CLAIMS

1. A method of maintaining ABCG2-positive phenotype of pluripotent stem cell-derived corneal limbal stem cells, wherein the method comprises culturing pluripotent stem cell-derived ABCG2-positive corneal limbal stem cells in a culture medium comprising EGF and at least one Wnt activator and wherein the pluripotent stem cell-derived ABCG2-positive corneal limbal stem cells do not express cytokeratin 3 or cytokeratin 12.

2. The method according to claim 1, wherein the Wnt activator is selected from the group consisting of GSK3 inhibitors, preferably CHIR99021, and proteins of R-spondin family, preferably R-spondin-1 or its supplement RS-246204.

3. The method according to claim 1 or 2, wherein the Wnt activator is CHIR99021.

4. The method according to any one claims 1-3, wherein the culture medium further comprises noggin or its supplement LDN-193189.

~~5. The method according to any one of claims 1-4, wherein the ABCG2-positive corneal limbal stem cells are ABCG2-positive primary corneal limbal stem cells or pluripotent stem cell-derived ABCG2-positive corneal limbal stem cells.~~

~~6~~5. A method of producing ABCG2-positive corneal limbal stem cells, the method comprising:

a) providing pluripotent stem cells;

b) culturing said cells in a cell culture medium comprising a TGF-beta inhibitor and a fibroblast growth factor (FGF), preferably basic FGF;

c) withdrawing the TGF-beta inhibitor and the FGF, and culturing the cells obtained in step b) in a cell culture medium comprising bone morphogenetic protein 4 (BMP-4) thereby producing eye precursor cells;

d) culturing said eye precursor cells in a corneal differentiation medium thereby producing ABCG2-positive corneal limbal stem cells; and

e) culturing said ABCG2-positive corneal limbal stem cells in a maintenance cell culture medium comprising EGF and at least one Wnt activator, thereby maintaining ABCG2-positive corneal limbal stem cell phenotype obtained via steps a-d).

wherein the ABCG2-positive corneal limbal stem cells do not express cytokeratin 3 or cytokeratin 12.

~~7~~6. The method according to claim ~~6~~5, wherein the Wnt activator is

selected from the group consisting of GSK3 inhibitors, preferably CHIR99021, and proteins of R-spondin family, preferably R-spondin~~g~~-1 or its supplement RS-246204.

~~8~~7. The method according to claim ~~6~~5 or ~~7~~6, wherein the Wnt activator is CHIR99021.

~~8~~9. The method according to any one claims ~~6~~5-7, wherein the maintenance culture medium in step e) further comprises noggin or its supplement LDN-193189.

~~10~~9. The method according to any one of claims ~~6~~5-~~9~~8, wherein the pluripotent stem cells are selected from induced pluripotent stem (iPS) cells and embryonic stem (ES) cells, with the proviso that if human embryonic stem (hES) cells are used, the method does not include the destruction of human embryos.

~~11~~10. The method according to any one claims ~~6~~5-~~10~~9, wherein step a) comprises forming embryoid bodies from said pluripotent stem cells.

~~12~~11. The method according to claim ~~11~~10, wherein said forming of embryoid bodies in step a) is carried out by a physical or chemical method, preferably selected from the group consisting of culturing cells in the presence of attachment-preventing agents, culturing cells in hanging drops, microfabrication techniques, forced aggregation e.g. by centrifugation, and culturing cells in the presence of one or more aggregation-promoting agents such as macromolecular crowders, blebbistatin and ROCK inhibitors.

~~13~~12. The method according to any one claims 5~~6~~-~~12~~11, wherein said culturing in step d) and e) is carried out on a substrate coated at least with collagen IV and laminin, preferably laminin-521 and/or laminin-511.

~~14~~13. The method according to any one of the preceding claims, which is performed in substantially xeno-free, substantially serum-free and/or defined conditions.

~~15~~14. ABCG2-positive corneal limbal stem cells, which do not express cytokeratin 3 or cytokeratin 12 and whose ABCG2-positive phenotype has been maintained by the method of any one of claims 1-~~5~~4 or which have been produced by the method of any one of claims ~~6~~5-~~14~~13, for use in treating ~~an eye disease, preferably~~ limbal stem cell deficiency (LSCD).

~~16~~15. The ABCG2-positive corneal limbal stem cells for use according to claim ~~15~~14, having capability to maintain their ABCG2-positive phenotype for at least 35 days.