Beyond T4: discovery and design of DNA ligases for use in biotechnological applications

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DNA ligases have facilitated the generation of recombinant DNA via the restriction-ligation cloning procedure for the last 20 years, and have also found utility in diagnostic profiling of genetic mutations. More recently, DNA ligases have been employed in Next Generation Sequencing (NGS) technologies, both in preparation of DNA libraries and in the sequencing reaction itself. A limitation of using wild-type DNA ligases is that the majority of these enzymes have evolved to seal single-stranded breaks (nicks) in double-stranded DNA, while the biotechnologically relevant substrate is a double-stranded break (DSB). Many DNA ligases also have temperature and salt optima that are not ideal for the biotechnological reaction conditions.

The DNA ligase from the bacteriophage T4 is the workhorse of ligation-based molecular cloning and NGS library preparation protocols as it is the only commercially available DNA ligase that can join blunt-ended DSBs in the absence of macromolecular crowding agents; however, this reaction occurs ~5 orders of magnitude less efficiently than its biologically-relevant nick sealing activity. Additionally, ligation of cohesive-ended DSBs requires incubation at suboptimal temperatures for T4 activity to stabilize the DNA duplex, and activity is inhibited by relatively low concentrations of NaCl.
Here we present a two-pronged approach to produce DNA ligases with better properties for biotechnological applications.

The first strategy involves structural and biochemical characterization of new DNA ligases from a variety of different environments. Previously we crystalized a minimal DNA ligase from the psychrophilic bacterium Psychromonas spp. SP041 and demonstrated its ability to seal both single and double-stranded DNA breaks, despite having no large DNA binding domains or loops, making it an ideal scaffold for building in additional structural features (Williamson et al. 2014, Acta Cryst. D). Further candidates have been identified by in silico analysis with a focus on bacteria and bacteriophage-derived enzymes, and these are currently the subject of high-throughput expression screening. Promising wild-type ligases will be improved by a combination of random mutagenesis and structure-guided engineering to tailor their activities to the requirements of the biotechnological application.

The second strategy involves improving the activity of characterized DNA ligases by fusing them with non-ligase DNA binding domains. For example, we showed previously that fusion of the eukaryotic transcription factor, NF-κB p50, to T4 DNA ligase improved its performance in adaptor ligation for Illumina sequencing by ~160% (Wilson et al. 2013, PEDS). We will present our on-going efforts to identify and optimize both the fusion partner, and the linker between it and T4 DNA ligase.

In addition to designing DNA ligases for biotechnology, we hope a combination of natural diversity and rational engineering can address the question of ‘what makes a ‘good’ DNA ligase’ both for human uses and nature.