Supporting Online Material for:

3'LIFE: A Functional Assay to Detect miRNA Targets in High-Throughput

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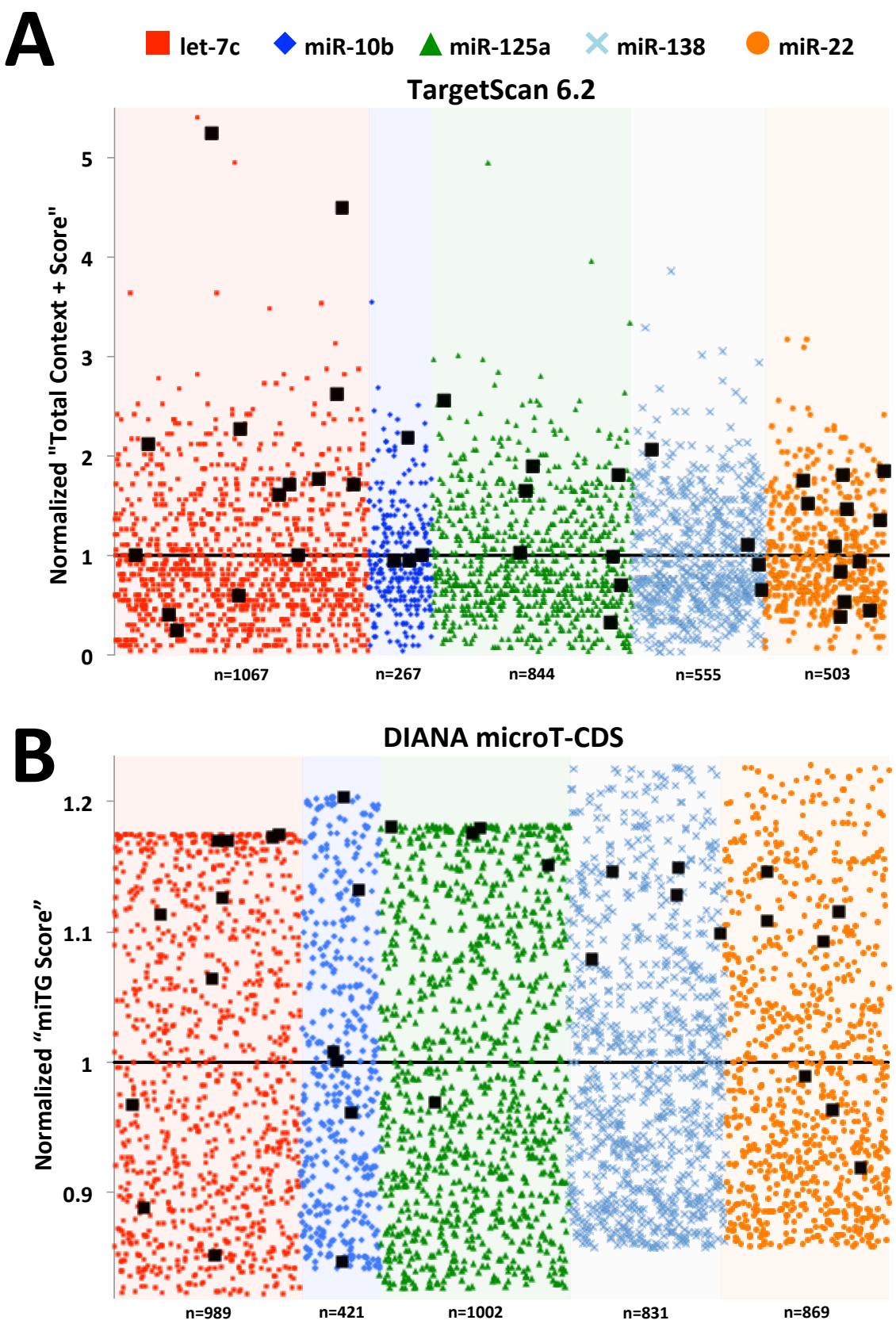
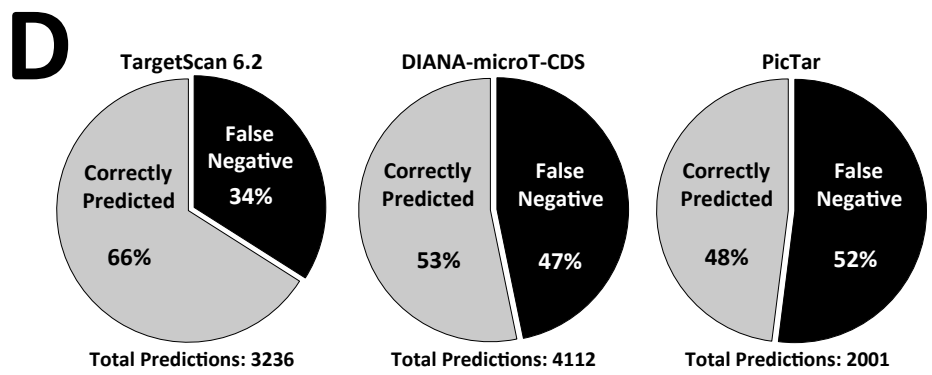
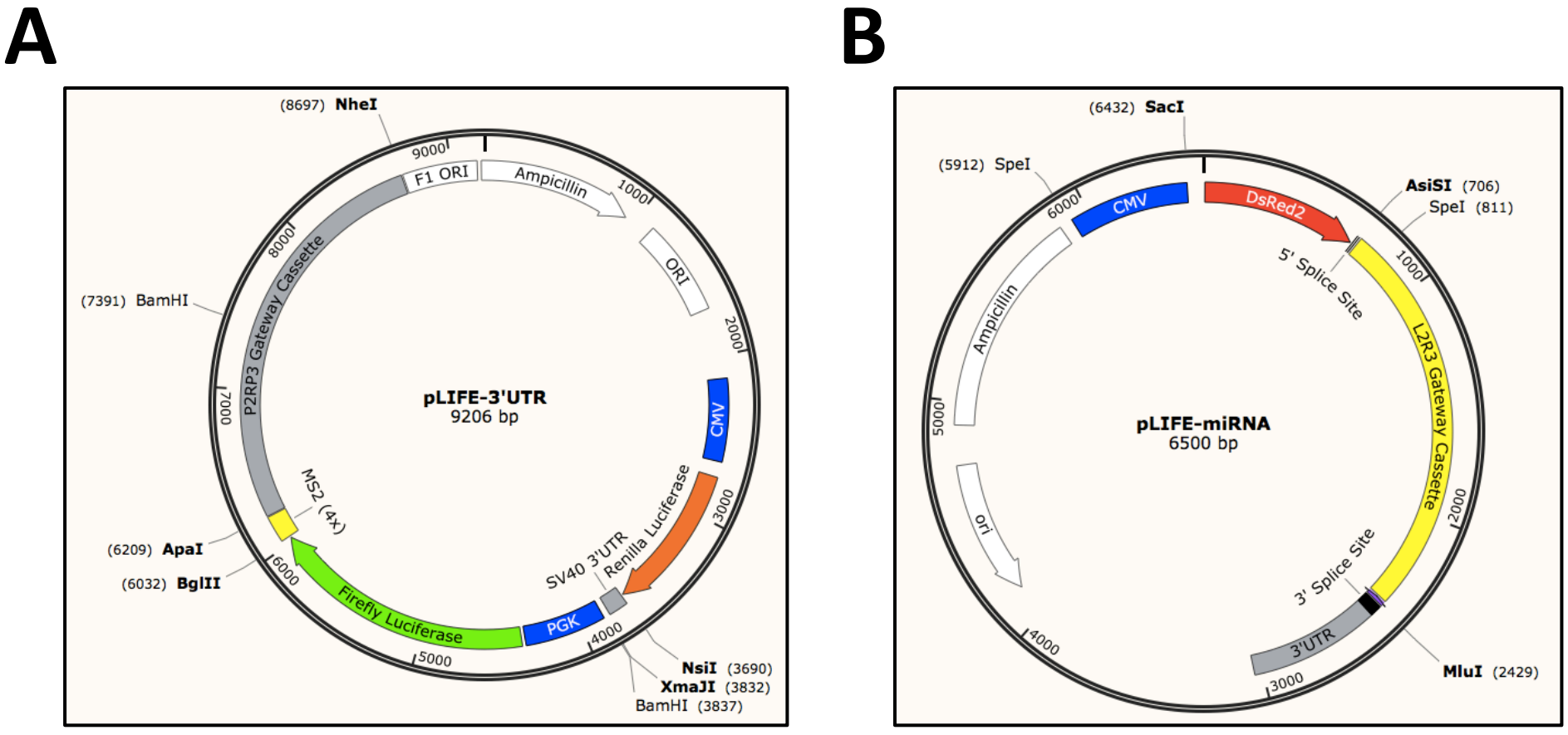
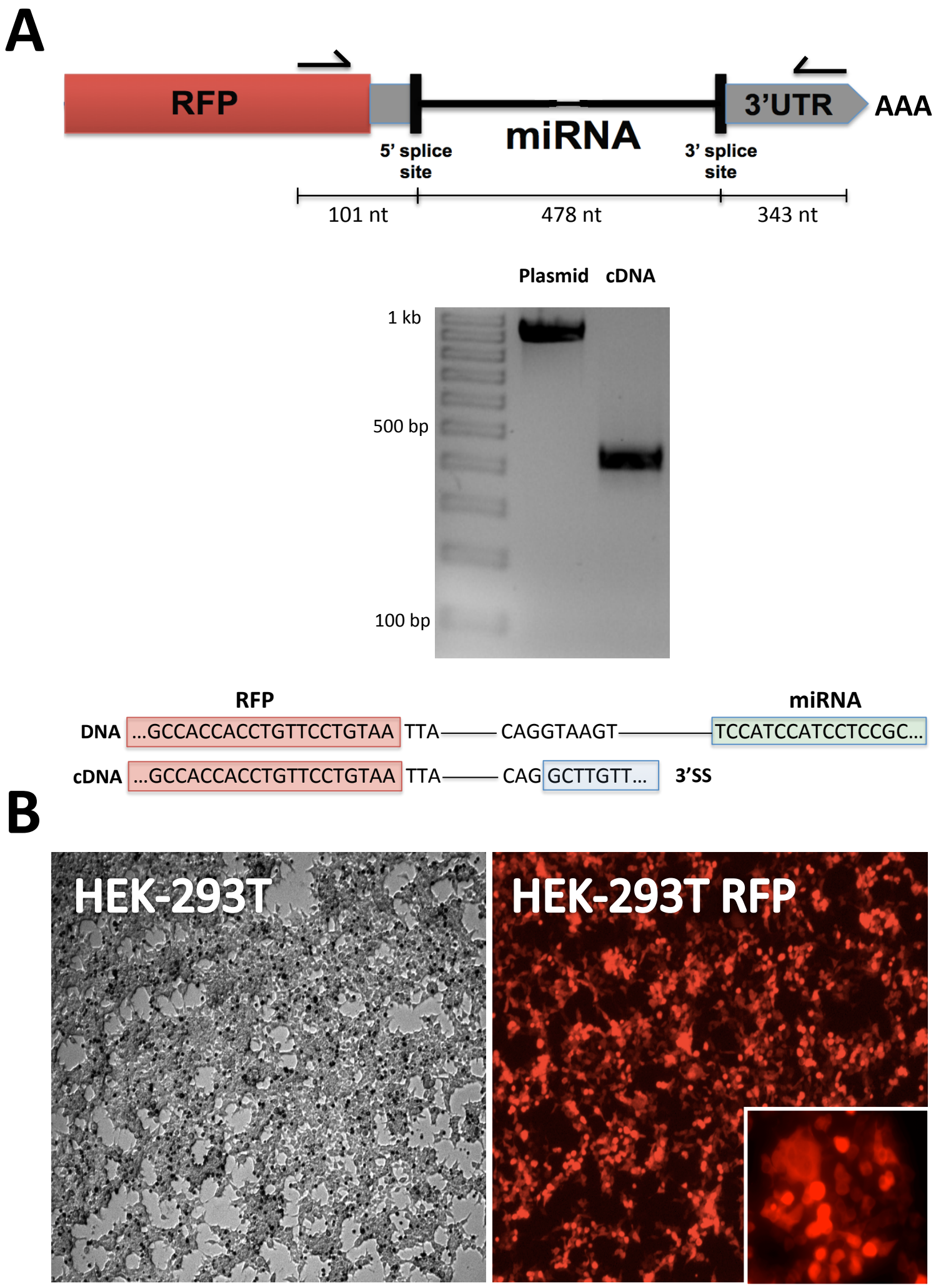




Supplementary Figure 1. Predicted miRNA targets have high false negative rates. We have superimposed all the experimentally validated miRNA targets of five cancer-related miRNAs (x-axis, black dots) to target predictions produced by a panel of widely utilized prediction algorithms, TargetScan (**A**) (5), DIANA microT-CDS (**B**) (42), and PicTar (**C**) (8). The Y-axis represents the normalized targeting score assigned to each prediction, with the black horizontal line representing the normalized mean. We show the same five representative miRNAs in each panel. These miRNAs were selected for this analysis based on 1) their significant presence in the literature, 2) their correlation with oncogenic pathways, and 3) their uncharacteristic high number of validated gene targets when compared to other less studied miRNAs. (**D**) Among 67 experimentally validated targets for these five miRNAs, 34% were not predicted by TargetScan, 47% were not predicted by DIANA-microT-CDS, and 52% were not predicted by PicTar. Of note, these algorithms produce thousands of predictions that are yet to be tested, representing >98% of predicted targets. This comprehensive list was compiled utilizing miRTarbase (40) and by our own manual literature review of up-to-date resources.



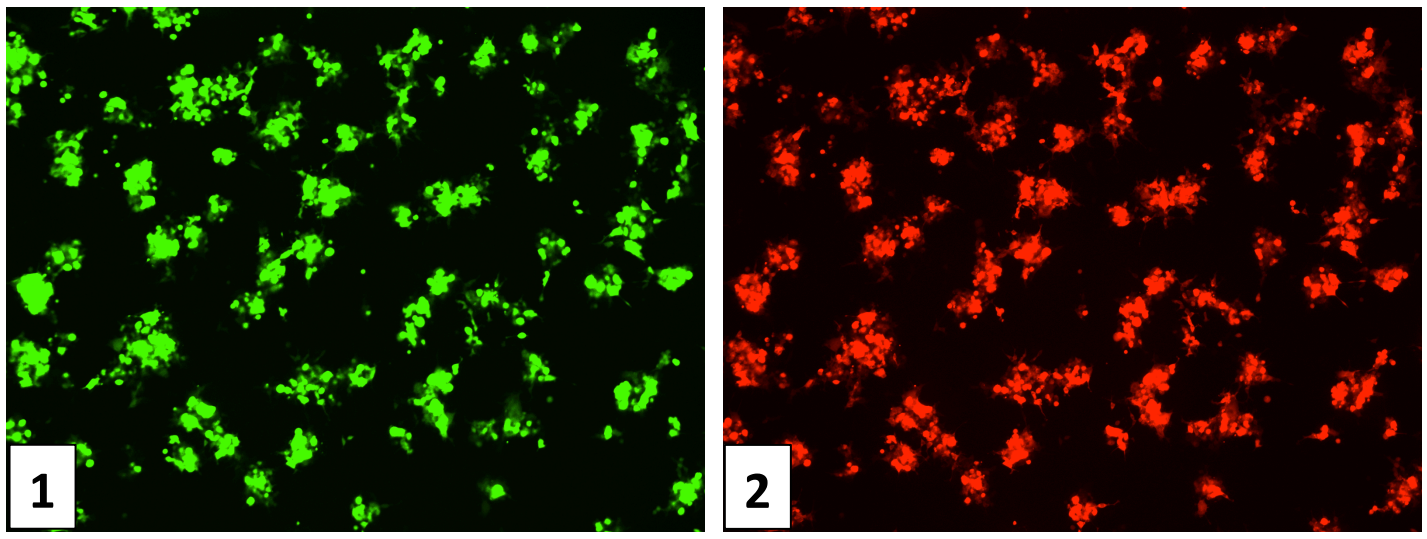
Supplementary Figure 2. 3'LIFE assay plasmid maps (**A**) pLIFE-3'UTR (**B**) pLIFE-miRNA. Positions of restriction sites used to clone various elements are noted. Plasmids are available through DNASU Plasmid Repository (www.DNASU.org, clone IDs:EvNO00601503 and EvNO00601504).
http://dnasu.org/DNASU/GetCloneDetail.do?cloneid=601503
http://dnasu.org/DNASU/GetCloneDetail.do?cloneid=601504



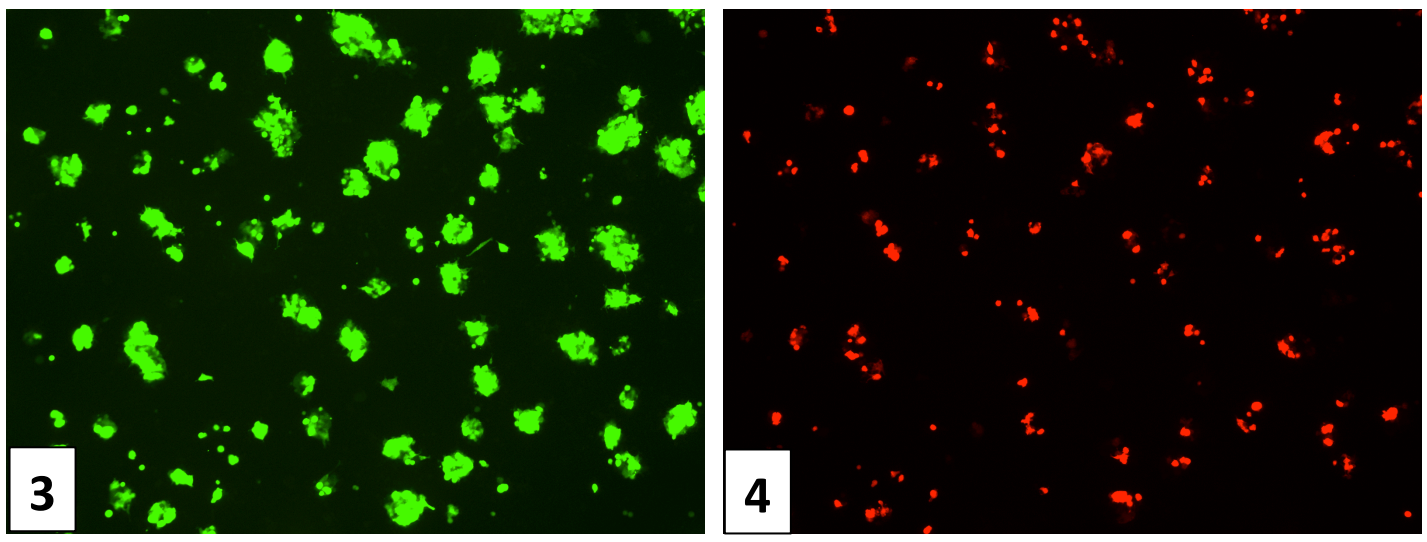
Supplementary Figure 3. Expression and splicing of pri-miRNA from pLIFE-miRNA vector (**A**) Top: Total RNA from HEK293T cells transfected with pLIFE-miRNA plasmid was extracted, and cDNA was synthesized using a polydT reverse primer. The second strand PCR reaction was performed using a forward primer that anneals in the open reading frame of RFP, and a reverse primer that anneals downstream of the 3' splice site in the 3'UTR. Middle: Gel electrophoresis depicts PCR of plasmid DNA and cDNA, with a shift of the expected size given proper intron/ miRNA splicing. Bottom: Sequencing of the PCR products confirmed that the miRNA is properly spliced out of the RFP mRNA transcript (**B**) Bright field (left) and fluorescent (right) images of HEK293T cells used in the above experiment. RFP expression functions both as a marker for transfection efficiency, and signals transcription of the primary miRNA transcript.

A

CMV Max-GFP + CMV-*dsRed2-miR-10b*



CMV Max-GFP + PGK-*dsRed2-miR-10b*



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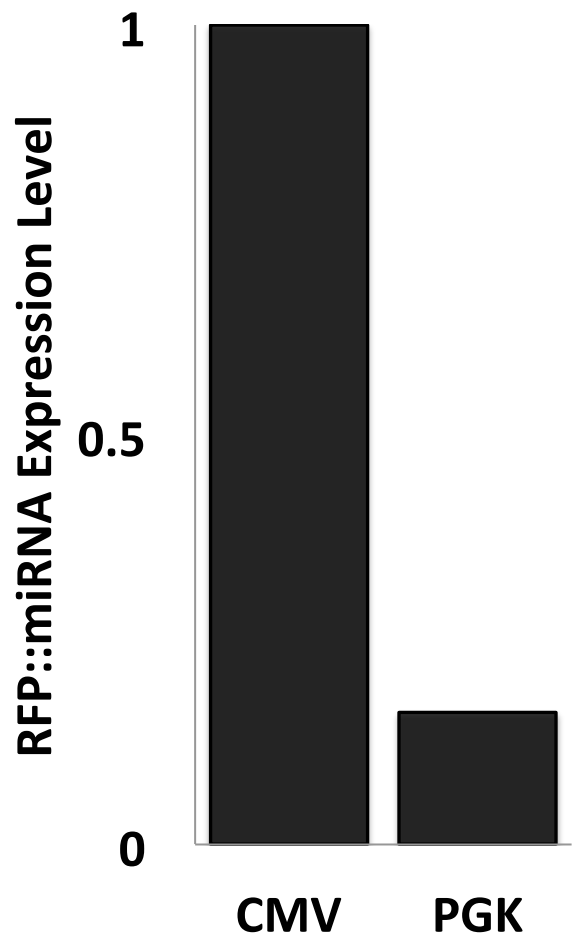
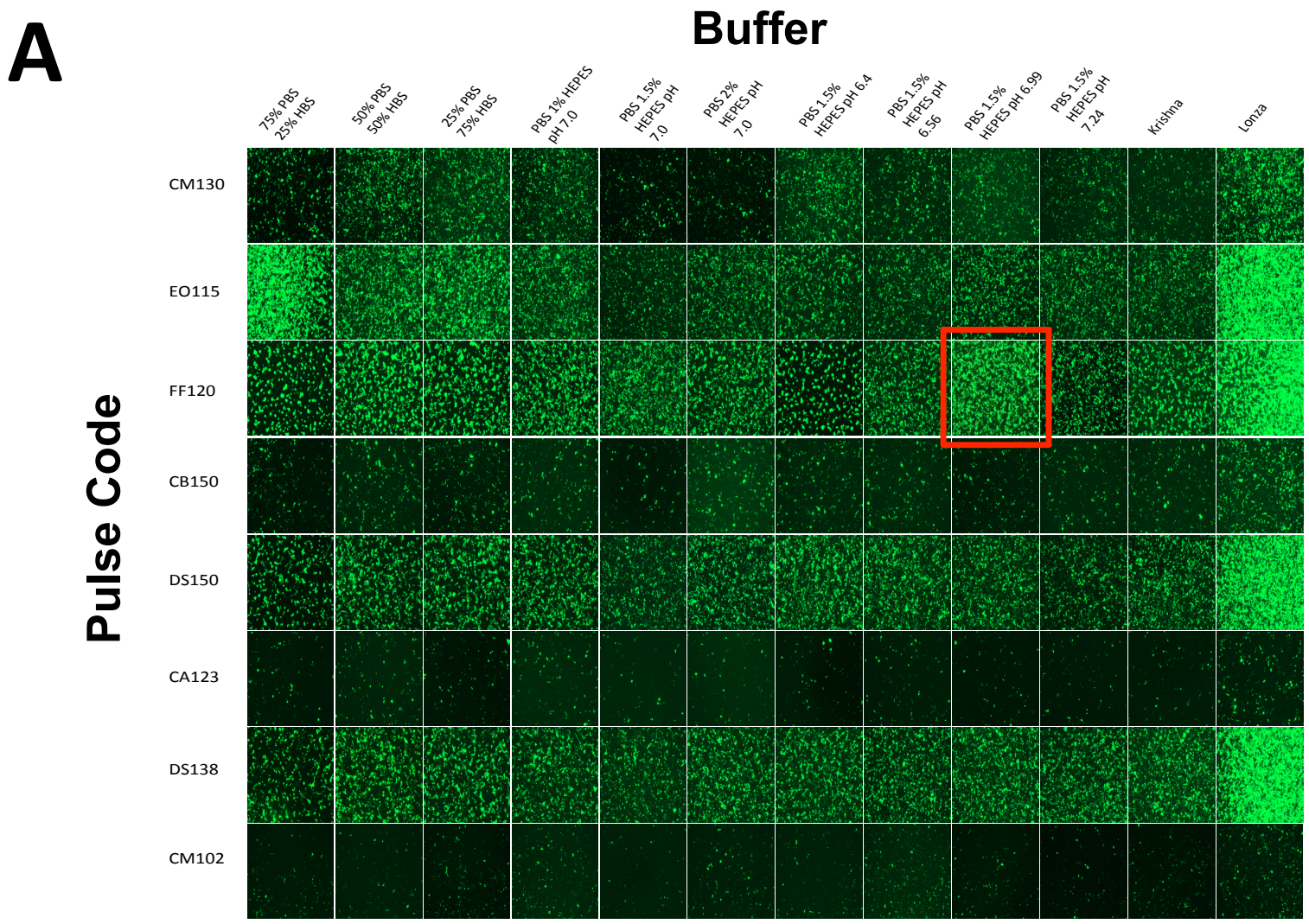
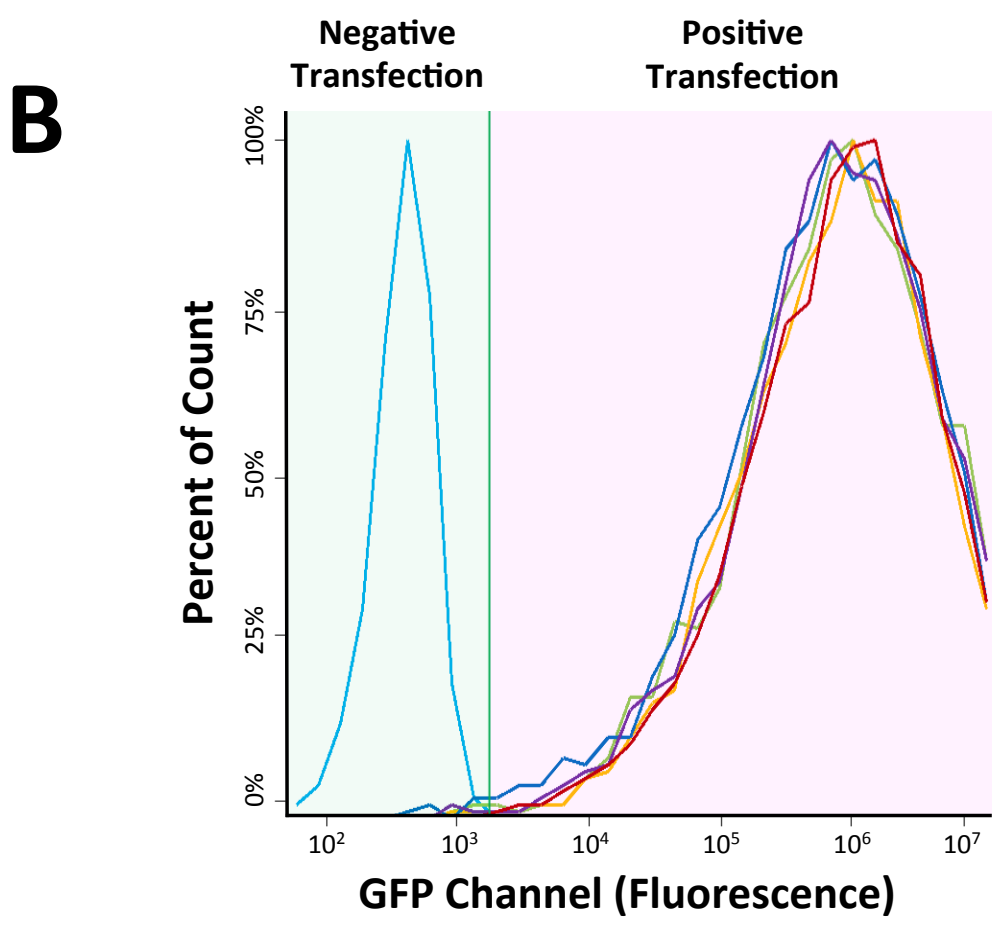
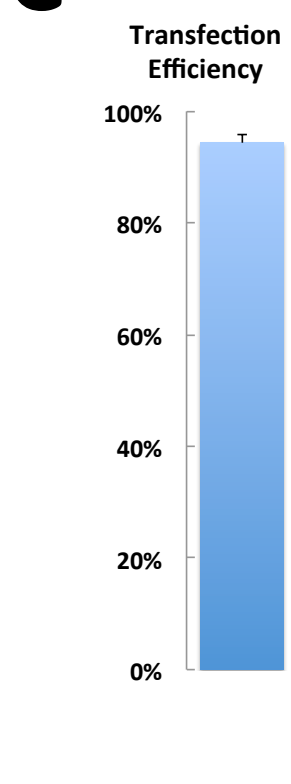


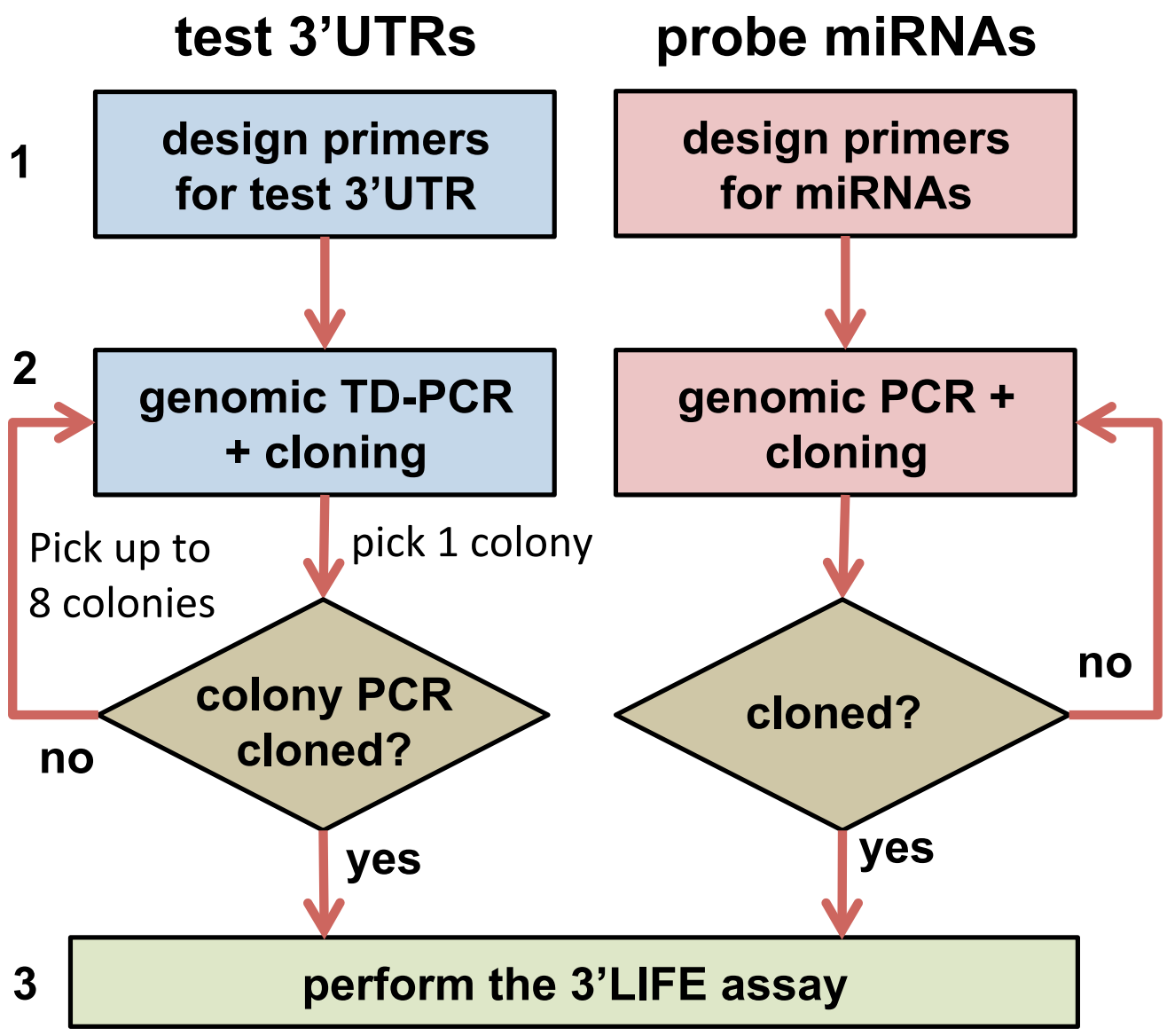
Supplementary Figure 4: PGK promoter is 80% weaker than CMV Promoter. (**A**) We have compared the strength of the CMV promoter related to the PGK promoter in co-transfection experiments using fluorescence microscopy. While the expression of GFP driven by a CMV promoter is constant in both experiments (compare panel 1 with 3), the expression of dsRed2 included in the pLIFE is much weaker using the PGK promoter (compare panel 2 with panel 4). (**B**) Quantification of fluorescence produced by these two promoters shows that CMV promoter is at least five times stronger than the PGK promoter, results comparable to those obtained by Qin, et al. 2010 (52).





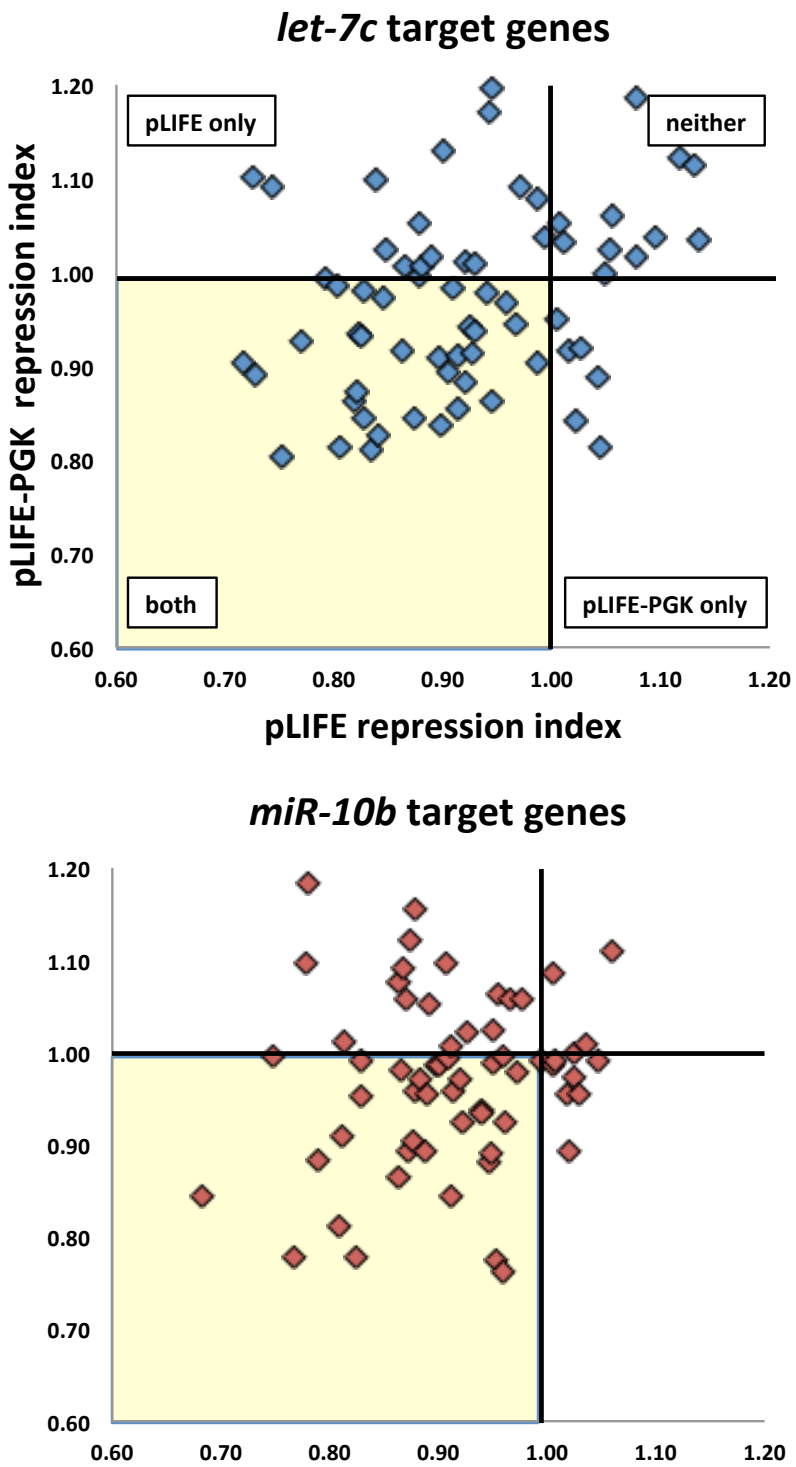


Supplementary Figure 5. Development of nucleofection transfection buffers. We tested 11 electroporation buffers against 8 different pulse codes. 100k HEK293T cells were transfected with 50 ng pmaxGFP plasmid (Lonza) and compared against the SF cell line solution (Lonza) (*data not shown*). (**A**) The highest performing buffers were each retested with various permutations of pH and buffer composition to determine optimal transfection conditions. Based on fluorescence and cell survival PBS 1.5% HEPES pH 7.0 and pulse code FF120 (red box) was chosen for the 3'LIFE assay. (**B**) HEK293T cells transfected with GFP in PBS 1.5% HEPES pH 7.0 buffer and pulse code FF120 performed in 6 replicates. Cells were cultured for 48 hours following transfection, and fluorescence was analyzed using flow cytometer. Light blue line is negative transfection control without GFP. The percentage of transfected cells is consistent between experiments. (**C**) Transfection efficiency of previous experiment (b).



Supplementary Figure 6. 3'LIFE cloning pipeline. This cloning pipeline was used to clone the 384 3'UTRs, and the miRNAs used in the 3'LIFE assay. 1. Primers used to amplify 3'UTRs from the human the genome anneal in the terminal exon of each gene and 150 nt downstream of the longest 3'UTR annotation in Refseq HG19 annotation. miRNA were amplified using primers that anneal ~200 nucleotides upstream and downstream from the pre-miRNA annotation from miRbase. All primers contain 5' universal Gateway elements to facilitate cloning in pLIFE Gateway compatible plasmids. 2. 3'UTRs were amplified using touchdown PCR cycling conditions (TD-PCR) in 96-well plates and used in BP cloning reaction. BP reactions were transformed in DH5α cells and plated in 48 well culture plates. Screens for successful clones were performed using colony PCR and size based selection for one colony. If first colony was not positive, up to eight additional colonies were picked and analyzed using colony PCR. 3. Successful 3'UTR clones are re-arrayed into 96-well plates and tested for targeting by each miRNA in the 3'LIFE assay.

A



B

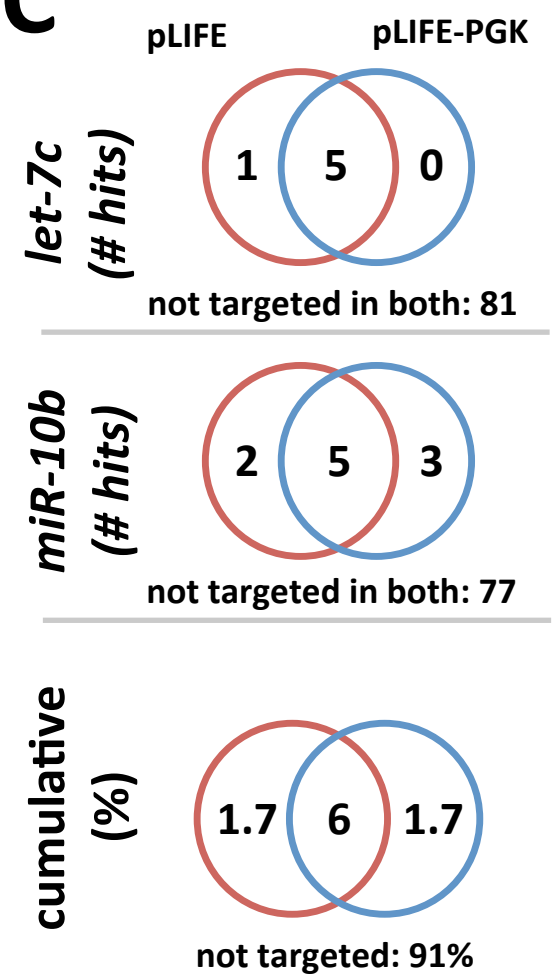
let-7c

Gene	pLIFE	pLIFE-PGK	Std Err
RHOB	0.58	0.77	0.00
HSF1	0.72	0.90	0.12
CNNM2	0.73	1.10	0.10
RARB	0.73	0.89	0.09
ARID3A	0.75	0.80	0.11
RNF7	0.80	0.81	0.04

miR-10b

Gene	pLIFE	pLIFE-PGK	Std Err
SDC1	0.47	0.65	0.12
LYL1	0.58	0.89	0.10
TCF15	0.68	0.84	0.05
FXR2	0.75	1.00	0.05
HOXD10	0.77	0.78	0.09
ASCL2	0.78	1.07	0.15
HIVEP2	0.79	0.88	0.03

C



D

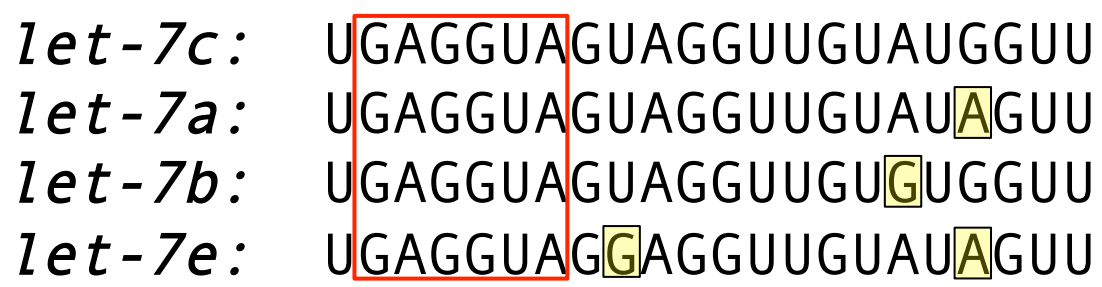
pLIFE Score	pLIFE-PGK Score	Refseq	Position	Alignment (top 3'UTR, bottom miRNA)
0.95	0.78	AATK	619-644	5' CCCGUAAGCCCACCCUUCCCACAGGGAACA 3' 3' GUGUUUAAGC---CAAGAUGUCCCAU 5'
0.96	0.76	HOXA1	584-606	5' AUGUUUAGGUUACUAAAACAGGGUGGG 3' 3' GUGUUUAAGCCAAGAUGUCCCAU 5'
0.86	0.76	KRT1	326-350	5' AGAGUAGGACCAGUUAGACCCAGGGUGUG 3' 3' GUGUUUAAGCCAAGAU--GUCCCAU 5'

Supplementary Figure 7. miRNA delivered using both weak and strong promoters identify comparable targets. (**A**) Comparison of 87 genes screened in the 3'LIFE assay using pLIFE (strong promoter) and pLIFE-PGK (weak promoter) for targeting by *let-7c* and *miR10b*. The yellow panel highlights the repression from both miRNA delivery methods. (**B**) Top hits produced with pLIFE compared with hits produced with pLIFE-PGK. Of the top hits produced using pLIFE-miRNA, 77% of them were also repressed using a weaker promoter, but to a lesser extent. (**C**) Venn diagram showing the overlap between hits produced using both miRNA delivery vectors. 97% of genes were either repressed or not repressed using both vectors. (**D**) We studied the seed region in three statistically significant hits detected using the pLIFE-PGK for *miR-10b* that were not targted by pLIFE (middel panel in C). Two of three have a perfect seed element in their 3'UTRs, suggesting that although at a low frequency (1.7%), some genes may exhibit dosage-dependent targeting.

A

CLASH 3'UTR Target	Targeting miRNA	Target Region	Interaction Type	# of chimeric reads	3'LIFE Repression Index	3'LIFE *p*-value
EZH2	*let-7a*	CDS, 3'UTR	7-mer	4	0.75	<0.01
EN2	*let-7e*	3'UTR	noncanonical	1	0.77	<0.025
CCND3	*let-7b*	3'UTR	none	1	0.83	<0.05
FXR2	*let-7b*	CDS, 3'UTR	none	1	0.85	
HSF2	*let-7a*	3'UTR (2x)	noncanonical	2	0.85	
SNX6	*let-7a*	3'UTR	noncanonical	1	0.91	
NUP153	*let-7b*	3'UTR	noncanonical	2	0.94	
MYC	*let-7b*	3'UTR	noncanonical	1	1.00	
HES1	*let-7b*	3'UTR	none	3	1.00	

B



Supplementary Figure 8: Comparative analysis of 3'LIFE with AGO-HITS-CLIP. (**A**) In 2011, Kudla, et al. (PNAS), developed a method to isolate RNA:RNA interactions using immunoprecipitation approach, followed by ligation of the two complementary RNAs, resulting in a chimeric sequence composed of the two interacting RNAs. Recently, this technique, termed cross-linking and sequencing of hybrids (CLASH) was applied to the AGO protein and miRNA:mRNA interactions (50), providing a potential solution to the issue of identifying which miRNA is targeting the mRNA footprint obtained from original AGO-HITS-CLIP approach. The CLASH dataset was also conducted in HEK293T cells, which provided a complementary approach to cross validate hits obtained from 3'LIFE. We compared our results with this dataset. Unfortunately, of the 272 chimeric reads obtained for *let-7c* and *miR-10b*, only one gene was present in the 3'LIFE library (EIF3A), and the target footprint mapped to the open reading, thus was not comparable to 3'LIFE. However, CLASH did identify 21 genes which were targeted by a family member of *let-7c* or *miR-10b* which were included in the 3'LIFE library. (**B**) Alignments of *let-7* family members shows that these miRNAs share identical seed regions (red box), and diverge by 1-2 nucleotides in the 3' end of the miRNA (yellow boxes). Of these 21 genes, 12 mapped to coding sequences, while 9 mapped to 3'UTRs. Of the 9 overlapping 3'UTRs in the two datasets, 3 of them were significantly repressed in the 3'LIFE ($p<0.05$). Of these 3, the gene with the highest repression in 3'LIFE, EZH2, was the only gene with a canonical seed target identified by CLASH and had the highest number of reads sequenced by CLASH. The remaining eight genes had either non-canonical, or unidentifiable target sites. The only gene with canonical seed was the top hit in both 3'LIFE and CLASH, and 7 out of 9 CLASH hits have some degree of repression in 3'LIFE, although only 3 significantly. While there may be subtle differences in miRNA targets by closely related miRNA family members, this comparison suggests that binding (as shown by CLASH) is not an accurate proxy for functional repression (as shown by 3'LIFE).

Supplementary Table 1: Primers used for cloning

Alias	RefSeq ID	Forward Primer	Reverse Primer
AATK	NM_001080395	GGTGAGAGTAAAGAGGCTTGA	CCTCCACCGGGGTGTGCC
AKT1	NM_005163	GCCAGCGGCACGGCCTGA	ACACAGCCTGTCCCCAAAC
ALX3	NM_006492	CTGAACTGGACCACGTGA	CCTTTACACCCTCCTTAGTGTCAGAC
ANXA7	NM_001156	CTGGCTATTGTGGGCCAGTAG	CTGAACACTTAAAATTTGCTAAGGTAGTAG
APC	NM_000038	TTCTGGGTCTTACCTTGTGACATCTGTTTA	ATTTGAATTAAAAGGCAAGTTTATCTAATT
APPL2	NM_018171	GCAGAATCCGAAGCATAA	CTACTCATGTTGACTGGAAGTATTTTTTGA
ARID1A	NM_139135	TGATGTACTGTTTTTGATTGGCCAGTCATG	TTTTGGAATATAGTAAATAAATGACAGGGT
ARID3A	NM_005224	CACATCTACCTCAAATAACTCGTTGCCTTA	AGTTCATTGGCTCCGCGG
ARID3B	NM_006465	CACCAGCTGGTCCCTCTG	TTGAAGAAGGCGTGGGTGAAG
ARNT	NM_178427	CTAACTATGTTTCCCCCCTTTTCAGAATAG	AGCCAAGATCGTGCCACTG
ASCL1	NM_004316	CGACTTCACCAACTGGTTCTG	TGGATGGGACTGGCCATAGC
ASCL2	NM_005170	TGGTTAGGGGGCTACTGA	CAAGGTGTCCCAATGGCTCC
ASXL1	NM_015338	TGTATTGTGCCTTGTGGTGAGATA	TAACCTCCCAAAAGAAGAGCTCCAAGTATA
ATF1	NM_005171	GAAGGATCTTTATTCCAATAAAAGTGTTTG	TCATAAAGTGCTGCCAAGTCAACAGA
ATF3	NM_001674	ACAGATAAAAGAAGGAACATTGCAGAGCTA	ACTATCCCATCTAGTGATGCCCCAAA
ATF5	NM_012068	GAGGACCCGTAGCTGCTA	GTATTTTCAGTAGAGATGGGGTTTCACCTT
ATOH7	NM_145178	TTCCAGATGGCCACCTAG	TCTCATCAGCTTTTTGAGGAACTACTACAA
ATXN2	NM_002973	CACCAACAGCAGTTGTAA	TCTAGAGATACCTTGAACCAGAACTAAGGG
BAG2	NM_004282	GCAACAAAATGCTGAAAGCAGATTCAATTA	TTTGTATATCAATAGGACTAGTTACTTTGA
BAG3	NM_004281	TAACCCAGCAGCACCGTA	CAATTCTCTCTTGAGCCGGGCTATT
BAZ2B	NM_013450	AAGTGGACAGATACTTTCAAAGTGAGCTGA	ACTTAAACTCACCCTCTTCCACCCA
BCCIP	NM_078469	GTCTGTTCCCCCAGTATTAGAATA	CACCAATCTATATATAGCCTTATTCCACAA
BCL11A	NM_018014	TCGAGAGCCCTTAAGTTCTGA	TCTCTTACTGATGTGGCCTCTGG
BCL2L1	NM_001191	TCACTCTTCAGTCGGAAATGA	TCACCGTACAGGTGGATAAATTCAG
BCL3	NM_005178	AGCTCCAGGAGGCAGCTG	AGAAGTCCCAGAGTGACACACAG
BCL6	NM_001706	CTCCCCAAAGCCTGCTGA	ACCTAGCCTCATCTCAAAAGACGC
BHLHB9	NM_001142530	GGAAGTTAAAGAGATTATTGAAACAATGTA	TAAGTGTTAAGAGGAAAAGGCAAGTCTCAA
BHLHE22	NM_152414	ACAGTGCACGGAGAAGCCTTA	TAAACAAGTAAATTAGGGCTACTTCTAATT
BICD1	NM_001714	CAAGCCTCCTCACCCCTA	ATGTCCAGTGAAGAACTCTAGTTAAAAATA
BMI1	NM_005180	ATCAGCAACTTCTTCTGGTTG	TTAAAGTTTTTAGCCTTTTAAAAATATTTT
BMP2	NM_001200	GGGTTGTGGGTGTCGCTA	TGACTTATCAAATAACTTGCCTGCAATTTT
BMP4	NM_130850	GGATGTGGGTGCCGCTGA	TACTTCCTGTCCCTACAACTTAACCAC
BMP6	NM_001718	AAGAGCTTGTGGATGCCACTA	ACTTTGCATCCAACACTCTTCACC
BMPR1A	NM_004329	GATGGTTGAATCCCAAGATGTAAAAATCTG	GATGTGATATAATCACATTTATTTTATGTT
BRCA1	NM_007299	AATTGGGCAGATGTGTGA	CAGGAAATACAAAAGGTATTTAAGCTGCCT
BRCA2	NM_000059	GGACACAATTACAACTAAAAAATATATCTA	ACAAAGCAAGACTCCATCTCCAAAAAAAAA
BTAF1	NM_003972	CCTGGAAAATTTTATGCATTCTCTCAAGTA	TAAAAAAATATTCAACTCAAATATACTGAA
BZW1	NM_014670	ATCTGAAGCTGAAGAAGGTGACTG	TTTAGCTACACCAGAATTCTGTGTAGGTGG
BZW2	NM_014038	AGAATCCGAATCGGAAGGTGAGGAAAATTA	CTGGGATTACAGGTGTGAGCAGAATAG
CASZ1	NM_017766	CTCCAGTTCCAGGAGAAGTGA	ACTCTGACCAGGCCAGGGAA
CCND3	NM_001136017	GATGTCACAGCCATACACCTGTAG	GAACTGACTATATTTGGGTCAGAGACCATG
CCNE2	NM_057749	GAAAAACCACCAGGAAAACACTAA	ATTTTTGACACACCTCTAATAACATCACTT
CDC25A	NM_001789	TACAGTCGTCTGAAGAAGCTCTGA	TCTTGCAAATCAATCTGAACCACAAGTTTT
CDK4	NM_000075	CTACATAAGGATGAAGGTAATCCGGAGTGA	TTCCTCTGTCCATATACTGGATCACCT
CDKN1A	NM_000389	CTTCTCCAAGAGGAAGCCCTA	CTGTTGTTTTTTGCAGCAGTCTTTCTGTTT
CDKN1C	NM_000076	CGCAAGAGGCTGCGGTGA	TGGGCCAGGCCCAGCCAG
CDKN2C	NM_001262	GGGAGCCACAAATCTTCAATA	ATCATCAAGAGAGTATGTCTAGTTTTCCAG
CDKN2D	NM_001800	ATGGTGGCCCCGCTGTGA	CCCAGCTCACTCACCCTG
CDNF	NM_001029954	CCCAAAACAGAGCTCTGA	GAAATGTTTTTTCAGTATTCCCCAGCTGTA
CDPF1	NM_207327	CCCGGTTCTCGGACGTGA	TGGAGGCTGTCTCCACCT
CDX1	NM_001804	GAAAGAGGAGTTTCTGCCATA	GCTTACAAAATGGACTCACATTTTTACGTA
CDX2	NM_001265	CCCACCGTCACCCAGTGA	GAGCCACGCATTCCAAGGC
CERS2	NM_022075	CTCAATAACAACCATCGTAAGAATGACTGA	CCAGCTAGATGTATTTTTAGTAGAGATGGG
CHMP4B	NM_176812	GAACTGGGCTGGATCCATGTA	ACTTCTGGTGAGACAAGCTCTGC
CNNM2	NM_017649	CAACGAAGGCGCCATCTA	AGAAAACGCTCTCTCCTTTCCTAGTGAT
COPS6	NM_006833	GCGCGGGCTCTTTTTCTG	GCTGCCTCTCCAAAATATGTAGTAAACG
COPS8	NM_006710	TTATGTGGCTTTCCTTGAAAACTG	CTCCTGATCCTCTGACTTCTTGAATTC
CPLX1	NM_006651	CAGGACATGCTCAAGAAGTAG	CTGGACGGCCTAGGGAAAATG
CREB3L3	NM_032607	GGCGGGAGACGAGCTGTG	TGCCCGGCGCCTCTCCCT

CREB3L4	NM_001255981	GCATGCAGATGAGATGTG	GGTTTCCTTCCCTTCTGTAACTTGGA
CREG1	NM_003851	TAATTCTTCTCTGTTCCCCTTTCTAGGTGA	GCTTTTTGCCAATTAGAAAGAGTGGTATTA
CREM	NM_182721	CAAAGATCTTTATTGCCATAAAGTAGAGTA	TCTCCCACCACACACTGATCAGAT
CRK	NM_016823	CCCGATGAGGACTTCAGCTGA	AGGAATCCTTAGGACTTGAGTAGCG
CTCF	NM_006565	CAGCATGATGGACCGGTG	CCCGCCAAGATCATATCGTCC
CTF1	NM_001142544	GCCCGGGGGCTCGGCCTG	GGGCTGGCAGAGGGCACC
CTNNB1	NM_001904	CTGGTTTGATACTGACCTGTA	AAGTAGGGCCCTCTCTATCGCTA
DEC1	NM_017418	GCTTTTTTTTTTTTTGTCTCCCAGCAGATTG	AGACCAAATCTTGAAACCAGAATAGGCA
DIDO1	NM_022105	GGAGTTGCAGCTTTGTGTTAA	TGCTTGCCTGCATGAGTG
DLL1	NM_005618	GTGGCCCCCCAGGTGTAA	AGGAGAGGAACGGGCTGCT
DLL4	NM_019074	CGCTTGCTCCCAGGTATA	ACATGCCAAATCCAAGTGTCTGC
DLX1	NM_001038493	GGGCGGCTCTGGAGGGTA	CTGACGATACCCTGGTGTTTCTG
DLX3	NM_005220	CCTGGGGCTGTGTACTGA	GGCCAGGCCCAGCCCAGC
DLX4	NM_001934	TTCGCCTCAGATGATGTG	ACAGAGCTAGTTAGTGGCCAAGACAA
DMRT1	NM_021951	CATCGAGGAGGACGAGTG	GCATTTAATAAAAAACAAGATGGTCAGGTT
DNMT1	NM_001379	GAGGAAGCTGCTAAGGACTAG	AGTTCTGAACAAACACAGACAGACACA
DNMT3B	NM_175848	GAAGGACTACTTTGCATGTGAATA	TAAGCTCCAGTCAGCTTGGGG
DOCK11	NM_144658	CCCAAGATACGCTGAAGTGTG	CTAATTGGCAAGGATTCAAATGATCTCCCC
DONSON	NM_017613	AGAGACTACATTTATAATTGGAGATCCTGA	TACAGTGGTATTCATATGCTATGTCTCTAA
DPF1	NM_004647	GCTTACATCACCCTCACCTAG	CAAAGCCGGGGAGAGGCA
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EBP	NM_006579	AAAAGCCAAGAGCAAGAAGAACTG	TCTGGCGGGGGGTCAGTG
EGR1	NM_001964	CTTTTCTCCCAGGACAATTGAAATTTGCTA	ATACTCAGTCCTCATAAATAACGAACTCCA
EGR2	NM_000399	CGGACCCGGACACCTTGA	CAGGCTAGCAAAGAAGATCTGGAGA
EGR4	NM_001965	TCCTTCGCTTCTCTCTGA	CGCAGTTCCTGGCAGGTGT
EHMT1	NM_024757	TGCCGCCGACCCCCTATG	CTGCCTGGAACGTCCCCT
EHMT2	NM_025256	CCCCCTGTCAACACATGA	CATAGTGGCCCCCCACCT
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ELF1	NM_172373	CTGCTGGAACCCAACTCTTTTTAG	TCTCAAAAACAAAACAAAACAAAAGTGTGT
ELF2	NM_201999	ACAGAAGGACTAGTGACATGTGAGAAATAA	CAAAAGGTCTGATTGTGAAACAAATGGTTT
ELF3	NM_004433	GGTTCTCCAGAGTCGGAACTG	TGTAAGTAAAAGCCTTTTTCCAAATGGCTC
ELF5	NM_001422	CAGGAAGACAAGCTATGA	CTTGGGCAACAAGCAAGAACC
ELK1	NM_005229	GGGCCCCAGAAGCCATGA	GATATGCAGTCCCTACTATTGTTTCTCACA
ELK3	NM_005230	ACTGCTTTCTTCAAACTCTCAGAAATCCTG	CCTGGAATAGCATGAACTTGATGTAGCTTT
EMX1	NM_004097	CATCGATGTCACCTCCAATGACTA	GGTCTGGATGTCGTAAGGGAAAGACTTA
EMX2	NM_004098	GGAGGAAATAGACGTGACCTCAGATGATTA	ATCTTTCTTTAAAATGCCTGAGAATCCATC
EN1	NM_001426	GACAAAGACGAGAGCGAGTAG	TCTGGGTCGCGCTTCCCC
EN2	NM_001427	CAAGTCGGACAGCGAGTA	TAATAATCACCTCTCGCTACAAAGGCA
EPHA8	NM_020526	GCCCCGCCGGCACCTCTG	GCACTAATCCTACCTCCCCAGACA
ERBB2	NM_004448	TCTGGACGTGCCAGTGTG	CCCCAACCCTGAAGCTGGA
ERBB3	NM_001982	GGCTAATGCCCAGAGAACGTA	GTTGTTCTAAAGAAATAGAAGTAATAGTAG
ERF	NM_006494	GAGCACCGAGACTCCTGA	GCCTGGATTTGGCCCATTCTGATTA
ESR2	NM_001214902	AAGCAATTCATTCATTTGAAGTTATCTTAG	GGTTTCGCCATGTTGGCCA
ETNK2	NM_018208	TTGGAGATGCCAAAGTGA	CGGAGCTAGGTGCACAGGA
ETV3	NM_005240	AAGATACAAACTCTTTTGGTAGGGAATTAA	GAAAACAGTTAAATACAATCTACAGTAGTC
EZH2	NM_001203247	GAAAGAGAAATGGAAATCCCTTGA	CAGTGTTCTTCATATATGTCTTCCACATAT
FAP	NM_004460	CTAAAGCAGTGTTTCTCTTTGTCAGACTAA	GAGTTCTCATGACAGAAATCCAAAACCACT
FHL1	NM_001449	CTGTGCCAAAAAGCTGTA	TGTGGTTTTTACAGGTTTGTTTATAATTCC
FIZ1	NM_032836	CACCGGGGCATGGACTGA	AGCCTAAACCGACTCTAATTTAAAAGGG
FOS	NM_005252	CACGCTGCTGGCCCTGTG	TAAAATCAGCTCTATAGTTTCCTTGTCCTC
FOSL1	NM_005438	ACCCTCCTCGCTTTGTGA	GCGGATGTAGCCCCACTTGT
FOXA1	NM_004496	CCCGTCCTAAACACTTCCTAG	ATGCAAAATAGCGGCTACCCTAAAATGT
FOXA2	NM_021784	CCCATTATGAACTCCTCTTAA	TTGAAGTGGATTTAGAGAAATAAGATGGAT
FOXC1	NM_001453	CGTCTACGACTGTAGCAAGTTTTG	CCAGCGAGATTTAAACGGGGC

FOXD2	NM_004474	TAGTGGCTGCCACTTCTG	CTTGCATCCTCTAAGCTCAAACCC
FOXE3	NM_012186	GCTGGAGCGCTACCTGTG	CCTGCTGGAGAATCACTTCCTAATC
FOXI1	NM_144769	GGAGGGCACCGAGGTCTA	AGAGGTGAAAAGAGTCTTGATATGCATTCT
FOXL1	NM_005250	CACGGTACTCCACTTCCAGTA	TCTCCACTCCTTCCCCTCCAT
FOXL2	NM_023067	TCGCGCCTCGATCTCTGA	ACAAAGCAGCAGCGACAG
FOXM1	NM_021953	CAGTTTATTCCTGAGCTACAGTAG	TGTGTGCCTGTGCAAGTGCT
FOXN1	NM_003593	GCCCGTGGCCCTGGCATG	TTTCTGGAGGAGGAGTGAGATTGTG
FOXO4	NM_005938	GTTTCTTCTTCCCACAGATCCCTG	CTCCACCCCCACACTCCA
FOXQ1	NM_033260	GGAGACGCTCCTAGCCTG	GGAAATCTGCAAGCCCATTCTATAATACCT
FST	NM_006350	ACACAAGAGCGCTTTTTATCTAATTTCAGG	AGTCAAGGTTCCTTGTAAACAAAACCC
FUT8	NM_004480	CACATATCCTGAGGCTGAGAAATA	CAATTTAGAAGGAAAAGAGACTAACTTAAT
FXR2	NM_004860	TTGGGTAGTATGGTGAATGGGGTTTCATAA	TTCCCAGAAGTAAGACCATCTTTGGG
GATA2	NM_032638	GTGACCGCCATGGGCTAG	TGGCAAAATCAGACCCAGGC
GATA3	NM_002051	GGTCACCGCCATGGGTTA	GACGTTGACTCTCTGGGGTTAGC
GATA6	NM_005257	CGCCCTGGCCCTGGCCTG	CACTGGCTATGGACACTGTCCC
GCFC1	NM_016631	GAATTTAAGTCTTTGATCGAAGGAAAATAG	AAGATAAAATTAACAGAGCAAATACAGTGC
GCM2	NM_004752	TTCTTTACCTACAACAATGAGGATTTTTGA	ACAACTGTTTCCCAGGTCATAATATGAACA
GGNBP2	NM_024835	GACAACGGCTGGAGCAAATTA	CAATTCTCATCAAAGGAAGATTTTTGTCTT
GNAI1	NM_002069	AAATAATCTAAAAGATTGTGGTCTCTTTTA	CCAAATGTGGCATCACATCTCATAGCT
GNAI2	NM_002070	GGACTGCGGCCTCTTCTG	TGAAGCTCAGAGCGTGGG
GNAQ	NM_002072	TTGAACCTGAAGGAGTACAATCTGGTCTAA	AACAATGTCATCTTAAGGACAAAGAAAAGA
GTF2H1	NM_005316	GCGTCTGATGAAGAAAACGTG	GCATGTGCTACCATGCCTG
GTF2I	NM_001518	ACCAGACCCCACGTGGTA	TCTGAGAAACACCGCAAGTGG
HDAC1	NM_004964	GGAGGTCAAGTTGGCCTG	AAACCTCAAGGGAGGAGTTAAGGC
HDAC3	NM_003883	AATGACAAGGAAAGCGATGTGGAGATTTAA	AATAATAAATGTAGAATACATACACAGGGC
HDAC5	NM_005474	CAGGAGCCTGCCCTGTGA	TACAGGACAGATCTTGCGCC
HDAC6	NM_006044	TATGCCCCACCCACACTA	TTCAGTTAGTTTTTTGGGGGCAATGGA
HES1	NM_005524	GAGGCCGTGGCGGAACTG	TAGTTCATGGAGGATTGGTGAAAAGTTTGA
HES5	NM_001010926	CTCTGGCGGCCCTGGTGA	GACCCCACCCTTCTTCCG
HES7	NM_001165967	TGGAGACCTTGGCCCTGA	AAGTAAGGAATGGGGCAAATCTTAAGAGTG
HHAT	NM_018194	GACCTACGCCACGGACTA	GCACCACCATTCAGAGTAACTACAAGATTT
HIF1A	NM_181054	CTTAGGTATCTCTTTTGTTTTTCAGATTTA	TACATTAAGGTGATGGCACTAAGATAAATG
HIVEP1	NM_002114	CAGGCTTGTGATAGCAACCTG	TTTCACATGGTAGAAAGGCGAGTAAAGTCT
HIVEP2	NM_006734	CCTTCATCAGAAAAGAGTCAGCTACATTGA	GACTGTCTTCTCACTTGTTAAAAGCATTCA
HLA-E	NM_005516	GTCTGAGTCTCACAGCTTGTA	GGGATTCACCAGGAAAACGGGAAA
HLTF	NM_003071	AATGAAATCAGAACATTAATTGACTTATAA	AATTGAGCAAAATAAAGTTAGTTAGGATAT
HLX	NM_021958	CGCGCTTGGCTGCTTATA	TGTCTGTAACAAGATGGCTCTGAGGT
HMBOX1	NM_024567	GGCCCTGGATGATGACTG	AAAAAGAAGCCGTGCTTAACATCCA
HNF1A	NM_000545	GGCCTCTTCCTCCCAGTA	TAGAGTCTCAGCAGAGCAAGAAAAGC
HOXA1	NM_005522	ACTCTGACTACCTCCCACTGA	TATATCAGAACTCTGCCTGACAATCCAAAA
HOXA10	NM_018951	GAGCTCACAGCCAACTTTAATTTTTCCTGA	CCAGGGCTATAGGGCCCAG
HOXA3	NM_030661	AAGCTCACCCACCTGTGA	AATTTGATTCCTTTCTCGAGGAATCCTTAA
HOXA9	NM_152739	GACCGAGCAAAAGACGAGTGA	GCGGACTGGTTGTGGCAG
HOXC11	NM_014212	TTTCTCGGGAAATCCTCTGCTGTA	CGGCTAGCACCGGCCTAT
HOXC4	NM_014620	AGAGGACATTACCAGGTTATA	CCTTGCTTGTTCTTCTAAGGACATTGGAAG
HOXC5	NM_018953	TTCCAAAATGAAAAGCAAAGAGGCTCTTTA	AATTTCACCTCCCTCTACTCACTG
HOXC6	NM_004503	AGAAGAGGAGAAGCAGAAAGAGTG	GAAGGCCGGGGCCGGGCG
HOXD1	NM_024501	GTCCCAAGAGCCTTCGTG	AACAGAGCAAGACTCCGTGTCAAAAAAAAA
HOXD10	NM_002148	CGCCAACCTCACGTTTTCTTA	CCTCCAGCTTTTCTCCCCCAT
HOXD11	NM_021192	GCAGTATTTCACTGGAAACCCCTTATTTTG	TCGATTTTCAGTTGCATGGGTTCTG
HOXD3	NM_006898	CAAACTGACGCATCTGTA	CCCGAGAACCAATTTATGCACTAGACT
HOXD8	NM_019558	AGCCGAAGGCCTGACAAATTA	TTTTTTTTTTTTTAAACAGCGCGGAATGTGT
HOXD9	NM_014213	GAAATGCCCCAAAGGAGACTG	GAGACACATCAGAGAGATCTGTCAGGT
HRAS	NM_005343	AAGTGTGTGCTCTCCTGA	GAGCACCACAGCCCAGAC
HSF1	NM_005526	GGACCCCACTGTCTCCTA	CTGGGGAGTCGGGCAGGC
HSF2	NM_001135564	GGATAGTGATATGCCACTTTTAGATAGCTA	TCCAGTGATAATATTCTTACACTATTTGGG
ID1	NM_181353	GAGATCCAGATCCGACCACTA	TTAAAGACACCGGAAACACTCATTCAG
ID2	NM_002166	CAAAGCACTGTGTGGCTG	GGGGTGCTGCTGGTGGGG
ID3	NM_002167	AAAAGGAGCTTTTGCCACTGA	CTTCTCTCTCCACCCCATCCC
IRF2	NM_002199	CGCGTCAAGAGCTGTTAA	AACTGAAGCCTCACAGTAAGAACCT
IRF5	NM_001242452	CCCAGCTGGCATGCAATA	TGAGATACTATCTCACCTGTCAGGTTG

ITFG2	NM_018463	CCTCCAGGATCCCACCTA	AGTATAATTGAGAGGAATTTGAAAGCAATG
JAK2	NM_004972	GGATCAAATAAGGGATAACATGGCTGGATG	GATTTCTGCATCCAGAACTATTAAAATGCT
JARID2	NM_004973	CAAAAGTGCTTCGAGCTCATCATG	ATGGAAACCATTAGGTTAGGCTGAAG
JUN	NM_002228	ACGCAGCAGTTGCAAACATTTTGA	TTCTAGTTTGACTCTTCCTAAATTCTTTGC
KL	NM_004795	CTCGAAGAAAGGCAGAAGAAGTTACAAATA	TGGGTCGCCCTCCCACCC
KLF4	NM_004235	CTCGCCTTACACATGAAGAGGCATTTTTAA	GTTTTTTTTTAATAAAAAAAGGTATTTTAA
KLF5	NM_001730	TATGAAGAGGCACCAGAACTG	CAGAGCGAGACTCCGTCTCAAAAAATAAAT
KRT1	NM_006121	TCTACCACTTATTCCGGAGTAACCAGATAA	GGTGAAATGTTGCCTGGTTGG
KRT12	NM_000223	CAAGTTCAGGAAATTGAAGAACTAATGTAA	CTTAATCCTCAAACCCTGACATCTTCATCC
LMX1A	NM_177398	TCCATGCAGAATTCTTACTTCACATCTTGA	TTGAAGACCCTTTTCTCCTTATGTTTTGCT
LRFN1	NM_020862	CTGGAGAGTACCGTGTGA	ATCTTCCCATCTTCCTTCAGTCTCACTTAG
LRFN2	NM_020737	ATGGAGAGCACGGTCTAG	TCTTGTCAATTGGCAACAATTACCCACTAC
LRRN1	NM_020873	CGACACATCCAGAAGCTATTACATGTGGTA	CACTGCCCATTAGGTGCTAGTCAGT
LYL1	NM_005583	AGCCCAGAGGTGCGGTGA	CCTCTGTCCAAGCGCCGG
MAB21L1	NM_005584	ACCAACCCGAAAAGTTTGGAAAAACTTTAG	ATGCACTGTATTTTATTTTAAAATAACTTA
MAF	NM_005360	CGTGTACTTACCAGTGTGTTCACAAAATGA	GTCAGATACATTGTAAAAAATTATTACATG
MAF1	NM_032272	GGTCCCAGTGATCTGTATTTG	CTCTGCCTCCATTTCAGCAACT
MAFA	NM_201589	GCCGACTTCTTCCTGTAG	GCTGCTCAGTTTCTCCCTGGT
MAFB	NM_005461	TCTCCCGAGTTCTTTCTGTGA	CAAGGAAGGGATTTGCTAGGGCTT
MAFF	NM_001161573	CCCGGCCTCCTGCTCCTA	GTCTCTGTCGCCATCTCCCC
MAG	NM_080600	GGTTTCTACCCTGGAATCTCACTG	GGCCCTGGGTTCAAGGCTAAAAT
MAX	NM_145112	CGGATGGAGGCCAGCTAA	GCAACTCTGGCTTATGGCTGCT
MDFI	NM_005586	GCTCTGCTTCTCCTCCTG	GGGCAGTTTCTGGGGTAAGAAAG
MED28	NM_025205	ACCTCTGAAGCCAACGTG	AAATGTTTCCCCTCCCAAAATGTAGAACAA
MICB	NM_005931	TTCCACTGAGGGCACCTA	GTCAGTCATTGGGAACTAGCAGGG
MLL3	NM_170606	CGGAAGTGGATGAACTGA	AACTTATTTGAAAATCTAAGAAAACCAAGT
MLL4	NM_014727	CCGTCGGTTCCTTAACTG	CCGTAACAGACCCCATGACTGAC
MLL5	NM_182931	CCATGGGTCAGGGTGGCATTA	CCACCACAGGAACAGCCC
MLPH	NM_024101	GGTGGCCCACCAGTCCTA	GAGAAACCACTGACTTCTGTCAATCTTTAG
MLX	NM_170607	CCAATTGAAAAACCAGCTTTACTG	GAGGCAGAAGTACTGTAAGGAGTGGA
MMP11	NM_005940	TGCCAACACTTTCCTCTG	GCAGAAGGAGCCCTGAGCA
MSH3	NM_002439	GGAAGAAACACAGACTTCTCTTCTTCATTA	GTTAATGAACAGAAAGGTCCCAAATGAACA
MSX2	NM_002449	TGGCATGTACCACCTGTCCTA	CCCCACCACCACCAATCAC
MTOR	NM_004958	CTTAGGTGCCCTTTCTGGTAA	TGGGAACAGTCTGAGGAAAGGGA
MYB	NM_005375	CCGGACGCTGGTCATGTG	TGTTCTTTCTTTTCCATTGTAATGATTCCT
MYBBP1A	NM_014520	AAGGCAGGGAAGCCCTGA	CAGGTGGAATACTCACCCACCAA
MYBL2	NM_002466	TCGGACCCTCATCTTGTCCTG	AAACAGGGTCAAGGGCTCAG
MYC	NM_002467	GCTACGGAACTCTTGTGCGTA	GAACTTAAAGACCTTAAGGCCCCCA
MYCBP	NM_012333	GAGGAGAAGCGTGCTGAATAG	TTTAGGCCCAGCAGTTTGGGTATT
MYCL1	NM_001033082	AAAAGAATTGCATACCTCACTGGCTACTAA	ATGTCTGAAATGGCATTTCAGGAACC
MYCN	NM_005378	AATTGAACACGCTCGGACTTGCTA	GGGACAATGACTCATGCCCC
MYF5	NM_005593	TAGTTCCAGGCTTATCTATCATGTGCTATG	CCTAAACTGGGTACATGAGAATGGTAAATA
MYF6	NM_002469	GGAGGAAGTGGTGGAGAAGTA	AGGAAAGCCAAATTTCCTTTCAATTGG
MYOD1	NM_002478	CCCGATATACCAGGTGCTCTG	TTTGCACCCCTCCTTCCTTC
MYOG	NM_002479	GATGAAACCATGCCCAACTGA	AAGAGCAGGGGTCCCCAG
MZF1	NM_001267033	AACGCAGCAACCTGCTGA	ATGCTAGGAGCCACCTCTCTCA
NAB2	NM_005967	TGAGGCCAGCCGGCAGTG	CCAGGGTGAGAGTCTGGGTC
NANOG	NM_024865	CATGCAACCTGAAGACGTGTG	AGGTTCAAGCGATTCTCCTGC
NCOA6	NM_014071	TCCAAGCGAAGAAAATCCAAGTAA	AAGCCAAGAATGAGGTGAGGGA
NCOR2	NM_006312	CTCTCCGACAGCGAGTGA	TTACCAAGGGTATAAATATTCAACTTGCAA
NFE2L2	NM_001145413	AGTAAGAAGCCAGATGTTAAGAAAAACTAG	TATTTCTCTGTAACCCTGGTACTAGAAATG
NFKB1	NM_003998	AGGACCTCTAGAAGGCAAAATTTA	AAACACTTTCCTTTTGATAATTATTTGTTC
NFXL1	NM_152995	TACATCACCCATGATGTCAATTAA	AACATATCTACTTTTTAGATACTTTTAGGT
NFYC	NM_014223	CCAGGTGACCGGCGACTG	AGGTACACAGCATTAAGTTCCCTTAGCT
NHLH1	NM_005598	CCACGTGCTGGACGTCTG	TGTGGCCCCTTTTCTCTGTCTT
NHLH2	NM_005599	CACGTCCTGGACGTGTAG	ATCAAATATTTCACTACATAAATATGTTTA
NKX2-1	NM_003317	TACGGTCGGACCTGGTGA	CCTGGCCTCCTTACCTCCTTAA
NKX2-5	NM_004387	GGTATCCGAGCCTGGTAG	AACCAGTATGGTTCCAGCAAGG
NRF1	NM_005011	GGTGGTGACATTGGAACAGTG	CTTTACGGAGGTCCCCAGC
NRL	NM_006177	TCCCACCTCTTCCTCTGA	GCCTGCCCCTCCACCCCA
NRM	NM_001270710	CCTGGCTCACGGGCTTGA	GGGGGCGGGTCTGCGGGG

NRN1L	NM_198443	CCTGAGGCCTCTGGCCTA	TGGGAAATGAGTGTTTGTAAGGAGGAAGAT
NUAK2	NM_030952	TGCTCAAAGCTCACCTGA	GAGCTACTGAGCTCACGTTTGTTTTGATTC
NUP153	NM_005124	ATAAAGACTGCTGTTAGACGCAGGAAATAA	CAGATACTTTCAGATACTTTCCCTTTCTCT
OGN	NM_014057	AAAAGATTACCGATAGGGTCATACTTTTAA	AATTTGAGAGTTATTTGATGGTGTTTTGCT
OLFM1	NM_014279	CCGCTCCGACGAGTTGTA	TGGAGATCCAACAGGGACCTGT
OLFM3	NM_058170	CATATCATCAAGACAGAGGATGACACATAG	GAATCTCTTAAATTTTCCAACTCCCAGTAG
OLFM4	NM_006418	TGTCTTGCAGAAGCCCCAGTA	TTATAAAAGTTATTGATGTGATCTGTTGTT
ORC5	NM_002553	ATAATAAAATACTTGTATGATTTCTTGTGA	TGGGTAGCTAATTTTAAGAAAGTGACCAGA
OTX2	NM_021728	TGGAAATTCCAGGTTTTGTGA	TCTATTTTATGCATAGATTAGCAAAAAAAA
PAX3	NM_001127366	TTTCATTATCTCAAGCCAGATATCGCGTAA	ATACAACTGTGGGTTGTGTAACCTTATTTG
PBX3	NM_006195	TGTGCACTCGGATACCTCTAACTA	GCAGCTGTTCCTTCCCTCC
PBXIP1	NM_020524	CACCACCACCGGGGCTGA	GAGCAAAATGGAGCCAGGGT
PITX1	NM_002653	TGCCAGTACAACAGCTGA	GAATGGTGGTGGGGAAGCG
PITX2	NM_000325	GTGGACCGGCCCGTGTGA	GCTATCATTTATTTCTACCAAATTTAAATA
POU2F3	NM_014352	GAATCATTCCACCTACCTCCACTG	CTTTAGCATCAGTTTGGGGAGTAATACACT
PPARD	NM_006238	GGAGATCTACAAGGACATGTACTA	GTCCACCTGACTGACAAGCG
PTHLH	NM_002820	GAGCTCGATTCACGGTAA	TTCTATTTTATAAAATGGGATTAATATTTG
PTMS	NM_002824	AAATGGGGCATCGGCGTG	ATCTTGTCTTCTTGAGTCGTGGGT
QSOX1	NM_002826	TGGCCACCCTGCAGCCTG	CAGACAGTTCTATGGGCAGTCTGT
RARA	NM_000964	GGCCACCCACTCCCCGTG	GGCAGCTTGGAAGGGTGC
RARB	NM_000965	TCAGTCACCACTCGTGCAATA	ATAGACTGCCGTGCATTAGCACA
RARG	NM_001042728	CTGAAGTCCCCAGCCTGA	TGTCCCCACCACAATCCAG
RB1	NM_000321	CATGGATACCTCAAACAAGGAAGAGAAATG	GAAGAAATAAATTTGGAAATCTCTAGCATA
RB1CC1	NM_014781	AAAGCCGTATCATGGAATAAGAAAGTATAA	GAAGGGTACCAATTAAGTCAAAATGGCCTA
REV1	NM_016316	ACTTATGGAAGCACATTAAAAGTTACATAA	TTCCTGATATTTGGGCTTAGTGCTTCTAAA
RFX1	NM_002918	GCGCTGCCCTCCAGCTAA	CCAGGCCGGCAACCTGGC
RFX2	NM_134433	TCCCTGCAGGGCATCTAG	GGGGGACAGGGCCTAGAG
RFX3	NM_002919	GACTGTGGAGTTATTGCAAGAGTTCCTTAA	GAAGTGGAAAAGATGAATATTTCAACTGAC
RFX5	NM_000449	AAAGCAACACCCCCATGA	TAAAAAACAAGCAAAATTAAACACATTTTT
RFX6	NM_173560	AGCAGCTGGAGGCACTTA	GGATATGATATGCAAATGTTTTTAAATTAT
RHEB	NM_005614	TCTTCATGCTCGGTGATGTGA	ACAGAGTGAGACCCTGTCTTTAAAAATGTA
RHO	NM_000539	CCAGGTGGCCCCGGCCTA	GCCTCCCAAAGTGCTGGGATTA
RHOA	NM_001664	AAATCTGGGTGCCTTGTCTTGTGA	CCAGATTAGCTGCTGGGTGG
RHOB	NM_004040	CAACTGCTGCAAGGTGCTATG	ATGCTCTCAAAGGGACTTCATCCTCATTTA
RHOC	NM_175744	GGCTGTCCCATTCTCTGA	GAGCCAGGCATGACCTCATC
RHOF	NM_019034	CTCTGCCTGCTGCTCTGA	GGCACCTGCGTTTGCCCT
RHOG	NM_001665	TCCTGCATCCTCTTGTGA	GGGAGCCTAGAGCTTTGTTAGGGA
RHOH	NM_004310	CTTCTCCATCAATGAGTGCAAGATCTTCTA	AAAGTGACACTCTCTTGCTTCTGTTC
RHOT1	NM_001033566	TATGTACAAAGCATTATTGAAACAGCGATG	CCAAAGTGCTGGGATTACACGATTGA
RHOV	NM_133639	AAGAAGTTCTTCTGCTTCGTTTGA	ATTACACTGCCCCCACCC
RIN3	NM_024832	GGAGCCCAACTTCCTGTG	AGGGTGGCTGAGAGTATGCC
RLF	NM_012421	GACAGATGAGCTTTGTGTAGGAAGTTCATA	AAAAAAAAAAAAAGAACCTTGTTCTGAATG
RNF152	NM_173557	ACTGTGATATCCTGTGGCTGA	TCAAGATGTCCAAGAAGTGGCCA
RNF43	NM_017763	TGTGAACAGGCTGTGTGA	TGCCAAGCCCTCTACTCTACC
RNF7	NM_014245	GGTCCAAAGAATCGGCAAATG	AGAAAGTTACTAAAACAGTTCACTGCACAA
ROCK2	NM_004850	TAACTTTATTTTTCCTTTCTTTTCAGCTAA	TAGGCCAGTAGCTACTCTTCAGTTTC
RORB	NM_006914	TGCCACCGGCTGCAAATG	GGAAACTTTACAAAGCAGGGGTTTGTATGT
RORC	NM_005060	GTGGGGCTGTCCAAGTGA	GCAGGGGTCGGGTGAGGG
RTCA	NM_003729	AGGAATTGGGATGACAAATCCAAATCTATA	AATTACACACTCTATTGTAGTACAACACAT
RXRB	NM_001270401	CCCCATCAACTGGCCTGA	CGACAAAGCTTGCCTTGTGTC
RYK	NM_002958	CTGGGGGCCTACGTCTGA	GGAGTCTGAGCCAGCTTAACTGT
SDC1	NM_002997	AAACAGGAGGAATTCTATGCCTGA	CTCTGTCTTCCACGACAGGAGG
SDC4	NM_002999	AATGAGTTCTACGCGTGA	CTCTGCCCCCACCCTCTG
SETDB1	NM_012432	TGAATGCAGAGGACGTCTTCTTTA	CGAGAACTCCATCTAAAAAAATTCACATGC
SIM2	NM_005069	CATCACCAACGGGAGGTG	AAAGGAATTCCTTTCCCTGGGAG
SIX1	NM_005982	GTGGACTTGGGGTCCTAA	GAACAGAAATAGGAAAGAGTAAGAAAGAAA
SIX2	NM_016932	GTGGACCTGGGCTCCTAG	ACCTAGCGACTGACACAAGTCG
SIX6	NM_007374	CAGCGAGTGCGACATCTG	GTTTAAAACATATACCAGGAAGCACCAAAG
SMAD6	NM_005585	CCTCCTCAACAACCCCAGATA	AGAGTAAATGTGACTTTCCTCCCTTTTCAA
SMARCE1	NM_003079	CCCATACCAGAAGATGAGAAAAAAGAATAA	AGTTGGGTGAGACAAGGGC
SNAI1	NM_005985	CTCAGGATGTCCCCGCTG	GTGACACCCAGGACTCCAAAG

SNAI2	NM_003068	TGCTGTGTAGCACACTGA	TTTTTGTTAAAGTGGTATGCTAACAAACCA
SNAPC1	NM_003082	ATCCAAGAAGAGGAGAAAACACTG	AGTGGGGCCAATAATGGATCTACA
SNF8	NM_007241	AGAGAAGCCCTCCCCTGA	TTTTCTTTCCTCCAATTTAGCCACGATTGG
SNX4	NM_003794	AATGCTAAGGAATGCTTTAGCAAGATGTAA	TGTGTACTCAATGTTTAGCTCTCACTGATA
SNX6	NM_021249	GCAGTGTTAAATGGAGACACATAA	AGGGGCCAAGGGATGCAC
SOHLH1	NM_001012415	GCTGGTCCCCCCGCGTAA	CTACCCCAGGGCACCAGG
SOLH	NM_005632	TGGGCCCCGACCGCTGTG	CTTGTGAGTCACCCCACCAC
SON	NM_138927	CAATTGTATGTTTTTCTTGAATAGGTATTG	GTGTGTGTGTGTGTGTGTGTGTG
SOX13	NM_005686	GGTGGTGCTCACAGACTG	GGATTCCCTTGCATTAAGCCAATTCC
SOX2	NM_003106	GCCCCTCTCACACATGTG	TTTTGCAGTTAACATTAGCACATGATGCTG
SOX3	NM_005634	CCGCTGACCCACATCTGA	TTAAAAGGAAACCATTTCAGATTATGCTTG
SOX8	NM_014587	CACCCTGACCAGGCCCTG	GGCTCGCGGCCGCCCTCC
SOX9	NM_000346	CACACAGCTCACTCGACCTTG	GTCTTTTCCTCAGCTTGTTTTCTAGGATTG
SP2	NM_003110	CCTGGTCACGAAGAACTTGTA	GGGCATGTGGCTTGGGTTT
SP7	NM_152860	AGCAACTTGCTGGAGATCTGA	CCCGGCCGATAAACTATTTTACCG
SP8	NM_182700	CGCAACGGCCTAGAGTGA	AGCTTTGCCAAATAGATCTTTCCATCTAAC
SPDEF	NM_012391	TTCGTGCACCCCATCTGA	CAATCAATTATCCCTTGCCCTTTAGGCT
SPEN	NM_015001	GATTGTCATTGCCTCCGTGTG	GGACTGACTGACTAGGAGGCAGAA
SPI1	NM_003120	CGCCACCCGCCCCACTGA	TGTGTCATTGGTCCTCATGTTCC
SREBF1	NM_001005291	ACTGTCACTTCCAGCTAG	GTAAACGTGTGTATTATATCTGGCCTCGTT
SREBF2	NM_004599	TGCCATTGCCGCCTCCTG	TGGGGTAGGGGGAGGAGG
SSRP1	NM_003146	GCGTCAGGATCCGATGAGTAG	AATGCTCGTCCAGCCGGG
SSX1	NM_005635	CCCTGAGGAAGATGACGAGTA	GCGCTCAGCACGAGGACA
STAT1	NM_007315	TTTGTCTTTTTACAGATGAACACAGTATAG	TAGGGCAGGTAAGGACAACATTTAGGAAAA
STAT2	NM_005419	TTGATGCCTTCTGACTTCTAG	AAGGGAAGAAGAAATATGACAAGAGGAATG
STAT5A	NM_003152	CAGAGGCTCCCTCTCATG	AAACCACAGCAAAGCGGG
STAT6	NM_003153	GCCAACCCCAGTTGGTGA	CCCCAAGTCCTTTGCAATTTCTTC
STRA13	NM_001271007	CAGCTCCTGGACTTCTAG	CCTGGGGGTGGGGCCGAG
SUV420H2	NM_032701	CGGCGGTGAAGAGCTGTG	CCTAGTCCATCACGCTCCCAAAT
SWI5	NM_001040011	TGGGCTGGACATGAATGACTG	TTATGCTAATTCCTCACAGGATGATTTTAA
TAF4	NM_003185	CTCTACAAAGCATTCCTTAAGTGA	CCTCACAGAGAAAGTTCTTTCTCCC
TAGLN2	NM_003564	CCACGCCAGATCCTCTGA	GAGAATGAGGATGGGGAGGAAACTCA
TAL2	NM_005421	AAGCCACCACATTCCTTA	TGATCAAATCTCATTTTTCAAAACAAACTT
TBX2	NM_005994	CCGGGAGTCGCCCAAGTG	CGCCCTGCCGCCGTGCCG
TBX5	NM_000192	GAGTGGAGCGACAATAGCTAA	CCCAAATTCTCCTGCGATGG
TBX6	NM_004608	TCCAAACCCATGTACTGA	ATTTTCCTGAAGAGGAGATGGGCCTA
TCERG1	NM_006706	CACGAGACGATCAACAAAATA	TGCATTATTATATTCAAACCTTTAAAAACT
TCF15	NM_004609	CGAGGGCCACGGAGATGA	TGAATGGGACATCACAGGAAACTG
TCF19	NM_007109	GGCTGGCATTCAGACCTA	GCCTGGGACACAGTAGATAGACAC
TCF7L2	NM_030756	GCTCGTCACCAAGTCTTTAGAATA	GAGTCCAAGACCACGTTCTGGTATCTAA
TEK	NM_000459	TGCTGAAGAAGCGGCCTA	AGGAGACCTTTTCAATCTGATGAGCTTC
TERT	NM_198253	TTCAAGACCATCCTGGACTGA	TGACCACAACCCCATTCACTCATAG
TFAP2A	NM_003220	AAAGAGGAGAAGCACAGAAAGTGA	GGGGGGCGGGGGGGGGGG
TFDP1	NM_007111	CGAGAATGACGAGGACGACTG	ACAACATGTGGTGGTGGGGTTT
TFE3	NM_006521	AGCATGGAAGAGGAGTCCTGA	TTTGCATATTGTCTTCCTCCATGCC
TFEB	NM_001167827	GAGGGCGATGTGCTGTGA	TGAGGCGTGTGACAAGCGT
TFG	NM_001195479	ACCTGGACCTGGTTATCGATA	ACCAAATGGCACTGTTTTCTCTGAAT
TGFBR3L	NM_001195259	GCCCAGGAGGTCCCAGTG	TGGGCATATGGGCCCCGT
TNFAIP3	NM_006290	TCAGTTCAAGCAGATGTATGGCTA	TTATTATTTATGAAAAGCTGCCTTGCTACC
TOPORS	NM_005802	TGTCTTGGTAGAGACTGTGATATGTCTTAA	AGGCAAAGGCAAACTGAAGGC
TP53	NM_000546	GGGCCTGACTCAGACTGA	CAGACTGACCCAGTCTCCAG
TP53BP2	NM_005426	CAAAGGAGCTTGGCCTGA	GCAAATGGGTTTGGAAACAATTCACAAA
TRIM39	NM_021253	CCCAACAGATTGGGAGTG	CTCTGTCTCATAAATAAAATGTAGATGTCC
TRIM71	NM_001039111	CAACAATCGAATCCTCGTCTTCTA	AGGAAATTTCTCTGTTGCAGCATTTAAAAA
TTF1	NM_007344	GGCCGGTGGATCATCTGA	CAGTACCCATATAGCTGAACTCACGTAATG
TTF2	NM_003594	CCTCAGAGTCCTTTTTGGCATCTA	GAGGGCCACCTAGAAGTGACTTACAT
TWIST1	NM_000474	ATGTCCGCGTCCCACTAG	CTTGTGCCTGTCAGTAGCTGCTTTATT
UBTF	NM_014233	TCAGACTCTGACTCCAACTGA	AACAATGTGAAGGTAGGTTGCCTATG
USF1	NM_207005	GTCATCAAGAATGACAGCAACTAA	CACACCGGTCTGGCCCAG
USF2	NM_003367	CGAGGGCACCCGGCAGTG	CCATCCAACCAACCACTGGGTAAT
USP6	NM_004505	TGATTACGAAAAGTACTCTATGTTACAGTA	GTACTGGTCTGTAGCCCAGGAGT

VAMP2	**NM_014232**	CCTCTCTCCACAGTTTACTTCAGCACTTAA	CTCTTGCAAAGTCCAGTCCATTACCAAT
VEGFA	**NM_001171627**	TGTTTTCCATTTCCCTCAGATGTG	ACAACAAGGTGGGTCACCC
VIM	**NM_003380**	AACTTCTCAGCATCACGATGACCTTGAATA	GTGAGGTCTATCAAAAATGACAAAATTAAG
VIMP	**NM_203472**	TCTGGCGGATGAGGCTAA	TCCCCTCCATTATCTCCTAATCATTCTC
WT1	**NM_024424**	CTCCAGCTGGCGCTTTGA	TGAAGTTTGACAGAGAGAAATAAATTGTGG
XBP1	**NM_005080**	AGCTGGAAGCCATTAATGAACTAA	AAAGCAGGCAGTAATTAAGGTGGAAAA
XRCC3	**NM_005432**	GGGACCCAGTCCCACTGA	AGCTACTTAACGGTTCTGGGCC
XRCC4	**NM_022550**	AGAAGACCTCTTTGATGAGATTTA	ATAAATCTTTTATCAATAATCCCTGAAAAA
XRCC5	**NM_021141**	TTGTTCTTGTTCACAGTTGGACATGATATA	TCCTAGAAGCCCAAAGTAAAGCACT
YBX2	**NM_015982**	ACCACCATCCTGGAGTGA	AACCCATGATAGGGGCACTCTTC
YY1	**NM_003403**	TAAGGCCAAAAACAACCAGTG	TCAATAATCACTGTAATTGAGTGCAAAATA
ZCCHC3	**NM_033089**	CGGCGTGGCCGGGCACTA	CAACGTAGTAGGTGCTAAGTGTTGC
ZFP36L1	**NM_004926**	TTCAGCAGACTTTCCATCTCAGATGACTAA	GCTATAGGCTTAGCTTATAGGGATGAGAGA
ZFYVE1	**NM_178441**	AATAAAAAGCCCGGTGACCTTTAA	GTAAAAGACTGCAGTATTTGCAGGTAAAAT
ZKSCAN5	**NM_014569**	TACCTTAAGTGTAGAGGGGTCTCTGTTGTA	ATTAACAGTGATGCGGGTCTTCACT
ZMYND11	**NM_006624**	CTGCCGCCGGAAAAGATG	GTTTAAGTGAACAAATGATGTTTAACACAC
ZNF282	**NM_003575**	GCCTCCTGAGCGAGACTA	CACTACCCCCCTCCCCCA
ZNF331	**NM_001079906**	ACATCAGAGGATCCACAACAGTTG	CTCTGGGCCCTGACCCCG
ZNF362	**NM_152493**	GCGAATCTCTCTCATCTG	CTCAGTTCAGCACCTGGAGTGA
ZSWIM4	**NM_023072**	GCGGGAGCGTTTTGGTTG	CTGGGCAACAGAGAAAGACCAAAAAAAAAA

Primer	Sequence
SV40 let-7c target mutagenesis F	CTTGTTTATTGCAGCTTATAATGACATGTAACCATACAACCTACTACCTCAGTTACAAATAAAGCAATAGCATCAC
SV40 let-7c target mutagenesis R	GTGATGCTATTGCTTTATTTGTAACTGAGGTAGTAGGTTGTATGGTTACATGTCATTATAAGCTGCAATAAACAAG
SV40 miR-10b target mutagenesis F	CTTGTTTATTGCAGCTTATAATGACATGTACAAATTCGGTTCTACAGGGTAGTTACAAATAAAGCAATAGCATCAC
SV40 miR-10b target mutagenesis R	GTGATGCTATTGCTTTATTTGTAACTACCCTGTAGAACCGAATTTGTACATGTCATTATAAGCTGCAATAAACAAG
SV40 gateway F	GGGGACAGCTTTCTTGTACAAAGTGGAACTTGTTTATTGCAGCTTATAATGGT
SV40 gateway R	GGGGACAACTTTGTATAATAAAGTTGGACGGTATACAGACATGATAAGATACA
pmiRint pro mut F	GTATCATATGCCAAGTACGCCCCCCTCGAGATGGCCTCCTCCGAGAACGTC
pmiRint pro mut R	GACGTTCTCGGAGGAGGCCATCTCGAGGGGGGCGTACTTGGCATATGATAC
CMV (SpeI)	ATGACTAGTAGTTCCGCGTTACATAACTTACGGT
CMV (SacI)	GAGCTCTGCTTATATAGACCTCCCACCGTA
L2R3 (AsiSI) F	ACGTGCGATCGCACCCAGCTTTCTTGTACAAAGTTGG
L2R3 (MluI) R	ACGTACGCGTGTAAAACGACGGCCAGTGAATTATC
miR10b 400 asisi F	ACGTGCGATCGCAAGAATATTCTGGTTGTTCGCC
mir10b 400 noti R	ACGTGCGGCCGCTCTTTCTTTCTTTTCAGCACCC
let7c 400 asisi F	ACGTGCGATCGCGACATTTTACGTGACCTATGCTG
let7c 400 noti R	ACTGGCGGCCGCCCATTAGAAATACCATTTTGACA
PGK BamHI F	CATGGATCCTGGTACCTACCGGGT
PGK PstI R	CATCTGCAGTGTCTAGAGTCGAAAGG
TIC mut PstI F	GGACGTGGTTTTCCTTTGAAAAACACCTGCAGTAATCCATGGAAGACGCC
TIC mut PstI R	GGCGTCTTCCATGGATTACTGCAGGTGTTTTTCAAAGGAAAACCACGTCC
SV40 pA For (NsiI)	ACGTATGCATAACTTGTTTATTGCAGCTTATAATGGT
SV40 pA Rev (XmaJI)	ACGTCCTAGGGACGGTATACAGACATGATAAGATACA
TIC P2RP3 mut F	GTCCAAATTGTAACTAGAGATCTCCGCGCGCTAGCGGGACGCGCCCTGTAGCGG
TIC P2RP3 mut R	CCGCTACAGGGCGCGTCCCGCTAGCGCGCGGAGATCTCTAGTTACAATTTGGAC
P2R-P3 BglII F	CATAGATCTGTAAAACGACGGCCAGTCTTAAGC
P2R-P3 NheI R	CATGCTAGCCAGGAAACAGCTATGACCATG
MS2 BglII F	ACGTACAGATCTACATGAGGATCACCCATGTCT
MS2 ApaI R	ACGTACGGGCCCACATGGGTGATCCTCATGTTT
pLIFE ampli F	GGCGGAAAGTCCAAATTGTAACTAGAGATC
pLIFE ampli R	CAGGAAACAGCTATGACCATGTAATACGACTCACTATAG

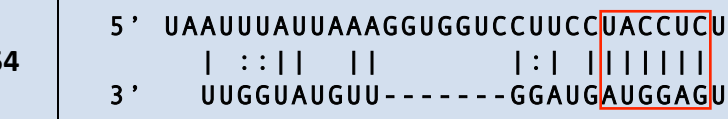
Supplementary Table 2a - 3'LIFE hits - let-7c

Rank	Score	Refseq	Position	Alignment (top 3'UTR, bottom miRNA)
1	0.52	E2F5	1-37	5' GAUUCCAUGGAAACUUGGGACUGUUAUCUACCUCUAA 3' ||||: || || ||||||| 3' UUGGUAUGUUGGAU----------GAUGGAGU 5'
2	0.58	CREM	779-804	5' AGUAAACCACAAAAAAAUACCUCAGG 3' 	||||	||||||| 3' UUGGUAUGUUGGAUGAUGGAGU 5'
3	0.58	RHOB	922-951	5' CUGACCACACUUGUACGCUGUAACCUCAUC 3' :|||| ||	||:: |||||| 3' UUGGUAUG----UUGGAUGAUGGAGU 5'
4	0.60	HOXD1	510-535	5' UUUAAAAAAGCGGUUUCUACCUCUCU 3' 			: :| ||||||| 3' UUGGUAUGUUGGAUGAUGGAGU 5'
5	0.60	XBP1	798-836	5' GGAACACCUGCUGAGGGGGCUCUUUCCCUCAUG 3' |||	|:| ::|: :| ||||| 3' UUG-GUAUG------UUGGAUGAUGGAGU 5'
6	0.65	SETDB1	330-351	5' GGAGCCUGUGUAUCUACUAUCUCCAG 3' |:|| |:||||||:||| 3' UUGGUAUGUUGGAUGAUGGAGU 5'
7	0.65	SREBF1	805-837	5' GGCCUCCAUGGGGUCAGUUGUCCCUUCUCACCUCCCA 3' ||||: ||: ||| || ||||| 3' UUGGUAU-----GUU-----GGAUGA-UGGAGU 5'
8	0.66	PBX3	892-923	5' UGUAGCUUAGAGUGCUCACUUACUACCUCUGA 3' 		||	||:|||||||| 3' UUGGUAUGU------UGGAUGAUGGAGU 5'
9	0.68	ID1	173-199	5' CGUCCCUUCCAACCCGCCGGUCUCAUU 3' ||	||||| :	::|||| 3' UUGGUAUGUUGGAU-GAUGGAGU 5'

10	**0.69**	**MAF1**	**246-274**	5’ ACUGCCCUGCCCAAAUGAACUGCCACAGC 3’ :||				||||:|| || 3’ UUGGUA-UGUUGGA--UGAUGGAGU 5’
11	**0.70**	**BHLHB9**	**1802-1825**	5’ CUUUGCAUGUCAAUAAAUAUGCCUCUAC 3’ |||: |||: |: |:|||| 3’ UUGGUAU-GUUGGAUG-AUGGAGU 5’
12	**0.70**	**NRM**	**-2-25**	5’ UGAUCAGCAAGACCUCCGCUACCUCCGG 3’ |	:	:|||| :||||||| 3’ UUGGUAUGUUGGA--UGAUGGAGU 5’
13	**0.70**	**BCCIP**	**215-247**	5’ UUUUCCUUUUCUAACCUAUUAAAAUACCUCACU 3’ ||		||||||: ||||||| 3’ UUGGUA-UG-UUGGAUG-----AUGGAGU 5’
14	**0.71**	**RFX6**	**450-474**	5’ AAAGUCAAAUGUGUAUGUUCUACCUCCAA 3’ |:::|| |:: |:	||||||| 3’ UUGGUAUGU---UGGAUGAUGGAGU 5’
15	**0.71**	**MICB**	**732-757**	5’ GGUUCAAGCACUUCUCGUACCUCAGA 3’ |	:|| ||||||| 3’ UUGGUAUGUUGGAUGAUGGAGU 5’
16	**0.72**	**HSF1**	**287-319**	5’ AGAAUUGUAUUUUGGAUUUUUACACAACUGUCCCGUU 3’ ||:::||| :|:::| |||:::| |: 3’ UUGGUAUG----UUGGA-------UGAUGGAGU 5’
17	**0.72**	**DLX4**	**165-196**	5’ CUAACCCUAACAGCUAAAUCAAGGACCUCAGC 3’ ||||	|||:|		|||||| 3’ UUGGUA-UGUUG-----GAUGAUGGAGU 5’
18	**0.73**	**RHOV**	**807-828**	5’ AAGGUCACACAGCCUAGAAGCUAGAG 3’ :::|| |||:|||| |||| : 3’ UUGGUAUGUUGGAUGAUGGAGU 5’

19	**0.73**	**CNNM2**	**1207-1232**	5’ GAGGGCUCUGUGCCUCCUGCCUCAGA 3’ :: | :||| ||:|||| 3’ UUGGUAUGUUGGAUGAUGGAGU 5’
20	**0.73**	**USF2**	**296-323**	5’ GAGGCCCUGCCACGUCCCGCUGCCUCCUG 3’ ::|| | ||: || :||:||| 3’ UUGGUA---UGUUGGAUGAUGGAGU 5’
21	**0.73**	**RARB**	**1026-1060**	5’ ACUCCCAAAGAAACAGGCAUAGAAUCUGCCUCCUU 3’ ||| | || | ||:||| 3’ UUGGUAUGUUGGA---------UGAUGGAGU 5’
22	**0.73**	**OLFM4**	**714-736**	5’ AAAGUCAGUAGAAUCUUCUACCUCAUA 3’ |::|| || ||:|| ||||||| 3’ UUGGU-AUGUUGGAUGAUGGAGU 5’
23	**0.73**	**CRK**	**1950-1971**	5’ GCUAAUUUAAUGUAUUUUACCUCACA 3’ | : || :: | :||||||| 3’ UUGGUAUGUUGGAUGAUGGAGU 5’
24	**0.74**	**DNMT1**	**293-317**	5’ GUAGUUUUUAUAUGUUGUAAUAUUUCUU 3’ |:: :|||:: : || ||:|| 3’ UUG---GUAUGUUGGAUGAUGGAGU 5’
25	**0.74**	**SMAD6**	**99-127**	5’ AAAACCCCCCAGAUAUCAUCUACCUAGAU 3’ |||| || |:| ||||| : 3’ UUGGUAUGU---UGGAUGAUGGAGU 5’
26	**0.75**	**ETNK2**	**387-413**	5’ GGAGGCGGGGAGGGCUCCUUUCUACCUCCAG 3’ |: |: : |: |:| |||||| 3’ UUGGUAUGUU-----GGAUGAUGGAGU 5’
27	**0.75**	**ARID3A**	**382-414**	5’ ACAGCAGUGUGGGCCGAUCCUGUUUACCUCAUA 3’ |:| :|:: | |||: :||||||| 3’ UUGGUAUG------UUGGAU-GAUGGAGU 5’

28	**0.76**	**EZH2**	**15-38**	5’ GAAAUCCCUUGACAUCUGCUACCUCCUC 3’ || || |: |:||:||||||| 3’ UU--GGUAUGUUGGAUGAUGGAGU 5’
29	**0.76**	**STAT2**	**1192-1214**	5’ GGGUUCAAGUGACUCUCCUGCCUCAGC 3’ : :|| :::| || ||:||||| 3’ UUGGUAUGUUG-GAUGAUGGAGU 5’
30	**0.76**	**HHAT**	**695-725**	5’ AGAGAAGUAUAACAUGGUAGUUCCUCUACCUUACA 3’ |: :||:|||	|||||||:| 3’ UUGGUAUGUUG---------GAUGAUGGAGU 5’
31	**0.76**	**MYCL1**	**548-569**	5’ UGAUCAACAUUGACCAUUACCUCACU 3’ 			|: :	|:||||||| 3’ UUGGUAUGUUGGAUGAUGGAGU 5’
32	**0.77**	**TRIM71**	**174-204**	5’ AGAAAAGUACAACAUUGCUUAAGUCCUACCUCAUC 3’ || :|||||| :|| ||||||| 3’ UUGGUAUGUUG-----GAU----GAUGGAGU 5’
33	**0.77**	**SPDEF**	**36-63**	5’ GCCUCUCUCCUGCCUGCCCUGCCUCAGC 3’ |:		:|||: ||:||||| 3’ UUGGUAUGUUGGAU--GAUGGAGU 5’
34	**0.77**	**EN2**	**1371-1396**	5’ ACAGUUCUGAAACAUGUGGCUACCUUGUC 3’ |::: |: |||	:||||||: 3’ UUGGUAUGUUGGA---UGAUGGAGU 5’
35	**0.78**	**TTF1**	**96-118**	5’ GUGGUAGUGCACACCUGUAAUUUCAAC 3’ ::: :|:|| ||||:: |::||| 3’ UUGGUAUGU-UGGAUGAUGGAGU 5’
36	**0.79**	**HES5**	**50-76**	5’ ACGACCAGAGGGCGAGCCUGCUCCUCUCGCC 3’ :|||| : |:|||:||	|||: 3’ UUGGUAUG----UUGGAUGAUG-GAGU 5’

37	**0.80**	**RNF7**	**226-254**	

Supplementary Table 2b: 3'LIFE hits - miR-10b

Rank	Score	Refseq	Position	Alignment (top 3'UTR, bottom miRNA)
1	0.44	ZMYND11	524-551	5' GGUAUAUGAUUGAAUUUAGGGAACAGGGUUGA 3' :|:| : |:| |:|| ||||| 3' GUGUUUAAGCCAAGAU-----GUCCCAU 5'
2	0.47	SDC1	734-759	5' GUCGCUCAUGUG-UGCAACAGGGUAUG 3' |:| || :| | | ||||||| 3' GUGUUUAAGCCAAGAUGUCCCAU 5'
3	0.57	RARG	439-473	5' GUGCCUAAUGCUGUGUGAUGCACCUGCAGGGUGUG 3' | ||| | || ||:||||||: 3' GUGUUUAAGCCA--------AGAUGUCCCAU 5'
4	0.58	LYL1	151-178	5' GGGGCAAGGUCUCGGGGGUCCGGAAGGGUGAU 3' :|||: || ||| | : |||||: 3' GUGUUUAAG-----CCAAGAUGUCCCAU 5'
5	0.64	OLFM3	30-67	5' AGGCAAAUGUGACAUGUUUUCAUUGAUUUAAACAGUGUGAU 3' :||||| :| |||:| ||||:||: 3' GUGUUUAAGC----CAAGA----------UGUCCCAU 5'
6	0.67	NCOR2	668-695	5' GAUGUAAAUGAUGUGUUGGUUUACAGGGUAUA 3' :::|||| || |:|||||||| 3' GUGUUUAAGCCA-----AGAUGUCCCAU 5'
7	0.68	TCF15	249-273	5' AAAACAAAGACUGUUGGUGACAGGGUGUG 3' ||||| | ||| : ||||||: 3' GUGUUUAAGCCAA--GAUGUCCCAU 5'
8	0.69	MYF5	278-306	5' AUUUCUGAUAGGGGGCCAUUGAUUGAGGGUAGC 3' : | :|| || | |: ||||| 3' GUGUUUAAGCCAAGAUG-----UCCCAU 5'
9	0.70	CRK	78-104	5' AGUCUUAAUUACCAUAUUCAGGGUACG 3' : : |||| | | |||||| 3' GUGUUUAAGCCAAGAUGUCCCAU 5'

10	0.71	USP6	1960-1988	5’ AUCAAAAGUAUUGGUAAUUGUAUAUGGGGUGUA 3’ || ||:| |:|||	||::|||||: 3’ GUGUUUA-AGCCA-----AGAUGUCCCAU 5’
11	0.71	DPF2	283-311	5’ GGCCCAGCCCCUGGUGAUCACAGGGUUCA 3’ | ||:			: ||||||| 3’ GUGUUUAAGCCAA--GAUGUCCCAU 5’
12	0.72	TCF19	1350-1382	5’ AGUAUAAAGCCAUUUAAGAAUUCCAGAGUAGGGUGGG 3’ :|:|||	|| :||||||: 3’ GUGUUUAAGCCAAGA----------UGUCCCAU 5’
13	0.72	SREBF1	717-760	5’ CUUAGUGGCUUUUUUCCUCCUGUGUACAGGGAAGA 3’ :| :: |: ||| ||||||| 3’ GUGUUUAAGCCAAG--------AUGUCCCAU 5’
14	0.73	DLX1	791-817	5’ UGAGGCUGUUUGCCAAUUCAGGGUUCU 3’ : :||:|	|||||| 3’ GUGUUUAAGCCAAGAUGUCCCAU 5’
15	0.73	ANXA7	94-133	5’ ACCGAAAGAGCUUUCUGUCAAGGACCGUAUCAGGGUAA 3’ | ||| ||||: ||||||| 3’ GUGUUUAAGCCAAGAU-----------GUCCCAU 5’
16	0.75	FXR2	174-205	5’ UCCAGGAGCUAGUGGAGGGGUGUGUAACAGGGUCAU 3’ || :|:		|| : ||||||| 3’ GUGUUUAAGC---------CAAGAUGUCCCAU 5’
17	0.76	HOXD11	382-409	5’ CCUUCCUCUUCGGUGAAUGCAGGUUAUU 3’ :	|||||| |:|||| || 3’ GUGUUUAAGCCAAG-AUGUCCCAU 5’
18	0.77	NCOA6	267-302	5’ UUCACAUUUCCUAAGCAGCCUAGAGUACAGGGUGAG 3’ ||||			||||||||: 3’ GUGUUUAAGCCAAG---------AUGUCCCAU 5’
19	0.77	HOXD10	257-285	5’ AUUAUUUUUUCAUCGUAAUGCAGGGUAAC 3’ :|: ||| || |:||||||| 3’ GUGUUUAAGC--CAAGAUGUCCCAU 5’

20	**0.77**	**HHAT**	**252-278**	5’ GUGUCUUACCCAGCUACACAGGGUGAC 3’ 3’ GUGUUUAAGCCAAGAUGUCCCAU 5’
21	**0.77**	**NUAK2**	**701-737**	5’ CCGGCUAAUUUUGUAUUUUUAGUAGAGACAGGGUUUC 3’ 3’ GUGUUUAAGCCAAGA----------UGUCCCAU 5’
22	**0.78**	**HOXD1**	**442-468**	5’ UUUUGAGAUGACCAAAGCUAGUUAGGGUCUC 3’ 3’ GUGUUUA--AGCCAAGAU--GUCCCAU 5’
23	**0.78**	**STAT6**	**920-955**	5’ UCCACACCUCCAAUGCUGCCUGGGAGCCAGGGUGAG 3’ 3’ GUGUUUAAGCCAAGAU---------GUCCCAU 5’
24	**0.78**	**ASCL2**	**1112-1138**	5’ GACACGAG--CAGUCCCUGAGGGGCGGGGUCCC 3’ 3’ GUGUUUAAGCCAAGA------UGUCCCAU 5’
25	**0.79**	**HIVEP2**	**1267-1289**	5’ AGCCGGUUUACAUGGGAACAGGGUUAA 3’ 3’ GUGUUUAAGCCAAGAUGUCCCAU 5’
26	**0.79**	**GATA3**	**240-262**	5’ CUCAUAUCCCCUAUUUAACAGGGUCUC 3’ 3’ GUGUUUAAGCCAAGAUGUCCCAU 5’

Supplementary table 3 - Literature review

The following references were used to define genes detected by 3'LIFE as either having a positive or a negative role in tumorigenesis.

let-7c Targets:

Gene	References:
E2F5	(Umemura, Shirane et al. 2009; Zhao, Wu et al. 2013)
CREM	(Passon, Puppin et al. 2012; Healey, Crow et al. 2013)
RHOB	(Marlow, D'Innocenzi et al. 2010; Zhou, Zhu et al. 2011; Kazerounian, Gerald et al. 2013; Medale-Giamarchi, Lajoie-Mazenc et al. 2013)
HOXD1	(Faryna, Konermann et al. 2012; Pussila, Sarantaus et al. 2013)
XBP1	(Doane, Danso et al. 2006; Sengupta, Sharma et al. 2010)
SETDB1	(Rodriguez-Paredes, Martinez de Paz et al. 2013; Noh, Kim et al. 2014)
SREBF1	(Furuta, Pai et al. 2008; Pandey, Xing et al. 2013)
PBX3	(Ramberg, Alshbib et al. 2011; Han, Gu et al. 2012)
ID1	(Kalas, Yu et al. 2005; Swarbrick, Roy et al. 2008; Pillai, Rizwani et al. 2011)
MAF1	
BHLHB9	
NRM	(Chen, Chen et al. 2012)
BCCIP	(Liu, Yuan et al. 2001; Meng, Liu et al. 2003; Lu, Yue et al. 2007)
RFX6	(Takata, Akamatsu et al. 2010; Huang, Whitington et al. 2014)
MICB	(Groh, Wu et al. 2002; Holdenrieder, Stieber et al. 2006; Kohga, Takehara et al. 2008)
HSF1	(Stanhill, Levin et al. 2006; Dai, Whitesell et al. 2007; Dai, Santagata et al. 2012; Xi, Hu et al. 2012)
DLX4	(Tomida, Yanagisawa et al. 2007; Zhang, Yang et al. 2012; Trinh, Ko et al. 2013)
RHOV	(Cavalli, Man et al. 2008; Peng, Zhou et al. 2011; Shepelev and Korobko 2013)
CNNM2	
USF2	(Ismail, Lu et al. 1999; Pawar, Szentirmay et al. 2004)
RARB	(Farias, Arapshian et al. 2002; Liu, Nugoli et al. 2011)
OLFM4	(Koshida, Kobayashi et al. 2007; Huang, Wang et al. 2012; Li, Rodriguez-Canales et al. 2013)
CRK	(Tsuda and Tanaka 2012; Kumar, Fajardo et al. 2014)
DNMT1	(Gazin, Wajapeyee et al. 2007; Huang, Stewart et al. 2013; Wajapeyee, Malonia et al. 2013)
SMAD6	(Kleeff, Maruyama et al. 1999; Jeon, Dracheva et al. 2008)
ETNK2	
ARID3A	(Peeper, Shvarts et al. 2002; Ma, Araki et al. 2003)
EZH2	(Chase and Cross 2011; Deb, Thakur et al. 2013)
STAT2	(Yu and Jove 2004)
HHAT	(Konitsiotis, Chang et al. 2014; Petrova, Matevossian et al. 2014)
MYCL1	(Kim, Girard et al. 2006; Xiong, Wu et al. 2011; Rudin, Durinck et al. 2012)
TRIM71	(Chen, Yuan et al. 2013)
SPDEF	(Steffan, Koul et al. 2012; Buchwalter, Hickey et al. 2013; Fletcher, Castro et al. 2013; Mukhopadhyay, Khoury et al. 2013; Noah, Lo et al. 2013)
EN2	(Martin, Saba-El-Leil et al. 2005; Bose, Bullard et al. 2008)
TTF1	(Lessard, Morin et al. 2010)

HES5	(Osipo, Patel et al. 2008; Mittal, Subramanyam et al. 2009; Aste-Amezaga, Zhang et al. 2010) also RAS implication
RNF7	(Tan, Li et al. 2011; Lazar, Suo et al. 2013; Yang, Huh et al. 2013)

miR-10b Targets:

ZMYND11	(Wen, Li et al. 2014)
SDC1	(Yang, MacLeod et al. 2007; Ishikawa and Kramer 2010; Nguyen, Grizzle et al. 2013)
RARG	(Chen, Goyette et al. 2004; Goranov, Campbell Hewson et al. 2006; Zhao, Graves et al. 2009; Yan, Wu et al. 2010; Huang, Luo et al. 2013)
LYL1	(Nagel, Venturini et al. 2010; McCormack, Shields et al. 2013)
OLFM3	
NCOR2	(Cheng and Kao 2009; van Agthoven, Sieuwerts et al. 2009; Varlakhanova, Hahm et al. 2011)
TCF15	
MYF5	
CRK	(Tsuda and Tanaka 2012; Kumar, Fajardo et al. 2014)
USP6	(Pringle, Young et al. 2012; Rueckert and Haucke 2012)
DPF2	
TCF19	
SREBF1	(Furuta, Pai et al. 2008; Pandey, Xing et al. 2013)
DLX1	
ANXA7	(Srivastava, Torosyan et al. 2007; Torosyan, Dobi et al. 2010; Jin, Wang et al. 2013)
FXR2	
HOXD11	(Shiraishi, Sekiguchi et al. 2002; Miyamoto, Fukutomi et al. 2005)
NCOA6	(Lee, Lee et al. 2006)
HOXD10	(Ma, Teruya-Feldstein et al. 2007; Ma, Reinhardt et al. 2010; Sekar, Bharti et al. 2014)
HHAT	(Konitsiotis, Chang et al. 2014; Petrova, Matevossian et al. 2014)
NUAK2	(Suzuki, Kusakai et al. 2003; Tsuchihara, Ogura et al. 2008)
HOXD1	(Faryna, Konermann et al. 2012; Pussila, Sarantaus et al. 2013)
STAT6	(Gooch, Christy et al. 2002; Wei, He et al. 2014)
ASCL2	(de Sousa, Colak et al. 2011; Reed, Tunster et al. 2012; Zhu, Yang et al. 2012)
HIVEP2	(Fujii, Gabrielson et al. 2005; Yin, Wang et al. 2010)
GATA3	(Chou, Lin et al. 2013; Chu, Lai et al. 2013; Li, Ishiguro et al. 2014)

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3'LIFE/High-throughput Nucleofection and Dual Luciferase Protocols V1
Developed by Justin Wolter, Mangone Lab, 5-21-2013

This portion of the protocol describes a method to transfect plasmid-DNA in 96-well format using the Lonza Nucleofector 96-well shuttle plates. This protocol is optimized for HEK293T cells. Each cell line will have optimal transfection efficiency/survival with individualized buffer conditions, pulse code, and number of cells. These conditions must be optimized for each cell line used.

General Comments:

- For consistent transfections, cells should be plated at a sufficient density that they are rapidly dividing, and not be more than 90% confluent at time of transfection. This is typically achieved by calculating the doubling time of the cell line, and seeding cells 24-48 hours prior to transfection at a density that will result in 70-90% confluency at the time of transfection. Cells should not be allowed to become 100% (and thus growth inhibited) at any time prior to transfection, as this will reduce transfection efficiency, growth rate, and thus expression of the miRNA and luciferase reporter.
- The Lonza Nucleofector and HEK293T cells are extremely sensitive to the buffer conditions used to transfect cells. Extreme accuracy when preparing buffers will ensure consistent performance of the equipment. Additionally, extra care must be taken when performing the assay to minimize evaporation of buffers. We have observed that this is the most significant source of errors when using the Nucleofector and in-house buffers. Specifically, special care must be taken to minimize evaporation following loading the buffer/DNA/cell mixture into the 96-well shuttle device. In our hands, loading the shuttle device immediately before transfection reduces errors in transfection.
- Owing to the sensitivity of the nucleofection buffer conditions, the total volume of transfected materials (including cells and plasmids) should not exceed 10% of the total liquid pipetted into each well of the 96-well shuttle device. To achieve this the pLIFE-miRNA plasmid should be at a concentration of least 500ng/uL.
- Transfection buffer is composed of PBS supplemented with 1.5% HEPES, pH to 7.00. This should typically be prepared fresh, although can be stored for up to 1 month at 4°.
- In formulating buffer and plasmid DNA volumes, we have found that assuming 120 reactions for each 96-well plate sufficiently accounts for errors in pipetting and volume lost using liquid reservoirs and multichannel pipettes.

The following protocol assumes transfecting 3 96-well plates in one experiment. Each plate will correspond to the same 96-well mini-prepped sample of pLIFE-3'UTR plasmids, and be treated with either pLIFE-miRNA-blank, pLIFE-miRNA-#1, pLIFE-miRNA-#2.

1. **(24-48 hrs prior to transfection):** Seed sufficient quantity of HEK293T cells based on the number of 96-well plates being transfected. Each well in a 96-well plate will require

75,000 cells, and each plate requires $9x10^6$ cells (equivalent of 120 wells to account for use of reservoir and multichannel pipette). In our lab, the doubling time of HEK293T cells is ~20 hrs, which can be used to calculate the seeding density based on the number of hours prior to transfection the seeding is occurring. We use 145mm circular culture plates, which, when grown to ~90% confluency, is sufficient for 3 96-well transfections, with ~10% of cells to spare to reseed a new plate.

2. Items to be prepared prior to transfection

a. Transfection Buffer: 18 uL per well, 120 wells/96-well plate = 2.16 mL per plate. Set this aside into an epindorf tube and set aside (being careful not to leave buffer exposed to prevent evaporation).
b. Plasmids:
    i. pLIFE-3'UTR: In 96-well format, resuspended to ~100 ng/uL per well. We typically observe insufficient luciferase signal if plasmid concentration falls below 40 ng/uL. 4 wells must be reserved for the following controls: 1) no pLIFE-3'UTR (to measure background of luciferase assay), 2) pLIFE-SV40 3'UTR (negative target control), 3) positive control for miRNA #1, 4) positive control for miRNA #2. We typically reserve wells A1-A4 for these controls.
    ii. pLIFE-miRNA: At a concentration of 500 ng/uL for each miRNA and Blank control plasmids.
c. 96-well cell culture plates: Each well should possess 200 uL of DMEM supplemented with 10% FBS, 1% Pen/Strep, and placed in a 37° incubator for use following transfection.
d. Warm media, trypsin (0.25%) to 37°.
e. Turn on all Nucleofector hardware followed by Nucleofector Shuttle software. Pulse code used for HEK293T cells and PBS/HEPES buffer should be set to FF120.

3. Preparation of Plasmid DNA and cells:

a. Prepare 3 stocks of pLIFE-miRNA + transfection buffer for each miRNA. This stock should account for 50% (10uL) of total volume of each well, multiplied by 120 wells. Thus, each stock should contain 1.08 mL buffer + 120 uL plasmid DNA (pLIFE-miRNA).
b. Remove cells from plate by eluting media, washing *gently* with PBS, and treating with ~5mLs 0.25% trypsin for 5 minutes. Neutralize trypsin with an equal volume of media, and pellet cells at 300g for 5 minutes.
c. Remove trypsin/media, and resuspend pellet in ~5-10 mLs media (depending on cell density).
d. Count cells. Mangone lab uses the Countess from Invitrogen. Cells should be >95% viable and within accurate range of machine. Inaccurate count can result from having extremely high cell concentrations ($>6.0x10^6$/mL). Transfecting too many cells can drastically reduce efficiency of miRNA targeting by reducing plasmid:cell ratio and/or decreasing transfection efficiency.
e. Aliquot three tubes of cells, each containing $9x10^6$ cells, corresponding to the cells required for transfection of one 96-well plate. Spin cells at 300g for 3 minutes.

f. Remove media. Be sure to remove as much as possible with minimal disturbance of the pellet. Excess media can impact transfection efficiency.
g. Resuspend cells in 1.2 mLs buffer/plasmid mixture, and set aside.
h. The following steps detail resuspension of pLIFE-3'UTR plasmid in transfection buffer. As this occurs in 96 well plates, care should be taken to avoid evaporation of buffer by covering plates at all times.
    i. Using a multichannel pipette, move 32.4 uL transfection buffer into each well of a 96 well PCR plate (9 uL [per transfection] * 3 [plates] * 1.2 [to account for pipette error]).
    ii. Add 3.6 uL of mini-prepped pLIFE-3'UTR plasmid.
    iii. Mix thoroughly.
    iv. Pipette 10 uL of this mixture into each well of the 96-well shuttle device. Cover 96-well shuttle device.

4. Transfection:

a. Move 1.2 mLs of first cell/buffer/pLIFE-miRNA plasmid mixture into reservoir. Mix well. Add 10 uL of this mixture into the first 96-well shuttle already containing 10 uL buffer/pLIFE-3'UTR. **Mix well by pipetting up and down several times.** Equal suspension of cells in the buffer will entail even and thorough passage of the electrical current through the cuvette and maximize transfection efficiency. Place 96-well shuttle on the Nucleofector device and initiate transfection.
b. Once transfection is complete, add 100 uL of prewarmed media from 96-well culture plate to each well of the 96-well shuttle and mix well. Move 100 uL from each well into the 96-well culture plate. Mix well, with pipette positioned vertically, as cells will tend to move to the sides of the well unless mixed properly.
c. Repeat 3a-3b for the remaining two plates.
d. Culture cells for 48-72 hours at 37°, followed by the dual luciferase assay (see below).

5. Cleaning 96-well shuttle plates: These plates can be reused following transfection and proper washing to ensure no carry over between nucleic acids between experiments. We perform two 70% EtOH washes using a spray bottle to completely fill each well, followed by wiping down excess EtOH on the electrode strips (bottom side) and allowing cuvettes to completely dry in the culture hood. We have tested for carry-over contamination by transfecting 12 wells with 2 ug pmaxGFP plasmid each into HEK293T cells, followed by a single wash with 70% EtOH, and a second transfection with no plasmid DNA. With this extremely high plasmid concentration, extremely bright reporter, and only one wash we observed no fluorescence in the second transfection for all 12 wells. We have reused each cuvette >30 times each with no detectable decrease in transfection efficiency.

Buffer preparation for dual-luciferase assay: The following buffer components can be prepared ahead of time and stored at room temperature (unless otherwise noted).

Firefly Luciferase Assay Buffer Components:
Stock solutions prepared separately and stored at 10x concentration:

Reagent:	Final Concentration (1x)

Glycylglycine	25mM
K_xPO_4 (pH 7.8)	15 mM
$MgSO_4$	15 mM
DTT (store at 4º)	1 mM
EGTA	4 mM

Note: EGTA will not go into solution at neutral pH. Slowly add NaOH to EGTA until it dissolves completely.

Reagents added just before luciferase assay:

ATP	2 mM
Beetle luciferin	200 uM

***Renilla* luciferase assay buffer:**

Renilla luciferase buffer can be prepared ahead of time to 1x concentration and stored at room temperature:

Reagent:	Final Concentration (1x)
NaCl	1.1 M
Na_2EDTA	2.2 mM
KH_2PO_4	.22 M
NaN_3	1.3 mM
pH to 5.0	

Reagents added just before luciferase assay:

BSA	.44 mg/mL
Coelenterazine	2.5 uM

Beetle Luciferin (firefly luciferase substrate) Reconstitution:

- 50 mg luciferin in 7.134 mLs H_20 (25 mM). Aliquot into 105 uL tubes.
- Store @ -80º. Per Promega technical support, this should be stable for >6 months, but may be light sensitive.

Coelenterazine (*Renilla* luciferase substrate) Reconstitution:

- Acidify methanol by adding HCl to final concentration of 5 mM (<3 pH).
- Reconstitute 250 ug coelenterazine with 2.36 mL acidified methanol (250 uM)
- Aliquot 100 uL into 1.5 mL tubes for use on 1 96-well plate.
- Store @ -80ºC (stable for at least 6 months at -80º, but may be light sensitive).

Protocol for buffer preparation and 96-well dual-luciferase assay using Promega Glomax 96-well dual injection luminometer

1. **Lysis buffer:** 4 parts water, 1 part 5x passive lysis buffer (Promega) in a reservoir. You need 26 uL/well, so calculate accordingly with ~20 wells extra to account for loss in the reservoir. Buffer is stored at -20º and can be extremely viscous, thus prior to allowing the

5x buffer to approach room temperature will improve accuracy. Mix 1x lysis buffer well prior to use.

2. **Analyze each well for transfection efficiency using fluorescence microscopy.** Note any inconsistent wells that did not transfect efficiently, or are expressing low levels of RFP. These can cause inconsistencies in data and should be removed from the analysis.
3. ***Completely* remove the media from the cells,** being careful not to elute too quickly and lose cells. Remaining media will dilute the lysate and cause fluctuations in values across experiments. Add 26 uL of lysis buffer to each well, and place on a plate shaker/rocker at low/moderate speed.
4. **Start the timer.** For consistency I allow 30 minutes from addition of lysis buffer to the start of measurement. This time is used to prepare buffers, wash and prime the luminometer(s), and transfer lysate to opaque measurement plates. Any of these steps can be completed beforehand (with the exception of the final steps of buffer preparation and lysate transfer) to ensure that you do not go exceed 30 minutes of lysis.

Wrap tubes (typically Falcon 15/50mL tubes) containing of the firefly and *Renilla* buffers with tinfoil as substrates may be light sensitive.

The following instructions are for 1 96-well plate. If multiple plates are being measured simultaneously (with multiple luminometers) then multiply all volumes by then number of luminometers/plates. If multiple plates are being measured sequentially on one luminometer, create buffer master mixes with everything except ATP and substrates, and add these reagents followed by pHing immediately before the beginning of the luciferase assay. ATP and substrates may degrade over time; consistency in the amount of time these reagents are in the buffer will improve consistency across multiple plates.

5. **Firefly luciferase buffer:** Have the five reagents for the firefly buffer prepared to 10x concentration. Prepare a master mix with the following:

- For each 96-well plate, add 1mL of each reagent to 5mLs water, adding EGTA last.
- Add .025 g ATP (powder) per plate just before you add the firefly substrate. Keep ATP on ice at all times. ATP will degrade over time, so if you are measuring more than one plate consecutively buffer must be made fresh beginning at this step for each additional plate.
- Add 100 uL beetle luciferin (substrate) (previously aliquoted and stored at 100x conc.) Buffer should change to yellowish color based on pH.

6. To Renilla buffer:

- Per 96-well plate, aliquot 10 mLs of "*Renilla* buffer".
- Add 100 uL of BSA (44 mg/mL stock) per plate
- If screening more than one plate, separate master mix into 10mL aliquots.
- Add 100 uL coelenterazine to buffer (previously aliquoted and stored at 100x conc.)

7. **pH Buffers:** pH the firefly to 8.0, followed by the Renilla to 5.0 using NaOH and HCl. The activity of each buffer, and the ability of the *Renilla* buffer to quench the Firefly luciferase activity is highly dependent on pH. For consistent results be extremely accurate in this step. Bring volume of each buffer (corresponding to 1 96-well plate) to 10.5 mLs to accommodate for luminometer priming.

8. **Transfer lysate to opaque white plates:** At this point the cells should be close to done lysing (~20 min has passed?), and are ready to be transferred to white opaque plates. Take 25 uL from each well using a multichannel pipette, be sure to pipet up and down vigorously to break up the clumps of cells and homogenizing the lysate. Once transferred to white plates, lysate may appear pinkish. This may be due to poor removal of culture media, but is also commonly observed with high-quality transfections (RFP).

9. **Setting up the luminometer:** Turn on the luminometer and select the program. The program is a promega protocol, is listed in the 'DLR' folder, and is called DLR with two injections, not column format. Select the wells you wish to test (all is the default), and make sure to extend the "Delay before measurement" setting to 5 seconds, with a 10 second measurement time (see Dyer, et al. 2000, *Analytical Biochem*, for explanation).

10. **Capillary wash steps**: water 3x, EtOH 3x, water 3x, dry 3x. Prime buffers once into the waste, and then prime a second time back into the buffer tubes to ensure mixing. Firefly buffer is injected first and should be primed in the left capillary, followed by *Renilla* in the right capillary.

11. **Luciferase assay**: Each plate takes ~48 minutes to read. After you are done save the file first, and then repeat the wash steps and shut off the luminometer.

12. **Reading multiple plates:** Multiple plates can be read and data stored on the same excel file, however we have encountered issues with multiple plate reads where the luminometer program will crash. Be sure to save all data between measurements and take screenshots if program crashes before save is possible! Replace old buffers with new, being sure to prime at least twice with new buffers before starting the new plate

13. **Considerations**: White opaque plates can be reused following a wash with DI water, 70% EtOH, DI water, and spun upside down in a centrifuge to remove any liquid before it can evaporate.