



## Rapid and Universal Genetic Modification of Bacteria: Xer-cise™

Cobra Biologics, with locations in Matfors and Södertälje, Sweden and Keele, UK, provides a full range of services from gene cloning to cGMP manufacturing for pre-clinical through to Phase III clinical trials and commercial supply. In support of customer programmes, the company has developed a range of genetic technologies. Xer-cise™ enables genetic modification of bacteria followed by the automatic removal of the antibiotic resistance gene, and works in a wide range of species.

### Features and Benefits

- Native Xer recombinases excise the antibiotic resistance gene after chromosomal insertion
- No exogenous recombinases are required
- Xer-cise™ works in a broad range of species
- Xer-cise™ enables multiple gene integration events in the same strain

### Applications

- Gene deletion: precise excision of target genes prevents reversion in mutant strains
- Gene insertion: new genes can be inserted to alter the phenotype, or for protein expression

### Background

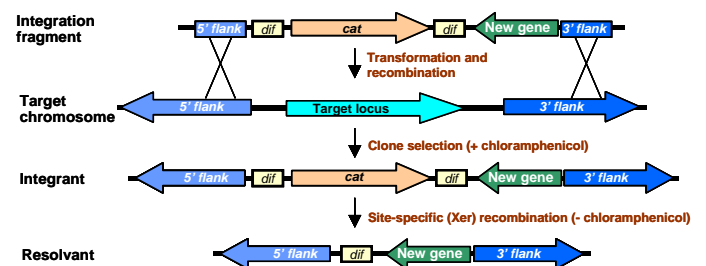
The deletion and insertion of genes in bacterial chromosomes has traditionally been accomplished by labeling the integration cassette with an antibiotic resistance gene, thus selecting the mutants by antibiotic resistance. The main disadvantage of this approach is that if an antibiotic resistance gene is present on the chromosome, the same gene cannot be used for plasmid maintenance, and gene integration events become progressively more difficult due to the limited number of suitable antibiotic resistance genes. Additionally, permanent insertion of a marker gene can alter the expression of surrounding genes, and certain antibiotic resistance genes are not permitted in biologics manufacture.

One approach to address this has involved using antibiotic resistance genes flanked by the sites for site-specific recombinase enzymes (e.g. Cre, Flp), which have to be supplied *in trans* on a plasmid. This requires an additional transformation step and further culturing to remove the plasmid. Also, this approach has only been optimised for a limited range of bacteria.

### Technical Design

The Xer-cise technology employs native Xer recombinases that normally function to restore the chromosomal and

plasmidal dimers generated by RecA back to monomers. These enzymes are ubiquitous in bacteria. An antibiotic resistance gene is flanked by *dif* sites, which are in turn flanked by chromosomal target homology. This cassette is either constructed on a plasmid and linearised, or assembled by PCR, and transformed into the target bacterium. Gene integration mutants are selected on agar plates containing the antibiotic. These are then cultured in antibiotic-free medium, and the Xer recombinases recombine the two *dif* sites to a single site, thereby excising the intervening antibiotic resistance gene to generate the new mutant strain (Figure 1).



**Figure 1:** A linear DNA cassette with the chloramphenicol resistance gene *cat* is used to delete a target chromosomal gene, and simultaneously insert a new gene, selected in the presence of chloramphenicol. Further culture in its absence enables *cat* deletion by Xer-cise™.

Xer-cise has been used successfully for gene insertions and deletions in *E. coli*, *Salmonella*, *Bacillus subtilis* and *Mycobacterium*. Cobra offers the technology for licensing, or fee-for-service bacterial genetic modification.

### Reference

Bloor and Cranenburgh 2006. An efficient method of selectable marker gene excision by Xer recombination for gene replacement in bacterial chromosomes. *Appl. Environ. Microbiol.* 72: 2520-2525.