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Supplemental Information

A Simple and Cost-Effective Approach for In Vitro Production of Sliced siRNAs as Potent Triggers for RNAi

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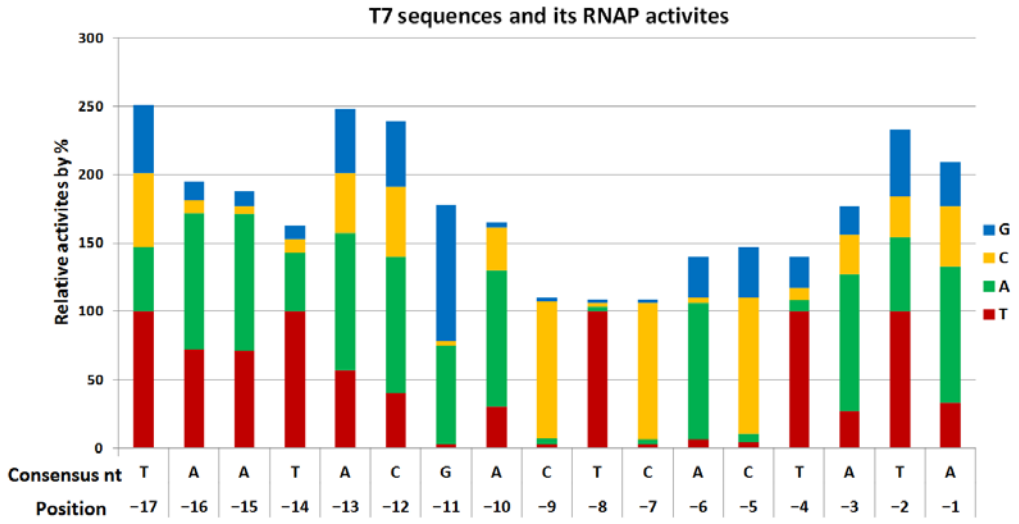
Supplementary data and figures:

I: Supplementary figures

Figure S1: T7 RNAP activities and its sequences or nts downstream of its promoter

The plot was based on the original data from the reference by Imburgio, D. et. al

a). T7 RNAP activities and its sequences



b). T7 RNAP activities and its downstream sequences

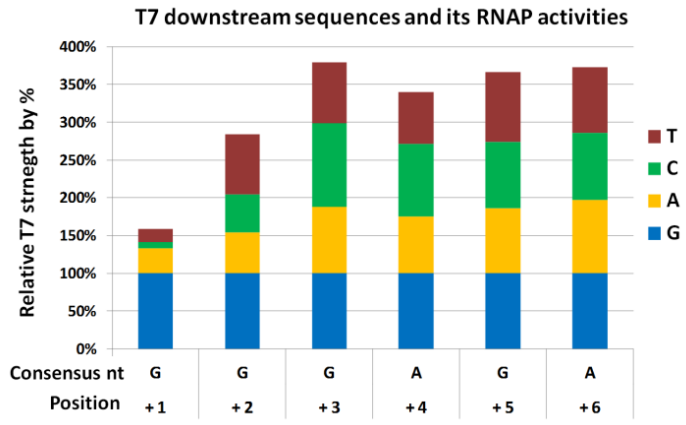


Figure S2: T7 *in vitro* transcription is affected by the +1 nts

A: an A was used at +1 position on the T7 transcripts; GA: A G was appended to the same sequence to make it +1 position as a G; A/G: the +1 A was replaced with a G.

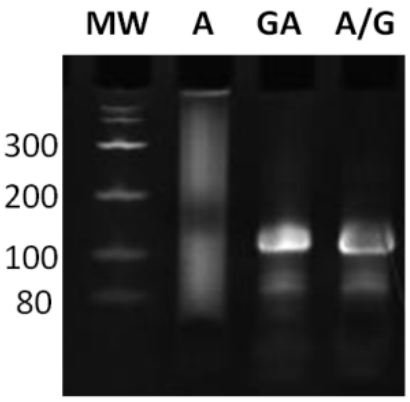


Figure S3: T7 transcripts yield per reaction by the amount of template used

The tsli-siRNA-control was used to examine the effect from the amount of template on the yields of tsli-. The amount of template was optimized as 200 to 300 ng per reaction.

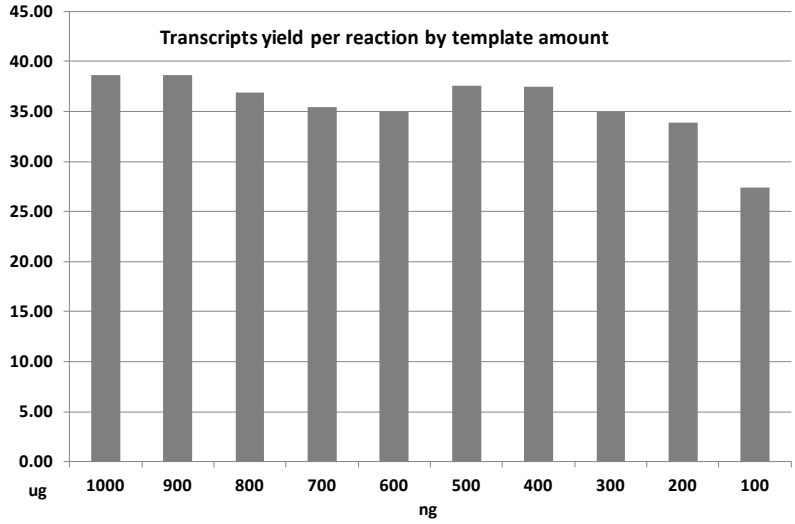
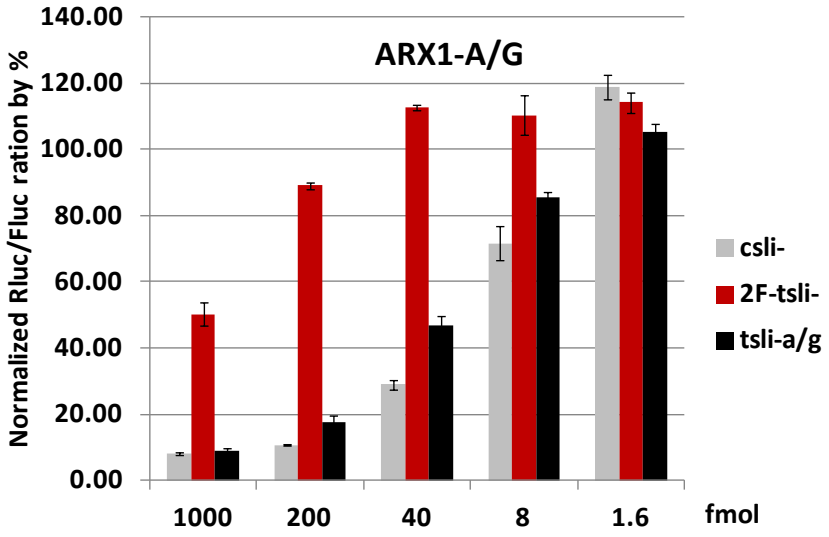


Figure S4. Reporter assay of anchor nt replacement tsli-siRNA variants

a. Knockdown ARX1 reporter in HEK-293 cells by csli-, tsli-, and 2F-tsli-siRNAs

b. Knockdown ARX3 reporter in HEK-293 cells by csli-, tsli-, and 2F-tsli-siRNAs

a.



b.

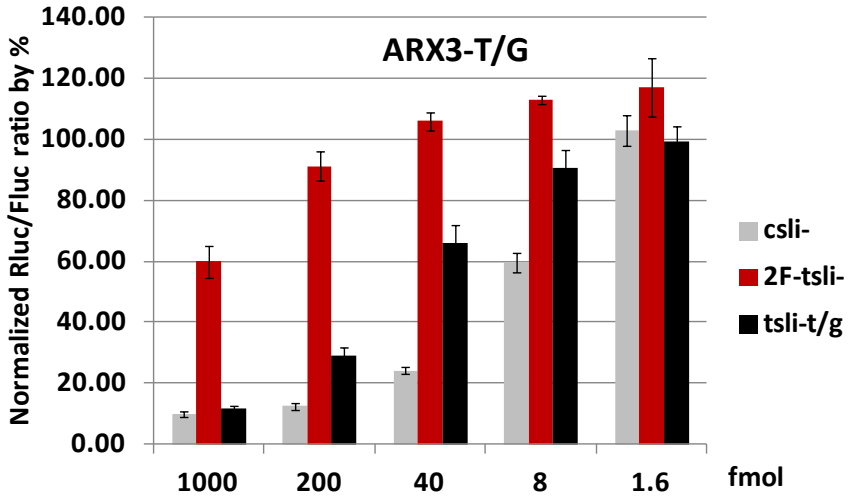


Figure S5. Visualize tsli-siRNA, 2F-tsli-siRNA, and Biotin-tsli-siRNA on PAGE gel

SiRNA (21-mer duplex), csli-siRNA, tsli-siRNA, 2F-tsli-siRNA, and Biotin-tsli-siRNA of control and STAT-3 were run on 10% PAGE gel, stained with Ethidium Bromide.

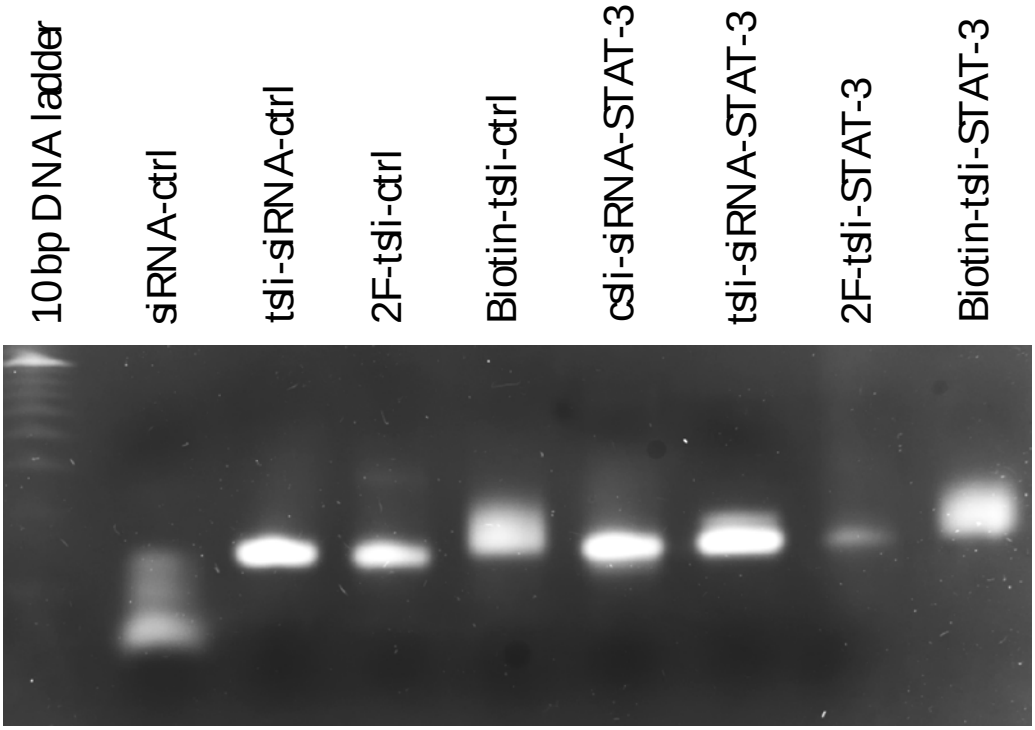
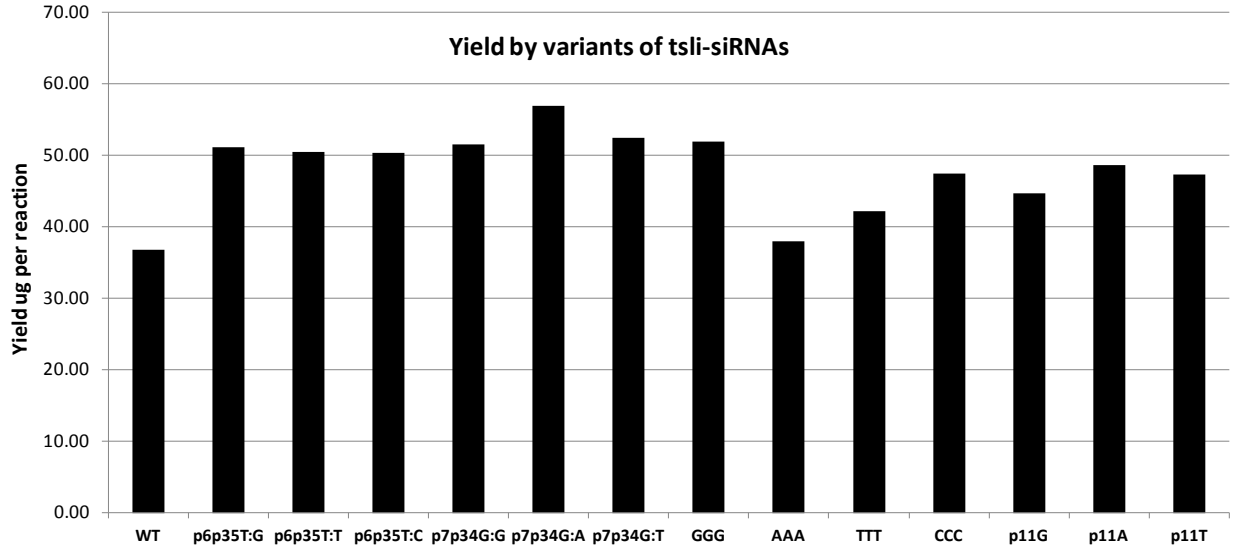
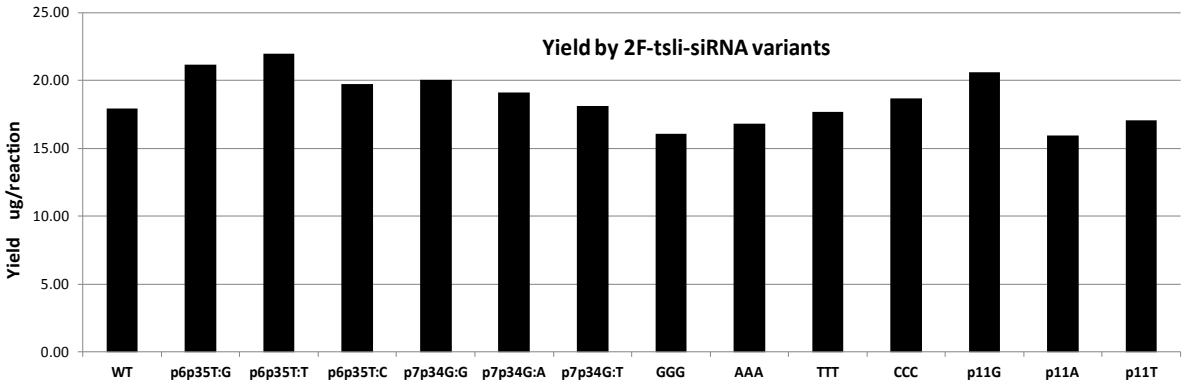


Figure S6. Yield by tsli-siRNA variants and Biotin-tsli-siRNAs

a. Yield by tsli-siRNA variants (100 ng of template DNA was used per reaction)



b. Yield by 2F-tsli-siRNA variants (1ug of template DNA was used per reaction)



c. Yield by biotin-tsli-siRNAs (1ug of template DNA was used per reaction)

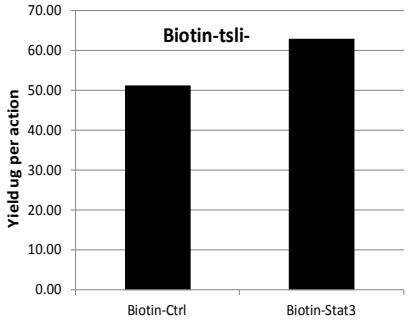
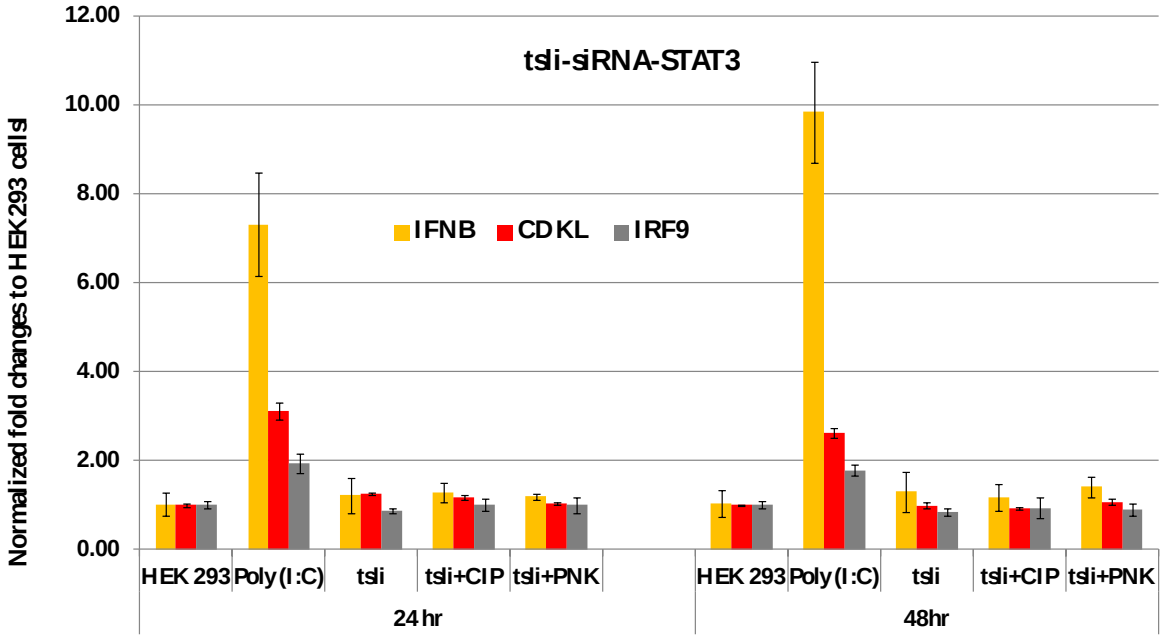


Figure S7. Manipulating 5'ppp triggered interferon response from tsli-STAT3

HEK 293 cells were transfected with Poly (I:C) or tsli-siRNA-STAT3. Gene expression level changes at 24 hours or 48 hours post transfection in IRF9, CDKL, and IFNB relative to GAPDH were measured by qPCR. CIP treatment or T4 PNK treatment were used to minimize or elevate the interferon response from tsli-STAT3. Fold changes in gene expression were normalized to untreated HEK293 cells. Details of qPCR procedure and results calculation were provided in Method section.



I: Supplementary data

1. Supplementary data table S1: tsil-siRNA template (all from 5'- to -3')

Name	Sequence
T7-17 (ϕ6.5)	TAATACGACTCACTATA
tsli-RRM2	TAATACGACTCACTATAgAATTCTCTGTTGGACTTGACATTAAGTCCAACAGAGAATC
tsli-STAT3	TAATACGACTCACTATAGAAGCTGTCACTGTAGAGCTGACTCTACAGTGACAGCTTA
tsli-STAT3-A	TAATACGACTCACTATAaAAGCTGTCACTGTAGAGCTGACTCTACAGTGACAGCTTA
tsli-STAT3-T	TAATACGACTCACTATAtAAGCTGTCACTGTAGAGCTGACTCTACAGTGACAGCTTA
tsli-STAT3-C	TAATACGACTCACTATAcAAGCTGTCACTGTAGAGCTGACTCTACAGTGACAGCTTA
G-tsli-STAT3	TAATACGACTCACTATAgGAAGCTGTCACTGTAGAGCTGACTCTACAGTGACAGCTTA
GG-tsli-STAT3	TAATACGACTCACTATAggGAAGCTGTCACTGTAGAGCTGACTCTACAGTGACAGCTTA
tsli-STAT3-p10-11-12 GGG	TAATACGACTCACTATAGAAGCTGTCgggGTAGAGCTGACTCTACCCCGACAGCTTa
tsli-STAT3-p10-11-12 CCC	TAATACGACTCACTATAGAAGCTGTCcccGTAGAGCTGACTCTACAGTGACAGCTTa
tsli-STAT3-p10-11-12 AAA	TAATACGACTCACTATAGAAGCTGTCaaaGTAGAGCTGACTCTACTTTGACAGCTTa
tsli-STAT3-p10-11-12 TTT	TAATACGACTCACTATAGAAGCTGTCtttGTAGAGCTGACTCTACGGGGACAGCTTa
tsli-STAT3-p11 G	TAATACGACTCACTATAGAAGCTGTCAgTGTAGAGCTGACTCTACACTGACAGCTTa
tsli-STAT3-p11 A	TAATACGACTCACTATAGAAGCTGTCAaTGTAGAGCTGACTCTACATTGACAGCTTa
tsli-STAT3-p11 T	TAATACGACTCACTATAGAAGCTGTCAtTGTAGAGCTGACTCTACAATGACAGCTTa
tsli-STAT3-p6:p35 T:G	TAATACGACTCACTATAGAAGCTGTCACTGTAGAGCTGACTCTACAGTGACgGCTTA
tsli-STAT3-p6:p35 T:T	TAATACGACTCACTATAGAAGCTGTCACTGTAGAGCTGACTCTACAGTGACtGCTTA
tsli-STAT3-p6:p35 T:C	TAATACGACTCACTATAGAAGCTGTCACTGTAGAGCTGACTCTACAGTGACcGCTTA
tsli-STAT3-p7:p34 G:G	TAATACGACTCACTATAGAAGCTGTCACTGTAGAGCTGACTCTACAGTGAgTGCTTA
tsli-STAT3-p7:p34 G:A	TAATACGACTCACTATAGAAGCTGTCACTGTAGAGCTGACTCTACAGTGAaTGCTTA
tsli-STAT3-p7:p34 G:T	TAATACGACTCACTATAGAAGCTGTCACTGTAGAGCTGACTCTACAGTGAtTGCTTA
tsli-Plk1	TAATACGACTCACTATAgAAGCACTTGGCAAAGCCGCCCttGGCTTTGCCAAGTGCTc
tsli-ARX1	TAATACGACTCACTATAgCTGGCTGATCTTGAGCGTGTCTGCTCAAGATCAGCCAGa
tsli-ARX3	TAATACGACTCACTATAgGAACGTGGTGCGGTAGCGCCTGCTACCGCACCACGTTCa
tsli-siRNA-ctrl (control)	TAATACGACTCACTATAgGCGTTCTACACTCGACGTACTtGTCGAGTGTAGAACGCa
T7-17 (ϕ2.5)	TAATACGACTCACTATT
tsli-A-ctrl (control)	TAATACGACTCACTATTaGCGTTCTACACTCGACGTACTtGTCGAGTGTAGAACGCc
tsli-A-ARX1	TAATACGACTCACTATTaCTGGCTGATCTTGAGCGTGTCTGCTCAAGATCAGCCAGc
tsli-A-Plk1	TAATACGACTCACTATTAAGCACTTGGCAAAGCCGCCCttGGCTTTGCCAAGTGCTc

2. Supplementary data table S2: csil-siRNA sequence (all from 5'- to -3')

Name	Sequence
csli-RRM2	AAUUCUCUGUUGGACUUGACAUUAAGUCCAACAGAGAAUC
csli-RRM2-G	gAAUUCUCUGUUGGACUUGACAUUAAGUCCAACAGAGAAUC
csli-RRM2-G/A	gAUUCUCUGUUGGACUUGACAUUAAGUCCAACAGAGAAUC
csli-RRM2-ΔA	AUUCUCUGUUGGACUUGACAUUAAGUCCAACAGAGAAUC
csli-RRM2-ΔΔA	UUCUCUGUUGGACUUGACAUUAAGUCCAACAGAGAAUC
csli-STAT3	GAAGCUGUCACUGUAGAGCUGACUCUACAGUGACAGCUUA
csli-Plk1	AAGCACUUGGCAAAGCCGCCCuuGGCUUUGCCAAGUGCUc
csli-ARX1	ACUGGCUGAUCUUGAGCGUGUCUGCUCAAGAUCAGCCAGc
csli-ARX3	UGAACGUGGUGCGGUAGCGCCUGCUACCGCACCACGUUCc
csli-ctrl (control)	AGCGUUCUACACUCGACGUACUUGUCGAGUGUAGAACGCCUU

3. Supplementary data table S3: qPCR primers (all from 5'- to -3')

Gene	Forward	Reverse
Plk1	GACAAGTACGGCCTTGGGTA	GTGCCGTCACGCTCTATGTA
RRM2	AAGAAGAAGGCAGACTGGGC	TATCGACGCAAAAGAACCGG
STAT3	GCCATCTTGAGCACTAAGCC	CCTTCTCCACCCAAGTGAAA
OAS1	AGGTGGTAAAGGGTGGCTCC	ACAACCAGGTCAGCGTCAGAT
CDKL	GCCTCCTTGGGTTCGTCTATAA	CTCAGGGCCCGCTCATAGTA
IRF9	GACTTGGTCAGGTACTTTCAGG	TCTACACCAGGGACAGAATG
IFNB	AGACTTACAGGTTACCTCCGAA	CAGTACATTCGCCATCAGTCA