BioSpotlight

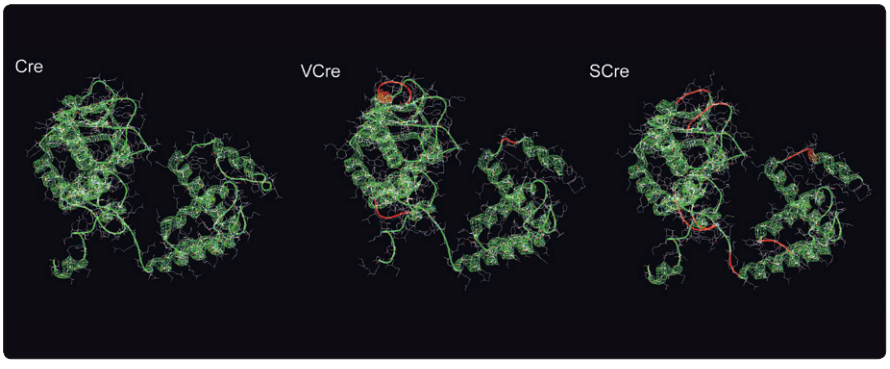
Cold Storage

Cell lines are routinely frozen to protect them from degradation during preservation, but upon thawing, the membrane integrity of the cells is often disrupted. Studying the onset of these preservation injuries can be challenging due to the limited availability of appropriate culture chambers for maintaining live cells at temperatures below 10°C during microscopic imaging. Writing in this issue of *BioTechniques*, Sigot et al. at the Universidad Nacional de Rosario (Rosario, Argentina) describe the construction and performance of an inexpensive modular perfused chamber (MPC) for imaging live cells at low temperatures using an inverted microscope. The MPC is composed of four lathed pieces of stainless steel assembled to form an open cylinder. The chamber is autoclavable so cells may be grown directly inside it, or the chamber can hold coverslips carrying cells cultured in other locations. Temperature within the MPC is controlled using a standard thermal water bath by circulating the water. When imaging at cold temperatures, condensation may form on the coverslip and interfere with the quality of image acquisitions, so the authors directed a stream of cool air onto the coverslip. The ability of the device to aid in biological studies was demonstrated by following the uptake of quantum dots coupled to epidermal growth factor (EGF) in rat hepatocytes as they recovered from hypothermic preservation. Activation of the EGF receptor results in clathrin-mediated endocytosis via coated pits, a process inhibited by low temperatures. Images were acquired from the endpoint of preservation, throughout rewarming of the cells, and for 40 min after the cells reached physiological temperature. Internalization of the quantum dots was observed after 30 min at 37°C, indicating restored endocytosis, basal recovery of the ATP pool, and viability of the recovering cells. This experiment shows the value of the MPC in evaluating the effect of cold storage on the recovery of endocytic and energy-dependent transport in hepatocytes and should also be useful in other studies of the onset of cold-induced injury or to examine cellular processes that occur during cooling to preservation temperatures.

See "A modular perfused chamber for low- and normal-temperature microscopy of living cells" on page 251.

Lots More loxP

Site-specific recombination is an important tool for various methods of genome engineering. Two of the most commonly used site-specific recombination systems are Cre recombinase, with its recognition site loxP, and Flp recombinase, with its recognition site Frt. The combination of these two systems has proven to be especially useful (e.g., for generating mouse conditional knockouts). Increasing the relatively limited number of site-specific recombination systems would facilitate the execution of more complicated genome engineering schemes involving greater numbers of cassettes and target sites. In this issue, S. Minorikawa and M. Nakayama at the Kazusa DNA Research Institute (Chiba, Japan) describe the application to genome engineering of their two recently developed site-specific recombination systems, VCre/VloxP and SCre/SloxP. These systems were isolated respectively from *Vibrio* and *Shewanella* and are members of the same tyrosine recombinase family as Cre and Flp. The VCre and SCre recombinases are both weakly homologous to Cre, and their recognition sites, VloxP and SloxP, are different from each other and loxP. These two novel systems had been previously demonstrated to not interact with the Cre/loxP nor Flp/FRT systems. Here, the authors first showed that recombinase-mediated cassette exchange (RMCE) could be carried out with VCre, VloxP, and the mutant recognition site Vlox2272, which, like the lox2272 site, can only recombine with itself and not with VloxP. Using a donor cassette flanked by a VloxP and a Vlox2272 site, RMCE with an appropriate recipient plasmid was easily induced in the presence of VCre expression. The analogous RMCE experiment using SCre, SloxP, and Slox2272 could also be easily done. Next, they demonstrated that VCre/VloxP could be combined with λ Red recombineering to engineer a BAC. A PCR cassette bearing a blasticidin resistance gene flanked by two VloxP sites, as well as a puromycin gene, was introduced by recombineering into a human Nanog BAC clone. Subsequent expression of VCre caused the elimination of the blasticidin resistance gene flanked by VloxP sites from the recombineered BAC in *Escherichia coli*. These two new site-specific recombination systems should aid in expanding the scope and complexity of genome engineering schemes.



Predicted three-dimensional structures of the VCre and SCre recombinases compared to that of Cre recombinase.

See "Recombinase-mediated cassette exchange (RMCE) and BAC engineering via VCre/VloxP and SCre/SloxPsystems" on page 235.

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