**SUPPLEMENTARY INFORMATION**

The following tables show the DNA fragments used for Gibson Assembly of each plasmid and the sequences of DNA oligos. PCR products are shown with the oligonucleotide primer pair used, and plasmid digests are shown with the restriction enzymes used.

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| **p3xP3-dsRedv2** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | 3xP3-dsRed | pBB2\_r\_F | pBB2\_r\_R |
| *PCR Product* | g3xP3v2 | 3xP3v2\_F | 3xP3v2\_R |
| *PCR Product* | 3xP3-dsRed | dsRed\_F | dsRed\_R |
| *PCR Product* | pJFRC81 | P10\_F | P10\_R |

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| **BHDgg1** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | none | GFPa\_gRNA1\_F | GFPa\_gRNA1\_R |
| *PCR Product* | pCFD3 | CFD\_1\_F | CFD\_1\_R |
| *PCR Product* | pCFD3 | CFD\_2\_F | CFD35\_2\_R |

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| **BHDrg1** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | none | Rosy\_gRNA1\_F | Rosy\_gRNA1\_R |
| *PCR Product* | pCFD3 | CFD\_1\_F | CFD\_1\_R |
| *PCR Product* | pCFD3 | CFD\_2\_F | CFD35\_2\_R |

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| --- | --- | --- | --- |
| **BHDaag1** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | none | Auto\_gRNA1\_F | Auto\_gRNA1\_R |
| *PCR Product* | pCFD3 | CFD\_1\_F | CFD\_1\_R |
| *PCR Product* | pCFD3 | CFD\_2\_F | CFD35\_2\_R |

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| --- | --- | --- | --- |
| **BHDabg1** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | pCFD3 | CFD\_1autoB\_F | CFD\_1\_R |
| *PCR Product* | pCFD3 | CFD\_2\_F | CFD\_2autoB\_R |

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| --- | --- | --- | --- |
| **BHDaeg1** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | none | AutoE\_gRNA\_F | AutoE\_gRNA\_R |
| *PCR Product* | pCFD3 | CFD\_1\_F | CFD\_1\_R |
| *PCR Product* | pCFD3 | CFD\_2\_F | CFD35\_2\_R |

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| --- | --- | --- | --- |
| **BHDxyg1** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | none | Xy\_gRNA\_F | Xy\_gRNA\_R |
| *PCR Product* | pCFD3 | CFD\_1\_F | CFD\_1\_R |
| *PCR Product* | pCFD3 | CFD\_2\_F | CFD35\_2\_R |

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| --- | --- | --- | --- |
| **BHDrN1i1** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | Genomic | RosyLeftN\_F | RosyLeftN\_R |
| *Plasmid Digest* | IHDyN1 | AvrII | SphI |

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| --- | --- | --- | --- |
| **BHDaai1** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | Genomic DNA | AutoLeft\_F | AutoLeft\_R |
| *Plasmid Digest* | 3xP3-EGFP | XmaI | EcoRI |

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| --- | --- | --- | --- |
| **BHDgN1ai1** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | BHDaa | GFPaLeft\_F | GFPaLeft\_R |
| *Plasmid Digest* | BHDrN1 | KpnI | XbaI |

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| --- | --- | --- | --- |
| **BHDgN1bi1** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | ATSabG | EGFPabLeft\_F | EGFPabLeft\_R |
| *PCR Product* | BHDgN1a | Cas9Nos3b\_F | Cas9Nos3\_R |
| *Plasmid Digest* | BHDgN1a | KpnI | AscI |

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| --- | --- | --- | --- |
| **BHDgN1ei1** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | ATSaeG | EGFPaeLeft\_F | EGFPgLeft\_R |
| *Plasmid Digest* | BHDgN1bv2 | KpnI | NheI |

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| **BHDgN1yi1** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | ATSxyG | EGFPxyLeft\_F | EGFPg2Left\_R |
| *Plasmid Digest* | BHDgN1bv2 | KpnI | NheI |

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| **ATSaaG** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | Genomic DNA | AutoRight\_F | AutoRight\_R |
| *Plasmid Digest* | BHDaai1 | SpeI | PstI |

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| **ATSabG** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | 3xP3-EGFP | pBB2\_b\_F | pBB2\_b\_R |
| *PCR Product* | Genomic | AutoB\_Left\_F | AutoB\_Left\_R |
| *PCR Product* | 3xP3-EGFP | EGFP\_b\_F | EGFP\_b\_R |
| *PCR Product* | Genomic | AutoB\_Right\_F | AutoB\_Right\_R |

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| --- | --- | --- | --- |
| **ATSaeG** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | 3xP3-EGFP | pBB2\_e\_F | pBB2\_e\_R |
| *PCR Product* | Genomic | AutoE\_Left\_F | AutoE\_Left\_R |
| *PCR Product* | 3xP3-EGFP | EGFP\_e\_F | EGFP\_e\_R |
| *PCR Product* | Genomic | AutoE\_Right\_F | AutoE\_Right\_R |

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| --- | --- | --- | --- |
| **ATSxyG** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | 3xP3-EGFP | pBB2\_y\_F | pBB2\_y\_R |
| *PCR Product* | Genomic | Xy\_Left\_F | Xy\_Left\_R |
| *PCR Product* | 3xP3-EGFP | EGFP\_y\_F | EGFP\_y\_R |
| *PCR Product* | Genomic | Xy\_Right\_F | Xy\_Right\_R |

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| **BHDaaN** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | pnos-Cas9-nos | NosCas9\_1b\_F | NosCas9\_1\_R |
| *PCR Product* | pnos-Cas9-nos | NosCas9\_2\_F | NosCas9\_2b\_R |
| *Plasmid Digest* | BHDaa | SphI | NotI |

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| **BHDrN1** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | BHDrg1 | U6\_3\_gRNA1\_F | Rosy\_U6\_3\_gRNA1\_R |
| *PCR Product* | Genomic DNA | RosyRight1\_F | RosyRight1N\_R |
| *Plasmid Digest* | BHDrNi1 | SpeI | DraIII |

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| **BHDgN1a** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | BHDgg1 | U6\_3\_gRNA1\_v2\_F | EGFPa\_U6\_3\_gRNA1\_R |
| *PCR Product* | BHDaa | EGFPaRight\_F | EGFPaRight\_R |
| *Plasmid Digest* | BHDgN1ai1 | SpeI | DraIII |

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| **BHDgN1b** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | BHDgg1 | U6\_3\_gRNA1\_v3\_F | EGFPb\_U6\_3\_gRNA1\_R |
| *PCR Product* | ATSabG | EGFPabRight\_F | EGFPabRight\_R |
| *Plasmid Digest* | BHDgN1bi1 | AgeI | DraIII |

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| **BHDgN1bv2** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | p3xP3-dsRedv2 | b1v2\_F | b1v2\_R |
| *Plasmid Digest* | BHDgN1b | NotI | MluI |

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| **BHDgN1e** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | ATSaeG | EGFPgRight\_F | EGFPaeRight\_R |
| *Plasmid Digest* | BHDgN1ei1 | XbaI | DraIII |

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| **BHDgN1e** | *Template* | *Oligo/Enzyme 1* | *Oligo/Enzyme 2* |
| *PCR Product* | ATSxyG | EGFPgRight\_F | EGFPxyRight\_R |
| *Plasmid Digest* | BHDgN1yi1 | XbaI | DraIII |

**Construction primers**

3xP3v2\_F: ATTCTGAACATTATCGCGAGCCGGATCTAATTC

3xP3v2\_R: CCTCGGAGGAGGCCATGCCTCGCTGCGGCTTC

Auto\_gRNA1\_F: TATATATAGACCTATTTTCAATTTAACGTCGAATCGATTAGCATCAACCA

Auto\_gRNA1\_R: ATTTTAACTTGCTATTTCTAGCTCTAAAACTGGTTGATGCTAATCGATTC

AutoB\_Left\_F: ACATTATCGCGAGCCCCCACATTCTCTCATCGCAGG

AutoB\_Left\_R: ATTAGATCCCGTACGGTGCGGATGCTGTTTGTTTGT

AutoB\_Right\_F: ATGTATCTTAACCGGAGGTTTCAAAGTGCAGCGATAAGA

AutoB\_Right\_R: CAGAAGGCCCCTGACATGAAGGTGGTCTCCAACGC

AutoE\_gRNA\_F: TATATATAGACCTATTTTCAATTTAACGTCGCAATTTAAAAGACAGCCGG

AutoE\_gRNA\_R: ATTTTAACTTGCTATTTCTAGCTCTAAAACCCGGCTGTCTTTTAAATTGC

AutoE\_Left\_F: ACATTATCGCGAGCCGAGTCCGGCCCAGGAATCGTC

AutoE\_Left\_R: ATTAGATCCCGTACGCGGCGGTCGCTTCTCCCA

AutoE\_Right\_F: ATGTATCTTAACCGGGCTGTCTTTTAAATTGCAGTGCC

AutoE\_Right\_R: CAGAAGGCCCCTGACCAGCAAAGACATGGTATCGCC

AutoLeft\_F: AAGGCTCAGTCGAAAGACTGGGCCTTTCGCCCGGTTGCGAACAAAACGCCCATCGAGGA

AutoLeft\_R: TGCATATGTCCGCGGCCGCTAGCATGCAAGAATTACCAGGGCTGCAAAGAGCGGG

AutoRight\_F: ATGCTATACGAAGTTATAGAAGAGCACTAGTTGATGCTAATCGATTCAATTCTGTAATGC

AutoRight\_R: TTGAACTCGATTGACGGAAGAGCCTCGAGCTGCATATGACGAAATCAAGGCTAAGGTCG

b1v2\_F: ATTTCGAGGTTAAAACGGTCGAAGCGCGGCCGCGGATCTAATTCAATTAGAGACTAATTC

b1v2\_R: GAGTAGGAGCAATCACAGGTGAGCAAAAAAACGCGTGTTAACTCGAATCGCTATCCA

Cas9Nos3\_R: TATCCACTTGTTTACTCTGACCAACT

Cas9Nos3b\_F: AGCTGACCCTGAGCTAGCTCCTTCCTGGCCCTTTTCGAG

CFD\_1\_F: GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG

CFD\_1\_R: GGCTATGCGTTGTTTGTTCTGC

CFD\_1autoB\_F: ACGTCGCTGCACTTTGAAACCTGTGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG

CFD\_2\_F: AACAGTAGGCAGAACAAACAACGC

CFD\_2autoB\_R: AAAACCACAGGTTTCAAAGTGCAGCGACGTTAAATTGAAAATAGGTCTATATATACGAA

CFD35\_2\_R: CGACGTTAAATTGAAAATAGGTCTATATATACG

dsRed\_F: AGCCGCAGCGAGGCATGGCCTCCTCCGAGGACGT

dsRed\_R: TTAAAAACGATTCATCTACAGGAACAGGTGGTGGCGG

EGFP\_b\_F: AAACAGCATCCGCACCGTACGGGATCTAATTCAATTAGAGACTAA

EGFP\_b\_R: TGCACTTTGAAACCTCCGGTTAAGATACATTGATGAGTTTGG

EGFP\_e\_F: GAGAAGCGACCGCCGCGTACGGGATCTAATTCAATTAGAGACTAA

EGFP\_e\_R: AATTTAAAAGACAGCCCGGTTAAGATACATTGATGAGTTTGG

EGFP\_y\_F: TACAATTTTCCTAATCGTACGGGATCTAATTCAATTAGAGACTAA

EGFP\_y\_R: AATACAAGTAAGGAACCGGTTAAGATACATTGATGAGTTTGG

EGFPa\_U6\_3\_gRNA1\_R: TGGTGCAGATGAACTATGCATACGCATTAAGCGAACA

EGFPabLeft\_F: ATTAACCAATTCTGAACATTATCGCCTAGGGTACCCCCACATTCTCTCATCGCAGG

EGFPabLeft\_R: GGCCAGGAAGGAGCTAGCTCAGGGTCAGCTTGCCGTA

EGFPabRight\_F: ATGCGTATGCATTCTAGAAGTTCATCTGCACCACCGGC

EGFPabRight\_R: TGATTGACGGAAGAGCCTCGAGCTGCACACACAGTGATGAAGGTGGTCTCCAACGCCT

EGFPaeLeft\_F: ATTAACCAATTCTGAACATTATCGCCTAGGGTACCGAGTCCGGCCCAGGAATCGTC

EGFPaeRight\_R: TGATTGACGGAAGAGCCTCGAGCTGCACACACAGTGCAGCAAAGACATGGTATCGCC

EGFPaRight\_F: TTAATGCGTATGCATAGTTCATCTGCACCACCGGCA

EGFPaRight\_R: GACGGAAGAGCCTCGAGCTGCACACACAGTGTGAGCATAACCAAGGTCACGAAAGGT

EGFPb\_U6\_3\_gRNA1\_R: TGCAGATGAACTTCTAGAATGCATACGCATTAAGCGAACA

EGFPg2Left\_R: CGGCGTTTCTCGAAAAGGGCCAGGAAGGAGCTAGCTCAGGGTCAGCTTGCCGTAG

EGFPgLeft\_R: CGGCGTTTCTCGAAAAGGGCCAGGAAGGAGCTAGCTCAGGGTCAGCTTGCCGTAGGT

EGFPgRight\_F: TTTTTAATGTTCGCTTAATGCGTATGCATTCTAGAAGTTCATCTGCACCACCGG

EGFPxyLeft\_F: TAACCAATTCTGAACATTATCGCCTAGGGTACCTTTCTTGCTGTCTGACTTGGATTATTC

EGFPxyRight\_R: GATTGACGGAAGAGCCTCGAGCTGCACACACAGTGCAAATTAGCCACACGTAGGATATTC

GFPa\_gRNA1\_F: TATATATAGACCTATTTTCAATTTAACGTCGGTGGTGCAGATGAACTTCA

GFPa\_gRNA1\_R: ATTTTAACTTGCTATTTCTAGCTCTAAAACTGAAGTTCATCTGCACCACC

GFPaLeft\_F: ATTAACCAATTCTGAACATTATCGCCTAGGGTACCGCGCAGCGCCAATGATAGGTACAAT

GFPaLeft\_R: TTTCTCGAAAAGGGCCAGGAAGGAGCATGTCTAGATCAGGGTCAGCTTGCCGTAGGTG

P10\_F: CACCTGTTCCTGTAGATGAATCGTTTTTAAAATAACAAATCAATT

P10\_R: CAGAAGGCCCCTGACGTTAACTCGAATCGCTATCCA

pBB2\_b\_F: GGAGACCACCTTCATGTCAGGGGCCTTCTGCTTAGT

pBB2\_b\_R: ATGAGAGAATGTGGGGGCTCGCGATAATGTTCAGAATTG

pBB2\_e\_F: ACCATGTCTTTGCTGGTCAGGGGCCTTCTGCTTAGT

pBB2\_e\_R: TCCTGGGCCGGACTCGGCTCGCGATAATGTTCAGAATTG

pBB2\_r\_F: GCGATTCGAGTTAACGTCAGGGGCCTTCTGCTTAGT

pBB2\_r\_R: TTAGATCCGGCTCGCGATAATGTTCAGAATTG

pBB2\_y\_F: CGTGTGGCTAATTTGGTCAGGGGCCTTCTGCTTAGT

pBB2\_y\_R: TCAGACAGCAAGAAAGGCTCGCGATAATGTTCAGAATTG

Rosy\_gRNA1\_F: TATATATAGACCTATTTTCAATTTAACGTCGATATGCTTCACTACTTTGC

Rosy\_gRNA1\_R: ATTTTAACTTGCTATTTCTAGCTCTAAAACGCAAAGTAGTGAAGCATATC

Rosy\_U6\_3\_gRNA1\_R: TGCCGGCAACGCGTCCTGCAGGATGCATACGCATTAAGCGAACATT

RosyLeftN\_F: ATTAACCAATTCTGAACATTATCGCCTAGGGTACCTTATTCCAGGTGACCGAAGTGTCG

RosyLeftN\_R: TTTCTCGAAAAGGGCCAGGAAGGAGCATGTCTAGAAAGTAGTGAAGCATATCCACGGTGC

RosyRight1\_F: GTATGCATCCTGCAGGACGCGTTGCCGGCAAGCAGATCCGCAA

RosyRight1N\_R: GATTGACGGAAGAGCCTCGAGCTGCACACACAGTGTGGCGCCCACTATCTGACCATAGCA

U6\_3\_gRNA1\_F: ATGCTATACGAAGTTATAGAAGAGCACTAGGCTAGCTTTTTTGCTCACCTGTGATTGCTC

U6\_3\_gRNA1\_v2\_F: GTATGCTATACGAAGTTATAGAAGAGCACTAGTTTTTTTGCTCACCTGTGATTGCTC

U6\_3\_gRNA1\_v3\_F: GTCCAAACTCATCAATGTATCTTAACCGGTACGCGTTTTTTTGCTCACCTGTGATTGCTC

Xy\_gRNA\_F: TATATATAGACCTATTTTCAATTTAACGTCGCAATACAAGTAAGGAAATTG

Xy\_gRNA\_R: ATTTTAACTTGCTATTTCTAGCTCTAAAACAATTTCCTTACTTGTATTGCG

Xy\_Left\_F: ACATTATCGCGAGCCTTTCTTGCTGTCTGACTTGGA

Xy\_Left\_R: ATTAGATCCCGTACGATTAGGAAAATTGTAAAAAAAACACGATG

Xy\_Right\_F: ATGTATCTTAACCGGTTCCTTACTTGTATTGCCTACTTT

Xy\_Right\_R: CAGAAGGCCCCTGACCAAATTAGCCACACGTAGGAT

**Sequencing primers**

AutoB\_Left\_S\_F: ATGACCTTTCGTGACCTTGG

AutoB\_Left\_S\_R: ACGACCAATTGAACTCAGACC

AutoB\_Right\_S\_F: ACATCGTTCCTTTGGGATTG

AutoB\_Right\_S\_R: AAAACTTGTGCATCGAATGAAG

AutoE\_Left\_S\_F: GTGACTTTGAATGTCAGAATATCAACT

AutoE\_Left\_S\_R: CGGCCATCTCACACATTGTTAC

AutoE\_Right\_S\_F: GTACATTTGTGTGGCTGGTCC

AutoE\_Right\_S\_R: TCGTCTATTTTCGATTTGGGCAC

AutoLeft\_S\_F: GCAGCGCCAATGATAGGTACA

AutoLeft\_S\_R: TTGCATGTCGGCATACTTCGG

AutoRight\_S\_F: TGCATATCTCAGCAAGTCCGC

AutoRight\_S\_R: GTTGAATAAAGTATCGCCCTGTTAC

EGFP\_S\_F: AGCGCACCATCTTCTTCAAGG

EGFP\_S\_R: AGTTGTACTCCAGCTTGTGCC

EGFPaLeft\_S\_F: ATCGATTTCGAACCCTCGACC

EGFPaLeft\_S\_R: GCTTGTTTATTTGCTTAGCTTTCGC

EGFPaRight\_S\_F: CCAGGAGCGCACCATCTTCTT

EGFPaRight\_S\_R: GTCCTCCTTGAAGTCGATGCC

Xy\_Left\_S\_F: GGGTTTCGCATCTTCTGGATC

Xy\_Left\_S\_R: GCTCTGACAACCCCAACAGAA

Xy\_Right\_S\_F: TGCATACTCTTGGTCACAGCA

Xy\_Right\_S\_R: AGCTCTGTTTCAAGAACATTTCCG

**gBlock**

g3xP3v2:

CATTATCGCGAGCCGGATCTAATTCAATTAGAGACTAATTCAATTAGAGCTAATTCAATTAGGCTAAGCCGTGTATAGAGAACAACCACACCCCGCCGGAGTATAAATAGAGGCGCTTCGTCTACGGAGCGACAATTTAATTCAAACAAGCAAAGTGAACACGTCGCTAAGCGAAAGCTAAGCAAATAAACAAGCGCAGCTGAACAAGCTAAACAATCGGTCCGAAGCCGCAGCGAGGCAT