# **hTERT cell culture**

Check out the “cell culture” methods here: <https://breast-cancer-research.biomedcentral.com/articles/10.1186/s13058-014-0510-y>

**Methods from paper:**

Passage 6 184-*hTERT* cells [[18](https://breast-cancer-research.biomedcentral.com/articles/10.1186/s13058-014-0510-y#ref-CR18)] were cloned in 96-well plates and subcultured in serum-free mammary epithelial cell basal media (MEBM; Lonza, Walkersville, MD, USA) supplemented with the mammary epithelial cell growth media in the SingleQuots kit (Lonza), 5 μg/ml transferrin (Sigma-Aldrich, St Louis, MO, USA) and 10−5 M isoproterenol (Sigma-Aldrich), referred to as *mammary epithelial cell growth medium* (MEGM).

The media is a bit more complicated than typical growth media. It’s a bottle of media, isoproterenol, transferrin, and tubes called “singlequots”. We omit the GA-1000 (antibiotic) from the singlequots pack. In regards to actually growing the cells, you can follow basic adherent cell culture techniques, but with a few tweaks:

* they do fairly poorly at low density, so don’t split them too sparse
* they will never reach “full” confluence and cover the entire plate. They will stop growing at about 70-80% confluence, so you should split them before then
* they take a very long time to trypsinize. Add the trypsin, then put them in the incubator for ~5-10mins, checking occasionally. They will take ages if left at room temp.
* the media does not contain FBS, so you will need to use a different type of media to neutralize the trypsin, then spin them down and resuspend in the correct media.

![A screenshot of a cell phone

Description automatically generated]()