

# Characterization of Conserved Regulatory Elements in the Telomerase Promoter in Primates

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**Background.** Telomerase, a ribonucleoprotein complex consisting of a telomerase RNA component (TR) and a telomerase reverse transcriptase (TERT), is believed to be reactivated in 90% of human tumor cells, allowing cancer cells to bypass senescence. Even though hTERT function can be regulated at the post-transcriptional and post-translational levels, the main regulatory step for telomerase activity is the transcriptional control of the hTERT gene. A better comprehension of the regulatory mechanisms for hTERT expression is necessary for understanding telomerase activity and subsequent telomere maintenance in human tumor cells.

**Approach.** mVISTA analysis on available species with TERT promoter regions was used to design primers to amplify in primates. A 300bp region, named TB, about 5000bp upstream of the TERT gene was identified and PCR amplified in primates. Amplified regions were cloned into pGEMT-Easy vectors and sequenced. Resulting sequences from primates were then aligned and compared using VISTA software.

## VISTA Plot of Telomerase Promoter Region

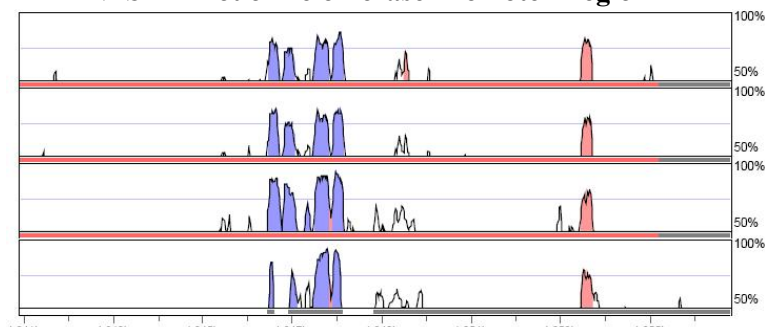


Figure 2. VISTA Browser plot with the promoter region displayed to the right of exon 1 showing a highly conserved peak (in pink) about 5000bp upstream of the telomerase exons (in blue). From the top to the bottom, the species being compared to the base genome human are mouse, rat, dog, cattle.

## VISTA Plot of TB Region in Primates

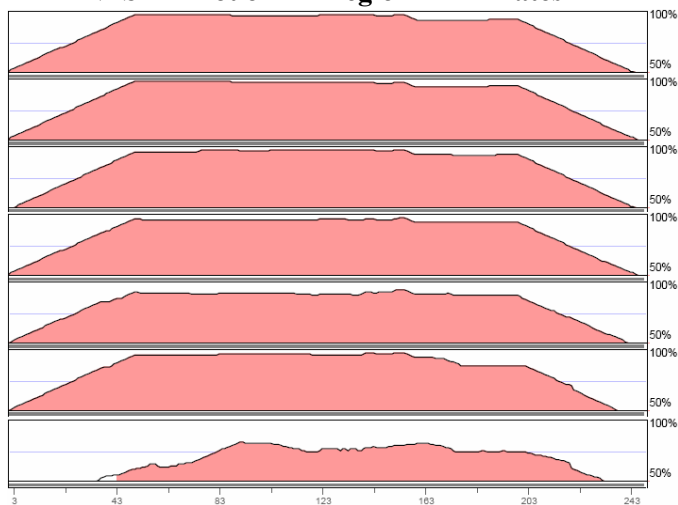


Figure 1. mVISTA plot of the TB region aligned with human as the base genome. The species from top to bottom are chimpanzee, pigmy chimpanzee, orangutan, spider monkey, rhesus monkey, lemur, and mouse. There are high levels of conservation across all these mammals.

**Results.** There are high levels of conservation between humans and the other primates: chimpanzee, pigmy chimpanzee, orangutan, spider monkey, rhesus monkey, and lemur (Figure 1). Even between human and mouse, the TB region has a level of conservation generally above 50%. This seems to suggest that the TB

region has some functional importance since sequence conservation is so high between distantly related species. Also, this region is approximately 5000bp in the 5' region from the start of exon 1. Even so, the telomerase gene is the closest gene to this region. It is possible that this fragment consists of an enhancer due to the high level of conservation among most mammals.

**Future Work.** We plan to build constructs that contain the TB sequences in a Promega pGL3 Luciferase Reporter Vector containing a promoter region. The vector can then be transfected into mammalian cell cultures and assayed for luminescence in a luminometer. This can give a quantitative analysis on possible regulatory elements that drive gene expression.

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