Supplemental protocol for “Mammalian cell penetration, siRNA transfection, and DNA transfection by supercharged proteins” (McNaughton, Cronican, Thompson, and Liu)

Since publication of our paper, we have developed the following protocol to improve the consistency of gene silencing using pyrene butyric acid, presumably by enhancing the consistency of endosomal escape (see Futaki, et al. ACS Chem. Biol. (2006) 1, 299). We recommend using this protocol for gene silencing applications.

siRNA transfection protocol using +36 GFP and pyrene butyric acid (PBA)

This protocol is for the treatment of 3 wells from a 12-well tissue culture plate.

1. HeLa cells were grown to ~ 80% confluency in a 12-well plate. DMEM / 10% FBS was removed and the cells were washed 3 times with PBS.

2. To each well was added 1 mL of a solution containing 50 µM PBA in PBS. The cells were incubated in this solution for 5 min. at 37 °C.

3. In a small plastic tube, 200 pmol of GAPDH-suppressing siRNA (2 µL of a 100 µM siRNA solution) and 800 pmol +36 GFP were pre-mixed and allowed to incubate for 5 min. at 25 °C.

4. ¼ of the total volume of the siRNA/+36 GFP complex was added to each well containing 1 mL 50 µM PBA in PBS. The tissue culture tray was agitated slightly to homogenize the solution in each well, resulting in a solution containing 50 nM siRNA and 200 nM +36 GFP. Cells were incubated under these conditions for 3 hours* at 37 °C.

5. The 50 µM PBA / PBS solution was removed and cells were washed three times with PBS, followed by the addition of 1 mL DMEM in 10% FBS. Cells were incubated under these conditions for 4 days, and knockdown of GAPDH expression was quantitated by Western blot.

* ~ 20 % cytotoxicity was observed after 3 hour incubation in 50 µM PBA/PBS. Much higher cytotoxicity (~80%) was observed when HeLa cells were incubated in 50 µM PBA/PBS for ≥4 hours. The cytotoxicity of PBA may vary by cell type.