Inducing RNAi in *Drosophila* Cells by Soaking with dsRNA

Rui Zhou, Stephanie Mohr, Gregory J. Hannon and Norbert Perrimon

*Cold Spring Harb Protoc*; doi: 10.1101/pdb.prot080747
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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.

RECIPIES: 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 H2O 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 Ca²⁺ or Mg²⁺)** <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).*


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Cite this protocol as Cold Spring Harb Protoc; doi:10.1101/pdb.prot080747
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).
RECIPIES

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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Quantity (for 500 mL)</th>
<th>Final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate-buffered saline (PBS) (10×)</td>
<td>50 mL</td>
<td>1×</td>
</tr>
<tr>
<td>EDTA (0.5 M, pH 8.0)</td>
<td>1 mL</td>
<td>1 mM</td>
</tr>
<tr>
<td>EGTA (0.1 M, pH 8.0)</td>
<td>5 mL</td>
<td>1 mM</td>
</tr>
<tr>
<td>H2O</td>
<td>to 500 mL</td>
<td></td>
</tr>
</tbody>
</table>

Phosphate-Buffered Saline (PBS)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount to add (for 1× solution)</th>
<th>Final concentration (1×)</th>
<th>Amount to add (for 10× stock)</th>
<th>Final concentration (10×)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>8 g</td>
<td>137 mM</td>
<td>80 g</td>
<td>1.37 M</td>
</tr>
<tr>
<td>KCl</td>
<td>0.2 g</td>
<td>2.7 mM</td>
<td>2 g</td>
<td>27 mM</td>
</tr>
<tr>
<td>Na2HPO4</td>
<td>1.44 g</td>
<td>10 mM</td>
<td>14.4 g</td>
<td>100 mM</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>0.24 g</td>
<td>1.8 mM</td>
<td>2.4 g</td>
<td>18 mM</td>
</tr>
</tbody>
</table>

If necessary, PBS may be supplemented with the following:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount to add (for 1× solution)</th>
<th>Final concentration (1×)</th>
<th>Amount to add (for 10× stock)</th>
<th>Final concentration (10×)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCl2·2H2O</td>
<td>0.133 g</td>
<td>1 mM</td>
<td>1.33 g</td>
<td>10 mM</td>
</tr>
<tr>
<td>MgCl2·6H2O</td>
<td>0.10 g</td>
<td>0.5 mM</td>
<td>1.0 g</td>
<td>5 mM</td>
</tr>
</tbody>
</table>

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 H2O. Adjust the pH to 7.4 (or 7.2, if required) with HCl, and then add H2O to 1 L. Dispense the solution into aliquots and sterilize them by autoclaving for 20 min at 15 psi (1.05 kg/cm2) 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 Ca2+ or Mg2+

REFERENCES


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