Box 2: Methodological approaches to telomerase activity measurement

As interest in telomere dynamics for ecology and evolution has increased so too has the need for simple and effective methods for estimating telomerase activity. Much of the early work for telomerase detection was borne from the fields of cancer research where accurate estimation of activity is essential for tumor diagnostics and the search for anti-tumor drugs with telomerase inhibition properties.

The methods for telomerase measurement basically fall into two separate categories: those based on the signals arising directly from telomerase products (no PCR); and others based on the detection of signals from DNA products of telomerase via an amplification step (PCR-based). All methods have advantages and disadvantages, particularly in terms of sensitivity, cost, and specialized equipment required. The decision of which method to adopt will vary according to the application and resources of individual labs. Although all of the methods below were initially developed to screen purified cell lines for telomerase activity, they have also been shown to be applicable to tissue lysate in species ranging from protozoans to mammals and from diverse starting materials including tumor biopsies, liver, muscle and even human urine [1]. Preparation typically involves homogenization of cells or tissues in lysis buffer with the lysate either used immediately or stored at -80°C for later measurement.

The non-PCR methods include the use of **surface plasmon resonance (SPR)** to detect small local changes in the refractive index of a surface with telomere oligonucleotides attached [2]. In this method the surface bound telomere oligonucleotides are treated with cell lysate. In the presence of active telomerase, the oligo nucleotides are extended and the refractive index of the chip surface is altered compared to the baseline control. The method has excellent sensitivity with the ability to detect 20-100 telomerase-active cells against a background of 1000-fold excess of telomerase-negative cells. In its present form however, it has the disadvantage that it requires the use of the specialized BIACORE™ system and expensive biotin-conjugated primers. A similar strategy is employed by the **silicon microring resonator** technique [3] which immobilizes the telomere oligonucleotides on silicon rings

housed in a phototonic chip, before lysate is applied and light resonance measurements are compared to baseline controls. This method has increased sensitivity (up to 10 active cells/ uL) and a simplified workflow but still requires the use of specialized equipment not necessarily available to all laboratories. An alternative non-PCR method is the use of **magnetic nanoparticles** that switch their magnetic state once they are annealed to telomerase-extended oligonucleotide repeats [4, 5]. The method is extremely sensitive with the ability to detect telomerase activity from a few as 10 active cells and can be automated for high-throughput processing in a 384 well format. A drawback once again however is the specialized equipment and reagents in the form of a NMR spectrometer and a set of nanosensors covalently bound to telomere oligonucleotides that are needed for the assay.

The PCR-based methods are dominated by myriad variants of the **telomeric repeat amplification protocol (TRAP).** In its original form [6], this assay involves three steps. First, cell lysate is added to a telomere-imitating oligonucleotide which will elongate if telomerase is present. Second, PCR amplification is performed with the oligonucleotides as a template and using primers specific to the elongated extension product. Different labelling methods can be incorporated at this stage to enable the final step of detection. Detection can be carried out via gel electrophoresis and radioactive or fluorescent imaging. Initial versions of the assay suffered from problems of non-specific primer binding leading to false positive signals and high background noise. Refinements and modifications [7, 8] have largely overcome these issues and the assay can now achieve particularly high sensitivity with detection limits between 10 and 50 telomerase active cells depending on the particular assay variant adopted. Commercial kits are available for TRAP assays including TRAPeze (EMD Millipore) and other ELISA-based methods (eg. Aviva Systems) which facilitate the routine adoption of telomerase measurement for most labs. This convenience can sometimes be a trade-off with sensitivity which can be compromised with universal type kits. A promising variant of the TRAP protocol is the **droplet digital TRAP (ddTRAP)** assay [9] which incorporates ddPCR technology into the detection step of the TRAP protocol and allows highly sensitive (as low as one telomerase-positive cell equivalent) and absolute

quantification of telomerase activity, doing away with the need for control cell lines as internal standards.

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