Hanna Lab - Human naïve iPSC microinjection protocol

1. Dissect oviducts of hormone primed and mated B6D2F1 females X B6D2F1 males, and extract zygotes (as routinely done with mouse micro-manipulation in our lab).
2. Culture zygotes for 2 days in KSOM medium droplets (Zenith Biotech KSOMaa Evolve cat # ZEKS-050) covered with mineral oil at 37C 20% O2 or 5%O2 incubator, until they develop to morula stage.
3. Grow human naïve cells to 70-90% confluence in NHSM medium on gelatin/vitronectin or gelatin/DR4 irradiated MEF coated plates.
4. The day before cell harvesting add 10μM ROCKi to the cells (in case not continuously used in the NHSM medium).
5. Trypsinize the cells for ~ 5 minutes with 0.05% trypsin, shake and pipette thoroughly to yield one cell suspension. Stop the reaction with DMEM+15% FBS and centrifuge at 1000RPM for 3 Minutes. Aspirate and discard medium.
6. Resuspend cells in 900μl NHSM medium, add 100μl filtered FBS (to reduce stickiness of cells) and 10μM ROCKi. Keep on ice until and during injections!!! It is preferable to inject the cells as soon as they are harvested.
7. Inject 5-12 human naïve cells to a mouse morula by using of Piezo (as routinely done with mouse ES injections). Include 10μM ROCKi also in M2 media throughout the injection period. (any drop that has naïve PSCs during injection should have ROCKi to increase cell survival during the process). (We use 15micron Piezo needles 15-15-MS for both mouse and human naïve injections).
8. After injection, incubate for 3-4 hours in KSOM droplets supplemented with 10μM ROCKi covered with mineral oil.
9. After 3-4 hours transfer the morulas to KSOM droplets (without ROCKi) covered with mineral oil, incubate over night. That way the morulas will develop into blastocyst.
10. The next day, most morulas should develop to blastocysts. Transfer 15-20 blastocysts to uterus of pseudo-pregnant B6D2F1 female mice.

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