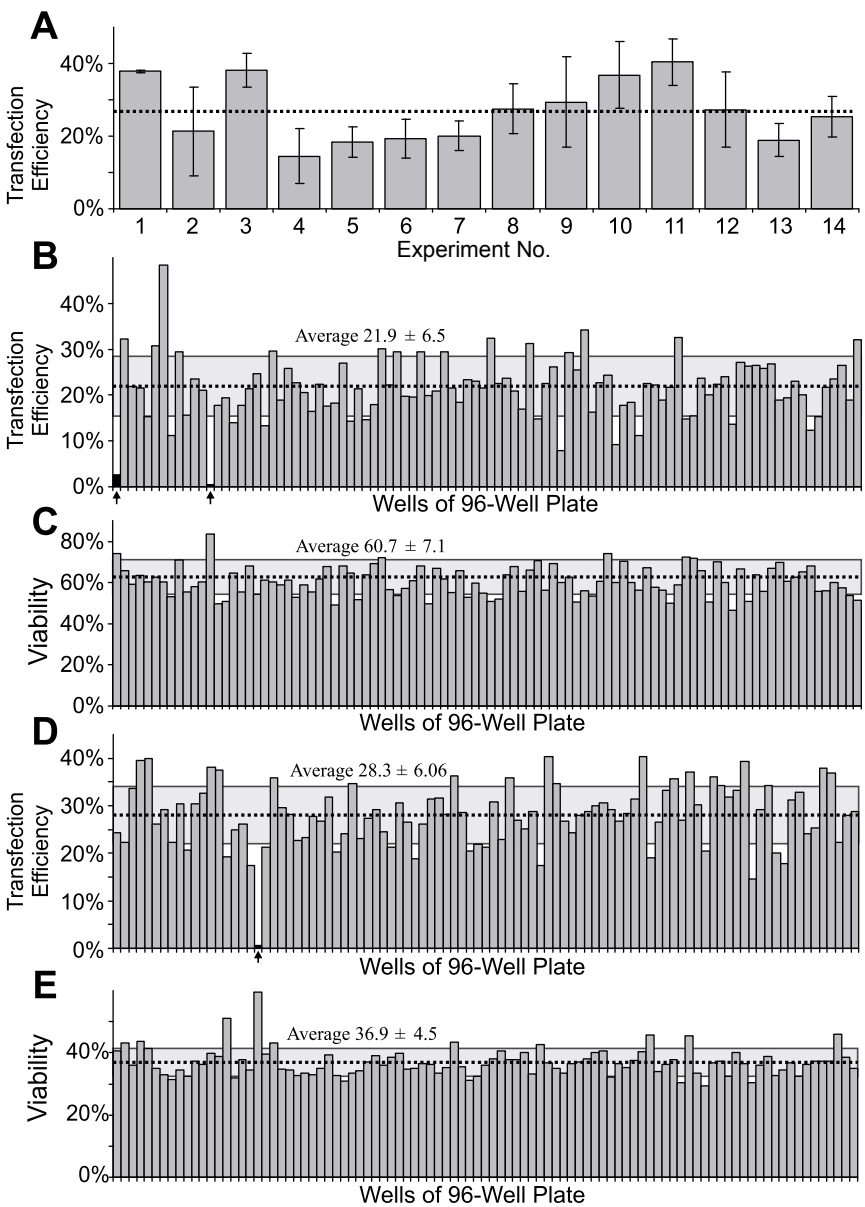
SUPPLEMENTARY MATERIAL FOR:

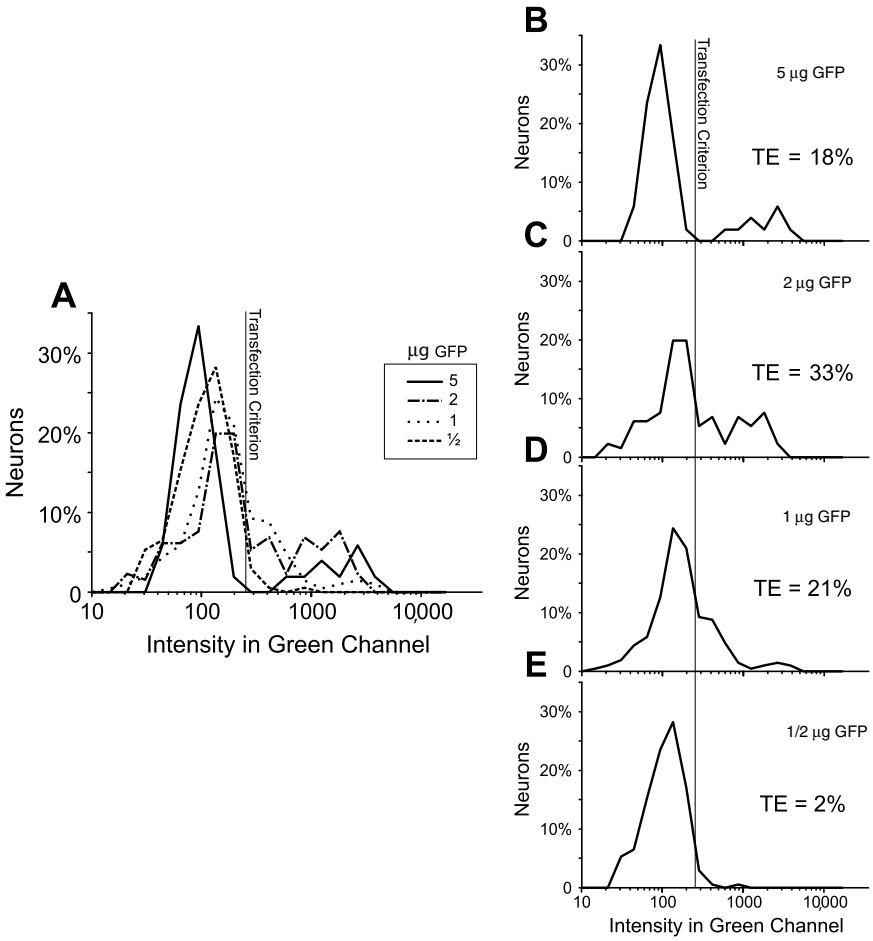
96-Well electroporation method for transfection of mammalian central neurons

William J. Buchser, Jose R. Pardinas, Yan Shi, John L. Bixby, and Vance P. Lemmon
University of Miami Miller School of Medicine, Miami, FL, USA

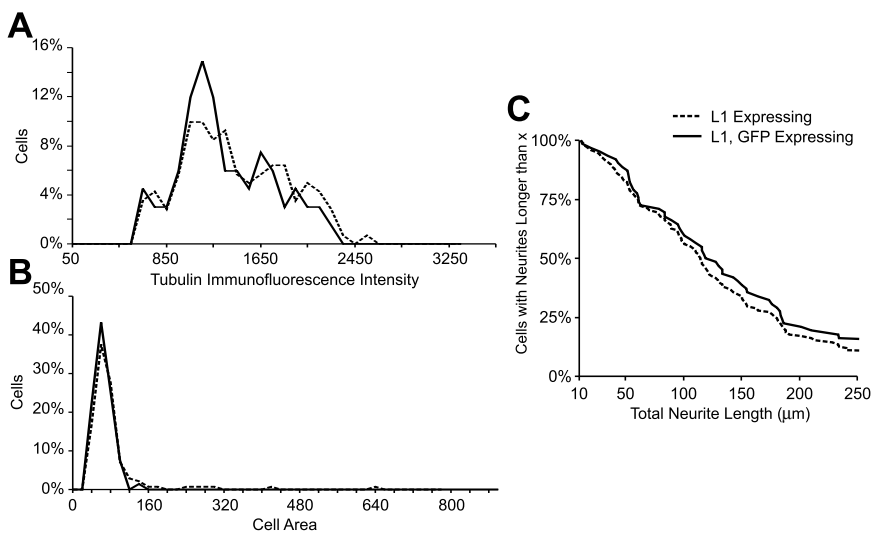
BioTechniques 41:XXX-XXX (November 2006)



Supplementary Figure S1. Transfection efficiency and variability. (A) The average transfection efficiency across 14 experiments using these conditions is 26% (dotted line). Error bars are SEM. (B and D) Transfection efficiencies for green fluorescent protein (GFP) plotted for each well of a 96-well plate**.** (C and E) Viability of cerebellar granule neurons (CGN) transfected with GFP plotted for each well of a 96-well plate. In panels B and C, 25,000 cells were electroporated per well and fixed after 24 h in culture, while 180,000 were electroporated in panels D and E and fixed after 48 h in culture. Dotted lines were drawn along the average for the plate. Shaded region behind the bars indicate the upper and lower extent of 1 SD. Control wells were electroporated but received no plasmid (black bars indicated with arrows) and, therefore, expressed no GFP.



Supplementary Figure S2. Green fluorescent protein (GFP) expression when plasmid amounts vary. GFP intensity, on a log scale, is plotted against the percentage of total neurons for experiments with different amounts of DNA. All neurons are plotted, both GFP positive and GFP negative. The cutoff for GFP positive is plotted as a vertical line (transfection criterion). (A) Four different amounts of GFP plasmid were electroporated. (B–E) individual graphs are plotted for each experiment. The transfection efficiencies (TE) corresponding to each amount of GFP added are also noted on each figure.



Supplementary Figure S3. Co-expression effect on neurons. (A) Tubulin immunofluorescence and (B) cell body area are plotted against the percentage of total neuron number for a co-transfection experiment. (C) Total neurite lengths are plotted as a cumulative probability histogram. In all plots, dashed lines represent neurons that were electroporated with human L1 plasmid and are expressing L1. Solid lines represent neurons that were electroporated with both green fluorescent protein (GFP) and L1 and are expressing both. Total neurite length was included only for values >10 µm. In the histograms, the y-axis reports percentage of total neurons, which normalizes for the different number of cells in the two populations.