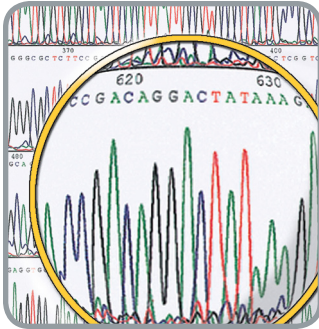


# Tetracycline Response Elements (TRE) Sequence comparison



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## TRE Promoter Alignment

```

          tetO                                tetO
PTRE2 (1)  TTACCACCTCCCTATCAGTGTAGAGAAAAGTAAAGTCGAGTTTACCACFCCCTATCAGTGTAGAGAAA-GTAAAGTC
PTight (1)  TTT---ACTCCCTATCAGTGTAGAGAAC---GTATGTCGAGTTA---CTCCCTATCAGTGTAGAGAACG-ATG---TC
PTRE3G (1)  TTT---ACTCCCTATCAGTGTAGAGAAC---GTATGAAAGTTA---CTCCCTATCAGTGTAGAGAACGTATG---CA

          tetO                                tetO
PTRE2 (82) GAGTTTACCACCTCCCTATCAGTGTAGAGAAAAGTAAAGTCGAGTTTACCACFCCCTATCAGTGTAGAGAAAAGTAAAG
PTight (69) GAGTTTAC---TCCCTATCAGTGTAGAGAAC---GTATGTCGAGTTA---CTCCCTATCAGTGTAGAGAAC---GTATG
PTRE3G (70) GACTTTAC---TCCCTATCAGTGTAGAGAAC---GTATAGGAGTTA---CTCCCTATCAGTGTAGAGAAC---GTATG

          tetO                                tetO
PTRE2 (164) TCGAGTTTACCACCTCCCTATCAGTGTAGAGAAAAGTAAAGTCGAGTTTACCACFCCCTATCAGTGTAGAGAAAAGTAAAG
PTight (139) TCGAGTTTAC---TCCCTATCAGTGTAGAGAAC---GTATGTCGAGTTA---CTCCCTATCAGTGTAGAGAAC---GTA
PTRE3G (140) ACCAGTTTAC---TCCCTATCAGTGTAGAGAAC---GTATCTACAGTTA---CTCCCTATCAGTGTAGAGAAC---GTA

          tetO                                Modified Minimal CMV Promoter
PTRE2 (246) AGTCGAGTTTACCACFCCCTATCAGTGTAGAGAAAAGTAAAGTCGAGCTCGGTACCCGGTCCGAGGTAGGCGGTACCGGT
PTight (208) TGTTCGAGTTTAC---TCCCTATCAGTGTAGAGAA--C-----GTAT---GTCGAGGTAGGCGGTACCGGT
PTRE3G (210) TATCCAGTTTAC---TCCCTATCAGTGTAGAGAA-----C-----GTAT---AAGCTTAGGCGGTACCGGT

          Modified Minimal CMV Promoter (cont.)
PTRE2 (328) GGGAGGCCTATAAAGCAGAGCTCGTTAGTGAACCGTCAGATCGCCTGGAACGCCATCCACGCTGTTTACCTCCATAG
PTight (266) GGGAGGCCTATAAAGCAGAGCTCGTTAGTGAACCGTCAGATCGCCTGGA-----GTAT---GTCGAGGTAGGCGGTACCGGT
PTRE3G (267) GGGCG-CCTATAAAGCAGAGCTCGTTAGTGAACCGTCAGATCGCCTGGAGCAAT--TCCACAACACTTTTGTCTTATAC

PTRE2 (410) AAGACACCGGGAACGATCC-----
PTight (317) -----
PTRE3G (346) AACTTTCCGTACCACCTCCCTACCCTCGTAA

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## Sequence comparison of the three generations of TRE promoters sold by Clontech®.

Each promoter consists of 7 identical repeats of the tet operator sequence (green), and a minimal CMV promoter—although this sequence has been mutated to consensus in  $P_{TRE3G}$ . Moreover, in  $P_{TRE3G}$  the spacer sequences between the *tetO* repeats are evenly spaced and contain randomized central sequences. (L6w, R., Heinz, N., Hampf, M., Bujard, H. & Gossen, M. (2010) Improved Tet-responsive promoters with minimized background expression. BMC Biotechnol. 10:81.)