

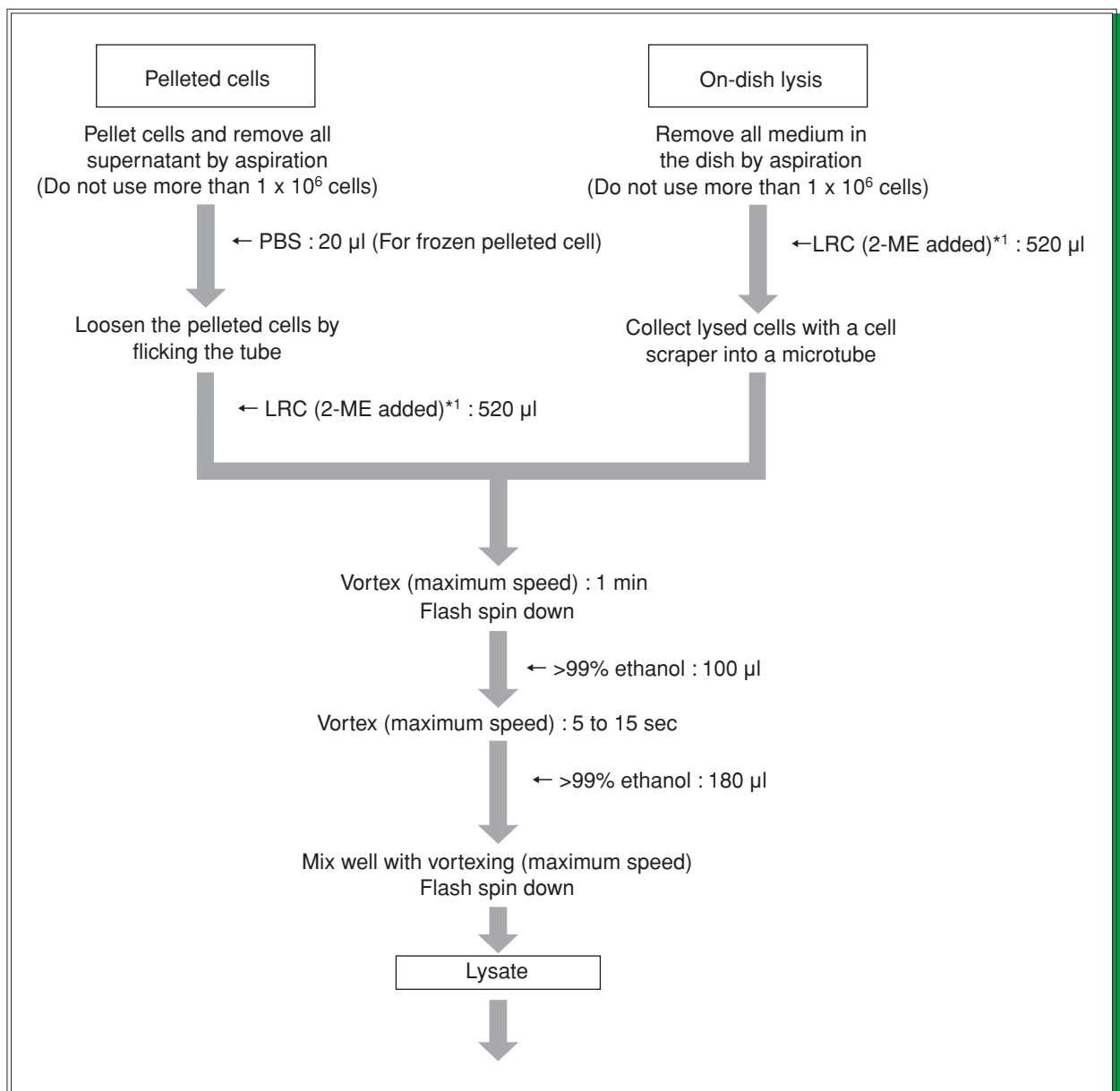


# QuickGene Series Application Guide

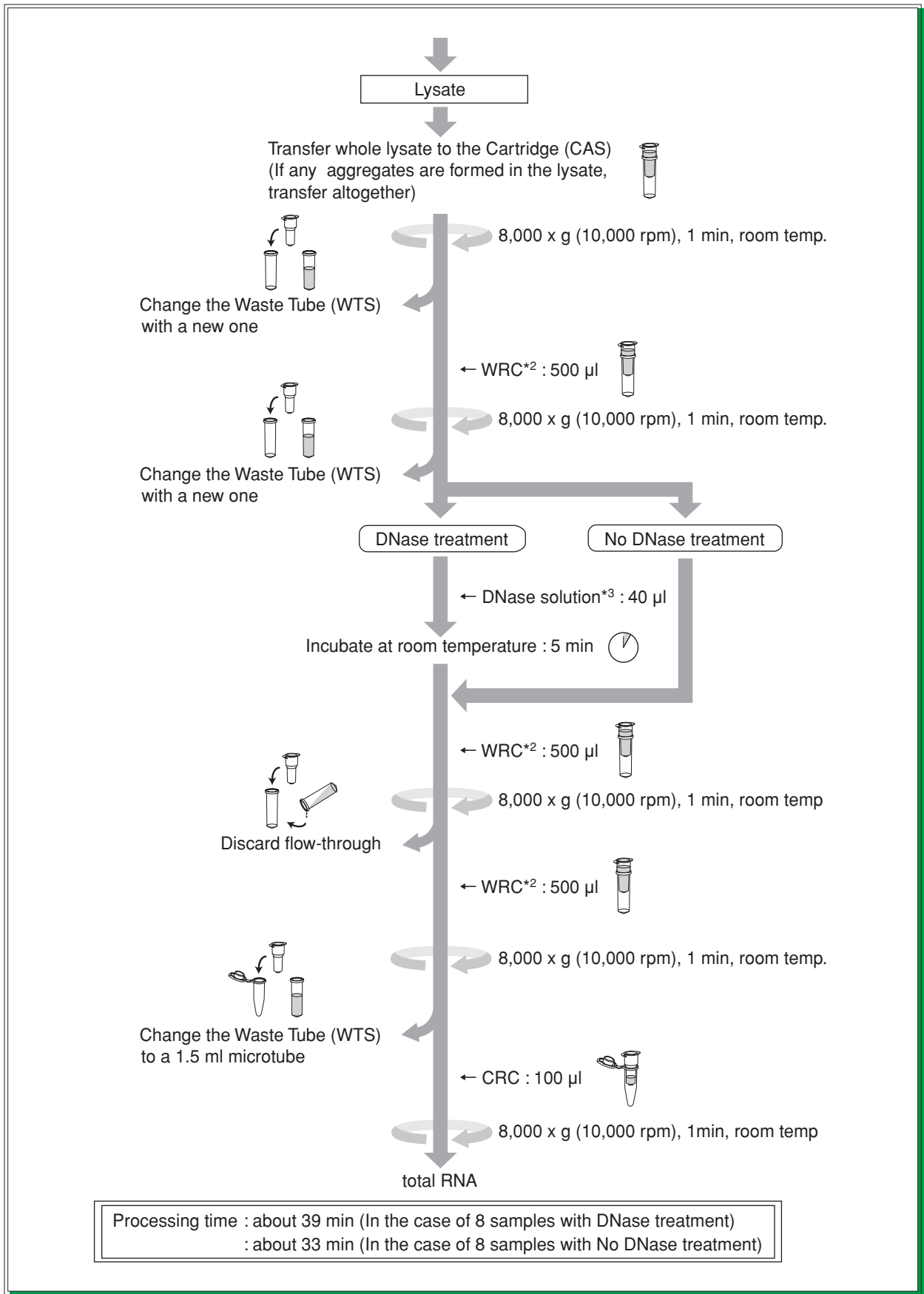
## Total RNA Extraction from Culture Cells

Kit : QuickGene SP kit RNA cultured cell (Spin Method)

### Protocol 1



\*1 : 2-Mercaptoethanol (2-ME) must be added to LRC before each use. Add 10 µl 2-ME per 1 ml of LRC.



\*2 : Add 75 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use.  
\*3 : DNase is not included in the kit, so prepare recommended product (p.3).

## Recommended DNase

- a) RQ1 RNase-Free DNase (Promega : Cat. No. M6101)  
 b) DNase I, Amplification Grade (Invitrogen : Cat. No. 18068-015)  
 c) DNase I, Amplification Grade (SIGMA : Cat. No. AMP-D1)  
 d) Deoxyribonuclease (RT Grade) (Nippon Gene : Cat. No. 313-03161)  
 e) DNase I, RNase-Free (Ambion : Cat. No. 2222)  
 f) RNase-Free DNase Set (QIAGEN : Cat. No. 79254)

In the case of DNase a)~d)

1 U / $\mu$ l DNase I	: 20 $\mu$ l
10 x Reaction Buffer	: 4 $\mu$ l
Nuclease-free water	: 16 $\mu$ l

In the case of DNase e)

2 U / $\mu$ l DNase I	: 20 $\mu$ l
10 x Reaction Buffer	: 4 $\mu$ l
Nuclease-free water	: 16 $\mu$ l

In the case of DNase f)

2.7 Kunitz unit / $\mu$ l DNase I	: 1.25 $\mu$ l
Buffer RDD	: 35 $\mu$ l
Nuclease-free water	: 3.75 $\mu$ l

## Results : Total RNA Extracted from various model cells

### ● The Yield and Purity of total RNA

QuickGene SP kit RNA cultured cell (SP-RC) for the extraction of total RNA from various model cells.

Cells		HL60	HEK293	HeLa	COS-7		NIH / 3T3	
Cell form		Pellet	3.5 cm dish	3.5 cm dish	3.5 cm dish	6 cm dish	3.5 cm dish	6 cm dish
Number of cells (x 10 <sup>6</sup> )		1.0	1.9~2.6	0.7~1.1	0.6	1.3	0.8~1.2	1.7~2.0
Homogenizing treatment after addition of LRC		Vortexing for 1 min	Needle*	Needle*	Needle*	Needle*	Vortexing for 1 min	Needle*
DNase(+)	Yield( $\mu$ g)	10.5	38.0	27.8	22.2	31.2	12.8	27.8
	A <sub>260/280</sub>	2.11	1.90	2.11	1.90	2.03	2.25	1.88
	A <sub>260/230</sub>	2.31	2.26	2.26	2.26	2.27	2.25	2.30
DNase(-)	Yield( $\mu$ g)	11.5	55.1	24.1	22.0	31.7	13.2	33.6
	A <sub>260/280</sub>	2.16	2.13	2.05	1.90	2.04	2.22	2.30
	A <sub>260/230</sub>	2.30	2.29	2.22	2.33	2.30	2.29	2.34

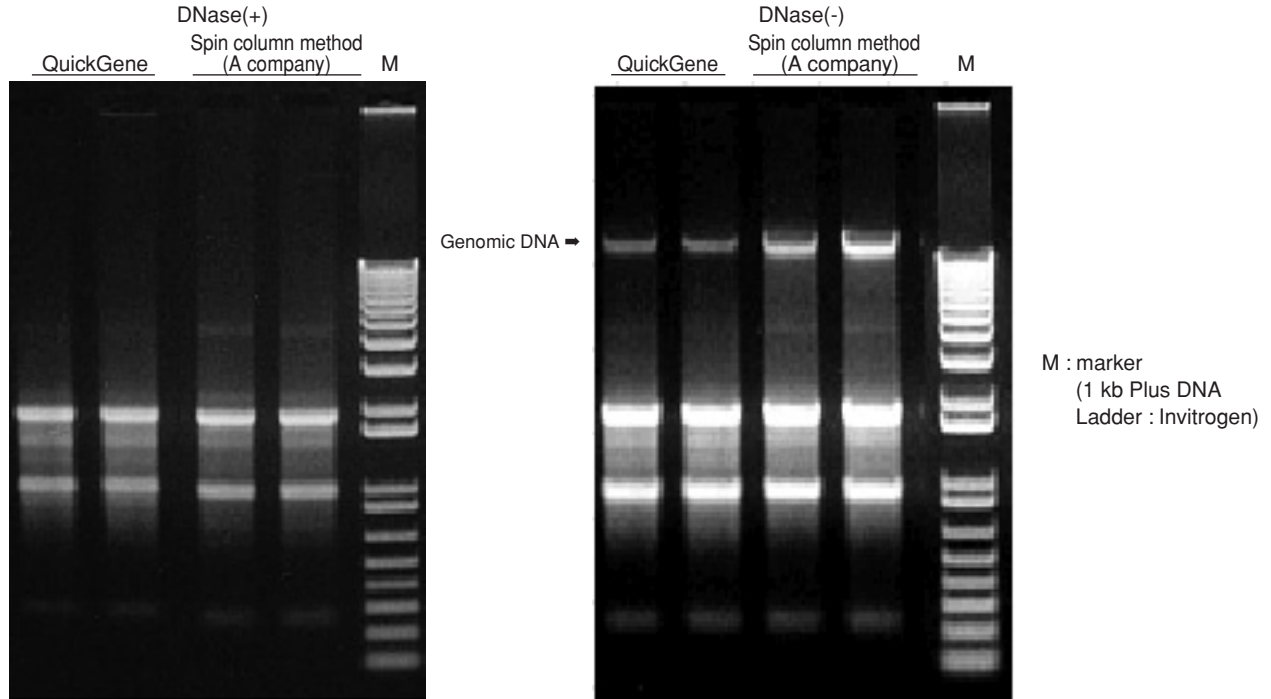
\* In the case of adherent Cells, homogenizing treatment was performed by passing the lysate 15 times through a 21-gauge needle fitted to 1 ml instead of vortexing for 1 min after addition of LRC.

A<sub>260/280</sub> : The ratio indicates the quality of nucleic acid from protein contamination (A<sub>260/280</sub> >1.7).  
 (Protein contamination decreases the ratio.)

A<sub>260/230</sub> : The ratio indicates the quality of nucleic acid from chaotropic salt (guanidium salt) contamination.  
 (Guanidium salt contamination decreases the ratio.)

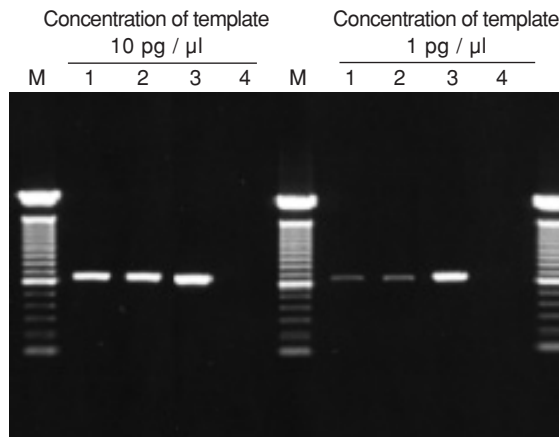
## ● Electrophoresis of total RNA

Non denaturing gel electrophoresis (1% Agarose gel / 1 x TAE Buffer) was performed on total RNA extracted from HL60 (1 x 10<sup>6</sup> cells) with QuickGene SP kit RNA cultured cell and Spin column method (A company).



## ● RT-PCR (with DNase treatment)

RT-PCR was performed using total RNA (with DNase treatment) extracted from HL60 (1 x 10<sup>6</sup> cells) with QuickGene SP kit RNA cultured cell and Spin column method (A company).



For RT-PCR performed on total RNA (1 pg / μl), electrophoretic bands of amplification products were detected.

### \* Trademark and exclusion item

Right to registered names etc. used in this Application Guide is protected by law especially even in the case of no denotation.

# FUJIFILM

FUJIFILM Corporation 7-3, Akasaka 9-Chome, Minato-ku, Tokyo 107-0052, Japan, Tel : +81-3-6271-2158, Fax : +81-3-6271-3136 • E-mail : sginfo@fujifilm.co.jp

FUJIFILM Europe GmbH Heesenstr.31, 40549 Dusseldorf, Germany, Tel:+49-211-5089-174, Fax:+49-211-5089-9144 • E-mail:lifescience@fujifilm-europe.de

FUJIFILM UK Ltd. Unit 12 St Martins Way, St Martins Business Centre, Bedford, MK42 0LF, U.K., Tel:+44-1234-245291, Fax:+44-1234-245293 • E-mail:lifesciences@fuji.co.uk

富士胶片(中国)投资有限公司 31st floor, Hong Kong New World Tower, No.300 Huai Hai Zhong Road, Shanghai, P.R China, Tel:+86-21-3302-4655 ext.363, Fax:+86-21-6384-3322 • E-mail:wgxiang@fujifilm.com.cn

FUJIFILM Medical Systems U.S.A., Inc. Tel:+1-866-902-3854 Fax:+1-203-327-6485 • E-mail:don.wilke@fujimed.com

<http://lifescience.fujifilm.com/>