Use of Metafectene for the Transfection of Mouse Embryonic Fibroblasts

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1. Transfection Protocol

MEFs were plated 24 hours prior to transfection and cultured in growth medium without antibiotics (DMEM + 15% FBS) at 37°C. For transfections in 6 well plates, 4µl/8µl /12µl of Metafectene was diluted into a total of 100µl of OptiMEM (Gibco) and 2µg of DNA was diluted into a total of 100µl of OptiMEM. The dilutions were mixed and incubated at room temperature for 30 minutes. Cells were washed with PBS and 1 ml of OptiMEM was added to each plate. Then Metafectene/DNA complex was added to plate dropwise. The transfections were incubated at 37. After 6 hours, the transfection mixture was aspirated and 2 ml of growth medium was added to each well. GFP signal was checked the next day.

2. Optimization of Metafectene Transfection

Primary mouse embryonic fibroblasts (MEF) were transfected with Metafectene under 3 different conditions in order to optimize the transfection efficiency. The ratio of DNA to Metafectene was varied. The condition of transfection is described below.

6-well plate (per well)

Cell type	Primary MEFs (P3)
Confluency	80-90%
DNA (pEGFP-C1)	2 µg
Metafectene	4µl / 8µl / 12µl

The transfections using 8µl or 12µl of Metafectene had **~40% GFP positive cells**.

3. Comparison of Transfection reagents

MEFs were also transfected with other lipid-based transfection reagents (e.g. Lipid L2). The same protocol was applied except the amount of Lipid L2 used.

6-well plate (per well)

Cell type	Primary MEFs (P3)
Confluency	80-90%
DNA (pEGFP-C1)	2 µg
Lipid L2	2µl / 4µl / 8µl

The transfections using Lipid L2 **had ~20% GFP positive cells**.

4. Conclusion

Metafectene can be used to transiently transfect primary MEFs. The reagent performed better than other lipid based transfection reagents tested. Usage of 4:1 or 6:1 Metafectene:DNA is recommended.