**Plasmid construction overview**

Construct with just DsRed (“no-Cas9\_no-gRNAs”):

|  |  |  |  |
| --- | --- | --- | --- |
| **FACacR** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | pDsRed | FACacR\_F | FACacR\_R |
| *Plasmid Digest* | ATSacG | NcoI | HincII |

Intermediate for the gRNAs:

|  |  |  |  |
| --- | --- | --- | --- |
| **TTTacU4** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | TTTgRNAtRNAi | Acg4\_41\_F | Acg4\_41\_R |
| *PCR Product* | TTTgRNAt | Acg4\_12\_F | Acg4\_12\_R |
| *PCR Product* | TTTgRNAt | Acg4\_23\_F | Acg4\_23\_R |
| *PCR Product* | TTTgRNAt | Acg4\_34\_F | Acg4\_34\_R |

Construct with Cas9 and no gRNAs (“Cas9\_no-gRNAs”):

|  |  |  |  |
| --- | --- | --- | --- |
| **FACacN** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | none | acN\_F | acN\_R |
| *Plasmid Digest* | BHDgN1c | StuI | XbaI |

Construct with Cas9 and four gRNAs (“Cas9\_gRNAs”):

|  |  |  |  |
| --- | --- | --- | --- |
| **FACacN4** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | none | U6\_3\_gRNA1\_v4\_F | gRNA\_f\_R |
| *Plasmid Digest* | BHDgN1c | StuI | XbaI |

Intermediate for Cas9HF1:

|  |  |  |  |
| --- | --- | --- | --- |
| **Nos-Cas9HF1** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | VP12 | Cas9HF1\_F | Cas9HF1\_R |
| *Plasmid Digest* | nos-Cas9-nos | Bsu36I | FspI |

Construct with Cas9HF1 and four gRNAs (“Cas9HF1\_gRNAs”):

|  |  |  |  |
| --- | --- | --- | --- |
| **FACacNf4** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | Nos-Cas9HF1 | HF1\_F | HF1\_R |
| *Plasmid Digest* | FACacN4 | Bsu36I | AscI |

Homing drive with Cas9HF1:

|  |  |  |  |
| --- | --- | --- | --- |
| **BHDgNf1v2** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | Nos-Cas9HF1 | HF1\_F | HF1\_R |
| *Plasmid Digest* | BHDgN1cv3 | Bsu36I | AscI |

**Construction oligonucleotides**

Acg4\_12\_F: GGCAATATATAGGAATGCACGTTTTAGAGCTAGAAATAGCAAGTTAAA

Acg4\_12\_R: AACACTCGGTATAAATTGGTTTATGCACCAGCCGGGAATCG

Acg4\_23\_F: GCATAAACCAATTTATACCGAGTGTTTTAGAGCTAGAAATAGCAAGTTAAA

Acg4\_23\_R: AACTCCCCGCAAGTTCTGTCCCTTGCACCAGCCGGGAATCG

Acg4\_34\_F: GCAAGGGACAGAACTTGCGGGGAGTTTTAGAGCTAGAAATAGCAAGTTAAA

Acg4\_34\_R: GGTGGTCTCCGTTTTCCACTTGCACCAGCCGGGAATCG

Acg4\_41\_F: GTGCAAGTGGAAAACGGAGACCACCGTTTTAGAGCTAGAAATAGCAAGTTAAA

Acg4\_41\_R: AAAACGTGCATTCCTATATATTGCCTGCATCGGCCGGGAATCG

acN\_F: CAAACTCATCAATGTATCTTAACCGGTAGGAGCAAGCTGCCCGTGCCCTGGCCCACCCTC

acN\_R: GAGGGTGGGCCAGGGCACGGGCAGCTTGCTCCTACCGGTTAAGATACATTGATGAGTTTG

Cas9HF1\_F: CACCTGGGCGAACTGCACGCTATCCTCAGGAGGCAGGAGGATTTTTATCCGT

Cas9HF1\_R: ACCACTGCATTCAGGTAGGCATCATGCGCATGGTGGTAGTTATTTATCTCCCTAACTTT

FACacR\_F: CTAAACAATCGGCTCGAAGC

FACacR\_R: GTAACCATTATAAGCTGCAATAAACAA

gRNA\_f\_R: GAGGGTGGGCCAGGGCACGGGCAGCTTGCTCTAGAATGCATACGCATTAAGCGAACA

HF1\_F: GGTGGTGTCGAAGTACTTGAAG

HF1\_R: AGATTCACCTGGGCGAACTG

U6\_3\_gRNA1\_v4\_F: GTCCAAACTCATCAATGTATCTTAACCGGTAGGCCTTTTTTTGCTCACCTGTGATTGCTC

**Sequencing oligonucleotides**

AutoDLeft\_S2\_F: CTTACGCTGAAGCCATTTCAA

AutoDRight\_S2\_R: ATCTGGTTCTCACTTCCATTTAAAT

Cas9\_HF1\_S1\_R: GGACTTTCTTGTCATCCATGCG

Cas9HF1\_S\_R: CTACCCCGGAGATCTCGACAG

Cas9mid\_S\_F: CGACCAGTACGCAGACCTTTT

DsRed\_S\_F: CTGAAGGGCGAGATCCACAAG

EGFP\_S\_R: AGTTGTACTCCAGCTTGTGCC

pCFD5\_S\_R: ACGTCAACGGAAAACCATTGTCTA

AutoC\_S2\_F: TCAAGCCATGTGACCCAGATT

EGFPaLeft\_S\_R: GCTTGTTTATTTGCTTAGCTTTCGC