Claims

1. A method of differentiating pluripotent stem cells into definitive endoderm (DE) lineage comprising
   i) contacting said pluripotent stem cells with:
   a. one or more activators of TGFβ/Nodal signaling; and
   b. one or more activators of Wnt signaling
   to produce cells of the anterior primitive streak (APS) lineage; and
   ii) contacting said APS cells with:
   a. one or more activators of TGFβ/Nodal signaling; and
   b. one or more inhibitors of BMP signaling or one or more inhibitors of Wnt signaling
   to produce cells of the DE lineage.

2. The method as claimed in claim 1, wherein the one or more activators of TGFβ/Nodal signaling is Activin A, TGFβ1, TGFβ2 or Nodal.

3. The method as claimed in claim 1 or 2, wherein the one or more activators of Wnt signaling are CHIR99201 or Wnt3a or other family members of the wnt signaling pathway.

4. The method as claimed in any one of the preceding claims, wherein the one or more inhibitors of BMP signaling is DM3189/LDN-193189, noggin, chordin, or dorsomorphin or DMH1.

5. The method as claimed in any one of the preceding claims, wherein the pluripotent stem cells are contacted with Activin A, and CHIR99201.

6. The method of claim 5, wherein the pluripotent stem cells are contacted with about 1ng/ml to 10µg/ml of Activin A and about 1nM to 100mM of CHIR99201.

7. The method of claim 6, wherein the pluripotent stem cells are contacted with about 100ng/ml of Activin Aand about 2µM of CHIR99201.

8. The method of any one of the preceding claims, wherein the cells of the anterior primitive streak lineage comprise elevated gene expression of anterior streak or pan-streak markers and decreased expression of posterior streak markers relative to undifferentiated cells.

9. The method as claimed in any one of the preceding claims, wherein the differentiation of pluripotent stem cells into APS cells is completed within 24 to 60 hours.

10. The method of any one of the preceding claims, wherein the APS cells are contacted with about 1ng/ml to 10µg/ml of Activin A and about 1nM to 100mM of LDN-193189.

11. The method of claim 10, wherein the APS cells are contacted with about 100ng/ml of Activin A and about 250nM of LDN-193189.

12. The method as claimed in any one of the preceding claims, wherein the one or more inhibitors of Wnt signaling is Iwp2, Dkk1, C-59, or Iwr-1 or XAV-939.

13. The method of claim 12, wherein the APS cells are contacted with about 1ng/ml to about 10µg/ml of Activin A and about 1nM to about 100mM of Iwp2 or about 1nM to about 100mM C-59.

14. The method of claim 13, wherein the APS cells are contacted with about 100ng/ml of Activin A and about 4μM of Iwp2 or about 1 μM of C-59.

15. The method of any one of the preceding claims, wherein the cells of the definitive endoderm lineage comprise elevated endoderm gene expression and decreased pluripotency gene expression relative to undifferentiated cells.

16. The method of any one of the preceding claims, wherein the cells of the definitive endoderm lineage comprise significantly reduced mesoderm gene expression.

17. The method as claimed in any one of the preceding claims, wherein the differentiation of cells from the APS lineage into cells of DE lineage is completed within 24 to 96 hours.

18. A cell culture medium for differentiating a pluripotent stem cell into APS lineage comprising one or more of the following factors: a matrix component, one or more activators of TGFβ/Nodal signaling, one or more activators of Wnt/β-catenin signaling.

19. A cell culture medium for differentiating an APS cell into DE lineages comprising one or more of the following factors: one or more activators of TGFβ/Nodal signaling, and one or more inhibitors of BMP signaling or one or more inhibitors of Wnt signaling.

20. A cell culture medium for differentiating a DE cell into a AFG cell comprising a TGFβ inhibitor and a BMP inhibitor.

21. A cell culture medium for differentiating a DE cell into a PFG cell comprising one or more of the following factors: retinoic acid, a BMP inhibitor, a Wnt inhibitor and a FGF/MAPK inhibitor.

22. A cell culture medium for differentiating a DE stem cell into a MHG cell comprising one or more of the following factors: a BMP activator, a Wnt activator and an FGF activator.

23. A cell culture medium for differentiating a PFG cell into a pancreatic progenitor cell comprising one or more of the following factors: one or more FGF/MAPK inhibitors; one or more Hedgehog inhibitors; one or more BMP inhibitors; one or more Wnt inhibitors; retinoic acid (RA), and Activin A.

24. A cell culture medium for differentiating a PFG cell into a liver progenitor cell comprising one or more of the following factors: one or more TGFβ inhibitors; one or more BMP activators, retinoic acid, and one or more Wnt inhibitors.

25. A kit when used in the method of any one of claims 1 to 17, comprising one or more containers of cell culture medium as claimed in any one of claims 18 to 24, together with instructions for use.