

Transfection of S2 Cell with DNA Using CellFectin Reagent

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[Abstract] This method provides a step-by-step guide to transfecting *Drosophila* S2 cells with the pRmHA-3 (or similar) vector with insert of choice (in this case SDF-1 β -FLAG) and generating a stable cell line. This cell line is then capable of producing the protein of interest under inducible conditions by addition of copper sulfate, which can then be purified and used as desired. This protocol provides an example to finding out when your peak protein production occurs and a method for determining optimal selection conditions.

Materials and Reagents

1. *Drosophila* Schneider 2 (S2) cells (Life Technologies, Invitrogen™, catalog number: R690-07)
2. Dros-SFM (Life Technologies, Invitrogen™, catalog number: 10797-025)
3. pHAS β AG (pRmHA-3 vector with SDF-1 β -FLAG inserted. Contains a metallothionein promoter inducible with Cu²⁺)
4. pUC-Hsneo (contains the neomycin-resistant cassette required for stable selection)
5. CellFectin reagent (Life Technologies, Invitrogen™, catalog number: 10362-010)
6. FBS (Life Technologies, Invitrogen™, catalog number: 10437-028)
7. Penicillin-Streptomycin (P/S) (Life Technologies, Invitrogen™, catalog number: 15140-122)
8. G418 sulphate (Life Technologies, Invitrogen™, catalog number: 11811-031)
9. CuSO₄ (Sigma Aldrich, catalog number: C8027)
10. HT supplement (Life Technologies, Invitrogen™, catalog number: 11067-030)
11. Transfection solutions (see Recipes)

Equipment

1. 6-well plate (Corning Incorporated, catalog number: 353046)
2. 24-well plate (Corning Incorporated, catalog number: 353047)

Procedure

1. *Drosophila* Schneider 2 (S2) cells are plated out at 1.6×10^6 cells/well (6-well plate, 2 ml /well) and incubated overnight at room temperature (RT).
Note: The optimal temperature of S2 cells is actually 28 °C and they do not need CO₂ to culture.
2. Following day, cells are washed 2x with Dros-SFM (no FBS, P/S or HT) 1 ml/well.
3. Prepare the 'DNA mix' and 'CellFectin Mix' transfection solutions.

6-well plate layout

0.5 mg/ml G418	1.0 mg/ml G418	1.5 mg/ml G418	0.5 mg/ml G418	1.0 mg/ml G418	1.5 mg/ml G418
Un-transfected	Untransfected	Untransfected	Transfected w. 4 µl CellFectin	Transfected w. 4 µl CellFectin	Transfected w. 4 µl CellFectin
No G418	No G418	No G418	0.5 mg/ml G418	1.0 mg/ml G418	1.5 mg/ml G418
Un-transfected	Transfected w. 4 µl CellFectin	Transfected w. 8 µl CellFectin	Transfected w. 8 µl CellFectin	Transfected w. 8 µl CellFectin	Transfected w. 8 µl CellFectin

4. 100 µl /sample (i.e. 400 µl total) of the DNA mix is added to 100 µl /sample (i.e. 400 µl total) CellFectin mix. Solution is gently mixed and left at RT for 30 min.
5. After 30 min, 800 µl /sample (3.2 ml total) of Dros-SFM (no FBS, P/S or HT) is added to the DNA/CellFectin mix.
6. 1 ml of this solution is then added to the appropriate wells containing the S2 cells and incubated at RT for 5 h.
7. Following the 5 h incubation, medium is aspirated off and Dros-SFM + 10% FBS + P/S + HT (= complete media) is added to each well (2 ml/well).
8. Plates are incubated at RT for 48 h.
9. At 48 h post-transfection the media is removed and replaced with 2 ml media containing G418 sulphate at indicated concentrations. The cells are transferred to 24-well plates (one 6-well ≥ eight 24-wells) supplemented with additional media + G418 to 1 ml.

24-well plate layout

No G418	No G418	No G418	0.5 mg/ml G418	1.0 mg/ml G418	1.5 mg/ml G418
Un-transfected	Transfected w. 4 µl CellFectin	Transfected w. 8 µl CellFectin	Un-transfected	Untransfected	Untransfected
0.5 mg/ml G418	1.0 mg/ml G418	1.5 mg/ml G418	0.5 mg/ml G418	1.0 mg/ml G418	1.5 mg/ml G418
Transfected w. 4 µl CellFectin	Transfected w. 4 µl CellFectin	Transfected w. 4 µl CellFectin	Transfected w. 8 µl CellFectin	Transfected w. 8 µl CellFectin	Transfected w. 8 µl CellFectin

10. Cells are incubated at RT with media changed every 5 days (1 ml/well).
11. After 3 weeks, untransfected cells should be killed by the G418 at least at the highest concentration. The transfected cells should be fine and healthy.

12. Cells within the 4 μ l and 8 μ l CellFectin groups are pooled and split 1:1 into two 24-well plates (*i.e.* 8 wells /plate). After 1 h, media is removed and replaced with either complete SFM + 1.5 mg/ml G418 or complete SFM + 1.5 mg/ml G418 + 1 mM CuSO₄.

24-well plate layout	1.5 mg/ml G418		1.5 mg/ml G418		1.5 mg/ml G418
	Transfected w. 4 μ l CellFectin		Transfected w. 8 μ l CellFectin		Transfected w. 8 μ l CellFectin

13. Cell lysates are taken at 2, 3, 4 and 5 days post-CuSO₄ addition to determine peak time of SDF-1 β -FLAG production.

Recipes

1. Transfection solutions
 - a. DNA mix (per sample):

4 μ g pHAS β AG = 1.67 μ l	Total (9 samples)
0.2 μ g pUC-Hsneo = 0.63 μ l	15.0 μ l
100 μ l Dros-SFM (no FBS or P/S)	5.7 μ l
	900 μ l
 - b. CellFectin Mix (per sample):

4 μ l or 8 μ l CellFectin reagent	Total (4 samples)
100 μ l Dros-SFM	16 or 32 μ l
	400 μ l

References

1. Bunch, T. A., Grinblat, Y. and Goldstein, L. S. (1988). [Characterization and use of the *Drosophila* metallothionein promoter in cultured *Drosophila melanogaster* cells.](#) *Nucleic Acids Res* 16(3): 1043-1061.