Inducing RNAi in *Drosophila* Cells by Soaking with dsRNA

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Protocol

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RNA interference (RNAi) triggered by synthetic long double-stranded RNAs (dsRNAs) has been applied in many *Drosophila* cell lines to study the functions of individual genes or for genome-wide scans. One contributor to the popularity of this approach is that many fly cell lines spontaneously take up dsRNAs from media, obviating the need for assisted uptake methods such as transfection. In this protocol, RNAi is induced in *Drosophila* S2 cells by soaking with dsRNA. Cell lines other than S2 can also be used, although the ability of each line to passively take up dsRNA does vary. Therefore, the efficiency of passive uptake should be carefully verified for each line.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Complete S2 medium <R>

It may be necessary to test different lots of fetal bovine serum to find one that works well with S2 or other fly cells.

Drosophila S2 cells in semiadherent culture or suspension culture

Cells that are healthy and growing exponentially give the best results. Doubling times of 18–24 h are normal for most lines. Note especially that many S2 lines are persistently virus infected (e.g., with flock house virus). Care must be taken to obtain and work with noninfected cells.

dsRNA at 0.5–10 µg/µL in nuclease-free H_2O or TE buffer (10 mM Tris [pH 8] and 1 mM EDTA)

dsRNAs derived from different portions of target genes differ in their silencing efficiency. Therefore, it is generally a good idea to test several different dsRNAs in parallel for each gene to be silenced.

Trypsin/EDTA in PBS (without Ca^{2+} or Mg^{2+}) <R>

Alternatively, use PBS/EDTA/EGTA <R> (see Step 1).

Equipment

Centrifuge (low speed)
Culture incubator (25°C)
Hemocytometer and microscope for cell counting
Tissue culture plates (six-well)

This protocol is designed for Schneider S2 cells in one well of a standard six-well tissue culture plate. The protocol can be scaled up or down easily for different plate formats simply by multiplying all volumes and cell numbers by the surface area of the desired plate divided by the surface area of the six-well plate (see Table 1).

Adapted from *RNA: A Laboratory Manual* by Donald C. Rio, Manuel Ares Jr, Gregory J. Hannon, and Timothy W. Nilsen. CSHL Press, Cold Spring Harbor, NY, USA, 2011.

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TABLE 1. Scaling up or down for different plate formats

Culture vessel	96 well	48 well	24 well	12 well	6 well	35 mm	60 mm	100 mm	150 mm	T25	T75
Surface area (cm^2)	0.3	0.7	2	4	10	10	20	60	140	25	75

Multiply all volumes and cell numbers by the surface area of the desired plate divided by the surface area of the six-well plate.

METHOD

1. Collect S2 cells from semiadherent culture using trypsin/EDTA (or by vigorous pipetting in PBS/EDTA/EGTA) or harvest from suspension culture. Pellet cells by centrifuging at 800–1000*g* for 5 min at room temperature.

   S2 cells adhere weakly to plates and can often be removed without the use of trypsin by incubation with PBS/EDTA/EGTA.

2. Resuspend the cells in culture medium lacking serum, count cells with a hemocytometer, and adjust to a concentration of 1×10^6 to 5×10^6 cells/mL.
3. Add 10–30 µg of dsRNA per tissue culture plate well.
4. Add 1.5 mL of cell suspension and mix by pipetting.
5. Incubate the mixture for 30–60 min at room temperature.
6. Add 3 mL of complete S2 medium to each well.
7. Culture the cells for 3–6 d at 25°C (or at room temperature).

   See Discussion.

8. Measure knockdown by quantitative PCR or western blotting and determine the phenotypic consequences of knockdown as desired.

   It is important wherever possible to measure knockdown by quantifying changes in protein levels. Although it is easier to measure RNA knockdown, RNA and protein are not always equally affected nor does their decline follow the same time course. See Troubleshooting.

TROUBLESHOOTING

Problem (Step 8): Achieving the desired knockdown is problematic.

Solution: A different dsRNA should be tested (although it is always wise to test several initially). Alternatively, a second round of soaking can be done 4 d after the initial treatment using this protocol. This may not only increase knockdown (although it does not in all cases), but it also affords the possibility of examining phenotypes that require longer times to become evident.

DISCUSSION

The length of incubation required varies depending on the target gene and the effectiveness of the dsRNA. Loss of mRNA, measured by semiquantitative reverse transcription–PCR (RT–PCR) or northern blotting, is generally evident after 2–3 d. The time required to observe loss of protein is more variable and depends on many factors, including the protein half-life. In the majority of cases, a knockdown of ≥80% or greater at both mRNA and protein levels is observed after 3–4 d, and this can persist until ~5–6 d.

RELATED INFORMATION

dsRNA-mediated RNAi in *Drosophila* cell culture (i.e., without assisted uptake) was first developed and used by Clemens et al. (2000). If the soaking protocol does not work for a particular cell line, one can almost always introduce dsRNA by transfection as described in **Inducing RNAi in *Drosophila* Cells by Transfection with dsRNA** (Zhou et al. 2013).

RECIPES

Complete S2 Medium

1× antibiotic-antimycotic solution (Invitrogen 15240–062)
10% fetal bovine serum

Prepare in Schneider's complete medium (Invitrogen 11720–034).

PBS/EDTA/EGTA

Reagent	Quantity (for 500 mL)	Final concentration
Phosphate-buffered saline (PBS) (10×)	50 mL	1×
EDTA (0.5 M, pH 8.0)	1 mL	1 mM
EGTA (0.1 M, pH 8.0)	5 mL	1 mM
H_2O	to 500 mL	

Phosphate-Buffered Saline (PBS)

Reagent	Amount to add (for 1× solution)	Final concentration (1×)	Amount to add (for 10× stock)	Final concentration (10×)
NaCl	8 g	137 mM	80 g	1.37 M
KCl	0.2 g	2.7 mM	2 g	27 mM
Na_2HPO_4	1.44 g	10 mM	14.4 g	100 mM
KH_2PO_4	0.24 g	1.8 mM	2.4 g	18 mM
If necessary, PBS may be supplemented with the following:				
$CaCl_2 \cdot 2H_2O$	0.133 g	1 mM	1.33 g	10 mM
$MgCl_2 \cdot 6H_2O$	0.10 g	0.5 mM	1.0 g	5 mM

PBS can be made as a 1× solution or as a 10× stock. To prepare 1 L of either 1× or 10× PBS, dissolve the reagents listed above in 800 mL of H_2O. Adjust the pH to 7.4 (or 7.2, if required) with HCl, and then add H_2O to 1 L. Dispense the solution into aliquots and sterilize them by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle or by filter sterilization. Store PBS at room temperature.

Trypsin/EDTA in PBS

0.25% trypsin
1 mM EDTA

Prepare in phosphate-buffered (PBS) without Ca^{2+} or Mg^{2+}

REFERENCES

Clemens JC, Worby CA, Simonson-Leff N, Muda M, Maehama T, Hemmings BA, Dixon JE. 2000. Use of double-stranded RNA interference in *Drosophila* cell lines to dissect signal transduction pathways. *Proc Natl Acad Sci* **97:** 6499–6503.

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