# GENOTYPING PCR OF THE HUMAN TERT PROMOTER (TPMS AND ATG)

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1. extract gDNA from human cells using Quick-DNA miniprep kit (11-317AC; Zymo Research)

2. 20 ul PCR reactions were performed with 50ng of gDNA, and Phusion HF DNA polymerase (SEA lab stock) supplemented with 7-Deaza-dGTP (Sigma 10988537001), which facilitates GC-rich PCRs.

- The order of 7-Deaza-dGTP comes in as a 200 ul, 10mM stock solution.
- In the Phusion PCR reaction, use it as 200uM working concentration by dilute it 1:50, in presence of 200uM of regular dNTPs.

3. Primer sequences:

- Pair1 (flanking **ATG**):
  - hTERT TS Hoffmeyer FWD agcccctccccttcctttcc
  - hTERT TS Hoffmeyer REV agcgcacggctcggcagc
  - agcccctccccttcctttccgcggccccgccctctcctcgcggcgcgagtttcaggcagcgctgcgtcctgctgcgcacgtgggaagccctggccccggccacccccgcg**ATG**ccgcgcgctccccgctgccgagccgtgcgct (144bp)
- Pair2 (flanking **ATG**)
  - mccance TERT FWD cctccccttcctttccgcgg
  - mccance TERT REV ggaaagccgccgggtccc
  - cctccccttcctttccgcggccccgccctctcctcgcggcgcgagtttcaggcagcgctgcgtcctgctgcgcacgtgggaagccctggccccggccacccccgcg**ATG**ccgcgcgctccccgctgccgagccgtgcgctccctgctgcgcagccactaccgcgaggtgctgccgctggccacgttcgtgcggcgcctggggccccagggctggcggctggtgcagcgcggggacccggcggctttcc (248bp)
- Pair3 (flanking hDIE1, hDIE2, and hPIE)
  - Stern2015 FWD gtcctgccccttcacctt
  - Stern2015 REV agcgctgcctgaaactcg
  - gtcctgccccttcaccttccagctccgcctcctccgcgcggaccccgccccgtcccgacccctCccggggtccccggcccagccccCtccgggccctcccagcccctcccctttcctttccgcggccccgccctctcctcgcggcgcgagtttcaggcagcgct (162bp)

4. PCR products were purified with EZNA Cycle Pure Kit (D492, omega bio-tek), and was subject to sanger sequencing.

**Reference:**


1. Rowland et al. Molecular Oncology 2020, DOI: 10.1002/1878-0261.12786

2. Hoffmeyer et al. Science 2012. DOI: 10.1126/science.1218370

3. McMurray and McCance, J Viol 2003. DOI: 10.1128/jvi.77.18.9852-9861.2003

4. Stern et al. Genes Dev 2015. DOI: 10.1101/gad.269498.115