CAMPBELL

METHODS: IN VITRO MUTAGENESIS

Researchers can use in vitro mutagenesis to introduce mutations into specific genes. It is first necessary to add the wild-type allele (lighter blue) to a plasmid or other appropriate vector, and clone the DNA. ① After cloning, the double-stranded plasmid is denatured to obtain a single-stranded DNA template that includes the wild-type version of the DNA of interest. ② A mutagenic primer is allowed to base-pair with the single-stranded template. The primer consists of two regions that are complementary to template regions on either side of a mismatched region—a region where the base sequence of the mutagenic primer is not complementary and will not pair with the base sequence in the template DNA. ③ Addition of DNA polymerase elongates the primer strand to produce a double-stranded plasmid, one strand representing the original genetic information and the other strand containing the new mutant DNA. ④ The vectors (plasmids) are then used to transform bacterial cells, which replicate both strands of the plasmid. When such a transformed cell first divides, one daughter cell gets a mutant version of the plasmid, while the other cell gets the wild-type version. ⑤ Thus, half the resulting colonies will provide a source of the cloned mutant allele and its protein product. The resulting defective protein provides a comparison to help identify the function of the normal protein. (The colonies are artificially color-coded in this diagram.)