Supplementary Table 3: Primers and templates for GG toolkit generation

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Construct** | **Primer** | **Primer Sequence 5'- 3'** | **Template** | **Comment** |
|  |  |  |  |  |
| **LI backbone and dummies** |  |  |  |  |
| pUC57 (Gent) + Esp3I | GentR BspHI + | GGTCATGAGACGCACACCGTGGAAACGG | pBBR-5-MCS [[1](#_ENREF_1)] | replaced *bla* resistance in pUC57 |
| GentR BspHI - | CCTCATGAGCGGCGTTGTGACAATTTACCG | pBBR-5-MCS [[1](#_ENREF_1)] |
| pUC57 (Gent) | Gentmut + | GGTCACAGCTTGTCTGTAAGC | pUC57 (Gent) + Esp3I | Esp3I site removal |
| Gentmut - | TTCTCCGGGAGCTGCAT | pUC57 (Gent) + Esp3I |
| pENTR-BsaI | GW BsaI CACC + | CACCTGAGACCTGCGGCCCCTCGAGGG | pENTR-D/TOPO (Invitrogen) | Introductio of BsaI sites and internal BsaI site removal |
| GW BsaI AAGG - | TGAGACCAGGTCGACCTGCAGAC | pENTR-D/TOPO (Invitrogen) |
| BsaI-del + | CAGTGTGCCGGTATCCGTTATCGGGGAAG | pENTR-D/TOPO (Invitrogen) |
| BsaI-del - | CTTCCCCGATAACGGATACCGGCACACTG | pENTR-D/TOPO (Invitrogen) |
| LI+Bpi | pUC+BpiI fw | TACGAAGTCTTCACAAGTTTGTACAAAAAAGCTGAACG | pENTR-BsaI | ligation into pUC57 (Gent) |
| pUC+BpiI rv | CAGAATGTCTTCCCAACCACTTTGTACAAGAAAGCTGAACG | pENTR-BsaI | ligation into pUC57 (Gent) |
| LI dy A-B | LI A-B dy + | ATGGTCTCAGCGGAACATCTGAGAGACCTGCAAGCTTGGCGTAATCAT | pUC57 (Gent) | amplified with LI dy - |
| LI dy B-C | LI B-C dy + | TTTGGTCTCTTCTGGAAGCACCTGAGACCGGGCCCGTCGACTGCAGAGG | pUC57 (Gent) | amplified with LI dy - |
| LI dy C-D | LI C-D dy + | TTTGGTCTCTCACCTGACCTAAGCTAGCCTAGAAGGTGAGACCGGGCCCGTCGACTGCAGAGG | pUC57 (Gent) | amplified with LI dy - |
| LI dy D-E | LI D-E dy + | TAGGTCTCTAAGGGCTAAGACCTAAGCTAGCCTAGAATCTGAGACCTGCAAGCTTGGCGTAATCAT | pUC57 (Gent) | amplified with LI dy - |
| LI dy F-G | LI F-G dy + | TTTGGTCTCTTGAGTGACCTAAGCTAGCCTAGTGTCTGAGACCGGGCCCGTCGACTGCAGAGG | pUC57 (Gent) | amplified with LI dy - |
|   | LI dy - | GGGATCCGATATCTAGATGC | pUC57 (Gent) |   |
|  |  |  |  |  |
| **Expression Vector Backbones** |  |   |  |  |
| Stage 1: LIIIa and LIIIb (both obsolete) vector backbones, were assembled as shown below | pUB 1 + | TTTGGTCTC TGATAGAAGACTTTCTGTTTAAACTATCAGTGTTTGACAGGATATATTGG  | pUB-GW-HYG [[2](#_ENREF_2)] |   |
| pUB 1 - | AAAGGTCTCGAAGGCTGCACTGAACGTCAGA | pUB-GW-HYG [[2](#_ENREF_2)] |  |
| pUB 2 + | TTTGGTCTCCCCTTCTTCTGAAAACGACATGTC | pUB-GW-HYG [[2](#_ENREF_2)] |  |
| pUB 2 - | AAAGGTCTCGATGTGCGCGGGCGGC | pUB-GW-HYG [[2](#_ENREF_2)] |  |
| pUB 3 + | TTTGGTCTCCACATCTCAACCGTGCGGC | pUB-GW-HYG [[2](#_ENREF_2)] |  |
| pUB 3 - | AAAGGTCTCTCGCCAGCTCGTCGGTCAC | pUB-GW-HYG [[2](#_ENREF_2)] |  |
| pUB 4 + | TTTGGTCTCCGGCGAGGTGATCCGCTACG | pUB-GW-HYG [[2](#_ENREF_2)] |  |
| pUB 4 - | AAAGGTCTCAGAGTCCTTTTCGACCTTTTTCCCCTG | pUB-GW-HYG [[2](#_ENREF_2)] |  |
| pUB 5 + | TTTGGTCTCTACTCTTTCCTGTGGATAGCACGTAC | pUB-GW-HYG [[2](#_ENREF_2)] |  |
| pUB 5 - | AAAGGTCTCGACTGTCTCCGGGAGCTGC | pUB-GW-HYG [[2](#_ENREF_2)] |  |
| pUB 6 + | TTTGGTCTCACAGTCACAGCTTGTCTGTAAGC | pUB-GW-HYG [[2](#_ENREF_2)] |  |
| pUB 6 - | AAAGGTCTCATACTCCATTTAAAGATCCGCGCG | pUB-GW-HYG [[2](#_ENREF_2)] |  |
| pUB 7 + | TTTGGTCTCGAGTATCTTCTTCCCAGTTTTCGC | pUB-GW-HYG [[2](#_ENREF_2)] |  |
| pUB 7 - | AAAGGTCTCTGCGCGAAGACAACGTAGTCTAAGCGTCAATTTGTTTACACCAC | pUB-GW-HYG [[2](#_ENREF_2)] |  |
|  | ccdb +  | TTTGGTCTCTGCGCACAAGTTTGTACAAAAAAGCTGAA | pENTR-BsaI |  |
|  | ccdb - | AAAGGTCTCATATCAACCACTTTGTACAAGAAAGCTGA | pENTR-BsaI |  |
|  | LIII bb + | TTTGGTCTCTGATAGAAGACTTTCTGGTTTAAACTATCAGTGTTTGACAGGA | pICH50505 (ICON Genetics) |  |
|  | LIII bb - | AAAGGTCTCTGCGCGAAGACAACGTAATCCACCCCAGTACATTAAAAA | pICH50505 (ICON Genetics) |  |
| LIIIa (obsolete) BsaI cut-ligation: 1 + 2 + 3 + 4 + 5 + 6 +7 + ccdb |  |  |
| LIIIb (obsolete) BsaI cut-ligation: bb + ccdb |  |   |   |
|  |  |  |  |  |
| Stage 2: Final Expression vector backbones were assembled as shown below | A fw | ATGGTCTCATGCCATTTTTGGGGTGAG | LIII b (obsolete) |  |
| A rv | TAGGTCTCAATCCACCCCAGTACATTAAAAA | LIII b (obsolete) |  |
| B + E fw | ATGGTCTCAGGATTACGTGAGACGATACGTCTCATCTGGTTTAAACTATCAGTGTTTGACAGGA | LIII b (obsolete) |  |
| B rv | TAGGTCTCAATGATCTAGGTGAAGATCCTTTTTGA | LIII b (obsolete) |  |
| C-s fw | ATGGTCTCATCATCGGAAGAACGGCAACTAAG | pK7WG2D,1 [[3](#_ENREF_3)] |  |
| C-s rv | TAGGTCTCAGGCAGAAGATCCTTTGATCTTTTCTACGG | pK7WG2D,1 [[3](#_ENREF_3)] |  |
| C-k1 fw | ATGGTCTCATCATAAAATCATTATTTGCCATCCA | pENTR-BsaI |  |
| C-k1 rv | TAGGTCTCAGCAAGACGAAATACGCGATC | pENTR-BsaI |  |
| C-k2 fw | ATGGTCTCATTGCTCAGGCGCAATCAC | pENTR-BsaI |  |
| C-k2 rv | TAGGTCTCAGGCAGGGGTCTGACGCTCAGTG | pENTR-BsaI |  |
| D fw | ATGGTCTCATGCCGGTAACATGAGCAAAGTCTG | LIII a (obsolete) |  |
| D rv | TAGGTCTCAATCCGTCTAAGCGTCAATTTGTTTACACC | LIII a (obsolete) |  |
| E rv | TAGGTCTCAATGAGCGTCAGACCCCGTAGAA | LIII a (obsolete) |  |
| Xpre-S BsaI cut-ligation: A + B + C-s  |  |  |  |
| Xpre-K BsaI cut-ligation: A + B + C-k1 + C-k2  |  |  |  |
| Xpre2-S BsaI cut-ligation D + E + C-s |  |  |  |
| Xpre2-K BsaI cut-ligation: D + E + C-k1 + C-k2  |  |   |   |
|  |  |   |  |  |
| **LII vector construction**  |  |  |  |  |
| ccdB F | L II F + | GCGGTGAGACCACAAGTTTGTACAAAAAAGCTGAA | pENTR-BsaI (ccdB cassette) |   |
| L II F - | GACATGAGACCCCAACCACTTTGTACAAGAAAGC | pENTR-BsaI (ccdB cassette) |   |
| LIIc F 1-2 | L II F 1-2 + | GAAGACAATACGGCGGTGAGACCACAAGTTTG | ccdB F | ligation into pAMP (Geneart) |
| L II F 1-2 - | GAAGACAAGCCCGACATGAGACCCCAACCACT | ccdB F |
| LIIc F 2-3 | LII F 2-3 + | GAAGACAAGGGCGCGGTGAGACCACAAGTTTG | ccdB F | ligation into pAMP (Geneart) |
| LII F 2-3 - | GAAGACAACAATGACATGAGACCCCAACCACT | ccdB F |
| LIIc F 3-4 | LII F 3-4 + | GAAGACAAATTGGCGGTGAGACCACAAGTTTG | ccdB F | ligation into pAMP (Geneart) |
| LII F 3-4 - | GAAGACAAGACAGACATGAGACCCCAACCACT | ccdB F |
| LIIc F 4-5 | LII F 4-5 + | GAAGACAATGTCGCGGTGAGACCACAAGTTTG | ccdB F | ligation into pAMP (Geneart) |
| LII F 4-5 - | GAAGACAACTCAGACATGAGACCCCAACCACT | ccdB F |
| LIIc F 5-6 | LII F 5-6 + | GAAGACAATGAGGCGGTGAGACCACAAGTTTG | ccdB F | ligation into pAMP (Geneart) |
| LII F 5-6 - | GAAGACAACAGAGACATGAGACCCCAACCACT | ccdB F |
|  |  |  |  |  |
| ccdB R | LII R + | GACATGAGACCACAAGTTTGTACAAAAAAGCTGAA | pENTR-BsaI (ccdB cassette) |   |
| LII R - | GCGGTGAGACCCCAACCACTTTGTACAAGAAAGC | pENTR-BsaI (ccdB cassette) |   |
| LIIc R 1-2 | LII R 1-2 + | GAAGACAATACGGACATGAGACCACAAGTTTGTACA | ccdB R | blunt ligation into pAMP (Geneart) |
| LII R 1-2 - | GAAGACAAGCCCGCGGTGAGACCCCAACC | ccdB R | blunt ligation into pAMP (Geneart) |
| LIIc R 3-4 | LII R 3-4 + | GAAGACAAATTGGACATGAGACCACAAGTTTGTACA | ccdB R | blunt ligation into pAMP (Geneart) |
| LII R 3-4 - | GAAGACAAGACAGCGGTGAGACCCCAACC | ccdB R | blunt ligation into pAMP (Geneart) |
| LIIc R 5-6 | LII R 5-6 + | GAAGACAATGAGGACATGAGACCACAAGTTTGTACA | ccdB R | blunt ligation into pAMP (Geneart) |
| LII R 5-6 - | GAAGACAACAGAGCGGTGAGACCCCAACC | ccdB R | blunt ligation into pAMP (Geneart) |
| LII F 1-2 | LII X 1-2 + | TTCGTCTCTTACGGAAGACAATACGGCGGTGAGACCAAAGCTGAACGAGAAACGTAAAA | LIIc F1-2 RNAi (ccdB) | Esp3I cut-ligation into Xpre-S / Xpre2-S |
| LII X 1-2 - | AACGTCTCACAGAGAAGACAAGCCCGACATGAGACCGTCGACCTGCAGACTGGC | LIIc F1-2 RNAi (ccdB) |
| LII R 1-2 | LIIx R 1-2 + | TTCGTCTCTTACGGAAGACAATACGGACATGAGACCAAAGCTGAACGAGAAACGTAAAA | LIIc F1-2 RNAi (ccdB) | Esp3I cut-ligation into Xpre-S / Xpre2-S |
| LIIx R 1-2 - | AACGTCTCACAGAGAAGACAAGCCCGCGGTGAGACCGTCGACCTGCAGACTGGC | LIIc F1-2 RNAi (ccdB) |
| LII F 2-3 | LIIx F 2-3 + | TTCGTCTCTTACGGAAGACAAGGGCGCGGTGAGACCAAAGCTGAACGAGAAACGTAAAA | LIIc F1-2 RNAi (ccdB) | Esp3I cut-ligation into Xpre-S / Xpre2-S |
| LIIx F 2-3 - | AACGTCTCACAGAGAAGACAACAATGACATGAGACCGTCGACCTGCAGACTGGC | LIIc F1-2 RNAi (ccdB) |
|  |  |  |  |  |
| LII R 2-3 | LIIx R 2-3+ | TTCGTCTCTTACGGAAGACAAGGGCGACATGAGACCAAAGCTGAACGAGAAACGTAAAA | LIIc F1-2 RNAi (ccdB) | Esp3I cut-ligation into Xpre-S / Xpre2-S |
| LIIx R 2-3- | AACGTCTCACAGAGAAGACAACAATGCGGTGAGACCGTCGACCTGCAGACTGGC | LIIc F1-2 RNAi (ccdB) |
| LII F 3-4 | LIIx F 3-4+ | TTCGTCTCTTACGGAAGACAAATTGGCGGTGAGACCAAAGCTGAACGAGAAACGTAAAA | LIIc F1-2 RNAi (ccdB) | Esp3I cut-ligation into Xpre-S / Xpre2-S |
| LIIx F 3-4- | AACGTCTCACAGAGAAGACAAGACAGACATGAGACCGTCGACCTGCAGACTGGC | LIIc F1-2 RNAi (ccdB) |
| LII R 3-4 | LIIx R 3-4+ | TTCGTCTCTTACGGAAGACAAATTGGACATGAGACCAAAGCTGAACGAGAAACGTAAAA | LIIc F1-2 RNAi (ccdB) | Esp3I cut-ligation into Xpre-S / Xpre2-S |
| LIIx R 3-4- | AACGTCTCACAGAGAAGACAAGACAGCGGTGAGACCGTCGACCTGCAGACTGGC | LIIc F1-2 RNAi (ccdB) |
| LII F 4-5 | LIIx F 4-5+ | TTCGTCTCTTACGGAAGACAATGTCGCGGTGAGACCAAAGCTGAACGAGAAACGTAAAA | LIIc F1-2 RNAi (ccdB) | Esp3I cut-ligation into Xpre-S / Xpre2-S |
| LIIx F 4-5- | AACGTCTCACAGAGAAGACAACTCAGACATGAGACCGTCGACCTGCAGACTGGC | LIIc F1-2 RNAi (ccdB) |
| LII R 4-5 | LIIx R 4-5+ | TTCGTCTCTTACGGAAGACAATGTCGACATGAGACCAAAGCTGAACGAGAAACGTAAAA | LIIc F1-2 RNAi (ccdB) | Esp3I cut-ligation into Xpre-S / Xpre2-S |
| LIIx R 4-5- | AACGTCTCACAGAGAAGACAACTCAGCGGTGAGACCGTCGACCTGCAGACTGGC | LIIc F1-2 RNAi (ccdB) |
| LII F 5-6 | LIIx F 5-6+ | TTCGTCTCTTACGGAAGACAATGAGGCGGTGAGACCAAAGCTGAACGAGAAACGTAAAA | LIIc F1-2 RNAi (ccdB) | Esp3I cut-ligation into Xpre-S / Xpre2-S |
| LIIx F 5-6- | AACGTCTCACAGAGAAGACAACAGAGACATGAGACCGTCGACCTGCAGACTGGC | LIIc F1-2 RNAi (ccdB) |
| LII R 5-6 | LIIx R 5-6+ | TTCGTCTCTTACGGAAGACAATGAGGACATGAGACCAAAGCTGAACGAGAAACGTAAAA | LIIc F1-2 RNAi (ccdB) | Esp3I cut-ligation into Xpre-S / Xpre2-S |
| LIIx R 5-6- | AACGTCTCACAGAGAAGACAACAGAGCGGTGAGACCGTCGACCTGCAGACTGGC | LIIc F1-2 RNAi (ccdB) |
| LII dy 2-3 | LII 2-3 dy + | GAAGACAAGGGCTAGATAATTAGGCTAAACTATCCTTAAGGATTGTTGTCTTCCCTCGAATTCCCAAGCTTATC | pAMP (Genart®) | amplified with LII dy- |
| LII dy 4-5 | LII 4-5 dy + | GAAGACAATGTCTAGATAATTAGGCTAAACTATCCTTAAGGTGAGTTGTCTTCCCTCGAATTCCCAAGCTTATC | pAMP (Genart®) | amplified with LII dy- |
| LII dy 4-6 | LII 4-6 dy + | GAAGACAATGTCTAGATAATTAGGCTAAACTATCCTTAAGGTCTGTTGTCTTCCCTCGAATTCCCAAGCTTATC | pAMP (Genart®) | amplified with LII dy- |
| LII dy 2-6 | LII 2-6 dy + | TTTGAAGACAAGGGCTAGATAATTAGGCTAAACTATCCTTAAGGTCTGTTGTCTTCCCTCGAATTCCCAAGCTTATC | pAMP (Genart®) | amplified with LII dy- |
| LII dy 1-2 | LII 1-2 dy + | TTTGAAGACAATACGTAGATAATTAGGCTAAACTATCCTTAAGGGGGCTTGTCTTCCCTCGAATTCCCAAGCTTATC | pAMP (Genart®) | amplified with LII dy- |
| LII dy 3-4 | LII 3-4 dy + | TTTGAAGACAAATTGTAGATAATTAGGCTAAACTATCCTTAAGGTGTCTTGTCTTCCCTCGAATTCCCAAGCTTATC | pAMP (Genart®) | amplified with LII dy- |
| LII dy 5-6 | LII 5-6 dy + | TTTGAAGACAATGAGTAGATAATTAGGCTAAACTATCCTTAAGGTCTGTTGTCTTCCCTCGAATTCCCAAGCTTATC | pAMP (Genart®) | amplified with LII dy- |
| LII dy 1-3 | LII 1-3 dy + | TTTGAAGACAATACGTAGATAATTAGGCTAAACTATCCTTAAGGATTGTTGTCTTCCCTCGAATTCCCAAGCTTATC | pAMP (Genart®) | amplified with LII dy- |
|   | LII dy - | AGCTCGAGCGAAGCTTTAGA | pAMP (Genart®) |   |
| LII ins 2-3 | LII ins 2-3 + | GAATATATATATATTCGAATATATATATATTCATTGTTGTCTTCCCTCGAATTC | LII dy 2-3 | amplified with LII ins- |
|  |  |  |  |  |
| LII ins 4-5 | LII ins 4-5 +  | GAATATATATATATTCGAATATATATATATTCTGAGTTGTCTTCCCTCGAATT | LII dy 4-5 | amplified with LII ins- |
|   | LII ins - | CCTTAAGGATAGTTTAGCCTAATTATCTA | LII dy 2-3 / LII dy 4-5 |   |
|  |  |   |  |  |
| **LIII vector construction**  |  |  |  |  |
| LIII fin | LIII fin + | TTCGTCTCTTACGTTGTCTTCAAAGCTGAACGAGAAACGTAAAA | LIIc F1-2 RNAi (ccdB) | Esp3I cut-ligation into Xpre-k or / Xpre2-k |
| LIII fin - | AACGTCTCACAGAAAGTCTTCGTCGACCTGCAGACTGGC | LIIc F1-2 RNAi (ccdB) |
| LIII F A-B | LIII X A-B + | TTCGTCTCTTACGGGTCTCAGCGGTACGTTGTCTTCAAAGCTGAACGAGAAACGTAAAA | LIIc F1-2 RNAi (ccdB) | Esp3I cut-ligation into Xpre-k or / Xpre2-k |
| LIII X A-B - | AACGTCTCACAGAGGTCTCACAGACAGAAAGTCTTCGTCGACCTGCAGACTGGC | LIIc F1-2 RNAi (ccdB) |
| LIII R A-B | LIIIx R A-B+ | TTCGTCTCTTACGGGTCTCAGCGGCAGATTGTCTTCAAAGCTGAACGAGAAACGTAAAA | LIIc F1-2 RNAi (ccdB) | Esp3I cut-ligation into Xpre-k or / Xpre2-k |
| LIIIx R A-B- | AACGTCTCACAGAGGTCTCACAGATACGAAGTCTTCGTCGACCTGCAGACTGGC | LIIc F1-2 RNAi (ccdB) |
| LIII F C-D | LIIIx F C-D+ | TTCGTCTCTTACGGGTCTCACACCTACGTTGTCTTCAAAGCTGAACGAGAAACGTAAAA | LIIc F1-2 RNAi (ccdB) | Esp3I cut-ligation into Xpre-k or / Xpre2-k |
| LIIIx F C-D- | AACGTCTCACAGAGGTCTCACCTTCAGAAAGTCTTCGTCGACCTGCAGACTGGC | LIIc F1-2 RNAi (ccdB) |
| LIII R C-D | LIIIx R C-D+ | TTCGTCTCTTACGGGTCTCACACCCAGATTGTCTTCAAAGCTGAACGAGAAACGTAAAA | LIIc F1-2 RNAi (ccdB) | Esp3I cut-ligation into Xpre-k or / Xpre2-k |
| LIIIx R C-D- | AACGTCTCACAGAGGTCTCACCTTTACGAAGTCTTCGTCGACCTGCAGACTGGC | LIIc F1-2 RNAi (ccdB) |
| LIII F E-F | LIIIx F E-F+ | TTCGTCTCTTACGGGTCTCAAATCTACGTTGTCTTCAAAGCTGAACGAGAAACGTAAAA | LIIc F1-2 RNAi (ccdB) | Esp3I cut-ligation into Xpre-k or / Xpre2-k |
| LIIIx F E-F- | AACGTCTCACAGAGGTCTCACTCACAGAAAGTCTTCGTCGACCTGCAGACTGGC | LIIc F1-2 RNAi (ccdB) |
| LIII R E-F | LIIIx R E-F+ | TTCGTCTCTTACGGGTCTCAAATCCAGATTGTCTTCAAAGCTGAACGAGAAACGTAAAA | LIIc F1-2 RNAi (ccdB) | Esp3I cut-ligation into Xpre-k or / Xpre2-k |
| LIIIx R E-F- | AACGTCTCACAGAGGTCTCACTCATACGAAGTCTTCGTCGACCTGCAGACTGGC | LIIc F1-2 RNAi (ccdB) |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |   |  |  |
| **LI constructs** |  |   |  |  |
| 35S mutagenesis | 35S BsaI + | GGGGTCTCTGCGGAGATTAGCCTTTTCAATTTCAGAAAGAATGC  | pGWB2 [[4](#_ENREF_4)] |   |
| 35S BsaI - | GGGGTCTCACAGACGTGTTCTCTCCAAATGAAATGAACTTCC | pGWB2 [[4](#_ENREF_4)] |  |
| 35S Bsa\* 1. + | GGCTTACGCAGCACGTCTCATCAAGACG | pGWB2 [[4](#_ENREF_4)] |  |
| 35S Bsa\* 1. - | CGTCTTGATGAGACGTGCTGCGTAAGCC | pGWB2 [[4](#_ENREF_4)] |  |
| 35S Bpi\* 2. + | CAGTCTCAGAAGAGCAAAGGGCAATTGAG | pGWB2 [[4](#_ENREF_4)] |  |
| 35S Bpi\* 2. - | CTCAATTGCCCTTTGCTCTTCTGAGACTG | pGWB2 [[4](#_ENREF_4)] |  |
| 35S Bsa/Bpi\* 3. + | CACGACTTCAAAGCAAGTGGATTGATGTG | pGWB2 [[4](#_ENREF_4)] |  |
| 35S Bsa\*/Bpi\* 3. - | GTTGGAACGTGTTCTTTTTCCACGATGC | pGWB2 [[4](#_ENREF_4)] |  |
| 35S Esp3I Mut + | CTTACGCAGCACGACTCATCAAGACGATC | pGWB2 [[4](#_ENREF_4)] |  |
| 35S Esp3I Mut - | GATCGTCTTGATGAGTCGTGCTGCGTAAG | pGWB2 [[4](#_ENREF_4)] |   |
| LI A-B p35S | A-B 35S + | ATGGTCTCTGCGGAGATTAGCC | 35S mutagenesis | ligation into pUC57 (Gent) |
| A-B 35S - | TAGGTCTCACAGACGTGTTCTCTCCAAATGAAATG | 35S mutagenesis |
| LI A-C p35S | A-B 35S + | ATGGTCTCTGCGGAGATTAGCC | 35S mutagenesis | ligation into pUC57 (Gent) |
| A-C 35S - | TAGGTCTCAGGTGCGTGTTCTCTCCAAATGAAATG | 35S mutagenesis |
|  |  |  |  |  |
| LI A-B pUbi | A-B Ubi+ | GGGTCTCTGCGGGGAGAGAGGATTTTGAGGAAA | pUB-GW-HYG [[2](#_ENREF_2)] | ligation into pUC57 (Gent) |
| A-B Ubi- | ATGGTCTCACAGACTGTAATCACATCAACAACAGATAAA | pUB-GW-HYG [[2](#_ENREF_2)] |
| LI A-B pEF1 | EF1 + | ATGGTCTCAGCGGGTGTATATCGTCCAAGTAAAACCTTC | *A. thaliana* Col-0 genomic DNA | ligation into pUC57 (Gent) |
| EF1 - | TAGGTCTCTCAGAGGTTAGAGACTGTCAAACAAATCTG | *A. thaliana* Col-0 genomic DNA |
| LI A-B pNOS  | pNos + | ATGGTCTCAGCGGGATCATGAGCGGAGAATTAAGG | pBI121 (Clontech) | ligation into pUC57 (Gent) |
| pNos - | TAGGTCTCTCAGAAGATCCGGTGCAGATTATTTG | pBI121 (Clontech) |
| L0 N-term linker | Link 1 + | ATGAAGACTTTACGGGTCTCAAAGGGAGGTGGAGGAGGTTCTGGAGGCGGTGGAAGT | PCR without template | N-Linker |
| Link 1 - | TAGAAGACAAGCTACCTCCGCCACCACTTCCACCGCCTCCAG | PCR without template | N-Linker  |
| LI D-E Ceruelan, GFP, YFP, T-Sapphire | C-GFP+ | TAGAAGACAATAGCGTGAGCAAGGGCGAGGAG | p35S\_GW\_T-Sapph[[5](#_ENREF_5)]; pUB-GW-GFP[[2](#_ENREF_2)],;pAM-PAT-YFP[[6](#_ENREF_6)]; p35S\_GW\_T-Sapph[[5](#_ENREF_5)] | BpiI cut-ligation with N-linker into LI+BpiI |
| C-GFP- | ATGAAGACTTCAGAGGTCTCAGATTTTACTTGTACAGCTCGTCCATG |
| LI D-E mOrange, mCherry  | C-GFP+ | TAGAAGACAATAGCGTGAGCAAGGGCGAGGAG | pC1625\_pCAG\_mCh\_GW [[7](#_ENREF_7)]; p35S\_GW\_mOrange[[5](#_ENREF_5)] | BpiI cut-ligation with N-linker into LI+BpiI |
| mOR 2- | ATGAAGACTTTTTCTTCTGCATTACGGGG |
| mOR 3+ | TAGAAGACAAGAAAACCATGGGCTGGG |
| C-GFP- | ATGAAGACTTCAGAGGTCTCAGATTTTACTTGTACAGCTCGTCCATG |
| LI C-D Cerulean, GFP, YFP, T-Sapphire , mOrange, mCherry | GFP+ | ATGAAGACTTTACGGGTCTCACACCATGGTGAGCAAGGGCGAG | LI D-E Cerulean, GFP, YFP, T-Sapphire, mOrange, mCherry | BpiI cut-ligation into LI+BpiI |
| GFP- nostop | TAGAAGACAACAGAGGTCTCACCTTCTTGTACAGCTCGTCCATGC |   |
| L0 C-term linker | Link 2 + | TAGAAGACAACAAGGGTGGAGGAGGTTCTGGAGGCGGTGGAAGT | PCR without template | C-Linker |
| Link 2 - | ATGAAGACTTCAGAGGTCTCAGGTGCCGCTACCTCCGCCACCACTTCCACCGCCTCCAG | PCR without template | C-Linker |
| LI B-C Cerulean, GFP, YFP, T-Saphhire, mOrange, mCherry | N-GFP-KOZAK + | ATGAAGACTTTACGGGTCTCA TCTGAACAATGGTGAGCAAGGGCGAG | LI C-D Cerulean; GFP; YFP; T-Sapphire; mOrange; mCherry  | BpiI cut-ligation with C-linker into LI+BpiI |
| N-GFP -  | TAGAAGACAACTTGTACAGCTCGTCCATGC |
| LI C-D noATG Cerulean, GFP, YFP, T-Saphhire, mOrange, mCherry | GFP+ noATG | ATGAAGACTTTACGGGTCTCACACCGTGAGCAAGGGCGAGGAG | LI C-D Cerulean, GFP, YFP, T-Sapphire, mOrange, mCherry  | BpiI cut-ligation into LI+BpiI |
| GFP- nostop | TAGAAGACAACAGAGGTCTCACCTTCTTGTACAGCTCGTCCATGC |
| LI C-D dGFP | desGFP +  | ATGGTCTCACACCATGGGCTTAATTAATATAATTAATAATCCA | pLV.CMV.dsEGFP [[8](#_ENREF_8)] | ligation into pUC57 (Gent) |
| des GFP- | TAGGTCTCACCTTCACATTGATCCTAGCAGAAGCAC | pLV.CMV.dsEGFP [[8](#_ENREF_8)] |
| LI C-D RNAi dGFP | desGFP +  | ATGGTCTCACACCATGGGCTTAATTAATATAATTAATAATCCA | pLV.CMV.dsEGFP [[8](#_ENREF_8)] | not subcloned |
| Si\_desGFP - | TAGGTCTCACCTTGAAGTCGATGCCCTTCAGCT | pLV.CMV.dsEGFP [[8](#_ENREF_8)] |
|  |  |  |  |  |
|  |  |  |  |  |
| LI C-D Plastid Prelim. | L0 Pla. 1+ | ATGAAGACTTTACGGGTCTCACACCATGGCAAGCATTGCTGGTTC | *L. japonicus* Gifu genomic DNA | BpiI cut-ligation into LI+BpiI |
| L0 Pla. 1-  | TTGAAGACTTTTCGATTTCAGGGCTCTCTTTGTTACCTGATGACAACAAGCACCCTTTTGG | *L. japonicus* Gifu genomic DNA |
| L0 Pla 2+ | TTGAAGACTTCGAAACCCTGATGATTATT | *L. japonicus* Gifu genomic DNA |
| L0 Pla 2- | ATGAAGACTTCAGAGGTCTCACCTTGGGCCTGAATGGAGCAA | *L. japonicus* Gifu genomic DNA |
| LI C-D Plastid-Marker | Pla\_mut + | GTTGTCATCAGGTAACAAAGAGAGC | LI C-D Plastid Prelim. | BpiI site removal |
| Pla\_mut - | AAGCACCCTTTTGGCATG | LI C-D Plastid Prelim. |
| LI B-E GUSi | GUSi Koz + | ATGGTCTCATCTGAACAATGGTAGATCTGAGGGTAAA | pIG121-Hm [[9](#_ENREF_9)] | ligation into pUC57 (Gent) |
| GUSi Koz - | AAGGTCTCAGATTTCATTGTTTGCC | pIG121-Hm [[9](#_ENREF_9)] |
| LI C-D 1-10GFP | GFP1-10 fw | ATGGTCTCACACCATGGTGTCTAAGGGCGAAGA | pUC-sGFPFL (Gene synthesis) | ligation into pUC57 (Gent) |
| GFP1-10 rv STOP | TAGGTCTCTCCTTTCACTTTTCGTTAGGGTCCTTGG |
| LI C-D 1-10GFP noATG | GFP1-10 noATG | ATGGTCTCACACCGTGTCTAAGGGCGAAGAACTC | pUC-sGFPFL (Gene synthesis) | ligation into pUC57 (Gent) |
| GFP1-10 rv STOP | TAGGTCTCTCCTTTCACTTTTCGTTAGGGTCCTTGG |
| LI D-E 1-10GFP | C-sGFP+ | TAGAAGACAATAGCGTGTCTAAGGGCGAAGAACTC | pUC-sGFPFL (Gene synthesis) | ligation into pUC57 (Gent) |
| C-sGFP- | ATGAAGACTTCAGAGGTCTCAGATTTTACTTTTCGTTAGGGTCCTTGG |
| LI C-D 11GFP | GFP11 fw | ATGGTCTCACACCATGGACTACAAGGACGACGATGACA | pUC-sGFPFL (Gene synthesis) | ligation into pUC57 (Gent) |
| GFP11 rv | TAGGTCTCTCCTTTTATGTGATTCCGGCGG |
| LI D-E 11GFP | C-GFP11 fw | ATGGTCTCAAAGGGAGACTACAAGGACGACGATGACA | pUC-sGFPFL (Gene synthesis) | ligation into pUC57 (Gent) |
| C-GFP11 rv | TAGGTCTCTGATTTTATGTGATTCCGGCGG |
| LI B-C N-NLS | N-NLS KOZ + | ATGGTCTCATCTGAACAATGCTGCAGCCTAAGAAGAAG | NLS-YC3.6 [[10](#_ENREF_10)] | ligation into pUC57 (Gent) |
| N-NLS rv | TAGGTCTCAGGTGGCGGCCGC | NLS-YC3.6 [[10](#_ENREF_10)] |
| LI B-C N-NES (I) | N-NES KOZ b+ | ATGGTCTCATCTGAACAATGCTGCAGAACGAGCTTG | NES-YC3.6 [[10](#_ENREF_10)] | ligation into pUC57 (Gent) |
| N-NES b rv | TAGGTCTCAGGTGGCGGCCGC | NES-YC3.6 [[10](#_ENREF_10)] |
| LI B-C N-NES (II) | N-NES KOZ a + | ATGGTCTCATCTGAACAATGGCAAATTGTTGCTCTCA | p35S\_CPK17G2A\_NES [[11](#_ENREF_11)] | ligation into pUC57 (Gent) |
| N-NES a rv | TAGGTCTCTGGTGCCTCCTCCAGTCTTGTTAATATCAAGTC | p35S\_CPK17G2A\_NES [[11](#_ENREF_11)] |
| LI D-E C-NLS | C-NLS fw | ATGGTCTCAAAGGGACTGCAGCCTAAGAAGAAGAGAAA | NLS-YC3.6 [[10](#_ENREF_10)] | ligation into pUC57 (Gent) |
| C-NLS rv | TAGGTCTCTGATTTTACGCACTCGAGTCGACTCC | NLS-YC3.6 [[10](#_ENREF_10)] |
| LI D-E C-NES | C-NES fw | ATGGTCTCAAAGGGACTGCAGAACGAGCTTGCTCT | NES-YC3.6 [[10](#_ENREF_10)] | ligation into pUC57 (Gent) |
| C-NES rv  | TAGGTCTCTGATTTTACGCACTCGAGTCGACTCC | NES-YC3.6 [[10](#_ENREF_10)] |
| LI E-F HSP-T | HSP-T fw | ATGGTCTCAAATCATATGAAGATGAAGATGAAA | *A. thaliana* Col-0 genomic DNA | ligation into pUC57 (Gent) |
| HSP-T rv | TAGGTCTCTCTCACTTATCTTTAATCATATTCC | *A. thaliana* Col-0 genomic DNA |
| LI E-F nos-T | Nos-Term BsaI + | GGGGTCTCTAATCGATCGTTCAAACATTTGGCAATAA | pBI121 (Clontech) | ligation into pUC57 (Gent) |
| Nos-Term BsaI - | GGGGTCTCACTCAGATCTAGTAACATAGATGACAC | pBI121 (Clontech) |
| LI E-F 35S-T  | 35S terminator + | ATGAAGACTTTACGGGTCTCAAATCCGGCCATGCTAGAGTCCG | pK7RWG2 [[3](#_ENREF_3)] | ligation into pUC57 (Gent) |
| 35S terminator -  | ATGAAGACTTCAGAGGTCTCACTCAAGGTCACTGGATTTTGGTTTT | pK7RWG2 [[3](#_ENREF_3)] |
| LI F-G Hygro / Prelim | hyg F1 + | ATGAAGACTTTACGGGTCTCATGAGAGATTAGCCTTTTCAATTTCAGAAA | pUB-GW-HYG [[2](#_ENREF_2)] | BpiI cut-ligation into LI+BpiI |
| hyg F1 - | TAGAAGACAAGGTGCGTGTTCTCTCCAAATGAAATG | pUB-GW-HYG [[2](#_ENREF_2)] |
| hyg F2+ | TAGAAGACAACACCATGAAAAAGCCTGAACTCACC | pUB-GW-HYG [[2](#_ENREF_2)] |
| hyg F2- | ATGAAGACTTCAGAGGTCTCAGACATGCGGACGTTTTTAATGTACTG | pUB-GW-HYG [[2](#_ENREF_2)] |
| LI F-G Hygro | G94 mut + | GTCCGACCTGATGCAGCTCT | LI F-G Hygro / Prelim | removal of Esp3I site |
| G94 mut- | ACGCTGTCGAACTTTTCGAT | LI F-G Hygro / Prelim |
| LI F-G neo | Neo + | GGTCTCTTGAGGATCATGAGCGGAGAATTAAG | pBI121 (Clontech) | ligation into pUC57 (Gent) |
| Neo - | GGTCTCAGACACCCGATCTAGTAACATAGATG | pBI121 (Clontech) |
| LI B-C 6xHIS | N-Term 6xHis + | ATGGTCTCATCTGaacaATGCATCACCATCACCATCACGGCGGCAGCGGCGGATCCAGCACCAGAGACCGGGCCCGTCGACTGC | pUC57 (Gent) |  amplified with LI dy- |
| LI D-E 6xHIS | C-Term 6xHis +  | ATGGTCTCAAAGGGCGGCAGCGGCGGATCCCATCACCATCACCATCACTAAAATCAGAGACCGGGCCCGTCGACTGC | pUC57 (Gent) |  amplified with LI dy- |
| LI B-C HA | N-Term HA +  | ATGGTCTCATCTGaacaATGTACCCATACGATGTTCCAGATTACGCTGGCGGCAGCGGCGGATCCAGCACCAGAGACCGGGCCCGTCGACTGC | pUC57 (Gent) |  amplified with LI dy- |
| LI D-E HA | C-Term HA +  | ATGGTCTCAAAGGGCGGCAGCGGCGGATCCTACCCATACGATGTTCCAGATTACGCTTAAAATCAGAGACCGGGCCCGTCGACTGC | pUC57 (Gent) |  amplified with LI dy- |
| LI B-C c-myc  | N-Term c-myc +  | ATGGTCTCATCTGaacaATGGAGCAAAAGTTGATTTCTGAGGAGGATCTTGGCGGCAGCGGCGGATCCAGCACCAGAGACCGGGCCCGTCGACTGC | pUC57 (Gent) |  amplified with LI dy- |
| LI D-E c-myc | C-Term c-myc + | ATGGTCTCAAAGGGCGGCAGCGGCGGATCCGAGCAAAAGTTGATTTCTGAGGAGGATCTTTAAAATCAGAGACCGGGCCCGTCGACTGC | pUC57 (Gent) |  amplified with LI dy- |
|   | LI dy - | GGGATCCGATATCTAGATGC | pUC57 (Gent) |   |
|  |  |  |  |  |
| **Custom backbone vector dummies**  |  |  |  |
| LI dy Esp3I-lacZ A-B | lacZ-Pro fw | TTGGTCTCTGCGGTGAGACGTACCGCCTTTGAGTGAGCTG | pUC57 (Genscript) | ligation into pUC57 (Gent) |
| lacZ-Pro rv | AAGGTCTCACAGAAGAGACGATGCCGGGAGCAGACA | pUC57 (Genscript) |
| LI dy Esp3I-lacZ C-D | lacZ-GOI fw | TTGGTCTCTCACCTGAGACGTACCGCCTTTGAGTGAGCTG | pUC57 (Genscript) | ligation into pUC57 (Gent) |
| lacZ GOI rv | AAGGTCTCACCTTAGAGACGATGCCGGGAGCAGACA | pUC57 (Genscript) |
| LI dy Esp3I-ccdB A-B | ccdB - Pro fw | TTCGTCTCTGCGGTGAGACCAAAGCTGAACGAGAAACGTAAAA | LIIc F 1-2 RNAi | ligation into pUC57 (Gent) |
| ccdB - Pro rv | AACGTCTCACAGAAGAGACCGTCGACCTGCAGACTGGC | LIIc F 1-2 RNAi |
| LI dy Esp3I-ccdB C-D | ccdb GOI fw | TTCGTCTCTCACCTGAGACCAAAGCTGAACGAGAAACGTAAAA | LIIc F 1-2 RNAi | ligation into pUC57 (Gent) |
| ccdB GOI rv | AACGTCTCACCTTAGAGACCGTCGACCTGCAGACTGGC | LIIc F 1-2 RNAi |
|  |  |  |  |  |
| **Silencing Vectors** |  |  |  |  |
| LI Intron  | Intron fw | ATGGTCTCAAAGGAATTCCTGTGGTTGGAGAAGC | pUB-GWS-GFP [[2](#_ENREF_2)] |   |
| Intron rv | TAGGTCTCTAAGGAGCTTCTCCTCCTCTGCTAACG | pUB-GWS-GFP [[2](#_ENREF_2)] |   |
| LI B-E Esp3I dy  | LI Esp3I dy fw | ATGGTCTCATCTGTGAGACGTTCGTCTCAAATCAGAGACCTGCAAGCTTGGCGTAATCAT | pUC57 (Genscript) | inserted into LII RNAi prelimiary vector  |
| LI dy - | P-GGGATCCGATATCTAGATGC | pUC57 (Genscript) | inserted into LII RNAi prelimiary vector  |
| Esp3I - ccdB C-C dy 1 of 2 | ccdb 1 fw | ATCGTCTCATCTGCACCTGAGACCAAAGCTGAACGAGAAACGTAAAA | pENTR-BsaI | inserted into LII RNAi final vector  |
| ccdb 2 rv | TACGTCTCTACACGAAAAACATATTCTCAATAAACC | pENTR-BsaI |
| Esp3I - ccdB C-C dy 2 of 2 | ccdb 2 fw | ATCGTCTCAGTGTCAGCCAATCCCTGG | pENTR-BsaI |
| ccdb 3 rv | TACGTCTCTGATTCACCTGAGACCGTCGACCTGCAGACTGGC | pENTR-BsaI |

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