Synchronize Human Embryonic Stem Cells at Different Cell Cycle Stages

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[Abstract] Pluripotency and the capability for self-renewal are essential characteristics of human embryonic stem cells (hESCs), which hold great potential as a cellular source for tissue replacement. Short cell cycle (15-16 h) compared to somatic cells is another property of hESCs. Efficient synchronization of hESCs at different cell cycle stages is important to elucidate the mechanistic link between cell cycle regulation and cell fate decision. This protocol describes how to establish synchronization of hESCs at different cell cycle stages.

Materials and Reagents

1. Human embryonic stem cells (hESCs) Undifferentiated hESCs were cultured on irradiated mouse embryonic fibroblast (MEF) feeders in DMEM/F12 medium supplemented with 20% KnockOut serum replacement, 0.1 mM nonessential amino acids (NEAA), 1 mM GlutaMAX^{TM-1}, 0.1 mM 2-mercaptoethanol (all from Invitrogen) and 8 ng/ml recombinant human FGF2 (Peprotech, catalog number: 100-18B).
2. Matrigel (BD Biosciences, catalog number: 354230)
3. Nocodazole (Sigma-Aldrich, catalog number: M1404)
4. Thymidine (Sigma-Aldrich, catalog number: T1895)
5. Aphidicholine (Sigma-Aldrich, catalog number: A0781)
6. Phosphate buffered saline (PBS)
7. Dimethyl sulfoxide (DMSO)
8. Nocodazole stock solution (see Recipes)
9. Thymidine stock solution (see Recipes)
10. Aphidicholine stock solution (see Recipes)

Equipment

1. Centrifuges (Eppendorf 5415D centrifuge)
2. Fluorescence activated cell sorter (FACS, FACScan machine, Stanford FACS facility)

Procedure

A. Synchronize hESCs at G2/M phase

1. Method I
   a. Passage hESCs to matrigel on the first day. On the second day, change culture medium.
   b. On the third day, add 200 ng/ml nocodazole into fresh medium and incubate for 16 h at 37 °C.
   c. After 16 h, harvest cells, and prepare a small aliquot for cell cycle analysis by FACS.
2. Method II
   a. Passage hESCs to matrigel on the first day. On the second day, change culture medium.
   b. On the third day, add 2 mM thymidine into fresh medium and incubate for 10 h at 37 °C.
   c. Remove the medium containing thymidine, wash with PBS (store at room temperature) twice to release cells from thymidine arrest.
   d. Add fresh culture medium and incubate for another 10 h at 37 °C.
   e. Add nocodazole to a final concentration at 200 ng/ml. Incubate for 24 h at 37 °C.
   f. Harvest cells, and prepare a small aliquot for cell cycle analysis by FACS.

B. Synchronize hESCs at G1 phase

1. Method I
   a. Passage hESCs to matrigel on the first day. On the second day, change culture medium.
   b. On the third day, add 200 ng/ml nocodazole into fresh medium and incubate for 16 h at 37 °C.
   c. Remove the medium containing nocodazole, wash with PBS (store at room temperature) twice.
   d. Add fresh medium supplemented with 10 μg/ml of aphidicholine and incubate for 9-10 h at 37 °C.
   e. Harvest cells, and prepare a small aliquot for cell cycle analysis by FACS.
2. Method II
   a. Passage hESCs to matrigel on the first day. On the second day, change culture medium.
   b. On the third day, add 10 μg/ml of aphidicholine into fresh medium and incubate for 20 h at 37 °C.
   c. Harvest cells, and prepare a small aliquot sample for cell cycle analysis by FACS.

C. Synchronize hESCs at S phase

1. Method I
   a. Synchronize hESCs at G2/M phase as described in A.
   b. Remove the medium containing nocodazole, wash with PBS (store at room temperature) twice.
   c. Add fresh medium to release cells, incubate hESCs at 37 °C. Collect cells every hour, and prepare a small aliquot for cell cycle analysis by FACS. Usually the S phase cell populations appear at 5-6 h after releasing from nocodazole arrest.
2. Method II
   a. Synchronize hESCs at G1 phase as described in B.
   b. Remove the medium containing aphidicholine, wash with PBS (store at room temperature) twice.
   c. Add fresh medium to release cells, incubate hESCs at 37 °C. Collect cells every hour, and prepare a small aliquot for cell cycle analysis by FACS. Usually the S phase cell populations appear at 2-3 h after releasing from aphidicholine arrest.

Recipes

1. Nocodazole stock solution
   Dissolve nocodazole in DMSO
   Stock concentration at 5 mg/ml
2. Thymidine stock solution
   Dissolve thymidine in ddH_2O
   Stock concentration at 100 mM
3. Aphidicholine stock solution
   Dissolve aphidicholine in DMSO
   Stock concentration at 10 mg/ml

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Reference

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