

Table 1. Description of inserts used in monocistronic constructs

Insert	Description
<p>DNA oligonucleotides for the generation of a multiple cloning site in the pGL3c vector</p>	<p>Oligonucleotide linkers used to generate a multiple cloning site in the pGL3-control vector. Annealed oligonucleotides generate a cassette with overhanging <i>HindIII</i>/<i>NcoI</i> sites which are then ligated into the pGL3-control vector restricted with the same endonucleases. The cassette introduces <i>SpeI</i> and <i>EcoRI</i> restriction so that the inserts described below can be inserted into the 5' UTR of monocistronic FF luciferase.</p> <p style="text-align: center;"><i>HindIII</i> <i>SpeI</i> <i>EcoRI</i> <i>NcoI</i></p> <p style="text-align: center;"><u>AGCTT</u> AAA <u>ACTAGT</u> AAA <u>GAATTC</u> AAA <u>C</u></p> <p style="text-align: center;">A TTT TGATCA TTT CTTAAG TTT GGTAC</p> <p><i>Forward</i> - AGCTT AAA ACTAGT AAA GAATTC AAA C</p> <p><i>Reverse</i> - CATGG TTT GAATTC TTT ACTAGT TTT A</p>
<p>(Gtx 9-nt)₅(SI)₅β</p>	<p>Five repeats of the Gtx IRES-module interspersed with 9-nt spacers based on a segment of the β-globin 5' UTR (SI). This sequence is spaced 25 nt upstream of the initiator AUG with the β-globin-5' UTR. The constructs that follow (m1–m15) are based on this format. In constructs in which the complementary match was increased, nucleotides in the 9-nt Gtx element were mutated to A (m1–m8), whereas in constructs in which the complementary match was increased, nucleotides in the flanking sequence were mutated accordingly (m9–m15).</p> <p style="text-align: center;"><u>GAATTC</u> <i>TTCTGACAT CCGGCGGGT TTCTGACAT</i></p> <p style="text-align: center;"><i>CCGGCGGGT TTCTGACAT CCGGCGGGT TTCTGACAT</i></p> <p style="text-align: center;"><i>CCGGCGGGT TTCTGACAT CCGGCGGGT</i></p> <p style="text-align: center;"><i>GACTCACAACCCAGAAACAGACAT</i> <u>CCATGG</u></p>

Insert	Description
m1	<p>Decrease the length of complementarity at the 3' end by 1 nt (<u>U</u> CCGGCGGGU <u>U</u> ⇒ <u>U</u> CCGGCGGGU <u>A</u>)</p> <p><i><u>GAATTC</u> TTCTGACAU CCGGCGGGT ATCTGACAU CCGGCGGGT ATCTGACAU CCGGCGGGT ATCTGACAU CCGGCGGGT ATCTGACAU CCGGCGGGT AACTCACAACCCAGAAACAGACAT <u>CCATGG</u></i></p>
m2	<p>Decrease the length of complementarity in the Gtx IRES-module at the 3' end by 1 nt (CCGGCGGGU ⇒ CCGGCGGGA)</p> <p><i><u>GAATTC</u> TTCTGACAT CCGGCGGGA TTCTGACAT CCGGCGGGA TTCTGACAT CCGGCGGGA TTCTGACAT CCGGCGGGA TTCTGACAT CCGGCGGGA GACTCACAACCCAGAAACAGACAT <u>CCATGG</u></i></p>
m3	<p>Decreases the length of complementarity in the Gtx IRES-module at the 3' end by 2 nt (CCGGCGGGU ⇒ CCGGCGGAA)</p> <p><i>SpeI</i></p> <p><i><u>ACTAGT</u> CAGCTG <u>GAATTC</u> TTCTGACAT CCGGCGGAA TTCTGACAT CCGGCGGAA TTCTGACAT CCGGCGGAA TTCTGACAT CCGGCGGAA TTCTGACAT CCGGCGGAA GACTCACAACCCAGAAACAGACAT <u>CCATGG</u></i></p> <p><i>NcoI</i></p>
m4	<p>Decrease the length of complementarity in the Gtx IRES-module at the 3' end by 3 nt (CCGGCGGGU ⇒ CCGGCGAAA)</p> <p><i><u>GAATTC</u> TTCTGACAT CCGGCGAAA TTCTGACAT CCGGCGAAA TTCTGACAT CCGGCGAAA TTCTGACAT</i></p>

Insert	Description
	<p>CCGGCGAAA TTCTGACAT CCGGCGAAA <i>GACTCACAACCCAGAAACAGACAT <u>CCATGG</u></i></p>
m5	<p>Decrease the length of complementarity in the Gtx IRES-module at the 3' end by 4 nt (CCGGCGGGU ⇒ CCGGCAAAA)</p> <p><i><u>GAATTC</u> TTCTGACAT CCGGCAAAA TTCTGACAT</i> <i>CCGGCAAAA TTCTGACAT CCGGCAAAA TTCTGACAT</i> <i>CCGGCAAAA TTCTGACAT CCGGCAAAA</i> <i>GACTCACAACCCAGAAACAGACAT <u>CCATGG</u></i></p>
m6	<p>Decrease the length of complementarity at the 5' end by 1 nt (<u>U</u> CCGGCGGGU <u>U</u> ⇒ <u>A</u> CCGGCGGGU <u>U</u>)</p> <p><i><u>GAATTC</u> TTCTGACAA CCGGCGGGT TTCTGACAA</i> <i>CCGGCGGGT TTCTGACAA CCGGCGGGT TTCTGACAA</i> <i>CCGGCGGGT TTCTGACAA CCGGCGGGT</i> <i>GACTCACAACCCAGAAACAGACAT <u>CCATGG</u></i></p>
m7	<p>Decrease the length of complementarity in the Gtx IRES-module at the 5' end by 1 nt (CCGGCGGGU ⇒ ACGGCGGGU)</p> <p><i><u>GAATTC</u> TTCTGACAT ACGGCGGGT TTCTGACAT</i> <i>ACGGCGGGT TTCTGACAT ACGGCGGGT TTCTGACAT</i> <i>ACGGCGGGT TTCTGACAT ACGGCGGGT</i> <i>GACTCACAACCCAGAAACAGACAT <u>CCATGG</u></i></p>
m8	<p>Five repeats of the β-globin spacer (I) interspersed with 9-nt poly(A) segments (SIII). Spaced 27 nt upstream of the initiator AUG with β-globin 5' UTR.</p> <p><i><u>GAATTC</u> TTCTGACAT AAAAAAAAAA TTCTGACAT</i> <i>AAAAAAAAAA TTCTGACAT AAAAAAAAAA TTCTGACAT</i> <i>AAAAAAAAAA TTCTGACAT AAAAAAAAAA</i></p>

Insert	Description
	<i>GACTCACAACCCCAGAAACAGACAT <u>CCATGG</u></i>
m9	<p>Increase the length of complementarity in the Gtx IRES-module at the 3' end by 1 nt (<i>GACAU CCGGCGGGU UUCUG</i> ⇒ <i>GACAU CCGGCGGGU CUCUG</i>)</p> <p><i><u>GAATTC</u> TTCTGACAT CCGGCGGGT CTCTGACAT CCGGCGGGT CTCTGACAT CCGGCGGGT CTCTGACAT CCGGCGGGT CTCTGACAT CCGGCGGGT CACTCACAACCCCAGAAACAGACAT <u>CCATGG</u></i></p>
m10	<p>Increase the length of complementarity in the Gtx IRES-module at the 3' end by 2 nt (<i>GACAU CCGGCGGGU UUCUG</i> ⇒ <i>GACAU CCGGCGGGU CACUG</i>)</p> <p><i><u>GAATTC</u> TTCTGACAT CCGGCGGGT CACTGACAT CCGGCGGGT CACTGACAT CCGGCGGGT CACTGACAT CCGGCGGGT CACTGACAT CCGGCGGGT CACTCACAACCCCAGAAACAGACAT <u>CCATGG</u></i></p>
m11	<p>Increase the length of complementarity in the Gtx IRES-module at the 3' end by 3-nt (<i>GACAU CCGGCGGGU UUCUG</i> ⇒ <i>GACAU CCGGCGGGU CAUUG</i>)</p> <p><i><u>GAATTC</u> TTCTGACAT CCGGCGGGT CATTGACAT CCGGCGGGT CATTGACAT CCGGCGGGT CATTGACAT CCGGCGGGT CATTGACAT CCGGCGGGT CATTACAACCCCAGAAACAGACAT <u>CCATGG</u></i></p>
m12	<p>Increase the length of complementarity in the Gtx IRES-module at the 3' end by 4 nt (<i>GACAU CCGGCGGGU UUCUG</i> ⇒ <i>GACAU CCGGCGGGU CAUGG</i>)</p> <p><i><u>GAATTC</u> TTCTGACAT CCGGCGGGT CATGGACAT</i></p>

Insert	Description
	<p>CCGGCGGGT CATGGACAT CCGGCGGGT CATGGACAT CCGGCGGGT CATGGACAT CCGGCGGGT CATGCACAACCCAGAAACAGACAT <u>CCATGG</u></p>
m13	<p>Increase the length of complementarity in the Gtx IRES-module at the 5' end by 1 nt (<i>GACAU CCGGCGGGU UUCUG</i> ⇒ <i>GACAC CCGGCGGGU UUCUG</i>)</p> <p><u><i>GAATTC TTCTGACAC CCGGCGGGT TTCTGACAC</i></u> <i>CCGGCGGGT TTCTGACAC CCGGCGGGT TTCTGACAC</i> <i>CCGGCGGGT TTCTGACAC CCGGCGGGT</i> <i>TACTACAACCCAGAAACAGACAT <u>CCATGG</u></i></p>
m14	<p>Increase the length of complementarity in the Gtx IRES-module at the 5' end by 2 nt (<i>GACAU CCGGCGGGU UUCUG</i> ⇒ <i>GACGC CCGGCGGGU UUCUG</i>)</p> <p><u><i>GAATTC TTCTGACGC CCGGCGGGT TTCTGACGC</i></u> <i>CCGGCGGGT TTCTGACGC CCGGCGGGT TTCTGACGC</i> <i>CCGGCGGGT TTCTGACGC CCGGCGGGT</i> <i>TACTACAACCCAGAAACAGACAT <u>CCATGG</u></i></p>
m15	<p>Increase the length of complementarity in the Gtx IRES-module at the 5' end by 4 nt (<i>GACAT CCGGCGGGT TTCTG</i> ⇒ <i>GCTGC CCGGCGGGT TTCTG</i>)</p> <p><u><i>GAATTC TTCTGCTGC CCGGCGGGT TTCTGCTGC</i></u> <i>CCGGCGGGT TTCTGCTGC CCGGCGGGT TTCTGCTGC</i> <i>CCGGCGGGT TTCTGCTGC CCGGCGGGT</i> <i>TACTACAACCCAGAAACAGACAT <u>CCATGG</u></i></p>
m16	<p>Decrease the length of complementarity in the Gtx IRES-module at the 5' end by 1 nt; however, the mutated sequence is contained</p>

Insert	Description
	<p>within the context of the full-length Gtx 5' leader (1). (CCCGAG CCGGCGGGU G ⇒ CCCGAG CCGGCGGGGA G)</p> <p><i>BsePI</i> <u>GCGCGC</u> AAACTTCCCGAG CCGGCGGGGA GCGGGCGGTGGCAGC <u>GGGCCC</u></p> <p><i>ApaI</i></p>
m17	<p>Decrease the length of complementarity in the Gtx IRES-module at the 3' end by 2 nt; however, the mutated sequence is contained within the context of the full-length Gtx 5' leader (1). (CCCGAG CCGGCGGGU G ⇒ CCGAG CCGGCGGGGAA G)</p> <p><i>BsePI</i> <u>GCGCGC</u> AAACTTCCCGAG CCGGCGGGGAA GCGGGCGGTGGCAGC <u>GGGCCC</u></p> <p><i>ApaI</i></p>
m18	<p>Decrease the length of complementarity in the Gtx IRES-module at the 3' end by 4 nt; however, the mutated sequence is contained within the context of the full-length Gtx 5' leader (1). (CCCGAG CCGGCGGGU G ⇒ CCGAG CCGGCGGGCAAAA G)</p> <p><i>BsePI</i> <u>GCGCGC</u> AAACTTCCCGAG CCGGCGGGCAAAA GCGGGCGGTGGCAGC <u>GGGCCC</u></p> <p><i>ApaI</i></p>