Hot Start, Fidelity and Cloning Improvements for PCR

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We demonstrate two ways to achieve a hot start for PCR.
(1) Precipitate the magnesium with "too much" phosphate; the mineral resuspends during thermal cycling and starts the PCR.
(2) Cold-sensitive mutations of Klentaq DNA polymerase have been identified which have reduced activity during bench temperature reaction setup, yet are active and stable at PCR temperatures of 65-95°. The mutants cluster at the hinge point ("knuckle") of the fingers that are believed to move at each polymerase incorporation cycle. The mutant enzymes provide an automatic hot start for PCR, and the fidelity of the resulting amplicons is slightly improved.

Fidelity of DNA polymerase is increased in non-standard buffers and dNTP concentrations (Eckert & Kunkel); we show progress in determining how extreme the conditions can be, while still allowing effective PCR-amplification of 4 kb of DNA (with lacZ) which is of resulting higher fidelity. Betaine (1.3 M) does not hurt fidelity.

To clone and recombine the PCR products, we start with "riboprimers" which have one 3'-ribo base. We have identified conditions and RNAses that can cleave the single ribo base when it is surrounded by double-stranded DNA. After removal of the primer, 3'-sticky ends of the length of the primers (25-30) are left, and they readily recombine *in vitro* with the vector which was prepared the same way with complementary riboprimers. Using long and accurate PCR, our largest vector and target combination so far has been 12 kb vector and 4 kb target.

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