**Genetic Background affects Induced Pluripotent Stem (iPS) Cell Generation.**
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**Introduction:** There is growing evidence that the genomic signature of an individual significantly affects not only their susceptibility to disease, but also their response to therapy. The purpose of this study was to determine if the genetic background of an individual influences the ability to generate induced pluripotent stem (iPS) cells. Our hypotheses were that the efficiency of generating iPS cells and the pluripotent stability of the iPS cells would be strongly influenced by genetic background.

**Methods:** Mouse embryonic fibroblasts (MEFs) were isolated from six strains of mice (NON/LtJ; C57BL/6J; DBA/2J; BALB/cJ; 129S1/SvImJ; CAST/EiJ) selected based on genetic diversity and differences in ability to produce embryonic stem (ES) cell lines. The MEFs were reprogrammed via doxycycline-inducible lentiviral transduction of murine Oct4, Klf4, Sox2, and c-Myc. Culture media consisted of standard ES cell media with Knockout serum replacement and doxycycline was added to the media during reprogramming. Differences in efficiency to generate iPS cells were assessed on primary transformation plates by comparing the total number of colonies, the percentage of colonies positive for alkaline phosphatase (AP) staining and the percentage of cells positive for SSEA-1 (determined by FACS). Proliferation of parent MEFs from each strain was examined by calculating the number of population doublings over ten days during which time the MEFs were maintained in standard media and doxycycline to simulate reprogramming conditions. All experiments were performed using MEFs derived from two different embryos for each strain. iPS colonies from primary transformation plates were passaged onto 96-well plates and expanded to establish doxycycline-independent cell lines. The pluripotency of established cell lines was then evaluated via ability to form teratomas in SCID mice.

**Results:** Strain differences in efficiency to generate iPS cells were observed for all parameters measured on primary transformation plates. Notably, NON/LtJ and CAST/EiJ strains were more efficient than other strains (Figure 1). Similar results were obtained for percentage of colonies positive for alkaline phosphatase and percentage of cells positive on flow cytometry for SSEA-1 (data not shown).

**Discussion:** The results of this study suggest that genetic background does have an impact on iPS cell generation and pluripotent stability. A positive correlation was found between the proliferation of parent MEFs from each strain and the efficiency of generation of iPS cells on primary transformation plates. The finding that BALB/cJ iPS cells required a higher concentration of LIF than the other strains is consistent with the ES cell literature and suggests that BALB/cJ iPS cells may have reduced pluripotent stability.

**Significance:** Knowledge gained from this study will likely lead to further investigations into the molecular mechanisms regulating iPS cells derivation and to development of new methods for the generation of therapeutically useful iPS cells from all individuals.

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**Figure 1.** Total number of colonies on primary transformation plates eight days after the start of MEF reprogramming. Experiments were performed using MEFs derived from two different embryos for each strain of mouse. Bars represent mean ± s.d.

**Figure 2.** Number of MEF population doublings over 10 days (mean ± s.d.). MEFs derived from two different embryos were evaluated for each strain of mouse.

**Figure 3.** Gross (left) and histologic (right) images of teratomas four weeks post injection of 129S1/SvImJ iPS cells into the flanks of a SCID mouse.