CLAIMS

1. A method for obtaining a liver organoid by expanding epithelial stem cells, wherein said method comprises:

culturing at least one epithelial stem cell from a liver fragment or a liver biliary duct in contact with an extracellular matrix in the presence of a medium, the medium comprising a basal medium for animal or human cells to which is added: Epidermal Growth Factor (EGF), a Wnt agonist, hepatocyte growth factor (HGF) and Nicotinamide.

2. A method according to claim 1, wherein one or two from the group: a bone morphogenetic protein (BMP) inhibitor and a TGF-beta inhibitor are added to the medium.

3. A method according to claim 1 or claim 2, wherein after at least one day, the medium is changed to a second medium.

4. A method according to claim 3, wherein the second medium is a differentiation medium comprising a basal medium for animal or human cells to which is added EGF, FGF and/or HGF, a TGF-beta inhibitor and a Notch inhibitor.

5. A method according to any one of claims 1 to 4, wherein the method comprises culturing said epithelial stem cells in a cell culture medium comprising a basal medium for animal or human cells to which is added EGF, a BMP inhibitor, R-spondin and Wnt; and subsequently in a second expansion medium which comprises a basal medium for animal or human cells to which is added: EGF, and HGF, Nicotinamide, and an R-spondin; and subsequently in a differentiation medium which comprises or consists of a basal medium for animal or human cells to which is added: EGF, FGF and/or HGF, a TGF-beta inhibitor, and a Notch inhibitor.

6. A cell culture medium for expanding liver epithelial stem cells to obtain an organoid comprising a basal medium for animal or human cells to which is added: EGF, a Wnt agonist, HGF and Nicotinamide.

7. The cell culture medium according to claim 6, wherein one or two from the group: a BMP inhibitor and a TGF-inhibitor are added to the medium.

8. A method according to any one of claims 1 to 5 or a cell culture medium according to claims 6 or 7 wherein the Wnt agonist is selected from the group consisting of: a Wnt family member, R-spondin 1-4, Norrin, and a GSK-inhibitor.

9. A method according to any one of claims 1 to 5 or a cell culture medium according to claim 7, wherein the TGF-beta inhibitor is a small molecule inhibitor

selected from the group consisting of: A83-01, SB-431542, SB-505124, SB-525334, LY 364947, SD-208, SJN 2511.

10. A method according to any one of claims 4, 5, 8 or 9, wherein the FGF is an FGF able to bind to FGFR2 or FGFR4.

11. A method or a cell culture medium according to claim 10 wherein the FGF is FGF10.

12. A method according to any one of claims 1 to 5 or a cell culture medium according to claim 6 or claim 7, wherein one, two, three, four or more from the group gastrin, B27, N2, and N-Acetylcysteine are added to the basal medium.