CRISPR Bacterial Gene Knock-in Service (Cat. No. C425)

Case Study: CRISPR-assisted knock-in of the mCherry cassette in *E. coli*.

Summary

- The mCherry cassette was knocked into the chromosome using CRISPR-assisted genome editing.
- Transformants were screened using colony PCR.
- mCherry knock-in was confirmed by sequencing.

Phase 1: Cas9 and sgRNA Design and Cloning

- To improve recombination rates in bacteria, phage-derived (λ red) recombinases were employed alongside Cas9 in pCas to carry out enhanced homologus recombination **(Figure 1)**.
- sgRNAs were designed against the *yeeR* locus (accession number: NP_416505). Each sgRNA was individually cloned into pTarget **(Figure 1)**.
- Repair template was designed as double-stranded DNA containing the mCherry cassette flanked by homologies to the *yeeR* locus **(Figure 2)**.

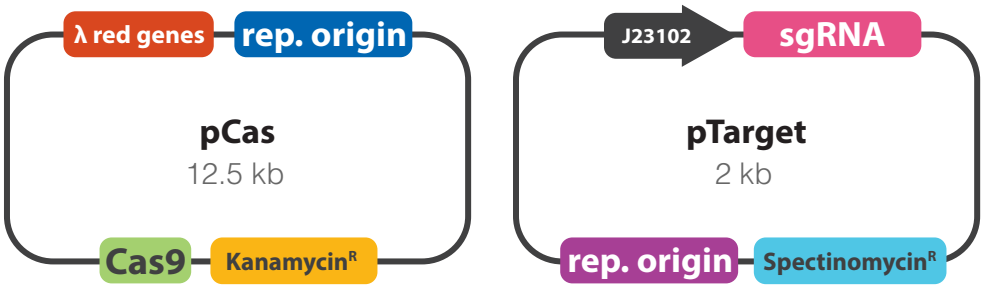

Figure 1 *Vector maps of pCas and pTarget. pCas9 constitutively expresses Cas9, whereas the λ red genes are inducible. pTarget constitutively expresses the sgRNA to guide Cas9 to the target locus.*

Phase 2: Preparation of λ red-induced electrocompetent cells and transformation

- pCas, carrying the λ red genes and Cas9, was transformed into *E. coli* cells. These cells were then made electrocompetent and the λ red genes were induced prior to co-transformation of pTarget and the repair template.

Phase 3: Screening and Sequencing - Knock-in of a chromosomal mCherry cassette

A) Colony PCR screening for insertion of chromosomal mCherry cassette

- Colonies were subjected to PCR using one primer specific to the upstream region of the integration site on the chromosome and one primer specific to the mCherry cassette **(Figure 2)**.

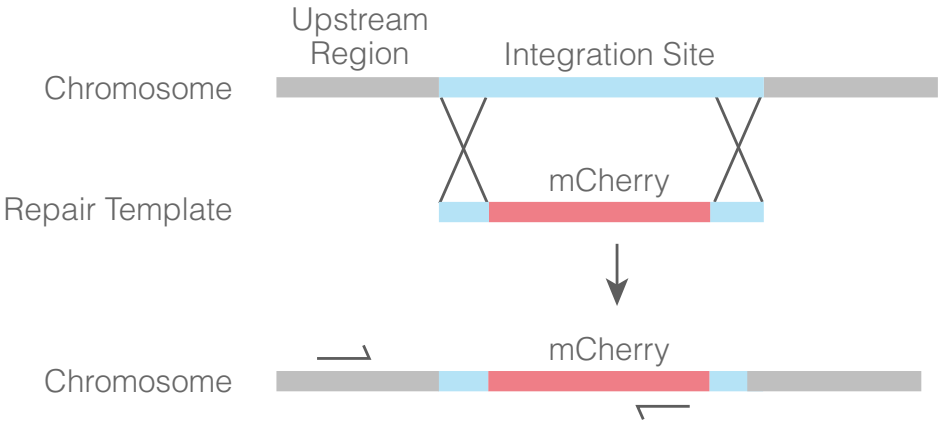

Figure 2 *Schematic of mCherry cassette chromosomal knock-in and location of specific primers for colony PCR screening.*

- The resulting PCR products were run on an agarose gel to confirm correct chromosomal insertion **(Figure 3)**.

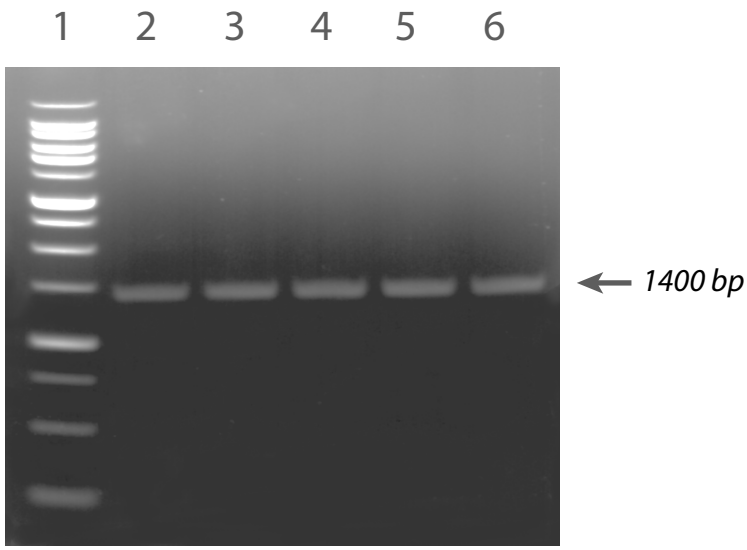

Figure 3 *Agarose gel depicting colony PCR screen for positive mCherry cassette integrants. An amplicon of 1400 bp is consistent with correct chromosomal integration. Lane 1: 1 kb Plus Opti-DNA Marker. Lane 2-6: Colonies #1-5.*

B) Confirmation of mCherry integration

- Positive screened colonies were grown in liquid media and pelleted to reveal mCherry expression **(Figure 4)**.

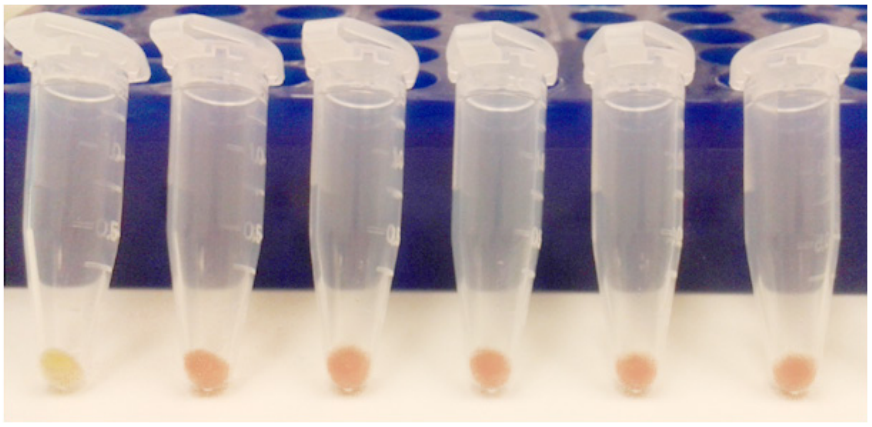

Figure 4 *mCherry positive clones express red fluorescent protein thus producing a pink-red phenotype. The wildtype E. coli strain is depicted in the first tube on the left.*

C) Sequencing of mCherry positive colonies

- PCR products were subjected to Sanger sequencing to confirm correct insertion and knock-in of the mCherry cassette **(Figure 5)**.

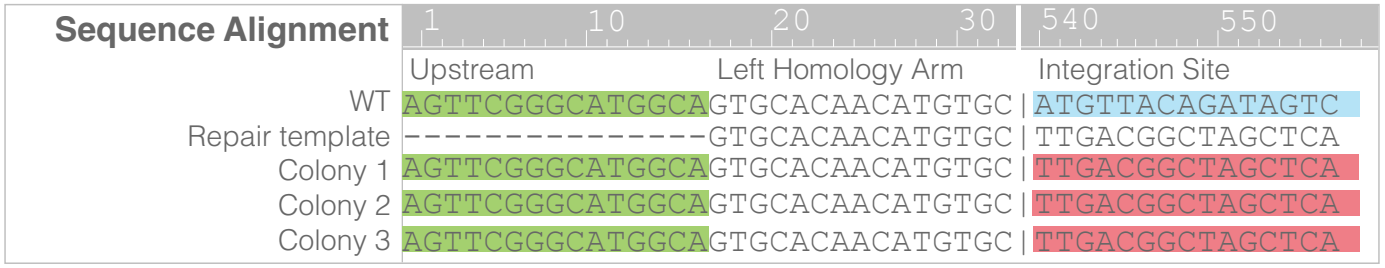

Figure 5 *Sequence alignment of mCherry positive colonies compared to wildtype and repair template sequences. The upstream sequence (green) confirms correct integration and a portion of the mCherry cassette sequence (red) is shown to differ from the wildtype sequence (blue).*