**File S3. Gibson and SapTrap cloning instructions**

*Gibson cloning into SEC vectors*

Gibson cloning into SEC vectors was performed according to the detailed protocol provided in (Dickinson *et al.* 2015). To design primers to amplify 5’ and 3’ homology arms, we included the following sequence on the 5’ end of our primers.

*Oligo design for Gibson cloning into SEC vectors*

**N-terminal mNeonGreen::AID\*::3xFLAG**

|  |  |
| --- | --- |
| *Digest pJW1582 vector with ClaI and SpeI* | |
| 5' arm forward primer | 5'-acgttgtaaaacgacggccagtcgccggca‐(Homology arm sequence)-3' |
| 5' arm reverse primer | 5’-­CATGTTGTCCTCCTCTCCCTTGGAGACCAT‐(Homology arm sequence)-3' |
| 3' arm forward primer | 5’-CGTGATTACAAGGATGACGATGACAAGAGA-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm reverse primer | 5’-tcacacaggaaacagctatgaccatgttat-(Homology arm sequence)-3' |

**C-terminal mNeonGreen::AID\*::3xFLAG with flexible linker**

|  |  |
| --- | --- |
| *Digest pJW1582 vector with AvrII and SpeI* | |
| 5' arm forward primer | 5'-acgttgtaaaacgacggccagtcgccggca‐(Homology arm sequence)-3' |
| 5' arm reverse primer | 5’-­CATCGATGCTCCTGAGGCTCCCGATGCTCC-­‐(Cas9 target mutations)-(Homology arm sequence)-­‐3' |
| 3' arm forward primer | 5’-CGTGATTACAAGGATGACGATGACAAGAGA-(Cas9 target mutations)-(Homology arm sequence)-­‐3' |
| 3' arm reverse primer | 5’-ggaaacagctatgaccatgttatcgatttc-(Homology arm sequence)-3' |

**N-terminal GFP::AID\*::3xFLAG**

|  |  |
| --- | --- |
| *Digest pJW1583 vector with ClaI and SpeI* | |
| 5' arm forward primer | 5'-acgttgtaaaacgacggccagtcgccggca-(Homology arm sequence)-3' |
| 5' arm reverse primer | 5-TCCAGTGAACAATTCTTCTCCTTTACTCAT­-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm forward primer | 5-CGTGATTACAAGGATGACGATGACAAGAGA-­-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm reverse primer | 5-ttcacacaggaaacagctatgaccatttat-(Homology arm sequence)-3' |

**C-terminal GFP::AID\*::3xFLAG with flexible linker**

|  |  |
| --- | --- |
| *Digest pJW1583 vector with AvrII and SpeI* | |
| 5' arm forward primer | 5'-acgttgtaaaacgacggccagtcgccggca-(Homology arm sequence)-3' |
| 5' arm reverse primer | 5-CATCGATGCTCCTGAGGCTCCCGATGCTCC­‐(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm forward primer | 5-CGTGATTACAAGGATGACGATGACAAGAGA-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm reverse primer | 5- aggaaacagctatgaccatttatcgatttc -(Homology arm sequence)-3' |

**N-terminal YPET::AID\*::3xFLAG**

|  |  |
| --- | --- |
| *Digest pJW1584 vector with ClaI and SpeI* | |
| 5' arm forward primer | 5'-acgttgtaaaacgacggccagtcgccggca-(Homology arm sequence)-3' |
| 5' arm reverse primer | 5-TCCTGTAAATAACTCTTCTCCTTTTGACAT-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm forward primer | 5-CGTGATTACAAGGATGACGATGACAAGAGA-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm reverse primer | 5-ttcacacaggaaacagctatgaccatttat-(Homology arm sequence)-3' |

**C-terminal YPET::AID\*::3xFLAG with flexible linker**

|  |  |
| --- | --- |
| *Digest pJW1584 vector with AvrII and SpeI* | |
| 5' arm forward primer | 5'-acgttgtaaaacgacggccagtcgccggca-(Homology arm sequence)-3' |
| 5' arm reverse primer | 5-CATCGATGCTCCTGAGGCTCCCGATGCTCC­-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm forward primer | 5-CGTGATTACAAGGATGACGATGACAAGAGA-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm reverse primer | 5- aggaaacagctatgaccatttatcgatttc -(Homology arm sequence)-3' |

**N-terminal mKate2::AID\*::3xFLAG or N-terminal mKate2::BioTag::AID\*::3xFLAG**

|  |  |
| --- | --- |
| *Digest pJW1586 or pJW1595 vector with ClaI and SpeI* | |
| 5' arm forward primer | 5'-acgttgtaaaacgacggccagtcgccggca-(Homology arm sequence)-3' |
| 5' arm reverse primer | 5-CATGTTTTCTTTAATGAGCTCGGAGACCAT-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm forward primer | 5-CGTGATTACAAGGATGACGATGACAAGAGA-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm reverse primer | 5-ttcacacaggaaacagctatgaccatttat-(Homology arm sequence)-3' |

**C-terminal mKate2::AID\*::3xFLAG with flexible linker or C-terminal mKate2::BioTag::AID\*::3xFLAG with flexible linker**

|  |  |
| --- | --- |
| *Digest pJW1586 or pJW1595 vector with AvrII and SpeI* | |
| 5' arm forward primer | 5'-acgttgtaaaacgacggccagtcgccggca-(Homology arm sequence)-'' |
| 5' arm reverse primer | 5-CATCGATGCTCCTGAGGCTCCCGATGCTCC­-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm forward primer | 5-CGTGATTACAAGGATGACGATGACAAGAGA-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm reverse primer | 5- aggaaacagctatgaccatttatcgatttc -(Homology arm sequence)-3' |

**N-terminal TagRFP-T:: 3xFLAG::AID\***

|  |  |
| --- | --- |
| *Digest pTNM063 vector with ClaI and SpeI* | |
| 5' arm forward primer | 5'-acgttgtaaaacgacggccagtcgccggca-(Homology arm sequence)-3' |
| 5' arm reverse primer | 5-TTGATGAGCTCCTCTCCCTTGGAGACCAT-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm forward primer | 5-CGGTGGCCCGGAGGCGGCGGCGTTCGTGAAA-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm reverse primer | 5-acacaggaaacagctatgaccatgttatcg-(Homology arm sequence)-3' |

**C-terminal TagRFP-T:: 3xFLAG::AID\* without flexible linker** (note linker sequence could be added to the homology arm by PCR or gene synthesis)

|  |  |
| --- | --- |
| *Digest pTNM063 vector with AvrII and SpeI* | |
| 5' arm forward primer | 5'-acgttgtaaaacgacggccagtcgccggca-(Homology arm sequence)-3' |
| 5' arm reverse primer | 5-CCATCGATGCTCCTGAGGCTCCCGATGCTC-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm forward primer | 5-CGGTGGCCCGGAGGCGGCGGCGTTCGTGAA-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm reverse primer | 5-acagctatgaccatgttatcgatttcctag-(Homology arm sequence)-3' |

**N-terminal TagRFP-T::BioTag::AID\*::3xFLAG** (note loss of AvrII site means C-terminal fusions cannot be generated by AvrII+ClaI digestions. However. linker sequence could be added to the homology arm by PCR or gene synthesis)

|  |  |
| --- | --- |
| *Digest pJW1594 vector with ClaI and SpeI* | |
| 5' arm forward primer | 5'-acgttgtaaaacgacggccagtcgccggca-(Homology arm sequence)-3' |
| 5' arm reverse primer | 5-CTTGATGAGCTCCTCTCCCTTGGAGACCA-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm forward primer | 5-CGTGATTACAAGGATGACGATGACAAGAGA-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm reverse primer | 5-ttcacacaggaaacagctatgaccatttat-(Homology arm sequence)-3' |

**N-terminal GFP::BioTag::AID\*::3xFLAG** (note vector backbone is inverted compared to other vectors, but vector still is functional. Different vector homology sequences are used.)

|  |  |
| --- | --- |
| *Digest pJW1592 vector with ClaI and SpeI* | |
| 5' arm forward primer | 5'-ttcacacaggaaacagctatgaccatttat-(Homology arm sequence)-3' |
| 5' arm reverse primer | 5-TCCAGTGAACAATTCTTCTCCTTTACTCAT­-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm forward primer | 5-CGTGATTACAAGGATGACGATGACAAGAGA-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm reverse primer | 5-acgttgtaaaacgacggccagtcgccggca-(Homology arm sequence)-3' |

**C-terminal GFP::BioTag::AID\*::3xFLAG with flexible linker**

|  |  |
| --- | --- |
| *Digest pJW1592 vector with AvrII and SpeI* | |
| 5' arm forward primer | 5'-aggaaacagctatgaccatttatcgatttc-(Homology arm sequence)-3' |
| 5' arm reverse primer | 5-CATCGATGCTCCTGAGGCTCCCGATGCTCC­-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm forward primer | 5-CGTGATTACAAGGATGACGATGACAAGAGA-(Cas9 target mutations) ‐(Homology arm sequence)-3' |
| 3' arm reverse primer | 5-acgttgtaaaacgacggccagtcgccggca-(Homology arm sequence)-3' |

*SapTrap cloning*

SapTrap reactions were performed as previously described

(Schwartz and Jorgensen 2016). The original protocol recommended incubating the SapTrap reaction at room temperature overnight, however for inefficient assemblies we found extending the incubation time to 30-44 hours improved efficiency.

*5’ homology arms*

All 5’ homology arms used the same forward primer, designed to connect to the vector 5’TGG connector.

Forward primer: 5’-GGCTGCTCTTCgTGG-(Homology arm sequence)-3’

To connect to a CT slot vector, or any of our multi-cassettes starting with a CT slot cassette (pJW1816, pJW1822, pJW1827, pJW1846, pJW1886), used the following reverse primer design to connect to the 5’GCG connector:

5’ GGGTGCTCTTCgCGC-(Homology arm sequence)-3’

To connect to an FP slot vector, or our multi-cassette starting with an FP slot cassette (pJW1820), we used the following reverse primer design to connect to the 5’ATG connector:

5’ GGGTGCTCTTCgCAT-(Homology arm sequence)-3’

*3’ Homology arms*

All 3’ homology arms used the same reverse primer, designed to connect to the vector 5’GTA connector.

Reverse primer: 5’GGGTGCTCTTCgTAC-(Homology arm sequence)-3’

To connect to our NT slot vectors, or multi-cassettes ending with a NT slot cassette (pJW1816, pJW1820, pJW1846, pJW1886) we used the following forward primer design to connect to the 5’ACG connector:

5’ GGCTGCTCTTCgACG-(Homology arm sequence)-3’

**Literature Cited**

Dickinson D. J., Pani A. M., Heppert J. K., Higgins C. D., Goldstein B., 2015 Streamlined Genome Engineering with a Self-Excising Drug Selection Cassette. Genetics **200**: 1035–1049.

Schwartz M. L., Jorgensen E. M., 2016 SapTrap, a Toolkit for High-Throughput CRISPR/Cas9 Gene Modification in *Caenorhabditis elegans*. Genetics **202**: 1277–1288.