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Efficient transfection of murine embryonic motor neurons via magnetofection

1.) Primary embryonic motor neurons are grown on poly-ornithine/laminine coated glass coverslips in 12-well plates for 48-72h.
2.) Pre-incubation: Collect motor neuron growth medium from cells, transfer it to a cell culture dish and keep it in the incubator for later. Replace medium with glia-conditioned Neurobasal+B27 medium (500-750ul) and incubate motor neurons for 1h.
3.) Complex preparation: During pre-incubation, dilute 0.5ug purified endotoxin free plasmid DNA in 100ul MEM medium (w/o serum or supplements). Add 1.75ul of resuspended NeuroMag beads (NM51000; Oz Biosciences), vortex briefly and incubate for 15-20 min at RT.
4.) Magnetofection: Add DNA/NeuroMag mix dropwise to motor neurons. Place dish on magnetic plate (MF10000; Oz Biosciences) inside the incubator for 15 min.
5.) Remove magnetic plate and incubate motor neurons for another 45 min.
6.) Place motor neurons briefly on the magnetic plate, aspirate off conditioned medium + DNA/NeuroMag and replace it with motor neuron growth medium collected before. Return motor neurons to incubator.
7.) Expression of fluorescent proteins should be visible 24h after magnetofection.

Important factors:

Make sure that motor neurons look healthy with normal morphology.

DNA should be pure, free of contaminants, and with low endotoxin levels.