

Q&A ATCC® Excellence in Research Webinar “hTERT-immortalized cell lines – Unique tools for tissue relevant research”

General Questions

1. Will we be able to download the presentation?
This presentation will be available to watch on demand [here](#).
2. Does ATCC provide culture media for all hTERT-immortalized cell lines?
Yes, you can find the recommended culture media on the webpage and on the product sheets for all hTERT-immortalized cell lines.
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 with atmospheric oxygen level.
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. Hence, introducing hTERT would effectively reconstitute the activity of telomerase and help to establish an immortalized cell line.
5. Do you have a cell line from the endothelial cells of a mouse kidney?
Unfortunately, our current collection does not include an hTERT-immortalized endothelial cell line from mouse kidney. ATCC provides a mouse mesangial cell line ([ATCC® CRL-1927™](#)) derived from the kidney of transgenic mouse line expressing the early region of simian virus 40 (SV40).

How to Generate an hTERT immortalized Cell Line Questions

6. What type of license is required to make my own hTERT lines with your plasmid?
In addition to the ATCC MTA that is required for obtaining biomaterials from ATCC, you also need to sign an addendum for hTERT that you can find on [ATCC's website](#). Once the document is completed, the ATCC licensing department quickly completes the approval process.

7. Can T-cells or B-cells be immortalized with hTERT?

T-cells are resistant to lentivirus or retrovirus transduction and are hard to transfect, thus it is very difficult to get immortalized T-cells or B-cells. EB virus can be used to make transformed cell lines from B-cells.

8. 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 widely used method is using retroviral or lentiviral vectors to transduce primary cells. Alternatively, electroporation and transfection may also work for some cell types.

9. Can the plasmid pGRN145 (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.

10. 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/electroporation 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 would recommend using 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 might be 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.

11. Can I immortalize high passage fibroblasts, or is it best to use low passage?

The BJ-5ta line was actually immortalized at late passage (around population doubling 58), so it is possible to immortalize high passage fibroblasts. Still, we recommend immortalizing fibroblasts as early as possible, since the cells might accumulate genetic drift during extended culture that might affect their functions.

12. I saw commercial hTERT lentivirus. Is there a big difference using hTERT plasmid vs. lentivirus?
Compared with plasmid transfection, lentiviral and retroviral vectors can efficiently transduce the primary cell, deliver the hTERT and facilitate integration into the host genome, thus increase the chance to establish immortalized cell lines.
13. 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 immortalizing rat or mouse cells using human TERT, e.g., rat pancreatic beta cell (PMID: 21167227), rat arachnoid cell lines (PMID: 21195136), mouse osteoblast (PMID: 20686067) and mouse principal cells from collecting duct (PMID: 20926633).
14. Can you use lipofectamine to transfect primary cells with hTERT?
Primary cells are difficult to transfect; we recommend testing several different transfection reagents on the primary cells of interest to identify the best ones. A higher transfection efficiency will increase the chance that you will be able to establish an immortalized cell line.
15. What are the parameters used to confirm the cells have become completely immortalized?
Immortalized cells possess 1) extended proliferative capacity, 2) stable genotype, 3) phenotypic markers from the tissue of interest and 4) continued expression of hTERT.

Cell Line Specific Questions

16. Do [RPTEC/TERT1](#) cells express OAT1, OAT3, and OCT2?
We did not see expression of these important transporters. In our analysis of many primary and immortal renal cell lines, we did not see OAT1, OAT3 expression in any of the cell lines. As the RPTEC/TERT1 cell can be propagated for much longer time than primary cells and retain relevant phenotypic features, it is possible to create stable cell lines that express the transporters by genetic engineering.
17. Do [TIME](#) cells express VEGF-R2 (KDR)?
Yes, [this paper](#) showed that TIME cells express VEGFR2 and demonstrated an association between Shb and the VEGF-R2 in the TIME cells.

