[0024a] According to another aspect there is provided a method of differentiating pluripotent stem cells into definitive endoderm lineage (DE) 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; and

c. one or more inhibitors of PI3K/mTOR signaling

to produce cells of the anterior primitive streak (APS) lineage; and

ii) contacting said anterior primitive streak 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 definitive endoderm (DE) lineage.

CLAIMS:

1. A method of differentiating pluripotent stem cells into definitive endoderm lineage (DE) 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; and

c. one or more inhibitors of PI3K/mTOR signaling

to produce cells of the anterior primitive streak (APS) lineage; and

ii) contacting said anterior primitive streak 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 definitive endoderm (DE) lineage.

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

3. The method as claimed in 1 or 2, wherein the one or more inhibitors of BMP signaling is selected from the group consisting of DM3189/LDN-193189, noggin, chordin, dorsomorphin and DMH1.

4. The method as claimed in any one of claims 1 to 3, wherein the cells of the APS lineage are contacted with 100ng/ml of Activin A and 250nM of LDN-193189.

19. The method as claimed in any one of claims 1 to 18, wherein the differentiation of pluripotent stem cells to cells of the APS lineage is completed within 24 to 60 hours.