Protocol

RNAi in *Drosophila* S2 Cells by dsRNA Soaking

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This is a simple method for inducing RNAi in *Drosophila* S2 cells by soaking the cells in medium containing dsRNAs. In comparison with transfection, soaking requires fewer procedures and avoids the cost of transfection reagents, making it the method of choice for high-throughput RNAi screening. Moreover, soaking avoids potential toxicity associated with transfection reagents. However, delivery of dsRNA to S2 cells by soaking is less efficient than transfection, and genes whose expression has proved difficult to repress using soaked dsRNA often can be suppressed by transfecting the dsRNA multiple times.

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.

Reagents

Drosophila Schneider 2 (S2) cells (e.g., Life Technologies, catalog no. R690-07)
dsRNAs (1 µg/µL) (see Protocol: **Preparation of dsRNAs for RNAi by In Vitro Transcription** [Li and Zamore 2019])
Fetal bovine serum (FBS), heat-inactivated
Schneider's *Drosophila* medium (e.g., Life Technologies, catalog no. 11720-034)

Equipment

Conical tubes (50 mL)
Immunofluorescence and western blotting, quantitative RT-PCR, or northern hybridization equipment (see Step 8)
Laminar flow hood (Class II)
Stereomicroscope
Tissue culture dishes (10 cm)
Tissue culture incubator (25°C), humidified
Tissue culture plates (six well)

METHOD

The soaking protocol here is designed for cells grown in six-well plates. If other multiwell plates, flasks, or dishes of a different diameter are used, scale the cell density and reagent volumes according to the surface area of the well (see Table 1).

From the Molecular Cloning collection, edited by Michael R. Green and Joseph Sambrook.

Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot097477

TABLE 1. Volumes of cells and dsRNA used for soaking cultured *Drosophila* cells

Culture plate or dish	24 well	12 well	6 well	6 cm	10 cm
Surface area per well (cm^2)	2	4	10	20	60
Soaking medium					
Schneider's *Drosophila* medium (mL)	0.2	0.4	1	2	3
1 µg/µL dsRNAs (µL)	3	6	15	30	45
Schneider's *Drosophila* medium + 10% FBS (mL)	0.4	0.8	2	4	6
Total growth medium (mL)	0.6	1.2	3	6	9

Preparation of Cells for Soaking

1. Grow S2 cells at 25°C in Schneider's *Drosophila* medium supplemented with 10% FBS to a density of 5×10^6 to 10×10^6 cells/mL.
2. Centrifuge the cells at 1000*g* for 3 min at room temperature. Discard the supernatant and dilute cells in serum-free Schneider's *Drosophila* medium to a density of 1×10^6 cells/mL.
3. Transfer 1 mL of the cell suspension (1×10^6 cells) into each well of a six-well cell culture plate.
4. Incubate the cultured cells at 25°C in a humidified incubator.

Soaking Cells with dsRNAs and Analysis of Knockdown

5. Add 15–30 µg of dsRNA into each well of the diluted cells. Mix by gently moving the plate back and forth in straight lines rather than circles to avoid aggregation of cells in the middle, which reduces the efficiency of the uptake of dsRNA into cells.
6. Incubate the cultured cells for 30 min at 25°C in a humidified incubator.
7. Add 2 mL of Schneider's *Drosophila* medium supplemented with 10% FBS into each well.
8. Incubate the culture for 2–6 d at 25°C in a humidified incubator. Analyze knockdown effects by assessing the reduction of the target protein level by immunofluorescence and western blotting using antibody that specifically recognizes the target protein and measuring the reduction of the target mRNA level by quantitative reverse transcription-polymerase chain reaction (RT-PCR) or northern hybridization.

See Troubleshooting.

TROUBLESHOOTING

Problem (Step 8): There is no gene silencing.
Solution: dsRNAs are degraded. Repeat the transfection with freshly prepared dsRNAs.

REFERENCES

Li C, Zamore PD. 2019. Preparation of dsRNAs for RNAi by in vitro transcription. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot097469.

Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot097477

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