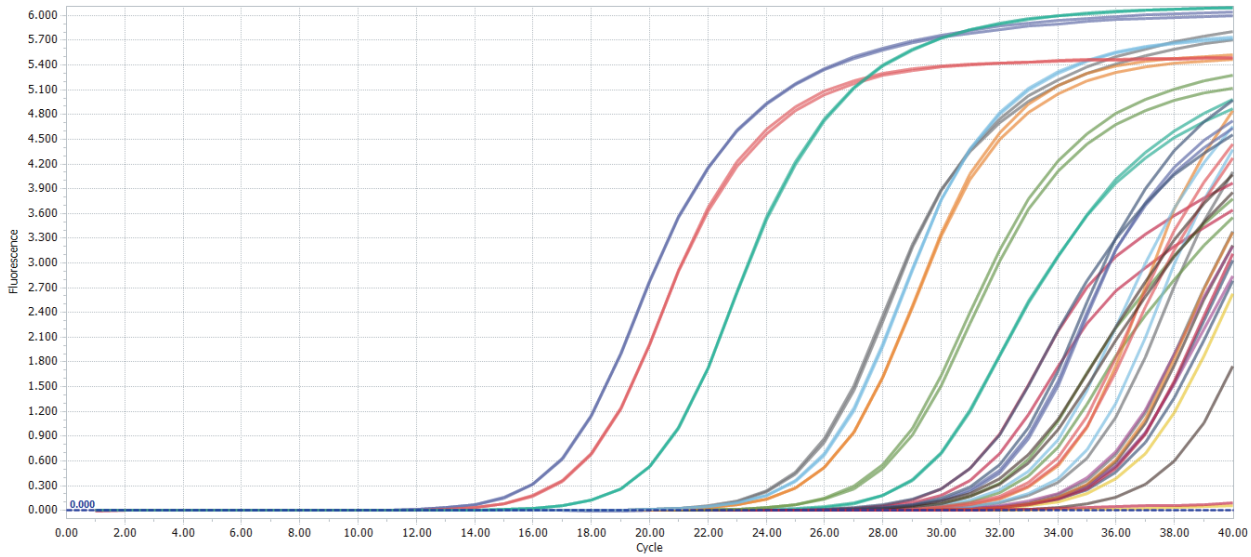
Primers	No.	Cq value	Result
Col2 9/4_2	1	27.30	Positive
	2	27.30	Positive
Col2 9/4_3	1	31.76	Positive
	2	31.53	Positive
Col2 9/4_4	1	31.32	Positive
	2	30.95	Positive
Col2 8/30_1	1	39.03	Positive
	2	31.74	Positive
Col2 8/30_2	1	31.43	Positive
	2	32.34	Positive
Col2 8/30_3	1	35.11	Positive
	2	37.93	Positive

Primers	No.	Cq value	Result
Col2 A	1	28.41	Positive
	2	28.39	Positive
Col2 B	1	32.38	Positive
	2	34.27	Positive
Col2 C	1	33.62	Positive
	2	33.17	Positive
Col2 D	1	33.48	Positive
	2	33.41	Positive

Sample B (RNA 1.0 µg)

As shown below, most of the primers detected respective targets.

● Amplification curve



● Cq values and positive/negative judgements

Primers	No.	Cq value	Result
GAPDH	1	18.66	Positive
	2	18.67	Positive
ACTB	1	16.24	Positive
	2	16.22	Positive
RUNX2_1	1	24.15	Positive
	2	24.21	Positive
RUNX2_2	1	24.60	Positive
	2	24.61	Positive
ALPL	1	26.45	Positive
	2	26.61	Positive
Col1	1	15.39	Positive
	2	15.37	Positive

Primers	No.	Cq value	Result
SP7	1	34.60	Positive
	2	-	Negative
Bglap1	1	30.51	Positive
	2	29.61	Positive
Bglap3	1	30.95	Positive
	2	31.28	Positive
SOX9	1	23.90	Positive
	2	23.81	Positive
Aggrecan	1	34.99	Positive
	2	-	Negative
Col2 9/4_1	1	30.70	Positive
	2	30.85	Positive

Primers	No.	Cq value	Result
Col2 9/4_2	1	28.12	Positive
	2	28.17	Positive
Col2 9/4_3	1	32.22	Positive
	2	32.47	Positive
Col2 9/4_4	1	33.04	Positive
	2	31.69	Positive
Col2 8/30_1	1	32.56	Positive
	2	34.34	Positive
Col2 8/30_2	1	31.92	Positive
	2	31.29	Positive
Col2 8/30_3	1	34.37	Positive
	2	33.96	Positive

Primers	No.	Cq value	Result
Col2 A	1	29.66	Positive
	2	30.15	Positive
Col2 B	1	34.47	Positive
	2	34.67	Positive
Col2 C	1	36.38	Positive
	2	34.41	Positive
Col2 D	1	34.11	Positive
	2	33.22	Positive

General evaluation

- LightCycler® 96 system (high performance, simple operation)
  → · Because its melt curve analysis does not take time, the experimental time can be reduced, which is good.
  · Because of its simple operation using a touchscreen, the machine is easy for beginners to use.
  · Its software is easy to use, for example it is compatible with Excel.
- FastGene™ RNA Premium Kit (low price, high quality)
  → · To be honest, I felt it burdensome to eliminate gDNA after RNA was eluted, instead of doing it on column. However, because you do it carefully, even when you are not accustomed to it, there is a sense of security that gDNA can be sufficiently eliminated.
  · Although there are multiple columns, they were color-coded in the protocol and easy to identify.
  · Reaction time is much reduced than the previous products, and it became easy to process many samples.
  · I am satisfied because its quality is equal to or better than Qiagen's RNeasy Mini Kit and its price is cheaper.
- Transcriptor Universal cDNA Master (convenience, high performance)
  → · cDNA can be easily synthesized by mixing 2 premixed reagents and RNA, and is high-quality. I am very satisfied. Because all of our laboratory members are beginners of PCR, I am thankful that it can be done by simple operation.
- KAPA SYBR® FAST qPCR Kit (low price, high sensitivity)
  → · Clearer calibration curve can be drawn than Cells to Ct kit

Customers' comments

When we purchased LightCycler®, we consulted Nippon Genetics, which is selling reagents from Roche, and they recommended these products because of their low price and high quality. Although the RNA purification step is burdensome, I am satisfied because RNA has been sufficiently purified in our laboratory which has many beginners.

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