pCFD3(v+)

Vector usage: express single sgRNA for in vitro and in vivo

Rules:

1. Forward primer: add "gtcg" to 5'-end of sgRNA

2. Reverse primer: add "aaac" to 5'-end of reverse complement of sgRNA

Examples:

Ex 1) starts with G

sgRNA sequence	GTTAATAAAATAGCTTATTA
Forward primer	gtcgGTTAATAAAATAGCTTATTA
Reverse primer	aaacTAATAAGCTATTTTATTAAC

Annealed oligo:

```
gtcgGTTAATAAAATAGCTTATTA
     CAATTATTTTATCGAATAATcaaa
```

Ex 2) does not start with G

sgRNA sequence	ATATACATTTCTAAAGTTAA
Forward primer	gtcgATATACATTTCTAAAGTTAA
Reverse primer	aaacTTAACTTTAGAAATGTATAT

Annealed oligo:

```
gtcgATATACATTTCTAAAGTTAA
     TATATGTAAAGATTTCAATTcaaa
```

---

pCFD4(v+)

Vector usage: express double sgRNAs for in vitro and in vivo

Rules:

1. Forward primer: if the sgRNA1 does not start with "G", add a "G" before the sgRNA1 to generate the modified sgRNA1 sequence. The primer sequence will be

   TATATAGGAAAGATATCCGGGTGAACTTC + modified sgRNA1 + GTTTTAGAGCTAGAAATAGCAAG

2. Reverse primer: if the sgRNA2 does not start with "G", add a "G" before the sgRNA2 to generate the modified sgRNA2 sequence. The primer sequence will be

   ATTTTAACTTGCTATTTCTAGCTCTAAAAC + reverse complement of modified sgRNA2 + GACGTTAAATTGAAAATAGGTC

Examples:
Forward primer:
Ex 1) protospacer starts with G
TATATAGGAAAGATATCCGGGTGAACTTCGCTGCTGACAAACGCAGAGTGTTTTAGAGCTAGAAATAGCAAG

Ex 2) protospacer does not start with G
TATATAGGAAAGATATCCGGGTGAACTTCGCTACTTGCCCCTACCGCCGCGTTTTAGAGCTAGAAATAGCAAG

Reverse primer:
Ex 1) protospacer starts with G
ATTTTAACTTGCTATTTCTAGCTCTAAAACGACTCCTGCATTGCAGCTGCGACGTTAAATTGAAAATAGGTC

Ex 2) protospacer does not start with G
ATTTTAACTTGCTATTTCTAGCTCTAAAACTATAAACTCCAACTGCGCCTCGACGTTAAATTGAAAATAGGTC

---

pCFD4-MS2(v+)

Vector usage: express double sgRNAs for in vitro and in vivo

Rules:

1. Forward primer: if the sgRNA1 does not start with "G", add a "G" before the sgRNA1 to generate the modified sgRNA1 sequence. The primer sequence will be

   TATATAGGAAAGATATCCGGGTGAACTTC + modified sgRNA1 + GTTTTAGAGCTAGAAATAGCAAG

2. Reverse primer: if the sgRNA2 does not start with "G", add a "G" before the sgRNA2 to generate the modified sgRNA2 sequence. The primer sequence will be

   GTGATCCTCATGTTGGCCTAGCTCTAAAAC + reverse complement of modified sgRNA2 + GACGTTAAATTGAAAATAGGTC

Examples:
Forward primer:
Ex 1) protospacer starts with G
TATATAGGAAAGATATCCGGGTGAACTTCGCGCAAATAGGATTACACATGTTTTAGAGCTAGGCCAACATGA

Ex 2) protospacer does not start with G
TATATAGGAAAGATATCCGGGTGAACTTCGCCCCGATCCGATCGCATCGTGTTTTAGAGCTAGGCCAACATGA

Reverse primer:
Ex 1) protospacer starts with G
GTGATCCTCATGTTGGCCTAGCTCTAAAACATGAAAGATGAGTACGGCCCGACGTTAAATTGAAAATAGGTC

Ex 2) protospacer does not start with G
GTGATCCTCATGTTGGCCTAGCTCTAAAACTCTCATTCTTCGTTTCAAATCGACGTTAAATTGAAAATAGGTC

---

pl100

Vector usage: express single sgRNA for in vitro and in vivo

Rules:

1. Forward primer: add "cttcg" to 5'-end of sgRNA
2. Reverse primer: add "aaac" to 5'-end and "c" to 3'-end of reverse complement of sgRNA

Example:

sgRNA sequence	GGTGAAGAAGAAACGGGGAA
Forward primer	cttcgGGTGAAGAAGAAACGGGGAA
Reverse primer	aaacTTCCCCGTTTCTTCTTCACCc

---

pl18

Vector usage: express single sgRNA for in vitro only

Rules:

1. Forward primer: add "gttcg" to 5'-end of sgRNA
2. Reverse primer: add "aaac" to 5'-end and "c" to 3'-end of reverse complement of sgRNA

Example:

sgRNA sequence	CGGCGTCACCAAGGTGATCA
Forward primer	gttcgCGGCGTCACCAAGGTGATCA
Reverse primer	aaacTGATCACCTTGGTGACGCCGc

VTPHG

Vector usage: CRISPR activation with single sgRNA target site for in vivo only

Rules:

1. Forward primer: add "ttcg" to 5'-end of sgRNA
2. Reverse primer: add "aaac" to 5'end of reverse complement of sgRNA

Example:

sgRNA sequence	CATGCAAGCGGCTCGGAGCC
Forward primer	ttcgCATGCAAGCGGCTCGGAGCC
Reverse primer	aaacGGCTCCGAGCCGCTTGCATG