6th April 2018

Real time qPCR protocol - FSQ-301 -

【Purpose】

Removal of contaminated genomic DNA in total RNA prior to the cDNA synthesis.

【Material】

cDNA synthesis reagent

・ReverTra Ace® qPCR RT Master Mix with gDNA Remover （FSQ-301）

Real-time PCR reagent

・THUNDERBIRD® SYBR® qPCR Mix（QPS-201）

【Method】

1. RNA extraction from cells

Total RNA is extracted and purified from HeLa cells using RNA isolation kits

HeLa cell RNA （ ng/uL）

2. cDNA synthesis

cDNA is synthesized using FSQ-301.
Negative control is added No-RT contorol.

Dilution of RNA （ ng/uL）→ 100ng/uL (RNA： 50 uL + Water： uL)

FSQ-301

	(+)	(−)
4×DN Master Mix	4	4
RNA(100ng/uL)	5	5
Water	7	7
total volume	16	16

↓

37℃, 5min
(4℃, hold)

↓

	(+)	(−)
Reaction mix	16	16
5×RT Master Mix Ⅱ	4	0
5×RT Master Mix Ⅱ no-RT Contr	0	4
total volume	20	20

↓

37℃, 15min
50℃, 5min
98℃, 5min
(4℃, hold)

↓

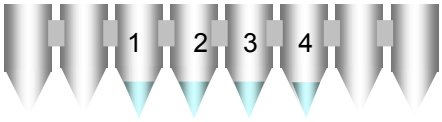
cDNA

3. Real-time PCR

RNA detection using QPS-201

QPS-201

	x1	x15
QPS-201	10	150
50×ROX	0.4	6
10uM Forward Primer	0.6	9
10uM Reverse Primer	0.6	9
cDNA	2	–
Water	6.4	96
	20	300



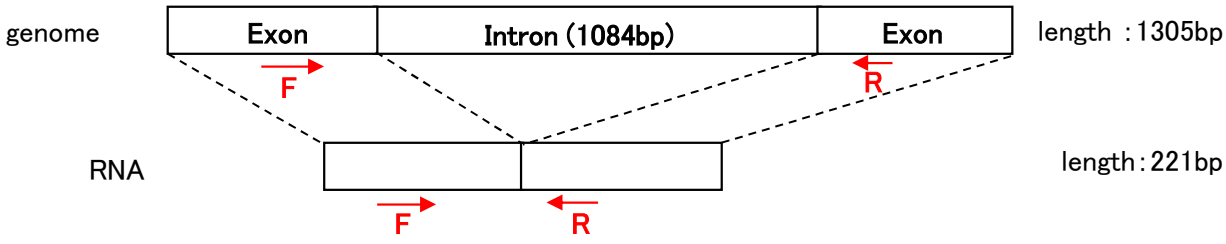
1 FSQ-201(+)
2 FSQ-201(−)
3 FSQ-301(+)
4 FSQ-301(−)

↓ Dispense 18uL reaction mix to each tube
(The test is perfomed in dupricate.)
↓ Add 2uL each synthesized cDNA

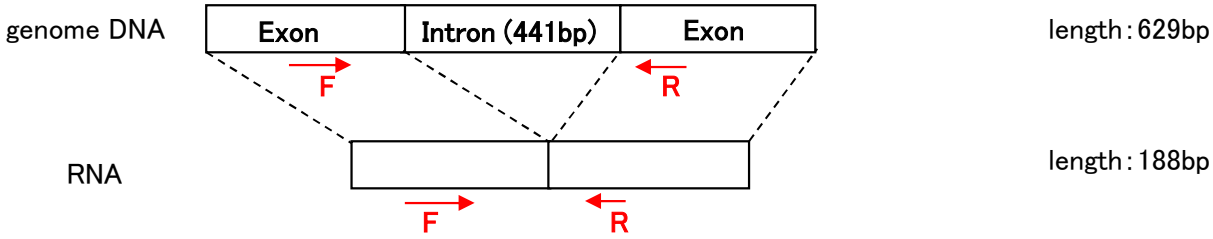
PCR cycle condition
95℃, 60sec → 95℃, 15sec / 60℃, 45sec (40 cycles) → Melting Curve

【Primer Design】

①TNF (Homo sapiens tumor necrosis facot)



②ACTB (Homo sapiens actin, beta)



③AMIGO3 (Homo sapiens adhesion molecule with Ig-like domain 3)