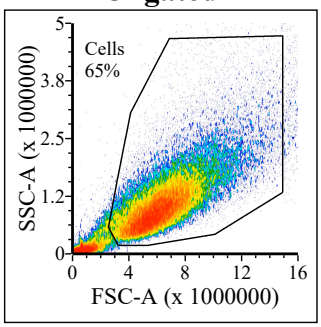
Undifferentiated Status Assessment: Flow Cytometry Data Analysis Gating Scheme

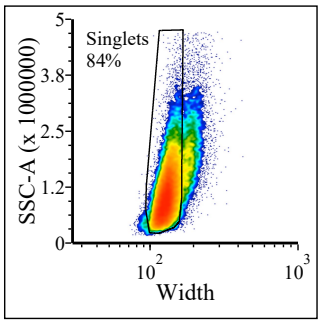
A. Restrict data analysis to Live, Single Cells

All data files are gated first for Cells (excluding debris), then Singlets, and finally Live cells.

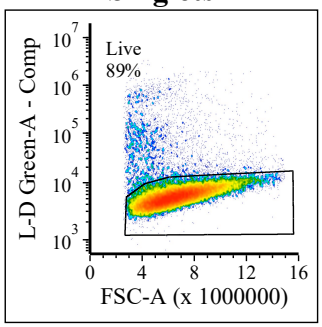
Ungated



Cells



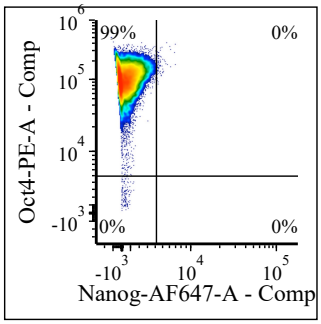
Singlets



B. Gate for dual expression of Oct4 and Nanog

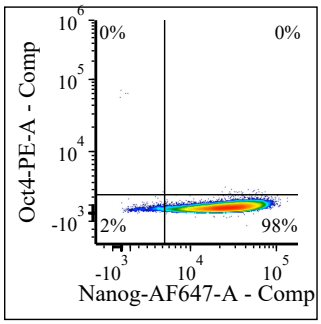
On the plots of Live, Single Cells, axes of the quadrant gate are first sequentially adjusted using the Fluorescence Minus One (FMO) stain data files, then the final gate placement is applied to the Full Stain data file.

FMO_Nanog-AF647
(Nanog omitted from Stain)



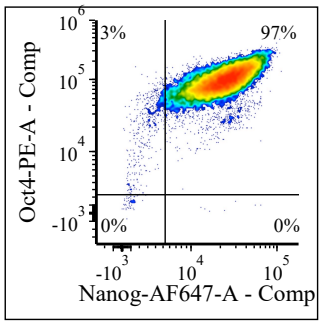
1. The vertical axis of the quadrant gate is set to exclude 99.9% of cells from the upper and lower right quadrants combined.

FMO_Oct4-PE
(Oct4 omitted from Stain)



2. Without moving the vertical axis of the quadrant gate set first using the FMO-Nanog-AF647 stain, the horizontal axis of the quadrant is set to exclude 99.9% of cells from the left and right upper quadrants combined.

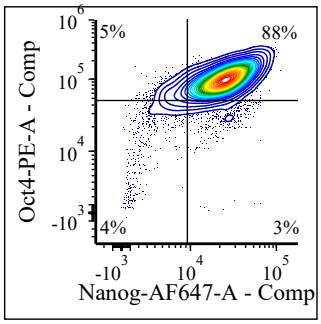
Full Stain



3. The Full Stain is analyzed without moving the position of the quadrant gate previously set on the FMO stains.

C. Gate for dual high expression of Oct4 and Nanog

Gate drawn on the contour plot of Live, Single Cells from the Full Stain data file.



The vertical and horizontal borders of the upper right quadrant are placed where the lines of the dominant double positive population begin to noticeably separate at the leftmost and lowest points.