

# Determination of the transfection efficiency of mouse embryonic fibroblasts (MEFs)

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# Introduction:

MCL-1 is an anti-apoptotic BCL-2 homologue that regulates cytochrome c release from mitochondria. In order to determine the role of the N-terminus of MCL-1 in the anti-apoptotic function of MCL-1, we analyzed the effect of various truncation mutants in MCL-1 null MEFs. As these MEFs proved rather difficult to transfect, we tested several transfections reagents.

### Materials and methods:

Wild type and MCL-1 knockout MEFs were cultured in DMEM supplemented with 10% heat-inactivated foetal bovine serum, 20 mM L-glutamine, 100 mM ß-mercaptoethanol and 0.1 mM non-essential amino acids, as described (1)

# **Experimental:**

Transfections reagents were used according to the manufacturers' instructions.

### Results and discussion:

MEFs were first transfected with GFP using Lipofectamine LTX, Lipofectamine 2000, Lipofectine, Effectene or Metafectene Pro and analyzed by fluorescence microscopy. While a few green cells were observed with effectene, no transfected cells were observed when using Lipofectamine-based reagents. On the other hand, between 30-50% of the cells transfected using Metafectene Pro were positive for GFP signal.

# **Conclusion:**

Metafectene Pro was thus used for all subsequent experiments using MEFs (1)

References: M Germain and V Duronio (2007) J Biol Chem 282, 32233-32242