

| Name | Primers | Sequence (5' -> 3') | Key Features | Key Restriction Sites |
|--------------------------------|---------|--|--|--|
| gRFP-2 | | GGTGAAGGTAGACCATATGA | RFP guide RNA | |
| gLEU2-1 | | TGGCAGCACCACCAATCAAG | LEU2 gRNA | |
| P _{ADH} HHgRFP HDV | | <p> GTCGACATGTCAAAGGATTCAACGGTTGAGATGGAGCCGT CAAATATCCAAAACACTCTTCTTTCAACTAATTTGCCGTAAA CTATCTCCATCGTACGATTAAGATAAAAAAAAAACAATTATTT CTTACAATATTTGATAGAGACCCAATGCAAAGCCAGCGAG AAAGAGGAGTATTGGCATTGTTGGGTAGTAGATGAATGAG TAATCTTCTAGCCAAAACAAAATAAACCAACAAGAACAGTAG GCAAACTGAGTACTTGATAGCGCTTGTAGTCTTTGTATTT AAAAAACCTAATTGTCCAAAATTGAAAATCATGAGTTGC AAATTTGTTGAAATCACCAGGTAATAGATCATTTAGCTATA TCGCCTTATCATTCTTCGTACAAGAATTATACACAAATACC ACCACCACACCACCAGAAATTTTACACGTGTTTTCTAAAT TATTACTTTCCGTGGCCAATCAAATTGAAATTTTCTTGTTT GAAACGGCAGTGCCTCAAACATTATAGTAGCAAGTATTGC CTAAAGAGTTTCCGTCTCGTGCCCCCCCCCTATCCCCTTTT GTAATTTGCCAAAATAGCTCAAAAAGGATAATCCCAGCACTT TTTTTTTCCGAGTTCTCGTCATGCAAGCAAGCTTATTCAGA ATTTTCAGAGGTGCGAGTCTCACCCTACCCTGCAGCTG CATCTTTTACAGCAACAGCAACAGCAGGTGCCACCACGGC AAAGACATTGTCTGGATCCACTGTGCTCCGAAAACGTATA AAAGGACCTATTCATCCCTGGTCTTATCTTCTCCAGAATTA TTTTTTTTTTCATCAGTTTAAACAACAACAACGTTATTGTCAT ACAACAACAACAACAATAACAAAACAATTagatcTTTACCCct gatgagtcccgtgaggacgaaacgagtaagctcgtcGGTGAAGGTAGACCA TATGAgttttagagctagaaatagcAagttaaataaggctagtccgttatcaactg aaaagtggcaccgagtcggtgcttttGCCGGCATGGTCCCAGCCTC CTCGCTGGCGCCGGCTGGGCAACATGCTTCGGCATGGCG AATGGGACacgctg </p> | <p> Red: <i>ADH1</i> Promoter; Green: Hammer-head; Blue: 20bp gRFP; Purple: Cas9 binding Motif; Orange: HDV </p> | <p> <i>Sall</i>, <i>BglII</i>, <i>MluI</i> </p> |

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| <p>P_{ADHt}AgRFP HDV</p> | | <p>GTCGACATGTCAAAGGATTCAACGGTTGAGATGGAGCCGT CAAATATCCAAAACACTCTTCTTTCAACTAATTTGCCGTA CTATCTCCATCGTACGATTAAGATAAAAAAAAAACAATTTT CTTACAATATTTGATAGAGACCCAATGCAAAGCCAGCGAG AAAGAGGAGTATTGGCATTGTTGGGTAGTAGATGAATGAG TAATCTTCTAGCCAAAACAAAATAAACCAACAAGAACAGTAG GCAAAACTGAGTACTTGATAGCGCTTGTAGTCTTTGTATTT AAAAAACCTAATTGTCCAAAAATTGAAAATCATGAGTTGC AAATTTGTTGAAATCACCAGGTAATAGATCATTAGCTATA TCGCCTTATCATTCTTCGTACAAGAATTATACACAAATACC ACCACCACACCACCAGAAATTTTACACGTGTTTTCTAAAT TATTACTTTCCGTGGCCAATCAAATTGAAATTTTTCTTGTT GAAACGGCAGTGCCTCAACATTATAGTAGCAAGTATTGC CTAAAGAGTTTCCGTCTCGTGCCCCCCCCCTATCCCCTTTT GTAATTTGCCAAAATAGCTCAAAAAGGATAATCCCGCACTT TTTTTTCCGAGTTCTCGTCATGCAAGCAAGCTTATTCAGA ATTTTCAGAGGTGCGAGTCTCACCCTACCCTGCAGCTG CATCTTTTACAGCAACAGCAACAGCAGGTGCCACCACGGC AAAGACATTGTCTGGATCCACTGTGCTCCGAAAACGTATA AAAGGACCTATTCATCCCTGGTCTTATCTTCTCCAGAATTA TTTTTTTTTTCATCAGTTTAAACAACAACAACGTTATTGTCAT ACAACAACAACAACAATACAAACAACAAGAGCTCAAACCG GGCGTGTGGCGTAGTTGGTAGCGCGTTCCCTTAGCATGG GAAAGGTCATGAGTTCGACTCTTATCTCGTCCAGGTGAAG BTAGACCATATGAGTTTTAGAGCTAGAAATAGCAAGTTAAA ATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGA GTCGGTGCTTTTGGCCGGCATGGTCCCAGCCTCCTCGCT GGCGCCGGCTGGGCAACATGCTTCGGCATGGCGAATGGG ACACGCGT</p> | <p>Red: <i>ADH1</i> Promoter; Green: tRNA^{Ala}; Blue: 20bp gRFP; Purple: Cas9 binding Motif; Orange: HDV</p> | <p><i>Sall</i>, <i>SacI</i>, <i>MluI</i></p> |
| <p>PtAgRFPHD V</p> | | <p>gtcgacgctctccctatgcgactcctgcattaggaagcagcccagtagtaggtgagg ccggtgagcaccgccgcccgaaggaatggtgcatgcaaggagatggcgccaaca gtccccggccacgggctgccaccatacccacgcccgaacaagcgctcatgag cccgaagtggcgagcccgatctcccatcggatgctggcgatataggcgccagc aaccgcacctgtggcgccggtgatccggccacgatgctccggcgtagaggatca gcgccGATCTcaaacGGCGTGTGGCGTAGTTGGTAGCGC GTTCCCTTAGCATGGAAAGGTCATGAGTTCGACTCTTAT</p> | <p>Grey: YPB vector; Green: tRNA^{Ala}; Blue: 20bp gRFP; Purple: Cas9 binding Motif; Orange: HDV</p> | <p><i>Sall</i>, <i>MluI</i></p> |

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| | | <p>CTCGTCCAGGTGAAGGTAGACCATATGAGTTTTAGAGCTA GAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCAGTCGGTGCCTTTGGCCGGCATGGT CCCAGCCTCCTCGCTGGCGCCGGCTGGGCAACATGCTTC GGCATGGCGAATGGGACACGCGT</p> | | |
| P _{SNR52} gRFP | 984/1002 | <p>gtcgacAAGTGATTAGACTTAGTCCGTTCAAATCAAGCACAA CTCTGTTCAATTGTTTCAACAAGAATTAATTCAAAAACAGGTT CGGTGCATAATTTGCAAAAAAATATTGCAGCTTCTGTGGCT CGAACACAGTACCTCCAGATTTTCAGGTTTGAATACTTCAG TCTGACGCTCTCCAGATGAGCTAAAGCTGCAATAAGAAA ACCCACGCCGGATTGCAACCCGGAATCCTTTGATTAGAA GTCAAAAGCGATAACCATTTGCCACGCAGGCCTACTTGA TGGGTTTGTAATGGTCTACTTTTTTCAGACCTAACAGAAAT TTAATGAAAGTCATATTCTTATACAATAAACTGTGTCATA AAAGCAGATATTCGACTTTCTAGATTATATAGGACCCAAG AACTAAAATTTAATGCCATATTATGCATTTTTAATCTGTA AGTGTGTTTCCAACCTATCACAAGTACGTTCTTGTA GTGTTTGTAGGGTTGCAAAATGAATCATAACAACATCTCAAC AGAACATGTATAGCAAAGCTTAGTATAAAATCAGTGT AGAGGCAATCCAAGAATGTTTACATCAAAGTTTCAATAAAT ATCGACCGAAACTGAAAATCTTTTTAGGTTATTGTTCACTTT TTTGTAATATTTAAACATTTTTGGACCTAAAAAATA ACACCAATTACGTACCAAGAAGCATCTAATCAACTCCCAGA TCACCACTATACATTTAAAAGTCATTGGTCAATAACTATACT CGAGTATTGCCTCATCAAAGAAACAATCAAATATTATAGAT ACTCACTCCATCACGTGATAATTTCACTGGTATGGAAAAGT GGAAAATTTTATAAAAAAATTTGATGCCTTTGGCATAGC TGAAACTTCGGCCCAATAGGATTGGAGAATATGTTTTCGCA GCGTTCTTACAATTAATTGTGGTGGAAAGTTCGAGACTTGC GTAAACTATTTTTAATTTgGTGAAGGTAGACCATATGAgtttag agctagaaatagcAagttaaaataaggctagccgttatcaactgaaaaagtgga ccgagtcggtgctttacgct</p> | <p>Red: SNR52 Promoter; Blue: 20bp gRFP; Purple: Cas9 binding motif</p> | Sall, MluI |
| P _{ADH1A} "SapI cassette"HDV | | <p>GTGACATGTCAAAGGATTCAACGGTTGAGATGGAGCCGT CAAATATCCAAAACACTCTTCTTTCAACTAATTTGCCGTAAA CTATCTCCATCGTACGATTAAGATAAAAAAACAATTATT</p> | <p>Red:ADH1 Promoter; Green tRNA^{Ala} ; Blue: SapI Cassette; Purple:</p> | Sall, SacI, SapI, ClaI, |

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| | | <p>CTTACAATATTTGATAGAGACCCAATGCAAAGCCAGCGAG AAAGAGGAGTATTGGCATTGTTGGGTAGTAGATGAATGAG TAATCTTCTAGCCAAAACAAAATAAACCAAGAAGCAGTAG GCAAAACTGAGTACTTGATAGCGCTTGTAGTCTTTGTATTT AAAAAACCTAATTGTCCAAAATTGAAAATCATGAGTTGC AAATTTGTTGAAATCACCAGGTAAATAGATCATTTAGCTATA TCGCCTTATCATTCTTCGTACAAGAATTATACACAAATACC ACCACCACACCACCAGAAATTTTACACGTGTTTTCTAAAT TATTACTTTCCGTGGCCAATCAAATTGAAATTTTCTTGTT GAAACGGCAGTGCCTCAACATTATAGTAGCAAGTATTGC CTAAAGAGTTTCCGTCTCGTGCCCCCCCCCTATCCCCTTTT GTAATTTGCCAAAATAGCTCAAAAAGGATAATCCCGCACTT TTTTTTCCGAGTTCTCGTCATGCAAGCAAGCTTATTCAGA ATTTTCAGAGGTGCGAGTCTCACCCTACCCTGCAGCTG CATCTTTTACAGCAACAGCAACAGCAGGTGCCACCACGGC AAAGACATTGTCTGGATCCACTGTGCTCCGAAAACGTATA AAAGGACCTATTCATCCCTGGTCTTATCTTCTCCAGAATTA TTTTTTTTTTCATCAGTTTAAACAACAACAAACGTTATTGTCAT ACAACAACAACAACAATACAAACAACAAGAGCTCAAAACG GGCGTGTGGCGTAGTTGGTAGCGGTTCCCTTAGCATGG GAAAGGTCATGAGTTCGACTCTTATCTCGTCCAGGAAGAG CATCGATGCTCTTCCGTTTTAGAGCTAGAAATAGCAAGTTA AAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACC GAGTCGGTGTCTTTGGCCGGCATGGTCCCAGCCTCCTCG CTGGCGCCGGCTGGGCAACATGCTTCGGCATGGCGAATG GGAC</p> | <p>Cas9 binding Motif; Orange: HDV</p> | <p><i>SapI</i>,</p> |
| <p>P_{ADH}RFP</p> | <p>701/629</p> | <p>GTGCTCGGTACCGTCGACATGTCAAAGGATTCAACGGTTG AGATGGAGCCGTCAAATATCCAAAACACTCTTCTTTCAACT AATTTGCCGTAAACTATCTCCATCGTACGATTAAGATAAAA AAAAACAATTATTTCTTACAATATTTGATAGAGACCCAATGC AAAGCCAGCGAGAAAGAGGAGTATTGGCATTGTTGGGTAG TAGATGAATGAGTAATCTTCTAGCCAAAACAAAATAAACCA CAAGAACAGTAGGCAAACTGAGTACTTGATAGCGCTTGT AGTCTTTGTATTTAAAAAACCTAATTGTCCAAAATTGAAA ATCATGAGTTGCAAATTTGTTGAAATCACCAGGTAAATAGA TCATTTAGCTATATCGCCTTATCATTCTTCGTACAAGAATTA</p> | | <p><i>KpnI, BglII,</i> <i>MluI</i></p> |

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| | | <p>TACACAAATACCACCACCACACCACCCAGAAATTTTACACG TGTTTTCTAAATTACTTTCCGTGGCCAATCAAATTGAAA TTTTTCTTGTTCGAAACGGCAGTGCCTCAAACATTATAGTA GCAAGTATTGCCTAAAGAGTTTCCGTCTCGTGCCCCCCCC CTATCCCCTTTTGTAAATTTGCCAAAATAGCTCAAAAAGGAT AATCCCGCACTTTTTTTTTCCGAGTTCTCGTCATGCAAGCA AGCTTATTCAGAATTTTCAGAGGTGCGAGTCTCACCCTAC CACTGCAGCTGCATCTTTTACAGCAACAGCAACAGCAGGT GCCACCACGGCAAAGACATTGTCTGGATCCACTGTGCTCC GAAAAGTGTATAAAAGGACCTATTCATCCCTGGTCTTATCT TCTCCAGAATTATTTTTTTTTTTCATCAGTTTAACAACAACAAA CGTTATTGTCATACAACAACAACAATAACAAAAACAAT Tagatctatggtttcaaaaggtgaagaagataatggctattataaagaattatgag atftaaagttcatatggaaggttcagttaatggctatgaattgaaattgaaggtgaaggt gaaggtagaccatatgaaggtactcaaactgctaaattgaaagttactaaaggtggtc cattaccattgctgggatattttgcaccacaattatgtatggttcaaaagcttatgttaa acatccagctgatattccagattatttaaattgtcattccagaaggtttaaatgggaaa gagttatgaatttgaagatgggtggtgttactgttactcaagattcatcattacaagatg gtgaattattataaagttaaattgagaggtactaatttccatcagatggtccagttatgc aaaaaaaaactatgggtgggaagcttcatcagaaagaatgatccagaagatgggtg ctftaaaggtgaaattaaacaaagattgaaattaaaagatgggtggtcattatgatgctg aagttaaaactactataaagctaaaaaccagttcaattaccaggtgcttataatgta atattaaattggatattacttcacataatgaagattatactattgttgaacaatatgaaaga gctgaaggttagacattcaactgggtgatggatgaattatataaataaACGCGT</p> | | |
| P _{ADH} RFP "Full Length" | 920/629 | <p>GCTTATTCAGAATTTTTCAGAGGTGCGAGTCTCACCCTACC ACTGCAGCTGCATCTTTTACAGCAACAGCAACAGCAGGTG CCACCACGGCAAAGACATTGTCTGGATCCACTGTGCTCCG AAAAGTGTATAAAAGGACCTATTCATCCCTGGTCTTATCTT CTCCAGAATTATTTTTTTTTTTCATCAGTTTAACAACAACAAC GTTATTGTCATACAACAACAACAATAACAAAAACAATTa gatctatggtttcaaaaggtgaagaagataatggctattataaagaattatgagatt taaagttcatatggaaggttcagttaatggctatgaattgaaattgaaggtgaaggtga aggttagaccatatgaaggtactcaaactgctaaattgaaagttactaaaggtggtccat taccattgctgggatattttgcaccacaattatgtatggttcaaaagcttatgttaaaca tccagctgatattccagattatttaaattgtcattccagaaggtttaaatgggaaaga gttatgaatttgaagatgggtggtgttactgttactcaagattcatcattacaagatggt</p> | | <i>Bam</i> HI, <i>Mlu</i> I |

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| | | gaattattataaagttaaattgagaggtactaatttccatcagatggtccagttatgca aaaaaaaaactatgggtgggaagcttcatcagaaagaatgtatccagaagatggtg ttaaaaggtaaaataaacaagattgaaataaaagatggtggcattatgatgctg aagtaaaactactataaagctaaaaaccagttcaattaccaggtgcttataatgta atattaaattggatattacttcacataatgaagattatactattgttgaacaatatgaaaga gctgaaggtagacattcaactggtggtatggatgaattatataaataaACGCGT | | |
| RFP fusion fragment-top | 920/954 | GCTTATTCAGAATTTTCAGAGGTGCGAGTCTCACCACTACC ACTGCAGCTGCATCTTTTACAGCAACAGCAACAGCAGGTG CCACCACGGCAAAGACATTGTCTGGATCCACTGTGCTCCG AAAAGTGTATAAAAGGACCTATTCATCCCTGGTCTTATCTT CTCCAGAATTATTTTTTTTTCATCAGTTTAACAACAACAAC GTTATTGTCATACAACAACAACAATAACAAAAACAATTa gatctatggtttcaaaaggtaagaagataatggcCCATCAGATGGTCCA GTT | Red: sequence homology between oligonucleotides | |
| RFP fusion fragment bottom | 953/629 | GGCCCTACGCGTtatttatataattcatccataaccaccagttgaatgtctacctc agctcttcatattgttcaacaatagataatcttcattatgtaagtaataccaatttaatat taacattataagcacctgtaattgaactggttttagctttataagtagtttaactcagc atcataatgaccacatcttttaattcaatcttgttaattcaccttttaagcaccatctc tgatacattcttctgatgaagctccaaccatagttttttgcataactggaccatct gatgg | Red: sequence homology between oligonucleotides | |
| RFP Healing Fragment | 920/954 | GCTTATTCAGAATTTTCAGAGGTGCGAGTCTCACCACTACC ACTGCAGCTGCATCTTTTACAGCAACAGCAACAGCAGGTG CCACCACGGCAAAGACATTGTCTGGATCCACTGTGCTCCG AAAAGTGTATAAAAGGACCTATTCATCCCTGGTCTTATCTT CTCCAGAATTATTTTTTTTTCATCAGTTTAACAACAACAAC GTTATTGTCATACAACAACAACAATAACAAAAACAATTa gatctatggtttcaaaaggtaagaagataatggccatcagatggtccagttatgc aaaaaaaaactatgggtgggaagcttcatcagaaagaatgtatccagaagatggtg ctttaaaggtaaaataaacaagattgaaataaaagatggtggcattatgatgctg aagtaaaactactataaagctaaaaaccagttcaattaccaggtgcttataatgta atattaaattggatattacttcacataatgaagattatactattgttgaacaatatgaaaga gctgaaggtagacattcaactggtggtatggatgaattatataaataaACGCGTA GGCC | Red: sequence homology between oligonucleotides | |

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| <i>LEU2</i> healing oligo- top | 976 | ACTGTCTTGCCTGGTGACCATGTCGGTACCGAAATTGTCA ATGAAGCgaattcCTGTTGA | Purple, Red: homologous sequence. Blue- <i>EcoRI</i> site | |
| <i>LEU2</i> healing oligo-bottom | 977 | AGCCATAAATGCTGCCATACGAGTGATACGAGTAACTTCG TCAACAGgaattcGCTTCAT | Purple, Red: homologous sequence. Blue- <i>EcoRI</i> site | <i>EcoRI</i> |
| <i>LEU2</i> healing fragment | 976+977 | ACTGTCTTGCCTGGTGACCATGTCGGTACCGAAATTGTCA ATGAAGCGAATTCCTGTTGACGAAGTTACTCGTATCACTCG TATGGCAGCATTATGGCT | Blue- <i>EcoRI</i> site | <i>EcoRI</i> |