En passant Mutagenesis – Playing with DNA Virus Sequences

Bacterial artificial chromosomes (BAC) are widely used vehicles to handle large DNA sequences in *Escherichia coli*. For instance, they allow the maintenance and modification of viral genomes independently of functional selection in infected cells. Due to the size of BAC constructs, modification of such sequences by classical methods, such as restriction enzyme digestion and ligation, is mostly impossible. Recombination techniques make BAC sequences accessible, but usually foreign inserts, such as resistance genes or recombinase recognition sites, are left behind. Our group developed a Red recombination system (en passant mutagenesis), which allows markerless manipulation of BACs in *E. coli*. In a first step a linear DNA fragment (e.g. a PCR-product) of modified sequences is introduced into the target site via Red recombination. In the second step, the BAC is cleaved in *E. coli* by a homing endonuclease and the selection marker consequently removed by an intra-molecular Red recombination.

The student training will give the theoretical background of the method in a step-by-step manner. In parallel, a complete *en passant mutagenesis* of a target BAC sequence will be performed in the laboratory. To allow the necessary incubation times, the training will be held on Monday, Wednesday and Friday at the end of February 2011.

Due to limited space, early registration is recommended.

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