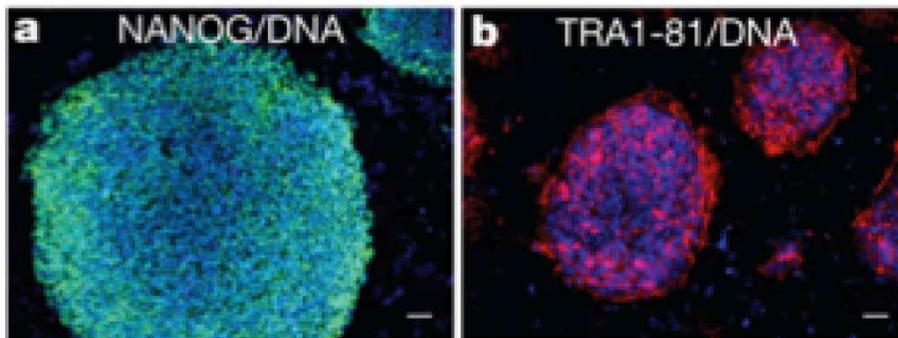
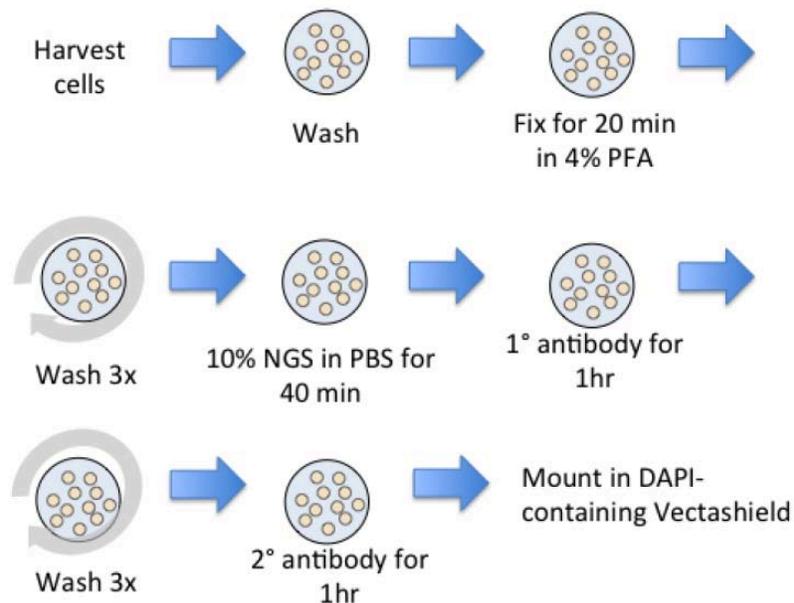


Title	Immunostaining
Date Submitted	May 5, 2012
Submitted by -	Efthymiou, Anastasia - anastasia.efthymiou@nih.gov
Adapted from -	Gibco Protocol
Contributors -	Efthymiou, Anastasia
Affiliation(s) -	NIH CRM - NIAMS – Laboratory of Stem Cell Biology

### ❖ Introduction:



iPSC lines express NANOG and TRA1-81<sup>1</sup>

### ❖ Protocol:

1. Wash cells with PBS
2. Fix for 20min with 4% paraformaldehyde
3. Wash 3x 5min with PBS at RT
4. Saturate cells in 10% normal goat serum (NGS) in PBS for 40min at RT

- Add Additional 0.3% triton-100 for intracellular epitopes
- 5. Dilute antibodies in 5% NGS/PBS, and incubate for 1hr at RT
- 6. Wash 3x 5min with PBS
- 7. Dilute secondary antibodies in 5% NGS/PBS, and incubate for 1hr in dark at RT
- 8. Mount in DAPI-containing Vectashield

❖ **Materials:**

PBS
4% PFA
10% NGS
5% NGS
primary antibody
secondary antibody
DAPI-containing Vectashield

❖ **Troubleshooting:**

❖ **References:**

1. Mason A Israel, Shauna H. Yuan, et al. Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. Nature. 1-5 (2012).