Stem Cell Research 16 (2016) 184–188

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab resource: stem cell line

# Generation of KCL034 clinical grade human embryonic stem cell line

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ARTICLE INFO

*Article history:*
Received 28 December 2015
Accepted 29 December 2015
Available online 3 January 2016

ABSTRACT

The KCL034 human embryonic stem cell line was derived from a normal healthy blastocyst donated for research. The ICM was isolated using laser microsurgery and plated on γ-irradiated human foreskin fibroblasts. Both the derivation and cell line propagation were performed in an animal product-free environment and under current Good Manufacturing Practice (cGMP) standards. Pluripotent state and differentiation potential were confirmed by in vitro assays. The line was also validated for sterility, specific and non-specific human pathogens.



## 1. Resource table

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|---|---|
| Name of stem cell line | KCL034 |
| Institution | King's College London, London UK |
| Derivation team | Neli Kadeva, Victoria Wood, Glenda Cornwell, Stefano Codognotto, Emma Stephenson |
| Contact person and email | Dusko Ilic, email: dusko.ilic@kcl.ac.uk |
| Date archived/ stock date | Aug. 08, 2011 |
| Type of resource | Biological reagent: cell line |
| Sub-type | Human pluripotent stem cell line |
| Origin | Human embryo |
| Key marker expression | Pluripotent stem cell markers: NANOG, OCT4, TRA-1-60, TRA-1-81, alkaline phosphatase (AP) activity |
| Authentication | Identity and purity of line confirmed |
| Link to related literature (direct URL links and full references) | 1) Jacquet, L., Stephenson, E., Collins, R., Patel, H., Trussler, J., Al-Bedaery, R., Renwick, P., Ogilvie, C., Vaughan, R., Ilic, D., 2013. Strategy for the creation of clinical grade hESC line banks that HLA-match a target population. EMBO Mol. Med. 5 (1), 10–17. doi: 10.1002/emmm.201201973 http://www.ncbi.nlm.nih.gov/pubmed/23161805<br>2) Canham, A., Van Deusen, A., Brison, D.R., De Sousa, P., Downie, J., Devito, L., Hewitt, Z.A., Ilic, D., Kimber, S.J., Moore, H.D., Murray, H., Kunath, T., 2015. The molecular karyotype of 25 clinical-grade human embryonic stem cells lines. *Sci. Rep.* 5, 17258. doi: 10.1038/srep17258 http://www.ncbi.nlm.nih.gov/pubmed/26607962<br>3) Ilic, D., Stephenson, E., Wood, V., Jacquet, L., Stevenson, D., Petrova, A., Kadeva, N., Codognotto, S., Patel, H., Semple, M., Cornwell, G., Ogilvie, C., Braude, P., 2012. Derivation and feeder-free propagation of human embryonic stem cells under xeno-free conditions. Cytotherapy. 14 (1), 122–128. doi: 10.3109/14653249.2011.623692 http://www.ncbi.nlm.nih.gov/pubmed/22029654<br>4) Stephenson, E., Jacquet, L., Miere, C., Wood, V., Kadeva, N., Cornwell, G., Codognotto, S., Dajani, Y., Braude, P., Ilic, D., 2012. Derivation and propagation of human embryonic stem cell lines from frozen embryos in an animal product-free environment. Nat. Protoc. 7 (7), 1366–1381. doi: 10.1038/nprot.2012.080 http://www.ncbi.nlm.nih.gov/pubmed/22722371<br>5) Devito, L., Petrova, A., Miere, C., Codognottom S., Blakely, N., Lovatt, A., Ogilvie, C., Khalaf, Y., Ilic, D., 2014. Cost-effective master cell bank validation of multiple clinical-grade human pluripotent stem cell lines from a single donor. Stem Cells Transl. Med. 3(10), 1116–1124. doi: 10.5966/sctm.2014–0015 http://www.ncbi.nlm.nih.gov/pubmed/25122690<br>6) Devito, L., Petrova, A., Miere, C., Codognottom S., Blakely, N., Lovatt, A., Ogilvie, C., Khalaf, Y., Ilic, D., 2014. Cost-effective master cell bank validation of multiple clinical-grade human pluripotent stem cell lines from a single donor. Stem Cells Transl. Med. 3(10), 1116–1124. doi: 10.5966/sctm.2014–0015 http://www.ncbi.nlm.nih.gov/pubmed/25122690<br>7) Petrova, A., Celli, A., Jacquet, L., Dafou, D., Crumrine, D., Hupe, M., Arno, M., Hobbs, C., Cvoro, A., Karagiannis, P., Devito, L., Sun, R., Adame, L.C., Vaughan, R., McGrath, J.A., Mauro, T.M., Ilic, D., 2014. 3D In vitro model of a functional epidermal permeability barrier from human embryonic stem cells and induced pluripotent stem cells. Stem Cell Reports. 2(5), 675–689. doi: 10.1016/j.stemcr.2014.03.009 http://www.ncbi.nlm.nih.gov/pubmed/24936454<br>8) Cvoro, A., Devito, L., Milton, F.A., Noli, L., Zhang, A., Filippi, C., Sakai, K., Suh, J.H., Sieglaff, D., Dhawan, A., Sakai, T., Ilic, D., Webb, P., 2015. A thyroid hormone receptor/KLF9 axis in human hepatocytes and pluripotent stem cells. Stem Cells. 33(2), 416–428. doi: 10.1002/stem.1875 http://www.ncbi.nlm.nih.gov/pubmed/25330987 |
| Information in public databases | KCL034 is a National Institutes of Health (NIH) registered hESC line NIH Registration Number: NIHhESC-14-0268 |

(*continued on next page*)

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http://dx.doi.org/10.1016/j.scr.2015.12.034

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| Ethics | http://grants.nih.gov/stem_cells/registry/current.htm?id=654<br>The hESC line KCL034 is derived under licence from the UK Human Fertilisation and Embryology Authority (research licence numbers: R0075 and R0133) and also has local ethical approval (UK National Health Service Research Ethics Committee Reference: 06/Q0702/90).<br>Informed consent was obtained from all subjects and the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the NIH Belmont Report. No financial inducements are offered for donation. |

## 2. Resource details

| | |
|---|---|
| Consent signed | May 26, 2009 |
| Embryo thawed | Jul. 11, 2011 |
| UK Stem Cell Bank Deposit Approval | Mar. 08, 2012<br>Reference: SCSC12-54 |
| Sex | Male 46, XY |
| Grade | Clinical |
| Disease status | Healthy/unaffected |
| Karyotype (aCGH) | No copy number changes detected. |
| SNP Array | Gain in region 6p22.1 (Canham et al., 2015) |
| DNA fingerprint | Allele sizes (in bp) of 16 microsatellite markers specific for chromosomes 13, 18 and 21 (Jacquet et al., 2013) |
| HLA typing | HLA-A 11,29; B 44,51; Bw 4; C 04,16; DRB1 04,07; DRB4 01; DQB1 02,03 (Jacquet et al., 2013; Canham et al., 2015) |
| Viability testing | Pass |
| Mycoplasma | Negative |
| Sterility | Pass |
| Pluripotent markers (immunostaining) (Fig. 1) | NANOG, OCT4, TRA-1-60, TRA-1-81, AP activity |
| Three germ layers differentiation in vitro (immunostaining) (Fig. 2) | Endoderm: AFP<br>Ectoderm: TUBB3 (tubulin, beta 3 class III)<br>Mesoderm: ACTA2 (actin, alpha 2, smooth muscle) |
| Three germ layer differentiation in vivo (teratomas) (Fig. 3) | Endoderm: AFP, GATA4<br>Ectoderm: TUBB3, GFAP (glial fibrillary acidic protein)<br>Mesoderm: DES (desmin), Alcian Blue and periodic acid–Schiff (PAS)-stained cartilage |
| Targeted differentiation (Fig. 4) | Endoderm: definitive endoderm — GATA4 (Cvoro et al., 2015).<br>Ectoderm: keratinocytes — p63, KRT14 (Petrova et al., 2014)<br>Mesoderm: cardiomyocytes — TNNT2 |
| Sibling lines available | KCL032, KCL033 |

We generated KCL034 clinical grade hESC line following protocols, established previously (Ilic et al., 2012; Stephenson et al., 2012), and now adapted to cGMP conditions. The expression of the pluripotency markers was tested after freeze/thaw cycle (Fig. 1). Differentiation potential into three germ layers was verified in vitro (Fig. 2), in vivo (Fig. 3) and with targeted differentiation into specific endoderm, ectoderm and mesoderm cell types (Fig. 4).

Molecular karyotyping identified a gain on chromosome 6p22.1. The gain on chromosome 5p14.3 containing the following genes: *HIST1H2BL*, *HIST1H2AI*, *HIST1H3H*, *HIST1H2AJ*, *HIST1H2BM*, *HIST1H4J*, *HIST1H4K*, *HIST1H2AK*, *HIST1H2BN*, *HIST1H2AL*, *HIST1H1B*, *HIST1H3I*, *HIST1H4L*, *HIST1H3J*, *HIST1H2AM*, *HIST1H2BO*, *OR2B2* and *OR2B6* (Canham et al., 2015). The 330.8 kb gain starts at bp 27627265 and ends at bp 27958049 as referred to Human Genome Build 38. This duplication that contained part of the Histone 1 gene cluster was not fully present on the database of genomic variants (DGV; http://dgv.tcag.ca), which has collected structural variations in more than 14,000 healthy individuals from worldwide population (MacDonald et al., 2014). It is probable that this gain represents a benign event as other histone clusters have been shown to be preferentially duplicated during evolution (Canham et al., 2015; Braastad et al., 2004).

Validation for sterility and specific and non-specific human pathogens (Devito et al., 2014) conformed that the cells in Master Bank were sterile, mycoplasma-free, and negative as well as for Treponema pallidum, Chlamydia, Neisseria gonorrhoeae, Human immunodeficiency virus-1 and 2 (HIV-1 and -2), Human T-lymphotropic virus type-1 and 2 (HTLV-1 and 2), Hepatitis A, B and C (HAV, HBV and HCV), Human herpes simplex virus HHV-4 (Epstein–Barr virus, EBV), -6, -7, and -8, Human cytomegalovirus (hCMV), human parvovirus B19, SV40, JCV, BKV, Enterovirus, HAV, HCV, nonspecific viral and other adventitious contaminants.

We also generated research grade of KCL034 line that is adapted to feeder-free conditions.

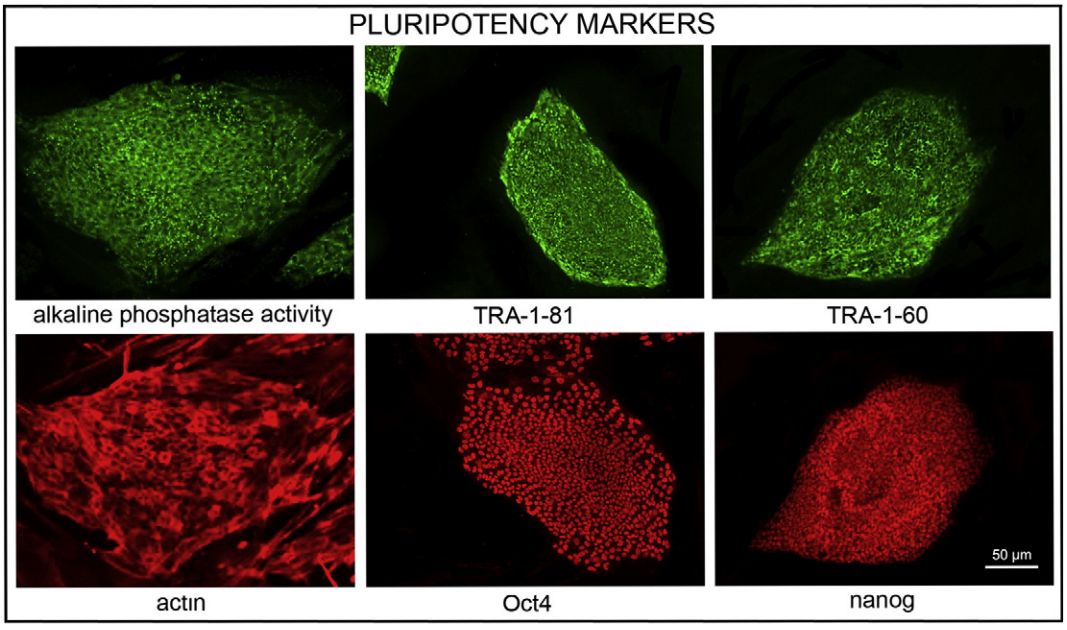


**Fig. 1.** Expression of pluripotency markers. Pluripotency is confirmed by immunostaining (Oct4, Nanog, TRA-1-60, TRA-1-81) and alkaline phosphatase (AP) activity assay. Actin stress fibers, visualized with rhodamine–phalloidin (red), are present in both feeders and hES cell colonies, whereas AP activity (green) is detected only in hES cells. Scale bar, 50 µm.