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Supplementary information

Purification of mammalian telomeric DNA for single-molecule analysis

In the format provided by the authors and unedited

Supplementary methods

Southern blot

Psoralen-crosslinked DNA is exposed to 254 nm UV for 10 min in a stratalinker (UVP CL1000 Ultra-violet crosslinker) to revert the crosslinking and allow DNA denaturation, before blotting. At the end of the run, the gel was treated as follows: 2 × 30 min with depurination solution (HCl 0.25 N), 2 × 30 min with denaturing solution, 2 × 30 min with neutralizing solution. The DNA was then transferred by capillarity in SSC 20X onto a Hybond-XL membrane. At the end of the transfer, the membrane DNA was crosslinked on the membrane by exposure to 254 nm UV, using the "autocrosslink" function of the stratalinker.

Probe sequences:

See Box 1 for the preparation of radiolabeled probes**.**

The 800 bp EcoRI fragment of the pSTY11 plasmid (Addgene no. 12401) was used as a probe for the detection of telomeric repeats. The sequence is available at: https://www.addgene.org/12401/sequences/

Full sequence of the EcoRV fragment used as the mouse L1-probe.

Note that the first three and last three nucleotides (lowercase) are removed after the EcoRV digestion.

gatATCGCCCATCGGCCGAGTTGTCATCACGAGAAAGGCAGAACACATGGCGGGAAAACTGCCC
CTGCACGTGTGCAGATTATGTTTACCACTTAGAACACAGCTGTCAGCGCCATCTTATAATGGCAA
ATGTGAGGGCGGCTCCCAACACTATTTGGAGTTCTGTAGGATTCTTGTATGTTCATGGGCATTTC
TTTCTTTAGGTTTGGGAAGTTTTCTTCTATAATTTTGTTGAAGATATTTGCTGGCCCTTTAAGTTGA
AAATCTTCATTCTCATCTACTCCTATTATCCGTAGGTTTGGTCTTCTCGTTGTGTCCTGGATTTCCT
GGATGGTTTGAGTTAGGATCTTTTTGCATTTTGCATTTTCTTTGATTGTTGTGATGATGTTCTCTAT
GGAATCTTCTGCACCTGACATTCTCTCTTCCATCTCTTGAATTCTATTGCTGATGCTCGCATCTAT
GATTCCAGATTTCTTTCCTAGGTTTTCTATCTCCAGCGTTGCCTCACTTTGGGCTGGATCGGATCT
CACTTTGGGTTTTCTTTATTGTGTCTACTTCCCTTTTTAGGTCTAGTATGGTTTTGTTCATTTCCAT
CACCTGCTTGGATGTGTTTTCCTGTTTTTCTTTAAGGACTTGCAACTCTTTAGCAGTGTTCTCCTG
TATTTCCTTAAGTGAGTTATTAAAGTCCTTCTTGATGTCTTCTACCATCATCCTGAGATATGCCTTT
AAATCCGGGTCTAGATTTTCAGGTGTGTTGTGGTGCCCAGGACTGGATGAGGTGGGAGTGCTGG
GTTCTGGTGATGGTTCTTGGTTTCTGTTAGTAAGATTCTTACGTCTGCTTTTCGCCATCTGGTAAT
CTCTGGAGTCAGTTGTTATAGTTGTCTCTGGTTAGAGCTTCTTCCTCTCGCGATTCTGTTATTCTC
TACCAGCAGACGTGGGAGACTAGGTCTCTCCTGAATTTCAGTGGTCAGAGCACTCTCTGCAGATa
tc-3'

Materials and equipment for the single-molecule analysis of telomere enrichment:

Reagents:

- MES hydrate (2-(N-morpholino)ethanesulfonic acid $C_6H1_3NO_4S$) (Sigma, cat. no. M2933)
- Formamide (Thermo Scientific, cat. no. 17899)
- Maleic acid (Sigma cat. no. M0375)
- Anti-single-stranded DNA antibody (Sigma, MAB3034) RRID:AB_94645
- Alexa-488-labeled anti-mouse IgG secondary antibody (Invitrogen, A1101) RRID:AB_2534069
- Cy3-labeled TTAGGG3 PNA probe (PNA Bio, F1006)
- Blocking reagent (Roche 11096176001)
- ProLong Gold (Invitrogen, P36930)

Reagent setup:

- MES buffer (50 mM MES buffer (2-(N-Morpholino) ethanesulfonic acid) pH 5.6)
- Maleic acid buffer (100 mM maleic acid, 150 mM NaCl, adjust pH to 7.5 with NaOH) Store at 4˚C for up to one year.
- Blocking reagent 10% (wt/vol) in maleic acid buffer. Dissolve by stirring overnight at RT. Store at 4˚C for up to one year.
- Hybridization buffer: 70% (vol/vol) formamide, 0.5% (wt/vol) blocking reagent and 10 mM Tris-HCl pH 7.4.

Equipment:

- Combing reservoir (Genomic Vision cat. no. RES-001).
- Silanized coverslip (Genomic Vision, cat. no. COV-002)
- FiberComb apparatus (Genomic Vision, Version 3, REF: MSC-001)
- Coverslip holder, bench reservoir holder (Genomic Vision, cat. no. POR-001)