DNA CLONING AND AMPLIFICATION

Amplifying the ancients

Using *in vitro* evolution, researchers generate DNA polymerases more efficient at amplifying ancient DNA.

Until about 20,000 years ago, the cave bear lived in what is now Europe, its territory overlapping with that of early man and the Neandertals. Bear and hominid remains are often found together in caves, and attempts are ongoing to recover and to decode the genomes of these creatures of the last Ice Age. But ancient DNA contains lesions that make it recalcitrant to amplification by PCR. In a recent collaborative effort to get around this problem, the laboratories of Philipp Holliger at the Laboratory of Molecular Biology, Cambridge, and of Svante Paabo and Michael Hofreiter at the Max Planck Institute, Leipzig, have evolved new polymerases that are better at amplifying damaged DNA.

The project came out of Holliger's ongoing effort to evolve DNA polymerases with expanded substrate specificity. It became clear that the ability of the enzyme to amplify damaged DNA could be evolved as well. "While there are specialized translesion synthetases in the genome," he says, "they are nonprocessive and error-prone, and usually can't be used for PCR. We asked ourselves, could one evolve good translesion synthesis while retaining processivity and some fidelity?" One exciting application of such an enzyme would be the amplification of ancient DNA.

As the spectrum of damage in ancient DNA is not entirely understood, the researchers chose to evolve a polymerase with the general ability to extend mismatches. They used a combination of molecular breeding and compartmentalized self-replication (CSR), starting with polymerases from three thermophilic bacteria.

CSR involves a positive feedback loop, in which a polymerase sequestered in a single aqueous droplet of a water-in-oil emulsion amplifies only its own encoding gene, such that adaptive gains in polymerase function result in selective amplification of the gene (**Fig. 1**). "Law number one is you get what you select for," says Holliger, "and what makes CSR so powerful for polymerases is that we can directly select for activity in PCR."

The researchers then applied a set of the newly evolved enzymes to samples of bear DNA found in caves in Austria, dating to 47,000 and 60,000 years ago. The blend of new polymerases resulted in statistically significantly more success with the ancient samples, and the researchers could recover amplicons from more highly diluted template than was possible with *Taq*. Although, as Holliger emphasizes, the work represents only a step, the hope is that such polymerases will render previously unproductive ancient samples productive, and allow the recovery of longer stretches of sequence.

Further, polymerases evolved by this method are pre-adapted to emulsion PCR, so they may be useful in direct sequencing efforts on cave bear as well as—the ultimate goal—on ancient hominid genomes. So we will perhaps one day know, at the genomic level, who the members of the clan of the cave bear really were.

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RESEARCH PAPERS

d'Abbadie, M. *et al.* Molecular breeding of polymerases for amplification of ancient DNA. *Nat. Biotechnol.* **25**, 939–943 (2007).

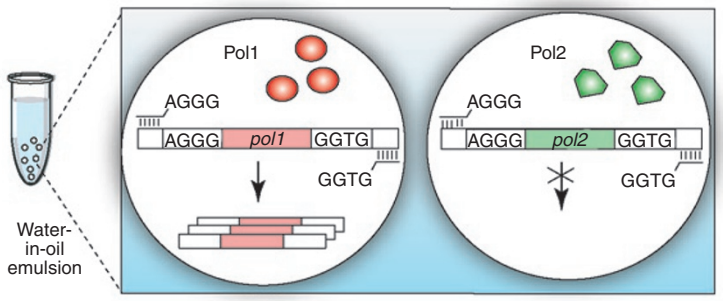

Figure 1 | CSR selection. A polymerase that can use mismatched primers (left) replicates its own encoding gene; a polymerase that is unable to do so (right) disappears from the pool. Reprinted from *Nature Biotechnology*.