RiboCluster Profiler™

Ribonomics

MBL

For Research Use Only

BRIC Kit

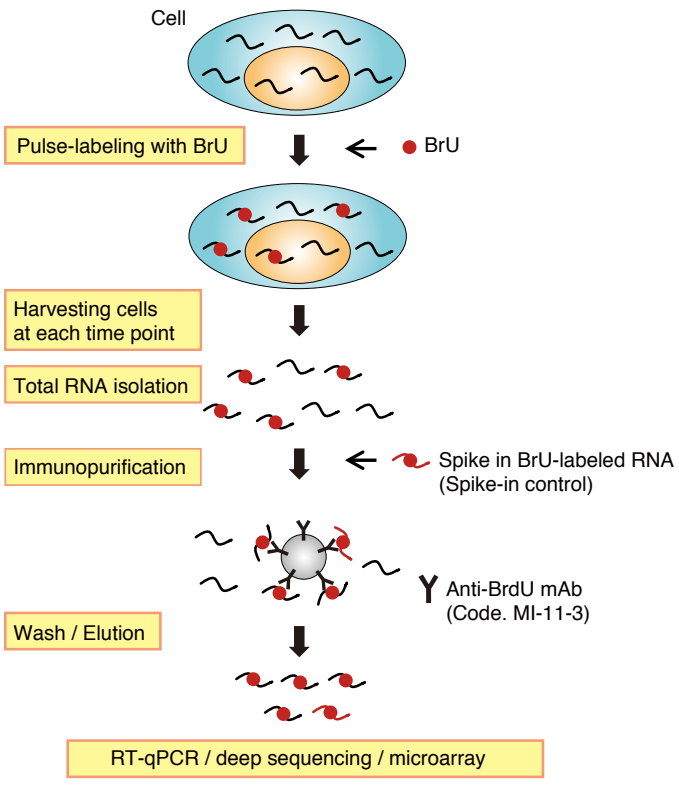
5-Bromouridine Immunoprecipitation Chase

- **The optimum kit for analyzing stability/half-life of mRNA and non-coding RNA (ncRNA).**
- **Useful tools to identify novel ncRNA and transcripts based on their half-lives.**

For characterization of mRNA and ncRNA

For searcing new ncRNA biomarkers and drug target

For discovering new RNA degradation control pathways



Transcriptional inhibitors such as actinomycin D have been used in the analysis of stability and half-life of RNA; however, the use of transcriptional inhibitors alters the stability and localization of RNA, and it has been shown to interfere with the results of analysis.

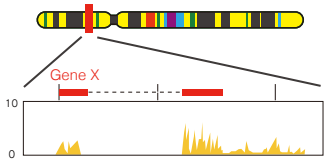
BRIC Kit enables determination of RNA stability by chasing chronological decreases of BrU-labeled RNA under physiologically undisturbed conditions. In the BRIC protocol, cells are pulse-labeled with BrU for constant period and washed with PBS to remove the BrU-containing medium, and then cells are chronologically harvested, followed by preparation of total RNA including newly BrU-labeled RNA. The BrU-labeled RNA can be specifically immunoprecipitated with Anti-BrdU mAb provided by MBL, followed by isolation of BrU-labeled RNA from immunocomplex on carrier material, such as protein G magnetic beads. The isolated BrU-labeled RNA can be analyzed to determine its own stability and half-life by various methods in molecular biology – RT-qPCR, deep sequencing or microarray.

<Reference>

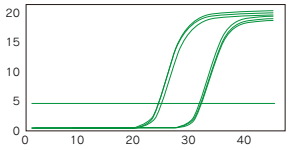
Tani, H *et al.* Genome Res. 22, 947-956 (2012)

Analytical methods that can be applied following BRIC

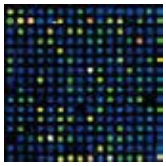
Deep sequencing



RT-qPCR



Microarray



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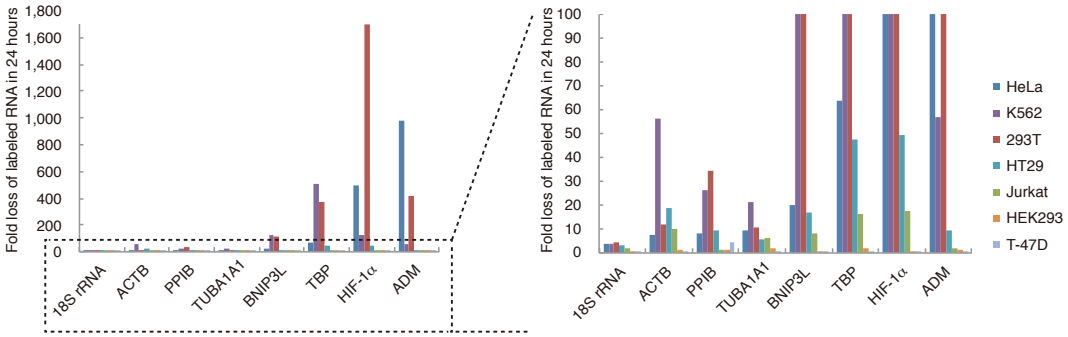
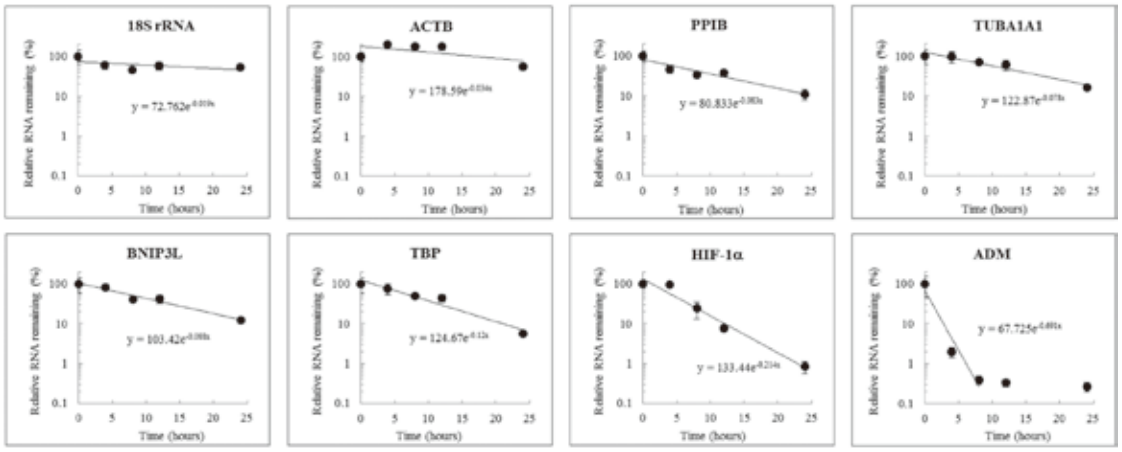
Target RNA decay in 24 hours



HeLa, K562, 293T, HT29, Jurkat, HEK293 and T-47D cells were pulse-labeled with 150 μM BrU for 24 hours. Then, the cells were washed and harvested at chase time 0 and 24 hours. After RNA extraction, BrU-labeled RNA was isolated by BRIC Kit. The isolated BrU-labeled RNAs were analyzed by RT-qPCR. The RNAs derived from housekeeping genes such as 18S rRNA and ACTB were stable in most of cell lines, while HIF-1α and ADM were unstable. The labeling efficiency was low in Jurkat, HEK293 and T-47D cells, which indicates labeling efficiency varies depending on the cell lines.

Measurement of half-life of target RNA by RT-qPCR



	18S rRNA	ACTB	PPIB	TUBA1A1	BNIP3L	TBP	HIF-1α	ADM
$T_{1/2}$ (hours)	36.5	20.4	8.4	8.9	7.9	5.8	3.2	1.0

HeLa cells were pulse-labeled with 150 μM BrU for 24 hours. Then, cells were washed and harvested at chase time 0, 4, 8, 12 and 24 hours. After RNA extraction, BrU-labeled RNA was isolated by BRIC Kit. Isolated BrU-labeled RNA was analyzed by RT-qPCR.
As expected, the transcripts derived from housekeeping genes, such as 18S rRNA and ACTB, showed relatively long half-lives, while HIF-1α and ADM* showed much shorter half-lives.

*ADM gene encodes a potent hypotensive peptide which plays important roles in both normal and disease conditions.

Code	Product	Size	Storage temp.
RN1007	BRIC Kit	20 assays	–20°C
RN1008*			2-8°C

*RN1007 and RN1008 are sold as a set. RN1007 and RN1008 should be stored at different temperature.

[Kit components] Code RN1008 (Storage temp. 2-8°C)	
1. RNA-IP buffer	18 mL × 2 bottles
2. Wash buffer	41 mL × 3 bottles
3. mi-Solution I	240 μL × 1 vial: enzyme solution
4. mi-Solution II	5.8 mL × 1 vial: diluent for Solution I
5. mi-Solution III	3.6 mL × 1 vial: protein dissolvent Solution III can dissolve proteins and dissociate immunocomplex.
6. mi-Solution IV	90 μL× 1 vial: co-precipitator Solution IV can increase RNA precipitation efficiently.
7. Protein G-Magnetic beads	1.5 mL × 4 vials 1% beads slurry (mouse IgG binding capacity: 7 μg/mg beads)

[Kit components] Code RN1007 (Storage temp. -20°C)	
8. BrU solution (100 mM)	1.1 mL × 2 vials
9. Anti-BrdU mAb	450 μL × 1 vial
10. Spike-in control	80 μL × 1 vial

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Produced by
MBL
MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
KDX Nagoya Sakae Bldg. 10F
4-5-3 Sakae, Naka-ku, Nagoya,
Aichi 460-0008, JAPAN
TEL: +81-52-238-1904 FAX: +81-52-238-1441
URL: http://ruo.mbl.co.jp