An all-in-one, Tet-On 3G inducible PiggyBac system for human pluripotent stem cells and derivatives

Lauren N. Randolph^{1,3,#}, Xiaoping Bao^{4,#}, Chikai Zhou⁵, Xiaojun Lian^{1,2,3,*}

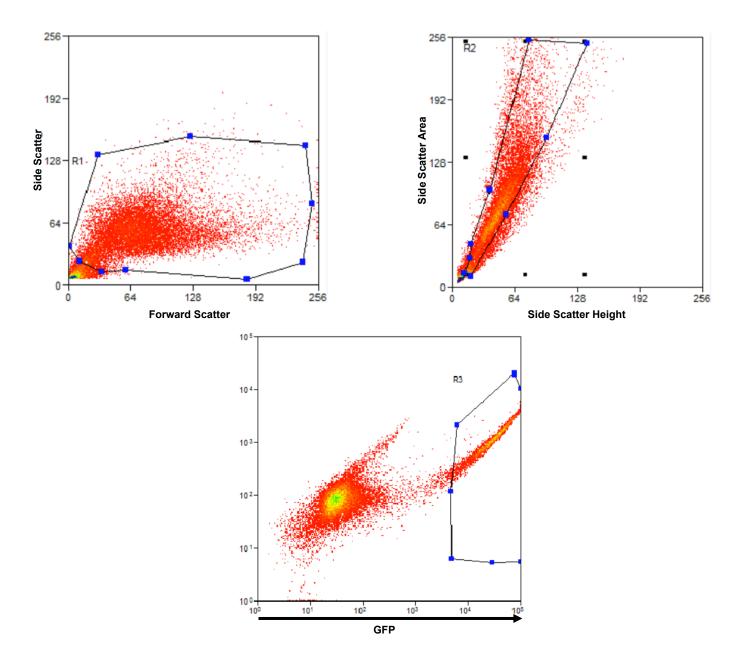
¹Department of Biomedical Engineering, ²Department of Biology, ³The Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, PA, 16802, USA

⁴Department of Chemical and Biological Engineering, University of Wisconsin, Madison, WI, 53706, USA

⁵Department of Cell and Molecular Biology, Karolinska Institutet, 17177, Stockholm, Sweden

^{*} Correspondence should be addressed to: Xiaojun Lian (Lian@psu.edu)

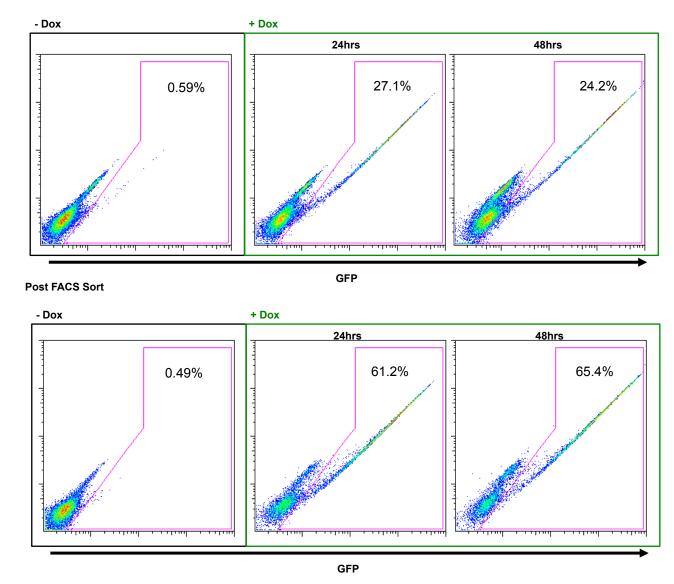
[#] These authors contributed equally to this work.



Supp. Fig. 1: FACS sort gating for population with highest GFP expression (R3).

A Post 2nd Bsd Selection

В



Supp. Fig. 2: Comparison of GFP expression at 24 and 48 hours in cells after A) two rounds of Bsd selection and B) FACS sorting.

Antibody	Source	Application
Oct3/4	Santa Cruz, mouse IgG _{2b} , Clone: C-10, sc-5279	1:100 (IS)
Nanog	Thermo Fisher Scientific, rabbit IgG, PA1-097	1:500 (IS)
cTnT	Lab Vision, mouse IgG1, Clone: 13-11, ms: 295-p1	1:200 (IS)
SSEA-4	DSHB, mouse IgG3, MC-813-70	1:20 (IS)
cTnl	Santa Cruz, rabbit IgG, sc-15368/H-170	1:100 (IS)
Nkx2.5	Santa Cruz, rabbit IgG, sc-14033/H-114	1:75 (IS)
Secondary Antibody	Alexa 555 Goat anti Rb IgG, A-21428	1:1000
Secondary Antibody	Alexa 647 Goat anti Rb IgG, A-21244	1:1000
Secondary Antibody	Alexa 647 Goat anti Ms IgG, A-21235	1:1000

Supp. Table 1: Antibodies used in immunostaining analysis.