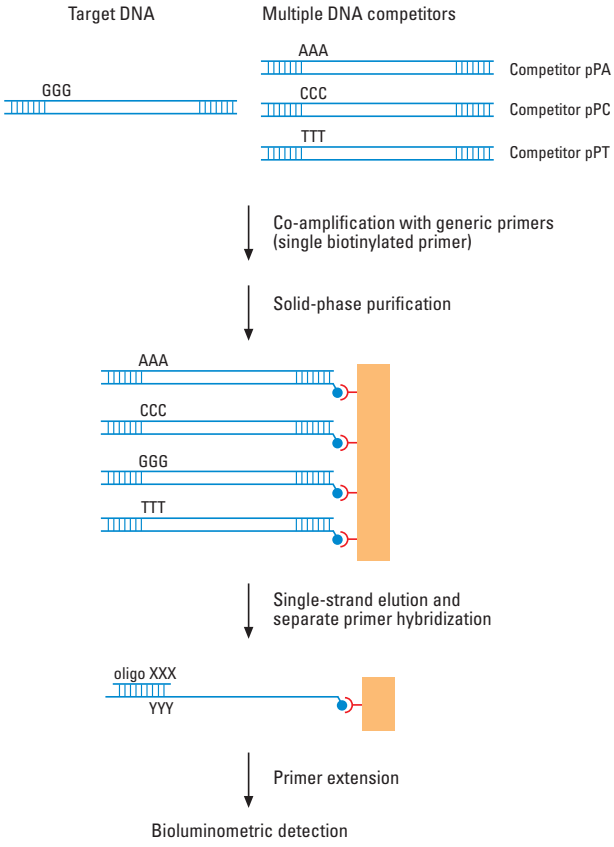
ANALYTICAL CURRENTS

Quantifying HIV infection levels



Schematic illustrating the bioluminometric detection technique for quantification of PCR products. (Adapted with permission. Copyright 2001 Academic Press.)

Determining the degree of human immunodeficiency virus (HIV) infection, also known as the viral load, has been a long-standing priority in health care. Now, Malin Nygren, Joakim Lundeberg, and colleagues at the Royal Institute of Technology and the Karolinska Institute (both in Sweden) describe a PCR-based assay that uses multiple quantitative standards and replaces gel electrophoresis with rapid bioluminometric detection.

The new method is a form of competitive PCR, in which one or more control templates, which are very similar to the test template, are amplified. The final amounts of all PCR products are compared, usually by running them on an electrophoretic gel and comparing band intensities. Because the initial amount of control template is known, the initial amount of test template can be determined.

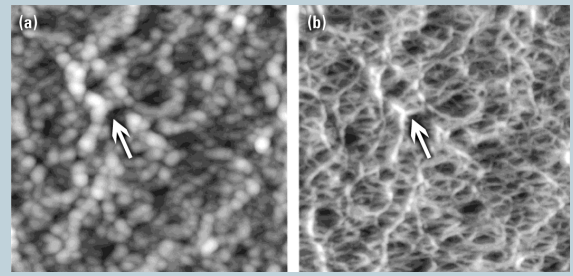
One drawback to competitive PCR methods has been the need to perform many parallel amplification reactions with various dilutions of each control template to generate a standard curve. The new method reduces the number of parallel PCR reactions by employing three control templates, which differ from the test template at only three or four nucleotides and produce PCR products of the same length, in the reaction with the test template. The four PCR products are immobilized onto beads, denatured to produce single-stranded DNA, and divided into four aliquots.

Each aliquot is then subjected to another reaction in which a modified DNA polymerase extends a primer if the primer and target match. Successful extension releases inorganic pyrophosphate, which is converted into ATP. A luciferase enzyme then takes up the ATP and converts it to a proportional amount of visible light. (*Anal. Biochem.* **2001,** *288,* 28–38)

Check those AFM tips

Here is a simple and easy method to ensure that your atomic force microscopy (AFM) tip is free of contaminants and is undamaged. H.-Y. Nie and N. S. McIntyre of the University of Western Ontario (Canada) propose testing the tip's resolution with biaxially oriented polypropylene (BOPP), a polymer film with nanometer-sized fiber structures.

AFM tips contaminated with impurities the size of the features being studied can yield images that mix surface features with contaminant structures. BOPP films are a good way to test the tip before a measurement. Not only does the polymer help check resolution, but the material's high hydrophobicity and low surface energy keep BOPP's own surface contaminant-free, thereby preventing new tip contamination. BOPP is also soft, and in this paper, that property was used to remove tip contaminants by pressing the tip repeatedly into the polymer without damaging the AFM probe. (*Langmuir* **2001,** *17,* 432–436)



Before and after. An AFM image of BOPP film with (a) a dirty tip and (b) a cleaned tip.