Droplet Digital™ PCR: TRAPing Telomerase Activity Using Droplet Digital PCR

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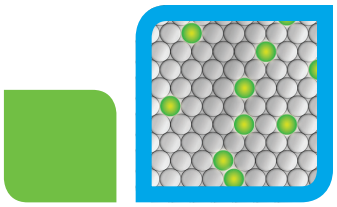

Droplet Digital PCR

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Abstract

Telomerase is responsible for adding hexameric repeats to telomeres, which rewinds the mitotic clock and enables a cell to continuously divide. Abundant telomerase activity is found in fetal and adult stem cells, germ cells, and cancer. It is also present at much lower levels in nonpluripotent 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. The current most sensitive method of detection still uses radioactivity and laborious PAGE sequencing gels followed by densitometry to quantify telomerase.

Here we explore using Droplet Digital PCR (ddPCR™) technology and EvaGreen chemistry to provide absolute quantification of telomerase activity. This was achieved through single-molecule counting of telomerase-extended templates that were partitioned into droplets, amplified by PCR, and detected by fluorescence droplet flow cytometry. Analysis of control samples suggests ddPCR is at least an order of magnitude more sensitive than TRAP radiography and is more amenable to higher-throughput analysis and lower input sample. This method extends throughput, sensitivity, and the range of biological samples that can be analyzed for telomerase activity.

Introduction

Telomeres are the protective structures at the ends of chromosomes and consist of 6 bp repeat sequences. In young cells, these regions can be as long as 15 kb and the telomeres act as caps, which protect the DNA ends. These ends naturally degrade with each passing cell division, usually losing 25–200 base pairs per division. Once they are shortened below a critical length (estimated to be 200–300 bp) the cells arrest and become senescent, or "old." Telomeres can be thought of as a cellular or mitotic clock. Once the clock has wound down, the cells either die or pass through crisis and become immortal.

One of the mechanisms of immortality is activation of the enzyme telomerase. Telomerase is a reverse transcriptase enzyme that adds hexameric repeats to the ends of telomeres using an internal RNA template. Extension of telomeres allows cells to proliferate. The vast majority of adult cells do not express telomerase. Adult stem cells, germ cells, and cancer cells are the exceptions.

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

Methods and Results

Complementary DNA was made from cell lysates containing active telomerase enzyme and a starting template, the TS oligonucleotide. Telomerase-extended templates were partitioned into droplets, amplified by PCR, and detected by fluorescence droplet flow cytometry using the QX200™ Droplet Digital PCR system and EvaGreen. We investigated two commonly used cancer cell lines, HeLa and 293T, for telomerase activity in parallel fivefold dilution series in duplicate. Templates were counted by ddPCR, and units of telomerase were calculated based on 1 293T cell = 1 unit of

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telomerase (Figure 1). Quantities of telomerase ranged from 0.77 units in 0.8 cells per reaction to 156 units in 100 cells per reaction. R^2 values for each series ranged from 0.9914 to 0.9998, demonstrating excellent linearity.

To further test the range of the system, lysates from purified normal immune cells were examined in two sets of twofold dilution series from two patient samples. Again linearity and separation from controls were excellent. R^2 values for each series ranged from 0.9859 at the lowest levels to 0.9982 at the higher telomerase levels. Patient sample B expressed 3–4 units of telomerase per 10,000 cells while patient A exhibited 21–24 units per 10,000 cells. We were able to clearly detect 0.043 units of telomerase in 125 cells of patient B, demonstrating at least 5 orders of sensitivity (Figure 2).

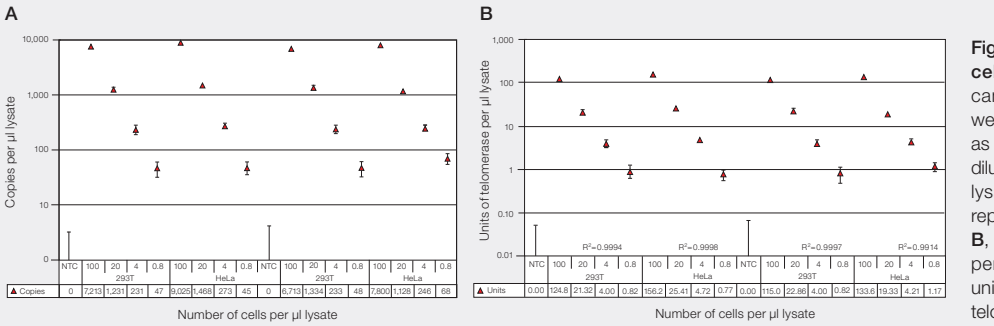

Fig. 1. Telomerase activity in cancer cell lysates. A, extracts from two cancer cell lines, 293T and HeLa, were incubated with the TS primer as template for telomerase addition, diluted to 100, 20, 4, and 0.8 cells/µl lysate concentrations in biological replicates, and measured by ddPCR; **B**, same samples reporting copies per µl converted to telomerase units, assigning 1 293T cell = 1 unit telomerase. NTC, no template control.

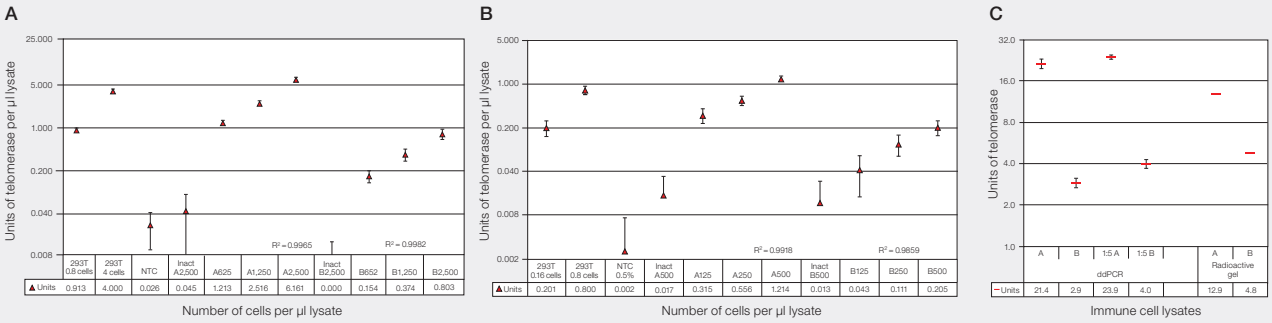

Fig. 2. Telomerase activity in immune cell lysates. A, immune cells were purified from blood, counted by trypan blue exclusion, and lysed in 1x CHAPS buffer. Two different series of twofold cell lysate dilutions are shown (A625, A1,250, A2,500 and B625, B1,250, B2,500). **B**, same lysates further diluted 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. NTC, no template control.

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
- This method extends throughput, sensitivity, and the range of biological samples that can be analyzed for telomerase activity

For more information, visit
www.bio-rad.com/web/ddPCRTRAP.

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