PCR amplification of repetitive DNA: a limitation to genome editing technologies and many other applications

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Supplementary information

Generation of dTALE12

For the generation of dTALE12, a Transcription Activator-like Effector containing 12 and a half repeats we followed the protocol of Golden Gate assembly as described previously [1]. The TALE repeat sequence is HD1, HD2, NI3, NG4, NN5, NN6, HD7, HD8, NI9, NI10, HD1, NI2, LR-HD. The final destination vector is pTAL2 (Addgene plasmid 31033).

Sequence data

TALE repeat in pTAL2 vector (only sequence between primers 534 and 535 is given)

GTTGGCGTCGGCAAACAGTGGTCCGGCGCACGCGCCCTGGAGGCCTTGCTCACGGATGCGGGGGAGTTGAGAGGTCCGCCGTTACAGTTG
GACACAGGCCAACTTGTGAAGATTGCAAAACGTGGCGGCGTGACCGCAATGGAGGCAGTGCATGCATCGCGCAATGCACTGACGGGTGCC
CCCCTGAACCTGACCCCGGACCAAGTGGTGGCTATCGCCAGCCACGATGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCG
GTGCTGTGCCAGGACCATGGCCTGACTCCGGACCAAGTGGTGGCTATCGCCAGCCACGATGGCGGCAAGCAAGCGCTCGAAACGGTGCAG
CGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATCGCCAGCAACATTGGCGGCAAGCAAGCGCTC
GAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATCGCCAGCAACGGTGGCGGC
AAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATCGCCAGC
AACAATGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTGGTG
GCTATCGCCAGCAACAATGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACTCCG
GACCAAGTGGTGGCTATCGCCAGCCACGATGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCAT
GGCCTGACTCCGGACCAAGTGGTGGCTATCGCCAGCCACGATGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTG
TGCCAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATCGCCAGCAACATTGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTG
TTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATCGCCAGCAACATTGGCGGCAAGCAAGCGCTCGAAACG
GTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATCGCCAGCCACGATGGCGGCAAGCAA
GCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATCGCCAGCAACATT
GGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATC
GCCAGCCACGATGGCGGCAAGCAAGCGCTCGAAAGCATTGTGGCCCAGCTGAGCCGGCCTGATCCGGCGTTGGCCGCGTTGACCAACGAC
CACCTCGTCGCCTTGGCCT

TALE repeat artifact products

Clone 1 M13F:

GGGCGATTGGGCCCTCTAGATGCATGCTCGAGCGGCCGCCAGTGTGATGGATATCTGCAGAATTCGCCCTTGATCGGCGCGCCAGGCCAA
GGCGACGAGGTGGTCGTTGGTCAACGCGGCCAACGCCGGATCAGGCCGGCTCAGCTGGGCCACAATGCTTTCGAGCGCTTGCTTGCCGCC
ATTGTTGCTGGCGATAGCCACCACTTGGTCCGGGGTCAGGTTCAGGGGGGCACCCGTCAGTGCATTGCGCGATGCATGCACTGCCTCCAT
TGCGGTCACGCCGCCACGTTTTGCAATCTTCACAAGTTGGCCTGTGTCCAACTGTAACGGCGGACCTCTCAACTCCCCCGCATCCGTGAG
CAAGGCCTCCAGGGCGCGTGCGCCGGACCACTGTTTGCCGACGCCAACCCCGGGGATCAAGGGCGAATTCCAGCACACTGGCGGCCGTTA
CTAGTGGATCCGAGCTCGGTACCAAGCTTGATGCATAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAG
CTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGA
GTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAA
CGCGCGGGGAGAGGCGGTTTGCGTATTGNGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCG
GTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCANGGGATAACGCNNGAAAGAACATGTGAGCAAAANGCCAGCAAAAG

GNCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAG
AGGTGGCGAAACCCGACAGGACTAT

Clone 2 M13F:

NNNNNNNNNNNNNNNNGANTGGGCCNCTAGATGCATGCTCGAGCGGCCGCCAGTGTGATGGATATCTGCAGAATTCGCCCTTGATCGGCG
CGCCAGGCCAAGGCGACGAGGTGGTCGTTGGTCAACGCGGCCAACGCCGGATCAGGCCGGCTCAGCTGGGCCACAATGCTTTCGAGCGCT
TGCTTGCTGCCATCGTGGCTGGCGATAGCCACCACTTGGTCCGGGGTCAGGTTCAGGGGGGCACCCGTCAGTGCATTGCGCGATGCATGC
ACTGCCTCCATTGCGGTCACGCCGCCACGTTTTGCAATCTTCACAAGTTGGCCTGTGTCCAACTGTAACGGCGGACCTCTCAACTCCCCC
GCATCCGTGAGCAAGGCCTCCAGGGCGCGTGCGCCGGACCACTGTTTGCCGACGCCAACCCCGGGGATCAAGGGCGAATTCCAGCACACT
GGCGGCCGTTACTAGTGGATCCGAGCTCGGTACCAAGCTTGATGCATAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAAT
CATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGG
GTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAAT
GAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGC
TGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCANGAAAGAACATGTGAGCAAAAG
GCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGAC
GCTCAAGTCANANGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGNAGCTCCCTCGTGCGCTCTCCTGTTCCGA
CCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGNANNGNNGNGCTTTCTCNNAGCTCACGCTGTAGGTATCTCANTCGGNG
TNGTCNNNGCTCCAANCTGGGNTGNGNGCACNAACCCCCNNNCAGCCCGACCGCTGNNCCTNATCCNGNANNNNTCGTCTNANNNCNNCC
NGNANNNNNACTNATNNNNNNNGNNANCANCNNTGNNANCNGATANCNANNNNGNNATNNNNGCGNNNNNNNNANNNNTNNNNNNNNNA
CNNNNGNTNNCTNAANANNNNNTNNNNNTGNNCNNNNNNTNNNCNNNNNACNTTNNNNANANNNNNNGNNNNCNTNNNNNN

Clone 3 M13F:

CTAGATGCATGCTCGAGCGGCCGCCAGTGTGATGGATATCTGCAGAATTCGCCCTTGGATCCCCGGGGTTGGCGTCGGCAAACAGTGGTC
CGGCGCACGCGCCCTGGAGGCCTTGCTCACGGATGCGGGGGAGTTGAGAGGTCCGCCGTTACAGTTGGACACAGGCCAACTTGTGAAGAT
TGCAAAACGTGGCGGCGTGACCGCAATGGAGGCAGTGCATGCATCGCGCAATGCACTGACGGGTGCCCCCCTGAACCTGACCCCGGACCA
AGTGGTGGCTATCGCCAGCCACATTGGCGGCAAGCAAGCGCTCGAAAGCATTGTGGCCCAGCTGAGCCGGCCTGATCCGGCGTTGGCCGC
GTTGACCAACGACCACCTCGTCGCCTTGGCCTGGCGCGCCGATCAAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGTGGATCCGAG
CTCGGTACCAAGCTTGATGCATAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTG
AAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCAC
ATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGG
CGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTC
AAAGGCGGTAATACGGTTATCCACAGAATCANGGGATAACGCNNAAAGAACATGTGAGCAAAAGGNCAGCAAAAGGCCAGGAACCGTAAA
AAGGCCGCGTTGCTGGCGTTTTTCCATNNCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCANANGTGGCGAAACCCGA
CAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCG
CCTTTCTCCCTTCGGGAAGCGTG

Clone 4 M13F:

CTATAGGGCGAATTGGGCCCTCTAGATGCATGCTCGAGCGGCCGCCAGTGTGATGGATATCTGCAGAATTCGCCCTTGATCGGCGCGCCA
GGCCAAGGCGACGAGGTGGTCGTTGGTCAACGCGGCCAACGCCGGATCAGGCCGGCTCAGCTGGGCCACAATGCTTTCGAGCGCTTGCTT
GCCGCCATCGTGGCTGGCGATAGCCACCACTTGGTCCGGGGTCAGGCCATGGTCCTGGCACAGCACCGGCAACAGCCGCTGCACCGTTTC
GAGCGCTTGCTTGCCGCCATCGTGGCTGGCGATAGCCACCACTTGGTCCGGGGTCAGGTTCAGGGGGGCACCCGTCAGTGCATTGCGCGA
TGCATGCACTGCCTCCATTGCGGTCACGCCGCCACGTTTTGCAATCTTCACAAGTTGGCCTGTGTCCAACTGTAACGGCGGACCTCTCAA
CTCCCCCGCATCCGTGAGCAAGGCCTCCAGGGCGCGTGCGCCGGACCACTGTTTGCCGACGCCAACCCCGGGGATCAAGGGCGAATTCCAG
CACACTGGCGGCCGTTACTAGTGGATCCGAGCTCGGTACCAAGCTTGATGCATAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGG
CGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAG
CCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGC
ATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCG
TTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATC

Clone 5 M13F:

GATTGGGCCCTCTAGATGCATGCTCGAGCGGCCGCCAGTGTGATGGATATCTGCAGAATTCGCCCTTGATCGGCGCGCCAGGCCAAGGCG
ACGAGGTGGTCGTTGGTCAACGCGGCCAACGCCGGATCAGGCCGGCTCAGCTGGGCCACAATGCTTTCGAGCGCTTGCTTGCCGCCATCG
TGGCTGGCGATAGCCACCACTTGGTCCGGAGTCAGGCCATGGTCCTGGCACAGCACCGGCAACAGCCGCTGCACCGTTTCGAGCGCTTGC
TTGCCGCCATCGTGGCTGGCGATAGCCACCACTTGGTCCGGGGTCAGGTTCAGGGGGGCACCCGTCAGTGCATTGCGCGATGCATGCACT
GCCTCCATTGCGGTCACGCCGCCACGTTTTGCAATCTTCACAAGTTGGCCTGTGTCCAACTGTAACGGCGGACCTCTCAACTCCCCCGCA
TCCGTGAGCAAGGCCTCCAGGGCGCGTGCGCCGGACCACTGTTTGCCGACGCCAACCCCGGGGATCAAGGGCGAATTCCAGCACACTGGC
GGCCGTTACTAGTGGATCCGAGCTCGGTACCAAGCTTGATGCATAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCAT
GGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTG
CCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAA
TCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGC
GGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGC

Clone 6 M13F:

TTGTATACGACTCACTATAGGGCGAATTGGGCCCTCTAGATGCATGCTCGAGCGGCCGCCAGTGTGATGGATATCTGCAGAATTCGCCCT
TGATCGGCGCGCCAGGCCAAGGCGACGAGGTGGTCGTTGGTCAACGCGGCCAACGCCGGATCAGGCCGGCTCAGCTGGGCCACAATGCTT
TCGAGCGCTTGCTTGCCGCCATCGTGGCTGGCGATAGCCACCACTTGGTCCGGGGTCAGGCCATGGTCCTGGCACAGCACCGGCAACAGC
CGCTGCACCGTTTCGAGCGCTTGCTTGCCGCCAATGTTGCTGGCGATAGCCACCACTTGGTCCGGGGTCAGGTTCAGGGGGGCACCCGTC
AGTGCATTGCGCGATGCATGCACTGCCTCCATTGCGGTCACGCCGCCACGTTTTGCAATCTTCACAAGTTGGCCTGTGTCCAACTGTAAC
GGCGGACCTCTCAACTCCCCCGCATCCGTGAGCAAGGCCTCCAGGGCGCGTGCGCCGGACCACTGTTTGCCGACGCCAACCCCGGGGATC
AAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGTGGATCCGAGCTCGGTACCAAGCTTGATGCATAGCTTGAGTATTCTATAGTGTC
ACCTAAATAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAA
GCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACC
TGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACT
CGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGC

Clone 7 M13F:

TGGGCCCTCTAGATGCATGCTCGAGCGGCCGCCAGTGTGATGGATATCTGCAGAATTCNCCCTTGATCGGCGCGCCAGGCCAAGGCGACG
AGGTGGTCGTTGGTCAACGCGGCCAACGCCGGATCAGGCCGGCTCAGCTGGGCCACAATGCTTTCGAGCGCTTGCTTGCCGCCATCGTGG
CTGGCGATAGCCACCACTTGGTCCGGGGTCAGGCCATGGTCCTGGCACAGCACCGGCAACAGCCGCTGCACCGTTTCGAGCGCTTGCTTG
CCGCCAATGTTGCTGGCGATAGCCACCACTTGGTCCGGAGTCAGGCCATGGTCCTGGCACAGCACCGGCAACAGCCGCTGCACCGTTTCG
AGCGCTTGCTTGCCGCCATCGTGGCTGGCGATAGCCACCACTTGGTCCGGGGTCAGGTTCAGGGGGGCACCCGTCAGTGCATTGCGCGAT
GCATGCACTGCCTCCATTGCGGTCACGCCGCCACGTTTTGCAATCTTCACAAGTTGGCCTGTGTCCAACTGTAACGGCGGACCTCTCAAC
TCCCCCGCATCCGTGAGCAAGGCCTCCAGGGCGCGTGCGCCGGACCACTGTTTGCCGACGCCAACCCCGGGGATCAAGGGCGAATTCCAG
CACACTGGCGGCCGTTACTAGTGGATCCGAGCTCGGTACCAAGCTTGATGCATAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGG
CGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAG
CCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGC
ATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCG
TTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATA

Clone 8 M13F:

TCTAGATGCATGCTCGAGCGGCCGCCAGTGTGATGGATATCTGCAGAATTCGCCCTTGATCGGCGCGCCAGGCCAAGGCGACGAGGTGGT
CGTTGGTCAACGCGGCCAACGCCGGATCAGGCCGGCTCAGCTGGGCCACAATGCTTTCGAGCGCTTGCTTGCCGCCATCGTGGCTGGCGA
TAGCCACCACTTGGTCCGGGGTCAGGCCATGGTCCTGGCACAGCACCGGCAACAGCCGCTGCACCGTTTCGAGCGCTTGCTTGCCGCCAA
TGTTGCTGGCGATAGCCACCACTTGGTCCGGGGTCAGGCCATGGTCCTGGCACAGCACCGGCAACAGCCGCTGCACCGTTTCGAGCGCTT
GCTTGCCGCCATCGTGGCTGGCGATAGCCACCACTTGGTCCGGAGTCAGGCCATGGTCCTGGCACAGCACCGGCAACAGCCGCTGCACCG
TTTCGAGCGCTTGCTTGCCGCCATCGTGGCTGGCGATAGCCACCACTTGGTCCGGGGTCAGGTTCAGGGGGGCACCCGTCAGTGCATTGC
GCGATGCATGCACTGCCTCCATTGCGGTCACGCCGCCACGTTTTGCAATCTTCACAAGTTGGCCTGTGTCCAACTGTAACGGCGGACCTC
TCAACTCCCCCGCATCCGTGAGCAAGGCCTCCAGGGCGCGTGCGCCGGACCACTGTTTGCCGACGCCAACCCCGGGGATCAAGGGCGAAT
TCCAGCACACTGGCGGCCGTTACTAGTGGATCCGAGCTCGGTACCAAGCTTGATGCATAGCTTGAGTATTCTATAGTGTCACCTAAATAG
CTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTG
TAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCA
GCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTC
GGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTA

Clone 9 M13F:

CTAGATGCATGCTCGAGCGGCCGCCAGTGTGATGGATATCTGCAGAATTCGCCCTTGATCCCGGGGTTGGCGTCGGCAAACAGTGGTCCG
GCGCACGCGCCCTGGAGGCCTTGCTCACGGATGCGGGGGAGTTGAGAGGTCCGCCGTTACAGTTGGACACAGGCCAACTTGTGAAGATTG
CAAAACGTGGCGGCGTGACCGCAATGGAGGCAGTGCATGCATCGCGCAATGCACTGACGGGTGCCCCCCTGAACCTGACCCCGGACCAAG
TGGTGGCTATCGCCAGCCACGATGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGA
CTCCGGACCAAGTGGTGGCTATCGCCAGCCACGATGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGG
ACCATGGCCTGACCCCGGACCAAGTGGTGGCTATCGCCAGCAACATTGGCGGCAAGCAAGCGCTCGAAACGGTGCAGCGGCTGTTGCCGG
TGCTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATCGCCAGCAACATTGGCGGCAAGCAAGCGCTCGAAACGGTGCAGC
GGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATCGCCAGCCACGATGGCGGCAAGCAAGCGCTCG
AAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCTATCGCCAGCCACGATGGCGGCA
AGCAAGCGCTCGAAAGCATTGTGGCCCAGCTGAGCCGGCCTGATCCGGCGTTGGCCGCGTTGACCAACGACCACCTCGTCGCCTTGGCCT
GGCGCGCCGATCAAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGTGGATCCGANCTCGGTACCAAGCTTGATGCATAGCTTGAGTA
TTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACA
TACGAGCC

Clone 10 M13F:

GGAAATCTATAGGGCGATTGGGCCTCTAGATGCATGCTCGAGCGGCCGCCAGTGTGATGGATATCTGCAGAATTCGCCCTTGATCGGCGC
GCCAGGCCAAGGCGACGAGGTGGTCGTTGGTCAACGCGGCCAACGCCGGATCAGGCCGGCTCAGCTGGGCCACAATGCTTTCGAGCGCTT
GCTTGCCGCCATCGTGGCTGGCGATAGCCACCACTTGGTCCGGGGTCAGGCCATGGTCCTGGCACAGCACCGGCAACAGCCGCTGCACCG
TTTCGAGCGCTTGCTTGCCGCCAATGTTGCTGGCGATAGCCACCACTTGGTCCGGGGTCAGGCCATGGTCCTGGCACAGCACCGGCAACA
GCCGCTGCACCGTTTCGAGCGCTTGCTTGCCGCCATCGTGGCTGGCGATAGCCACCACTTGGTCCGGGGTCAGGCCATGGTCCTGGCACA
GCACCGGCAACAGCCGCTGCACCGTTTCGAGCGCTTGCTTGCCGCCAATGTTGCTGGCGATAGCCACCACTTGGTCCGGGGTCAGGCCAT
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GGGTCAGGCCATGGTCCTGGCACAGCACCGGCAACAGCCGCTGCACCGTTTCGAGCGCTTGCTTGCCGCCATCGTGGCTGGCGATAGCCA
CCACTTGGTCCGGAGTCAGGCCATGGTCCTGGCACAGCACCGGCAACAGCCGCTGCACCGTTTCGAGCGCTTGCTTGCCGCCATCGTGGC
TGGCGATAGCCACCACTTGGTCCGGAGTCAGGCCATGGTCCTGGCACAGCACCGGCAACAGCCGCTGCACCGTTTCGAGCGCTTGCTTGC
CGCCATTGTTGCTGGCGATAGCCACCACTTGGTCCGGGGTCAGGCCATGGTCCTGGCACAGCACCGGCAACAGCCGCTGCACCGTTTCGA
GCGCTTGCTTGCCGCCATTGTTGCTGGCGATAGCCACCACTTGGTCCGGAGTCAGGCCATGGTCCTGGCACAGCACCGGCAACAGCCGCT
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CATTGCCCGATGCATGCATTGCCTCCATTGCGGTCACGCCGCCACGTTTTGCAATCTTCACAGGTTGGCCTGGGTCAAATGGAAACGGCG
GACCTCTCAACTCCCCCGGATCCGTGAGCAAGGCCTCAAGGGCCCTGGCGCCGGAACCATGTTTGCCAACCCCAACCCCGGGGGACAAAG
GCAAATTTCAGCAAATTGGGCGCCGTAACAATGGGATCCAAGCTCGTTAACAACTTGTAGCCAAACCTTGGATTTCTAAAAGTGCACCAA
ATTTACTGGGGGAAAAAATGGAACAAAGGTTTTCTTTGGGGAATATTGTTCGCCCCCAATTCTCCACAATAACACGCCGAGAAAAATAAG
AGAAACCGGGGGGGGCAAAAGAAAGAAACAACAATAATTGTTGGGCCCCACGGCCCTTCTTTCAGAAAAACTTCCGCGTCAAAGTTAATA
ACACCCCCGGGAGGGGAGGGTTTTGTTTTGGGCTTCCCTTTA

Clone 10 M13R:

GGGCGCTAATTTTAGGTGACCTATAGATACTCAGCTATGCATCAAGCTTGGTACCGAGCTCGGATCCACTAGTAACGGCCGCCAGTGTGC
TGGAATTCGCCCTTGATCCCCGGGGTTGGCGTCGGCAAACAGTGGTCCGGCGCACGCGCCCTGGAGGCCTTGCTCACGGATGCGGGGGAG
TTGAGAGGTCCGCCGTTACAGTTGGACACAGGCCAACTTGTGAAGATTGCAAAACGTGGCGGCGTGACCGCAATGGAGGCAGTGCATGCA
TCGCGCAATGCACTGACGGGTGCCCCCCTGAACCTGACCCCGGACCAAGTGGTGGCTATCGCCAGCCACGATGGCGGCAAGCAAGCGCTC
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GCGCTCGAAACGGTGCACCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACAAATGGTGGCTATCGCCACCACCAATG
GCGGCAAGCAACCGTCCAAAGCATTGGGGCCCACTTGACCCGCCCGATCCGGGTTTGGCCCGTTGACAAAAGACACCTTCGCCCTTTGGC
CTTGGCGCCCATCAAGGGGAATTTTCGAAAATTCCTACCATTGGGGGCCGCTCGAAATAGCATTAGAGGGCACATTCCCCCTTATGGAGT
CTTATACAATTCATGGCCGCTGTTTTACGCCTGGCAGGGGAAACCCGGGGTTCCACAATAAACCGTTTGGCAACACCCCTTTCTCGCTGT
GGGTTAAAAAGAAGGCCCCCCATGCCTCTCCAAAGG

pBasicS1-GFPss plasmid sequence (sequences only between primer binding sites 572 and 390 are shown)

GAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCAGCTTGGTACCGAGCTCGGATCCACTAGTAACGGCCGCC
AGTGTGCTGGAATTCTGCAGATAGCATGCTAACTCGAGATGGGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAAT
TAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTT
GCACTACTGGAAAACTACCTGTTCCTTGGCCAACACTTGTCACTACTTTCTGTTATGGTGTACAATGCTTTTCAAGATACCCAGATCATA
TGAAGCAGCACGACTTCTTCAAGAGCGCCATGCCTGAGGGATACGTACAGGAGAGGACCATCTTCTTCAAGGACGACGGGAACTACAAGA
CACGTGCTGAAGTCAAGTTTGAGGGAGACACCCTCGTCAACAGGATCGAGCTTAAGGGAATCGATTTCAAGGAGGACGGAAACATCCTCG
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ACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTG
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GATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGA
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TAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGA
GGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCTTGGCCAACACTTGTCAC
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CGTACAGGAGAGGACCATCTTCTTCAAGGACGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAGGGAGACACCCTCGTCAACAG
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TCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAA
CGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGAACTATACAAATAACTCGACAT
ACAGGCAGCCCATCAGTCCGGGACGGCGTCAGCGGGAGAGCCGTTGTAAGGCGGCAGACTTTGCTCATGTTACCGATGCTATTCGGAAGA
ACGGCAACTAAGCTGCCGGGTTTGAAACACGGATGATCTCG

***GFP* repeat artifact product**

1Kb artifact product (gel isolated from figure 10 and sequenced using primer 572)

GCGACACGCACCCGCCTCTCAGCTTGGTACCGAGCTCGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTCTGCAGATAGCATGCTA
ACTCGAGATGGGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTC
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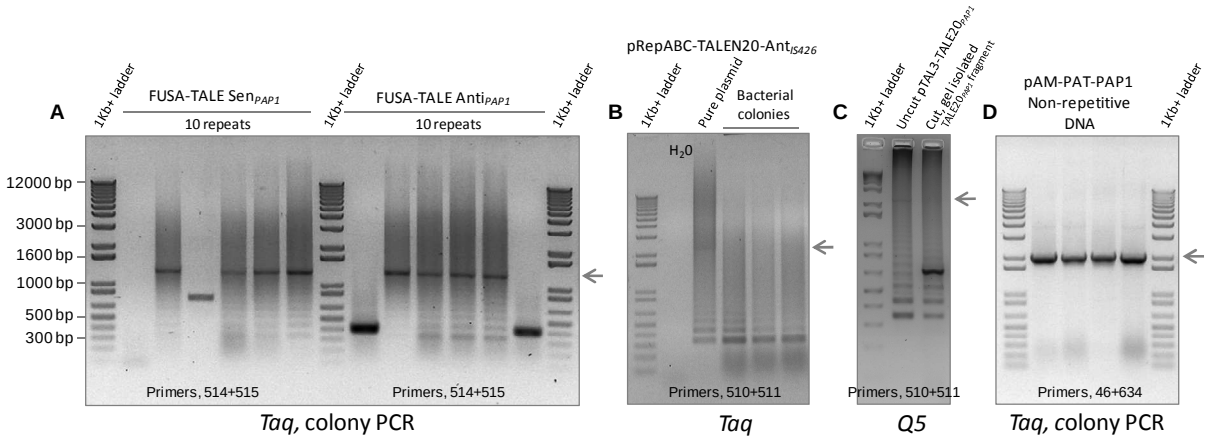
TCTTTGTGTGTAGTGTTATATATTAAGAATAGTGTAGTGGATAATTAT

Supplementary Tables

Supplementary Table 1. Primers used

Primer name	Sequence (5’-3’)
46	AACACATGAGCGAAACCCTATAA
239	GATCGGAATCGACTAACAGAACA
284	GCCTTTCACGTAGTGGACAAAT
310	AGAACTCTGTAGCACCGCCTAC
314	ATCGAGCTCAAGTTATCATTCGC
390	CGAGATCATCCGTGTTTCAA
510	GATCCCCGGGTTGGCGTCGGCAAACAGTGG
511	TAATGGCGCGCCGGCGACGAGGTGGTCGTTGG
514 (pCR8_F1)	TTGATGCCTGGCAGTTCCCT
515 (pCR8_R1)	CGAACCGAACAGGCTTATGT
534	GATCCCCGGGGTTGGCGTCGGCAAACAGTGG
535	GATCGGCGCGCCAGGCCAAGGCGACGAGGTGGTC
572	GAGTCAGTGAGCGAGGAAGC
634	AAGCGGATCCTTGACCAATTTACTTATACCTTT
825	GATCTGTACAAGAACCACCACCACC

Supplementary Figures



Supplementary Figure 1. Primers and vector backbones are not the reason for PCR artifacts. (A) Typical colony PCR results obtained from *E. coli* colonies containing assembled 10 TALE repeats in pFusA vector backbone and using recommended primers (514+515) by the Golden Gate protocol. If the assembly of desired repeats is successful, a laddering effect is observed. The rest of the colonies amplifiying distinct fragments are either empty or contain re-arrangements lacking TALE repeats. (B) Such laddering effects are observed in colony PCRs from different vector backbones as well, indicating that artifacts are generated independent of plasmid backbones. Similarly, the use of pure plasmids does not make a difference as well. (C) TALE DNA binding repeats were excised from TAL3-TALE20$_{PAP1}$ vector by restriction digestion (NotI-AatII fragment) and the fragment containing 20 TALE DNA binding repeats is gel isolated. PCR analysis was carried out using either the uncut vector or the gel isolated TALE fragment as template using primers 510 and 511. Laddering effect was observed in both templates, indicating that not the vector backbone but the cloned repetitive DNA is the reason. (D) Such laddering effects were not observed in colony PCRs with bacteria containing non-repetitive DNAs. PCR conditions are given below.

Supplementary Figure 1A, *Taq* DNA polymerase colony PCR condition

Component	**Stock**		**Final**		**25µl reaction**	
dd H2O	-	-	-	-	20,88	µl
Polymerase reaction buffer+2mM MgSO4	10	X	1	X	2,5	µl
dNTP-mix	10	mM	0,2	mM	0,5	µl
Primer –Frw 514	10	µM	0,2	µM	0,5	µl
Primer –Rev 515	10	µM	0,2	µM	0,5	µl
Template *E. coli* colonies containing either pFUSA-TALE-sense or pFUSA-TALE-Antisense TALE repeat assemblies						µl
Taq DNA polymerase (NE Biolabs, M0273S)	5	U/µl	0,025	U/µl	0,12	µl

Cycling conditions

Denature	95°C	5 min	
35 cycles of			
Denature	95°C	15 sec	
Anneal	60°C	30 sec	
Extend	68°C	1 min/Kb	1 min 45 sec
Final extension	68°C	5 min	

Supplementary Figure 1B, *Taq* DNA polymerase colony PCR condition

Component	**Stock**		**Final**		**25µl reaction**	
dd H2O	-	-	-	-	20,88	µl
Polymerase reaction buffer+2mM MgSO4	10	X	1	X	2,5	µl
dNTP-mix	10	mM	0,2	mM	0,5	µl
Primer –Frw 510	10	µM	0,2	µM	0,5	µl
Primer –Rev 511	10	µM	0,2	µM	0,5	µl
Template bacterial colonies containing pRepABC-TALEN-Ant.$_{IS426}$ plasmid or the pure plasmid						µl
Taq DNA polymerase (NE Biolabs, M0273S)	5	U/µl	0,025	U/µl	0,12	µl

Cycling conditions

Denature	95°C	5 min	
30 cycles of			
Denature	95°C	30 sec	
Anneal	60°C	30 sec	
Extend	68°C	1 min/Kb	2 min
Final extension	68°C	2 min	

Supplementary Figure 1C, *Q5* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	31,5	µl
Polymerase reaction buffer	5	X	1	X	10	µl
dNTP-mix	10	mM	0,2	mM	1	µl
Primer -Frw	10	µM	0,5	µM	2,5	µl
Primer -Rev	10	µM	0,5	µM	2,5	µl
Template DNA (gel isolated TALE fragment from pTAL2 plasmid)	5	ng/µl	0,2	ng/µl	2	µl
Q5 DNA polymerase (NE Biolabs, M04478G)	2	U/µl	0,02	U/µl	0,5	µl

Cycling conditions

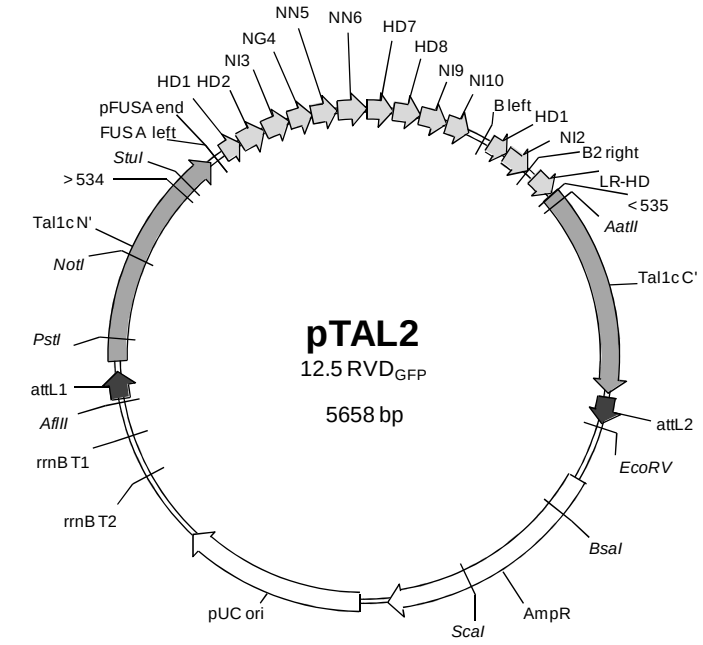
Denature	98°C	2 min	
34 cycles of			
Denature	98°C	10 sec	
Anneal	60°C	10 sec	
Extend	72°C	30 sec/Kb	45 sec
Final extension	72°C	2 min	

Supplementary Figure 1D, *Taq* DNA polymerase colony PCR condition

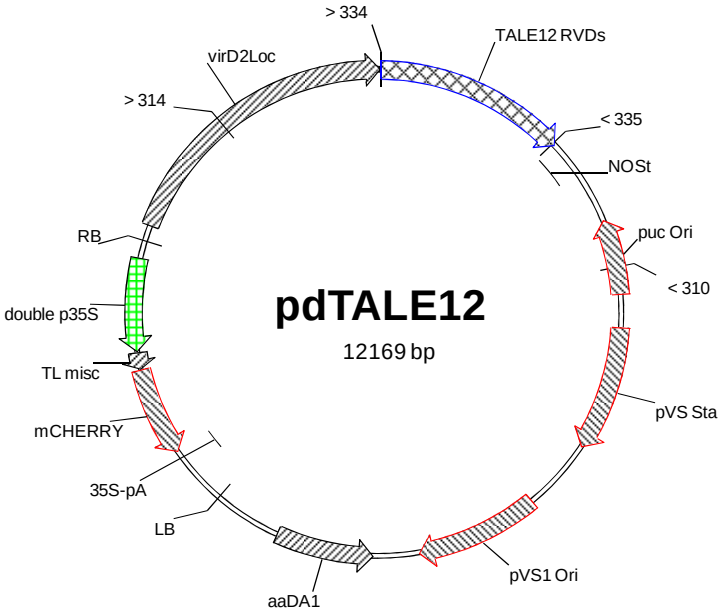
Component	**Stock**		**Final**		**25µl reaction**	
dd H2O	-	-	-	-	20,88	µl
Polymerase reaction buffer+2mM MgSO4	10	X	1	X	2,5	µl
dNTP-mix	10	mM	0,2	mM	0,5	µl
Primer –Frw 46	10	µM	0,2	µM	0,5	µl
Primer –Rev 634	10	µM	0,2	µM	0,5	µl
Template *E. coli* colonies containing pAM-PAT-PAP1 plasmid						µl
Taq DNA polymerase (NE Biolabs, M0273S)	5	U/µl	0,025	U/µl	0,12	µl

Cycling conditions

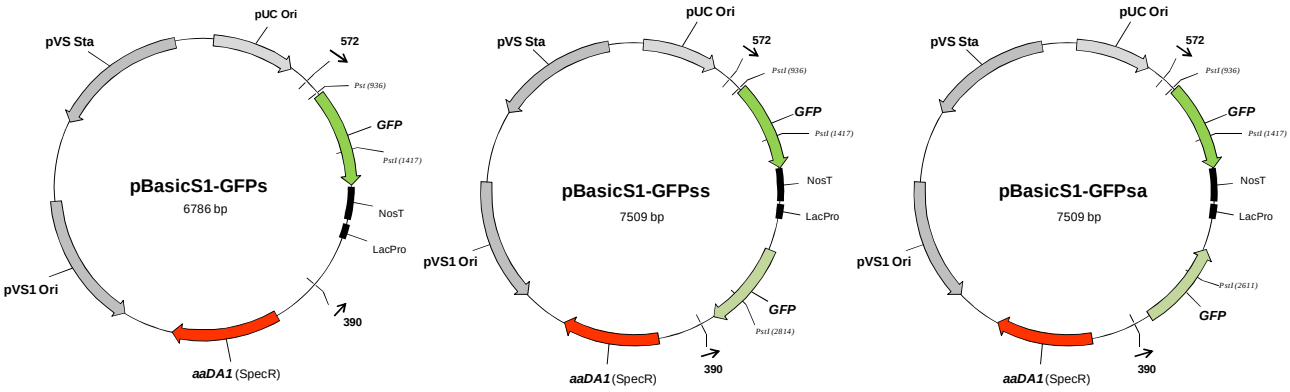
Denature	95°C	5 min	
30 cycles of			
Denature	95°C	15 sec	
Anneal	60°C	30 sec	
Extend	68°C	1 min/Kb	2 min
Final extension	68°C	5 min	



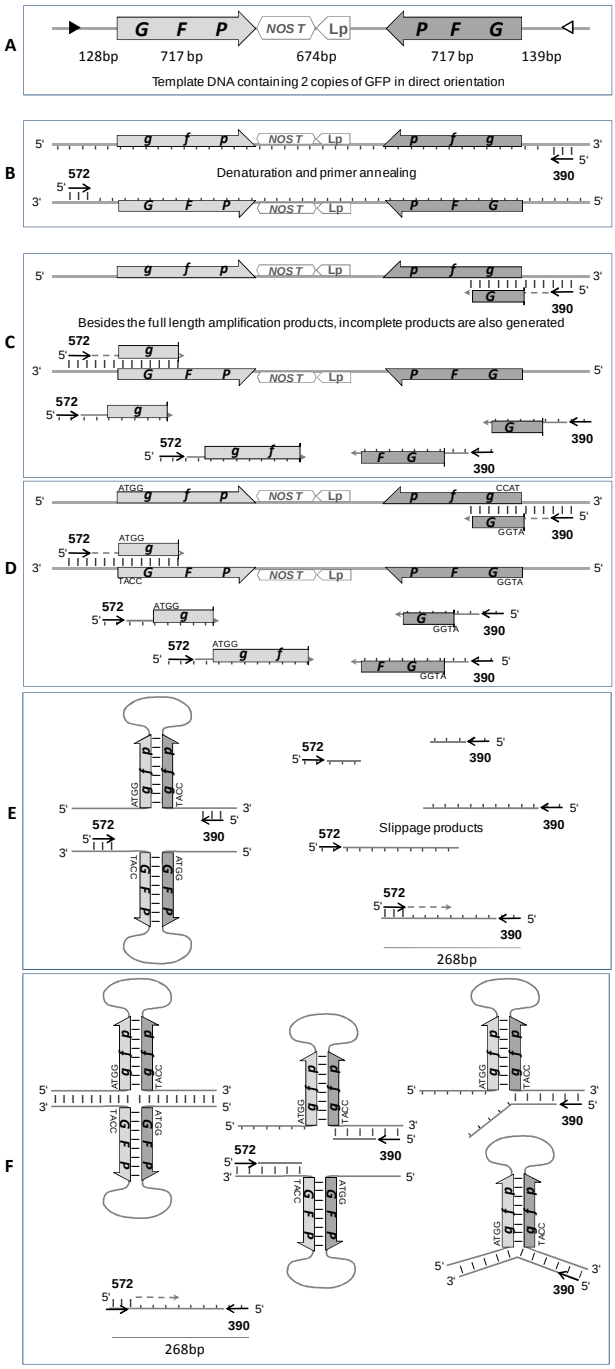
Supplementary Figure 2. Plamid map of pTAL2 vector with 12.5 RVD_{GFP}.



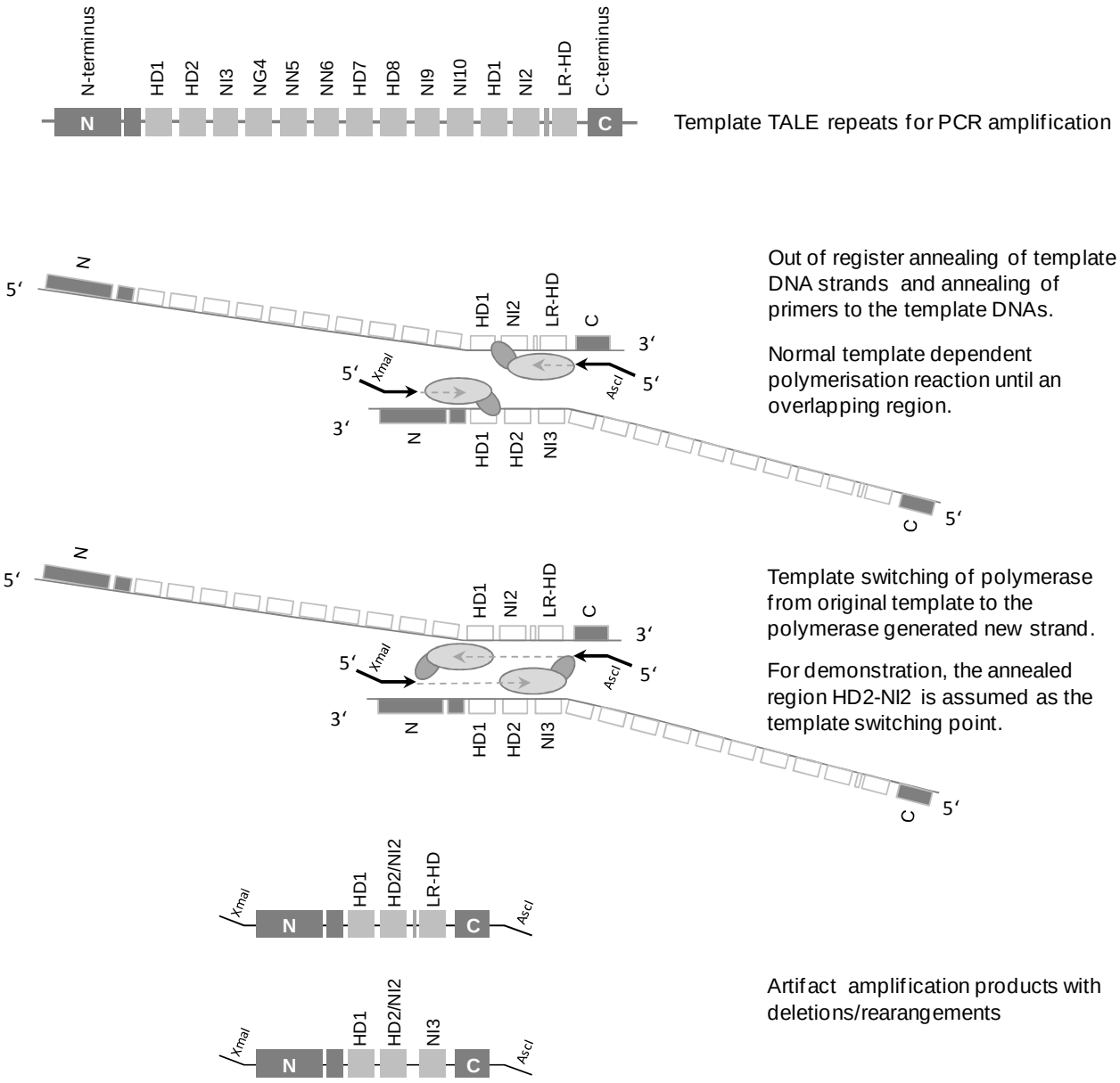
Supplementary Figure 3. Plamid map of pdTALE12.



Supplementary Figure 4. pBasicS1-GFP plasmid maps used in experiments.



Supplementary Figure 5. The model of *GFP* sequences arranged as inverted repeats separated by a linker DNA.



Supplementary Figure 6. Template switching by DNA polymerase could also explain how the truncated PCR artifacts are generated.

Supplementary material and methods

PCR conditions

Figure 1, *Pfu* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	40	µl
Polymerase reaction buffer	10	X	1	X	5	µl
dNTP-mix	10	mM	0,2	mM	1	µl
Primer -Frw	10	µM	0,2	µM	1	µl
Primer -Rev	10	µM	0,2	µM	1	µl
Template DNA (pTAL2 plasmid)	5	ng/µl	0,2	ng/µl	1	µl
Pfu DNA polymerase (Bioline)	2,5	U/µl	0,05	U/µl	1	µl

Cycling conditions

Denature	95°C	2 min	
33 cycles of			
Denature	95°C	15 sec	
Anneal	60°C	30 sec	
Extend	72°C	1 min/Kb	3 min (extended)
Final extension	72°C	5 min	

Figure 1, *Taq* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	39,75	µl
Polymerase reaction buffer+2mM MgSO4	10	X	1	X	5	µl
dNTP-mix	10	mM	0,2	mM	1	µl
Primer -Frw	10	µM	0,2	µM	1	µl
Primer -Rev	10	µM	0,2	µM	1	µl
Template DNA (pTAL2 plasmid)	5	ng/µl	0,2	ng/µl	2	µl
Taq DNA polymerase (NE Biolabs, M0273S)	5	U/µl	0,025	U/µl	0,25	µl

Cycling conditions

Denature	95°C	2 min	
33 cycles of			
Denature	95°C	15 sec	
Anneal	60°C	30 sec	
Extend	68°C	1 min/Kb	2 min
Final extension	68°C	5 min	

Figure 2A, *Q5* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	31,5	µl
Polymerase reaction buffer	5	X	1	X	10	µl
dNTP-mix	10	mM	0,2	mM	1	µl
Primer -Frw	10	µM	0,5	µM	2,5	µl
Primer -Rev	10	µM	0,5	µM	2,5	µl
Template DNA (pTAL2 plasmid)	1,755	ng/µl	0,07	ng/µl	2	µl
Q5 DNA polymerase (NE Biolabs, M04478G)	2	U/µl	0,02	U/µl	0,5	µl

Cycling conditions

Denature	98°C	2 min	
34 cycles of			
Denature	98°C	10 sec	
Anneal	60°C	10 sec	
Extend	72°C	30 sec/Kb	45 sec
Final extension	72°C	2 min	

Figure 5, *Phusion* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	30	µl
Polymerase reaction buffer	5	X	1	X	10	µl
MgCl2 (supplied with enzyme)	25	mM	0,75	mM	1,5	µl
dNTP-mix	10	mM	0,2	mM	1	µl
Primer -Frw	10	µM	0,5	µM	2,5	µl
Primer -Rev	10	µM	0,5	µM	2,5	µl
Template DNA (pTAL2 plasmid)	5	ng/µl	0,2	ng/µl	2	µl
Phusion DNA polymerase (NE Biolabs, M0530S)	2	U/µl	0,02	U/µl	0,5	µl

Cycling conditions

Denature	98°C	2 min	
33 cycles of			
Denature	98°C	15 sec	
Anneal	60°C	15 sec	
Extend	72°C	15 sec/Kb	25 sec
Final extension	72°C	2 min	

Figure 5, *Q5* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	31,5	µl
Polymerase reaction buffer	5	X	1	X	10	µl
dNTP-mix	10	mM	0,2	mM	1	µl
Primer -Frw	10	µM	0,5	µM	2,5	µl
Primer -Rev	10	µM	0,5	µM	2,5	µl
Template DNA (pTAL2 plasmid)	1,755	ng/µl	0,07	ng/µl	2	µl
Q5 DNA polymerase (NE Biolabs, M04478G)	2	U/µl	0,02	U/µl	0,5	µl

Cycling conditions

Denature	98°C	2 min	
34 cycles of			
Denature	98°C	10 sec	
Anneal	60°C	10 sec	
Extend	72°C	30 sec/Kb	45 sec
Final extension	72°C	2 min	

Figure 5, *Primestar GXL* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	31,5	µl
Polymerase reaction buffer	5	X	1	X	10	µl
dNTP-mix	2,5	mM	0,2	mM	4	µl
Primer -Frw	10	µM	0,5	µM	2,5	µl
Primer -Rev	10	µM	0,5	µM	2,5	µl
Template DNA (pTAL2 plasmid)	1,755	ng/µl	0,07	ng/µl	2	µl
Primestar GXL DNA polymerase (TAKARA, R050A)	1,25	U/µl	0,025	U/µl	1	µl

Cycling conditions

Denature	98°C	2 min	
34 cycles of			
Denature	98°C	10 sec	
Anneal	60°C	15 sec	
Extend	68°C	1 min/Kb	1 min 30 sec
Final extension	68°C	2 min	

Figure 5, *PrimeSTAR GXL* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	31,5	µl
Polymerase reaction buffer	5	X	1	X	10	µl
dNTP-mix (supplied with the polymerase)	2,5	mM	0,2	mM	4	µl
Primer -Frw	10	µM	0,5	µM	2,5	µl
Primer -Rev	10	µM	0,5	µM	2,5	µl
Template DNA (pTAL2 plasmid)	1,755	ng/µl	0,07	ng/µl	2	µl
PrimeSTAR GXL DNA polymerase (TAKARA, R050A)	1,25	U/µl	0,025	U/µl	1	µl

Cycling conditions

Denature	98°C	2 min	
34 cycles of			
Denature	98°C	10 sec	
Anneal	60°C	15 sec	
Extend	68°C	1 min/Kb	1 min 30 sec
Final extension	68°C	2 min	

Figure 5, *PrimeSTAR Max* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	21	µl
PrimeSTAR® Max **Premix**, TAKARA, R045A	2	X	1	X	25	µl
Primer -Frw	10	µM	0,2	µM	1	µl
Primer -Rev	10	µM	0,2	µM	1	µl
Template DNA (pTAL2 plasmid)	1,755	ng/µl	0,07	ng/µl	2	µl

Cycling conditions

Denature	98°C	2 min	
34 cycles of			
Denature	98°C	10 sec	
Anneal	60°C	5 sec	
Extend	72°C	5 sec/Kb	10 sec
Final extension	72°C	2 min	

Figure 5, *PrimeSTAR HS* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	31,5	µl
Polymerase reaction buffer	5	X	1	X	10	µl
dNTP-mix (supplied with the polymerase)	2,5	mM	0,2	mM	4	µl
Primer -Frw	10	µM	0,2	µM	1	µl
Primer -Rev	10	µM	0,2	µM	1	µl
Template DNA (pTAL2 plasmid)	1,755	ng/µl	0,07	ng/µl	2	µl
PrimeSTAR HS DNA polymerase (TAKARA, R010A)	2, 5	U/µl	0,025	U/µl	0,5	µl

Cycling conditions

Denature	98°C	2 min	
34 cycles of			
Denature	98°C	10 sec	
Anneal	60°C	10 sec	
Extend	72°C	1 min/Kb	1 min 30 sec
Final extension	72°C	2 min	

Figure 5, *AccuPrime Pfx* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	39,5	µl
Polymerase reaction buffer+dNTPs (premix)	10	X	1	X	5	µl
Primer -Frw	10	µM	0,3	µM	1,5	µl
Primer -Rev	10	µM	0,3	µM	1,5	µl
Template DNA (pTAL2 plasmid)	1,755	ng/µl	0,07	ng/µl	2	µl
AccuPrime Pfx DNA polymerase (Life Technologies, 12344-024)	2,5	U/µl	0,025	U/µl	0,5	µl

Cycling conditions

Denature	95°C	2 min	
34 cycles of			
Denature	95°C	15 sec	
Anneal	60°C	30 sec	
Extend	68°C	1 min/Kb	1 min 30 sec
Final extension	68°C	2 min	

Figure 6, *DeepVentR* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	31,65	µl
Polymerase reaction buffer	5	X	1	X	10	µl
MgSO4	100	mM	2	mM	1	µl
dNTP-mix	10	mM	0,2	mM	1	µl
Primer -Frw	10	µM	0,4	µM	2	µl
Primer -Rev	10	µM	0,4	µM	2	µl
Template DNA (pTAL2 plasmid)	1,75	ng/µl	0,07 (0,14)	ng/µl	2-(4)	µl
DeepVentR DNA polymerase (NE Biolabs, M0258S)	2	U/µl	0,014 (0,004-0,03)	U/µl	0,35 (varied)	µl

Cycling conditions

Denature	95°C	2 min	
34 cycles of			
Denature	95°C	15 sec	
Anneal	60°C	15 sec	
Extend	72°C	1 min/Kb	1 min 30 sec
Final extension	72°C	2 min	

Figure 7, *DeepVentR* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	29,65	µl
Polymerase reaction buffer	5	X	1	X	10	µl
MgSO4	100	mM	2	mM	1	µl
BSA (NE Biolabs, B9000S, various dilutions) or RNAseA (Life technologies, 12091-039, various dilutions) or hexamer oligo (Life technologies, various dilutions) or ET SSB (NE Biolabs M2401S, various dilutions)					2	µl
dNTP-mix	10	mM	0,2	mM	1	µl
Primer -Frw	10	µM	0,4	µM	2	µl
Primer -Rev	10	µM	0,4	µM	2	µl
Template DNA (pTAL2 plasmid)	1,75	ng/µl	0,07	ng/µl	2	µl
DeepVentR DNA polymerase (NE Biolabs, M0258S)	2	U/µl	0,014	U/µl	0,35	µl

Cycling conditions

Denature	95°C	2 min	
34 cycles of			
Denature	95°C	15 sec	
Anneal	60°C	15 sec	
Extend	72°C	1 min/Kb	1 min 30 sec
Final extension	72°C	2 min	

Figure 8, *Taq* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	38,75	µl
Polymerase reaction buffer+2mM MgSO4	10	X	1	X	5	µl
ET SSB , NE Biolabs M2401S (50ng/ul)	50	ng/µl	2	ng/µl	2	µl
dNTP-mix	10	mM	0,2	mM	1	µl
Primer -Frw	10	µM	0,2	µM	1	µl
Primer -Rev	10	µM	0,2	µM	1	µl
Template DNA (pTAL2 plasmid)	5	ng/µl	0,2	ng/µl	2	µl
Taq DNA polymerase (NE Biolabs, M0273S)	5	U/µl	0,025	U/µl	0,25	µl

Cycling conditions

Denature	95°C	2 min	
34 cycles of			
Denature	95°C	15 sec	
Anneal	60°C	30 sec	
Extend	68°C	1 min/Kb	2 min
Final extension	68°C	5 min	

Figure 8, *AccuPrime Pfx* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	38,5	µl
Polymerase reaction buffer+dNTPs (premix)	10	X	1	X	5	µl
ET SSB , NE Biolabs M2401S (50ng/ul)	50	ng/µl	2	ng/µl	2	µl
Primer -Frw	10	µM	0,3	µM	1,5	µl
Primer -Rev	10	µM	0,3	µM	1,5	µl
Template DNA (pTAL2 plasmid)	1,755	ng/µl	0,07	ng/µl	2	µl
AccuPrime Pfx DNA polymerase (Life Technologies, 12344-024)	2,5	U/µl	0,025	U/µl	0,5	µl

Cycling conditions

Denature	95°C	2 min	
34 cycles of			
Denature	95°C	15 sec	
Anneal	60°C	30 sec	
Extend	68°C	1 min/Kb	1 min 30 sec
Final extension	68°C	2 min	

Figure 9, *Taq* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	39,75	µl
Polymerase reaction buffer+2mM MgSO4	10	X	1	X	5	µl
dNTP-mix	10	mM	0,2	mM	1	µl
Primer -Frw	10	µM	0,2	µM	1	µl
Primer -Rev	10	µM	0,2	µM	1	µl
Template DNA (pdTALE12 plasmid)	1,75	ng/µl	0,07	ng/µl	2	µl
Taq DNA polymerase (NE Biolabs, M0273S)	5	U/µl	0,025	U/µl	0,25	µl

Cycling conditions

Denature	94°C	4 min	
34 cycles of			
Denature	94°C	30 sec	
Anneal	60°C	30 sec	
Extend	68°C	1 min/Kb	4 min 30 sec
Final extension	68°C	10 min	

Figure 10, *Taq* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	39,75	µl
Polymerase reaction buffer+2mM MgSO4	10	X	1	X	5	µl
dNTP-mix	10	mM	0,2	mM	1	µl
Primer -Frw	10	µM	0,2	µM	1	µl
Primer -Rev	10	µM	0,2	µM	1	µl
Template DNA (pBasicS1-GFP plasmids)	5	ng/µl	0,2	ng/µl	2	µl
Taq DNA polymerase (NE Biolabs, M0273S)	5	U/µl	0,025	U/µl	0,25	µl

Cycling conditions

Denature	94°C	4 min	
34 cycles of			
Denature	94°C	30 sec	
Anneal	60°C	30 sec	
Extend	68°C	1 min/Kb	2 min 40 sec
Final extension	68°C	10 min	

Figure 12, *PrimeSTAR HS* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	31,5	µl
Polymerase reaction buffer	5	X	1	X	10	µl
dNTP-mix (supplied with the polymerase)	2,5	mM	0,2	mM	4	µl
Primer -Frw	10	µM	0,2	µM	1	µl
Primer -Rev	10	µM	0,2	µM	1	µl
Template DNA (pBasicS1-GFP plasmids)	5	ng/µl	0,2	ng/µl	2	µl
PrimeSTAR HS DNA polymerase (TAKARA, R010A)	2, 5	U/µl	0,025	U/µl	0,5	µl

Cycling conditions

Denature	98°C	2 min	
34 cycles of			
Denature	98°C	10 sec	
Anneal	60°C	10 sec	
Extend	72°C	1 min/Kb	2 min 40 sec
Final extension	72°C	5 min	

Figure 12, *DeepVentR* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	31,65	µl
Polymerase reaction buffer	5	X	1	X	10	µl
MgSO4	100	mM	2	mM	1	µl
dNTP-mix	10	mM	0,2	mM	1	µl
Primer -Frw	10	µM	0,4	µM	2	µl
Primer -Rev	10	µM	0,4	µM	2	µl
Template DNA (pBasicS1-GFP plasmids)	5	ng/µl	0,2	ng/µl	2	µl
DeepVentR DNA polymerase (NE Biolabs, M0258S)	2	U/µl	0,014	U/µl	0,35	µl

Cycling conditions

Denature	95°C	5 min	
34 cycles of			
Denature	95°C	30 sec	
Anneal	60°C	15 sec	
Extend	72°C	1 min/Kb	2 min 40 sec
Final extension	72°C	5 min	

Figure 13, *Taq* DNA polymerase PCR condition

Component	**Stock**		**Final**		**50µl reaction**	
dd H2O	-	-	-	-	39,75	µl
Polymerase reaction buffer+2mM MgSO4	10	X	1	X	5	µl
dNTP-mix	10	mM	0,2	mM	1	µl
Primer -Frw	10	µM	0,2	µM	1	µl
Primer -Rev	10	µM	0,2	µM	1	µl
Template DNA (pBasicS1-GFP plasmids)	5	ng/µl	0,2	ng/µl	2	µl
Taq DNA polymerase (NE Biolabs, M0273S)	5	U/µl	0,025	U/µl	0,25	µl

Cycling conditions

Denature	94°C	4 min	
34 cycles of			
Denature	94°C	30 sec	
Anneal	60°C	30 sec	
Extend	68°C	1 min/Kb	5 min
Final extension	68°C	10 min	

dNTP-mix, if not supplied with the individual polymerases, was obtained from NE Biolabs (N0447S).

1. Cermak, T. et al. Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic Acids Res* **39**, e82 (2011).