Video Article

Modified Mouse Embryonic Stem Cell based Assay for Quantifying Cardiogenic Induction Efficiency

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Abstract

Differentiation of pluripotent stem cells is tightly controlled by temporal and spatial regulation of multiple key signaling pathways. One of the hurdles to its understanding has been the varied methods in correlating changes of key signaling events to differentiation efficiency. We describe here the use of a mouse embryonic stem (ES) cell based assay to identify critical time windows for Wnt/β-catenin and BMP signal activation during cardiogenic induction. By scoring for contracting embryonic bodies (EBs) in a 96-well plate format, we can quickly quantify cardiogenic efficiency and identify crucial time windows for Wnt/β-catenin and BMP signal activation in a time course following specific modulator treatments. The principal outlined here is not limited to cardiac induction alone, and can be applied towards the study of many other cell lineages. In addition, the 96-well format has the potential to be further developed as a high throughput, automated assay to allow for the testing of more sophisticated experimental hypotheses.

Video Link

The video component of this article can be found at http://www.jove.com/video/2656/

Protocol

1. Embryonic Body (EB) formation using 96-round bottom well microtiter plates

1. Grow mouse ES cells in 10cm cell culture plates with mouse ES cell medium supplemented with LIF.
2. When ES cells are ready for use (generally at 50-70% confluence), remove ES media and rinse cells once with 5ml sterile PBS.
3. Add 2ml 0.05% trypsin / EDTA to each plate and incubate plates at 37°C for 3˜5 minutes, Quench trypsin EDTA with 3 ml ES media.
4. Transfer cells to 50ml falcon tubes and spin at 1000 rpm for 3 minutes.
5. Remove supernatant, and resuspend cell pellet with desired amount of EB media.
6. Count the cell number and dilute cells to 5 x10^3 cells/ml in EB media.
7. Use multichannel pipette to add 100µl of EB media containing cells into each well of 96- round well microtiter plates.
8. Place 96- round well microtiter plates into an incubator.

2. Identification of critical time windows of the key signaling pathway(s) for cardiogenesis

1. Add modulators of specific signaling pathways and vehicle control into each well of 96-well microtiter plates containing ES cells at various time points such as day 0, day 1, day 2, day 3 and day 4, etc. Half of wells in each 96-well plate (48 wells) are used for each starting time point of treatment.
2. Wash out the modulators by changing EB media for every 48 wells at different stopping time points. For example, to get two day treatment for ES cells starting from day 0, wash out modulators at day 2. For any treatment longer than 48 hours, change EB media supplemented with fresh modulators every two days until the desired stop time points.
3. Examine EB contraction under a microscope after day 7. Score the wells with contracting EBs as positive ones to get percentages of contracting EBs for each different treatment time courses.
4. The critical time points for ES cardiogenesis are the time frames in which contained the highest percentage of contracting EBs treated by signaling modulators when compared to the vehicle control.

3. Verification of time windows of signaling for cardiogenesis in EBs made from hanging droplets

1. Prepare Petri dishes by adding with 3-4 ml of PBS to prevent evaporation.
2. Follow steps 1.1 ~ 1.5 to prepare ES cells at $2.5x10^4$ cells / ml final cell density.
3. Pour cell mixture into bacterial dish for easy access. Using multichannel pipette, add 20 µl drops (containing about 500 cells) onto inverted lids. Do not allow the individual droplets to touch. Generally one lid can fit up to 80 drops. Flip the lid back over the dish containing PBS. Incubate for 24 hr or 48 hours to allow EB formation in an incubator.
4. Wash down EB formed from hanging droplets from each lid with 3 ml of EB media and pool 2 lids of EBs to a new Petri dish. Add EB media to a total volume of 10ml.
5. Transfer EBs in suspension onto 0.2% gelatin coated 6-well plates at day 4. In general 30 EBs are transferred to each well. Add EB media to get the 2 ml final volume for each well.
6. Incubate signaling modulators at the time period identified from 96-well plate experiments.
7. Observe the EB contraction under microscope after day 7 and cardiogenesis may be further characterized by RT-PCR, immunostaining and others, if necessary.

Discussion

The hanging drop method has been the conventional method used for EB formation and in vitro differentiation. However, it is laborious and limits experimental flexibility due to logistical concerns. By the same token, results are also more difficult to validate as the skill of the experimenter is crucial to successful EB formation and manipulation as hanging drops. A simpler method is to form EBs in a round-bottomed 96-well plate as a single-step process. This format allows in vitro differentiation to be standardized and can accommodate more experimental conditions due to the ease of EB formation and downstream manipulation (e.g. modulator addition or removal). Furthermore, the 96-well plate format can be optimized to be an efficient screening tool in mouse ES cells.

Disclosures

No conflicts of interest declared.

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