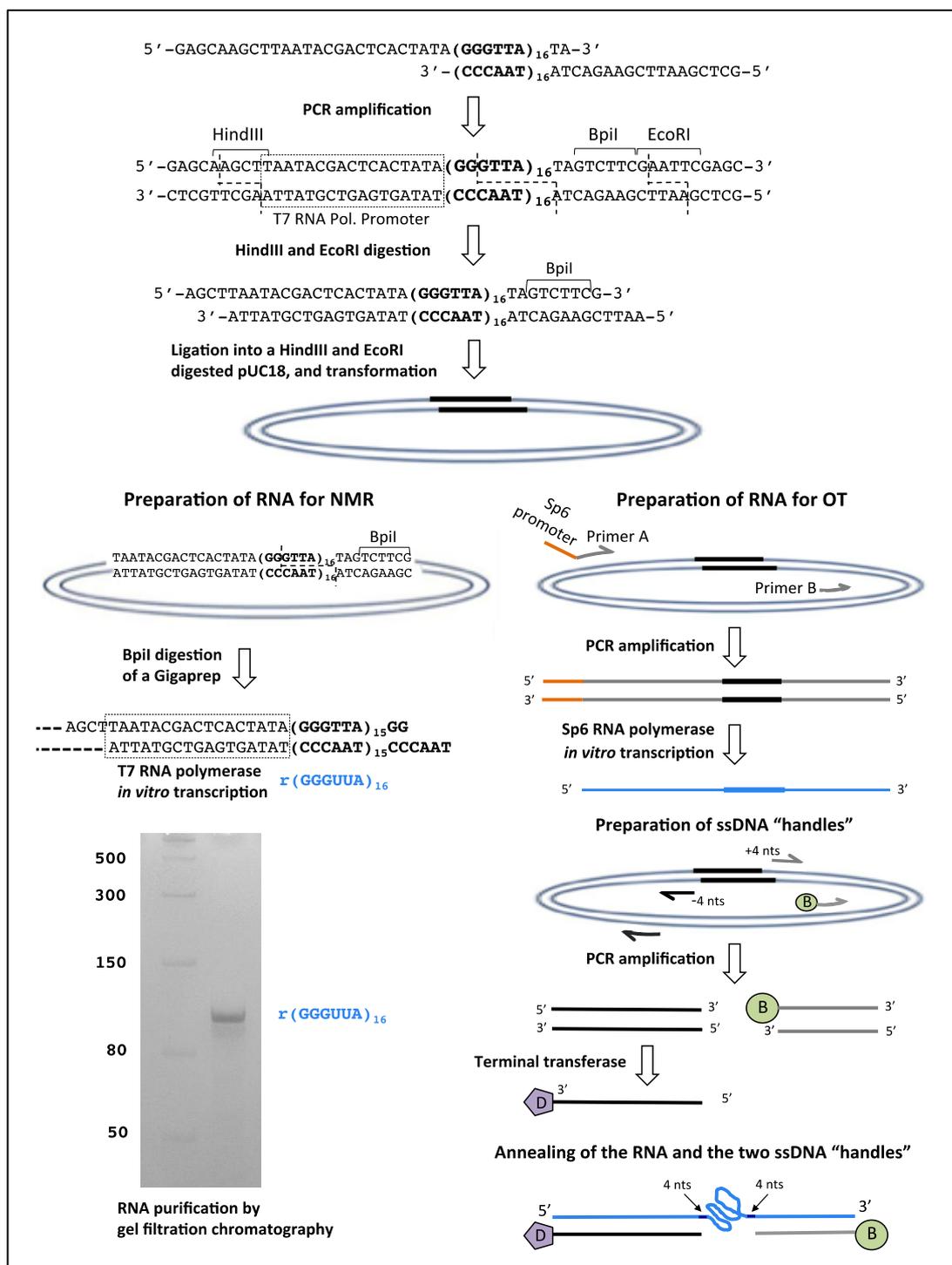


Supplementary DATA FOR:

## Mechanical unfolding of long human telomeric RNA (TERRA)

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### SUPPLEMENTARY FIGURES



**Figure S1.** Schematic representation of the steps involved in the preparation of long TERRA samples for NMR experiments and RNA constructions for optical tweezers. RNA appears in blue. For further details see Experimental Methods section.

## EXPERIMENTAL METHODS

### RNA preparations

The RNA sequences used for NMR, CD and optical-tweezers studies were obtained by *in vitro* transcription. The DNA template was generated by standard PCR methods using the following overlapping primers:

5'-GAGCAAGCTTAATACGACTCACTATA(GGGTTA)<sub>16</sub>TA-3' and

5'-GCTCGAATTCGAAGACTA(TAACCC)<sub>16</sub>TA-3'.<sup>1</sup> The resulting sequence was ligated into the pUC18 vector and transformed into *E. coli* DH5 $\alpha$  competent cells. Given the instability of repeated sequences in *E. coli*, different mutations (deletions, amplifications and single point mutations) frequently occur in the telomeric repeats during plasmid cloning. Nevertheless, this disadvantage has been used to obtain plasmid constructions containing variable number of telomeric repeats. These plasmids were then used for the synthesis of the RNA required for NMR of optical-tweezers studies.

To prepare large quantities of RNA for NMR, plasmid DNA was purified from 2 L of cell culture using Qiagen Giga-prep columns. The purified plasmid was resuspended in deionized water at 500  $\mu$ g/mL and linearized with the Fermentas restriction enzyme BpI (12 h, 37 °C, 1 U of enzyme per 50  $\mu$ g plasmid DNA). The linearized plasmid was used in a 10 mL *in vitro* transcription reaction, where the concentration of MgCl<sub>2</sub> and T7 RNA polymerase have been optimized for the highest yield using 25  $\mu$ L reactions assays. After 2 h at 37 °C, the reaction was stopped by the addition of EDTA to a final concentration of 2x the concentration of MgCl<sub>2</sub>, and the T7 polymerase was removed from the mixture by 3 successive extractions with Phenol:Chloroform:Isoamyl Alcohol (25:14:1, v/v) (Sigma Aldrich). The aqueous phase was desalted using 10-DG desalting columns (BioRad) and then purified by FPLC gel filtration chromatography (HILOAD Superdex 75 P 26/60 GE Healthcare Bio-Sciences Corp.). The purified RNA was concentrated and resuspended in 25 mM KPi pH=7 buffer using Amicon Ultra-0.5 mL, 10 kDa centrifugal filters (Millipore).

The optical-tweezers constructions Q1, Q4 and Q6 have the telomeric RNA between two handles made of DNA:RNA hybrid duplexes. The RNA of these constructions was synthesized by *in vitro* transcription using Sp6 RNA polymerase (New England Biolabs) whose promoter sequence was incorporated in the 5' end of the PCR primer. PCR amplification of three pUC18 plasmids containing the desired number of repeats were performed to obtain the DNA templates 1232 bp, 1178 bp and 1112 bp of Q6, Q4 and Q1 transcripts respectively. These DNAs were used, in 25  $\mu$ L *in vitro* transcription reactions (2 h, 40 °C) to synthesized RNAs having the telomeric sequences flanked by 677 nt at the 5' end and 405 nt at the 3' end. The DNAs of the handles were obtained by PCR amplification using two pairs of primers. The primers flanking the telomeric repeats start at +4 and -4 nucleotides far from the repeats respectively. The ssDNA of the 3' handle (401 nt) was labelled with biotin using a 5'-biotinylated primer (Integrated DNA Technologies, IDT, Coralville, IA) during PCR amplification. The ssDNA of the 5' handle (673 nt) was labelled at its 3' end with a very short tail of 2-3 digoxigenin-dUTP using DIG Oligonucleotide Tailing Kit, 2<sup>nd</sup> Generation (Roche) and terminal transferase (Fermentas). The DNA handles were purified by diatomaceous earth. Equimolar amounts of the RNA and the two ssDNA handles were mixed and annealed in a PCR apparatus using the following annealing procedure: 90 °C 5 min, 85 °C 10 min, 62 °C 90 min, 52 °C 90 min (being the ramp of temperature between steps 0.1 °C/seg).

### Circular Dichroism

CD spectra were recorded on a Jasco 8 J-810 Spectropolarimeter using a 1-mm path-length quartz cuvette. The annealed RNA Q4 was diluted at 2  $\mu$ M concentration in a volume of 200  $\mu$ L of 25 mM KPi pH=7 buffer or 25 mM KPi pH=7, 50 mM KCl buffer. Scans from 320 to 220 nm were performed with a 50nm/min scanning speed. For each spectrum, an average of three spectra was taken and the spectrum of the corresponding buffer was subtracted for baseline correction. The melting curves were obtained by recording the change of the molar ellipticity at 263.5 nm in a range of temperatures from 5 °C to 85 °C. The temperature was controlled using a Jasco peltier, being the rate of temperature rising 40 °C/h. The resulting melting temperatures were calculated by fitting the denaturing curves with the program Origin Pro 6.0.

### Optical Tweezers

Dual, counterpropagating laser beam ( $\lambda = 835$  nm) Optical Tweezers were used to measure force from changes in light momentum flux.<sup>2</sup> Single RNA constructs were tethered by opposite hybrid duplex ends between two dielectric polystyrene microspheres: an anti-digoxigenin antibody-coated bead, optically trapped, and a streptavidin-coated bead, held by suction on top of a micropipette. Force on the studied molecule was exerted by moving the micropipette relative to the optically trapped bead through a piezo-controlled stage, and its extension was measured from the distance between the centers of the beads. Stretch-relax cycles were performed at 100 nm/s. All experiments were carried out at room temperature ( $23 \pm 1$  °C) in 10 mM Tris Cl (pH 7.8) 100 mM KCl, 1 mM EDTA, in the absence of multivalent ions that may produce condensation.<sup>3-5</sup>

### References

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