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Using Tet-off cells and RNAi knockdown to assay mRNA decay

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Abstract

Cellular mRNA levels are determined by the competing forces of transcription and decay. A wide array of cellular mRNA decay pathways carry out RNA turnover either on a constitutive basis or in response to changing cellular conditions. Here, we outline a method to investigate mRNA decay that employs RNAi knockdown of known or putative decay factors in commercially-available Tet-off cell systems. Reporter mRNAs of interest are expressed under the control of a tetracycline-regulated promoter, allowing pulse-chase mRNA decay assays to be conducted. Levels of reporter and constitutively expressed control RNAs throughout the decay assay timecourse are detected by traditional northern blot analysis and used to calculate mRNA half-lives. We describe the utility of this approach to study nonsense-mediated mRNA decay substrates and factors, but it can be readily adapted to investigate key mechanistic features that dictate the specificity and functions of any mRNA decay pathway.

Keywords

mRNA decay; Nonsense-mediated mRNA decay (NMD); RNA stability; UPF1; Tet-off cells; RNA half-life; 3' untranslated region

1. Introduction

mRNA decay is a highly dynamic process central to the regulation of gene expression and maintenance of cellular homeostasis [1,2]. A major contributor to this regulation is the nonsense-mediated mRNA decay (NMD) pathway, which degrades diverse mRNAs in all eukaryotes. In addition to serving as a quality control pathway for aberrant transcripts containing premature termination codons (PTCs) resulting from genetic mutations or errors during mRNA biogenesis, the NMD pathway degrades 5–10% of apparently normal human mRNAs [3].

To investigate the activities and regulation of the mammalian NMD pathway, we describe an approach coupling RNAi-mediated protein depletion with a tetracycline-regulated reporter mRNA decay assay in human cells (Figure 1). In this assay, siRNAs targeting the essential NMD factor UPF1 are reverse-transfected into HeLa cells engineered to express a Tet repressor-transcriptional activator fusion protein, rendering the NMD surveillance pathway inoperative and stabilizing NMD mRNA target substrates. Following transfection with

siRNAs, plasmid DNAs encoding tetracycline-regulated reporter mRNAs and constitutively expressed transfection control mRNAs are chemically transfected into the cells.

As the reporter RNA is transcriptionally repressed in the presence of tetracycline (“Tet-off”), cells are initially grown in media supplemented with low concentrations of tetracycline or the more stable analog doxycycline. The day of sample collection, the cells are washed with 1X PBS and supplemented with tetracycline-free media to allow a brief pulse of transcription (typically four hours). Immediately following the induction period, tetracycline is supplemented back into the culture media to halt mRNA synthesis, and RNA samples are subsequently harvested over a range of time points. This “pulse-chase” approach allows for the relative levels of the reporter RNAs to be quantified by traditional northern blot assays, using the constitutively expressed control mRNA as a normalization control. RNA half-lives can then be calculated on the basis of the ratio of these values over time, and the comparison of decay kinetics between non-targeting siRNA and siUPF1 treatments provides insight into any potential role of the NMD pathway on reporter RNA stability.

To illustrate, we describe the use of dual-fluorescent GFP-mCherry fusion reporter constructs containing either an efficient termination codon (UAA) or no termination codon (CAA) between the GFP and mCherry open reading frames. The mRNAs expressing a full-length GFP-mCherry fusion protein are highly stable, while mRNAs containing a termination codon following the GFP ORF are degraded by NMD as a result of the extended mCherry-containing 3'-UTR [4,5]. The procedures described here are equally applicable to other commonly used NMD reporters, such as those derived from the β -globin gene [6].

While we specifically focus on NMD, this technique can easily be adapted to investigate alternative RNA decay pathways. In fact, two major utilities of the described method are the ability to both determine whether an RNA substrate (*i.e.*, the tetracycline-regulated reporter) is subject to degradation through a specific pathway, and to investigate whether the protein depleted by RNAi is a novel regulator of a characterized RNA decay process.

2. Materials

1. Tetracycline-regulated plasmids expressing experimental mRNAs (pcTET2 2FP) and plasmids containing the CMV promoter for constitutive expression of control mRNAs (pcGFP-bGH).
2. Tetracycline-regulated Tet-off cell lines (Clontech).
3. Complete DMEM media for standard cell propagation: high-glucose Dulbecco's Modified Eagle Medium, 1% penicillin/streptomycin (pen/strep) with L-glutamine, 10% heat-inactivated fetal bovine serum.
4. Complete DMEM media for siRNA reverse-transfection: high-glucose Dulbecco's Modified Eagle Medium, 1% pen/strep with L-glutamine, 20% heat-inactivated fetal bovine serum.

5. Complete DMEM media for transcriptional induction: high-glucose Dulbecco's Modified Eagle Medium, 1% pen/strep with L-glutamine, 10% Tet System Approved fetal bovine serum (Clontech).
6. Opti-MEM® reduced serum media (Gibco).
7. Sterile 1X PBS: phosphate buffered saline solution, pH 7.4.
8. siRNA (20 µM) targeting gene of interest and a non-targeting control.
9. Lipofectamine RNAiMAX® transfection reagent (ThermoFisher Scientific) or your chemical transfection reagent of choice for delivery of siRNA.
10. TurboFect® transfection reagent (ThermoFisher Scientific) or your chemical transfection reagent of choice for delivery of reporter plasmid DNA.
11. Doxycycline hyclate: 1 mg/mL stock solution dissolved in ultrapure H₂O and sterile-filtered.
12. Tissue culture plates, 24 well, flat bottom with low evaporation lid.
13. TRIzol® reagent (Ambion/ThermoFisher Scientific).
14. Sterile, RNase-free microfuge tubes.
15. Parafilm M laboratory film (Bemis).
16. Molecular-grade chloroform.
17. Molecular-grade isopropanol.
18. Molecular-grade ethanol.
19. GlycoBlue™ Coprecipitant (15 mg/mL) (ThermoFisher Scientific).
20. Formamide gel loading buffer: Deionized formamide, 15 mM EDTA, 0.1% Bromophenol blue, 0.15% Xylene cyanol.
21. Molecular-grade 37% formaldehyde solution.
22. 10X MOPS gel running buffer: 0.2 M MOPS, 40 mM sodium acetate, 5 mM EDTA, pH 7.0.
23. Horizontal gel electrophoresis system with power supply.
24. 2X MOPS/formaldehyde gel loading buffer.
25. 20X SSC: 3 M sodium citrate, 0.3 M sodium chloride, pH 7.0.
26. T7 *in vitro* transcription kit: including buffer, dNTPs, and T7 RNA polymerase.
27. α³²P-UTP (10 mCi/mL).
28. SUPERase-In™ RNase Inhibitor (ThermoFisher Scientific).
29. illustra™ Probe Quant™ G-50 micro columns (GE).
30. Geiger counter.
31. Whatman™ 3MM chromatography paper.

32. Amersham Hybond™ - XL blotting membrane (GE).
33. 265 nm UV Crosslinker (*e.g.*, Spectrolinker XL-1500 UV Crosslinker, Spectronics Corporation).
34. Hybridization oven mesh sheets (*e.g.*, HYBAID, ThermoFisher Scientific).
35. Hybridization bottles (*e.g.*, HYBAID, ThermoFisher Scientific).
36. ULTRAhyb™ Ultrasensitive Hybridization Buffer (ThermoFisher Scientific).
37. Hybridization Oven (*e.g.*, HB-1000 Hybridizer, UVP).
38. Typhoon Trio Plus variable mode imager (GE), or comparable scanner for sensitive quantitation of ³²P radioisotope.
39. ImageQuant (GE), or comparable imaging software for quantification of northern blot signals.

3. Methods

3.1 siRNA treatment and reporter transfection

All cell culture work should be performed in a sterile tissue culture hood using aseptic technique. Cells are grown throughout the duration of the experiment at 37°C and 5% CO₂ in DMEM with 10% FBS and 1% pen/strep.

Day 1

1. A reverse transfection is performed to deplete cells of endogenous gene expression. Pipette 1.5 µL (stock concentration = 20 µM) non-targeting (AN2, Ambion/ThermoFisher) or UPF1-specific siRNA in each well of a 24-well plate (*see* Note 1).
2. Make a transfection master mix consisting of 250 µL OptiMEM® and 1.5 µL RNAiMAX® per well. Account for technical replicates and pipetting error in calculating the final volume for each sample.
3. Add the above transfection mix to each well, thoroughly mixing by gently rocking the plate back and forth a few times. Allow the siRNA, OptiMEM®, and RNAiMAX® to complex at room temperature for 30–40 mins.
4. During the incubation (about 10 mins before the end), prepare the HeLa Tet-off cells for plating (*see* Note 2). Remove the normal growth media (10% FBS DMEM), rinse with 1X PBS, and trypsinize the cells thoroughly. Resuspend the cells in DMEM with 20% FBS and count (*see* Note 3). Add 2×10⁴ cells in 250

¹-To disrupt NMD, we use a previously characterized siRNA specific to UPF1 [7]. The use of other commercially-available targeting siRNAs and non-targeting controls can be substituted as necessary.

²-Our method describes using the HeLa Tet-Off® cell line, but there are several other commercial examples of both human and murine lines that express the tetracycline-regulated Tet-Off® system. For example, the HEK-293 Tet-Off® line is useful for achieving high transfection efficiency. It is important to note, however, that subtle changes to the protocol may be required when using alternative lines. It is our experience that certain lines including HEK-293 require an additional re-plating step on coated cell culture plates (*e.g.*, Pure-Coat amine) prior to transfection with the reporter mRNAs. Such modifications for enhanced cell attachment and growth may be necessary for other cell types, as well.

μL DMEM with 20% FBS to each well. Rock the plate gently to distribute evenly and incubate the cells with the siRNA and transfection reagent for 10 mins at room temperature. After the 10 min incubation, return plates to the cell culture incubator.

Day 2

1. In a tissue culture hood, co-transfect cells with plasmid DNAs expressing the experimental tetracycline-regulated mRNAs (pcTET2 2FP) and a constitutively expressed control (pcGFP-bGH) (see Note 4). For consistency in delivery, make a transfection mix consisting of 100 ng experimental pcTET2 2FP DNA, 25 ng control pcGFP-bGH DNA, 75 ng empty vector (*e.g.*, pcDNA3.1), 100 μL OptiMEM, and 0.4 μL TurboFect per well. Calculate the master mix to include all of the time points being tested, as well as additional samples to allow for loss from pipetting error. It is important to add gently vortexed TurboFect to the transfection mix as the last component.
2. Incubate the transfection mix for 15–20 mins at room temperature in the tissue culture hood.
3. During the incubation process, dilute a 1 mg/mL freshly-made doxycycline stock 1:1000 in OptiMEM.
4. Calculate the total volume required for a final concentration of 2 ng/mL doxycycline in each well and add this to the transfection mix (*see* Note 5).
5. At the end of the incubation period, carefully add the transfection mix dropwise to each well, rock the plate gently to mix, and return the plates to the cell culture incubator.

Day 3

1. Inspect cells under a light microscope to verify even distribution and healthy morphology. Fresh media and doxycycline may be added at this step, though this is not required for robust transcriptional repression and may lead to loss of cells in lines that adhere poorly.

Day 4

1. In a tissue culture hood, aspirate off the media, gently wash each well with 0.5 mL 1X PBS, and replace media with 0.5 mL DMEM + 10% tetracycline-free FBS

³.DMEM with 20% FBS (twice the normal) is required to account for the lack of serum in the OptiMEM® media.

⁴.The pcTET2 constructs used in this protocol were based on constructs originally introduced by Lykke-Andersen and Steitz [6] and were previously described [5]. We find these reporters to be useful as they provide a platform to study mRNA decay, translational readthrough, and mRNP composition in parallel. However, any tetracycline-regulated expression plasmid can be employed with this system to discern RNA stability and dependency on trans-acting factors. High-quality plasmid DNA that is endotoxin-free should be used for optimal transfection efficiency.

⁵.We prefer to use the tetracycline analogue doxycycline in these experiments for its enhanced stability. Tetracycline may be used, but requires supplementing the media with fresh drug on a daily basis. Doxycycline stock solution should be stored at 4°C, protected from light, and used within 7 days of preparation. For HeLa Tet-off cells, we use 2 ng/mL doxycycline to repress transcription. For different cell types, it may be necessary to titrate doxycycline to optimize the repression prior to the pulse and the magnitude and kinetics of activation following removal of doxycycline.

(Clontech) (see Note 6). Incubate the cells at 37°C for 4 h in tetracycline-free media to de-repress transcription of reporter mRNAs (pcTET2 2FP).

2. After the 4 h incubation period, supplement each well with doxycycline at a final concentration of 1 µg/mL (*i.e.*, dilute 1 mg/mL doxycycline stock 1:10 in OptiMEM and add 5 µL to each well).
3. Start the experimental time course 20–30 mins following the addition of doxycycline to provide sufficient time to repress all transcription of the reporter mRNAs.
4. Collect time points at 0, 3, 6, and 9 h by harvesting the cells in the hood with 500 µL TRIzol/well (*see* Note 7). Following the TRIzol harvest, rinse empty wells twice with 1 mL 1X PBS to remove residual TRIzol from harvested wells and return to incubator for later time points.
5. Store TRIzol samples in RNase-free microfuge tubes at –20°C for short term storage (*e.g.*, 2 weeks) or –80°C for longer periods of time.

3.2 RNA extraction and purification of TRIzol samples

Before proceeding to the RNA isolation step, chill a tabletop microcentrifuge to 4°C and label new microfuge tubes in advance.

1. Following a slightly-adapted version of the manufacturer's protocol, add 100 µL of chloroform to the 500 µL thawed TRIzol sample and cap the tube securely. Shake the tube vigorously by hand for 15 secs, and incubate at room temperature (25°C) for 2–3 mins.
2. Centrifuge the samples at 12,000 × g for 15 mins at 4°C.
3. Remove the aqueous phase of the sample by angling the tube at 45 degrees and pipetting the solution into a new labeled tube (*see* Note 8). Importantly, avoid drawing any of the interphase or organic layer into the pipette when removing the aqueous phase. Dispose of the organic phenol phase according to your institution's waste disposal guidelines.
4. Add 250 µL of 100% isopropanol to the aqueous phase (per 500 µL TRIzol used for homogenization). When precipitating RNA from small sample quantities, add 2 µL GlycoBlue™ coprecipitant as a carrier. This step increases both pellet mass and visibility.
5. Incubate at room temperature for 10 mins.
6. Centrifuge samples at 12,000 × g for 10 mins at 4°C. The RNA should be visible following centrifugation as a small gel-like blue pellet on the side and bottom of

⁶Standard fetal bovine serum often contains trace amounts of tetracycline contaminants, which can lead to undesirable background repression in the Tet-Off lines.

⁷These time points capture the dynamics of decay for the pcTET2 2FP experimental mRNAs described. For other experimental constructs, an appropriate time course will need to be determined empirically.

⁸The mixture separates into a lower red organic phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The upper aqueous phase is ~50% of the total volume.

the tube. During subsequent steps, carefully monitor the presence of this pellet when cautiously removing supernatant.

7. Carefully pipette the supernatant from the tube, leaving only the RNA pellet.
8. Wash the pellet with 500 μ L of 75% ethanol (per 500 μ L TRIzol used in the initial homogenization). Vortex the sample briefly, then centrifuge the tube at $7500 \times g$ for 5 mins at 4°C.
9. Carefully pipette the wash supernatant from the tube, leaving only the RNA pellet, and centrifuge the tube again at $7500 \times g$ for 1 min at 4°C to allow removal of residual ethanol (see Note 9).
10. Air dry the pellet for 3–5 mins at room temperature. Do not allow the RNA to dry completely, as this can result in a decrease in pellet solubility.
11. Resuspend RNA pellet by adding 10 μ L Formamide LB to each sample. Shake samples at 50°C for 5–10 mins, which can then either be stored at –80°C for future detection or used immediately for northern blotting.

3.3 Detection and quantification of reporter mRNAs by northern blot

Preparing, running, and transferring the gel

1. The evening before running the gel, prepare 2 L of 1X MOPS gel running buffer and refrigerate at 4°C.
2. Prepare a 1.2% agarose/formaldehyde gel. Combine the appropriate amount of agarose with ultrapure H₂O in a glass Erlenmeyer flask and microwave in 15 sec intervals to melt. Once all of the agarose is in solution, carefully swirl the contents and place the flask in a 60°C H₂O bath to equilibrate the temperature.
3. After equilibrating the agarose solution to 60°C, add 10X MOPS at 12% of the volume of H₂O used to dissolve the agarose (*e.g.*, if 100 mL of H₂O was used to dissolve agarose, add 12 mL of 10X MOPS) (see Note 10).
4. Add 37% formaldehyde at 7% of the volume of H₂O used to dissolve the agarose (*e.g.*, if 100 mL of H₂O was used to dissolve agarose, add 7 mL of 37% formaldehyde). Swirl the solution and cast the agarose/formaldehyde gels in a chemical hood (see Note 11).
5. To further prepare the RNA samples for electrophoresis, mix the RNA samples in formamide LB 1:1 with 10 μ L 2X MOPS/formaldehyde LB. Heat the samples at 80°C for 2–5 mins (see Note 12).

⁹. This additional centrifuge step pools residual ethanol from the wash into the bottom of the tube, which can then be gently pipetted away, allowing for the pellet to air dry more quickly and efficiently.

¹⁰. To make 1 L of 10X MOPS, dissolve 41.8 g of MOPS, 3.29 g of sodium acetate, and 1.46 g of EDTA to 900 mL of ddH₂O. Adjust the pH to 7.0, and bring the final volume to 1 L. Filter sterilize the solution, protect from light by covering the bottle with aluminum foil, and store at 4°C.

¹¹. If the formaldehyde solution is cloudy, use a new bottle for all of the steps described in this protocol.

¹². To make 2X MOPS/formaldehyde LB (prepare fresh every time), prepare a 4:1 solution of formaldehyde:10X MOPS, and use 0.5 M EDTA stock to achieve a final concentration of ~15 mM EDTA.

6. While the samples are incubating, add 50 mL formaldehyde to the 2 L of cold 1X MOPS buffer. Gently pour this solution over the agarose/formaldehyde gel in the electrophoresis chamber (see Note 13).
7. Before loading the samples, it is important to clean each well by pipetting 1X MOPS/formaldehyde buffer in and out of each well, preventing band smearing. Once the wells are all rinsed, load 20 μ L of each sample into each well in the desired order for the final northern blot image. Run the gel at 5 V/cm for approximately 3 h or until the two dye fronts have adequately separated.
8. Once the run is complete, begin the gel transfer by first trimming off the elevated lips bordering the gel without cutting into sample lanes. This will allow the gel to lay as flat as possible during the transfer. In an appropriately sized Pyrex (or comparable) glass dish, rinse the gel on a rotator 3X in ultrapure H₂O.
9. After the third wash, replace the water with 10X SSC (diluted from 20X SSC with ultrapure H₂O), and slowly agitate on rotator for 30–60 min.
10. While the gel is equilibrating in 10X SSC, place a glass plate over a large Pyrex (or comparable) glass tray filled with 600–800 mL of 10X SSC. Place two pieces of 3MM chromatography paper over the glass plate in such a way they dip into the 10X SSC buffer. Wet the paper with 10X SSC, and use a 25 mL plastic pipette to gently roll out any puddles, bubbles, or wrinkles before proceeding to the next step.
11. Place the equilibrated gel upside down on top of the 3MM chromatography paper, again ensuring that no bubbles or wrinkles are introduced. Using the gel as a template for size, cut a piece of Amersham Hybond™ - XL blotting membrane of comparable size, situate on top of the gel, and wet the membrane with the 10X SSC buffer clearing bubbles by rolling with the plastic pipette.
12. Wet a piece of 3MM chromatography paper with the 10X SSC and place on top of the membrane (see Note 14). Roll this with the pipette to remove any bubbles, and repeat with two additional pieces of 3MM paper (total of three).
13. To block buffer from transferring outside of the gel, place 4 pieces of Parafilm, one on each side of the gel, effectively creating a dam around the transfer sandwich. Finally, add three pieces of dry 15 $\times$ 20 cm 3MM papers on top of the stack, followed by about 10 cm worth of dry paper towels, and lastly a Pyrex tray or other flat surface weighed down with a 500 mL bottle of liquid. Leave overnight for capillary transfer.

Preparation of the riboprobe

1. To prepare the radioactive riboprobe used for northern blotting, first linearize the plasmid DNA serving as the transcription template using the proper restriction

¹³. We have found it is good practice to thoroughly rinse both the casting box and combs prior to casting the gel. Allow sufficient time for combs to air dry for best well formation.

¹⁴. We suggest marking a designated membrane corner with a pencil to establish orientation for future reference.

enzyme (*e.g.*, to linearize the pcTET2 constructs, digest with SpeI at 37°C overnight). Purify the linearized DNA by phenol:chloroform extraction and ethanol precipitation or spin column method of choice, and resuspend the DNA in ultrapure H₂O to a final concentration of 0.5 µg/µl. Alternatively, the desired probe fragment can be generated by PCR, using a reverse primer containing the T7 promoter sequence.

2. Following your institution's approved radioactive materials guidelines, prepare the *in vitro* transcription reaction by assembling the following reaction:

1 µL 10X T7 buffer
1 µL 50 mM DTT
1 µL 5 mM ATP, GTP, CTP, and 0.1 mM UTP
1 µL linearized plasmid (0.5 µg/µl)
4.0 µL α-³²P-UTP (10 mCi/mL)
0.5 µL SUPERase-In™ ribonuclease inhibitor
0.5 µL T7 RNA polymerase
-- µL ddH₂O to make 10 µL total

Incubate at 37°C for 1 h and dilute final reaction to 50 µL by adding 40 µL TE buffer.

3. To remove unincorporated dNTPs, first place a Sephadex G50 column in an open microfuge tube and centrifuge at 3,000 RPM for 1 min. Transfer the spin column to a new tube and add the probe mix directly to the center of the column. Centrifuge at 3,000 RPM for 2 mins and transfer eluate containing the probe to a new tube. Use a Geiger or scintillation counter to ensure the probe is radioactive, and dilute the eluate to 500 µL with TE-buffer. As only 100 µL of this probe is required per each membrane hybridization, the remaining reaction can be stored in a radioactive material storage box at -20°C.

Membrane hybridization and imaging

1. The morning after the transfer, wash the membrane gently with ultrapure H₂O to remove all salt. Set the membrane on 3MM paper and let dry at room temperature for approximately 30 mins.
2. Place the membrane on top of 3MM paper with the RNA sample-side facing up, and crosslink the RNA to the membrane using a 254 nm UV crosslinker set to deliver 1200 × 100 µJ/cm². Wet the blot in a bath of 2X SSC to avoid background, and carefully roll the blot in a nylon hybridization mesh before inserting into a hybridization bottle. It is crucial that during the rolling incubation the RNA sample-side of the membrane is facing towards the inner cavity of the tube with the surface area completely exposed to the hybridization buffer.

3. Add 10–20 mL ULTRAhyb™ Ultrasensitive Hybridization Buffer pre-warmed to 65°C, and screw on lids without over tightening. Place tubes in the hybridization oven ensuring that tube weight is balanced equally across the rotisserie rotor (simply match an unpaired tube with one filled with H₂O of an equal mass). Incubate in a hybridization oven for at least 60 mins at 68°C.
4. Add the RNA probe directly to the hybridization buffer in the bottom of the tube, being careful to avoid exposing any of the probe to the membrane at this step.
5. Incubate the blot at 68°C for at least 4 hours to overnight.
6. Decant the hybridization solution in an appropriate radioactive liquid waste container and rinse the hybridization tube with ~30 mL 2xSSC, 0.1% SDS solution. Decant the rinse and wash the blot twice for 5 min in 2xSSC, 0.1% SDS and twice for 15 min in 0.1xSSC, 0.1% SDS.
7. Following the final wash, remove the blot from the hybridization tube with forceps or hemostats. Lay the blot on plastic wrap to briefly dry, before wrapping it in a second piece of plastic wrap. Expose to a storage phosphor screen for a few hours to overnight to detect mRNAs.
8. Image storage phosphor screen using a Typhoon Trio Plus variable mode imager (GE), or comparable phosphorimager. Adjust exposure time as necessary to increase sensitivity to allow visualization of faint bands or decrease sensitivity to avoid saturation of the probe signal.
9. Use ImageQuant or equivalent image analysis software to quantify the signals derived from the experimental and control mRNAs at each timepoint. Normalize the experimental signal to the control signal and set the initial abundance of the experimental mRNA to 1 to calculate the fraction of experimental mRNA remaining at each timepoint. Plot the fraction mRNA remaining versus time on a semi-log plot. The slope of the best-fit line can then be used to calculate the mRNA half-life, using the equation $t_{1/2} = -0.43\ln(2)/slope$.

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Figure 1: Schematic of the workflow described to quantify mRNA decay using RNAi knockdown in Tet-off cell lines.