Evolution at the Lab Bench

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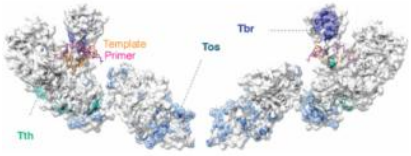
What if it were possible to easily design enhanced or more efficient enzymes than those found in nature, or specialized enzymes that suit your particular needs? Nathan Blow takes a look at the many ways directed evolution is enhancing protein function and changing life in the biochemistry lab.

We've all been through it—a blank gel following two hours of PCR cycling. What happened? Was it the primers? Maybe too little template? Or perhaps some molecule inhibited the reaction? For those working with more exotic samples, blood or soil for instance, many times it comes down to that last point—enzyme inhibition. While a great number of PCR workarounds to overcome inhibition have been described, including the use of additives such as betaine or BSA, why not look at the enzyme itself? Might there be a way to actually improve upon the natural design, and subsequently the performance, of Taq polymerase to enhance efficiency, and thereby prevent inhibition?

At the MRC Laboratory of Molecular Biology in Cambridge, Philipp Holliger and colleagues decided to explore this very question in 2011 (1). Interested in overcoming the PCR inhibitors often present in complex environmental samples, like those that frequently plague scientists working with soil samples, Holliger's team decided the best solution to the problem was to in fact re-engineer a better polymerase—an "evolved" polymerase that was resistance to those environmental inhibitors.



Using CSR, Holliger's team scanned through thousands of possible polymerases in combination and found one in particular, known as 2D9, displayed a broad range of resistance to the tested inhibitors, including humic acid bone dust, peat extract, and clay rich soils. Source: *Nucleic Acids Research*

"Initially, the idea was to evolve polymerases that could be used for ancient DNA research," explains Holliger who at the time was collaborating with paleogenetics researcher Svante Pääbo of the Department of Evolutionary Genetics at the Max Planck Institute. Ancient DNA samples are often rife with inhibitors and contaminants, making PCR amplification a challenge.

Working in the field of directed evolution for more than 10 years now, Holliger developed an approach to evolve biomolecules called compartmentalized self-replication (CSR). In CSR, initially described by Holliger in a 2001 *Proceedings of the National Academy of Sciences* article (2), directed evolution takes place in small microdroplets wherein the polymerase replicates only its encoding gene leading to a feedback loop. A variety of substances can be combined with the polymerase in the microdroplet allowing for selection of desired enzyme characteristics.

Liu's latest continuous system, which he termed PACE for phage-assisted continuous evolution, is focused around the life cycle of the M13 filamentous bacteriophage. Source: Harvard.edu

To identify a novel polymerase with broad resistance to environmental inhibitors, Holliger's group incorporated a molecular breeding program for polymerases with CSR. The idea here is to generate a molecular breeding library where components of the genes from various polymerases (eight in this case) are switched and swapped to create a collection of novel polymerases. From here, the newly generated polymerases have to be expressed and tested for their abilities to withstand harsh environmental inhibitors. For the screening, CSR was employed, enabling Holliger's team to scan through thousands of possible polymerases in combination with a series of inhibitors in the hunt for the one evolved polymerase that would solve the inhibitor challenge and generate that band on the gel after PCR cycling. In the end, the team identified several chimeric polymerases, but one in particular, known as 2D9, displayed a broad range of resistance to the tested inhibitors, including humic acid bone dust, peat extract, and clay rich soils.

While Holliger's team successfully employed what is often termed a conventional directed evolution approach for their polymerase study, there are other ways to evolve proteins in the lab as well—ways that take advantage of natural life cycles, only sped up hundreds of fold.

Improving upon Mother Nature without intervention

David Liu has also been working in field of directed evolution for many years now. Based at Harvard University in Cambridge, Massachusetts, members of Liu's lab have been focused on developing methods to evolve proteins and other biomolecules in an effort to enhance upon the designs of mother nature. One of Liu's major interests lies in developing systems that can support *continuous* laboratory evolution, in which molecules rapidly self-evolve without