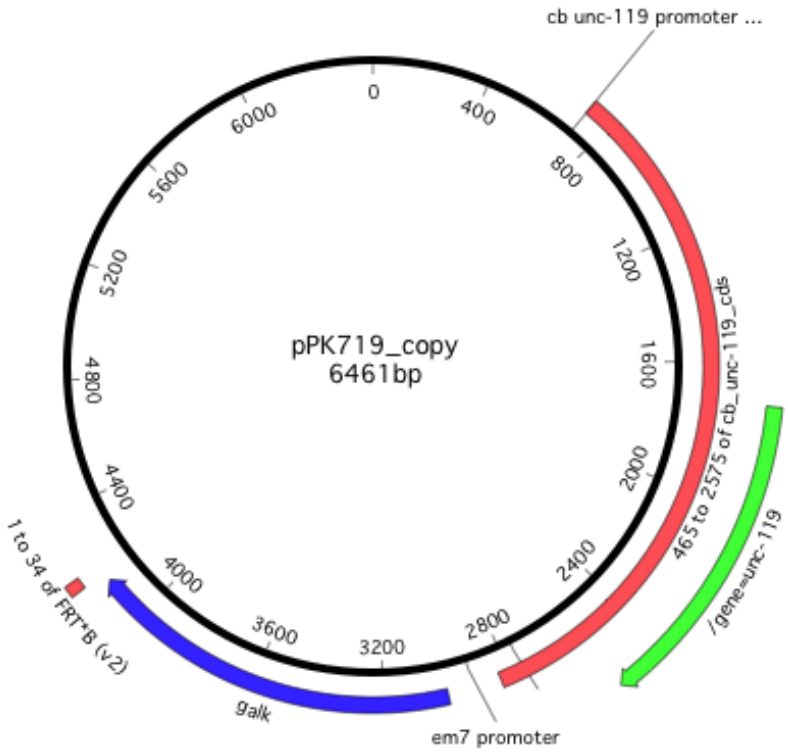
pPK719: ***Cbr-unc-119::frt*::galK::frt****

Insert: Rescuing *Cbr-unc-119* gene with *FRT*::galk*::*FRT** 3' UTR
Species: *Caenorhabditis* (nematode)
Size: 6461 bp (currently being re-sequenced)
Vector: pBluescript KS II (+) T/A cloning into EcoRV site
Antibiotic resistance: Ampicillin
Cloning method: Recombineering
Principal Investigator: Patricia Kuwabara

Sequence will be submitted.



From Worm Breeder's Gazette **(June 2012)**
A universal *Cbr-unc-119::FRT*::galk::FRT** template for recombineering and biolistics

Fosmid based recombineering has become a popular method for generating fluorescent expression reporters that retain the *cis*-acting regulatory features of a gene of interest. To facilitate the identification of successfully targeted recombination events, Tursun *et al.* (2009) have developed a toolkit of recombineering cassettes carrying fluorescent gene

sequences and the selectable *galK* marker flanked by *FRT* sites. To expand the versatility of such recombineered fosmids for use in biolistics (Praitis *et al.*, 2001), we have generated the pPK719 plasmid, which carries a complementary cassette consisting of a genomic copy of the *C. briggsae unc-119* gene and the selectable *galK* marker flanked by *FRT** sites in the presumptive 3' UTR of the *Cbr-unc-119* gene (Fig. 1). The *Cbr-unc-119* gene region was cloned for this purpose because it is more compact than the *Ce-unc-119* gene, yet remains capable of rescuing the *Ce-unc-119* gene (Maduro and Pilgrim, 1996). The inclusion of *FRT** sites in the *Cbr-unc-119::FRT*::galk::FRT** cassette also minimizes the occurrence of unwanted recombination events when performing sequential rounds of recombineering using *galK* flanked by other *FRT* sequences.

PK951	Cbr-unc-119_frt*F	[GATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCAC]AAGATCTATGCTTGCGCTTTGAGCC
PK952	Cbr-unc-119_frt*R	[GCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTC]GAAAATTTAAATATGTATGGTTAGTTAG

Table 1: Primers for amplifying the pPK719 cassette; pCC1FOS homology arms are shown in brackets. Note that the pCC1Fos homology sequence in PK951 is also present in the pBSKSII(+) vector backbone.

The 3.6 kb *Cbr-unc-119::FRT*::galk::FRT** cassette, which is PCR amplified from pPK719 using the PK951/PK952 primer pair (Table 1), can be targeted by recombineering to insert between nts 281:282 in the pCC1FOS fosmid vector backbone. In other words, this cassette can be readily inserted in all fosmids contained in the *C. elegans* library developed by Don Moerman and colleagues. We have tested the construct in fosmid recombineering and have shown that it rescues *unc-119(ed3)* mutants. The clone will be made available at http://www.Addgene.com.

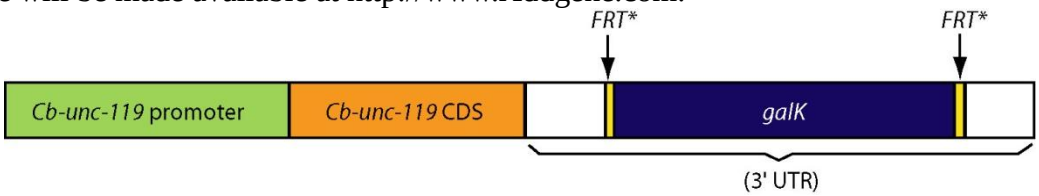


Figure 1: *Cbr-unc-119::FRT*::galk::FRT** cassette contained in plasmid pPK719.

References:

Maduro M and Pilgrim D. (1996). Conservation of function and expression of *unc-119* from two *Caenorhabditis* species despite divergence of non-coding DNA. Gene 183, 77-85. PMID: 8996090

Praitis V, Casey E, Collar D and Austin J. (2001). Creation of low-copy integrated transgenic lines in *Caenorhabditis elegans*. Genetics 157, 1217-26. PMID: 11238406

Tursun B, Cochella L, Carrera I and Hobert O. (2009). A toolkit and robust pipeline for generation of fosmid-based reporter genes in *C. elegans*. PLoS One 4, e4625. PMID: 19259264