# TRAPing Telomerase Activity Using Droplet Digital™ PCR (ddPCR™)

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## Introduction

The aim of this work was to develop a more sensitive and high throughput assay for measuring telomerase activity.

Abundant telomerase activity is found in fetal and adult stem cells, germ cells, and cancer cells. It is also present at much lower levels in non-pluripotent cells, such as immune cells, but these levels are difficult to measure using current methods.

The telomerase repeat amplification protocol (TRAP) measures the presence of active telomerase by measuring the activity of the enzyme on a starting template, which is then amplified by PCR. For samples with abundant telomerase activity, SYBR® Green qPCR assays provide high throughput. However, the current most sensitive method of detection still uses radioactivity and laborious PAGE sequencing gels followed by densitometry to quantify telomerase.

In this study, the PAGE detection method is replaced by single molecule counting of telomerase-extended templates using Droplet Digital PCR technology.

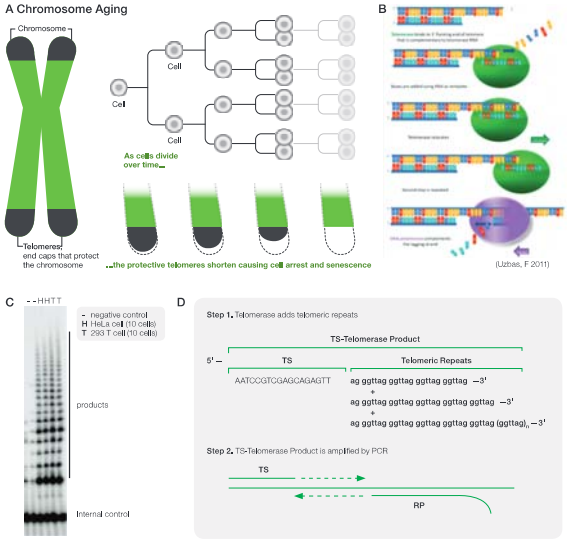



**Fig. 1. Telomeres and telomerase: winding the mitotic clock. A**, the process of cell division shortens telomeres in cells with no telomerase; **B**, telomerase uses an RNA template to add 6 bp repeats to the chromosome ends; **C**, typical example of radioactive quantitative TRAP assay results; **D**, overview of TRAP schema.

## Methods

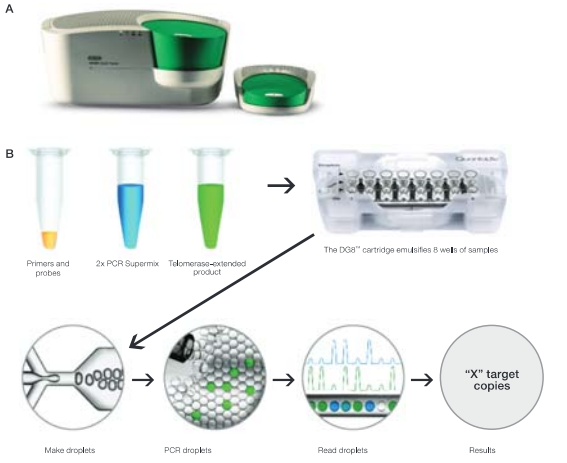



**Fig. 2. A**, QX200 Droplet Digital PCR system; **B**, workflow for Droplet Digital PCR.

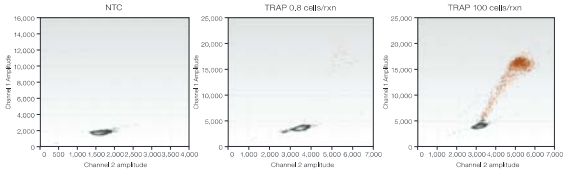
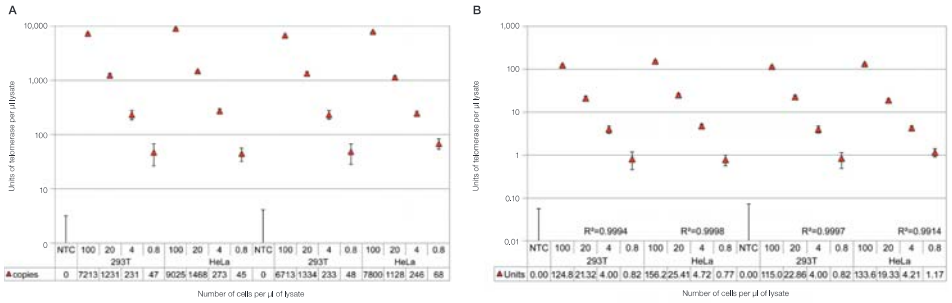


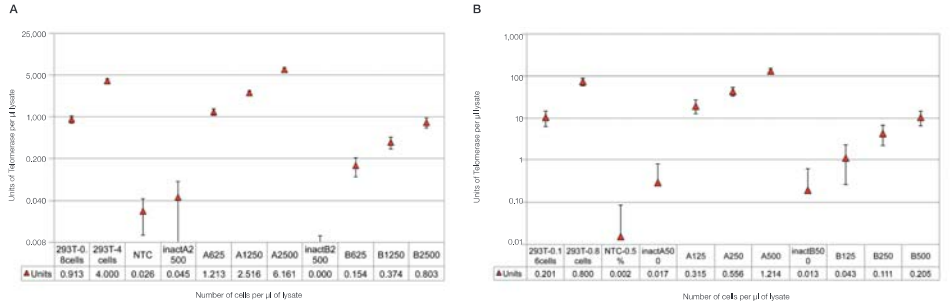

**Fig. 3. Optimization for ddTRAP assay.** Two-dimensional images of ddTRAP assay with 0, 0.8, and 100 293T cells/reactions.

## Results



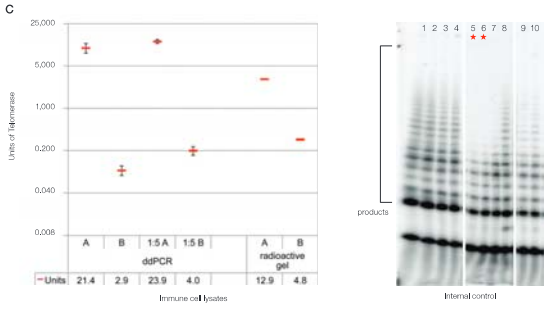

| | NTC | 100 | 20 | 4 | 0.8 | 100 | 20 | 4 | 0.8 | NTC | 100 | 20 | 4 | 0.8 | 100 | 20 | 4 | 0.8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 293T | | | | HeLa | | | | | 293T | | | | HeLa | | | |
| copies | 0 | 7213 | 1231 | 231 | 47 | 9025 | 1468 | 273 | 45 | 0 | 6713 | 1334 | 233 | 48 | 7800 | 1128 | 246 | 58 |
| Units | 0.00 | 124.8 | 21.32 | 4.00 | 0.82 | 156.2 | 25.41 | 4.72 | 0.77 | 0.00 | 115.0 | 22.86 | 4.00 | 0.82 | 133.6 | 19.33 | 4.21 | 1.17 |

**Fig. 4. Telomerase activity in cancer cell lysates. A**, extracts from two cancer cell lines, 293T and HeLa, were incubated with the TS primer as template for telomerase addition, diluted to 100, 20, 4, and 0.8 cells/µl lysate concentrations in biological replicates, and measured by ddPCR. **B**, same samples reporting copies per µl converted to telomerase units, assigning 1 293T cell = 1 unit telomerase.




| | 293T-0.8cells | 293T-4 cells | NTC | inactA2 500 | A625 | A1250 | A2500 | inactB2 500 | B625 | B1250 | B2500 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Units | 0.913 | 4.000 | 0.026 | 0.045 | 1.213 | 2.516 | 6.161 | 0.000 | 0.154 | 0.374 | 0.803 |

| | 293T-0.1 6cells | 293T-0.8 cells | NTC-0.5 % | inactA50 0 | A125 | A250 | A500 | inactB50 0 | B125 | B250 | B500 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Units | 0.201 | 0.800 | 0.002 | 0.017 | 0.315 | 0.556 | 1.214 | 0.013 | 0.043 | 0.111 | 0.205 |




| Lane | 1 | 2 | 3 | 4 | 5 ★ | 6 ★ | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample type | HeLa | HeLa | 293T | 293T | NTC | NTC | Immune A | Immune A | Immune B | Immune B |
| Cell # | 10 | 10 | 10 | 10 | 0 | 0 | 2500 | 5000 | 2500 | 5000 |

**Fig. 5. Telomerase activity in immune cell lysates. A**, immune cells were purified from blood, counted by trypan blue exclusion, and lysed in 1x CHAPS buffer. Two different series of twofold cell lysate dilutions are shown (A625, A1250, A2500 and B625, B1250, B2500); **B**, same lysates as above further diluted fivefold. Inactivated samples (inact) were extracts heated to 95°C for 5 minutes; **C**, direct comparison of radioactive TRAP and ddPCR results for 10,000 immune cells.

## Conclusions

- Droplet Digital PCR provides precise and accurate concentration measurements of telomerase activity on an absolute scale
- Analysis of control samples suggests ddPCR is more sensitive and linear than TRAP radiography
- We extended throughput, sensitivity, and the range of biological samples that can be analyzed for telomerase activity

## Reference

Uzbas, F (2011). Image retreived from Wikipedia. http://en.wikipedia.org/wiki/File:Working_principle_of_telomerase.png. Accessed October 9, 2013.



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