

syn-mRNA-Cre transfection into the various cell lines

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Abstract

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Protocol

Step 1.

The Cre-responsive cell lines were grown for several days in their respective culture media to reach 10% confluence prior to the addition of syn-mRNA-Cre.

Step 2.

The Lipofectamine Messenger MAX Transfection Reagent (Thermo Fisher Scientific, Waltham, MA) is diluted with Opti-MEM medium (Thermo Fisher Scientific) at the 1:33 – 2:33 volume ratio (depends on the cell type) and is incubated for 5-10 min. in room temperature.

Step 3.

syn-mRNA-Cre diluted in Opti-MEM medium (20 ng/μl) is added to Lipofectamine Messenger MAX Transfection Reagent at 1:1 volume ratio and is incubated for 5 min. in room temperature.

Step 4.

The syn-mRNA-Cre (10 ng/μl) is diluted in fresh culture media to final concentration 1 ng/μl

and is used as a maximal concentration without visible toxic effects. The two subsequent decimal dilutions are prepared to find out the optimal concentration for each cell line.