Q&A ATCC® Excellence in Research Webinar - **2014**
"hTERT immortalized cell lines – Unique tools for physiologically-relevant research"

1. Will we be able to download the presentation?
   Yes, the recorded presentation will be available on our website here.

2. Does ATCC provide culture media for all hTERT-immortalized cell lines?
   Yes, ATCC provides culture media for all our hTERT-immortalized cell lines. You can find the recommended culture media for each cell line of interest on the specific ATCC webpage and on the product sheets.

3. Do immortalized fibroblasts have to be maintained in a low oxygen environment?
   It is not necessary to maintain immortalized fibroblasts in a low oxygen environment, they can be cultured under regular culture conditions (95% air; 5% CO_2, 37°C) with atmospheric oxygen.

4. Why don't you have to transfect both the RNA and the protein component of telomerase to immortalize the cells?
   The RNA component of telomerase is ubiquitously expressed in all cells, and the only limiting factor is the protein component. Introducing hTERT would effectively reconstitute the activity of telomerase.

5. What is the most efficient way to introduce hTERT into primary cells, in order to create an immortalized cell line?
   There are several ways to introduce hTERT into primary cells with high efficiency. The most commonly used methods are to use retroviral or lentiviral vectors to transduce primary cells or transfection with hTERT-expressing plasmid DNA (ATCC® MBA-141). Alternatively, electroporation may also work for some cell types.

6. Can the plasmid pGRN145 (ATCC® MBA-141) be packed into lentivirus or retrovirus?
   MBA-141 contains the coding sequence for hTERT, but the plasmid does not have viral packaging sequences and integration sequences, so it cannot be used directly to make lenti- or retroviruses. You will need to clone the hTERT coding sequence into either lenti- or retroviral vectors and use appropriate packaging systems to make viruses.

7. Some protocols suggest co-infecting a GFP-expressing vector. Does this cause stress to the immortalized cells? Which is better for the experiment and for cell viability, to run the experiment with or without an infection marker?
   Including a marker like GFP will be useful to help assess the transduction/transfection efficiency into primary cells, which are notorious for their resistance to transfection. Even though cells that do not pick up hTERT will die out during extended culture, we recommend using antibiotic drug

selection to establish the stable cell lines for a couple of reasons. First, you will obtain clones having higher hTERT expression under drug selection, which is beneficial for immortalization. Second, keeping selection pressure will prevent the loss/silencing of exogenous gene expression in rare scenarios. You can omit the antibiotics from an established immortalized cell line if you are concerned the antibiotic will interfere with your experiments. ATCC provides the TIME-GFP cells (ATCC® CRL-4045™) that retain angiogenic potential and stably expresses GFP for at least 12 passages. The stable expression of GFP in these cells enables detection and analysis of the fragile endothelial structures to occur without post-assay fixation and/or staining.

8. Can I immortalize primary cells at high passage, or is it better to use cells at low passage?
   The BJ-5ta normal neonatal foreskin fibroblast cell line (ATCC® CRL-4001™) was actually immortalized at a late passage (around population doubling 58), so it is possible to immortalize high-passage fibroblasts. However, we do recommend introducing hTERT and other necessary ancillary factors into the cells-of-interest as early as possible since the cells might undergo a genetic drift or accumulate acquired mutations following extended culture, which could affect their functions.

9. Some companies provide commercial hTERT lentivirus. Is there a big difference between using hTERT plasmid vs. lentivirus?
   Lentiviruses and retroviruses can be efficiently transduced into primary cells and facilitate integration of the gene of interest into the target cell's genome, which is helpful for establishing stable expression of the cargo gene. But commercially available lentiviruses can be quite expensive, and work with such viruses requires very careful laboratory handling as titers and efficiency may decrease significantly if not handled properly. In contrast, transfection of hTERT cDNA plasmid is a simpler method of immortalization and gives the users more flexibility with usage. ATCC offers hTERT-expressing plasmid DNA (ATCC® MBA-141).

10. Does ATCC plan to offer a vector that encodes mouse TERT, for the immortalization of mouse cells?
    ATCC does not offer a vector with mouse TERT gene, however, there are several reports on successful immortalization of rat and mouse cells using the human TERT gene, e.g., rat pancreatic β cells (PMID: 21167227), rat arachnoid cell lines (PMID: 21195136), and mouse osteoblast cells (PMID: 20686067).

11. Can I use Lipofectamine™ to transfect primary cells with hTERT cDNA plasmid?
    Yes, Lipofectamine™ may be used to transfect primary cells with hTERT cDNA plasmid. However, in general, primary cells are much more difficult to transfect than continuous cell lines. There is no single transfection reagent that can transfect all kinds of primary cells with high efficiencies. ATCC provides the transfection reagent, TransfeX (ATCC® ACS-4005), which is designed for transfection of primary cells and hard-to-transfect cell lines. We have tested TransfeX on many primary cells and hard-to-transfect cell lines. You can visit the product webpage to learn more about this product.

12. What are the parameters used to confirm the cells have become completely immortalized?
    ATCC quality tests the immortalized cell lines for several important features including, 1) extended proliferative capacity (population doublings) beyond the Hayflick limit in the primary cell strains, 2) maintaining a stable genotype over an extended culture period via karyotyping, 3) retention of essential phenotypic markers and functions representative of the tissue source, and 4) continued expression of telomerase using the TRAP assay.

13. Do RPTEC/TERT1 cells express OAT1, OAT3, and OCT2?
    We did not see expression of these transporters in the RPTEC/TERT1 (ATCC® CRL-4031™) cells. In our analysis of many primary and immortalized renal cell lines, we did not see OAT1 or OAT3 expression in any of the cell lines examined. Since the RPTEC/TERT1 cells can be propagated for a much longer time than primary renal cells and retain relevant phenotypic features, it is possible to create stable cell lines that express these transporters through genetic engineering.

14. Do TIME cells express VEGF-R2 (KDR)?
    Yes, ATCC TIME cells (ATCC® CRL-4025™) express VEGFR-2 and Tie-2.

15. Can Ker-CT cells be differentiated into skin-equivalent structures?
    Yes, ATCC has tested the differentiation capacity of Ker-CT keratinocyte cells (ATCC® CRL-4048™) and found that it can be differentiated into skin-equivalent structures using the well-established air-liquid interface differentiation protocol. H&E staining revealed a well-organized multi-layered structure composed of a basal layer, spinous layer, granular layer, and cornified layer on the top. Immunofluorescence staining showed typical involucrin and filaggrin expression in the differentiated structure.

16. Are there any differences between hTERT cell lines and cancer cell lines from proliferation and cell markers to xenograft levels?
    Generally, hTERT cell lines do not have many of the genetic aberrations observed in cancer cell lines. There will be some differences in in vitro proliferation, growth rate, molecular and cellular phenotypic markers, and in the formation of xenograft tumors, depending on the type of cells and any acquired mutations following adaptation to culture conditions.

17. What is the main benefit of using hTERT cells compared to cells that have been immortalized by any other techniques such as HPV or SV40?
    There are many different methods for immortalizing mammalian cells. One method is to use viral genes, such as the simian virus 40 (SV40) T antigen or human papilloma virus type 16 (HPV16) E6/E7, as opposed to the expression of the Telomerase Reverse Transcriptase protein (TERT) for cell immortalization. The main benefit of cells immortalized by hTERT over-expression is that they maintain a more stable genotype after extended culture and retain their characteristic phenotype. Viruses can interfere with the p53 pathway, which may result in the interruption or inhibition of p53 function which could contribute to genetic instability, especially

after extended culture.

18. Are TeloHAEC cells from the same donor?
    Yes, TeloHAEC aortic endothelial cells (ATCC® CRL-4052™) were acquired from a single donor and immortalized by introducing telomerase.

19. Does ATCC offer kidney cells from healthy and diseased donors?
    Yes, we have three hTERT cell lines that were isolated from healthy or diseased human kidneys. The RPTEC/TERT1 (ATCC® CRL-4031™) cell line is from a healthy adult donor. ATCC also offers SV7tert PDGF tumor-1 (ATCC® CRL-4008™) and UMB19849 (ATCC® CRL-4004™), which were sourced from patients with renal angiomyolipomas. For more information on these cells lines, please visit our website here.

20. What are the advantages of NFκB-TIME Cells?
    NFκB-TIME (ATCC® CRL-4049™) cells were established after hygromycin selection of TIME (ATCC® CRL-4025™) cells that have integrated the NanoLuc® vector pNL3.2.NF-κB-RE[NlucP/NF-κB-RE/Hygro] that contains five copies of an NF-κB response element (NF-κB-RE) that drives transcription of a destabilized form of NanoLuc® luciferase. The primary advantage of these cells is the luciferase reporter to study activation of the NFkB signaling pathway. Moreover, this assay system has high sensitivity, excellent signal/background ratio and can be used as a simple single-addition assay.

21. Are the hTERT immortalized cells good for drug selection after transfection?
    TIME-GFP (ATCC® CRL-4045™), a clonal cell line with stable expression of the Vivid Colors™ EmGFP fluorescence protein is established after G418 selection of TIME cells. NFKB-TIME (ATCC® CRL-4049™) cells are established after hygromycin selection of TIME cells that have integrated the NanoLuc® vector, which contains an NF-κB response element (NF-κB-RE) that drives transcription of NanoLuc® luciferase.

22. How can the renal hTERT-immortalized cells be used to create stable cell lines? Are there any recommendations for transfection methods or subsequent treatment? What are the limitations?
    As ATCC has done for the stable generation of TIME-GFP (ATCC® CRL-4045™) and NFκB-TIME (ATCC® CRL-4049™) cell lines, renal hTERT-immortalized clonal cell lines stably expressing a gene of interest may be developed by integration of the transfected plasmid into the target cell's genome followed by antibiotic selection. ATCC has successfully transfected plasmid DNA into primary renal proximal tubule epithelial cells (ATCC® PCS-400-010) using our TransfeX™ reagent (ATCC® ACS-4005) and has achieved transfection efficiencies of approximately 70%. In general, primary cells are much more difficult to transfect than continuous cell lines and different methods and transfection reagents may need to be experimented with to find the optimal transfection conditions.

23. Do you have any data about proliferation of TIME cells with or without VEGF in the medium?
    TIME cells can be grown in medium with or without VEGF. The base medium for this cell line is Vascular Cell Basal Medium (ATCC® PCS-100-030). We provide endothelial cell culture media supplements with (Microvascular Endothelial Cell Growth Kit-VEGF (ATCC® PCS-110-041) or without VEGF (Microvascular Endothelial Cell Growth Kit-BBE (ATCC® PCS-110-040).

24. How many hTERT immortalized cells have been used for screening purposes? Are these cells good for transfection with siRNAs or DNAs?
    The Geron Corporation has used cells immortalized with telomerase for drug screening (Refer to US Patent 6,617,110). ATCC has tested the TIME cells (ATCC® CRL-4025™) for transfection with plasmid DNA (EF1α-eGFP) and has achieved transfection efficiencies of approximately 50% using our TransfeX™ reagent (ATCC® ACS-4005). The transfection protocol for these cells is available on the ATCC website.

25. Are there any ongoing efforts, for example, by deep sequencing of DNA and RNA, to compare the hTERT cell lines with their original ancestors?
    We are not aware of ongoing efforts to comprehensively compare genetic information from different hTERT-immortalized cell lines with their primary ancestors, however, there several recent research studies describing TERT promoter mutations which lead to elevated expression of telomerase, that are frequently found in specific types of human cancers (Refs - PMID: 23887589, 23348503, 23348506, 23887712 and 24101484).

26. Has the hTERT technology been applied to neonatal cardiac myocytes?
    There is published literature regarding the application of hTERT technology to neonatal cardiac myocytes.