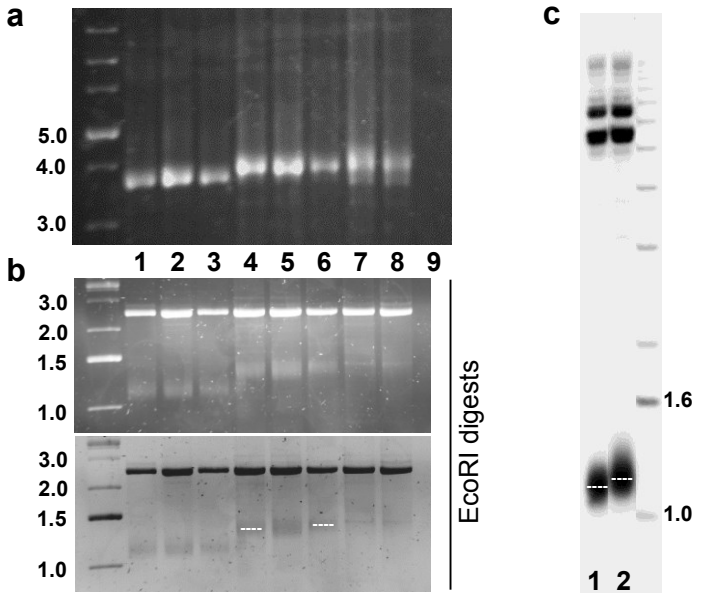
Supplementary information

ATLAS: an advanced PCR-method for routine visualization of telomere length in *Saccharomyces cerevisiae*

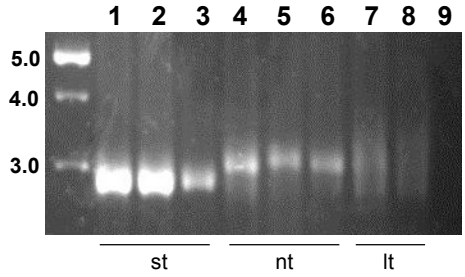
Elena I. Zubko, Jennifer L. Shackleton & Mikhajlo K. Zubko

Supplementary Figure 1. Enhanced resolution of TL differences after digestion of large DNA fragments with a restriction enzyme.



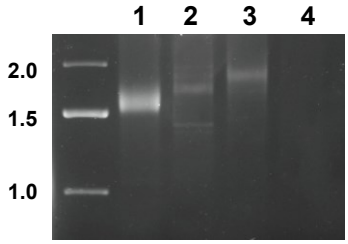
(a) Telomeric DNA fragments amplified from Chr I-RA were **(b)** digested with EcoRI (the panel includes direct and inverted images of the gel). Template DNAs were from the following strains: 1- DLY1331(*mre11*Δ), 2 – DLY1481 (*mre11*Δ), 3 – DLY1479 (*mre11*Δ), 4 – DLY640 (WT), 5 – DLY641 (WT), 6 – DLY1296 (*exo1*Δ *cdc13-1*), 7 – DLY5395 (*pif1*Δ *exo1*Δ), 8 – DLY5396 (*pif1*Δ *exo1*Δ), 9 – NTC. MW marker was 1 kb plus (Thermo Scientific). Electrophoresis conditions: 0.5 x TBE, 0.8% (a) and 1.2% (b) agarose gels. **(c)** Minor increase of TL in mutant DLY1296 (*exo1*Δ *cdc13-1*) [lane 2] as compared to DLY640 (WT) [lane 1] detected by SBH. The result is reproduced by ATLAS with EcoRI digestion (lanes 4-6 in the panel b). White dashed lines show positions for average lengths of telomeric fragments in strains DLY640 and DLY1296 (panels b and c).

Supplementary Figure 2. Amplification of telomeric DNA from Chr II.



The gel presents separation of amplified fragments corresponding to short (st), normal (nt) and long (lt) telomeres. Telomeric DNA from Chr II-RA was amplified using primers Chr. 2-F/dG(18) and template DNAs from the following strains: 1-DLY1331(*mre11*Δ), 2 – DLY1481 (*mre11*Δ), 3 – DLY1479 (*mre11*Δ), 4 – 640 (WT), 5 – DLY641 (WT), 6 – DLY1296 (*exo1*Δ *cdc13-1*), 7 – DLY5395 (*pif1*Δ *exo1*Δ), 8 – DLY5396 (*pif1*Δ *exo1*Δ), 9 – NTC.

Supplementary Figure 3. Detection of differences between TL in strains with long and very long telomeres by using ATLAS .

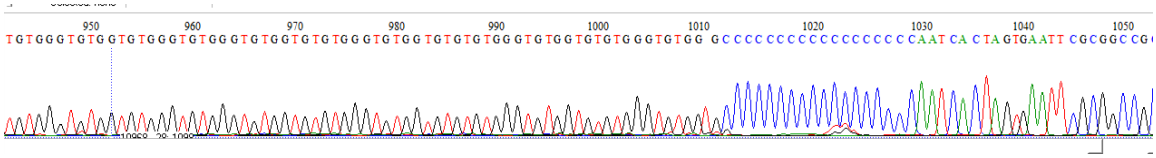


C-tailed DNAs were used for PCR amplification of telomeric DNA with primer Chr.11-F: 1 – DLY640 (WT), 2 – DLY5395 (*pif1*Δ *exo1*Δ mutant with long telomeres), 3 – DLY1171 (*rif2*Δ mutant with very long telomeres), 4 – NTC. MW marker is 1 kb plus (Thermo Scientific). Conditions for electrophoresis: 1% agarose gel, 0.5 x TBE, 100v.

Supplementary Figure 4. Examples of expanded sequences of telomeric DNA cloned from PCR fragments (Chr XI).

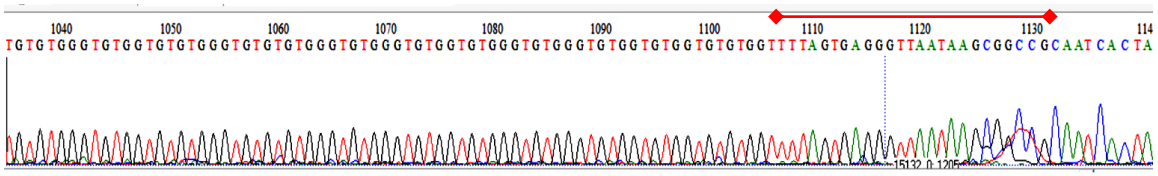
>clone 2 (strain DLY 1479)

gggtatagaccgctgaggcaagtgccgtgcataatgatgtgggtgcatttggtactgatttaatgagaatgggccatggattggagtg
tgagagtagggtaagtttgagagtggtatatactgtagcatccgtgtgcgtatgaccgatcagaatacaagtgaggatggctatggca
tgtggtagtgggattagagtggtagggtaagtatgtatgtattatttacgatcatttgttaacgtttcatatggtgggtagaacaacagtat
ggtgagtaacagatggctgatggtagggtaatggcagggtaagtggtggagttggatatgggtaattggagggtaacggttatgatg
gatggtggttgggattggtagggttgaatggcacagggtaacgaatgatgagttgggtaatggagggtaagttgtgagacaggttcat
cagggttggagtagggtagtgtta**GGGTTGTGGGTGTGTGGGTGTGGTGTGGGTGTGGGT**
GTGGTGTGTGGGTGTGGTGTGTGTGGGTGTGGTGTGTGGGTGTGGGCCCCC
CCCCCCCCCCCCC*aatcactagtgaattcgcggccgcctgcaggtcgaccatatgg*



>clone 5 (strain DLY 641)

gggtatagaccgctgaggcaagtgccgtgcataatgatgtgggtgcatttggtactgatttaatgagaatgggccatggattggagtgt
gagagtagggtaagtttgagagtggtatatactgtagcatccgtgtgcgtatgaccgatcagaatacaagtgaggatggctatggcat
gtggtagtgggattagagtggtagggtaagtatgtatgtattatttacgatcatttgttaacgtttcatatggtgggtagaacaacagtatg
gtgagtaacagatggctgatggtagggtaatggcagggtaagtggtggagttggatatgggtaattggagggtaacggttatgatgga
tggtggttgggattggtagggttgaatggcacagggtaacgaatgatgagttgggcaatggagggtaagttgtgagacaggttcatca
gggttggagtagggtagtgtta**GGGTTGTGGGTGTGTGGGTGTGGTGTGGGTGTGGGTGT**
GGTGTGGGTGTGGTGTGGGTGTGGTGTGGGTGTGTGGGTGTGGTGTGTGG
GTGTGTGGTGTGTGGGTGTGGGTGTGGGTGTGGTGTGGGTGTGGGTGTGG
TGTGGGTGTGGTGTGTGGGTGTGGGTGTGTGTGGGTGTGGTGTGTGGGTGT
GTGTGGGTGTGGGTGTGGTGTGGGTGTGGGTGTGGTGTGGTGTGTGGTTTT
AGTGAGGGTTAATAAGCGGCCGC *aatcactagtgaattcgcggccgcctgcaggtcgaccatatgg*



Lower case – chromosome XI region adjacent to telomeric repeats (coordinates 666136 bp to 666603 bp).
Upper case in bold – sequences of telomeric repeats.
Upper case underlined – poly-C tail generated by terminal transferase (clone 2) or a part the ligated adaptor sequence complementary to the primer r-ad2 (clone 5).
Small case italic – a part of the cloning vector sequence.
Fragments of DNA sequencing chromatograms are shown under the sequences. The red line indicates the sequence of the adaptor.

Supplementary Note 1. Reproducibility and reliability of results on TL analysis by using ATLAS.

In general, six pairs of primers that we used to amplify chromosomal ends of strains with different TL gave consistently reproducible results allowing to detect the differences in TL. Sizes of large amplicons (from Chr I, Chr II, Chr VI [forward primer Chr6-F2] and Chr IX) were resolved better on long gels (15-20 cm, long run **(Figure 3)** than on small gels **(Supplementary Figures 1, 2).** This advantage of longer gels was particularly obvious in case of discrimination between TL differences in strains possessing long and normal telomeres. All sizes of short amplicons (from Chr VI [forward primer Chr6-F1] and Chr XI) were highly resolvable on small gels **(Figures 2b; 4, 5)**. In this respect, using forward primers Chr.6-F1 and/or Chr.11-F for these chromosomes is the most effective approach for express-analysis of TL within a few hours (taking into account the shortest time for the amplification of short fragments and for running small gels). We did not find any differences in size of amplified fragments and efficiency of amplification when using dG18 primer and telomere anchored primers $dG_{(18)}M_{(1)}$ or $dG_{(18)}M_{(2)}$ (**Table 2**). DNA isolated by different methods was equally suitable for PCR amplifications.

Using digestion of amplified telomeric DNA with restriction enzymes enhanced the resolution of detecting TL differences, especially for large amplicons **(Figure 2d** and **Supplementary Figure 1a,b)**. This was achieved by a better control of migration of variable in size telomeric repeats in a gel, due to aligning them with the straight line formed by the migration of the same size bands of sub-telomeric DNA at the top of the gel. The smaller size of telomeric repeats after digestion allowed us to detect the difference in TL about 55-60 nucleotides (determined in a SBH experiment by using a graph for molecular weight markers; data not shown) that took place in comparison of WT to a mutant strain *exo1*Δ *cdc13-1* **(Supplementary Figure 1b,c)**.

In some experiments (depending on PCR cycler used and the number of cycles) we observed extra-bands of unexpected size. They did not affect interpretation of results on TL because: 1) predictable sizes of telomeric fragments could be easily recognised; 2) the extra-bands were located far away from telomeric bands. Extra-bands were observed also in other studies that used telomere PCR (1). We found that the extra-bands could be dismissed by slight increase of annealing temperature (in the range of 1-2^{o}C) or by reducing extension time to minimum, or by decreasing the number of cycles - suggesting that these bands are unspecific.

Supplementary Note 2. Advantages of ATLAS method over SBH.

Our numerous experiments showed the strong correlation between ATLAS data on TL and those obtained by SBH, when using DNAs from the same sets of different mutant strains and wild type cells of *S. cerevisiae* covering a wide range of TL (see examples in **Figures 2a, 4, 5, 7** and **Supplementary Figure 1a-c)**. Even minor differences in TL were detected by ATLAS **(Figures 4, 5** and **Supplementary Figure 1b,c)** suggesting high resolution of the method. This implies that SBH method in its main advantageous feature (namely visualisation of TL) could be replaced by ATLAS in many routine experiments on TL analyses in *S. cerevisiae.* There are a few beneficial aspects of using ATLAS. First, the direct visualisation of TL by using ATLAS makes this PCR method very convincing because: a) TL results are visually observable from gels and b) errors potentially caused by quantitation of relative TL (e.g. in RT-PCR in mammals and humans) are excluded. The method is also simple, cost-effective and rapid. From our experience, ATLAS results on TL could be obtained within one working day if DNA is available. From the same starting point, SBH requires 3-4 days (depending on the strength of workload, hybridisation conditions, detection system and visualisation of hybridisation signals). Amplification of telomeric DNA from different chromosomes allows in depth comparative investigation of their precise sequences in different mutants as well as in cells with TL affected by various exogenous factors. PCR-amplified telomeric DNA could be easily cloned and sequenced if necessary **(Figure 8; Supplementary Figure 4)**.

Supplementary Note 3. Scaled approaches for analysing TL by ATLAS.

Simplicity and time effectiveness of ATLAS make this method convenient and easy for high throughput experimentations on genetic and environmental deregulation of TL in *S. cerevisiae.* The whole experimental cycle of ATLAS includes 4 core stages: 1) isolation of DNA; 2) transferase reaction; 3) PCR amplification of telomeric DNA; 4) analysis of PCR products by gel-electropheresis or bio-analyser. Potentially all four steps or (optionally) any step/s could be performed in 96-well plates (or strip PCR tubes) using automatic and multichannel pipettes.

1. Large-scale isolation of yeast DNA is a routine procedure (2) based on using available kits for 96-well format:

http://www.mobio.com/microbial-dna-isolation/ultraclean-htp-96-well-microbial-dna-kit.html

https://norgenbiotek.com/display-product.php?ID=631

http://www.bosterbio.com/96-well-plate-yeast-dna-mini-preps-kit.html

2. The volume of transferase reaction for each DNA could be reduced up to 10 µl if necessary (3). First, required volume of the master mix for the reaction is placed into a multichannel pipette reservoir, and then it is added to wells of a 96-well plate. Denatured DNA samples are added to the master mix with another set of tips and mixed up gently with the tips. After reaction, tailed DNA could be used for a few series of PCR reactions.

3. For PCR amplification of telomeric regions, a PCR master mix (placed initially into a multichannel pipette reservoir) is loaded into wells of a PCR 96-well plate. Aliquots of products of the transferase reactions are loaded at a required volume directly from the 96-well plate (see stage 2) into a PCR 96-well plate with the PCR master mix and mixed gently with tips.

4. After completion of the PCR reactions, all products or some proportion of them are loaded with multichannel pipette into wells of agarose gels (prepared in 96-well format) and electrophoresed at the required regime. TL in the investigated strains are compared to those in wild-type cells after exposure of stained for DNA gels to UV **(Figures 2b,c; 3-5, 7; Supplementary Figures 1a, 2)**. If necessary (see **Supplementary Note 1)** PCR products are subjected to digestion with a restriction enzyme (see **Figure 1**) before electrophoresis.

References

1. Gupta, A., Sharma, S., Reichenbach, P., Marjavaara, L., Nilsson, A.K., Lingner, J., Chabes, A., Rothstein, R. and Chang, M. (2013) Telomere Length Homeostasis Responds to Changes in Intracellular dNTP Pools. Genetics, 193, 1095-+.
2. Drumonde-Neves, J., Vieira, E., Lima, M.T., Araujo, I., Casal, M. and Schuller, D. (2013) An easy, quick and cheap high-throughput method for yeast DNA extraction from microwell plates. Journal of Microbiological Methods, 93, 206-208.
3. Förstemann, K., Hoss, M. and Lingner, J. (2000) Telomerase-dependent repeat divergence at the 3 ' ends of yeast telomeres. Nucleic Acids Research, 28, 2690-2694.