

C57BL/6N-Tg(Arc-tTA/EGFP/Capzb<*>2)100Mkyph

AiCE-Tg

9875 bp Transgene Sall cut

GTCGACTAGCGCTACCGG

GGGGGGCCATCTGCTTTCTCCTGGAACCAGAGCTCTAGGGTACAAGTAAACAAA
TACCTGATACAAGATCTTTGTGCAGGTCTGGTTTAAGAGTCACAAAGCCACCAAT
GGCAGGGACAATGTCACCAGTCTCCAGCCAGGGACTCTGGTCCCCACCTCTCCT
TCAGGGCAGGGGCTGAGCAGGTGTAGGGCAGGGTGGGCCCTGGGTGGTGGCAG
GCTCAGCGCACAGAGCCTTCCTGCGTGGGGAAGCTCCTTGCTGCGTCATGGCTC
AGCTATTCTCAGCCTCTCTCCTTTTATGGTGCCGGAAGCAGGCAGGCTGCTGCTG
GGCTGGCTCTGGGAGGTATTTAAATGGAATTGCAGAGAACAGCAGGAAAACAC
AGCCGCCAGGGTGGGGGTGGCAGCCTTGAACGCCACCCTCTAACTGGCATGGG
CATCTGAACTCTCAGCTCTGGGGGTGTCTCATCGGCCAGCTGGCAGTAGCCCCC
CCAACCCCAACCCTGATGGAGAACTGCTCCTGTATGATCCAGCCCAGTGGGCGC
ACAGGGAGTTGGTAGGTGGGCACTCAGACTGGCTAGGGCCCCAGTGGAGCAGA
GATGAGGGGTGGCTGCCAGCCTCCACCTCAGCCCAGCCTCCCATGGCTCTTAC
TCATTCCTGGTTCGTAACTCTGCCCCCTACCCCCAGCTCAGCACTGGCTACCTGGA
TATAGGGAACCCTAATAGTCTTCATTCTTGTCCCTTTGGTAGCCCCAAATTCCTGGG
CCATCTCTCCAAAGCAGGGACAGCTCACACATCCAGAACACCAGCCACCAAATC
ATCTCCCCATATCCTGATTCTTTTCTACCCTCTCTACCCTCCATGTCCTCCCCAGGC
TGAGGAGGTCCTGGGTACAATGGCCCTGGCTCAAGGCTGGTGTCTGCTAGAA
TAAGGGAGACCTGACTCACCTTGATACCTGGTCTCTGCTACCATCCATGTTCTGC
CCCCACATGGACAAAACCCACATGGCCATGTCCTTGGCCTGGAGTAGCACCCCTC
ACACATCTGGCAGTCCCTCTTTATGTCTGGCTGTTGGCTCCTGCATTCCAGTGAT
GGCTGGAAGCAGGTACACTAGGAAAAGCTAAAGGGGGCTACGACTGTGACATA
TATGGTTGCACACAAAACACATTTCCAGACCTGTGCAGATACCAAGATTGACCA
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CTCACCTTGATGTTCTCCTTCAGTCTCAGCCCCCAGATCACTGTGCATCATTGTG
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ACTCACGGATGTTGACTGTTACGCCTTAGAGAAAACATCTGCTGGGAAGACCAT
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CACCAGAAGTCTTGGGTTGGGAAGAGGATGAGGAGAACAGTGCTAGAGGTTCC
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[illegible]

CCACGGGAGGGGAGCGAGTAGGCGCGGAAGGCGGGGCCTGCGGCAGGAGAGG
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TTCGGTGCAGAGCTCAAGCGAGTTCTCCCGCAGCCGCAGTCTCTGGGCCTCTCT
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ACCTGCCACTGCCGCTGCAGCCGCC
ATCAAGCTTATCGATAACCACC
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GAAGTCGGTATCGAAGGCCTGACGACAAGGAACTCGCTCAAAAGCTGGGAGT
TGAGCAGCCTACCCTGTACTGGCACGTGAAGAACAAGCGGGGCCCTGCTCGATGC
CCTGGCAATCGAGATGCTGGACAGGCATCATACCCACTTCTGCCCCCTGGAAGG
CGAGTCATGGCAAGACTTTCTGCGGAACAACGCCAAGTCATTCCGCTGTGCTCT
CCTCTCACATCGCGACGGGGCTAAAGTGCATCTCGGCAACCGCCCAACAGAGA
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CTGCTGACGCTCTTGACGATTTTGACCTTGACATGCTCCCCGGG
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CGGCATGGACGAGCTGTACAAGTCCGGCCGGA
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GAAAAACAAAGGACATCGTCAACGGGCTGAGGTCTGTGCAGACGTTTGCAGAC
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TACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTG
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ATAATGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATTTTTT
TCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTAACGCGTCTT
AAGTCGAC

Image showing the position of 3' terminal (1.1kbp) of used Arc7K Promotor in the Ch15 cloneRP23~

Download GenBank Graphics		Mus musculus chromosome 15, clone RP23-459I23, complete sequence		
Sequence ID: AC118022.12		Length: 190653		Number of Matches: 1
Range 1: 102266 to 103392		GenBank Graphics	Next Match Previous Match	
Score	Expect	Identities	Gaps	E
2167 bits(1127)	0.0	1127/1127(100%)	0/1127(0%)	P
Query 1	GTTGGCTGTGCCAGGGAATCGGAAGGGGCTCCCTCTAGAGGGAATCTCAGCTTCCGGA	60		
Sbjct 103392	GTTGGCTGTGCCAGGGAATCGGAAGGGGCTCCCTCTAGAGGGAATCTCAGCTTCCGGA	103		
Query 61	GCCCCATTCTTATATGGCATCTTGCTTTCTCTGCAGGctccttccctccttccctcct	120		
Sbjct 103332	GCCCCATTCTTATATGGCATCTTGCTTTCTCTGCAGGCTCCTCCCTCCTTCTCCT	103		
Query 121	tctccctccctccttccctccGGACTAACCTCTTGAAGGCTTGAGAAGCTTCTGGTCCAG	180		
Sbjct 103272	TCTCCCTCCCTCCTTCTCCGGACTAACCTCTTGAAGGCTTGAGAAGCTTCTGGTCCAG	103		
Query 181	TTGTGGCTGGGTCTAGGGACTTCAGGGTTCAGCAATGTTGCCTACACATCTTGATATCC	240		
Sbjct 103212	TTGTGGCTGGGTCTAGGGACTTCAGGGTTCAGCAATGTTGCCTACACATCTTGATATCC	103		
Query 241	CTATGCCCTACCCAGTGTCTGTACAGGTTCAAGTGATACATCTGTTTCAGGGCACTGCG	300		
Sbjct 103152	CTATGCCCTACCCAGTGTCTGTACAGGTTCAAGTGATACATCTGTTTCAGGGCACTGCG	103		
Query 301	GCCCTGTTCAGCCACTGACACACAGACAGGGCCACTCAGTGTCCACAACTATGAAGGG	360		
Sbjct 103092	GCCCTGTTCAGCCACTGACACACAGACAGGGCCACTCAGTGTCCACAACTATGAAGGG	103		
Query 361	GTCCAGAAAAAGCCTTGCTGAGTGGAGTCCCGAACAATATTGGACAGGCTAGGATTT	420		
Sbjct 103032	GTCCAGAAAAAGCCTTGCTGAGTGGAGTCCCGAACAATATTGGACAGGCTAGGATTT	102		
Query 421	GGACTTATGACTCTTGGGGCAAGGTTGGGCAGGAAGTAGgtgtgtgtgtgtgtgtgtgt	480		
Sbjct 102972	GGACTTATGACTCTTGGGGCAAGGTTGGGCAGGAAGTAGGTGTGTGTGTGTGTGTGTG	102		
Query 481	tggt	540		
Sbjct 102912	TG	102		
Query 541	GAGTGAGAGATTCTACAGGCACTTCTGAAGTTGCATAGTGTGTGTGTGTGTGTGTGTG	600		
Sbjct 102852	GAGTGAGAGATTCTACAGGCACTTCTGAAGTTGCATAGTGTGTGTGTGTGTGTGTGTG	102		
Query 601	GAGTCAGGATGGGCGCATCAAGGATTGAGGGGGTAACGTCCAGCTGCTCCCTAAGAAAA	660		
Sbjct 102792	GAGTCAGGATGGGCGCATCAAGGATTGAGGGGGTAACGTCCAGCTGCTCCCTAAGAAAA	102		
Query 661	GAGATCATGAGGCCCCAGGGCAACAGTAGGTAATAACCTGCCTTAGCCTCATCCTCGCT	720		
Sbjct 102732	GAGATCATGAGGCCCCAGGGCAACAGTAGGTAATAACCTGCCTTAGCCTCATCCTCGCT	102		
Query 721	GCCCAGGACTAGGTAGAGGAGAGTGTCTCCGGTCCCCAAATCCCTCCGGTGGGAGGCG	780		
Sbjct 102672	GCCCAGGACTAGGTAGAGGAGAGTGTCTCCGGTCCCCAAATCCCTCCGGTGGGAGGCG	102		
Query 781	CGCAGCAGAGCACATTAGTCACTCGGGCTGTGAAGGGGCGGGTCTTGAGGGCACCCA	840		
Sbjct 102612	CGCAGCAGAGCACATTAGTCACTCGGGCTGTGAAGGGGCGGGTCTTGAGGGCACCCA	102		
Query 841	gg	900		
Sbjct 102552	GGGAGGGGAGCGAGTAGGCGCGGAGGCGGGGCTGCGGCAGGAGAGGGCGCGGGCGGGC	102		
Query 901	tctgg	960		
Sbjct 102492	TCTGGCGCGGAGCTGGGCGCGGCAATGGGAGCCAGGGCTCCACGAGCTGCCGCCACG	102		
Query 961	GGCCCCGCGAGCATAAATAGCGCTGGTGGCGGTTTCGGTGCAGAGCTCAAGCGAGTTC	102		
Sbjct 102432	GGCCCCGCGAGCATAAATAGCGCTGGTGGCGGTTTCGGTGCAGAGCTCAAGCGAGTTC	102		
Query 1021	TCCCAGAGCCGAGCTCTTGGGCTCTCTAGCTTCAGCGGCGAGGCTGCCACACTCG	108		
Sbjct 102372	TCCCAGAGCCGAGCTCTTGGGCTCTCTAGCTTCAGCGGCGAGGCTGCCACACTCG	102		
Query 1081	CTAAGCTCTCCGGCACCACACCTGCCACTGCCGCTGCAGCCGCC	1127		
Sbjct 102312	CTAAGCTCTCCGGCACCACACCTGCCACTGCCGCTGCAGCCGCC	102266		

Transgene with annotation

≥ SmaI CCCGGG 1-7140 mouse Arc promotor

>SmaI (blunted-lost) ligated to AgeI (ACCGG|T blunted-lost) [Eco47III](#) [SalI](#) of a vector.

>Arc-SARE is underlined.

[GTCGACTAGCGCT](#)ACCGG

GGGGGGCCATCTGCTTTCTCCTGGAACCAGAGCTCTAGGGTACAAGTAAACAAA
TACCTGATACAAGATCTTTGTGCAGGTCTGGTTTAAGAGTCACAAAGCCACCAAT
GGCAGGGACAATGTCACCAGTCTCCAGCCAGGGACTCTGGTCCCCACCTCTCCT
TCAGGGCAGGGGCTGAGCAGGTGTAGGGCAGGGTGGGCCCTGGGTGGTGGCAG
GCTCAGCGCACAGAGCCTTCCTGCGTGGGGAAGCTCCTTGCTGCGTCATGGCTC
AGCTATTCTCAGCCTCTCTCCTTTTATGGTGCCGGAAGCAGGCAGGCTGCTGCTG
GGCTGGCTCTGGGAGGTATTTAAATGGAATTGCAGAGAACAGCAGGAAAACAC
AGCCGCCAGGGTGGGGGTGGCAGCCTTGAACGCCACCCTCTAACTGGCATGGG
CATCTGAACTCTCAGCTCTGGGGGTGTCTCATCGGCCAGCTGGCAGTAGCCCCC
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TCATTCCTGGTTCGTA ACTCTGCCCCCTACCCCCAGCTCAGCACTGGCTACCTGGA
TATAGGGAACCCTAATAGTCTTTCATTCTTGTCCCTTTGGTAGCCCAAATTCCTGGG
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ACACATCTGGCAGTCCCTCTTTATGTCTGGCTGTTGGCTCCTGCATTCCAGTGAT
GGCTGGAAGCAGGTACACTAGGAAAAGCTAAAGGGGGCTACGACTGTGACATA
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[illegible]

CCTTGTCCCATGCTGACACCAAGAAAGGACTCCAGCCACCTTCAGCTTGGTGGAG
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TAAAAAGGGATGTGGAAAGGCTCTTCTCTTTCCCTCCCTCTGATCTCTGTCAAAG
GACTGCTCTGGACCCCTGGTTTCAACCCATAGCATAGACCTGGAGTTGGACAGG
GTACCTGGGCATCAGCCAGGAGCTAGGACCAGGCAGCAAAAGACATCTGTGCA
AAGTTGGGTAGCAGGCAGCTGTCTAGAGATTTGACGAAAGCACCTGGGCCAGG
GGCTGGGCGGGAGGTCCTGCTGCTCGGCCAGTCAAGGGGCAGAAGCCTATACC
CTTTCTCCTGCGTCCACACAGATGGCAAGTCTGTGGGCAGATGAGAGCAGGAA
GTCCAACCAGGTCCCTGCTGCATCCACCTCTGCTGCCCTGGATTCTGTCTTCATG
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CACCAGAAGTCTTGGGTGGGAAGAGGATGAGGAGAACAGTGCTAGAGGTTCC
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CGCTCTAAAGATTACCCAGACCTGCCCCATTGCTGAGGACTTGCGCTTTTCCTC
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CCCTCGCATGGGCAGGATTAGTGGGGCGGGTACTGGAAGAGTGTGGGTAGAA
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CTGTGCCTGTCACCTGTCACTGGGCTCCTGTGTTTCGTGGCTTCTGAAGACCCCG
GAGGAGGAAGCAGGGCTTCCTGTGACTTTTCACCCAGTGGGGCTCAGAGTAG
TGTCTCGGGGTCAGTGGCCTGGATGGACTGCCCTCCATGGAGATAGGAAATGCA
GTGGTCAGAGGGTCAGAGCACTTCAGCCTCGGAAACTTCTCCATTTGTGCCCAA

[illegible]

AGCAGAGCACATTAGTCACTCGGGGCTGTGAAGGGGCGGGTCCTTGAGGGCAC
CCACGGGAGGGGAGCGAGTAGGCGCGGAAGGCGGGGCCTGCGGCAGGAGAGG
GCGCGGGCGGGCTCTGGCGCGGAGCCTGGGCGCCGCCAATGGGAGCCAGGGCT
CCACGAGCTGCCGCCCACGGGCCCCGCGCAGCATAAATAGCCGCTGGTGGCGGT
TTCGGT

>+1 = GCA of transcript1.

>Reading frame starts at +199-downstream (ATG); according to

https://www.ncbi.nlm.nih.gov/nuccore/NM_018790.3

>Thus, Arc5' region (GCA to 126GCC, 126bp) without any known signal is included

>A homologous sequence was found in the following underlined CTG to GCC, Sequence
ID: XM_021198619.2 engrailed 100%

GCAGAGCTCAAGCGAGTTCTCCCGCAGCCGCAGTCTCTGGGCCTCTCTAGCTTC
AGCGGCGACGAGCCTGCCACACTCGCTAAGCTCCTCCGGCACCGCACAC
CTGCCACTGCCGCTGCAGCCGCC
ATCAAGCTATCGATACCACC

> HindIII ClaI, neighboring XbaI (Southern probe Fr7179-9619 of 9875) then tTA at 959 of
GenBank: MH325109.1

ATGTCTAGACTGGACAAGAGCAAAGTCATAAACTCTGCTCTGGAATTACTCAAT
GAAGTCGGTATCGAAGGCCTGACGACAAGGAACTCGCTCAAAAGCTGGGAGT
TGAGCAGCCTACCCTGTACTGGCACGTGAAGAACAAGCGGGCCCTGCTCGATGC
CCTGGCAATCGAGATGCTGGACAGGCATCATACCCACTTCTGCCCCCTGGAAGG
CGAGTCATGGCAAGACTTTCTGCGGAACAACGCCAAGTCATTCCGCTGTGCTCT
CCTCTCACATCGCGACGGGGCTAAAGTGCATCTCGGCAACCGCCCAACAGAGA
AACAGTACGAAACCCTGGAAAATCAGCTCGCGTTCCTGTGTCAGCAAGGCTTCT
CCCTGGAGAACGCACTGTACGCTCTGTCCGCGGTGGGCCACTTTACACTGGGCT
GCGTATTGGAGGATCAGGAGCATCAAGTAGCAAAAGAGGAAAGAGAGACACCT
ACCACCGATTCTATGCCCCCACTTCTGAGACAAGCAATTGAGCTGTTTCGACCATC
AGGGAGCCGAACCTGCCTTCCTTTTCGGCCTGGAACATAATCATATGTGGCCTGG
AGAAACAGCTAAAGTGCGAAAGCGGCGGGCCGGCCGACGCCCTTGACGATTTT
GACTTAGACATGCTCCCAGCCGATGCCCTTGACGACTTTGACCTTGATATGCTGC

CTGCTGACGCTCTTGACGATTTTGACCTTGACATGCTCCCCGGG

> 1702 of tTA GenBank MH325109.1, devoid of stop codon , then, a synthetic seq for P2A self-cleaving seq: TSA-----PVAT

ACTAGTGCCACTAACTTTTCCCTGCTGAAGCAGGCTGGCGACGTGGAGGAAAAT
CCCGGACCGGTCGCCACC

> EGFP ATG reading frame

ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCTGA
GCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGG
GCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGC
TGCCCGTGCCCTGGCCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCT
TCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGC
CCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTAC
AAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGA
GCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGG
AGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACG
GCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAG
CTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTG
CCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGA
GAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCGGGGATCACTCT
CGGCATGGACGAGCTGTACAAGTCCGGCCCGGA

> Here is XhoI CTCGA|G

CTCAGATCTCGAGC

> HAtagseq=(S)YPYDVPDYA

TCAAGCTATCCTTACGATGTGCCAGACTATGCC

>=SFEFT=Spacer between HA-tag to CapZ

AGCTTCGAATTCACC

ATGAGCGATCAGCAGCTGGACTGCGCCTTGGACCTGATGAGGCGCCTGCCTCCA
CAGCAGATTGAGAAGAACCTCAGCGATCTGATCGACCTGGTCCCCAGTCTGTGT
GAAGATCTCCTGTCATCTGTTGACCAGCCCCTGAAAATTGCCAGAGACAAGGTG
GTGGGCAAGGATTACCTTTTGTGTGACTACAACAGAGACGGGGACTCCTATAGG
TCACCGTGGAGTAACAAGTATGACCCTCCTTTGGAAGATGGGGCCATGCCATCT
GCTCGGCTCAGAAAGCTGGAGGTAGAGGCCAACAATGCCTTCGACCAATACCG

AGACCTGTATTTTGAAGGTGGGGTCTCATCAGTCTACCTCTGGGATCTTGATCAT
 GGCTTTGCTGGAGTGATCCTCATAAAGAAAGCTGGAGATGGATCCAAGAAGATC
 AAAGGCTGCTGGGATTCCATCCACGTGGTGGAAAGTGCAGGAGAAGTCCAGCGG
 CCGTACTGCCATTACAAGTTGACCTCCACGGTGATGCTATGGCTGCAAACCAAC
 AAATCCGGCTCGGGCACCATGAACCTGGGAGGCAGCCTAACCAGACAGATGGA
 GAAAGACGAAACTGTGAGTGACTGTTCCCCACACATAGCCAACATCGGGCGCCT
 GGTGGAGGACATGGAAAACAAAATCCGAAGCACGCTGAATGAGATCTACTTTG
 GAAAAACAAAGGACATCGTCAACGGGCTGAGGTCTGTGCAGACGTTTGCAGAC
 AAATCAAAGCAAGAAGCGCTTAAGAACGACCTGGTGGAGGCCTTGAAGAGAAA
 GCAGCAGTGTGA

> TGA=StopCodon, then smaI-pEGFP-C1 which includes polyA site

CCCGGGATCCACCGGATCTAGATAACTGATCATAATCAGCCATACCACATTTGTAG
AGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAA
AATGAATGCAATTGTTGTTGTTAACTTGTATTATGCAGCTTATAATGGTTACAAAT
AAAGCAATAGCATCACAAATTCACAAATAAAGCATTTTTTTCACTGCATTCTAG
 TTGTGGTTTGTCCAAACTCATCAATGTATCTTAACGCGTCTTAAGTCGAC

>MluIACGCGT from pEGFP-C1 ligated to short synthetic oligo nucleotide having SalI,
 then ligated to pBlueScriptII KS. SalIGTCGAC was used to cutout whole transgene from
 the vector (pBlueScriptII).

SalI fragments of transgene were heated then incubated at low temperature to anneal at SalI
 before oocyte injection. I aimed at making TG with high-copy number.

1. linker SalI to AgeI(BI) 1-18
2. Promotor: mouse Arc SmaI NaeI Fr (7143bp, -7017to +126, Arc reading frame should starts at +199)
3. tTA seq from pTet-off advanced Tet-Off system Clontech
<https://www.novoprolabs.com/vector/V11072>
4. Synthetic P2A seq
5. EGFP-seq from pEGFP-C1

6. SyntheticOligo for HA tag
7. CapZ beta2 seq TGA=stop
8. pEGFP-C1 SmaI(CCCGGG)-SV40 polyA signal-MluI
9. linker MluI AflII SalI

Inframe AA seq

tTA 248aminoacids

MSRLDKSKVINSALELLNEVGIEGLTTRKLAQKLGVEQPTLYWHVKNKRALLDAL
AIEMLDRIHHTHFCPLEGESWQDFLRNNAKSFRCALLSHRDGAKVHLGTRPTEKQY
ETLENQLAFLCQQGFSLLENALYALSAVGHFTLGCVLEDQEHQVAKEERETPTTDSM
PPLLRQAIELFDHQGAEP AFLFGLELIICGLEKQLKCESGGPADALDDFDLDMLPAD
ALDDFDLDMLPADALDDFDLDMLPG

P2Aseq & Spacer 24aa

TSATNFSLLKQAGDVEENPGPVAT=

ACTAGTGCCACTAACTTTTCCCTGCTGAAGCAGGCTGGCGACGTGGAGGAAAAT
CCCGGACCGGTCGCCACC

EGFP244aa

MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICT
TGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIF
FKDDGNYKTRAIEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHN
VYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNH
YLSTQSALS KDPNEKRDHMLLEFVTAAGITLGMDELYKSGRTQ

Spacer 5aa; **XhoI** then a meaningless alpha-helix spacer in front of HA tag

ISSSS=AT**CTCGAGCT**CAAGC

HAtag 9aa

YPYDVDPDYA = TATCCTTACGATGTGCCAGACTATGCC

Spacer 5aa; After HAtag, TTC- is for the meaningless spacer PheGluPhe = FEF while it includes [EcoRI](#))

SFEFT = AGCTTTCGAATTCACC

CapZbeta2 272aa

MSDQQQLDCALDLMRRLPPQQIEKNLSDLIDLVPSLCEDLLSSVDQPLKIARDKVVG
KDYLLCDYNRDGDSYRSPWSNKYDPPLIEDGAMP SARLRKLEVEANNAFDQYRDL
YFEGGVSSVYLVWLDHGFAGVILIKKAGDGSKKIKGCWDSIHVVEVQEKSSGRTAH
YKLTSTVMLWLQTNKSGSGTMNLGGSLTRQMEKDETVSDCSPHIANIGRLVEDME
NKIRSTLNEIYFGKTKDIVNGLRSVQTFADKSKQEALKNDLVEALKRKQQC*

pink: tTA
orange: p2A seq
yellow: spacer
green: EGFP
Red: HA
pale yellow: spacer
orange: CapZ beta2

10	20	30	40	50	60
MSRLDKSKVI	NSALELLNEV	GIEGLTTRKL	AQKLGVEQPT	LYWHVKNKRA	LLDALAIEML
70	80	90	100	110	120
DRHHTHFCPL	EGESWQDFLR	NNAKSFRCAL	LSHRDGAKVH	LGTRPTEKQY	ETLENQLAFL
130	140	150	160	170	180
CQQGFSLENA	LYALSAVGHF	TLGCVLEDQE	HQVAKEERET	PTTDSMPPLL	RQAIELFDHQ
190	200	210	220	230	240
GAEPAPLFGL	ELIICGLEKQ	LKCESGGPAD	ALDDFDLDML	PADALDDFDL	DMLPADALDD
250	260	270	280	290	300
FDLDMLPGTS	ATNFSLLKQA	GDVEENPGPV	ATHVSKGEEL	FTGVVPILVE	LDGDVNGHKF
310	320	330	340	350	360
SVSGEGEGBA	TYGKLTLEFI	CTTGKLPVPM	PTLVTTLTYG	VQCFSRYPDH	MKQHDFFKSA
370	380	390	400	410	420
MPEGYVQERT	IFFKDDGNYK	TRAEVKFEGD	TLVNRIELKG	IDFKEDGNIL	GHKLEYNYNIS
430	440	450	460	470	480
HNVYIMADKQ	KNGIKVNFKI	RHNIEDGSVQ	LADHYQONTF	IGDGPVLLPD	NHYLSTQSAL
490	500	510	520	530	540
SKDPNEKRDE	MVLEFVTA	GITLGMDELY	KSGRTQISSS	SYPYDVDPDYA	SFEFTMSDQQ
550	560	570	580	590	600
LDCALDLMRR	LPPQQIEKNL	SDLIDLVPSL	CEDLLSSVDQ	PLKIARDKVV	GKDYLLCDYN
610	620	630	640	650	660
RDGDSYRSPW	SNKYDPPLIED	GAMP SARLRK	LEVEANNAFD	QYRDLYFEGG	VSSVYLVWLDL
670	680	690	700	710	720
HGFAGVILIK	KAGDGSKKIK	GCWDSIHVVE	VQEKSSGRTA	HYKLTSTVML	WLQTNKSGSG
730	740	750	760	770	780
TMNLGGSLTR	QMEKDETVSD	CSPHIANIGR	LVEDMENKIR	STLNEIYFGK	TKDIVNGLRS
790	800	810	820	830	840
VQTFADKSKQ	EALKNDLVEA	LKRKQQC*			

