Messenger RNA half-life measurements in mammalian Cells

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mRNA half-life can be determined using Northern blot analysis or quantitative RT-PCR (qRT-PCR) analysis. Depending on the amount of RNA required, one should choose the number of cells to be seeded.

Our experience is that HEK293 cells should not be used for mRNA half-life measurements, as these cells seem to have reduced post-transcriptional control.

This protocol is standardized for measuring reporter mRNA half-lives after transient transfection, but it can also be used for measuring endogenous mRNA half-lives. Typically, we use HeLa cells in a 10-cm dish format, from which we make 4 time points and obtain sufficient RNA for a Northern blot or qRT-PCR.

1 2.8×10^6 HeLa cells maintained in DMEM containing 10% FCS are seeded in a 10-cm culture dish and kept in an 5 % CO_2 incubator for 12 -16 hours, allowing the cells to reach 70 -80 % confluency.

2 Transfect the cells with your reporter gene plasmid using Lipofectamine 2000, Polyethyleneimine (PEI) or any reagent of your choice according to standard protocols.

3 18 – 48 hrs after transfection, cells are divided into 4 samples to obtain 4 time points, and Actinomycin D is added to stop transcription. This can be done in two ways:

A) The cells from one 10-cm dish are re-seeded into four 6-cm dishes the day before addition of Actinomycin D.

B) The cells from one 10-cm dish are collected, distributed into four 15-ml falcon tubes, and Actinomycin D is added immediately. In this case, the tubes are returned to the incubator. By tilting the tubes, one can avoid that all cells will settle into a dense pellet.

4 At t = 0, inhibit transcription by adding 5 µg/ml Actinomycin D (1:1000 dilution from 5mg/ml stock in DMSO).

5 Harvest cells at required time points. The time points will depend on the expected half-life of the mRNA. Typically, we do 0-30-60-90 minutes, 0-1-2-3 hours or 0-2-4-6 hours. Treatment with Actinomycin D should not exceed 6 hours because of cell death.

6 RNA isolation can be done using an RNA isolation kit of your choice or any other method.

7 After RNA isolation, one can choose to do a Northern blot or qRT-PCR to measure the mRNA level at the different time points.

Calculating mRNA half-life:
(from C.Y.A. Chen, N. Ezzedine and Ann-Bin Shyu, Methods Enzymology, 2008 ;448: 335-357)

Before calculating the half-life of an mRNA, one has to calculate the decay rate constant. Assuming complete transcription shut off, mRNA decay follows first order kinetics.
The rate by which the mRNA concentration changes over a given time (dC/dt) is proportional to both the rate constant for decay (k_{decay}) and the cytoplasmic concentration of the mRNA (C). This relation is described by the following equation:

$$dC/dt = -k_{decay}\, C$$

The minus symbol indicates that the mRNA is being degraded rather than synthesized. This relationship leads to the derivation of the equation:

$$\ln (C/C_0) = -k_{decay}\, t$$

where C_0 is the concentration of the mRNA at time 0.
Because we want to determine the half-life ($t_{1/2}$), this means that C/C_0 = 50%/100% = 1/2.

Rearrangement of the equation leads to following equation:

$$\ln (½) = -k_{decay}\, t_{1/2}$$

from where: $t_{1/2} = \ln(2)/k_{decay}$

After the theory, this is what you have to do: Plot the mRNA signal intensity (which is proportional to the mRNA concentration) on the y-axis versus time on the x-axis. Tell your program (e.g. Excel) to fit the best exponential curve to your data points.
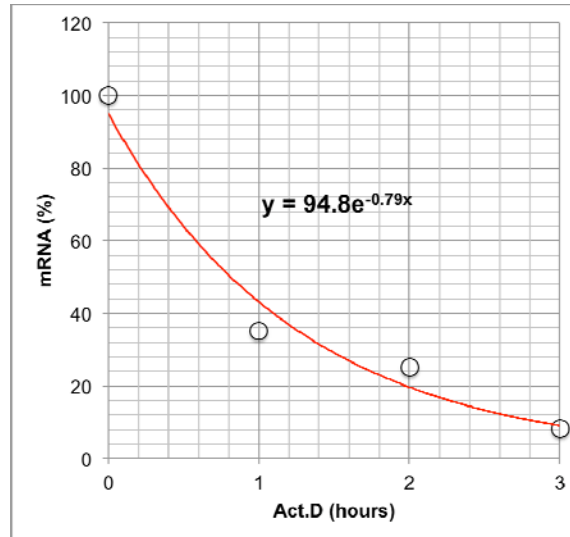
The curve will have the following general equation:

$$C = C_0 \, e^{-k_{decay} \, t}$$

In our case, the best fit exponential curve has the following values:

$$y = 94.8^{-0,79 \, x}$$

whereby the amount of mRNA y corresponds to C and x corresponds to the time t after Actinomycin D treatment.

k_{decay} is 0.79 and can be put in the final equation:

$$t_{1/2} = \ln(2) \, / \, k_{decay} = 0.69 \, / \, 0.79 = 0.88 \text{ hours}$$

It is important to note that the half-life of an mRNA ($t_{1/2}$) is inversely proportional to its decay rate constant (k_{decay}).