Improved telomere detection using a telomere repeat probe $(TTAGGG)_n$ generated by PCR

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We report the rapid generation of human telomere repeat sequence $(TTAGGG)_n$, with fragment sizes up to 25 kb (Figure 1), using the polymerase chain reaction (PCR). This probe can be labeled with biotin-11-dUTP or a mixture of modified deoxynucleotides in the same PCR reaction. Fluorescence *in situ* hybridisation shows signal at all telomeres with a signal intensity significantly stronger than seen using an oligonucleotide probe (1) of the same sequence (Figure 2). The $(TTAGGG)_n$ probe is also a useful tool as an anchor point in fluorescence *in situ* experiments in which several probes are used simultaneously. It allows merging of the different images obtained with several probes labeled with different fluorochromes at the same time.

PCR is carried out *in the absence of template* using primers $(TTAGGG)_5$ and $(CCCTAA)_5$. Staggered annealing of the primers provides a single strand template for extension by *Taq* polymerase. The primers serve as template in the early PCR cycles whereas the newly formed templates serve as primer and template in subsequent stages of the PCR, resulting in a heterogeneous population of molecules consisting of repeat arrays of various lengths. Clearly, reducing the initial primer concentration increases the average length of the products. PCR reactions were performed in 100 μl volumes containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin, 200 μM of each dNTP, 0.1 μM of each primer and 2 units of *Taq* polymerase (NB: it is important that the primer concentration be 0.1 μM, which is roughly 10-fold less than is commonly used. Changes in primer concentration will affect the average product size). Amplification consisted of first 10 cycles each 1 min at 94°C, 30 sec at 55°C, and 1 min at 72°C, followed by 30 cycles each 1 min at 94°C, 30 sec at 60°C, 90 sec at 72°C, and one final step of 5 min at 72°C. After amplification 5 μl of the PCR products were analysed by agarose gel electrophoresis. Fluorescence *in situ* hybridisation on banded chromosomes was performed as previously described (2).

REFERENCES

1. Moyzis,R.K., Buckingham,J.M., Cram,L.S., Dani,M., Deaven,L.L., Jones,M.D., Meyne,J., Ratliff,R.L. and Wu,J.R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6622–6626.
2. Baldini,A. and Ward,D.C. (1991) *Genomics* 9, 770–774.

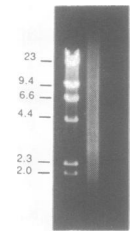

Figure 1. Agarose gel electrophoresis of 5 μl of the PCR products consisting of $(TTAGGG)_n$. The lambda *Hin*dIII size marker (lane 1) indicates that product sizes are up to 25 kb.

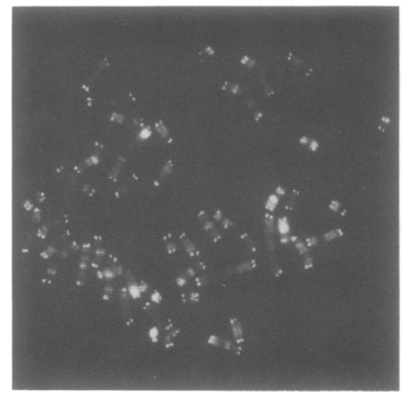

Figure 2. Fluorescence *in situ* hybridisation of biotinylated $(TTAGGG)_n$ on metaphase chromosomes, showing signal on all telomeres.