

Table 2. Troubleshooting advice.

Step	Problem	Possible reasons	Solution
5	Poor attachment of hPSCs on Matrigel-coated plates	No ROCK inhibitor included on Step 4	Add Y27632 to the medium
15, 16	Cell death or low cell density	Not enough medium was included on Step 14	Be sure to use 4 mL per well
19, 21	Low cell density	Excessive shearing during pipetting in Step 18 or 19	Pipette cells up and down no more than 3 times on any step or reduce the split ratio
19-25, 42	Loss of SOX2 expression	CHIR99021 concentration is too high	Test lower concentrations of CHIR99021
35, 41	Low % of PAX6 expression	Seeding density is not high enough in Step 14; hPSC line is of poor quality	Increase seeding density so that cells are nearly confluent in Step 15; thaw a new hPSC vial or test a different clone if one exists
46, 47	<i>HOXD10</i> was not detected	The concentration of GDF11 was not high enough	Titrate GDF11 in Steps 23-25 from 30-50 ng/mL, isolate RNA at Step 25, and perform qPCR to determine a working range of GDF11 concentrations
48	Low % of PAX6 expression	hPSC line is of poor quality; the concentration of GDF11 was too high or the concentration of DM was too low in Steps 23-25; the DM has expired	Thaw a new hPSC vial or test a different clone if one exists; in Steps 23-25, increase the concentration of DM or decrease the concentration of GDF11; prepare new DM