# PCaSpeR5 plasmids from the Beitel lab

Contact Greg Beitel (beitel@northwestern.edu or 847-467-7776) if there are questions

Reference:

Le T., Yu M. H., Williams B., Goel S., Paul S.M. and **Beitel G. J.**, 2007, "CaSpeR5, a family of *Drosophila* transgenesis and shuttle vectors with improved multiple cloning sites", BioTechniques, **42**, 164-166.

Abstract from 2007 International Fly Meeting:

Although the pCasper1-4 and pUAST vectors have been the mainstay of Drosophila transformation vectors, their multiple cloning sites (MCS) have a limited number of unique restriction sites. Further, neither of the MCSs in pCaSpeR or pUAST are present in small shuttle or cloning vectors, which is problematic because the large size (> 8 kb) of the transgenesis vectors requires sequence manipulations such as site-directed mutagenesis or deletion dropouts to be done in small plasmid vectors, and the modified DNA to be moved to the transgenesis vectors. The lack of matching shuttle vectors further constrains the usable cloning sites and can complicate moving large genomic fragments between a cloning vector and a transgenesis vector. To overcome the above limitations, **we engineered a new MCS based on the pCaSpeR4 MCS that adds five new six-base cutter sites, but most importantly flanks the entire MCS by two eight-base cutters on each side.** We call this improved vector pCaSpeR5 since the new vector retains all the restriction sites of the pCaSpeR4 MCS in their original order. **We also created pUAS-C5 by replacing the MCS of pUAST with a modified version of the pCaSpeR5 MCS (C5 MCS) that lacked the ATG-containing SphI site.** Although the pUAST vector has many common six-cutter sites in its backbone, the C5 MCS nonetheless adds five new six-cutter and the flanking four eight-cutter sites to the pUAS expression vector. **To facilitate clone manipulation, we created small ampicillin- and kanamycin-resistant shuttle vectors** by replacing the MCSs of pBlueScript and pHSX with the C5 MCS (. The availability of cloning vectors with different drug resistance offers the possibility of "gel-less cloning", although further characterization of the procedure would be required for high efficiency yields in this procedure.

Maps of the MCSs of representative plasmids are shown on next page.

| Plasmid | Genbank accession number |
|---|---|
| pCaSpeR5 | EF090407 |
| pUAS-C5 | EF090406 |
| pC5-Kan | EF090404 |
| pC5-KanΔSphI | EF090405 |
| pBS-C5 | EF090402 |
| pBS-C5ΔSphI | EF090403 |

# Maps and Sequencing primer information for the PCaSpeR5 MCS from the Beitel lab

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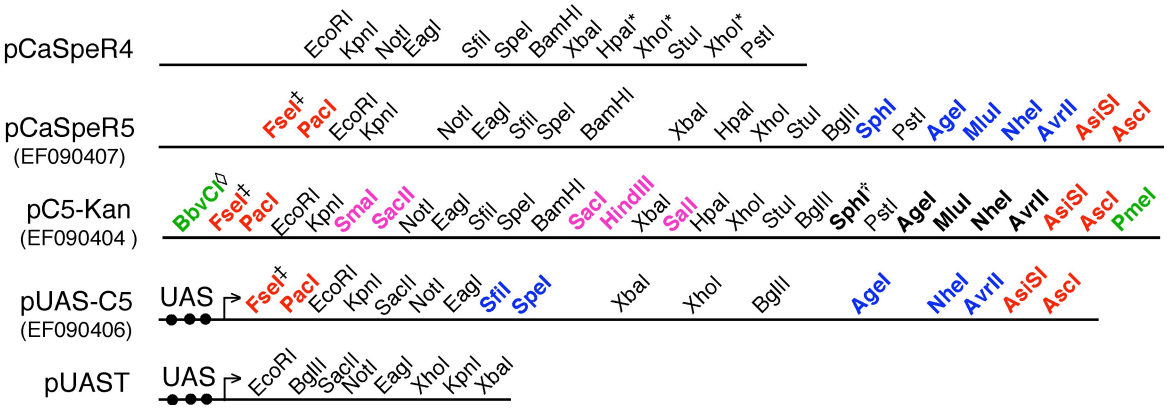


**Fig. 1. Comparison of the pCaSpeR5, pUAS-C5, pC5-Kan vectors with the pCaSpeR4 and pUAST vectors.** Maps comparing the restriction sites in the new and standard transgenesis vectors are shown. GenBank accession numbers for the CaSper5 vectors are shown in parenthesis below the vector names. The accession number for PBS-C5 is EF090402. Red text, new 8-cutter sites for moving fragments from pC5-Kan or pBS-C5 to pCaSper5 or pUAS-C5; blue text, six-cutter sites present in pCaSpeR5 but not pCaSpeR4, or in pUAS-C5 but not pUAST (however, note there is a different order of sites common to pUAS-C5 and pUAST); bold text, six-cutter sites present in pC5-Kan but not pCaSpeR4; pink text, six-cutter sites present in pCaSpeR5 or pUAS-C5 but only unique in pC5-Kan and pBS-C5; green text, blunt or imperfect 8- and 7-cutter sites in pC5-Kan and pBS-C5 that can be used to prevent ligation of the pC5-Kan or pBS-C5 into the target vector in ligations done without gel purification ("gel-less cloning" see text); ◊, 95% of BbvCI ends can not be religated (New England Biolabs product information); *, multiple sites for these restriction enzymes are present in the MCS, but these sites are still pseudo-unique because they are not present in the vector backbone; †, the SphI recognition site contains an ATG which has been eliminated in pC5-KanΔSphI (EF090405) and pBS-C5ΔSphI (EF090403); ‡, FseI is not stable at -20°C necessitating storage at -70°C for periods >30 days. Inserts into the MCSs can be sequenced using the following primers: pC5-Kan, "pC5-Kan FseI" 5' GCCATCACGAGATTTCGATTCC and "pC5-Kan AscI" 5'AAGCAGCAGATTACGCGCAG; pCaSpeR5, "pCasper-MCS-PstI" 5' CACGGACATGCTAAGGGTTA and "pCasper-MCS-EcoRI" 5' AGTTCAATGATATCCAGTGCAG; pUAS-C5 "pUAST-5737" 5' CCAGCAACCAAGTAAATCAACTGC and "pUAST-5432" 5' CCCATTCATCAGTTCCATAGGTTG; pBS-C5, standard T3, T7 and M13 primers.