**Table S1:**

**Primer sequences**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Primer | Primer name | sequence | Primer position | function |
| P1 | psgRNA-LT-Ⅰ-F | CTAGACTAGT*AAGCTTGGAGAGAAGAACCC*GTTTTAGAGCTAGAAATAGC | pTargetF 247-266 | Amplification of N20 (*eltⅠ*) + sgRNA |
| P2 | psgRNA- LT-Ⅰ-R | GGATAAAATGTGTAGTGAAAGTCGACTCTAGAGAATTCAAAAAAAGCACCG | *eltⅠ* 1199-1218pTargetF 317-347 |
| P3 | LT-Ⅰ DonorL-F | TAGAGTCGACTTTCACTACACATTTTATCCTCG | pTargetF 338-347*eltⅠ* 1199-1221 | Amplification of *eltⅠ* DonorL |
| P4 | LT-Ⅰ DonorL-R | AGTTGTTATATAGGCTCCTAGC CGAGGAAAACTTATATATCATACAA | *eltⅠ* 2507-25281311-1335 |
| P5 | LT-Ⅰ DonorR-F | GCTAGGAGCCTATATAACAACT | *eltⅠ* 2507-2528 | Amplification of *eltⅠ* DonorR |
| P6 | LT-Ⅰ DonorR-R | CCGCTCGAGTAACAGCTCCCTGTAGTGGA | *eltⅠ* 2619-2638 |
| P7 | LT-Ⅰ-F | CTAATAAAAATAATCAGGTTGCC | *eltⅠ* 1109-1131 | Identification primer |
| P8 | LT-Ⅰ-R | CCTCAGACCTGTCACATTTAT | *eltⅠ* 2746-2766 |
| P9 | psgRNA-STb-F | CTAGACTAGT*CAAATAATGGTTGCAGCAAA*GTTTTAGAGCTAGAAATAGC | pTargrtF 247-266 | Amplification of N20 (*eltⅡc1*) + sgRNA |
| P10 | psgRNA-STb-R | GTATTTACATGTCGACTCTAGAGAATTCAAAAAAAGCACCG | *estB* 287-296pTargrtF 317-347 |
| P11 | STb DonorL-F | TAGAGTCGACATGTAAATACCTACAACGGGTGAT | pTargetF 338-347*estB* 287-310 | Amplification of *estB* DonorL |
| P12 | STb DonorL-R | ATTGATAAAT AAATCACCTCAACCTTATTGCTAT | *estB* 674-683*estB* 430-453 |
| P13 | STb DonorR-F | GAGGTGATTT ATTTATCAATAGCATTCAGCACC | *estB* 444-453*estB* 674-696 | Amplification of *estB* DonorR |
| P14 | STb DonorR-R | CCGCTCGAGGCACCACTTAATTCCAAAGAAC | *estB* 878-899 |
| P15 | STb-F | CCCTCTCTTTTGCACTTCTTTC | *estB* 209-230 | Identification primer |
| P16 | STb-R | GGCACACTATTAACCAAACCAA | *estB* 947-968 |
| P17 | psgRNA-LT-Ⅱ-F400 | CTAGACTAGT*TCTTTTGGTGCGATAGAAGG*GTTTTAGAGCTAGAAATAGC | pTargetF 247-266 | Amplification of N20 (*eltⅡc1*) + sgRNA |
| P18 | psgRNA-LT-Ⅱ-R400 | GACAGGTTTGGTCGACTCTAGAGAATTCAAAAAAAGCACCG | *eltⅡc1* 218-227pTargetF 317-347 |
| P19 | LT-Ⅱ DonorL-F400 | TAGAGTCGACCAAACCTGTCTCCGGATATT | pTargetF 338-347*eltⅡc1* 218-237 | Amplification of *eltⅡc1* DonorL |
| P20 | LT-Ⅱ DonorL-R400 | CCAATGACTA TTTAATTTTTCACAACACCCT | *eltⅡc1* 1757-1766601-621 |
| P21 | LT-Ⅱ DonorR-F400 | AAAAATTAAA TAGTCATTGGTGTTTTAGTTTTT | *eltⅡc1* 612-621*eltⅡc1* 1757-1772 | Amplification of *eltⅡc1* DonorR |
| P22 | LT-Ⅱ DonorR-R400 | CCGCTCGAGCCAAATTGCTTAATGCTTTCT | *eltⅡc1* 2202-2222 |
| P23 | LT-Ⅱ-F1 | GTTCTGGGAAAAGAGGGAGG | *eltⅡc1* 31-50 | Identification primer |
| P24 | LT-Ⅱ-R1 | GGGGCGTGAACGTAATAGT | *eltⅡc1* 2735-2753 |
| P25 | psgRNA*-*STa-F | CTAGACTAGT*TGTTGTAATCCTGCCTGTGC*GTTTTAGAGCTAGAAATAGC | pTargetF 247-266 | Amplification of N20 (*estA*) + sgRNA |
| P26 | psgRNA-STa-R | GTACACTCGCGTCGACTCTAGAGAATTCAAAAAAAGCACCG | *estA* 555-564pTargetF 317-347 |
| P27 | STa DonorL-F | TAGAGTCGACGCGAGTGTACCTCGACATATA | pTargetF 338-347*estA* 174-194 | Amplification of *estA* DonorL |
| P28 | STa DonorL-R | TCTATGCTTT GTTACCTCCCGTCATGTTGT | *estA* 505-514266-285 |
| P29 | STa DonorR F | GGGAGGTAAC AAAGCATAGAGGGAATCTTTAT | *estA* 276-285505-526 | Amplification of *estA* DonorR |
| P30 | STa DonorR-R | CCGCTCGAGTGGAGTCATTACCTATAAAAGC | *estA* 584-605 |
| P31 | STa-F1 | CATTACCCGAACAAGAAAAG | *estA* 60-79 | Identification primer |
| P32 | STa -R2 | TGAACATAAAACACTATCAATAAGT | *estA* 606-630 |
| P33 | psgRNA-K88-F | GGACTAGT*GCCGGTGTGTTCGGGAAAGG*GTTTTAGAGCTAGAAATAGC | pTargetF 247-266 | Amplification of N20 (*faeG*) + sgRNA |
| P34 | psgRNA-K88-R | TCCACAAATAATAAACCTAGAGAATTCAAAAAAAGCA | *faeG* 60-75pTargetF 320-340 |
| P35 | K88 DonorL-F | TGCTTTTTTTGAATTCTCTAGGTTTATTATTTGTGGA | pTargetF 320-340*faeG* 60-75 | Amplification of *faeG* DonorL |
| P36 | K88 DonorL-R | TTACTCTTTGAATCTGTCC TCCATTAAGACCTGTACC | *faeG* 736-755*faeG* 245-262 |
| P37 | K88 DonorR-F | GGTACAGGTCTTAATGGA GGACAGATTCAAAGAGTAA | *faeG* 245-262737-755 | Amplification of *faeG* DonorR |
| P38 | K88 DonorR-R | CCCTCGAGGTTGCCTCAATAGTCTGACCGTT | *faeG* 860-882 |
| P39 | K88-F | GGGATGGTTTTACGGTAATTC | *faeG* 13-33 | Identification primer |
| P40 | K88-R | ATTGCTACGTTCAGCGGAGCG | *faeG* 919-939 |
| P41 | STb DonorL-R-STa | TCGGGTAATG AAATCACCTCAACCTTATTGCTAT | *estA* 60-69*estB* 430-453 | Amplification of *estB* DonorL with STb DonorL-F |
| P42 | r-STa F1 | GAGGTGATTT CATTACCCGAACAAGAAAAG | *estB* 444-453*estA* 60-79 | Amplification of the fragment of the inserted *estA* |
| P43 | r-STa R1 | ATTGATAAAT TTCTCAGCACCAATACATATAAT | *estA* 542-564*estB* 674-683 |
| P44 | STb DonorR-F-STa | GTGCTGAGAA ATTTATCAATAGCATTCAGCACC | *estA* 555-564*estB* 674-696 | Amplification of *estB* DonorR with STb DonorR-R |
| P45 | psgRNA-LT-Ⅱ-R1 | GAGTCTGCTCTAAAGAAATCGCTAGAGAATTCAAAAAAAGCA | *eltⅡc1* 678-698pTargetF 320-340 | Amplification of N20 (*eltⅡc1*) + sgRNA with psgRNA-LT-Ⅱ-F400 |
| P46 | LT-Ⅱ DonorL-F1 | TGCTTTTTTTGAATTCTCTAG CGATTTCTTTAGAGCAGACTC | pTargetF 320-340*eltⅡc1* 678-698 | Amplification of *eltⅡc1* DonorL |
| P47 | LT-Ⅱ DonorL-R1-K88 | GCTCCTTCATAGTCAGTAAAT GGACTATACCGCCCTAACAC | *faeG* 409-429*eltⅡc1* 967-986 |
| P48 | rK88-F | GTGTTAGGGCGGTATAGTCC ATTTACTGACTATGAAGGAGC | *eltⅡc1* 967-986*faeG* 409-429 | Amplification of the fragment of the inserted *faeG* |
| P49 | rK88-R | ATCCTTAAAAGTTTTACTTACG AAACCACCATAAAAGATAGCG | *eltⅡc1* 1462-1483*faeG* 622-642 |
| P50 | LT-Ⅱ DonorR-F1-K88 | CGCTATCTTTTATGGTGGTTT CGTAAGTAAAACTTTTAAGGAT | *faeG* 622-642*eltⅡc1*1462-1485 | Amplification of *eltⅡc1* DonorR |
| P51 | LT-Ⅱ DonorR-R1 | CCTCGAGCGGTAGGTTTCCACATAGT | *eltⅡc1*1870-1888 |
| P52 | LT-ⅡDonorL-R2 | GAGCAAATGCACTCCATGGGAGCTCTCGCCATGC | *eltⅡc1*1186-1219 | Amplification of *eltⅡc1* DonorL with LT-Ⅱ DonorL-F1 |
| P53 | LT-Ⅱ DonorR-F2 | GCATGGCGAGAGCTCCCATGGAGTGCATTTGCTC | *eltⅡc1*1186-1219 | Amplification of *eltⅡc1* DonorR with LT-Ⅱ DonorR-R1 |
| P54 | LT-Ⅱ-F2 | AATGGATAGGGTGTTGTG | *eltⅡc1* 594-611 | Identification primer |
| P55 | LT-Ⅱ-R2 | TGCTCAGGGCTGTGGGTCCT | *eltⅡc1*2167-2186 |
| P56 | psgRNA-LT-Ⅱ-R300 | CTTTTGATTAGTCGACTCTAGAGAATTCAAAAAAAGCACCG | *eltⅡc1* 318-327pTargetF 317-347 | Amplification of N20 (*eltⅡc1*) + sgRNA with psgRNA-LT-Ⅱ-F400 |
| P57 | LT-Ⅱ DonorL-F300 | TAGAGTCGACTAATCAAAAGGGGACGTTAT | pTargetF 338-347*eltⅡc1* 318-337 | Amplification of *eltⅡc1* Donor |
| P58 | LT-Ⅱ DonorR-R300 | CCGCTCGAGATGAATAAAAAAGCTCACCAT | *eltⅡc1*2067-2087 |
| P59 | psgRNA-LT-Ⅱ-R200 | GGCGCGTAACGTCGACTCTAGAGAATTCAAAAAAAGCACCG | *eltⅡc1* 387-396pTargetF 317-347 | Amplification of N20 (*eltⅡc1*) + sgRNA with psgRNA-LT-Ⅱ-F400 |
| P60 | LT-Ⅱ DonorL-F200 | TAGAGTCGACGTTACGCGCCTATCTGTCG | pTargetF 338-347*eltⅡc1* 387-405 | Amplification of *eltⅡc1* Donor |
| P61 | LT-Ⅱ DonorR-R200 | CCGCTCGAGTCCTTACCGTTAGTTTCAATAC | *eltⅡc1*1937-1958 |
| P62 | psgRNA-LT-Ⅱ-R100 | TGACTGGTTGGTCGACTCTAGAGAATTCAAAAAAAGCACCG | *eltⅡc1* 498-507pTargetF 317-347 | Amplification of N20 (*eltⅡc1*) + sgRNA with psgRNA-LT-Ⅱ-F400 |
| P63 | LT-Ⅱ DonorL-F100 | TAGAGTCGACCAACCAGTCATTTGTCTACG | pTargetF 338-347*eltⅡc1* 498-517 | Amplification of *eltⅡc1* Donor |
| P64 | LT-Ⅱ DonorR-R100 | CCGCTCGAGTTTAAATTAGGAACGTGTCAA | *eltⅡc1*1839-1859 |
| P65 | psgRNA-LT-Ⅱ-R50 | ATTAAAGGTGGTCGACTCTAGAGAATTCAAAAAAAGCACCG | *eltⅡc1* 573-582pTargetF 317-347 | Amplification of N20 (*eltⅡc1*) + sgRNA with psgRNA-LT-Ⅱ-F400 |
| P66 | LT-Ⅱ DonorL-F50 | TAGAGTCGACCACCTTTAATAATATTTTTTAAATG | pTargetF 338-347*eltⅡc1* 573-597 | Amplification of *eltⅡc1* Donor |
| P67 | LT-Ⅱ DonorR-R50 | CCGCTCGAGAAATACAGAATAAAATTGAATGAA | *eltⅡc1*1782-1805 |
| P68 | L4440\* | AGCGAGTCAGTGAGCGAG | pTargetF 98-115 | Identification primer |
| P69 | pTarget-R | CCTCTGATAGTTGAGTCGATACTTC | pTargetF 529-553 |
| P70 | LacI-R\* | GGCATACTCTGCGACATCGT | pCas 1448-1467 | Identification primer |
| P71 | pCasR1 | CCTTTTGTAATACTGCGGAACTG | pCas 2930-2952 |

a The underline is the enzyme cleavage site, the italic is N20, and the double underline is the part of the pTarget plasmid.

\*Primers are from <http://www.addgene.org/>.