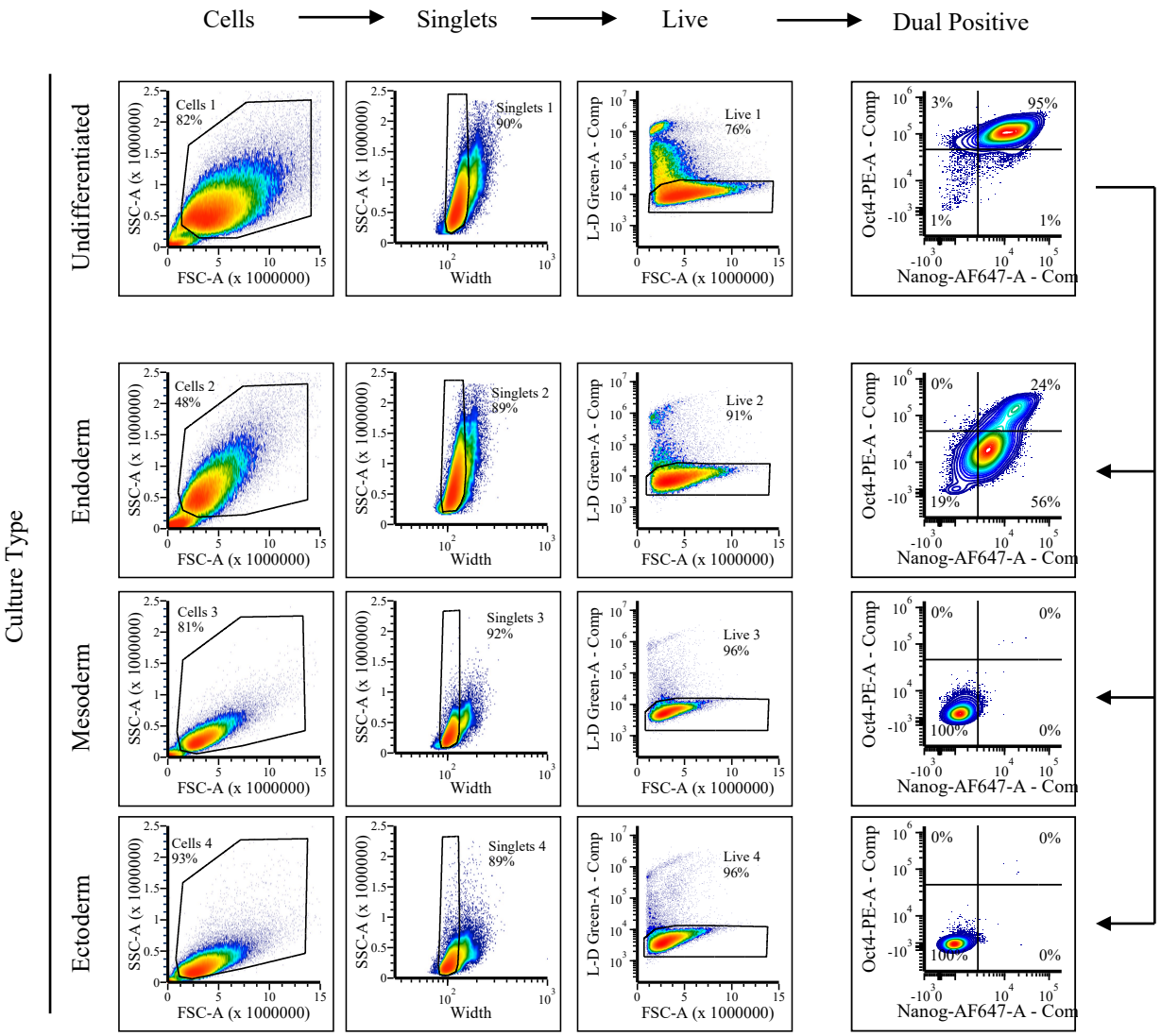
Pluripotency Assessment:
Flow Cytometry Data Analysis Gating Scheme

A. Gating for Undifferentiated markers Oct4 and Nanog

1. Cells, Singlets, and Live gates are set independently for each culture type.

2. Using the undifferentiated culture data, the 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.



3. Each lineage type is analyzed using the dual positive gate placement from the undifferentiated culture.

Pluripotency Assessment:
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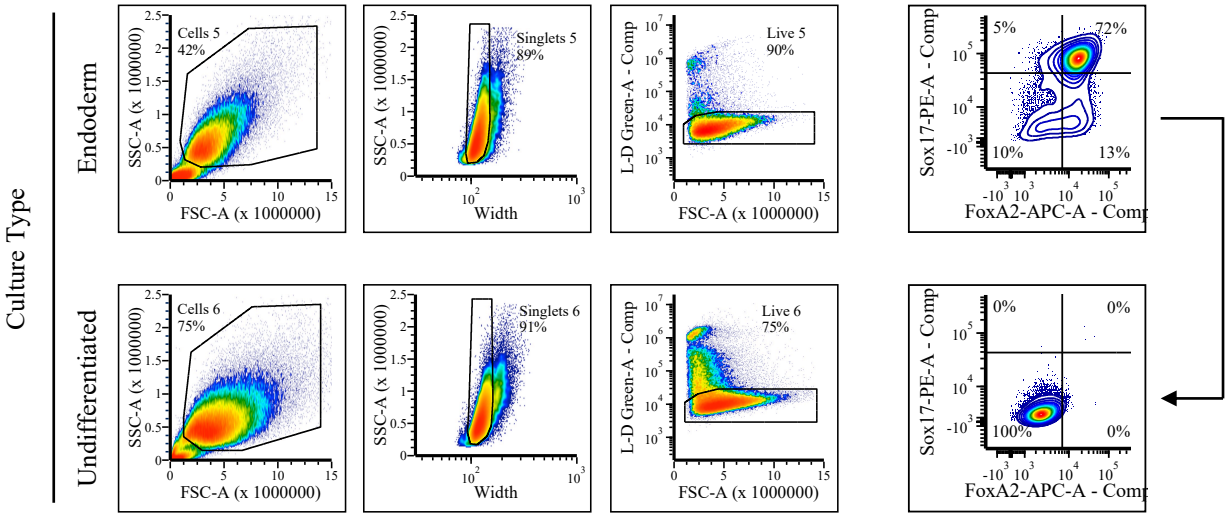
B. Gating for Endoderm markers Sox17 and FoxA2

1. Cells, Singlets, and Live gates are set independently for each culture type.

2. On the Endoderm culture data, the 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.

Cells → Singlets → Live → Dual Positive



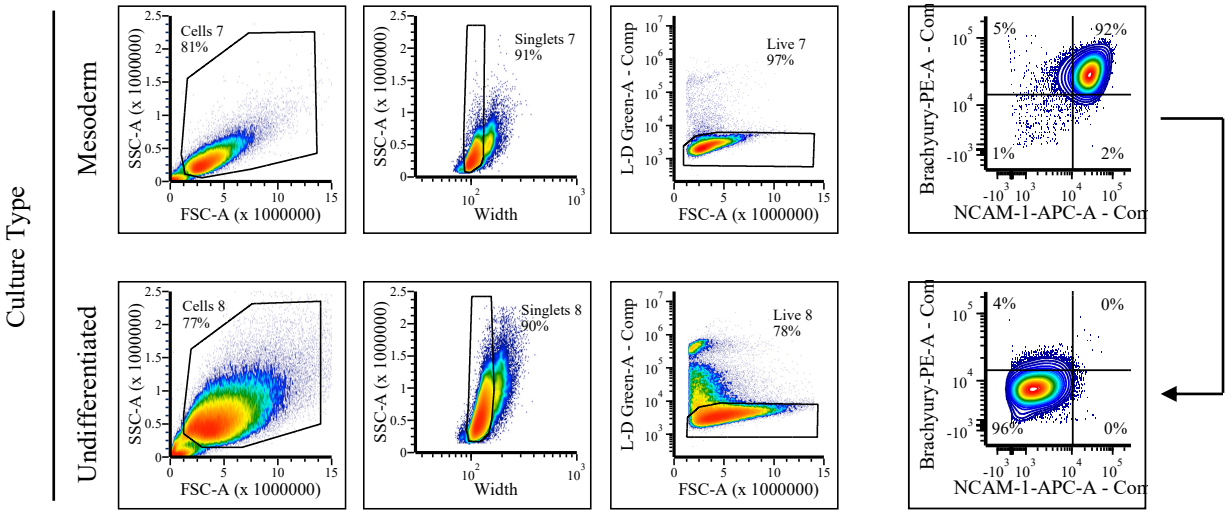
3. Undifferentiated culture is analyzed using the dual positive gate placement from the Endoderm cultures.

C. Gating for Mesoderm markers Brachyury and NCAM-1

1. Cells, Singlets, and Live gates are set independently for each culture type.

2. On the Mesoderm culture data, the 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.

Cells → Singlets → Live → Dual Positive



3. Undifferentiated culture is analyzed using the dual positive gate placement from the Mesoderm culture.

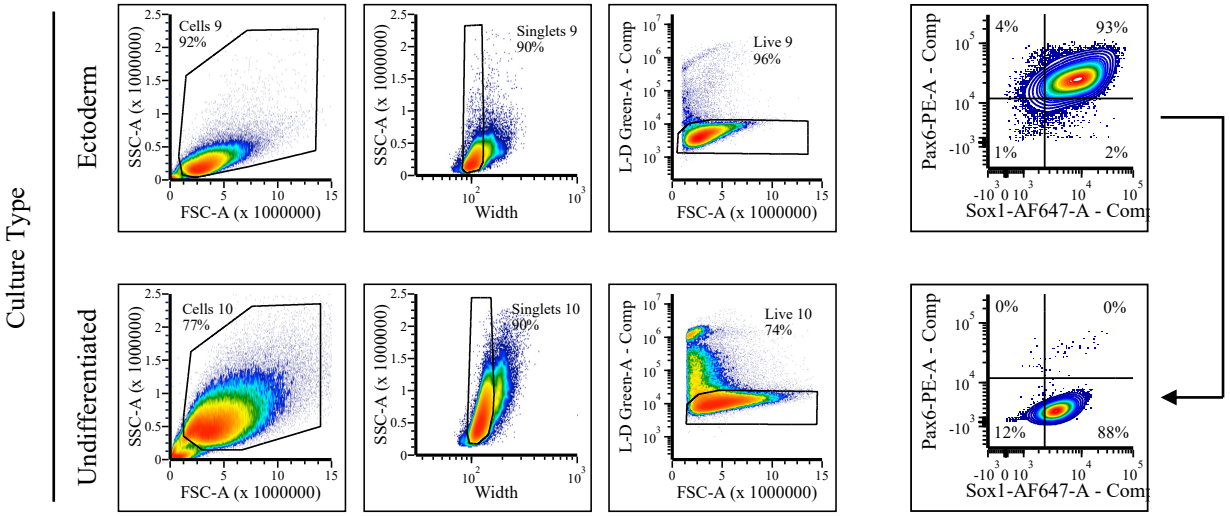
Pluripotency Assessment:
Flow Cytometry Data Analysis Gating Scheme

D. Gating for Ectoderm markers Pax6 and Sox1

1. Cells, Singlets, and Live gates are set independently for each culture type.

2. On the Ectoderm culture data, the 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.

Cells → Singlets → Live → Dual Positive



3. Undifferentiated culture is analyzed using the dual positive gate placement from the Ectoderm culture.