

## Analysis of Human Endogenous Retrovirus Expression in Multiple Sclerosis Plaques

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### Supplementary Data

#### Details of qPCR experiments

qPCR experiments conform to the MIQE guidelines as recommended by:

Bustin SA, Benes V, Garson JA, Hellems J, Huggett J, et al. (2009) The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* 55: 611-622.

#### EXPERIMENTAL DESIGN

- Definition of experimental and control groups:
- Cryopreserved normal white matter control brain specimens from patients who did not have any brain diseases (N=9), and Cryopreserved white matter from MS plaques (N=33) and were obtained from the Rocky Mountain MS Center Tissue Bank (Westminster, CO) and the UCLA Human Brain and Spinal Fluid Resource Center (Los Angeles, CA) (Table 1). Number within each group:
- Control group=9 and Experimental group=33 [chronic progressive (n=5), primary progressive (n=4), secondary progressive (n=14), relapsing remitting (n=3) and unclassified confirmed MS cases (n=7)].
- Assay carried out by core lab or investigator's lab? : investigator's lab.
- SAMPLE
- Description: Volume/mass of sample processed: approximately 100 mg specimens of frozen brain were used for RNA extractions.
- Processing procedure: The samples were received frozen from the brain banks. A cold chain was maintained such that the samples were never thawed until being prepared for homogenization. This was performed by chopping the tissue on dry ice and making fine paste. Then the homogenized tissue was added to a Qiagen lysis buffer with four 20second pulses on a vortex.

#### NUCLEIC ACID EXTRACTION

- Procedure and/or instrumentation: Name of kit and details of any modifications: RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany and Germantown, MD).
- Source of additional reagents used: QiA lysis buffer.
- Details of DNase or RNase treatment: TURBO DNA-free™ Kit (Life Technologies, CA).
- Nucleic acid quantification & Contamination assessment (DNA or RNA): RNA was quantified by Qubit® RNA HS Assay Kit and on Qubit® 2.0 Fluorometer instrument (Invitrogen/Thermo Fischer Scientific, Carlsbad, CA, USA). DNA in the prep was quantified by Qubit® dsDNA HS Assay Kit.
- Yield: 5-30 µg RNA was extracted from the samples.

Component	Final concentration of	Volume/ reaction	Final concentration of Master mix
10x Buffer RT		2 µl	1x
dNTP Mix (5 mM each dNTP)		2 µl	0.5 mM each dNTP
Random Hexamer primer (10 µM)		2 µl	1 µM
RNase inhibitor (10 units/µl)		1 µl	10 units (per 20 µl reaction)
Omniscrypt Reverse Transcriptase		1 µl	4 units (per 20 µl reaction)
RNase-free water		7 µl	variable
Template RNA		5 µl	500 ng
Total Volume		20 µl	

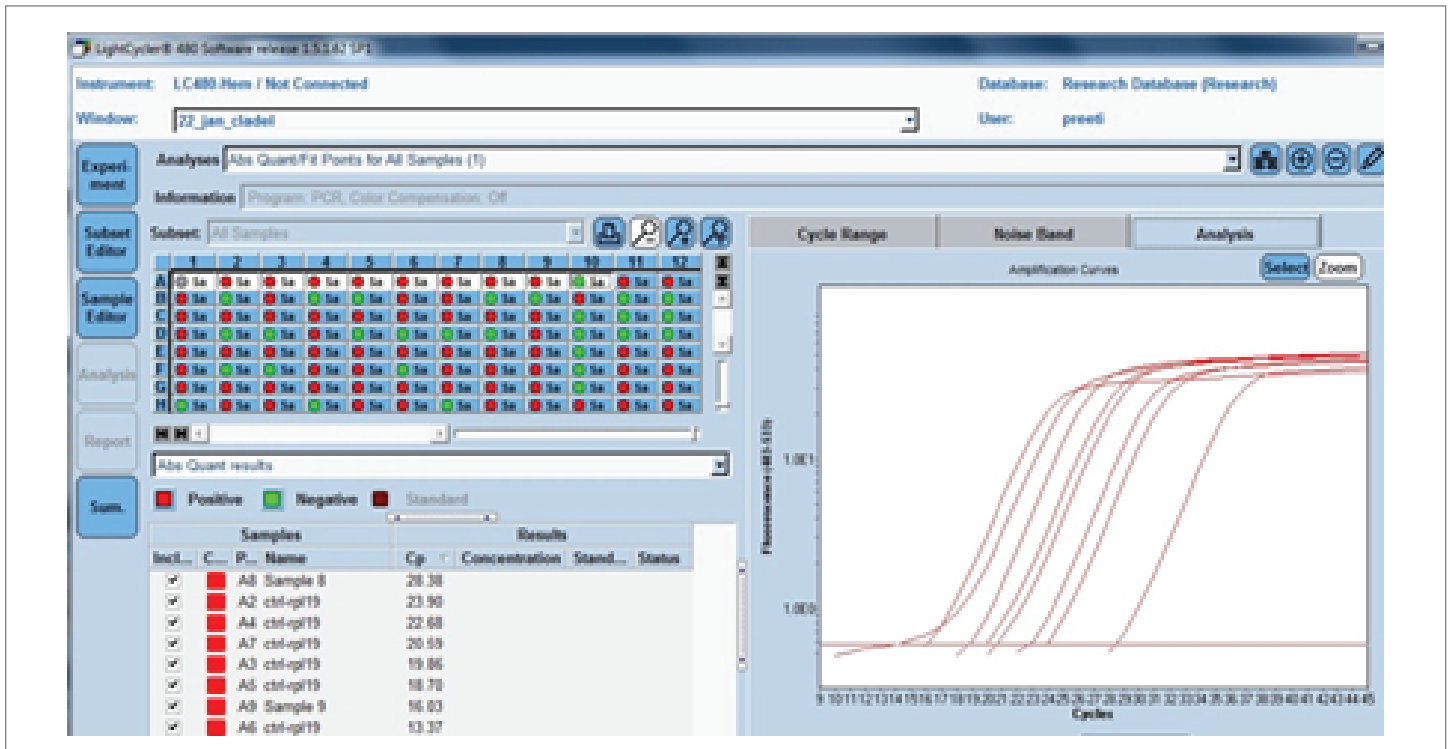
- Inhibition testing (Ct dilutions, spike or other): The samples were pooled and used in series two fold of dilution to plot standard graph.

#### REVERSE TRANSCRIPTION

- Per instruction manual of Omniscript RT kit (Qiagen) Cat No./ID 205111.
- Complete reaction conditions for RT: Amount of RNA and reaction volume: A total of 500 ng of RNA from each sample was used for RT reaction. The reverse transcription (RT) reaction was performed using Omniscript RT (Qiagen, Hilden, Germany and Germantown, MD).
- Priming oligonucleotide concentration: random hexamers were used in a final concentration of 10 µM in the reverse-transcription reaction.
- Reverse transcriptase components and concentrations in reaction:
- Temperature and time: 59 for 30 s.
- Storage conditions of cDNA : -20°C.

#### qPCR TARGET INFORMATION

- Sequence accession numbers: provided in supplementary Table
- Location of amplicons: provided in supplementary Table S2.
- Amplicon length : 120-130 bp.
- In silico specificity screen (BLAST, etc) : Yes, Blast against human genome build 37.p13.
- Pseudogenes, retropseudogenes or other homologs?: Due to the redundancy and duplication of retroviral sequences (GAG and ENV) in NCBI, a HERV phylogeny approach was used to derive primers that bind to many similar loci produced throughout the genome. An in silico analysis of the expected amplicons using these primers was performed before proceeding with invitro experiments and given in Table S2.
- Secondary structure analysis of amplicon: Primers were designed using Primer3 (primer3.sourceforge.net/). Amplicons with "No Secondary Structures" were selected.



Dilution	1:05	1:10	1:20	1:40	1:80	1:160	1:320	1:640
Unit value	0.2	0.1	0.025	0.0125	0.00625	0.00312	0.00156	0.00078
Log2 Dilution	-2.32193	-3.32193	-5.32193	-6.32193	-7.32193	-8.32193	-9.32193	-10.3219
Expression value $x=(y-c)/m$	2.23049	3.23485	4.23920	5.24355	6.24790	7.25226	8.25661	9.26096

- qPCR OLIGONUCLEOTIDES: 20-24bp in length (sequences are specified in Table 2).

**qPCR PROTOCOL :**

- Complete reaction conditions: Reaction volume and amount of cDNA/DNA: cDNA of MS and control samples was diluted to 1: 100 and 5ul from each sample was used as a template in total reaction volumes of 20 µl containing Fast SYBR Green mix
- qPCR kit used: Fast SYBR Green mix (Roche Diagnostics, Indianapolis, IN, USA) Cat number 04707516001.

Buffer and dNTP concentration : Per manufacturer’s instructions.

- Above, is a snap shot of a representative standard curve based on pool dilutions for one primer pair (Reference genes and GAG clade primers). Standard curves for each primer pair are given in the supplementary figures.
- Specificity: Dilutions resulting into Ct values <40 were considered as cut-off. Calibration curves with a slope in range of .95-1.27 (according to 2 fold dilution) and  $R^2 < 0.95$  were considered for analysis. Based on the dilution, gene expression values were calculated from the standard curve for each control and MS specimen.
- Ct of the no template control (NTC): >42
- Standard curves with slope and y-intercept: Using the linear equation  $y = mx + c$ ; where m is slope and c is Y intercept which is concentration of cDNA dilution converted into log2 scale. Y is the Ct Value and x is the arbitrary concentration value. Gene expression values of unknown (MS and Control) was derived by using the Ct Value from each of these reactions.

- Table showing the Dilution and arbitrary concentration value of dilution in log2.
- Slope Value m, was 0.95-1.29 for the 2-fold dilutions on each plate (see Supplementary Figures, slide 11).
- PCR efficiency calculated from slope: For each plate PCR amplification efficiency was determined from standard curve using the slope using equation:  $PCR\ efficiency = (-1 + 2^{-1/slope}) \times 100\%$ . (detailed in Supplementary Figures, slide 11)
- Confidence interval for PCR efficiency or standard error.
- r2 of standard curve : .95-.99.

Evidence for limit of detection: Standard graph from each primer pair with Pooled sample dilution. Lowest Ct Value for 1:05 dilution was 17.

**DATA ANALYSIS**

- qPCR analysis program (source, version): LightCycler® 480 Software, Version 1.5. Ct values were plotted in Excel. Expression values were calculated using the standard curve formulas for each gene of interest.
- Ct method determination: Per LightCycler® 480 Software, Version 1.5.
- Outlier identification and disposition : Per As per LightCycler® 480 Software, Version 1.5.
- Results of NTCs: >42 Ct value.
- Justification of number and choice of reference genes: ubiquitin C (UBC), RNA polymerase II polypeptide (RPL-19), β-2-microglobulin (B2M), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

were used as reference genes. Gene expression among the total MS sample and also each subtypes and control specimens were determined by qPCR for each of the candidate reference genes. The geNorm application was used to rank the least variable candidate reference gene. We identified RPL19 as the best reference gene for the data-set, as it had the lowest expression variation among all the samples we evaluated.

- Description of normalization method: The relative target gene expression was normalized with the reference gene RPL-19. The median expression value for each gene/primer set was derived for the control and MS groups. The expression changes in terms of

Fold change (log<sub>2</sub>) for each primer set were derived by dividing the median expression value of the MS group by the median of the CTRL group.

- Number and concordance of biological replicates: Duplicates.
- Number and stage (RT or qPCR) of technical replicates: Duplicates.
- Statistical methods for result significance: Mann-Whitney tests were performed on the dataset to detect differences among the variables. We considered the difference to be statistically significant when  $p < 0.05$ .

Software (source, version) : <http://vassarstats.net/>

**Table S1:** HERV sequences used to develop qPCR primers.

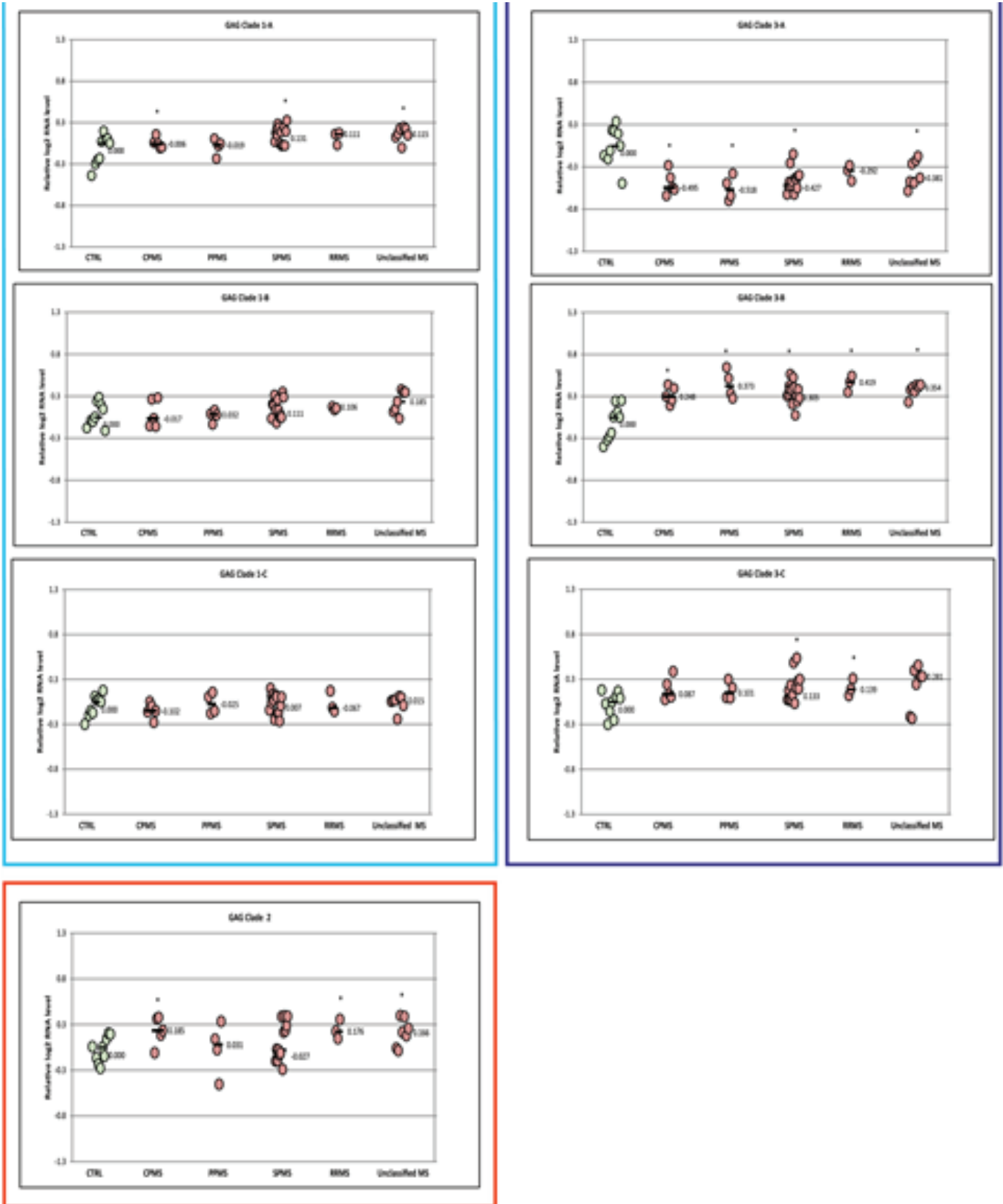
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GAG	1	K-HERV	224589800	20481865	816
GAG	2	HERV-E	224589800	75844419	1287
GAG	3	HERV-E	224589801	35258727	1224
GAG	4	HERV-E	224589802	60862212	1245
GAG	5	HERV-E	224589803	17450946	804
GAG	6	HERV-K10	224589803	32095804	756
GAG	7	HERV-E	224589803	58727764	1287
GAG	8	HERV-E	224589804	41453173	1296
GAG	9	HERV-K10	224589804	67972360	804
GAG	11	HERV-K10	224589810	20699104	765
GAG	12	HERV-E	224589810	28135395	1287
GAG	13	HERV-K10	224589811	71273545	765
GAG	14	K-HERV	224589813	19939279	1059
GAG	15	HERV-K10	224589814	18927835	1287
GAG	16	HERV-K10	224589814	23881091	1824
GAG	17	K-HERV	224589815	9893352	1917
GAG	18	HERV-K10	224589815	75601277	1050
GAG	20	HERV-K10	224589816	9661433	1023
GAG	21	K-HERV	224589817	30493375	1200
GAG	22	HERV-K10	224589817	46006565	1563
GAG	23	HERV-K10	224589818	78433157	1287
GAG	24	HERV-K10	224589819	4628594	1287
GAG	25	HERV-K10	224589819	4637097	1287
GAG	26	HERV-E	224589820	7361933	1287
GAG	27	HERV-E	224589820	11770051	765
GAG	28	HERV-E	224589820	11792634	762
GAG	29	HERV-E	224589820	35810060	933
GAG	30	HERV-E	224589823	59307943	1284
ENV	1	HERV-K10	224589800	13465685	1008
ENV	2	SMRV-H	224589800	13686521	1008
ENV	3	K-HERV	224589800	66883587	564
ENV	4	HERV-K10	224589800	75846956	1263
ENV	5	RTVL-Ia	224589801	6867069	1065
ENV	6	HERV-K10	224589801	85879093	249
ENV	7	RTVL-Ia	224589802	3469637	735
ENV	8	RTVL-Ia	224589802	4484932	294
ENV	9	HERV-K10	224589802	23014944	270
ENV	10	K-HERV	224589802	51354150	720
ENV	11	HERV-E	224589802	58771057	1188
ENV	12	RTVL-Ia	224589802	59958681	1467
ENV	13	HERV-K10	224589802	61990042	234
ENV	14	HERV-E	224589802	62137132	945
ENV	15	HERV-K10	224589802	71458171	1014
ENV	16	RTVL-Ia	224589802	71475117	750
ENV	17	RTVL-Ia	224589803	10056039	270
ENV	18	HERV-K10	224589803	32180481	234
ENV	19	RTVL-Ia	224589803	34773426	804
ENV	20	HERV-K10	224589803	48995457	192
ENV	21	RTVL-Ia	224589803	58722167	1263
ENV	22	RTVL-Ia	224589803	78878127	210
ENV	23	RTVL-Ia	224589804	69463668	213

ENV	24	RTVL-Ia	224589805	53643304	174
ENV	25	HERV-K10	224589806	34447718	261
ENV	26	RTVL-Ia	224589806	40979271	906
ENV	27	K-HERV	224589807	10536437	222
ENV	28	RTVL-Ia	224589807	34231965	1263
ENV	29	HERV-E	224589807	72106212	333
ENV	30	RTVL-Ia	224589808	26558819	1284
ENV	31	RTVL-Ia	224589808	75164629	225
ENV	32	RTVL-Ia	224589809	41088888	198
ENV	33	RTVL-Ia	224589809	44456395	180
ENV	34	RTVL-Ia	224589810	11996337	201
ENV	35	HERV-E	224589810	20010094	174
ENV	36	K-HERV	224589810	20930917	1284
ENV	37	HERV-K10	224589810	22762530	1107
ENV	38	HERV-K10	224589810	23721803	795
ENV	39	HERV-K10	224589810	28129450	1263
ENV	40	MMTV	224589810	36063297	1017
ENV	41	RTVL-Ia	224589810	52483588	540
ENV	42	RTVL-Ia	224589810	53172676	258
ENV	43	HERV-K10	224589812	47898460	195
ENV	44	K-HERV	224589813	19934352	717
ENV	45	RTVL-Ia	224589814	18933178	1263
ENV	46	RTVL-Ia	224589814	22812684	270
ENV	47	RTVL-Ia	224589814	42501252	243
ENV	48	K-HERV	224589815	1933483	300
ENV	49	RTVL-Ia	224589815	9890692	1293
ENV	50	HERV-E	224589815	14081270	213
ENV	51	K-HERV	224589815	74878232	1440
ENV	52	RTVL-Ia	224589815	75606975	1188
ENV	53	HERV-E	224589815	87656965	306
ENV	54	HERV-K10	224589815	87783703	1044
ENV	55	HERV-K10	224589816	3980231	1011
ENV	56	HERV-K10	224589816	4045256	630
ENV	57	HERV-K10	224589816	9131379	735
ENV	58	RTVL-Ia	224589816	9667002	1011
ENV	59	RTVL-Ia	224589816	42321620	261
ENV	60	HERV-K10	224589816	99935359	231
ENV	61	HERV-E	224589817	30487677	1263
ENV	62	RTVL-Ia	224589817	81564365	1284
ENV	63	RTVL-Ia	224589818	31297132	243
ENV	64	RTVL-Ia	224589818	35529505	234
ENV	65	HERV-K10	224589818	66872395	192
ENV	66	K-HERV	224589818	78427579	2094
ENV	67	HERV-K10	224589819	4622982	1263
ENV	68	RTVL-Ia	224589819	4631486	1263
ENV	69	RTVL-Ia	224589819	43858596	249
ENV	70	HERV-K10	224589819	43862514	246
ENV	71	RTVL-Ia	224589819	65470824	765
ENV	72	HERV-K10	224589819	70314099	207
ENV	73	HERV-K10	224589820	7356322	1263
ENV	74	HERV-K10	224589820	8062506	735
ENV	75	HERV-K10	224589820	12074951	744
ENV	76	K-HERV	224589820	12317473	735
ENV	77	HERV-E	224589820	47176633	1182
ENV	78	RTVL-Ia	224589820	56626581	1326
ENV	79	HERV-E	224589820	80035781	270
ENV	80	RTVL-Ia	224589821	29382787	1368
ENV	81	HERV-K10	224589821	90653107	261
ENV	82	RTVL-Ia	224589822	61960909	759
ENV	83	K-HERV	224589823	6825896	261
ENV	84	RTVL-Ia	224589823	6827412	1125
ENV	85	RTVL-Ia	224589823	7046287	174
ENV	86	RTVL-Ia	224589823	14581657	261
ENV	87	RTVL-Ia	224589823	21613337	192
ENV	88	HERV-K10	224589823	23746172	768

<sup>1</sup>U numbers, for clarity, derived from the Gypsy 2.0 database.

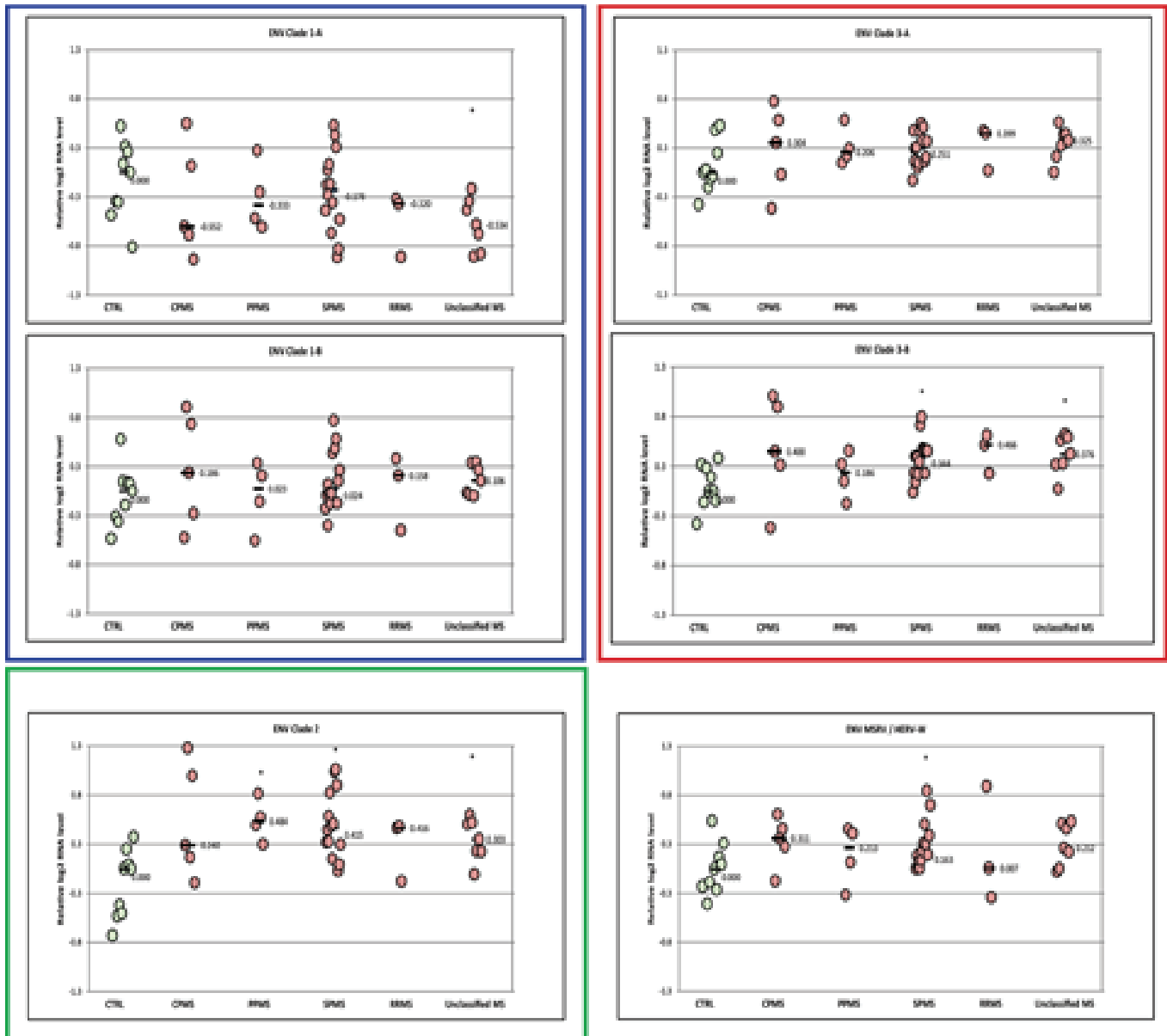
<sup>2</sup>HERV annotations as described in the Gypsy 2.0 database.

<sup>3</sup>GenBank/NCBI identifier number and start site.



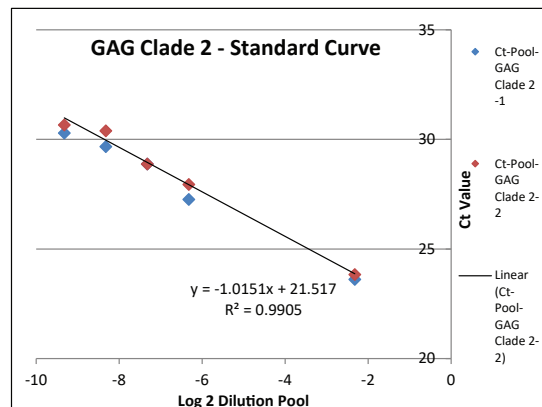
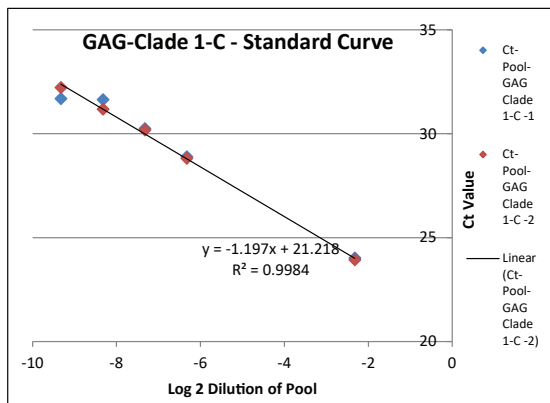
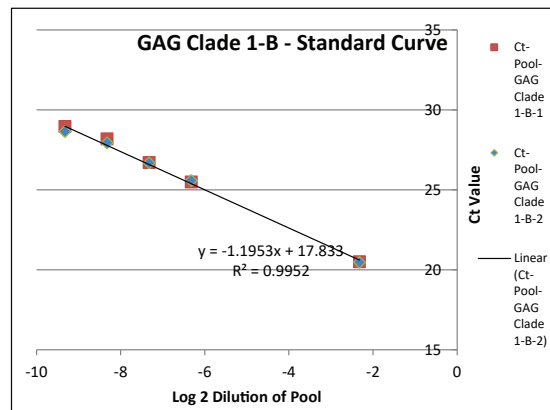
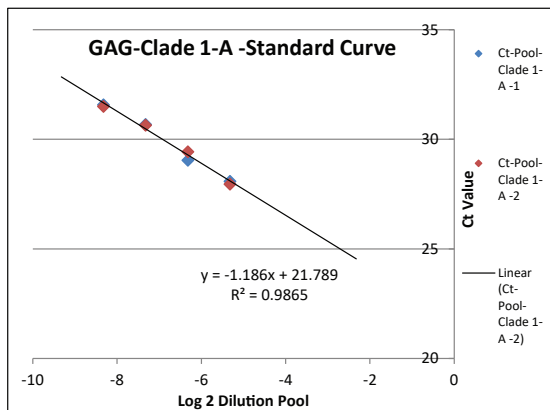
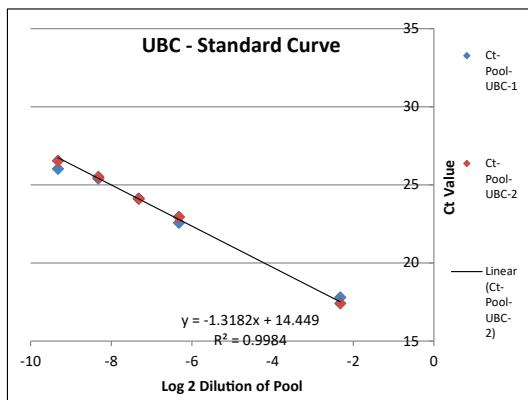
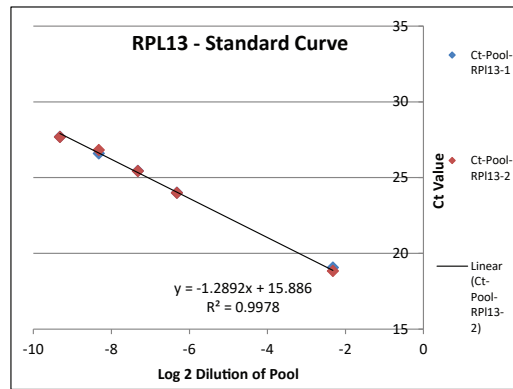
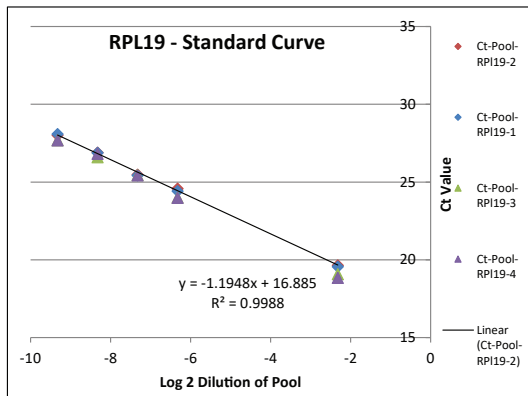
**Figure S1:** GAG domain expressions in Brain Tissue-Differences in normalized expression for each primer pair are shown as  $\text{Log}_2$ . Significance in expression differences was determined using the Mann-Whitney test comparing expression values for the Control patients (N=9) to the Chronic Progressive MS [CPMS (N=5)], primary progressive MS [PPMS, N=4], secondary progressive [SPMS, N=14], relapsing remitting MS [RRMS, N=3] and unclassified MS (N=7). Statistically significant groups ( $p < 0.05$ ) are noted by \*.

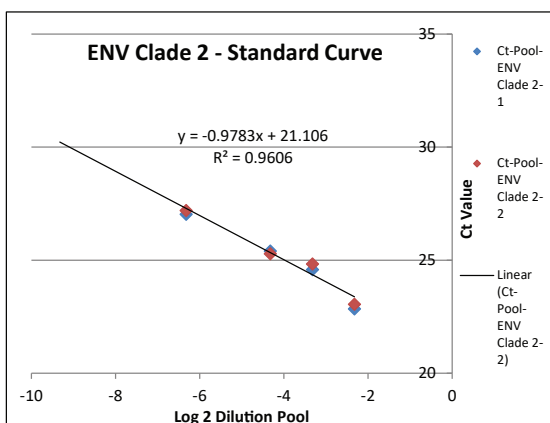
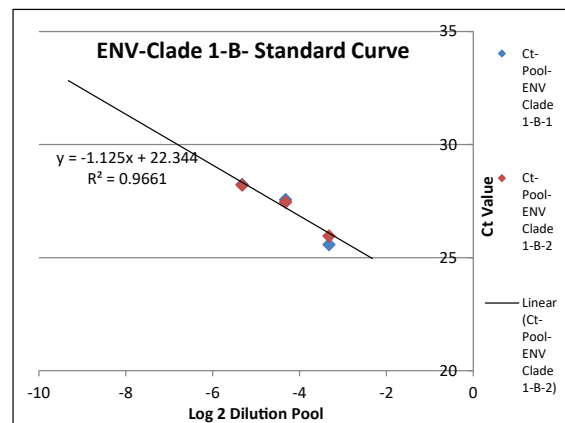
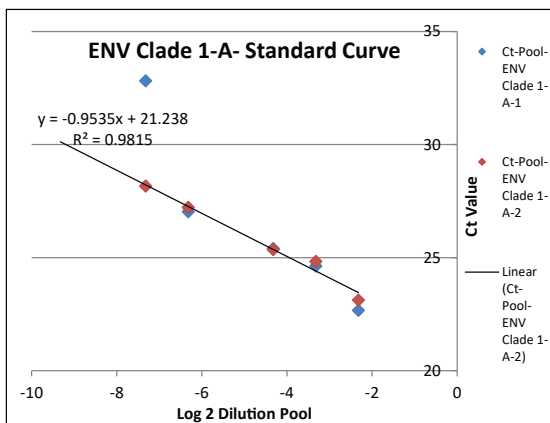
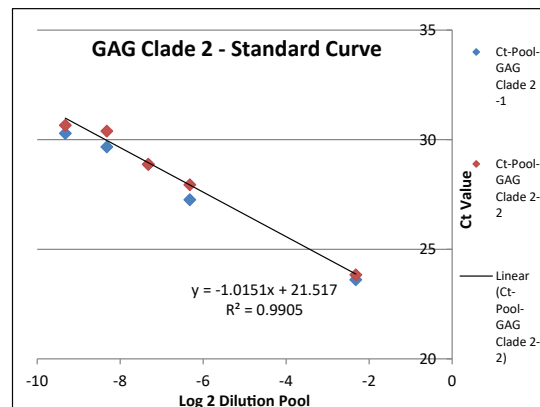
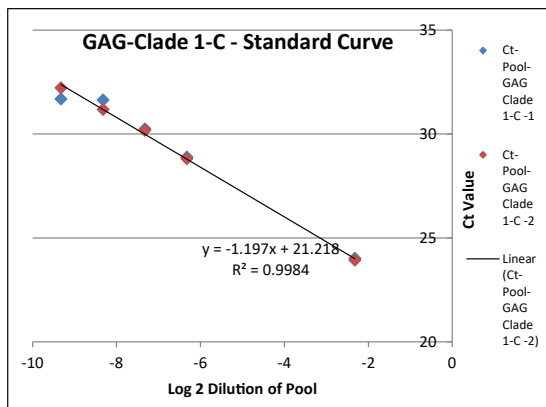
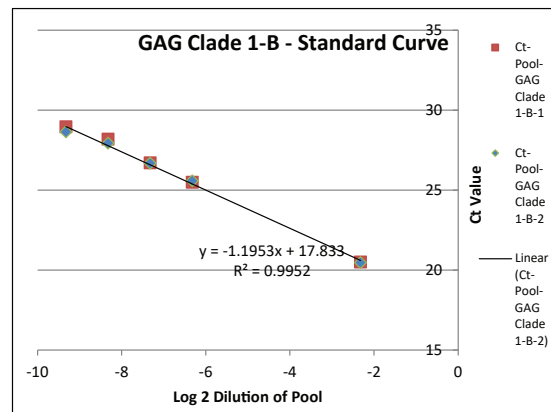
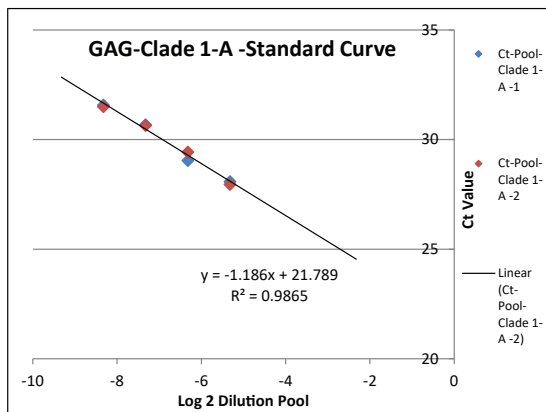
Fig S2 : ENV Expression in Brain Tissues



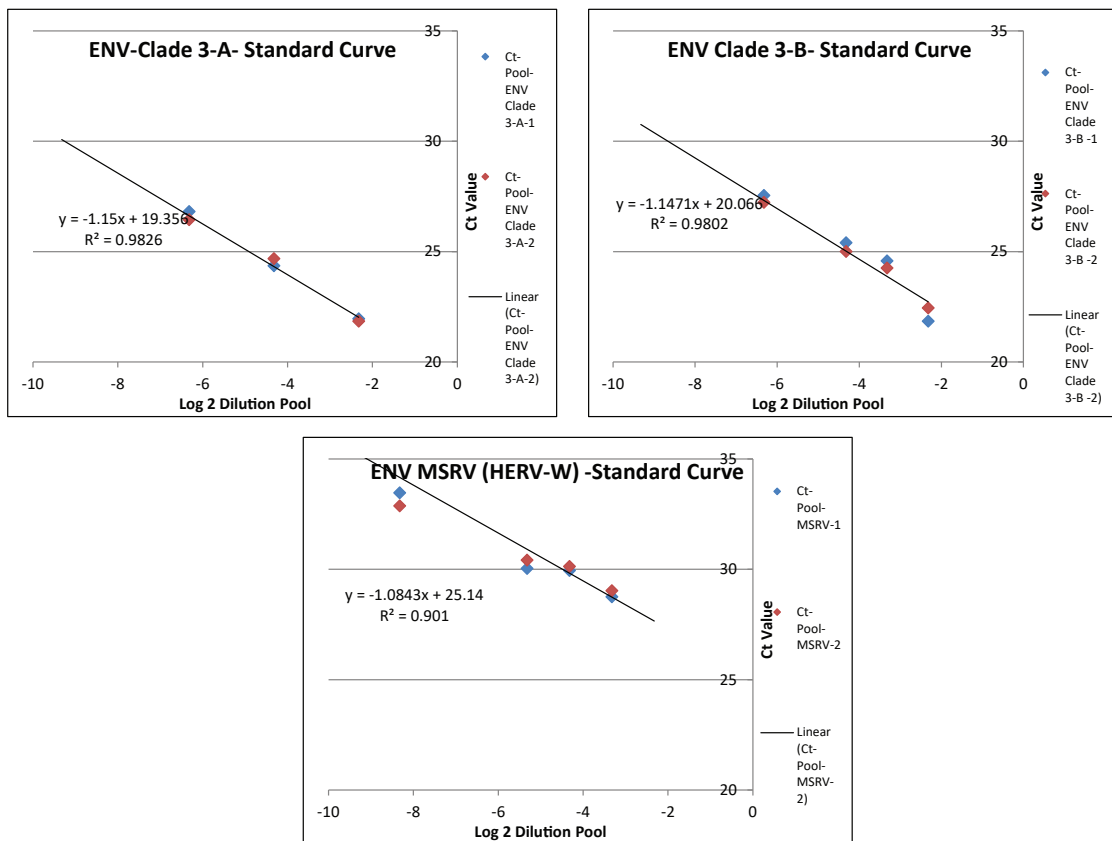
**Figure S2:** ENV domain expressions in Brain Tissues-Differences in normalized expression for each primer pair are shown as  $\text{Log}_2$ . Significance in expression differences was determined using the Mann-Whitney test comparing expression values for the Control patients (N=9) to the Chronic Progressive MS [CPMS (N=5)], primary progressive MS [PPMS, N=4], secondary progressive [SPMS, N=14], relapsing remitting MS [RRMS, N=3] and unclassified MS (N=7). Statistically significant groups ( $p < 0.05$ ) are noted by \*.

Graphs:







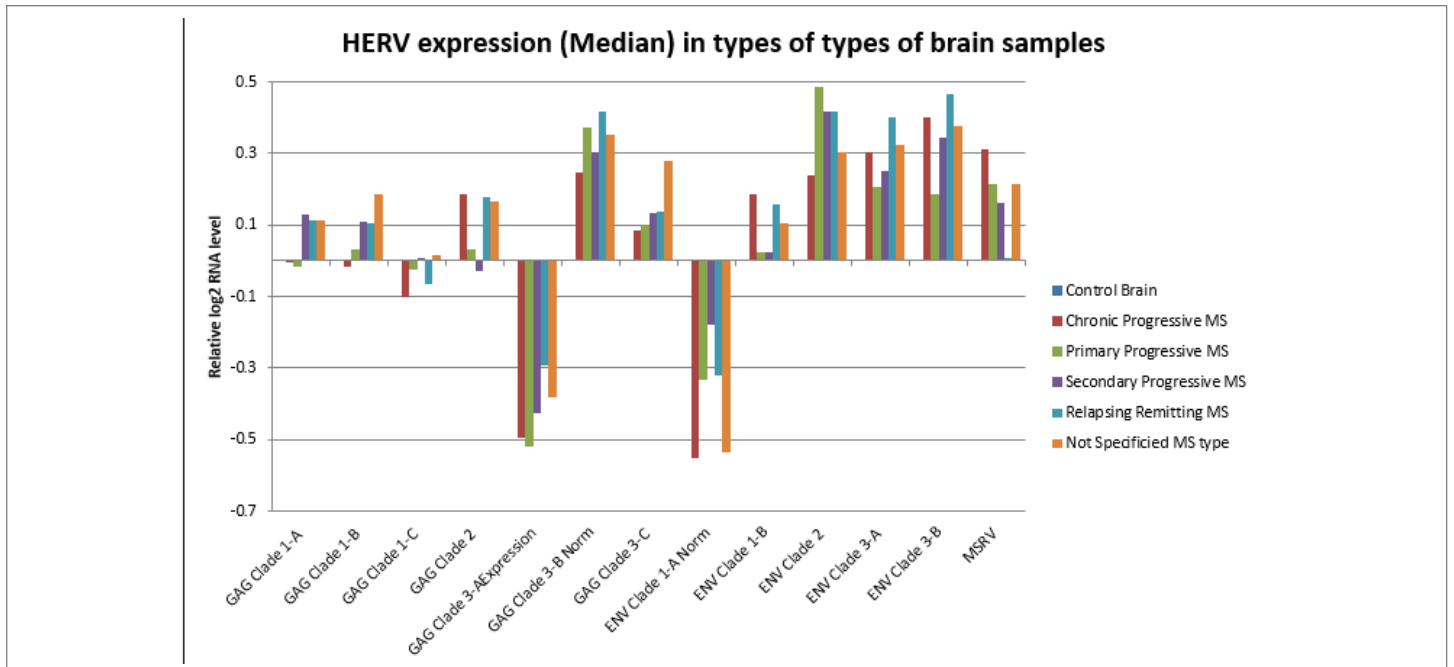
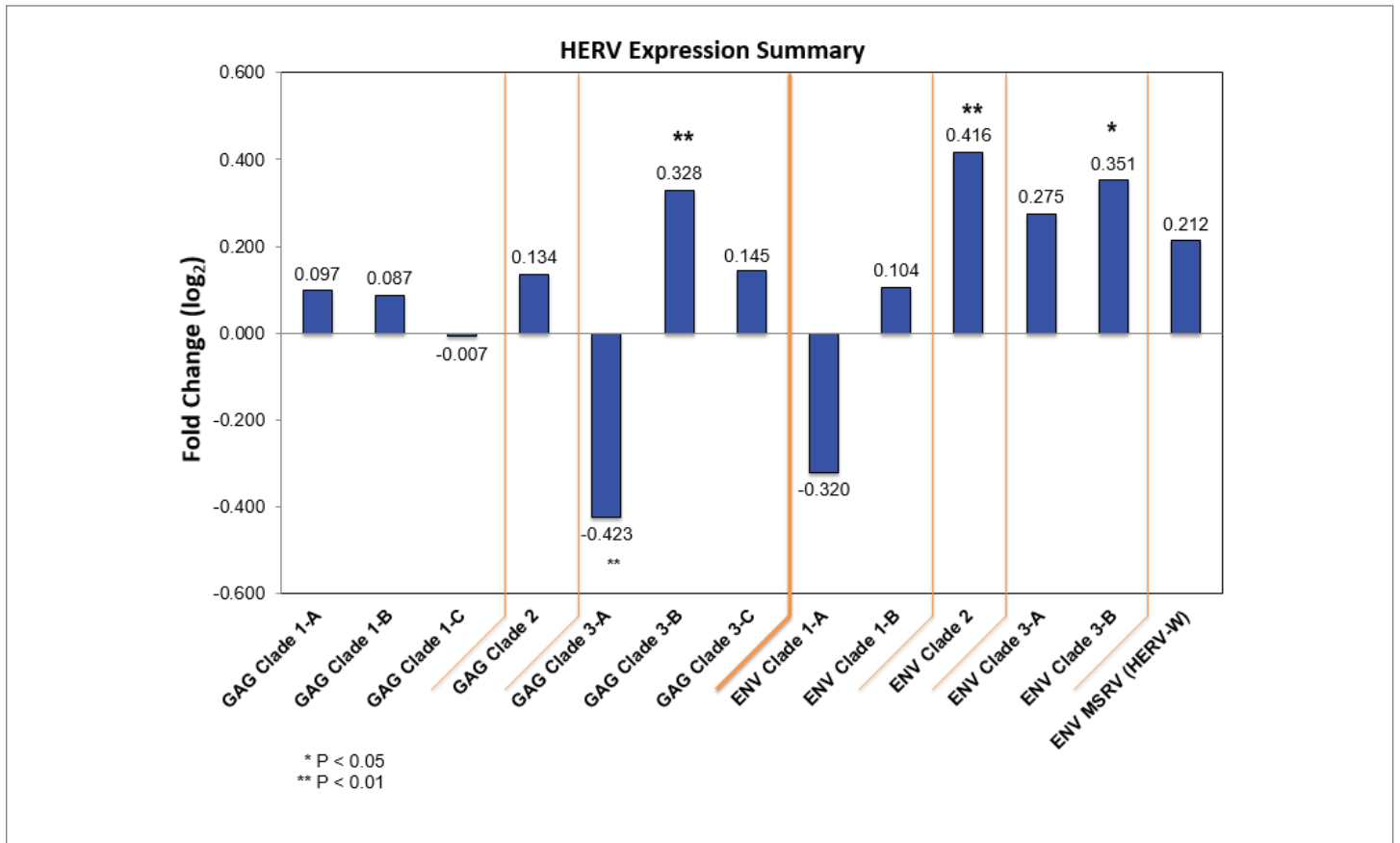


## PCR Efficiency for Reactions

Primer Pair	Standard Curve Slope	R-Square	PCR Efficiency (%)
RPL19*	-1.19	0.998	79.0
RPL-13**	-1.28	0.997	71.9
UBC**	-1.31	0.998	69.7
GAG-Clade-1A	-1.18	0.986	79.9
GAG-Clade-1B	-1.19	0.995	79.0
GAG-Clade-1C	-1.19	0.998	79.0
GAG-Clade-2	-1.01	0.990	98.6
GAG-Clade-3A	-1.29	0.998	71.1
GAG-Clade-3B	-1.27	0.983	72.6
GAG-Clade-3C	-1.01	0.950	98.6
ENV-Clade-1A	-0.95	0.981	107.4
ENV-Clade-1B	-1.12	0.966	85.7
ENV-Clade-2	-0.97	0.960	104.3
ENV-Clade-3A	-1.15	0.982	82.7
ENV-Clade-3B	-1.14	0.980	83.7
ENV MSRV HERV-W	-1.08	0.901	90.0

\* Selected as reference gene for expression determinations

\*\* Not selected as reference gene



**Table S2 Predicted Amplicons**

Primer Name	gi	NCBI accession no	Chromosome no.	start nucleotide	Amplicon Length
GAG Clade 1-A	224589800	NC_000001.10	1	75845145	117
GAG Clade 1-A	224589800	NC_000001.10	1	155603146	117
GAG Clade 1-A	224589800	NC_000001.10	1	160662939	117
GAG Clade 1-A	224589801	NC_000010.10	10	101586193	117
GAG Clade 1-A	224589802	NC_000011.9	11	101568168	117
GAG Clade 1-A	224589802	NC_000011.9	11	118598398	117
GAG Clade 1-A	224589803	NC_000012.11	12	58728208	117
GAG Clade 1-A	224589810	NC_000019.9	19	28135839	117
GAG Clade 1-A	224589814	NC_000022.10	22	18928561	117
GAG Clade 1-A	224589815	NC_000003.11	3	9893799	114
GAG Clade 1-A	224589815	NC_000003.11	3	112749800	117
GAG Clade 1-A	224589815	NC_000003.11	3	125611511	117
GAG Clade 1-A	224589815	NC_000003.11	3	148283874	117
GAG Clade 1-A	224589815	NC_000003.11	3	185287029	117
GAG Clade 1-A	224589817	NC_000005.9	5	30493732	117
GAG Clade 1-A	224589817	NC_000005.9	5	156091406	117
GAG Clade 1-A	224589818	NC_000006.11	6	78433601	117
GAG Clade 1-A	224589819	NC_000007.13	7	4629038	117
GAG Clade 1-A	224589819	NC_000007.13	7	4637541	117
GAG Clade 1-A	224589819	NC_000007.13	7	104391747	117
GAG Clade 1-A	224589820	NC_000008.10	8	7362377	117
GAG Clade 1-A	224589821	NC_000009.11	9	139681659	118
GAG Clade 1-A	303308482	NW_003315965.1	19	70520	117
GAG Clade 1-A	374341059	NW_003315964.2	19	17435	117
GAG Clade 1-A	394726933	NW_003871072.1	11	353127	117
GAG Clade 1-B	224589800	NC_000001.10	1	75845145	120
GAG Clade 1-B	224589800	NC_000001.10	1	155603143	120
GAG Clade 1-B	224589800	NC_000001.10	1	160662939	120
GAG Clade 1-B	224589801	NC_000010.10	10	101586190	120
GAG Clade 1-B	224589802	NC_000011.9	11	101568168	120
GAG Clade 1-B	224589802	NC_000011.9	11	118598395	120
GAG Clade 1-B	224589803	NC_000012.11	12	58728205	120
GAG Clade 1-B	224589810	NC_000019.9	19	28135836	120
GAG Clade 1-B	224589813	NC_000021.8	21	19939633	120
GAG Clade 1-B	224589814	NC_000022.10	22	18928561	120
GAG Clade 1-B	224589815	NC_000003.11	3	9893796	117
GAG Clade 1-B	224589815	NC_000003.11	3	112749797	120
GAG Clade 1-B	224589815	NC_000003.11	3	125611511	120
GAG Clade 1-B	224589815	NC_000003.11	3	148283871	120
GAG Clade 1-B	224589815	NC_000003.11	3	185287026	120
GAG Clade 1-B	224589817	NC_000005.9	5	30493729	120
GAG Clade 1-B	224589817	NC_000005.9	5	156091403	120
GAG Clade 1-B	224589818	NC_000006.11	6	78433598	120
GAG Clade 1-B	224589819	NC_000007.13	7	4629035	120
GAG Clade 1-B	224589819	NC_000007.13	7	4637538	120
GAG Clade 1-B	224589819	NC_000007.13	7	104391744	120
GAG Clade 1-B	224589820	NC_000008.10	8	7362374	120

GAG Clade 1-B	224589821	NC_000009.11	9	139681658	119
GAG Clade 1-B	303308482	NW_003315965.1	19	70520	120
GAG Clade 1-B	374341059	NW_003315964.2	19	17432	120
GAG Clade 1-B	394726933	NW_003871072.1	11	353124	120
GAG Clade 1-C	224589800	NC_000001.10	1	75845145	116
GAG Clade 1-C	224589800	NC_000001.10	1	155603147	116
GAG Clade 1-C	224589800	NC_000001.10	1	160662939	116
GAG Clade 1-C	224589801	NC_000010.10	10	101586194	116
GAG Clade 1-C	224589802	NC_000011.9	11	101568168	116
GAG Clade 1-C	224589802	NC_000011.9	11	118598399	116
GAG Clade 1-C	224589803	NC_000012.11	12	58728209	116
GAG Clade 1-C	224589810	NC_000019.9	19	28135840	116
GAG Clade 1-C	224589814	NC_000022.10	22	18928561	116
GAG Clade 1-C	224589815	NC_000003.11	3	9893800	113
GAG Clade 1-C	224589815	NC_000003.11	3	112749801	116
GAG Clade 1-C	224589815	NC_000003.11	3	125611511	116
GAG Clade 1-C	224589815	NC_000003.11	3	148283875	116
GAG Clade 1-C	224589815	NC_000003.11	3	185287030	116
GAG Clade 1-C	224589817	NC_000005.9	5	30493733	116
GAG Clade 1-C	224589817	NC_000005.9	5	156091407	116
GAG Clade 1-C	224589818	NC_000006.11	6	78433602	116
GAG Clade 1-C	224589819	NC_000007.13	7	4629039	116
GAG Clade 1-C	224589819	NC_000007.13	7	4637542	116
GAG Clade 1-C	224589819	NC_000007.13	7	104391748	116
GAG Clade 1-C	224589820	NC_000008.10	8	7362378	116
GAG Clade 1-C	224589821	NC_000009.11	9	139681660	117
GAG Clade 1-C	303308482	NW_003315965.1	19	70520	116
GAG Clade 1-C	374341059	NW_003315964.2	19	17436	116
GAG Clade 1-C	394726933	NW_003871072.1	11	353128	116
GAG Clade 2	224589800	NC_000001.10	1	12843290	167
GAG Clade 2	224589800	NC_000001.10	1	13460915	167
GAG Clade 2	224589800	NC_000001.10	1	13681751	167
GAG Clade 2	224589802	NC_000011.9	11	3475496	166
GAG Clade 2	224589805	NC_000014.8	14	24482572	170
GAG Clade 2	224589815	NC_000003.11	3	75602060	170
GAG Clade 2	224589816	NC_000004.11	4	3985943	170
GAG Clade 2	224589816	NC_000004.11	4	9126062	170
GAG Clade 2	224589816	NC_000004.11	4	9662123	170
GAG Clade 2	224589817	NC_000005.9	5	154021447	170
GAG Clade 2	224589818	NC_000006.11	6	42868602	170
GAG Clade 2	224589820	NC_000008.10	8	8057181	167
GAG Clade 2	224589820	NC_000008.10	8	12080846	167
GAG Clade 2	224589820	NC_000008.10	8	12323356	167
GAG Clade 3-A	224589800	NC_000001.10	1	186890648	126
GAG Clade 3-A	224589801	NC_000010.10	10	85869690	128
GAG Clade 3-A	224589802	NC_000011.9	11	60862669	127
GAG Clade 3-A	224589816	NC_000004.11	4	138400944	128
GAG Clade 3-A	224589817	NC_000005.9	5	111861076	128
GAG Clade 3-A	224589822	NC_000023.10	X	155205742	128

GAG Clade 3-A	224589823	NC_000024.9	Y	59308748	128
GAG Clade 3-B	224589800	NC_000001.10	1	186890754	222
GAG Clade 3-B	224589801	NC_000010.10	10	85869486	220
GAG Clade 3-B	224589802	NC_000011.9	11	60862464	225
GAG Clade 3-B	224589816	NC_000004.11	4	138400740	224
GAG Clade 3-B	224589817	NC_000005.9	5	111860872	221
GAG Clade 3-B	224589818	NC_000006.11	6	123068900	224
GAG Clade 3-B	224589822	NC_000023.10	X	155205850	224
GAG Clade 3-B	224589823	NC_000024.9	Y	8672330	224
GAG Clade 3-B	224589823	NC_000024.9	Y	59308856	224
ENV Clade 1-A	224589823	NC_000024.9	Y	6828037	161
ENV Clade 1-A	224589800	NC_000001.10	1	13466087	161
ENV Clade 1-A	224589800	NC_000001.10	1	13686923	161
ENV Clade 1-B	303308482	NW_003315965.1	19	76007	123
ENV Clade 1-B	224589803	NC_000012.11	12	58722427	123
ENV Clade 1-B	224589810	NC_000019.9	19	28129710	123
ENV Clade 1-B	224589810	NC_000019.9	19	36063557	123
ENV Clade 1-B	224589810	NC_000019.9	19	53866715	122
ENV Clade 1-B	224589816	NC_000004.11	4	165922769	123
ENV Clade 1-B	224589820	NC_000008.10	8	7356582	123
ENV Clade 1-B	224589820	NC_000008.10	8	140473334	123
ENV Clade 1-B	224589813	NC_000021.8	21	19934137	118
ENV Clade 1-B	224589815	NC_000003.11	3	9890548	123
ENV Clade 1-B	224589815	NC_000003.11	3	101418551	123
ENV Clade 1-B	224589815	NC_000003.11	3	112744301	123
ENV Clade 1-B	224589815	NC_000003.11	3	185281521	123
ENV Clade 1-B	224589815	NC_000003.11	3	125617302	122
ENV Clade 1-B	224589818	NC_000006.11	6	78427839	123
ENV Clade 1-B	224589818	NC_000006.11	6	28659434	123
ENV Clade 1-B	224589818	NC_000006.11	6	151182263	121
ENV Clade 1-B	224589811	NC_000002.11	2	130720716	123
ENV Clade 1-B	224515582	NT_167249.1	6	292	123
ENV Clade 1-B	224515577	NT_113891.2	6	181584	123
ENV Clade 1-B	394726933	NW_003871072.1	11	347637	123
ENV Clade 1-B	224589817	NC_000005.9	5	156085902	123
ENV Clade 1-B	224589817	NC_000005.9	5	30487937	123
ENV Clade 1-B	224589801	NC_000010.10	10	6867326	123
ENV Clade 1-B	224589819	NC_000007.13	7	4623242	123
ENV Clade 1-B	224589819	NC_000007.13	7	4631746	123
ENV Clade 1-B	224589807	NC_000016.9	16	34232845	123
ENV Clade 1-B	224589802	NC_000011.9	11	101573952	123
ENV Clade 1-B	224589802	NC_000011.9	11	62137137	123
ENV Clade 1-B	224589802	NC_000011.9	11	118592908	123
ENV Clade 1-B	224589800	NC_000001.10	1	36955747	123
ENV Clade 1-B	224589800	NC_000001.10	1	75847836	123
ENV Clade 1-B	224589800	NC_000001.10	1	155597642	123
ENV Clade 1-B	224589800	NC_000001.10	1	160668499	123
ENV Clade 1-B	374341059	NW_003315964.2	19	11639	123
ENV Clade 1-B	224589814	NC_000022.10	22	18934058	123

ENV Clade 1-B	224589814	NC_000022.10	22	23888125	123
ENV Clade 2	224589807	NC_000016.9	16	72106260	143
ENV Clade 3-A	224589810	NC_000019.9	19	11996341	136
ENV Clade 3-B	224589818	NC_000006.11	6	35529534	168
ENV MSRV (HERV-W)	224589812	NC_000020.10	20	53969008	164
ENV MSRV (HERV-W)	224589803	NC_000012.11	12	51297128	167
ENV MSRV (HERV-W)	224589803	NC_000012.11	12	105337239	167
ENV MSRV (HERV-W)	224589803	NC_000012.11	12	32429027	165
ENV MSRV (HERV-W)	224589803	NC_000012.11	12	34255312	161
ENV MSRV (HERV-W)	224589803	NC_000012.11	12	132358603	167
ENV MSRV (HERV-W)	224589810	NC_000019.9	19	41493028	161
ENV MSRV (HERV-W)	224589810	NC_000019.9	19	40577979	166
ENV MSRV (HERV-W)	224589810	NC_000019.9	19	21817054	108
ENV MSRV (HERV-W)	224589810	NC_000019.9	19	22934293	167
ENV MSRV (HERV-W)	224589821	NC_000009.11	9	94743779	167
ENV MSRV (HERV-W)	224589816	NC_000004.11	4	83400233	165
ENV MSRV (HERV-W)	224589816	NC_000004.11	4	139543782	166
ENV MSRV (HERV-W)	224589805	NC_000014.8	14	53829140	168
ENV MSRV (HERV-W)	224589805	NC_000014.8	14	26729146	167
ENV MSRV (HERV-W)	303308491	NW_003315956.1	18	157829	167
ENV MSRV (HERV-W)	224589820	NC_000008.10	8	74734257	165
ENV MSRV (HERV-W)	224589820	NC_000008.10	8	49148917	167
ENV MSRV (HERV-W)	224589815	NC_000003.11	3	96386086	167
ENV MSRV (HERV-W)	224589815	NC_000003.11	3	149474665	167
ENV MSRV (HERV-W)	224589815	NC_000003.11	3	141539789	167
ENV MSRV (HERV-W)	224589815	NC_000003.11	3	130916132	167
ENV MSRV (HERV-W)	224589806	NC_000015.9	15	55597499	163
ENV MSRV (HERV-W)	224589818	NC_000006.11	6	13883371	167
ENV MSRV (HERV-W)	224589818	NC_000006.11	6	84159539	166
ENV MSRV (HERV-W)	224589818	NC_000006.11	6	89124328	166
ENV MSRV (HERV-W)	224589811	NC_000002.11	2	176190675	166
ENV MSRV (HERV-W)	224589811	NC_000002.11	2	30742110	167
ENV MSRV (HERV-W)	224589817	NC_000005.9	5	44114141	167
ENV MSRV (HERV-W)	224589801	NC_000010.10	10	62794301	167
ENV MSRV (HERV-W)	224589801	NC_000010.10	10	96594741	167
ENV MSRV (HERV-W)	224589801	NC_000010.10	10	99050439	167
ENV MSRV (HERV-W)	224589819	NC_000007.13	7	149373276	166
ENV MSRV (HERV-W)	224589819	NC_000007.13	7	114019561	167
ENV MSRV (HERV-W)	224589802	NC_000011.9	11	107852228	167
ENV MSRV (HERV-W)	224589802	NC_000011.9	11	117907911	149
ENV MSRV (HERV-W)	224589800	NC_000001.10	1	55377530	167
ENV MSRV (HERV-W)	224589822	NC_000023.10	X	106295769	167
ENV MSRV (HERV-W)	224589822	NC_000023.10	X	65517335	167
ENV MSRV (HERV-W)	224589822	NC_000023.10	X	79218011	167
ENV MSRV (HERV-W)	224589804	NC_000013.10	13	37532540	161