Positive selection of primate *TRIM5α* identifies a critical species-specific retroviral restriction domain

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Primate genomes encode a variety of innate immune strategies to defend themselves against retroviruses. One of these, TRIM5α, can restrict diverse retroviruses in a species-specific manner. Thus, whereas rhesus TRIM5α can strongly restrict HIV-1, human TRIM5α only has weak HIV-1 restriction. The biology of TRIM5α restriction suggests that it is locked in an antagonistic conflict with the proteins encoding the viral capsid. Such antagonistic interactions frequently result in rapid amino acid replacements at the protein–protein interface, as each genetic entity vies for evolutionary dominance. By analyzing its evolutionary history, we find strong evidence for ancient positive selection in the primate *TRIM5α* gene. This selection is strikingly variable with some of the strongest selection occurring in the human lineage. This history suggests that *TRIM5α* evolution has been driven by antagonistic interactions with a wide variety of viruses and endogenous retroviruses that predate the origin of primate lentiviruses. A 13-aa "patch" in the SPRY protein domain bears a dense concentration of positively selected residues, potentially implicating it as an antiviral interface. By using functional studies of chimeric *TRIM5α* genes, we show that this patch is generally essential for retroviral restriction and is responsible for most of the species-specific antiretroviral restriction activity. Our study highlights the power of evolutionary analyses, in which positive selection identifies not only the age of genetic conflict but also the interaction interface where this conflict plays out.

capsid | human endogenous retroviruses | HIV type 1 | SPRY

Innate immune strategies that defend primates against retroviruses are of considerable medical and evolutionary importance. Two distinct cellular antiviral defense systems, APOBEC3G/F (1–3) and TRIM5α (4), that limit HIV infection have been described recently. APOBEC3G and APOBEC3F are cytidine deaminases that can cause hypermutation in the viral genome, but a viral accessory protein, Vif, can counteract their antiviral effect. TRIM5α is a postentry restriction factor that accounts for the resistance to HIV type 1 (HIV-1) observed in rhesus monkey cells. It is not yet known how TRIM5α mediates viral restriction, although a shorter, alternate transcript of the *TRIM5* gene has been shown to be an ubiquitin ligase (5). TRIM5α restriction depends on the viral capsid, and its effect is saturable (4), although direct physical interaction between TRIM5α and capsid has not been demonstrated. TRIM5α from human and nonhuman primates also can restrict other lentiviruses and some strains of murine leukemia virus, a distantly related gammaretrovirus (6–9).

Although host genomes benefit from TRIM5α's recognition of viruses, it is in the best interest of the virus to evade recognition. Such antagonistic interactions have been formalized as the "Red Queen" hypothesis (10) and lead to the rapid fixation of amino acid replacements (positive selection), most likely at the interaction interface. The history of positive selection is thus informative for determining how long genes have been participants in genetic conflict, for identifying the likely sources of this conflict, and even for defining interaction domains involved. We previously have performed such an analysis on the *APOBEC* genes to show that APOBEC3G's role in genome defense predates the origin of primate lentiviruses (11, 12) and that many other *APOBEC* cytidine deaminase genes likely participate in defending the primate genome against retroviruses.

Here, we show that the *TRIM5α* restriction factor has undergone multiple episodes of positive selection that predate the origin of primate lentiviruses. Selection pressures on *TRIM5α* vary widely among primate lineages, suggesting that distinct episodes of retroviral infection have dominated *TRIM5α* evolution. The positive selection in *TRIM5α* appears to be concentrated largely in the α-isoform-specific SPRY domain. We use this concentration of positively selected residues to propose and validate the presence of a SPRY "patch" that is essential for TRIM5α's general and species-specific restriction of retroviruses.

Materials and Methods

Primate Genomic DNA Sources. Genomic DNA samples obtained from Coriell Cell Repositories (Camden, NJ) are as follows (including Coriell repository numbers): *Pan troglodytes* (chimpanzee, NAO3448A), *Gorilla gorilla* (gorilla, NG05251B), *Pongo pygmaeus* (orangutan, NAO4272), *Erythrocebus patas* (patas monkey, NG06254), *Lagothrix lagotricha* (common woolly monkey, NG05356), *Ateles geoffroyi* (black-handed spider monkey, NGO5352), and *Saguinus labiatus* (red-chested mustached tamarin, NG05308). Genomic DNA from *Hylobates syndactylus* (island siamang, KB11539), *Colobus guereza kikuyuensis* (kikuyu colobus, OR160), *Pygathrix nemaeus* (douc langur, OR259), *Callithrix pygmaea* (pygmy marmoset, OR690), *Saimiri sciureus sciureus* (squirrel monkey, KB4544), *Callicebus donacophilus donacophilus* (Bolivian gray titi, OR1522), *Pithecia pithecia pithecia* (white-faced saki, KB5932), and *Alouatta sara* (Bolivian red howler, OR749) was obtained from the Center for Reproduction of Endangered Species FrozenZoo Project (San Diego Zoo, San Diego). *Papio anubis* (baboon) DNA was a personal gift from Trent Colbert (Fred Hutchinson Cancer Research Center). *Cercopithecus aethiops* (African green monkey) genomic DNA was prepared from Cos-7 cells with the QIAamp-DNA kit (Qiagen, Valencia, CA).

Sequencing of *TRIM5α* Exons from Primate Genomic DNA. *TRIM5α* was amplified and sequenced exon by exon from genomic DNA with PCR Supermix High Fidelity (Invitrogen) by using the PCR and sequencing primers shown in Table 2, which is published as supporting information on the PNAS web site. PCR products were sequenced directly, except in a few cases where they were first cloned into the TOPO TA cloning vector (Invitrogen), followed by sequencing of three independent clones. Exon reads

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Abbreviations: dS, synonymous changes per site; dN, replacement changes per site; HIV-1, HIV type 1; NWM, New World monkey; OWM, Old World monkey.

Data deposition: All sequences reported in this paper have been deposited in the GenBank database (accession nos. AY843504–AY843520).

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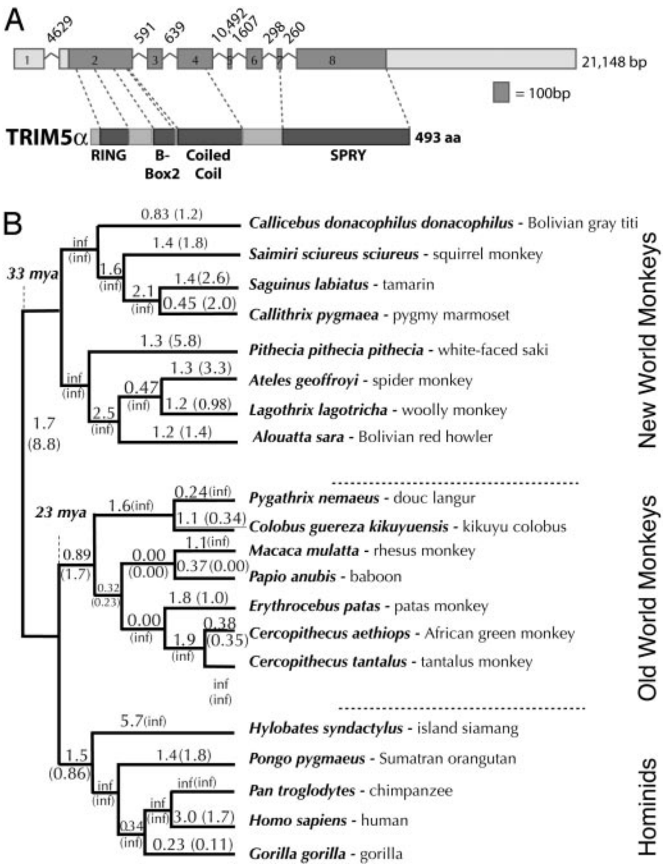

Fig. 1. Ancient and variable positive selection has shaped *TRIM5α* evolution. (*A*) TRIM5α is the longest of six reported transcripts of the eight-exon human *TRIM5* gene. It encodes a 493-aa protein consisting of a RING finger, a B-Box2, and a coiled-coil domain (signature domains of the TRIM family; ref. 24), as well as an α-isoform-specific SPRY domain. (*B*) *TRIM5α* was sequenced from a panel of primates representing 33 million years of evolutionary divergence. Values of dN/dS along each branch were calculated by using the free-ratio model of PAML for either the whole gene or for the SPRY domain alone (in parentheses), as shown on a cladogram of the accepted primate phylogeny (38). A dN/dS value of >1 suggests that positive selection has acted along that lineage. inf refers to cases where dS = 0.

were spliced together to create virtual transcripts for each primate, and have been entered into the GenBank database under the accession nos. AY843504–AY843520. The *TRIM5α* cDNA sequences for human (AY625000), *Macaca mulatta* (Rhesus macaque, AY523632.1), and *Cercopithecus tantalus* (tantalus monkey, AY593973.2) were obtained from the GenBank database. A phylogeny constructed by using the isolated *TRIM5α* genes is in good agreement with the accepted primate phylogeny, indicating that all sequences isolated by our PCR strategy are truly orthologous.

Confirmation of *TRIM5* Transcripts by Sequencing from RNA. The following monkey fibroblast cell lines were obtained from Coriell: *Ateles geoffroyi* (AG05352), *Lagothrix lagotricha* (AG05356), *Callicebus moloch* (AG06115A), and *Saguinus labiatus* (AG05308A). RNA was prepared from ≈1 million cells with the RNeasy kit (Qiagen). RT-PCR of the *TRIM5α* SPRY domain was performed with the primers shown in Table 2, by using the SuperScript One-Step kit (Invitrogen). PCR products were sequenced directly.

Sequence Analysis. DNA sequences were aligned by using CLUSTAL X (13), with hand alignment of small indels based on amino acid sequence. Maximum likelihood analysis was performed with CODEML in the PAML 3.14 software package (14). Global synonymous changes per site (dS)/replacement changes per site (dN) ratios for the tree (Fig. 1) were calculated by a free-ratio model, which allows dN/dS to vary along different branches. We tested whether dN/dS values were >1 in two lineages, those leading to gibbon and human, by using two methods as described in ref. 15 (see Appendix 1, which is published as supporting information on the PNAS web site). Briefly, likelihoods were compared when the lineage was fixed at dN/dS = 1 relative to when the lineage was allowed to have a dN/dS >1 (16). In the second method, we calculated the dN/dS ratios relative to the reconstructed ancestor and tested for significant deviations from dN/dS = 1 (16).

To detect selection in *TRIM5α*, multiple alignments were fitted to either the F3 × 4 or F61 models of codon frequencies. Likelihood ratio tests of the data were performed by using different sets of site-specific (NS sites) models as follows: M0 (one-ratio) to M3 (discrete); M1 (two-state, neutral, dN/dS > 1 disallowed) to M2 (selection, similar to model 1 but dN/dS > 1 allowed); and M7 (fit to a beta distribution, dN/dS > 1 disallowed) to M8 (similar to model 7 but dN/dS > 1 allowed). In all cases, permitting sites to evolve under positive selection gave a much better fit to the data (Table 1). These analyses also identified certain amino acid residues with high posterior probabilities (>0.95) of having evolved under positive selection (17, 18) (Fig. 2; see also Appendix 1).

Secondary structure predictions and associated confidence values for the entire human TRIM5α protein were made by using the PSIPRED (19) Protein Structure Prediction Server (http://bioinf.cs.ucl.ac.uk/psipred).

Table 1. Positive selection in the *TRIM5α* gene

Data set	−2(ln λ)	df	*P* value	dN/dS	Proportion of sites, %
All primates					
M0 vs. M3 ($k = 3$)	217.94	4	$P < 0.0001$	2.4, 7.2	33, 4
M1 vs. M2	109.88	2	$P < 0.0001$	4.5	16
M7 vs. M8	109.50	2	$P < 0.0001$	4.2	18
Hominids + OWMs					
M0 vs. M3 ($k = 3$)	57.42	4	$P < 0.0001$	1.8, 17	57, 2
M1 vs. M2	39.16	2	$P < 0.0001$	10	4
M7 vs. M8	40.21	2	$P < 0.0001$	10	4

We used likelihood ratio tests to determine whether any codon positions were associated with dN/dS significantly > 1 and hence possibly subject to positive Darwinian selection. Neutral models (M0, M1, and M7) were compared to selection models (M2 and M8), which allow a proportion of codons for which dN/dS exceeds 1, or models for heterogeneity of dN/dS among sites (M3). As indicated by the *P* values, all analyses find very strong evidence for the selection model. Note that in M3, two classes of sites are permitted to have dN/dS > 1. Also indicated are the proportion of codons that were found to have dN/dS > 1, with the associated dN/dS values shown. Analyses using the f61 model of codon frequencies are shown, but similar results were obtained by using the f3×4 frequency model (full PAML results are available in Appendix 1).

Viral Infection Assays. CRFK (feline renal fibroblast) cells were obtained from the American Type Culture Collection and grown in DMEM/10% FCS. The human and rhesus *TRIM5α* alleles with a hemagglutinin epitope tag (4) in the LPCX retroviral vector were obtained from the National Institutes of Health AIDS Reagent Program (donated by Joseph Sodroski). Site-directed mutagenesis of the rhesus or human genes was performed by the QuikChange kit (Stratagene) in a single step (oligonucleotide sequences available on request). Retroviral vectors were produced in 293T cells, and supernatants were used to infect CRFK cells. Twenty-four hours after infection, 3 mg/ml

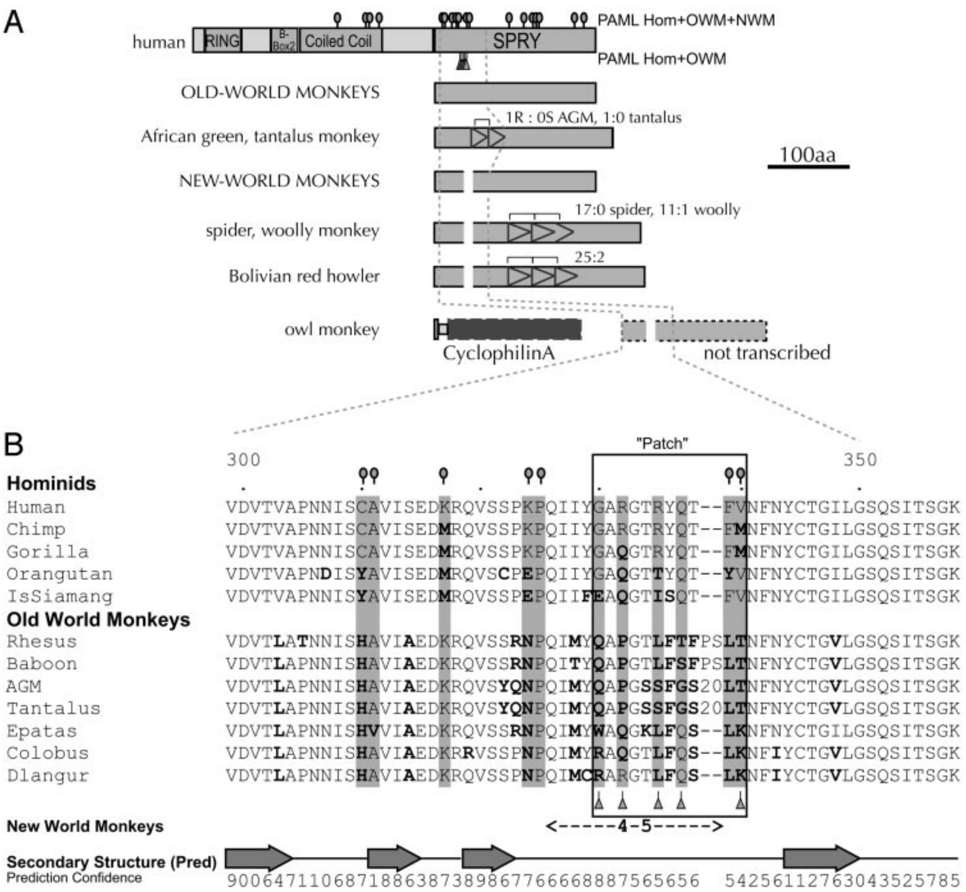

Fig. 2. The SPRY domain is a hot spot for insertions/deletions and positive selection. (*A*) Codons highlighted by PAML as being subject to positive selection with high posterior probabilities ($P > 0.95$) in an analysis of all primates are indicated as circles on stems above the TRIM5α protein schematic. In a more limited analysis of just the hominids and Old World monkeys (OWMs), only five residues in the entire protein were identified as evolving under positive selection (triangles on stems below the TRIM5α schematic). Representative SPRY domains from OWMs and New World monkeys are schematized, along with notable exceptions that have internal duplications (indicated by tandem arrowheads). These duplications appear to have accumulated more replacement (R) changes than synonymous (S) changes (R:S ratios indicated above). In owl monkeys, a CyclophilinA insertion (*TRIM-Cyp*) occurred between exons 7 and 8 (26), and it is believed that the 8th exon of *TRIM5* containing the SPRY domain is not transcribed. (*B*) Codons identified as being under positive selection are indicated in gray background and by using the same symbols as in *A*. Changes relative to human are indicated in bold. NWMs have a large deletion in the area of the patch (see Data Set 1, which is published as supporting information on the PNAS web site). Secondary structure predictions and confidence values (0, low; 9,high) were made by using the PSIPRED server (19). Arrows indicate β-strands, and lines indicate coils. According to this prediction, most of the TRIM5α protein is predicted to be comprised of α-helices, whereas the entire SPRY domain is predicted with high confidence to be β-strands and coils with no α-helices.

puromycin was added to the medium to select for transduced cells to obtain pools of cells that stably expressed exogenous *TRIM5α* genes. Expression was verified by Western blotting using a hemagglutinin antibody (Babco, Richmond, CA).

Single-cycle assays for HIV-1 were performed essentially as described in ref. 20. HIV-1 provirus was deleted for *env*, and GFP or luciferase was inserted into the *nef* region (20). SIVagmTAN virus (21) was a kind gift of Ned Landau (The Salk Institute, San Diego). Virus was produced in 293T cells by cotransfection of the provirus with the VSV-G gene, titrated on cells without restriction factors, and frozen. Infection of CRFK cells with HIV-1 (GFP) was performed in 24-well plates with 3×10^4 cells per well by using 5-fold dilutions of virus that had been titrated previously to give between 1% and 80% infection. Two days after infection, the cells were fixed with 2% paraformaldehyde and analyzed by flow cytometry for GFP expression. Luciferase assays were performed with virus stocks that had been titrated previously to be in the linear range of the assay (between 10,000 and 1 million relative light units). Infections were performed in triplicate in 24-well plates, assayed with the luciferase assay kit (Promega) in a 96-well plate, and read on a luminometer.

Results

We sequenced the *TRIM5α* gene (≈1,482 bp of coding sequence) from 17 primate genomes that represent 33 million years of evolution (Fig. 1). Comparison of the rates of nonsynonymous (that alter the encoded amino acid) and synonymous DNA changes between species can be used to assess the types of selective pressures that have acted on a gene (22). For most protein-coding genes, dS exceeds dN because amino acid replacements are generally detrimental to protein function and therefore are culled out of the population (purifying selection). We find that many branches of the primate phylogeny, including internal branches, show evidence for *TRIM5α* evolution under positive selection (defined as $dN/dS > 1.0$; Fig. 1*B*). Thus, *TRIM5α* has been subject to positive selection for at least 33 million years. This selection has been strikingly variable because dN/dS ratios along different branches are significantly different from each other ($P < 0.02$; Appendix 1). In contrast, the positive selection on *APOBEC3G* was found to be more constant (11, 12) because ω values along each branch did not differ significantly from each other ($P > 0.75$; Appendix 1). Positive selection of *TRIM5α* is especially strong in the hominid clade, with the highest whole-gene dN/dS values of 5.7 and 3.0 found in the lineages leading to island siamang (gibbon) and human, respectively. We tested for the presence of positive selection in the gibbon and human branches by both comparative two-ratio likelihood tests by using PAML (15) and Monte-Carlo simulation using K-ESTIMATOR (16) (see *Materials and Methods* and Appendix 1). The average dS in *TRIM5α* is not unusually low; it is 0.084 between hominids and Old World monkeys (OWMs) and 0.153 between hominids and New World monkeys (NWMs), compared with previous estimates of 0.08 and 0.15, respectively, for substitution rates in various intronic and noncoding regions of primate genomes (23). Thus, we can rule out the possibility that selection has led to deflated dS values in *TRIM5α*, resulting in artificially high dN/dS ratios.

TRIM5 is a member of the large tripartite motif family in primate genomes, characterized by having RING finger, B-box, and coiled-coil domains (24). The α isoform of *TRIM5* has an additional SPRY protein domain (Fig. 1*A*), which is found in many proteins including those in the Ig superfamily (25). This SPRY domain has been shown previously to be essential for the restriction of HIV-1 (4). Although little is known about SPRY function, we found that this domain had undergone the most intense positive selection. This finding is evidenced by the high dN/dS values obtained in an analysis of this domain alone (in parenthesis on cladogram, Fig. 1*B*), including a striking dN/dS of 8.8 in the branch separating NWM from OWM and hominids (see also Fig. 4, which is published as supporting information on the PNAS web site). In addition to the strong positive selection, the SPRY domain has undergone an unusual number of insertions and deletions (Fig. 2*A*). A small deletion has occurred in the lineage leading to the NWMs, whereas there have been two distinct instances of internal duplications, one in African green monkeys and close relatives and a different triplication in the lineage leading to spider, woolly, and howler monkeys. The African green monkeys and tantalus duplication has been verified in *TRIM5α* transcripts (GenBank accession nos. AY625003 and AY593973). We confirmed the woolly and spider monkey triplications by sequencing RT-PCR products (data not shown). When the duplicated and triplicated sequences within a single gene were aligned to each other, they showed an inflated number of nonsynonymous to synonymous changes (e.g., 25 replacement:2 synonymous changes for howler monkey; Fig. 2*A*). Thus, internal duplications in the SPRY domain followed by positive selection predict that these sequences are functionally important for the ability of TRIM5α to restrict different viruses. Finally, in owl monkey, the entire SPRY domain of TRIM5α has been replaced by a retrotransposed *CyclophilinA* gene, now referred to as *TRIM-Cyp* (26). CyclophilinA has been reported to directly interact with viral capsid (27), and, because the SPRY domain can be functionally replaced by CyclophilinA, this finding suggests that the SPRY also might be a capsid-interacting domain.

We used a maximum-likelihood approach, by using the PAML suite of programs (14), to determine whether specific codons of *TRIM5α* have been repeatedly subjected to positive selection in primates. Statistical results support the presence of positive selection with extraordinary confidence, regardless of whether we include or exclude NWMs in our analysis (Table 1). Analysis of the full data set identifies ≈18% of the codons as having evolved under positive selection with an average dN/dS ratio of >4 (Table 1, NSsites model 8 vs. 7). Of these, residues identified as being under positive selection with high confidence (>95% posterior probability) are illustrated on a schematic of the TRIM5α protein (circles on stems, Fig. 2) and fall in either the coiled-coil or SPRY domains. Some TRIM proteins have been shown to homomultimerize through their coiled-coil domains (24), but our results also suggest that the coiled-coil domain of TRIM5α may additionally participate in host defense. A secondary structure prediction of the SPRY domain suggests that the positively selected residues and insertions/deletions fall exclusively in predicted coils (Fig. 2*B*), which could represent specific interaction surfaces.

Because there are NWM-specific deletions in *TRIM5α*, excluding NWMs allows the opportunity to analyze all residues in hominids and OWMs, a more focused look at just the last 23 million years of primate phylogeny. Remarkably, the analysis of hominids and OWMs identified only five residues in the entire protein as being under positive selection with high confidence (triangles on stems, Fig. 2*A*). All five residues fall within an 11- to 13-aa segment of the SPRY domain (Fig. 2*B*), which we will refer to as the SPRY patch. Such tight clusters of positive selection are predicted to be points of physical contact between two proteins locked in genetic conflict. Similar PAML analyses have successfully highlighted the known binding surface between ZP3, an egg-receptor protein, and sperm (28). Additionally, the positive selection in the major histocompatibility complex (MHC) proteins is confined to small segments of the protein known to constitute the antigen-recognition site (29). In this case, we had no *a priori* knowledge of the TRIM5α interaction interface crucial for viral restriction, so we tested our computational prediction that the SPRY patch identifies such a domain. Because TRIM5α was originally identified because of the fact that it confers resistance to HIV-1 infection in rhesus, but not in human, cells (4) we used this species-specific example of TRIM5α restriction to investigate the functional importance of the patch.

We constructed chimeric proteins between human and rhesus *TRIM5α* that either differed only in the 11- to 13-aa patch region or had the patch region deleted altogether (Fig. 3*A*). CRFK cells (feline cells that have no known retroviral restriction) were stably transduced to express these proteins at approximately similar levels (Fig. 3*B*) and were tested for restriction of increasing titer of a recombinant HIV-1 virus that expresses GFP (20). As has been previously reported (4), human TRIM5α showed a weak restriction of HIV-1, whereas rhesus TRIM5α almost completely blocked infection (Fig. 3*C*). When the patch is deleted from rhesus TRIM5α (rhesus DEL), it loses restriction not only against HIV-1 (Fig. 3*C*) but also against SIV from African green monkeys and N-tropic murine leukemia virus (Fig. 3*E* and data not shown). Thus, the patch we have identified based on positive selection may define a protein surface that is generally necessary for broad retroviral restriction.

We next asked whether the patch is responsible for the species specificity of TRIM5α by substituting the human patch into rhesus TRIM5α and vice versa. Remarkably, we found that substitution of the human patch into the rhesus TRIM5α (rhesus SWAP) reduces its restrictive capability to a level that is close to that of human TRIM5α (Fig. 3*C*). Thus, the patch we have defined by using positive selection identifies a region necessary for species-specific restriction to HIV-1. We then tested the reciprocal chimera, human TRIM5α with the rhesus TRIM5α patch (human SWAP, Fig. 3*D*). The Human SWAP was significantly more restrictive against HIV-1 than human TRIM5α. Despite this gain-of-function effect, swapping the patch alone was not completely sufficient to recapitulate rhesus TRIM5α-like restriction, suggesting that other minor determinants of restriction also may exist.

We wanted to know whether the patterns seen with these chimeric proteins are specific to HIV-1 or are generally responsible for defining broad species-specific ability to restrict lentiviruses. To address this question, we challenged the TRIM5α-expressing CRFK cell lines with SIVagm and found that the reversal of species-specific restriction is the same as was observed for HIV-1 (Fig. 3*E*). Thus, human TRIM5α does not usually restrict SIVagm, but can acquire the ability to restrict SIVagm when the 13-aa patch from rhesus TRIM5α is added (human SWAP in Fig. 3*E*). Conversely, rhesus TRIM5α loses the ability to restrict SIVagm when its patch is replaced by the 11-aa patch from human TRIM5α (rhesus SWAP in Fig. 3*E*). Thus, the patch we have identified through positive selection determines the species-specificity of TRIM5α for restricting at least two highly divergent lentiviruses.

Discussion

Positive selection is a beacon for domains involved in genetic conflict. Based on analysis of positively selected codons, we were able to predict an important protein domain (potentially an interaction surface) that we then verified *in vivo*. In addition, we have illustrated strong positive selection of the *TRIM5α* gene throughout primate evolution. The ancient and intense selective pressures that have shaped the evolution of *APOBEC3G* (11) and *TRIM5α* indicate that the innate immune system is intricately evolved, just as the adaptive immune system is known to be (30). The tight clustering of positively selected residues in *TRIM5α* is in stark contrast to our earlier findings in *APOBEC3G*, where the codons that were identified as having been repeatedly subject to positive selection were scattered throughout the length of the gene, with no significant clustering (11). There are two possible reasons for this difference. First, the TRIM5α protein has a nonredundant domain structure, whereas APOBEC3G has resulted from two sequential duplications of a single cytidine deaminase domain to result in four potentially redundant structural copies of this domain (31, 32). Second, TRIM5α and APOBEC3G play different roles in their respective conflicts. Although it is in the host genome's best interest that TRIM5α improve binding to its target, it is in the host's interest that APOBEC3G avoid interactions with viral antagonists such as Vif in the case of HIV-1. Therefore, unlike TRIM5α, APOBEC3G is under pressure to mutate whichever domain is the current viral target.

What drives the evolution of *TRIM5α*? The antiquity of the positive selection rules out primate lentiviruses like HIV-1 as being the sole, or even the major, cause because they are believed to be <1 million years old (33). In addition, TRIM5α from human and OWM has been shown to be active against murine leukemia virus (6–9), a gammaretrovirus that is closely related to human endogenous retroviruses (34) that have episodically invaded primate genomes and continue to be active in the human genome (35–37). This finding suggests that *TRIM5α* evolution may have been strongly influenced by distinct episodes of endogenous retrovirus infection and subsequent retrotransposition events (36). HIV and other primate lentiviruses are likely to be newcomers to this conflict, with the OWM TRIM5α restriction against HIV-1 just an evolutionary coincidence.

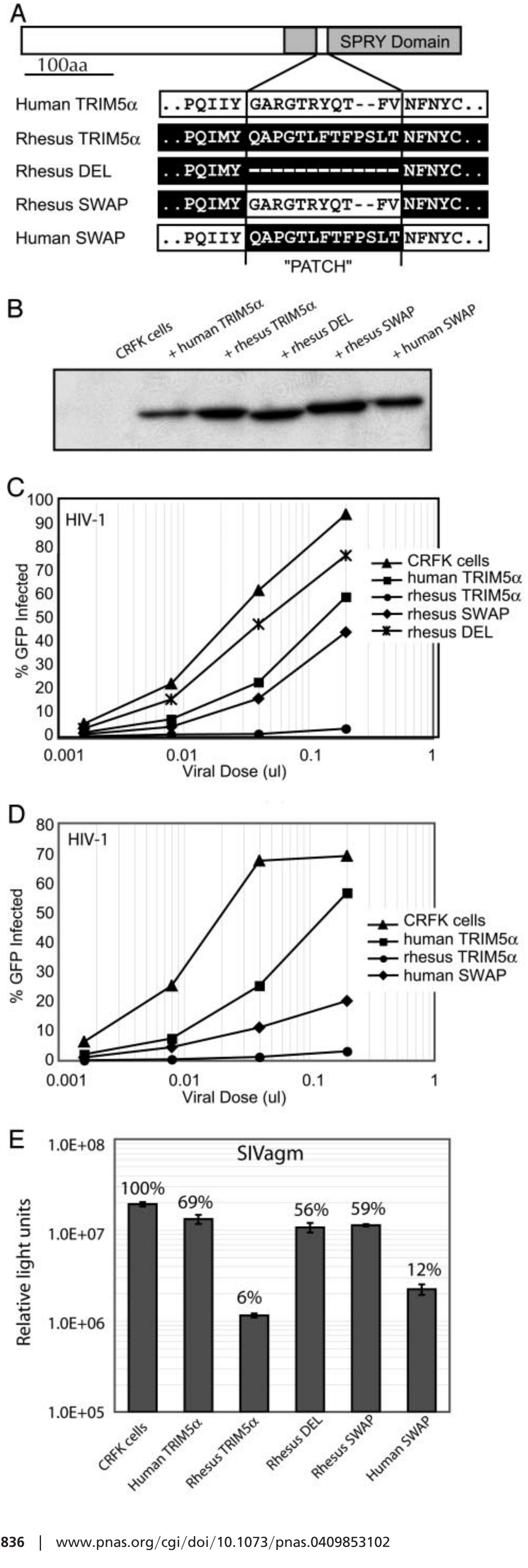

Fig. 3. The positively selected patch is critical for the species-specific retroviral restriction by TRIM5α. (*A*) Retroviral vectors (LPCX) containing hemagglutinin-tagged *TRIM5α* alleles from human and rhesus genomes were modified to delete the rhesus SPRY patch (Rhesus DEL), swap the human patch into rhesus TRIM5α (Rhesus SWAP), or swap the rhesus patch into the human TRIM5α (Human SWAP). The sequences in the region of the swaps are shown. (*B*) A Western blot (using a hemagglutinin antibody) shows stable CRFK lines expressing each construct at roughly equivalent levels. (*C*) Variants of the rhesus TRIM5α protein were assessed for their ability to restrict HIV-1. Single-cycle assays for HIV-1 infectivity were carried out in CRFK (feline renal fibroblasts) cells expressing exogenous *TRIM5α* genes. Experiments were performed by using 5-fold dilutions of an HIV-1 provirus that was deleted for *env* and where GFP was inserted into the *nef* region (20). HIV-positive cells were detected by flow cytometry for GFP expression. (*D*) A variant of the human TRIM5α (human SWAP) was tested in single-cycle assays for HIV-1 infectivity as in *C*. (*E*) CRFK cells that express the *TRIM5α* alleles were challenged with SIVagm virus, which expresses *luciferase* in place of the *nef* gene. Preliminary experiments were performed to establish a dose of virus within the linear range of infection (data not shown). All infections were performed in triplicate, and luciferase activity was measured 2 days after infection. The relative light units are shown on the *y*-axis, and the percent infection (compared with the CRFK control) is shown above the bar. SD of the data also are shown.

Nevertheless, as we have shown, the positive selection has had a profound impact on the species-specific restriction of both HIV-1 and SIVagm. Thus, the evolutionary histories of both *TRIM5* and *APOBEC3G* indicate ancient adaptation to endogenous retrovirus-like elements (11, 12), yet both restriction systems were discovered because of their incidental activity against HIV. These findings indicate that the cellular arsenal honed against endogenous retroviruses is large and mostly undiscovered and may strongly impact lentiviral restriction.

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1. Sheehy, A. M., Gaddis, N. C., Choi, J. D. & Malim, M. H. (2002) *Nature* **418,** 646–650.
2. Wiegand, H. L., Doehle, B. P., Bogerd, H. P. & Cullen, B. R. (2004) *EMBO J.* **23,** 2451–2458.
3. Zheng, Y.-H., Irwin, D., Kurosu, T., Tokunaga, K., Sata, T. & Peterlin, B. M. (2004) *J. Virol.* **78,** 6073–6076.
4. Stremlau, M., Owens, C. M., Perron, M. J., Kiessling, M., Autissier, P. & Sodroski, J. (2004) *Nature* **427,** 848–853.
5. Xu, L., Yang, L., Moitra, P. K., Hashimoto, K., Rallabhandi, P., Kaul, S., Meroni, G., Jensen, J. P., Weissman, A. M. & D'Arpa, P. (2003) *Exp. Cell Res.* **288,** 84–93.
6. Yap, M. W., Nisole, S., Lynch, C. & Stoye, J. P. (2004) *Proc. Natl. Acad. Sci. USA* **101,** 10786–10791.
7. Hatziioannou, T., Perez-Caballero, D., Yang, A., Cowan, S. & Bieniasz, P. D. (2004) *Proc. Natl. Acad. Sci. USA* **101,** 10774–10779.
8. Keckesova, Z., Ylinen, L. M. & Towers, G. J. (2004) *Proc. Natl. Acad. Sci. USA* **101,** 10780–10775.
9. Perron, M. J., Stremlau, M., Song, B., Ulm, W., Mulligan, R. C. & Sodroski, J. (2004) *Proc. Natl. Acad. Sci. USA* **101,** 11827–11832.
10. Van Valen, L. (1973) *Evol. Theory* **1,** 1–30.
11. Sawyer, S. L., Emerman, M. & Malik, H. S. (2004) *PLoS Biol.* **2,** 1278–1285.
12. Zhang, J. & Webb, D. M. (2004) *Hum. Mol. Genet.* **13,** 1785–1791.
13. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997) *Nucleic Acids Res.* **25,** 4876–4882.
14. Yang, Z. (1997) *Comput. Appl. Biosci.* **13,** 555–556.
15. Yang, Z. (1998) *Mol. Biol. Evol.* **15,** 568–573.
16. Comeron, J. M. (1999) *Bioinformatics* **15,** 763–764.
17. Nielsen, R. & Yang, Z. (1998) *Genetics* **148,** 929–936.
18. Yang, Z. & Bielawski, J. P. (2000) *Trends Ecol. Evol.* **15,** 496–503.
19. McGuffin, L. J., Bryson, K. & Jones, D. T. (2000) *Bioinformatics* **16,** 404–405.
20. Yamashita, M. & Emerman, M. (2004) *J. Virol.* **78,** 5670–5678.
21. Mariani, R., Chen, D., Schrofelbauer, B., Navarro, F., Konig, R., Bollman, B., Munk, C., Nymark-McMahon, H. & Landau, N. R. (2003) *Cell* **114,** 21–31.
22. Hurst, L. D. (2002) *Trends Genet.* **18,** 486–487.
23. Li, W.-H. (1997) *Molecular Evolution* (Sinauer, Sunderland, MA).
24. Reymond, A., Meroni, G., Fantozzi, A., Merla, G., Cairo, S., Luzi, L., Riganelli, D., Zanaria, E., Messali, S., Cainarca, S., *et al.* (2001) *EMBO J.* **20,** 2140–2151.
25. Henry, J., Mather, I. H., McDermott, M. F. & Pontarotti, P. (1998) *Mol. Biol. Evol.* **15,** 1696–1705.
26. Sayah, D. M., Sokolskaja, E., Berthoux, L. & Luban, J. (2004) *Nature* **430,** 569–573.
27. Luban, J., Bossolt, K. L., Franke, E. K., Kalpana, G. V. & Goff, S. P. (1993) *Cell* **73,** 1067–1078.
28. Swanson, W. J., Yang, Z., Wolfner, M. F. & Aquadro, C. F. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 2509–2514.
29. Hughes, A. L. & Nei, M. (1988) *Nature* **335,** 167–170.
30. Holmes, E. C. (2004) *PLoS Biol.* **2,** 1267–1269.
31. Jarmuz, A., Chester, A., Bayliss, J., Gisbourne, J., Dunham, I., Scott, J. & Navaratnam, N. (2002) *Genomics* **79,** 285–296.
32. Wedekind, J. E., Dance, G. S. C., Sowden, M. P. & Smith, H. C. (2003) *Trends Genet.* **19,** 207–216.
33. Sharp, P. M., Bailes, E., Robertson, D. L., Gao, F. & Hahn, B. H. (1999) *Biol. Bull.* **196,** 338–342.
34. Andersson, M. L., Lindeskog, M., Medstrand, P., Westley, B., May, F. & Blomberg, J. (1999) *J. Gen. Virol.* **80,** 255–260.
35. Berkhout, B., Jebbink, M. & Zsiros, J. (1999) *J. Virol.* **73,** 2365–2375.
36. Smit, A. F. (1999) *Curr. Opin. Genet. Dev.* **9,** 657–663.
37. Belshaw, R., Pereira, V., Katzourakis, A., Talbot, G., Paces, J., Burt, A. & Tristem, M. (2004) *Proc. Natl. Acad. Sci. USA* **101,** 4894–4899.
38. Purvis, A. (1995) *Philos. Trans. Biol. Sci.* **348,** 405–421.

Appendix 1. PAML summary results for TRIM5α

A. PAML (1) Analysis of Entire Data Set (hominids+OWM+NWM)

1. Log Likelihood Scores and Parameter Estimates for Four Models of Variable ω's Among Sites Assuming the F3x4 Model of Codon Frequencies.

Model	Parameter Estimates	Sites* with ω > 1	ℓ
M0: one ratio	Average dN/dS for each branch = 1.160 S = 1.55260		–5772.38
Site models			
M1: neutral	(ω_0 = 0) f_0= 0.354	Not allowed	–5,725.00
	(ω_1 = 1) (f_1 = 0. 646)		
	Average dN/dS for each branch = 0.646 S = 1.56259		
M2: selection	(ω_0 = 0) f_0 = 0.281 (ω_1 = 1) f_1 = 0.566 **ω_2 = 4.624 (f_2 = 0.153)** Average dN/dS for each branch = 1.273 S = 1.62074	**(47–51: gaps)** 175 Q 0.9958** 213 S 0.9635* 215 T 0.9955** 228 L 0.9942** 310 C 0.9924** 311 A 0.9603* 317 K 0.9757* 324 K 0.9995** 325 P 0.9933** **(326-338: gaps)** 339 F 0.9994** 340 V 0.9999** **(385-386: gaps)** 389 K 0.9953** 407 G 0.9974** 418 F 0.9997** 421 P 0.9769* 423 V 0.9633* 471 Q 0.9535*	–5,667.01

Model	Parameter Estimates	Sites* with $\omega > 1$	ℓ
		483 G 0.9936**	
M3:discrete	$\omega_0 = 0.342$ $f_0 = 0.662$ **$\omega_1 = 2.575$ ($f_1 = 0.305$)** **$\omega_2 = 7.922$ ($f_2 = 0.033$)** Average dN/dS for each branch = 1.273 S = 1.63377	Too many to list. Note: M3 vs. M0 is a test of heterogeneity among sites and not an explicit test of positive selection.	–5,664.25
M7:β	$p = 0.02830$ $q = 0.01638$ Average dN/dS for each branch = 0.6160 S = 1.55777	Not allowed	–5,724.62
M8:β and ω	$p = 0.2299$ $q = 0.14801$ $f_0 = 0.829$ **$\omega_1 = 4.308$ ($f_1 = 0.171$)** Average dN/dS for each branch = 1.2426 S = 1.62038	**(47-51: gaps)** 139 Q 0.9534* 175 Q 0.9967** 213 S 0.9739* 215 T 0.9966** 228 L 0.9958** 257 T 0.9511* 310 C 0.9943** 311 A 0.9717* 317 K 0.9830* 324 K 0.9996** 325 P 0.9946** **(326-338: gaps)** 339 F 0.9995** 340 V 0.9999** 384 D 0.9539* **(385-386: gaps)** 389 K 0.9964** 390 N 0.9611* 407 G 0.9979** 418 F 0.9998** 421 P 0.9830* 423 V 0.9737*	–5,666.71

Model	Parameter Estimates	Sites* with ω > 1	ℓ
		471 Q 0.9670* 483 G 0.9952**	

p and *q* are parameters of the β distribution. *f* is the proportion of sites assigned to an individual ω (= dN/dS) category or to a β distribution with shape parameters *p* and *q*. The proportion $f_{1,2}$ in parentheses is not a free parameter. Sites assigned to $\omega_{1,2}$ are those with posterior probabilities (P) > 0.95 , and those with $P > 0.99$ are in bold. Analyses were conducted using κ as a free parameter. S = tree length.

*Sites assigned to $\omega > 1$ are those with posterior probabilities $P > 0.95$ (*) or $P > 0.99$ (**).

2. Log Likelihood Scores and Parameter Estimates for Four Models of Variable ω's Among Sites Assuming the F61 Model of Codon Frequencies.

Model	Parameter estimates	Sites* with ω > 1	ℓ
M0: one ratio	Average dN/dS for each branch = 1.144 S = 1.55005		–5,748.06
Site models			
M1: neutral	$(\omega_0 = 0)\ f_0 = 0.359$	Not allowed	–5,696.78
	$(\omega_1 = 1)\ (f_1 = 0.641)$		
	Average dN/dS for each branch = 0.641 S = 1.56922		
M2: selection	$(\omega_0 = 0)\ f_0 = 0.291$ $(\omega_1 = 1)\ f_1 = 0.551$ **$\omega_2 = 4.451$ ($f_2 = 0.158$)** Average dN/dS for each branch = 1.2545 S = 1.62398	**(47-51: gaps)** 175 Q 0.9954** 213 S 0.9544* 215 T 0.9970** 228 L 0.9957** 310 C 0.9930** 311 A 0.9669* 317 K 0.9736* 324 K 0.9994** 325 P 0.9903**	–5,641.84

Model	Parameter estimates	Sites* with $\omega > 1$	ℓ
		(326-338: gaps) 339 F 0.9987** 340 V 0.9999** **(385-386: gaps)** 389 K 0.9935** 407 G 0.9958** 411 S 0.9536* 418 F 0.9994** 421 P 0.9784* 423 V 0.9680* 483 G 0.9909**	
M3:discrete	$\omega_0 = 0.301$ $f_0 = 0.631$ **$\omega_1 = 2.379$ ($f_1 = 0.328$)** **$\omega_2 = 7.224$ ($f_2 = 0.041$)** Average dN/dS for each branch = 1.2692 S = 1.63693	Too many to list. Note: M3 vs. M0 is a test of heterogeneity among sites and not an explicit test of positive selection.	–5,639.09
M7:β	$p = 0.029$ $q = 0.0167$	Not allowed	–5,696.39
	Average dN/dS for each branch = 0.6144 S = 1.56601		
M8:β and ω	$p = 0.16877$ $q = 0.11056$ $f_0 = 0.825$ **$\omega_1 = 4.190$ ($f_1 = 0.175$)** Average dN/dS for each branch = 1.2331 S = 1.62300	**(47-51: gaps)** 139 Q 0.9532* 175 Q 0.9963** 213 S 0.9659* 215 T 0.9975** 228 L 0.9967** 257 T 0.9607* 310 C 0.9945** 311 A 0.9749* 317 K 0.9805* 324 K 0.9995** 325 P 0.9920** **(326-338: gaps)** 339 F 0.9989** 340 V 0.9999** **(385-386: gaps)**	–5,641.64

Model	Parameter estimates	Sites* with ω > 1	ℓ
		389 K 0.9949** 390 N 0.9587* 407 G 0.9966** 411 S 0.9653* 418 F 0.9995** 421 P 0.9832* 423 V 0.9756* 471 Q 0.9570* 483 G 0.9929**	

p and *q* are parameters of the β distribution. *f* is the proportion of sites assigned to an individual ω (= dN/dS) category or to a β distribution with shape parameters *p* and *q*. The proportion $f_{1,2}$ in parentheses is not a free parameter. Sites assigned to $\omega_{1,2}$ are those with posterior probabilities $P > 0.95$; those with $P > 0.99$ are in bold. Analyses were conducted by using κ as a free parameter. S = tree length.

*Sites assigned to ω > 1 are those with posterior probabilities $P > 0.95$ (*) or $P > 0.99$ (**).

3. Likelihood Ratio Test Statistics (2δ) for Models of Variable Selective Pressure Among Sites.

	2δ	**df**	***P* value**
Model 0 (one dN/dS ratio for all branches)			
F3x4			
One ratio vs. M3 ($k = 3$)	216.26	4	$P < 0.0001$
M1 vs. M2	115.98	2	$P < 0.0001$
M7 vs. M8	115.82	2	$P < 0.0001$
F61			
One ratio vs. M3 ($k = 3$)	217.94	4	$P < 0.0001$
M1 vs. M2	109.88	2	$P < 0.0001$
M7 vs. M8	109.50	2	$P < 0.0001$

4. Likelihood Ratio Test Statistics (2δ) for Models of Variable Selective Pressure Along Branches.

	ℓ	**2δ**	**df***	***P* value**
TRIM5				
Model 0 (same dN/dS ratio for all branches)	–5,784.326			
Model 1 (different dN/dS ratio for each branch)	–5,755.534	57.584	37	$P < 0.02$
Apobec3G[†]				
Model 0 (same dN/dS ratio for all branches)	–4,209.048			
Model 1 (different dN/dS ratio for each branch)	–4,201.337	15.422	20	$P > 0.75$ (N.S.)

*df denotes degrees of freedom that is equal to one less than the total number of branches in the phylogeny. For a 20-taxa tree like in TRIM5, the total number of branches is 38, so df =37. N.S., not significant.

[†] Sawyer, S. L., Emerman, M. & Malik, H. S. (2004) *PLoS Biol.* **2,** e275.

5. Likelihood Ratio Test Statistics for Models of Selection Along the Human and Gibbon Lineages (OWM + hominids only).

	ω (dN/dS) (human or gibbon branch)	**ω (dN/dS) (remainder of tree)**	ℓ	**2δ**	**df***	***P* value**
Human						
Model 0 (two-ratio, human ω =1)	1.00	1.132	–5,748.233			
Model 1 [two-ratio, human ω >1 (estimated by PAML; ref. 1)]	2.985	1.133	–5,747.543	1.38	1	0.24 (N.S)
Gibbon						
Model 0 (two-ratio, gibbon ω =1)	1.00	1.087	–5,748.718			
Model 1 [two-ratio, gibbon ω >1 (estimated by PAML; ref. 1)]	5.594	1.091	–5,744.345	8.746	1	0.0031

df denotes degrees of freedom; here, $df = 1$.

6. *P* Value Statistics for Positive Selection Along the Human and Gibbon Lineages, Based on Monte Carlo Simulation.

	Confidence ancestral sequence (0-1) per site	**ω (dN/dS)**	***P* value**
Human vs. Ancestral Node	0.99971	3.6	$P < 10^{-4}$
Gibbon vs. Ancestral Node	0.99633	6.5	$P < 10^{-4}$

B. PAML (1) Analysis of Partial Data Set (hominids+OWM)

1. Log Likelihood Scores and Parameter Estimates for Four Models of Variable ω's Among Sites Assuming the F3x4 Model of Codon Frequencies.

Model	**Parameter estimates**	**Sites* with $\omega > 1$**	ℓ
M0: one ratio	Average dN/dS for each branch = 1.1923		**–3,519.244**
Site models			
M1: neutral	$(\omega_0 = 0)\, f_0 = 0.307$	Not allowed	**–3,513.813**
	$(\omega_1 = 1)\ (f_1 = 0.\ 693)$		
	Average dN/dS for each branch = 0.693		
M2: selection	$(\omega_0 = 0)\, f_0 = 0.185$ $(\omega_1 = 1)\, f_1 = 0.783$ **$\omega_2 = 11.283\ (f_2 = 0.032)$** Average dN/dS for each branch = 1.1429	**330 G 0.9960**** **332 R 0.9929**** **335 R 0.9890*** **337 Q 0.9981**** **340 V 0.9993****	**–3,492.320**
M3:discrete	ω_0 0.00001 $f_0 = 0.380$ **$\omega_1 = 1.667\ (f_1 = 0.601)$** **$\omega_2 = 16.866\ (f_2 = 0.019)$** average dN/dS for each branch = 1.3219	Too many to list. Note: M3 vs. M0 is a test of heterogeneity among sites and not an explicit test of positive selection.	**–3,490.526**
M7:β	$p = 0.00592$ $q = 0.00273$ Average dN/dS for each branch = 0.7	Not allowed	**–3,513.818**

Model	Parameter estimates	Sites* with $\omega > 1$	ℓ
M8:β and ω	$p = 0.01037$ $q = 0.00248$ $f_0 = 0.968$ **$\omega_1 = 11.191$ ($f_1 = 0.032$)** Average dN/dS for each branch = 1.1308	**330 G 0.9959**** **332 R 0.9927**** **335 R 0.9888*** **337 Q 0.9980**** **340 V 0.9992****	**–3,492.324**

p and q are parameters of the β distribution. f is the proportion of sites assigned to an individual ω (= dN/dS) category or to a β distribution with shape parameters p and q. The proportion $f_{1,2}$ in parentheses is not a free parameter. Sites assigned to $\omega_{1,2}$ are those with posterior probabilities $P > 0.95$; those with $P > 0.99$ are in bold. Analyses were conducted using κ as a free parameter. S = tree length.

*Sites assigned to $\omega > 1$ are those with posterior probabilities $P > 0.95$ (*) or $P > 0.99$ (**).

2. Log Likelihood Scores and Parameter Estimates for Four Models of Variable ω's Among Sites Assuming the F61 Model of Codon Frequencies

Model	Parameter estimates	Sites* with $\omega > 1$	ℓ
M0: one ratio	Average dN/dS for each branch = 1.1375		**–3,469.858**
Site models			
M1: neutral	($\omega_0 = 0$) $f_0 = 0.331$	Not allowed	**–3,462.818**
	($\omega_1 = 1$) ($f_1 = 0.669$)		
	Average dN/dS for each branch = 0.669		
M2: selection	($\omega_0 = 0$) $f_0 = 0.215$ ($\omega_1 = 1$) $f_1 = 0.749$ **$\omega_2 = 10.019$ ($f_2 = 0.036$)** Average dN/dS for each branch = 1.1057	**330 G 0.9914**** **332 R 0.9934**** **335 R 0.9840*** **337 Q 0.9984**** **338 T 0.6807** **340 V 0.9993****	**–3,443.237**
M3:discrete	$\omega_0 = 0.00001$ $f_0 = 0.415$ **$\omega_1 = 1.761$ ($f_1 = 0.568$)**	Too many to list. Note: M3 vs. M0 is a test	**–3,441.147**

Model	Parameter estimates	Sites* with $\omega > 1$	ℓ
	$\omega_2 = 17.069$ ($f_2 = 0.017$) Average dN/dS for each branch = 1.2835	of heterogeneity among sites and not an explicit test of positive selection.	
M7:β	$p = 0.00484$ $q = 0.00310$	Not allowed	**–3,463.372**
	Average dN/dS for each branch = 0.6		
M8:β and ω	$p = 0.00553$ $q = 0.00144$ $f_0 = 0.964$ **$\omega_1 = 10.263$ ($f_1 = 0.036$)** Average dN/dS for each branch = 1.2368	**330 G 0.9919**** **332 R 0.9938**** **335 R 0.9849*** **337 Q 0.9985**** **340 V 0.9994****	**–3,443.266**

p and *q* are parameters of the β distribution. *f* is the proportion of sites assigned to an individual ω (= dN/dS) category or to a β distribution with shape parameters *p* and *q*. The proportion $f_{1,2}$ in parentheses is not a free parameter. Sites assigned to $\omega_{1,2}$ are those with posterior probabilities $P > 0.95$; those with $P > 0.99$ are in bold. Analyses were conducted using κ as a free parameter. S = tree length.

* Sites assigned to $\omega > 1$ are those with posterior probabilities $P > 0.95$ (*) or $P > 0.99$ (**).

3. Likelihood Ratio Test Statistics (2δ) for Models of Variable Selective Pressure Among Sites.

	2δ	**df**	***P* value**
Model 0 (one ω for all branches)			
F3x4			
One ratio vs. M3 ($k = 3$)	57.436	4	$P < 0.0001$
M1 vs. M2	42.986	2	$P < 0.0001$
M7 vs. M8	42.988	2	$P < 0.0001$
F61			
One ratio vs. M3 ($k = 3$)	57.422	4	$P < 0.0001$
M1 vs. M2	39.162	2	$P < 0.0001$
M7 vs. M8	40.212	2	$P < 0.0001$

1. Yang, Z. (1997) *Comput. Appl. Biosci.* **13,** 555–556.

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                                                                 RING→
Human        ATGGCTTCTGGAATCCTGGTTAATGTAAAGGAGGAGGTGACCTGCCCCATCTGCCTGGAA(20)
Chimp        ATGGCTTCTGGAATCCTGGTTAATGTAAAGGAGGAGGTGACCTGCCCCATCTGCCTGGAA
Gorilla      ATGGCTTCTGGAATCCTGGTTAATGTAAAGGAGGAGGTGACCTGCCCCATCTGCCTGGAA
Orangutan    ATGGCTTCTGGAATCCTGGTTAATGTAAAGGAGGAGGTGACCTGCCCTATCTGCCTGGAA
Gibbon       ATGGCTTCTGGAATCCTGGTTAATGTAAAGGAGAAGGTGACCTGCCCCATCTGCCTGGAA
Rhes_cDNA    ATGGCTTCTGGAATCCTGCTTAATGTAAAGGAGGAGGTGACCTGTCCCATCTGCCTGGAA
Baboon       ATGGCTTCTGGAATCCTGCTTAATGTAAAGGAGGAGGTGACCTGTCCCATCTGCCTGGAA
AGM          ATGGCTTCTGGAATCCTGCTTAATGTAAAGGAGGAGGTGACCTGTCCCATCTGCCTGGAA
AGM_cDNA     ATGGCTTCTGGAATCCTGGTTAATGTAAAGGAGGAGGTGACCTGTCCCATCTGCCTGGAA
Tant_cDNA    ATGGCTTCTGGAATCCTGCTTAATGTAAAGGAGGAGGTGACCTGTCCCATCTGCCTGGAA
Patas        ATGGCTTCTGGAATCCTGCTTAATGTAAAGGAGGAGGTGACCTGTCCTATCTGCCTGGAA
Colobus      ATGGCTTCTGGAATCCTGGTTAATATAAAGGAGGAGGTGACCTGCCCCATCTGCCTGGAA
DLangur      ATGGCTTCTGGAATCCTGGTTAATATAAAGGAGGAGGTGACCTGCCCCATCTGCCTGGAA
PMarmoset    ATGGCTTCCAGAATCCTGGTGAATATAAAGGAGGAGGTAACCTGCCCCATCTGCCTGGAA
Tamarin      ATGGCTTCCAGAATCCTGGTGAATATAAAGGAGGAGGTGACCTGCCCCATCTGCCTGGAA
Squirrel     ATGGCTTCCAGAATCCTGGGGAGTATAAAGGAGGAGGTGACCTGCCCCATCTGCCTGGAA
Owl          ATGGCTTCCAGAATCCTGGTCAATATAAAGGAGGAGGTGACCTGCCCCATCTGCCTGGAA
Titi         ATGGCTTCCAGAATCCTGGTGAATATAAAGGAGGAGGTGACCTGCCCCATCTGCCTGGAA
Saki         ATGGCTTCCAGAATCCTGATGAACATAAAGGAGGAGGTGACCTGCCCCATCTGCCTGGAA
Howler       ATGGCTTCCAAAATCCTGGTGAATATAAAGGAGGAGGTGACCTGCCCCATCTGCCTGGAA
Spider       ATGGCTTCCGAAATCCTGTTGAATATAAAGGAGGAGGTGACCTGCCCCATCTGCCTGGAA
Woolly       ATGGCTTCCGAAATCCTGGTGAATATAAAGGAGGAGGTGACCTGCCCCATCTGCCTGGAC

Human        CTCCTGACACAACCCCTGAGCCTGGACTGCGGCCACAGCTTCTGCCAAGCATGCCTCACT(40)
Chimp        CTCCTGACACAACCCCTGAGCCTGGACTGCGGCCACAGCTTCTGCCAAGCATGCCTCACT
Gorilla      CTCCTGACACAACCCCTGAGCCTGGACTGCGGCCACAGCTTCTGCCAAGCATGCCTCACT
Orangutan    CTCCTGACACAACCCCTGAGTCTGGACTGCGGCCACAGCTTCTGCCAAGCATGCCTCACT
Gibbon       CTCCTGACACAACCCCTGAGTCTGGACTGCGGCCACAGCTTCTGCCAAGCATGCCTCACT
Rhes_cDNA    CTCCTGACAGAACCCCTGAGTCTGCACTGCGGCCACAGCTTCTGCCAAGCGTGCATCACT
Baboon       CTCCTGACAGAACCCCTGAGTCTGCCCTGTGGCCACAGCTTCTGCCAAGCGTGCATCACT
AGM          CTCCTGACAGAACCCCTGAGTCTGCCCTGCGGCCACAGCTTCTGCCAAGCGTGCATCACT
AGM_cDNA     CTCCTGACAGAACCCCTGAGTCTGCCCTGCGGCCACAGCTTCTGCCAAGCGTGCATCACT
Tant_cDNA    CTCCTGACAGAACCCCTGAGTCTGCCCTGCGGCCACAGCTTCTGCCAAGCGTGCATCACT
Patas        CTCCTGACAGAACCCCTGAGTCTGCCCTGCGGCCACAGCTTCTGCCAAGCGTGCATCACT
Colobus      CTCCTGACAGAACCCCTGAGTCTGCACTGCGGCCACAGCTTCTGCCAAGCGTGCATCACT
DLangur      CTCCTGACAGAACCCCTGAGTCTGCACTGCGGCCACAGCTTCTGCCAAGCGTGCATCACT
PMarmoset    CTCCTGACAGAACCTCTGAGCCTAGACTGTGGCCACAGCTTCTGCCAAGCCTGCATCACT
Tamarin      CTCCTGACAGAACCTCTGAGCCTAGACTGTGGCCACAGCTTCTGCCAAGCATGCATCACT
Squirrel     CTCCTGACAGAACCTCTGAGCCTAGACTGTGGCCACAGCTTCTGCCAAGCATGCATCACT
Owl          CTCCTGACAGAACCCCTGAGCCTGGACTGTGGCCATAGCTTCTGCCAAGCATGCATCACT
Titi         CTCCTGACAGAACCCCTGAGCCTAGACTGTGGCCACAGCTTCTGCCAAGCATGCATCACC
Saki         CTCCTGACAGAACCCCTGAGCCTAGACTGTGGCCACAGCTTCTGCCAAGCATGCATCACT
Howler       CTACTGACAGAACCTCTGAGCCTAGACTGTGGCCACAGCTTCTGCCAAGCATGCATCACT
Spider       CTACTGACAGAACCTCTGAGCCTAGACTGTGGCCACAGCTTCTGCCAAGCATGCATCACT
Woolly       CTACTGACAGAACCTCTGAGCCTAGACTGTGGCCACAGCTTCTGCCAAGCATGCATCACT
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Human        GCAAACCACAAGAAGTCCATGCTAGACAAAGGA---GAGAGTAGCTGCCCTGTGTGCCGG(59)
Chimp        GCAAACCACAAGAAGTCCATGCTAGACAAAGGA---GAGAGTAGCTGCCCTGTGTGCCGG
Gorilla      GCAAACCACAAGAAGTCCATGCTAGACAAAGGA---GAGAGTAGCTGCCCTGTGTGCCGG
Orangutan    GCAAACCACAAGAAGTCCACGCTAGACAAAGGA---GAGAGAAGCTGCCCTGTGTGCCGG
Gibbon       GCAAACCACAAAACGTCCATGCCAGACGAAGGA---GAGAGAAGCTGCCCTGTGTGCCGG
Rhes_cDNA    GCGAACCACAAGAAGTCCATGCTATACAAAGAAGGAGAGAGAAGCTGCCCTGTGTGCCGG
Baboon       GCAAACCACAGGAAGTCCATGCTATACAAAGAAGGAGAGAGAAGCTGCCCTGTGTGCCGG
AGM          GCAAACCACAAGGAGTCCATGCTATACAAAGAAGAAGAGAGAAGCTGCCCTGTGTGCCGG
AGM_cDNA     GCAAACCACAAGGAGTCCATGCTATACAAAGAAGAAGAGAGAAGCTGCCCTGTGTGCCGG
Tant_cDNA    GCAAACCACAAGGAGTCCATGCTATACAAAGAAGAAGAGAGAAGCTGCCCTGTGTGCCGG
Patas        GCAAACCACAAGAAGTCCATGCTATACAAAGAAGAAGAGAGAAGCTGCCCTGTGTGCCGG
Colobus      GCAAACCACAAGAAGTCCATGCTATACAAAGAAGGAGAGAGAAGCTGCCCTGTGTGCCGG
DLangur      GCAAACCACAAGAAGTCCATGCTATACAAAGAAGGAGAGAGAAGCTGCCCTGTGTGCCGG
PMarmoset    GCAAACCACAAAGAGTCTACGCTACACCAAGGA---GAGAGAAGCTGCCCTTTGTGCCGG
Tamarin      GCAAACCACAAAGAGTCTACGCCACACCAAGGA---GAGAGAAGCTGCCCCTTGTGCCGG
Squirrel     GCAAATCACAAAGAGTCTATGCTACACCAAGGA---GAGAGAAGCTGCCCTTTGTGCCGG
Owl          GCAAATCACAAAAAGTCTATGCCACACCAAGGA---GAGAGAAGCTGCCCTTTGTGCCGG
Titi         GCAAACCACAAAGAGTCTACGCTACACCAAGGA---GAGAGAAGCTGCCCTTTGTGCAGG
Saki         GCAAACCACAAAAAGTCTATGCTACACCAAGGA---GAGAGAAGCTGCCCTTTGTGCCGG
Howler       GCAAACCACAAAGAGTCC------------AGA---GAGAGAAGCTGCCCTTTGTGCCGG
Spider       GCAAACCACAAAGAGTCTACGCTACACCAAGGA---GAGAGAAGCTGCCCTTTGTGCCGG
Woolly       GCAGACCACAAAGAGTCTACGCTACACCAAGGA---GAGAGAAGCTGCCCTTTGTGCCGG


Human        ATCAGTTACCAGCCTGAGAACATACGGCCTAATCGGCATGTAGCCAACATAGTGGAGAAG(79)
Chimp        ATCAGTTACCAGCCTGAGAACATACGGCCTAATCGGCATGTAGCCAACATAGTGGAGAAG
Gorilla      ATCAGTTACCAGCCTGAGAACATACGGCCTAATCGGCATGTAGCCAACATAGTGGAGAAG
Orangutan    GTCAGTTACCAGCCTAAGAACATACGGCCTAATCGGCATGTAGCCAACATAGTGGAGAAG
Gibbon       ATCAGTTACCAGCATAAGAACATACGGCCTAATCGGCATGTAGCCAACATAGTGGAGAAG
Rhes_cDNA    ATCAGTTACCAGCCTGAGAACATACAGCCTAATCGGCATGTAGCCAACATAGTGGAGAAG
Baboon       ATCAGTTACCAGCCTGAGAACATACAGCCTAATCGGCATGTAGCCAACATAGTGGAGAAG
AGM          ATCAGTTACCAGCCTGAGAATATACAGCCTAATCGGCATGTAGCCAACATAGTGGAGAAG
AGM_cDNA     ATCAGTTACCAGCCTGAGAATATACAGCCTAATCGGCATGTAGCCAACATAGTGGAGAAG
Tant_cDNA    ATCAGTTACCAGCCTGAGAATATACAGCCTAATCGGCATGTAGCCAACATAGTGGAGAAG
Patas        ATCAGTTACCAGCCTGAGAACATACAGCCTAATCGGCATGTAGCCAACATAGTGGAGAAG
Colobus      ATCAGTTACCAGCCTGAGAACATACGGCCTAATCGGCATGTGGCCAACATAGTGGAGAAG
DLangur      ATCAGTTACCAGCCTGAGAACATACGGCCTAATCGGCATGTGGCCAACATAGTGGAGAAG
PMarmoset    ATGAGTTACCCGTCTGAGAACTTGCGGCCTAATCGGCATTTGGCCAATATAGTGGAGAGG
Tamarin      ATGAGTTACCCGTCTGAGAACTTGCGGCCTAATCGGCATTTGGCCAACATAGTGGAGAGG
Squirrel     CTCCCTTACCAGTCTGAGAACCTGCGGCCTAATCGGCATTTGGCCAGCATCGTGGAGAGG
Owl          ATCAGTTACTCGTCTGAGAACCTGCGGCCTAATCGGCATTTGGTCAACATAGTGGAGAGG
Titi         ATCAGTTACCCGTCTGAGAACCTGCGGCCTAATCGGCATTTGGCCAACATAGTGGAGAGG
Saki         ATCAGTTACCCATCTGAGAACCTGCGGCCTAATCGGCATTTGGCCAACATAGTGGAGAGG
Howler       GTCAGTTACCACTCTGAGAACCTGCGGCCTAATCGGCATTTGGCCAACATAGCGGAGAGG
Spider       GTCAGTTACCAGTCTGAGAACCTGCGGCCTAATCGGCATTTGGCAAACATAGCGGAGAGG
Woolly       GTCGGTTACCAGTCTGAGAACCTGCGGCCTAATCGGCATTTGGCAAACATAGCCGAGAGG
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                                                             B-box2→
Human        CTCAGGGAGGTCAAGTTGAGCCCA---GAGGGGCAGAAAGTTGATCATTGTGCACGCCAT(98)
Chimp        CTCAGGGAGGTCAAGTTGAGCCCA---GAGGGGCAGAAAGTTGATCATTGTGCACACCAT
Gorilla      CTTAGGGAGGTCAAGTTGAGCCCA---GAGGGGCAGAAAGTTGATCATTGTGCACGCCAT
Orangutan    CTCAGGGAGGTCAAATTGAGCCCA---GAGGGGCAGAAGGTTGATCACTGTGCACGCCAT
Gibbon       CTCAGGGAGGTCAAGTTGAGCCCAGAGGAGGGGCAGAAGGTTGATCACTGTGCACGCCAC
Rhes_cDNA    CTCAGGGAGGTCAAGTTGAGCCCAGAAGAGGGACAGAAGGTTGATCACTGTGCACGCCAT
Baboon       CTCAGGGAGGTCAAGTTGAGCCCAGAAGAGGGGCTGAAGGTTGATCACTGTGCACGCCAT
AGM          CTCAGAGAGGTCAAGTTGAGCCCAGAAGAGGGGCAGAAGGTTGATCACTGTGCACGCCAT
AGM_cDNA     CTCAGAGAGGTCAAGTTGAGCCCAGAAGAGGGGCAGAAGGTTGATCACTGTGCACGCCAT
Tant_cDNA    CTCAGAGAGGTCAAGTTGAGCCCAGAAGAGGGGCAGAAGGTTGATCACTGTGCACGCCAT
Patas        CTCAGAGAGGTCAAGTTGAGCCCAGAAGAGGGGCAGAAGGTTGATCACTGTGCACGCCAT
Colobus      CTCAGGGAGGTCAAGTTGAGCCCAGAAGAGGGGCAGAAGGTTGATCACTGTGCACGCCAT
DLangur      CTCAGGGAGGTCAAGTTGAGCCCAGAAGAGGGGCAGAAAGTTGATCACTGTGCACGCCAT
PMarmoset    CTCAAAGAGGTCATGCTGAGCCCAGAGGAGGGGCAGAAGGTTGATCACTGTGCACGCCAT
Tamarin      CTCAAAGAGGTCATGCTGAGCCCAGAGGAGGGGCAGAAGGTTGGTCACTGTGCACGCCAT
Squirrel     CTCAGGGAGGTCATGCTGAGACCAGAGGAAAGGCAGAACGTTGATCACTGTGCACGCCAT
Owl          CTCAGGGAGGTCATGCTGAGCCCAGAGGAGGGGCAGAAGGTTGATCACTGTGCACACCAT
Titi         CTCAGGGAGGTCGTGCTGAGCCCAGAGGAGGGGCAGAAGGTTGATCTCTGTGCACGCCAT
Saki         CTCAGGGAGGTCATGCTGAGCCCAGAGGAGGGGCAGAAGGTTGATCACTGTGCACGCCAT
Howler       CTCAGGGAGGTCATGTTGAGCCCAGAGGAGGGGCAGAAGGTTGATCGCTGTGCACGCCAT
Spider       CTCAGGGAGGTCATGTTGAGCCCAGAGGAAGGGCAGAAGGTTGATCGCTGTGCACGCCAT
Woolly       CTCAGGGAGGTCATGTTGAGCCCAGAGGAAGGGCAGAAGGTTGATCGCTGTGCACGCCAT


Human        GGAGAGAAACTTCTACTCTTCTGTCAGGAGGACGGGAAGGTCATTTGCTGGCTTTGTGAG(118)
Chimp        GGAGAGAAACTTCTACTCTTCTGTCAGGAGGACGGGAAGGTCATTTGCTGGCTTTGTGAG
Gorilla      GGAGAGAAACTTCTACTCTTCTGTCAGGAGGACGGGAAGGTCATTTGCTGGCTTTGCGAG
Orangutan    GGAGAGAAACTTCTACTCTTCTGTAAGGAGGACGGGAAGGTCATTTGCTGGCTTTGTGAG
Gibbon       GGAAAGAAACTTCTACTCTTCTGTCAGGAGGACAGGAAGGTCATTTGCTGGCTTTGTGAG
Rhes_cDNA    GGAGAGAAACTCCTACTCTTCTGTCAGGAGGACAGCAAGGTCATTTGCTGGCTTTGTGAG
Baboon       GGAGAGAAACTCCTACTCTTCTGTCAGGAGGACAGCAAGGTCATTTGCTGGCTTTGTGAG
AGM          GGAGAGAAACTCCTACTCTTCTGTCAGGAGGACAGCAAGGTCATTTGCTGGCTTTGTGAG
AGM_cDNA     GGAGAGAAACTCCTACTCTTCTGTCAGGAGGACAGCAAGGTCATTTGCTGGCTTTGTGAG
Tant_cDNA    GGAGAGAAACTCCTACTCTTCTGTCAGGAGGACAGCAAGGTCATTTGCTGGCTTTGTGAG
Patas        GGAGAGAAACTCCTACTCTTCTGTCAGGAGGACAGGAAGGTCATTTGCTGGCTTTGTGAG
Colobus      GGAGAGAAACTCCTACTCTTCTGTCAGGAGGACAGGAAGGTCATTTGCTGGCTTTGTGAG
DLangur      GGAGAGAAACTCCTACTCTTCTGTCAGGAGGACAGGAAGGTCATTTGCTGGCTTTGTGAG
PMarmoset    GGAGAGAAACTTCTACTCTTCTGTCAGCAGGATGGAAATGTCATTTGCTGGCTTTGTGAG
Tamarin      GGAGAGAAACTTCTACTCTTCTGTGAGCAGGATGGAAATGTCATTTGCTGGCTTTGTGAG
Squirrel     GGAGAGAAACTTCTACTCTTCTGTGAGCAGGATGGAAATATCATTTGCTGGCTTTGTGAG
Owl          GGAGAGAAACTTGTACTCTTCTGTCAGCAGGATGGAAATGTCATTTGCTGGCTTTGTGAG
Titi         GGAGAGAAACTTCTACTCTTCTGTCAGCAGGATGGAAATGTCATTTGCTGGCTTTGTGAG
Saki         GGAGAGAAACTTCTACTCTTCTGTCAGCAGGATGGAAATGTCATTTGCTGGCTTTGTGAG
Howler       GGAGAGAAACTTCTACTCTTCTGTCAGCAGCATGGAAATGTCATTTGCTGGCTTTGTGAG
Spider       GGAGAGAAACTTCTACTCTTCTGTCAGCAGCATGGAAATGTCATTTGCTGGCTTTGTGAG
Woolly       GGAGAGAAACTTCTACTCTTCTGTCAGCAGCATGGAAATGTCATTTGCTGGCTTTGTGAG
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                                              Coiled-Coil→
Human        CGGTCTCAGGAGCACCGTGGTCACCACACGTTCCTCACAGAGGAGGTTGCCCGGGAGTAC(138)
Chimp        CGGTCTCAGGAGCACCGTGGTCACCACACGTTCCTCACAGAGGAGGTTGCCCGGGAGTAC
Gorilla      CGGTCTCAGGAGCACCGTGGTCACCACACGTTCCTCACAGAGGAGGTTGCCCAGGAGTAC
Orangutan    CGGTCTCAGGAGCACCGTGGTCACCACACATTCCTCACGGAGGAGGTTGCCCAGAAGTAC
Gibbon       CGGTCTCAGGAGCACCGTGGTCACCACACATTCCTCACGGAGGAGGTTGCCCAGGAGTAC
Rhes_cDNA    CGGTCTCAGGAGCACCGTGGTCACCACACTTTCCTCATGGAGGAGGTTGCCCAGGAGTAC
Baboon       CGGTCTCAGGAGCACCGTGGTCACCACACTTTCCTCATGGAGGAGGTTGCCCAGGAGTAC
AGM          CGGTCTCAGGAGCACCGTGGTCACCACACTTTCCTCATGGAGGAGGTTGCCCAGGAGTAC
AGM_cDNA     CGGTCTCAGGAGCACCGTGGTCACCACACTTTCCTCATGGAGGAGGTTGCCCAGGAGTAC
Tant_cDNA    CGGTCTCAGGAGCACCGTGGTCACCACACTTTCCTCATGGAGGAGGTTGCCCAGGAGTAC
Patas        CGGTCTCAGGAGCACCGTGGTCACCACACTTTCCTCATGGAGGAGGTTGCCCAGGAGTAC
Colobus      CGGTCTCAGGAGCACCGTGGTCACCACACGTTCCTCATGGAGGAGGTTGCCCAGGAGTAC
DLangur      CGGTCTCAGGAGCACCGTGGTCACCACACGTTCCTCATGGAGGAAGTTGCCCAGGAGTAC
PMarmoset    CGGTCTCAAGAACACCGTGGTCACCACACATTCCTCGTGGAGGAGGTTGCAGAGAAATAC
Tamarin      CGGTCTCAAGAACATCGTGGTCACCACACATTACTCGTGGAGGAGGTTGCAGAGAAATAC
Squirrel     CGGTCTCAAGAACACCGTGGTCACAACACATTCCTCGTGGAGGAGGTTGCACAGAAATAC
Owl          CGGTCTCAAGAACACCGTGGGCACCAGACATTCCTTGTGGAGGAGGTTGCACAGAAATAC
Titi         CGGTCTCAAGAACACCGTGGTCACCACACATTCCTCGTGGAGGAGGTTGCACAGACATAC
Saki         CGGTCTCAAGAACACCGTGGTCACCACACATTACTCGTGGAGGAGGTTGCACAGACATAC
Howler       CGGTCTGAAGAACACCGTGGTCACCGCACATCCCTCGTGGAGGAGGTTGCACAGAAATAC
Spider       CGGTCTCAAGAACACCGTGGTCACAGCACATTCCTCGTGGAGGAGGTTGCACAGAAATAC
Woolly       CGGTCTCAAGAACACCGTGGTCACAGCACATTCCTCGTGGAGGAGGTTGCACAGAAATAC

                  Exon3→
Human        CAA|GTGAAGCTCCAGGCAGCTCTGGAGATGCTGAGGCAGAAGCAGCAGGAAGCTGAAGAG(158)
Chimp        CAA|GTGAAGCTCCAGGCAGCTCTGGAGATGCTGAGGCAGAAGCAGCAGGAAGCTGAAGAG
Gorilla      CAA|GTGAAGCTCCAGGCAGCTCTGGAGATGCTGAGGCAGAAGCAGCAGGAAGCTGAAGAG
Orangutan    CAA|GTGAAGCTCCAGGCAGCTCTGGAGATGCTGAGGCAGAAGCAGCAGGAAGCTGAAGAG
Gibbon       CAA|ATGAAGCTCCAGGCAGCTCTGCAGATGCTGAGGCAGAAGCAGCAGGAAGCTGAAGAG
Rhes_cDNA    CAT|GTGAAGCTCCAGACAGCTCTGGAGATGCTGAGGCAGAAGCAGCAGGAAGCTGAAAAG
Baboon       CAT|GTGAAGCTCCAGACAGCTCTGGAGATGCTGAGGCAGAAGCAGCAGGAAGCTGAAAAG
AGM          CAT|GTGAAGCTCCAGACAGCTCTGGAGATGCTGAGGCAGAAGCAGCAGGAAGCTGAAAAG
AGM_cDNA     CAT|GTGAAGCTCCAGACAGCTCTGGAGATGCTGAGGCAGAAGCAGCAGGAAGCTGAAAAG
Tant_cDNA    CAT|GTGAAGCTCCAGACAGCTCTGGAGATGCTGAGGCAGAAGCAGCAGGAAGCTGAAAAG
Patas        CAT|GTGAAGCTCCAGACAGCTCTGGAGATGCTGAGGCAGAAGCAGCAGGAAGCTGAAAAG
Colobus      CAC|GTGAAGCTCCAGACAGCTCTGGAGATGCTGAGGCAGAAGCAGCAGGAAGCTGAAAAG
DLangur      CAC|GTGAAGCTCCAGACAGCTCTGGAGATGCTGAGGCAGAAGCAGCAGGAAGCTGAAAAG
PMarmoset    CAA|GGAAAGCTCCAGGTAGCTCTGGAGATGATGAGGCAGAAGCAGCAGGATGCTGAAAAG
Tamarin      CAA|GAAAAGCTCCAGGTAGCTCTGGAGATGATGAGGCAGAAGCAGCAGGATGCTGAAAAG
Squirrel     CGA|GAAAAGCTCCAGGTAGCTCTGGAGACAATGAGGCAGAAGCAGCAGGATGCTGAAAAG
Owl          CGA|GAAAAGCTCCAGGTAGCTCTGGAGATGATGAGGCAGAAGCAGAAGGATGCTGAAAAG
Titi         CGA|GAAAATCTCCAGGTAGTTCTGGAGATGATGAGGCAGAAGCATCAGGATGCTGAAAAG
Saki         CGA|GAAAATCTCCAGGTAGCTCTGGAGACGATGAGGCAGAAGCAGCAGGATGCTGAAAAG
Howler       CGA|GAAAAGCTCCAGGCAGCTCTGGAGATGATGAGGCAGAAGGAGCAGGATGCTGAAATG
Spider       CAA|GAAAAGCTCCAGGTAGCTCTGGAGATGATGAGGCAGAAGCAGCAGGATGCTGAAAAG
Woolly       CGA|GAAAAGCTCCAGGTAGCTCTGGAAATGATGAGGGAGAAGCAGCAGGATGCTGAAAAG
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                                                      Exon4→
Human        TTGGAAGCTGACATCAGAGAAGAGAAAGCTTCCTGGAAG|ACTCAAATACAGTATGACAAA(178)
Chimp        TTGGAAGCTGACATCAGAGAAGAGAAAGCTTCCTGGAAG|ACTCAAATACAGTATGACAAA
Gorilla      TTGGAAGCTGACATCAGAGAAGAGAAAGCTTCCTGGAAG|ACTCAAATACAGTATGACAAA
Orangutan    TTGGAAGCTGACATCAGAGAAGAGAAAGCTTCCTGGAAG|ACTCAAATACAGTATGACAAA
Gibbon       TTGGAAGCTGACATCAGAGAAGAGAAAGCTTCCTGGAAG|ACTCAAATACAGTATGACAAA
Rhes_cDNA    TTGGAAGCTGACATCAGAGAAGAGAAAGCTTCCTGGAAG|ATTCAAATAGACTACGACAAA
Baboon       TTGGAAGCTGACATCAGAGAAGAGAAAGCTTCCTGGAAG|ATTCAAATAGACTACGACAAA
AGM          TTGGAAGCTGACATCAGAGAAGAGAAAGCTTCCTGGAAG|ATTCAAATAGACTACGACAAA
AGM_cDNA     TTGGAAGCTGACATCAGAGAAGAGAAAGCTTCCTGGAAG|ATTCAAATAGACTACGACAAA
Tant_cDNA    TTGGAAGCTGACATCAGAGAAGAGAAAGCTTCCTGGAAG|ATTCAAATAGACTACGACAAA
Patas        TTGGAAGCTGACATCAGAGAAGAGAAAGCTTCCTGGAAG|ATTCAAATAGACTACGACAAA
Colobus      TTGGAAGCTGACATCAGAGAAGAGAAAGCTTCCTGGAAG|ATTCAAATAGACTATGACAAA
DLangur      TTGGAAGCTGACATCAGAGAAGAGAAAGCTTCCTGGAAG|ATTCAAATAGACTGCGACAAA
PMarmoset    TTAGAAGCTGATGTCAGAGAAGAGCAAGCTTCCTGGAAG|ATTCAAATACAAAATGACAAA
Tamarin      TTGGAAGCTGACGTCAGAGAAGAGCAAGCTTCTTGGAAG|ATTCAAATACGAAATGACAAA
Squirrel     TTGGAAGCTGACGTCAGACAAGAGCAAGCTTCCTGGAAG|ATTCAAATACAAAATGACAAA
Owl          TTGGAAGCTGACGTCAGAGAAGAGCAAGCTTCCTGGAAG|ATTCAAATACAAAATGACAAA
Titi         TTGGAAGCTGACGTCAGAGAAGAGCAAGCTTCCTGGAAG|ATTCAAATACAAAATGACAAA
Saki         TTAGAAGCTGACGTCAGAGAAGAGCAAGCTTCCTGGAAG|ATTCAAATACGAGATGACAAA
Howler       TTGGAAGCTGACGTCAGAGAAGAGCAAGCTTCCTGGAAG|ATTCAAATAGAAAATGACAAA
Spider       TTGGAAGCTGATGTCAGAGAAGAGCAAGCTTCCTGGAAG|ATTCAAATAGAAAATGACAAA
Woolly       TTGGAAGCTGATGTCAGAGAAGAGCAAGCTTCCTGGAAG|ATTCAAATAAAAAACGACAAA

Human        ACCAACGTCTTGGCAGATTTTGAGCAACTGAGAGACATCCTGGACTGGGAGGAGAGCAAT(198)
Chimp        ACCAACGTCTTGGCAGATTTTGAGCAACTGAGAGACATCCTGGACTGGGAGGAGAGCAAT
Gorilla      ACCAACGTCTTGGCAGATTTTGAGCAACTGAGAGACATCCTGGACTGGGAGGAGAGCAAT
Orangutan    ACCAGCGTCTTGGCAGATTTTGAGCAACTGAGAGACATCCTGGACTGGGAGGAGAGCAAT
Gibbon       ACCAACATCTTGGCAGATTTTGAGCAACTGAGACACATCCTGGACTGGGTGGAGAGCAAT
Rhes_cDNA    ACCAACGTCTCGGCAGATTTTGAGCAACTGAGAGAGATCCTGGACTGGGAGGAGAGCAAT
Baboon       ACCAACGTCTCGGCAGATTTTGAGCAACTGAGAGAGATCCTGGACTGGGAGGAGAGCAAT
AGM          ACCAACGTCTCGGCAGATTTTGAGCAACTGAGAGAGATCCTGGACTGGGAGGAGAGCAAT
AGM_cDNA     ACCAACGTCTCGGCAGATTTTGAGCAACTGAGAGAGATCCTGGACTGGGAGGAGAGCAAT
Tant_cDNA    ACCAACGTCTCGGCAGATTTTGAGCAACTGAGAGAGATCCTGGACTGGGAGGAGAGCAAT
Patas        ACCAACGTCTTGGCAGATTTTGAGCAACTGAGAGAGATCCTGGACTGGGAGGAGAGCAAT
Colobus      ACCAACGTCTTGGCAGATTTTGAGCAACTGAGAGAGATCCTGGACTGGGAGGAGAGCAAT
DLangur      ACCAATGTCTTGGCAGATTTTGAGCAACTGAGAGAGATCCTGGACTGGGAGGAGAGCAAT
PMarmoset    ACCAACATCATGGCAGAGTTTAAGCAACTGAGAGACATCCTGGACTGTGAGGAGAGCAAA
Tamarin      ACCAACATCATGGCAGAGTTTAAGCAACTGAGAGACATCCTGGACTGTGAGGAGAGCAAA
Squirrel     ACCAACATCATGGCAGAGTTTAAGCAACTGAGAGACATCCTGGACTGTGAGGAGAGCAAT
Owl          ACCAACATCATGGCAGAGTTTAAAAAACGGAGAGACATCCTGGACTGTGAGGAGAGCAAA
Titi         ACCAACATCATGGCAGAGTTTAAGCAACTGAGAGACATCCTGGACTGTGAGGAGAGCAAT
Saki         ACCAACATTATGGCAGAGTTTAAGCAACTGAGAGACATCCTGGACTGTGAGGAGAGCAAT
Howler       ACCAGCACCCTGGCAGAGTTTAAGCAACTGAGAGACATCCTGGACTGTGAGGAGAGCAAC
Spider       ACCAACATCCTGGCAGAGTTTAAGCAACTGAGAGACATCCTGGACTGTGAGGAGAGCAAT
Woolly       ACCAACATCCTGGCAGAGTTTAAGCAACTGAGAGACATCCTGGACTGTGAGGAGAGCAAT
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Human        GAGCTGCAAAACCTGGAGAAGGAGGAGGAAGACATTCTGAAAAGCCTTACGAACTCTGAA(218)
Chimp        GAGCTGCAAAACCTGGAGAAGGAGGAGGAAGACATTCTGAAAAGCCTTACGAAGTCTGAA
Gorilla      GAGCTGCAAAACCTGGAGAAGGAGGAGGAAGACATTCTGAAACGCCTTACGAAGTCTGAA
Orangutan    GAGCTGCAAAACCTGGAGAAGGAGGAGGAAGACATTCTAAAAAGCCTTACGAAGTCTGAA
Gibbon       GAGCTGCAAAACCTGGAGAAGGAGGAGAAAGACGTTCTGAAAAGGCTTATGAGGTCTGAA
Rhes_cDNA    GAGCTGCAGAACCTGGAGAAGGAGGAAGAAGACATTCTGAAAAGCCTTACGAAGTCTGAA
Baboon       GAGCTGCAGAACCTGGAGAAGGAGGAAGAAGACATTCTGAAAAGCCTTACGAAGTCTGAA
AGM          GAGCTGCAGAACCTGGAGAAGGAGGAAGAAGACATTCTGAAAAGCCTTACGAAGTCTGAA
AGM_cDNA     GAGCTGCAGAACCTGGAGAAGGAGGAAGAAGACATTCTGAAAAGCCTTACGAAGTCTGAA
Tant_cDNA    GAGCTGCAGAACCTGGAGAAGGAGGAAGAAGACATTCTGAAAAGCCTTACGAAGTCTGAA
Patas        GAGCTGCAGTACCTGGAGAAGGAGGAAGAAGACATTCTGAAAAGCCTTACGAAGTCTGAA
Colobus      GAGCTGCAGAACCTGGAGAAGGAGGAGGAAGACATTCTGAAAAGCCTTACGAAGTCTGAA
DLangur      GAGCTGCAGAACCTGGAGAAGGAGGAGGAAGACATTCTGAAAAGCCTTACGAAGTCTGAA
PMarmoset    GAGCTGCAAAACCTGGAGAAGGAGGAGAAAAACATTCTGAAAAGACTTGTACAGTCGGAA
Tamarin      GAGCTGCAAAACCTGGAGAAGGAGGAGAAAAACATTCTGAAAAGACTTGTACAGTCTGAA
Squirrel     GAGCTGCAAAACCTGGAGAAGGAGGAGAAAAACATTCTGAAAAGACTTGTACAGTCTGAA
Owl          GAGTTGCAAAACCTGGAGAAGGAGGAGAAAAACATTCTGAAAAGACTTGTACAGTCTGAA
Titi         GAGCTGCAAAACCTAGAGAAGGAGGAGAAAAACATTCTGAAAAGACTTGTACAGTCTGAG
Saki         GAGCTGCAAATCCTAGAGAAGGAGGAGAAAAACATTCTGAAAAGACTTACACAGTCTGAA
Howler       GAGCTGCAAAAACTGGAGAAGGAGGAGGAAAACCTTCTGAAAAGACTTGTACAGTCTGAA
Spider       GAGCTACAAAACTTGGAGAAGGAGGAGGAAAACCTTCTGAAAACACTTGCACAGTCTGAA
Woolly       GAGCTGCAAAACCTGGAGAAGGAGGAGGAAAACCTTCTGAAAATACTTGCACAGTCTGAA


Human        ACTGAGATGGTGCAGCAGACCCAGTCCCTGAGAGAGCTCATCTCAGATCTGGAGCATCGG(238)
Chimp        ACTGAGATGGTGCAGCAGACCCAGTCCGTGAGAGAGCTCATCTCAGATCTGGAGCGTCGG
Gorilla      ACTGAGATGGTGCAGCAGACCCAGTCCGTGAGAGAGCTCATCTCAGATCTGGAGCATCGG
Orangutan    ACTGAGATGGTGCAGCAGACCCAGTCCGTGAGAGAGCTCATCTCAGATGTGGAGCATCGG
Gibbon       ATTGAGATGGTGCAGCAGACCCAGTCCGTGAGAGAGCTCATCTCAGATCTGGAGCATCGG
Rhes_cDNA    ACGGAGATGGTGCAGCAGACCCAGTACATGAGAGAGCTCATCTCAGAACTGGAGCATCGG
Baboon       ACGGAGATGGTGCAGCAGACCCAGTACATGAGAGAGCTCATCTCAGATCTGGAGCATCGG
AGM          ACGGAGATGGTGCAGCAGACCCAGTACATGAGAGAGCTCATCTCAGATCTGGAGCATCGG
AGM_cDNA     ACGGAGATGGTGCAGCAGACCCAGTACATGAGAGAGCTCATCTCAGATCTGGAGCATCGG
Tant_cDNA    ACGGAGATGGTGCAGCAGACCCAGTACATGAGAGAGCTCATCTCAGATCTGGAGCATCGG
Patas        ACGAAGATGGTGCGGCAGACCCAGTACGTGAGAGAGCTCATCTCAGATCTGGAGCATCGG
Colobus      ACTGAGATGGTGCAGCAGACCCAGTACATGAGAGAGCTCGTCTCAGATCTGGAGCATCGG
DLangur      ACTGAGATGGTGCAGCAGACCCAGTACATGAGAGAGCTCATCTCAGATCTGGAGCATCGG
PMarmoset    AGTGACATGGTGCTGCAGACCCAGTCCATTAGAGTGCTCATCTCAGATCTGGAGCGTCGC
Tamarin      AGTGACATGGTGCTGCAGACCCAGTCCATGAGAGTGCTCATCTCAGATCTGGAGCGTCGC
Squirrel     AATGACATGGTGCTGCAGACCCAGTCCGTGAGAGTGCTCATCTCAGATCTGGAGCGTCGC
Owl          AATGACATGGTGCTGCAGACCCAGTCCGTGAGAGTGCTCATCTCAGATCTGGAGCATCGC
Titi         AATGACATGGTGCTGCAGACCCAGTCCATAAGCGTGCTCATCTCGGATCTGGAGCATCGC
Saki         AATGACATGGTGCTGCAGACCCAGTCCATGGGAGTGCTCATCTCAGATCTGGAGCATCGC
Howler       AATGACATGGTGTTGCAGACCCAGTCCATAAGAGTGCTCATTGCAGACCTGGAGCGTCGC
Spider       AATGACATGGTGCTGCAGACCCAGTCCATGAGAGTGCTCATCGCAGATCTGGAGCACCGC
Woolly       AATGACATGGTGCTGCAGACCCAGTCCATGAGAGTGCTCATCGCAGATCTGGAGCATCGC
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                                           Exon5→                Exon6→
Human        CTGCAGGGGTCAGTGATGGAGCTGCTTCAG|GGTGTGGATGGCGTCATAAAAAG|GACGGAG(258)
Chimp        CTGCAGGGGTCAGTGATGGAGCTGCTTCAG|GGTGTGGATGGCGTCATAAAAAG|GATGGAG
Gorilla      CTGCAGGGGTCAGTGATGGAGCTGCTTCAG|GGTGTGGATGGCGTCATAAAAAG|GATGGAG
Orangutan    CTGCAGGGGTCAGTGATGGAGCTGCTTCAG|GGTGTGGATGGCATCATAAAAAG|GATGCAG
Gibbon       CTGCAGGGGTCAGTGATGGAGCTGCTTCAG|GGTGTGGATGGCGTCATAAAAAG|GATGAAG
Rhes_cDNA    TTGCAGGGGTCAATGATGGATCTACTGCAG|GGTGTGGATGGCATCATTAAAAG|GATTGAG
Baboon       TTGCAGGGGTCAATGATGGAGCTACTGCAG|GGTGTGGATGGCATCATTAAAAG|GATTGAG
AGM          TTGCAGGGGTCAATGATGGAGCTGCTGCAG|GGTGTGGATGGCATCATTAAAAG|GATTGAG
AGM_cDNA     TTGCAGGGGTCAATGATGGAGCTGCTGCAG|GGTGTGGATGGCATCATTAAAAG|GGTTGAG
Tant_cDNA    TTGCAGGGGTCAATGATGGAGCTGCTGCAG|GGTGTGGATGGCATCATTAAAAG|GATTGAG
Patas        TTGCAGGGGTCAATGATGGAGCTGCTGCAG|GGTGTGGATGGCATCATTAAAAG|GATTGAG
Colobus      TTGCAGGGGTCAGTGATGGAGCTGCTGCAG|GGTGTGGATGGCATCATAAAAAG|GATTGAG
DLangur      TTGCAGGGGTCAATGATGGAGCTGCTGCAG|GGTGTGGATGGCATCATAAAAAG|GATTGAG
PMarmoset    CTGCAGGGGTCAGTGATGGAGCTTTTACAG|GGTGTGGATGACGTCATAAAAAG|GATTGAG
Tamarin      CTGCAGGGGTCAGTGCTGGAGCTGTTACAG|GGTGTGGATGATGTCATAAAAAG|GATTGAG
Squirrel     CTGCAGGGGTCAGTGGTGGAGCTGTTACAG|GATGTGGATGGTGTCATAAAAAG|GATTGAG
Owl          CTGCAGGGGTCAGTGATGGAGCTGTTACAG|GGTGTGGATGGTGTCATAAAAAG|GATTGAG
Titi         CTGCAGGGGTCAGTGATGGAGCTGTTACAG|GGTGTGGATGGCGTCATAAAAAG|GGTTAAG
Saki         CTGCAGGGGTCAGTGATGGAGCTGTTACAG|GGTGTGGATGAAGTCATAAAAAG|GGTTAAG
Howler       CTGCAGGGGTCAGTTATGGAGCTGTTACAG|GGTGTGGAAGGCGTCATAAAAAG|GATTAAG
Spider       CTGCAGGGGTCAGTGATGGAGCTGTTACAG|GATGTGGAAGGCGTCATAAAAAG|GATTAAG
Woolly       CTGCAGGGGTCAGTGATGGAGCTGTTACAG|GGTGTGGAAGGCATCATAAAAAG|GACTACG

Human        AACGTGACCTTGAAGAAGCCAGAAACTTTTCCAAAAAATCAAAGGAGAGTGTTTCGAGCT(278)
Chimp        AACGTGACCTTGAAGAAGCCAGAAACTTTTCCAAAAAATCAAAGGAGAGTGTTTCGAGCT
Gorilla      AACGTGACCTTGAAGAAGCCAGAAACTTTTCCAAAAAATCGAAGGAGAGTGTTTCGAGCT
Orangutan    AACGTGACCTTGAAGAAGCCAGAAACTTTTCCAAAAAATCAAAGGAGAGTGTTTCGAGCT
Gibbon       AACGTGACCTTGAAGAAGCCAGAAACTTTTCCAAAAAATCGAAGGAGAGTGTTTCGAGCT
Rhes_cDNA    AACATGACCTTGAAGAAGCCAAAAACTTTTCACAAAAATCAAAGGAGAGTGTTTCGAGCT
Baboon       AACATGACCTTGAAGAAGCCAAAAACTTTTCACAAAAATCAAAGGAGAGTGTTTCGAGCT
AGM          AACATGACCTTGAAGAAGCCAAAAACATTTCACAAAAATCAAAGGAGAGTGTTTCGAGCT
AGM_cDNA     AACATGACCTTGAAGAAGCCAAAAACATTTCACAAAAATCAAAGGAGAGTGTTTCGAGCT
Tant_cDNA    AACATGACCTTGAAGAAGCCAAAAACATTTCACAAAAATCAAAGGAGAGTGTTTCGAGCT
Patas        AACATGACCTTGAAGAAGCCAGAAACATTTCACAAAAATCAAAGGAGAGTGTTTCGAGCT
Colobus      GACATGACCTTGAAGAAGCCAAAAACTTTTCCCAAAAATCAAAGGAGAGTGTTTCGAGCT
DLangur      AACATGACCTTGAAGAAGCCAAAAACTTTTCCCAAAAATCAAAGGAGAGTGTTTCGAGCT
PMarmoset    AAAGTTACTTTGCAGAAGCCAAAAACGTTTCTTAATGAAAAAAGGAGAGTATTTCGAGCT
Tamarin      ACAGTGACTTTGCAGAAGCCAAAAACCTTTCTTAATGAAAAAAGGAGAGTATTTCGAGCT
Squirrel     AAAGTGACTTTGCAGAAGCCAAAAACCTTCCTTAATGAAAAAAGGAGAGTATTTCGAGCT
Owl          AAAGTGACTTTGCAGAATCCAAAAACCTTTCTTAATGAAAAAAGGAGAATATTTCAAACT
Titi         AATGTGACTTTGCAGAAGCCAAAAACTTTTCTTAATGAAAAAAGGAGAGTATTTCGAGTT
Saki         AACGTGACTTTGCAGAAGCCGAAAACTTTTCTTAATGAAAAAAGGAGAGTATTTCGAGCT
Howler       AACGTGACTTTGCAGAAGCCAGAAACCTTTCTTAATGAAAAAAGGAGAGTATTTCAAGCT
Spider       AATGTGACTTTGCAGAAGCCAAAAACCTTTCTTAATGAAAAAAGGAGAGTGTTTCGAGCT
Woolly       AATGTGACTTTGCAGAAGCCAAAAACCTTTCTTAATGAAAAAAGGAGAGTGTTTCGAGCT
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                                                 Exon7→         SPRY→
Human        CCTGATCTGAAAGGAATGCTAGAAGTGTTTAGAG|AGCTGACAGATGTCCGACGCTACTGG(298)
Chimp        CCTGATCTGAAAGGAATGCTAGAAGTGTTTAGAG|AGCTGACAGATGTCCGACGCTACTGG
Gorilla      CCTGATCTGAAAGGAATGCTAGAAGTGTTTAGAG|AGCTGACAGATGTCCGACGCTACTGG
Orangutan    CCTAATCTGAAAGGAATGCTAGAAGTGTTTAGAG|AGCTGACAGATGTCCGACGCTACTGG
Gibbon       GCTGATCTGAAAGTAATGCTAGAAGTGTTGAGAG|AGCTGAGAGATGTCCGACGCTACTGG
Rhes_cDNA    CCTGATCTGAAAGGAATGCTAGACATGTTTAGAG|AGCTAACAGATGCCCGACGCTACTGG
Baboon       CCTGATCTGAAAGGAATGCTAGACATGTTTAGAG|AGCTAACAGATGTCCGACGCTACTGG
AGM          CCTGATCTGAAAGGAATGCTAGACATGTTTAGAG|AGCTAACAGATGTCCGACGCTACTGG
AGM_cDNA     CCTGATCTGAAAGGAATGCTAGACATGTTTAGAG|AGCTAACAGATGTCCGACGCTACTGG
Tant_cDNA    CCTGATCTGAAAGGAATGCTAGACATGTTTAGAG|AGCTAACAGATGTCCGACGCTACTGG
Patas        CCTGCTCTGAAAGGAATGCTAGACATGTTTAGAG|AGCTAACAGATGTCCGGCGCTACTGG
Colobus      CCTGATCTGAAAGGAATGCTAGACATGTTTAGAG|AGCTAACAGATGTCCGACGCTACTGG
DLangur      CCTGATCTGAAAGGAATCCTAGACATGTTTAGAG|AACTAACAGATGTCCGACGCTACTGG
PMarmoset    CCTGATCTGAAAGGAATGCTACAAGCATTTAAAG|AGCTGACAGAAGTCCAACGCTACTGG
Tamarin      CCTGATCTGAAAGCAATGCTACAAGCATTTAAAG|AGCTGACAGAAGTCCAACGCTACTGG
Squirrel     CCTGATCTGAAAAGAATGCTCCAAGTGTTAAAAG|AACTGACAGAAGTCCAACGCTACTGG
Owl          CCTGATCTGAAAGGAACACTACAAGTGTTTAAAG|AGCCGACAGAAGTCCAACGCTACTGG
Titi         CCTGATCTGAAAGGAATGCTACAAGTGTCTAAAG|AGTTGACAGAAGTCCAACGCTACTGG
Saki         CCTGATCTGAAAGGAATGCTACAAGTGTTCAAAG|AGCTGACAGAAGTCCAACGCTACTGG
Howler       CCTGATCTGAAAGGAATGCTACAAGTGTTTAAAG|AGCTGAAAGAAGTCCAGTGCTACTGG
Spider       CCTGATCTGAAAGGAATGCTACAAGTGTTTAAAG|AGCTGAAAGAAGTCCAGTGCTACTGG
Woolly       CCTAATCTGAAAGGAATGCTACAAGTGTTTAAAG|AGCTGAAAGAAGTCCAATGCTACTGG

                Exon8→                         C  A  V  I  S  E  D  K  R
Human        G|TTGATGTGACAGTGGCTCCAAACAACATTTCATGTGCTGTCATTTCTGAAGATAAGAGA(318)
Chimp        G|TTGATGTGACAGTGGCTCCAAACAACATTTCATGTGCTGTCATTTCTGAAGATATGAGA
Gorilla      G|TTGATGTGACAGTGGCTCCAAACAACATTTCATGTGCTGTCATTTCTGAAGATATGAGA
Orangutan    G|TTGATGTGACAGTGGCTCCAAACGACATTTCATATGCTGTCATTTCTGAAGATATGAGA
Gibbon       G|TTGATGTGACAGTGGCTCCAAACAACATTTCATATGCTGTCATTTCTGAAGATATGAGA
Rhes_cDNA    G|TTGATGTGACACTGGCTACAAACAACATTTCGCATGCTGTCATTGCTGAAGATAAGAGA
Baboon       G|TTGATGTGACACTGGCTCCAAACAACATTTCGCATGCTGTCATTGCTGAAGATAAGAGA
AGM          G|TTGATGTGACACTGGCTCCAAACAACATTTCGCATGCTGTCATTGCTGAAGATAAGAGA
AGM_cDNA     G|TTGATGTGACACTGGCTCCAAACAACATTTCGCATGCTGTCATTGCTGAAGATAAGAGA
Tant_cDNA    G|TTGATGTGACACTGGCTCCAAACAACATTTCGCATGCTGTCATTGCTGAAGATAAGAGA
Patas        G|TTGATGTGACACTGGCTCCAAACAACATTTCGCATGTTGTCATTGCTGAAGATAAGAGA
Colobus      G|TTGATGTGACACTGGCTCCAAACAACATTTCACATGCTGTCATTGCTGAAGATAAGAGA
DLangur      G|TTGATGTGACACTGGCTCCAAACAACATTTCACATGCTGTCATTGCTGAAGATAAGAGA
PMarmoset    G|CTCATGTGACACTGGTTCCAAGTCACCCTTCATGTACTGTCATTTCTGAAGATGAGAGA
Tamarin      G|CTCATGTGACACTGGTTCCAAGTCACCCTTCATATGCTGTTATTTCTGAAGATGAGAGA
Squirrel     G|CTCATGTGACACTGGTTCCAAGTCACCCTTCATATACTATCATTTCTGAAGATGGGAGA
Owl          G|CTCATGTGACACTGGTTCCAAGTCACCCTTCATGTACTGTCATTTCTGAAGATGAGAGA
Titi         G|CTCATGTGACACTGGTTGCAAGTCACCCTTCACGTGCTGTCATTTCTGAAGACGAAAGA
Saki         G|TTCATGTGACACTGGTTCCAAGTCACCTTTCATGTGCTGTCATTTCTGAAGATGAGAGA
Howler       G|CTCATGTGACACTGATTCCGAATCACCCTTCATGTACTGTCATTTCTGAAGATAAGAGA
Spider       G|CTCATGTGACACTGGTTCCAAGTCACCCTTCATGTACTGTCATTTCTGAAGATGAGAGA
Woolly       G|CTCATGTGACACTGGTTCCAAGTCACCCTTCATGTGCTGTCATTTCTGAAGATCAGAGA
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                                                      (Patch)→
               Q  V  S  S  P  K  P Q  I  I  Y   G  A R  G  T  R  Y  Q    T
Human        CAAGTGAGCTCTCCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAG---ACA(338)
Chimp        CAAGTGAGCTCTCCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATATCAG---ACA
Gorilla      CAAGTGAGCTCTCCGAAACCACAGATAATATATGGGGCACAAGGGACAAGATATCAG---ACA
Orangutan    CAAGTGAGCTGTCCGGAACCACAGATAATATATGGGGCACAAGGGACAACATATCAG---ACA
Gibbon       CAAGTGAGCTCTCCGGAACCACAGATAATATTTGAGGCACAAGGGACAATATCTCAG---ACA
Rhes_cDNA    CAAGTGAGCTCTCGGAACCCACAGATAATGTATCAGGCACCAGGGACATTATTTACG---TTT
Baboon       CAAGTGAGCTCTCGGAACCCACAGATAACGTATCAGGCACCAGGGACATTATTTTCG---TTT
AGM          CAAGTGAGCTATCAGAACCCACAGATAATGTATCAGGCACCAGGGTCATCATTTGGG---TCA
AGM_cDNA     CAAGTGAGCTATCGGAACCCACAGATAATGTATCAGTCACCAGGGTCATTATTTGGG---TCA
Tant_cDNA    CAAGTGAGCTATCAGAACCCACAGATAATGTATCAGGCACCAGGGTCATCATTTGGG---TCA
Patas        CAAGTGAGCTCTCGGAACCCACAGATAATGTATTGGGCACAAGGGAAATTATTTCAG---TCA
Colobus      CGAGTGAGCTCTCCGAACCCACAGATAATGTATCGGGCACAAGGGACATTATTTCAG---TCA
DLangur      CAAGTGAGCTCTCCGAACCCACAGATAATGTGTCGGGCACGAGGGACATTATTTCAG---TCA
PMarmoset    CAAGTGAGATATCAGGTTCCG---------------------------ATACATCAA---CCA
Tamarin      CAAGTGAGATATCAGTTTCAG---------------------------ATACATCAA---CCA
Squirrel     CAAGTGAGATATCAGAAACCT---------------------------ATACGTCAC---CTA
Owl          CAAGTGAGATATCAGAAACGG---------------------------ATATATCAA---CCA
Titi         CAAGTGAGATATCAGGAATGG---------------------------ATACATCAA---TCA
Saki         CAAGTGAGATATCAGGAACGG---------------------------ATACATCAA---TCA
Howler       GAAGTGAGATATCAGGAACAG---------------------------ATACATCATCACCCG
Spider       CAAGTGAGATATCAGGAACAG---------------------------ATACATCAA---CCA
Woolly       CAAGTGAGATATCAGAAACAG---------------------------AGACATCGA---CCA

Human           ------------------------------------------------------------
Chimp           ------------------------------------------------------------
Gorilla         ------------------------------------------------------------
Orangutan       ------------------------------------------------------------
Gibbon          ------------------------------------------------------------
Rhes_cDNA       CCGTCA------------------------------------------------------
Baboon          CCGTCA------------------------------------------------------
AGM             CTCACGAATTTCAATTATTGTACTGGCGTCCTGGGCTCCCAAAGTATCACATCAAGGAAA
AGM_cDNA        CTCACGAATTTCAGTTATTGTACTGGCGTCCCGGGCTCCCAAAGTATCACATCAGGGAAA
Tant_cDNA       CTCACGAATTTCAATTATTGTACTGGCGTCCTGGGCTCCCAAAGTATCACATCAAGGAAA
Patas           ------------------------------------------------------------
Colobus         ------------------------------------------------------------
DLangur         ------------------------------------------------------------
PMarmoset       ------------------------------------------------------------
Tamarin         ------------------------------------------------------------
Squirrel        ------------------------------------------------------------
Owl             ------------------------------------------------------------
Titi            ------------------------------------------------------------
Saki            ------------------------------------------------------------
Howler          ------------------------------------------------------------
Spider          ------------------------------------------------------------
Woolly          ------------------------------------------------------------
```

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                 F  V  N  F  N  Y  C  T  G  I
Human           TTTGTGAATTTCAATTATTGTACTGGCATCCTGGGCTCTCAAAGTATCACATCAGGGAAA(358)
Chimp           TTTATGAATTTCAATTATTGTACTGGCATCCTGGGCTCTCAAAGTATCACATCAGGGAAA
Gorilla         TTTATGAATTTCAATTATTGTACGGGCATCCTGGGCTCTCAAAGTATCACATCAGGGAAA
Orangutan       TATGTGAATTTCAATTATTGTACTGGCATCCTGGGCTCTCAAAGTATCACGTCAGGGAAA
Gibbon          TTTGTGAATTTCAATTATTGTACTGGCATCCTGGGCTCTCAAAGTATCACATCAGGGAAA
Rhes_cDNA       CTCACGAATTTCAATTATTGTACTGGCGTCCTGGGCTCCCAAAGTATCACATCAGGGAAG
Baboon          CTCACGAATTTCAATTATTGTACTGGCGTCCTGGGCTCCCAAAGTATCACATCAGGGAAG
AGM             CTCACGAATTTCAATTATTGTACTGGCGTCCTGGGCTCCCAAAGTATCACATCAGGGAAA
AGM_cDNA        CTCACGAATTTCAATTATTGTACTGGCGTCCTGGGCTCCCAAAGTATCACATCAGGGAAA
Tant_cDNA       CTCACGAATTTCAATTATTGTACTGGCGTCCTGGGCTCCCAAAGTATCACATCAGGGAAA
Patas           CTCAAGAATTTCAATTATTGTACTGGCATCCTGGGCTCCCAAAGTATCACATCAGGGAAA
Colobus         CTCAAGAATTTCATTTATTGTACTGGCGTCCTGGGCTCCCAAAGTATCACATCAGGGAAA
DLangur         CTCAAGAATTTCATTTATTGTACTGGCGTCCTGGGCTCCCAAAGTATCACATCAGGGAAA
PMarmoset       CTTGTGAAAGTCAAGTATTTTTATGGCGTCCTGGGCTCCCTAAGTATCACATCAGGGAAA
Tamarin         TCTGTGAAAGTCAACTATTTTTATGGCGTCCTGGGCTCCCCAAGTATCACATCAGGGAAA
Squirrel        CTTGTGAAAGTCCAGTATTTTTATGGCGTCCTGGGCTCCCCAAGTATCACATCAGGGAAA
Owl             TTTCTGAAAGTCAAGTATTTTTGTGGCGTCCTGGGCTCCCCAAGTATCACATCAGGGAAA
Titi            TCTGGGAGAGTCAAGTATTTTTATGGCGTCCTGGGCTCCCCAAGTATCACATCAGGGAAA
Saki            TTTGGGAAAGTCAAGTATTTTTATGGCGTCCTGGGCTCCCCAAGTATCAGATCAGGGAAA
Howler          TCTATGGAAGTCAAGTATTTTTATGGCATCCTGGGCTCCCCAAGTATCACATCAGGGAAA
Spider          TCTGTGAAAGTCAAGTATTTTTGTGGCGTCCTGGGCTCCCCAGGTTTCACATCAGGGAAA
Woolly          TCTGTGAAAGCCAAATATTTTTATGGCGTCCTGGGCTCCCCAAGTTTCACATCAGGGAAA

Human           CATTACTGGGAGGTAGACGTGTCCAAGAAAACTGCTTGGATCCTGGGGGTATGTGCTGGC(378)
Chimp           CATTACTGGGAGGTAGACGTGTCCAAGAAAAGTGCTTGGATCCTGGGGGTATGTGCTGGC
Gorilla         CATTACTGGGAGGTAGACGTGTCCAAGAAAAGTGCTTGGATCCTGGGGGTATGTGCTGGC
Orangutan       CATTACTGGGAGGTAGACGTGTCCAAGAAAAGTGCTTGGATCCTGGGGGTATGTGCTGGC
Gibbon          CATTACTGGGAGGTAGACGTGTCCAAGAAAAGTGCTTGGATCCTGGGGGTATGTGCTGGC
Rhes_cDNA       CATTACTGGGAGGTAGATGTGTCCAAGAAAAGTGCTTGGATCCTGGGGGTATGTGCTGGC
Baboon          CATTACTGGGAGGTAGATGTGTCCAAGAAAAGTGCTTGGATCCTGGGGGTATGTGCTGGC
AGM             CATTACTGGGAGGTAGATGTGTCCAAGAAAAGTGCTTGGATCCTGGGGGTATGTGCTGGC
AGM_cDNA        CATTACTGGGAGGTAGATGTGTCCAAGAAAAGTGCTTGGATCCTGGGGGTATGTGCTGGC
Tant_cDNA       CATTACTGGGAGGTAGATGTGTCCAAGAAAAGTGCTTGGATCCTGGGGGTATGTGCTGGC
Patas           CATTACTGGGAGGTAGATGTGTCCAAGAAAAGTGCTTGGATCCTGGGGGTATGTGCTGGC
Colobus         CATTACTGGGAGGTAGATGTGTCCAAGAAAAGTGCTTGGATCCTGGGGGTATGTGCTGGC
DLangur         CATTACTGGGAGGTAGATGTGTCCAAGAAAAGTGCTTGGATCCTGGGGGTATGTGCTGGC
PMarmoset       CATTACTGGGAAGTAGACGTGTCCAATAAAAGGGGTTGGATCCTGGGGGTATGTGGTAGC
Tamarin         CATTACTGGGAGGTAGACGTGACCAATAAAAGGGATTGGATCCTGGGGATATGTGTTAGC
Squirrel        CATTACTGGGAGGTAGACGTGTCCAATAAAAGGGCTTGGACCCTGGGGGTATGTGTTAGC
Owl             CATTACTGGGAGGTAGACGTGTCCAATAAAAGTGAGTGGATCCTGGGGGTATGTGTTAGC
Titi            CATTACTGGGAGGTAGACGTGTCCAATAAAAGTGCTTGGATCCTGGGGGTATGTGTTAGC
Saki            CATTACTGGGAGGTAGACGTGTCCAATAAAAGTGCTTGGATCCTGGGAGTATGTGTTAGC
Howler          CATTACTGGGAGGTAGACGTGTCCAATAAAAGTGCTTGGATCCTGGGGGTATGTGTCAGC
Spider          CATTACTGGGAGGTAGACGTGTCCGATAAAAGTGCTTGGATCCTGGGGGTATGTGTTAGC
Woolly          CATTACTGGGAGGTAGACGTGTCCAATAAAAGTGCTTGGATCCTGGGGGTATGTGTTAGC
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Human           TTCCAACCTGATGCAATG------------------------------------------(384)
Chimp           TTCCAACCTGATGCAATG------------------------------------------
Gorilla         TTCCAACCTGATGCAACG------------------------------------------
Orangutan       TTCCAACCTGATGCAATG------------------------------------------
Gibbon          TTGCAACCTGATGCAATG------------------------------------------
Rhes_cDNA       TTCCAATCCGATGCAATG------------------------------------------
Baboon          TTCCAACCTGATGCAATG------------------------------------------
AGM             TTCCAACCCGATGCAACG------------------------------------------
AGM_cDNA        TTCCAACCCGATGCAACG------------------------------------------
Tant_cDNA       TTCCAACCCGATGCAACG------------------------------------------
Patas           TTCCAACCCGATGCAATG------------------------------------------
Colobus         TTCCAACCCGATGCAATG------------------------------------------
DLangur         TTCCAACCCGATGCAATG------------------------------------------
PMarmoset       TGGAAATGCAATGCAAAA------------------------------------------
Tamarin         TTTAAATGCAATGCAAAA------------------------------------------
Squirrel        TTGAAATGTACTGCAAAT------------------------------------------
Owl             TTGAAGCGCACTGCAAGT------------------------------------------
Titi            TTGAAATGCGCTGCAAAT------------------------------------------
Saki            TTGAAATGCACTGCAAAT------------------------------------------
Howler          TTGAAATGCATTGGAAATTTTCCAGGAATTGAAAATTATCAACCTCAAAATGGCTACTGG
Spider          TTGAAATGCACTGCAAATGTTCCAGGAATTGAAAATTATCAACCTAAAAATGGCTACTGG
Woolly          TTGAAATGCACTGCAAATGTTCCAGGAATTGAAAATTATCAACCTAAAAATGGCTACTGG

Human           ------------TGTAAT------------------------------ATTGAAAAAAAT(390)
Chimp           ------------TGTAAT------------------------------ATTGAAAAAAAT
Gorilla         ------------TGTAAT------------------------------ATTGAAAAAAAT
Orangutan       ------------TATAAT------------------------------ATTGAACAAAAT
Gibbon          ------------TATAAT------------------------------ATTGAACAAAAT
Rhes_cDNA       ------------TATAAT------------------------------ATTGAACAAAAT
Baboon          ------------TATAAT------------------------------ATTGAACAAAAT
AGM             ------------TATAAT------------------------------ATTGAACAAAAT
AGM_cDNA        ------------TATAAT------------------------------ATTGAACAAAAT
Tant_cDNA       ------------TATAAT------------------------------ATTGAACAAAAT
Patas           ------------TATGAT------------------------------GTTGAACAAAAT
Colobus         ------------TATAAT------------------------------ATTGAACAAAAT
DLangur         ------------TATAAT------------------------------ATTGAACAAAAT
PMarmoset       ------------TGGAAT------------------------------GTTCTAAGACCT
Tamarin         ------------TGGAAT------------------------------GTTCTAAGACCT
Squirrel        ------------CAGAGT------------------------------GTTTCAGGAACT
Owl             ------------TGTAGT------------------------------GTTCCAAGAATT
Titi            ------------CGGAAT------------------------------GGTCCAGGAGTT
Saki            ------------CGGAAT------------------------------GGTCCAAGAATT
Howler          GTTATAGGGTTACGGAATGCAGATAACTATAGTGCTTTCCAAGATGCAGTTCCAGAAACT
Spider          GTTATAGGGTTACAGAATGCAAATAACTATAGTGCTTTCCAGGATGCAGTTCCAGGAACT
Woolly          GTTATAGGGTTACAGAATGCAGATAACTATAGTGCTTTCCAGGATGCAGTTCCAGGAACT
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Human        GAAAATTATCAACCTAAATACGGC---------------------------------------(398)
Chimp        GAAAATTATCAACCTAAATATGGC---------------------------------------
Gorilla      GAAAATTATCAACCTAAATATGGC---------------------------------------
Orangutan    GAAAATTATCAACCTCAATATGGC---------------------------------------
Gibbon       GAAAATTATCAACCTAAATATGGC---------------------------------------
Rhes_cDNA    GAAAATTATCAACCTAAATATGGC---------------------------------------
Baboon       GAAAATTATCAACCTAAATATGGC---------------------------------------
AGM          GAAAATTATCAACCTAAATATGGA---------------------------------------
AGM_cDNA     GAAAATTATCAACCTAAATATGGC---------------------------------------
Tant_cDNA    GAAAATTATCAACCTAAATATGGC---------------------------------------
Patas        GAAAATTATCAACCTAAATATGGC---------------------------------------
Colobus      GAAAATTATCAACCTAAATATGGC---------------------------------------
DLangur      GAAAATTATCAACCTAAATATGGC---------------------------------------
PMarmoset    GAAAATTATCAACCTAAAAATGGC---------------------------------------
Tamarin      GAAAATTATCAACCTAAAAATGGC---------------------------------------
Squirrel     GAAAATTATCAACCTAAAAATGGC---------------------------------------
Owl          GAAAATGATCAACCTAAAAATGGC---------------------------------------
Titi         GAAAACTATCAACCTAAAAATGGC---------------------------------------
Saki         GAAAATTATCAACCTAAAAATGGC---------------------------------------
Howler       GAAAATTATCAACCTAAAAATCGCAACCGG---TTTACAGGGTTACAGAATGCAGATAATTGT
Spider       GAAAATTATCAACCTAAAAATGGCAACCGGAGGAATAAAGGGTTACGGAATGCAGATAACTAT
Woolly       GAAGATTATCAACCTAAAAATGGCTGCTGGAGGAATACAGGGTTACGGAATGCAGATAACTAT

Human           ------------------------------------------------------TACTGG(400)
Chimp           ------------------------------------------------------TACTGG
Gorilla         ------------------------------------------------------TACTGG
Orangutan       ------------------------------------------------------TACTGG
Gibbon          ------------------------------------------------------TACTGG
Rhes_cDNA       ------------------------------------------------------TACTGG
Baboon          ------------------------------------------------------TACTGG
AGM             ------------------------------------------------------TACTGG
AGM_cDNA        ------------------------------------------------------TACTGG
Tant_cDNA       ------------------------------------------------------TACTGG
Patas           ------------------------------------------------------TACTGG
Colobus         ------------------------------------------------------TACTGG
DLangur         ------------------------------------------------------TACTGG
PMarmoset       ------------------------------------------------------TACTGG
Tamarin         ------------------------------------------------------TACTGG
Squirrel        ------------------------------------------------------TACTGG
Owl             ------------------------------------------------------TACTGG
Titi            ------------------------------------------------------TACTGG
Saki            ------------------------------------------------------TACTGG
Howler          AGTGCTTTCCAGAATGCATTTCCAGGAATTCAAAGTTATCAACCTAAAAAGAGCCACTTG
Spider          AGTGCTTTCCGGGATACATTT------------------CAACCTATAAATGACTCCTGG
Woolly          AGTGCTTTCCAGGATGTATTT------------------CAACCTAAAAATGACTACTGG
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Human           GTTATAGGGTTA---------------------------GAGGAAGGAGTTAAATGTAGT(411)
Chimp           GTTATAGGGTTA---------------------------GAGGAAGGAGTTAAATGTAGT
Gorilla         GTTATAGGGTTA---------------------------GAGGAAGGAGTTAAATGCAGT
Orangutan       GTTATAGGGTTA---------------------------GAGGAAGGAGTTAAATGTAGT
Gibbon          GTTATAGGGTTA---------------------------GAGGAAGGAGTTAAATGTAAT
Rhes_cDNA       GTTATAGGGTTA---------------------------CAGGAAGGAGTTAAATATAGT
Baboon          GTTATAGGGTTA---------------------------CAGGAAGGAGTTAAATATAGT
AGM             GTTATAGGGTTA---------------------------CAGGAAGGAGATAAATATAGT
AGM_cDNA        GTTATAGGGTTA---------------------------CAGGAAGGAGATAAATATAGT
Tant_cDNA       GTTATAGGGTTA---------------------------CAGGAAGGAGATAAATATAGT
Patas           GTTATAGGGTTA---------------------------CAGGAAGGAGTAAAATATAGT
Colobus         GTTATAGGGTTA---------------------------CAGGAAGGAGTTAAATATAGT
DLangur         GTTATAGGGTTA---------------------------CAGGAAGGAGTTAAATATAAT
PMarmoset       GTTATAGGGTTACGGAATACAGATAACTATAGTGCTTTCCAGGATGCAGTTAAATATAGT
Tamarin         GTTATAGGGTTACAGAATACAAATAACTATAGTGCTTTCCAGGATGCAGTTAAATATAGT
Squirrel        GTTATAGGGTTACGGAATGCAGGTAACTATAGGGCTTTCCAGAGTTCATTTGAATTTCGT
Owl             GTTATAGGGTTACGGAATGCAGATAACTATAGTGCTTTCCAGGATGCAGTTGAATATAGT
Titi            GTGATAGGGTTACGGAATGCAGATAACTATAGTGCTTTCCAGGATTCAGTTAAATATAAT
Saki            GTTATAGGGTTATGGAATGCAGGTAACTATAGTGCTTTCCAGGATTCAGTTAAATATAGT
Howler          TTTACAGGGTTACAGAATCTAAGTAACTATAATGCTTTCCAGAATAAAGTTCAATATAAC
Spider          GTTACAGGGTTACGGAATGTAGATAACTATAATGCTTTCCAGGATGCAGTTAAATATAGT
Woolly          GTTACAGGGTTATGGAACGCAGATAACTATAATGCTTTCCAGGATGCAGGTAAATATAGT


Human           ------GCTTTCCAGGATAGTTCCTTCCATACTCCTTCTGTTCCTTTCATTGTGCCCCTC(429)
Chimp           ------GCTTTCCAGGATGGTTCCTTCCATACTCCTTCTGCTCCTTTCATTGTGCCCCTC
Gorilla         ------GCTTTCCAGGATGGTTCCTTCCATACTCCTTCTGCTCCTTTCATTGTGCCCCTC
Orangutan       ------GCTTTCCAGGATGGTTCCTTCCATAATCCTTCTGCTCCTTTCATTGTGCCCCTC
Gibbon          ------GCTTTCCAGGATGGTTCCATCCATACTCCTTCTGCTCCTTTCGTTGTGCCCCTC
Rhes_cDNA       ------GTTTTCCAGGATGGTTCCTCACATACTCCTTTTGCTCCTTTCATTGTGCCCCTC
Baboon          ------GTTTTCCAGGATGGTTCCTCACATACTCCTTTTGCTCCTTTCATTGTGCCCCTC
AGM             ------GTTTTCCAGGATAGTTCCTCACATACTCCTTTTGCTCCTTTCATTGTGCCCCTC
AGM_cDNA        ------GTTTTCCAGGATGGTTCCTCACATACTCCTTTTGCTCCTTTCATTGTGCCCCTC
Tant_cDNA       ------GTTTTCCAGGATGGTTCCTCACATACTCCTTTTGCTCCTTTCATTGTGCCCCTC
Patas           ------GTTTTCCAGGATGGTTCCTCACATACTCCTTTTGCTCCTTTCATTGCGCCCCTC
Colobus         ------GTTTTCCAGGATGGTTCCTCACATACTCCTTTTGCTCCTTTCATTGTGCCCCTC
DLangur         ------GTTTTCCAGGATGGTTCCTCACATACTCCTTTTGCTCCTTTCATTGTGCCCCTC
PMarmoset       ------GATGTCCAGGATGGTTCTCGCTCTGTTTCTTCTGGTCCTTTGATCGTGCCCCTC
Tamarin         ------GATTTCCAGATTGGTTCCCGCTCTACTGCTTCTGTTCCTTTGATCGTGCCCCTC
Squirrel        ------GATTTCCTGGCTGGTTCCCGCCTTACTCTTTCTCCTCCTTTGATCGTGCCCCTC
Owl             ------GATTTCCAGGATGGTTCCCGCTCTACTCCTTCTGCTCCTTTGATCGTGCCCCTC
Titi            ------GATTTCCAGGATGGTTCCCGCTCTACTACTTATGCTCCTTTGATCGTGCCCCTC
Saki            ------GATTTCCAGGATGGTTCCCACTCTGCTACTTATGGTCCTTTGATCGTGCCCCTC
Howler          TATATTGATTTTCAGGATGATTCCCTCTCTACTCCTTCTGCTCCTTTGATCGTACCCCTC
Spider          ------GATTTCCAGGATGGTTCCTGCTCTACTCCTTCTGCTCCTTTGATGGTGCCCCTC
Woolly          ------GATTTCCAGGATGGTTCCTGCTCTACTCCTTTTGCTCCTTTGATTGTGCCCCTC
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Human          TCTGTGATTATTTGTCCTGATCGTGTTGGAGTTTTCCTAGACTATGAGGCTTGCACTGTC(449)
Chimp          TCTGTGATTATTTGTCCTGATCGTGTTGGAGTTTTCCTAGACTATGAGGCTTGCACTGTC
Gorilla        TCTGTGATTATTTGTCCTGATCGTGTTGGAGTTTTCCTAGACTATGAGGCTTGCACTGTC
Orangutan      TCTGTGATTATTTGTCCTGATCGTGTTGGAGTTTTCCTAGACTATGAGGCTTGCACTGTC
Gibbon         TCTGTGAATATTTGTCCTGATCGTGTTGGAGTTTTCCTAGACTATGAGGCTTGCACTGTC
Rhes_cDNA      TCTGTGATTATTTGTCCTGATCGTGTTGGAGTTTTCGTAGACTATGAGGCTTGCACTGTC
Baboon         TCTGTGATTATTTGTCCTGATCGTGTTGGAGTTTTCGTAGACTATGAGGCTTGCACTGTC
AGM            TCTGTGATTATTTGTCCTGATCGTGTTGGAGTTTTCGTAGACTATGAGGCTTGCACTGTC
AGM_cDNA       TCTGTGATTATTTGTCCTGATCGTGTTGGAGTTTTCGTAGACTATGAGGCTTGCACTGTC
Tant_cDNA      TCTGTGATTATTTGTCCTGATCGTGTTGGAGTTTTCGTAGACTATGAGGCTTGCACTGTC
Patas          TCTGTGATTTTTTGTCCTGATCGTGTTGGAGTTTTCGTAGACTATGAGGCTTGCACTGTC
Colobus        TCTGTGATCATTTGTCCTGATCGTGTTGGAGTTTTCGTAGACTATGAGGCTTGCACTGTC
DLangur        TCTGTGATTATTTGTCCTGATCGTGTTGGAGTTTTCGTAGACTATGAGGCTTGCACTGTC
PMarmoset      TTTATGACTATTTGTCCTAATCGTGTTGGAGTTTTCCTAGACTATGAGGCTTGCACTATC
Tamarin        TTTATGACTATTTATCCTAATCGTGTTGGAGTTTTCCTAGACTATGAGGCTTGCACTGTC
Squirrel       TTTATGACTATTTGTCCTAATCGGGTCGGAGTTTTCCTAGACTATGAGGCTCGCACTATC
Owl            TTTATGACTATTTGTCCTAATCGTGTTGGAGTTTTCCTAGACTATGAGGCTTGCACTGTC
Titi           TTTATGACTATTTGTCCTAATCGTGTTGGAGTTTTCCTAGACTATGAGGCTTGCACTGTC
Saki           TTTATGACTATTTGTCCTAATCGTGTTGGAGTTTTCCTAGACTATGAGGCTTGCACTGTC
Howler         TTTATGACTATTTGTCCTAAACGTGTTGGAGTTTTCCTAGACTATGAGGCTTGCACTGTC
Spider         TTTATGACTATTTGTCCTAAACGTGTTGGAGTTTTCCTAGACTGTAAGGCTTGCACTGTC
Woolly         TTTATGACTATTCGTCCTAAACGTGTTGGCGTTTTCCTAGACTATGAGGCTTGCACTGTC

Human          TCATTCTTCAATATCACAAACCATGGATTTCTCATCTATAAGTTTTCTCACTGTTCTTTT(469)
Chimp          TCATTCTTCAATATCACAAACCATGGATCTCTCATCTATAAGTTTTCTCACTGTTCTTTT
Gorilla        TCATTCTTCAATATCACAAACCATGGATTTCTCATCTATAAGTTTTCTCACTGTTCTTTT
Orangutan      TCATTCTTCAATATCACAAACCATGGATTTCTCATCTATAAGTTTTCTCACTGTTCTTTT
Gibbon         TCATTCTTCAATATCACAGACCATGGATTTCTCATCTATAAGTTTTCTCACTGTTCTTTT
Rhes_cDNA      TCATTCTTCAATATCACAAACCATGGATTTCTCATCTATAAGTTTTCTCAGTGTTCTTTT
Baboon         TCATTCTTCAATATCACAAACCATGGATTTCTCATCTATAAGTTTTCTCAGTGTTCTTTT
AGM            TCATTCTTCAATATCACAAACCATGGATTTCTCATCTATAAGTTTTCTCAGTGTTCTTTT
AGM_cDNA       TCATTCTTCAATATCACAAACCATGGATTTCTCATCTATAAGTTTTCTCAGTGTTCTTTT
Tant_cDNA      TCATTCTTCAATATCACAAACCATGGATTTCTCATCTATAAGTTTTCTCAGTGTTCTTTT
Patas          TCATTCTTCAATATCACAAACCATGGATTTCTCATCTATAAGTTTTCTCAGTGTTCTTTT
Colobus        TCATTCTTCAATATCACAAACCATGGATTTCTCATCTATAAGTTTTCTCAGTGTTCTTTT
DLangur        TCATTCTTCAATATCACAAACCATGGATTTCTCATCTATAAGTTTTCTCAGTGTTCTTTT
PMarmoset      TCATTCTTCAATGTCACAAGCAATGGATTTCTCATCTATAAGTTTTCTAACTGTCATTTT
Tamarin        TCATTCTTCAATGTCACAAACAATGGATTTCTCATCTATAAGTTTTCTAACTGTCATTTT
Squirrel       TCATTCTTCAATGTCACAAGCAATGGATTTCTCATCTACAAGTTTTCTGACTGTCATTTT
Owl            TCATTCTTCAATGTCACAAACAATGGATTTCTCATCTATAAGTTTTCTAACTGTCATTTT
Titi           TCATTCTTCAATGTCACAAGCAATGGATTTCTCATCTATAAGTTTTCTAACTGTCATTTT
Saki           TCATTCTTCAATGTCACAAGCAATGGATTTCTCATCTATAAGTTTTCTAACTGTCGTTTT
Howler         TCATTCTTCAATGTCACAAGCAATGGATATCTCATCTATAAGTTTTCTAACTGTCAGTTT
Spider         TCATTCTTCAATGTCACAAGCAATGGATGTCTCATCTATAAGTTTTCTAAGTGTCATTTT
Woolly         TCATTCTTCAATGTCACAAGCAATGGATGTCTCATCTATAAGTTTTCTAACTGTCATTTT
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Human           TCTCAGCCTGTATTTCCATATTTAAATCCTAGAAAATGTGGAGTCCCCATGACTCTGTGC(489)
Chimp           TCTCAGCCTGTATTTCCATATTTAAATCCTAGAAAATGTGGAGTCCCCATGACTCTGTGC
Gorilla         TCTCAGCCTGTATTTCCATATTTAAATCCTAGAAAATGTAGAGTCCCCATGACTCTGTGC
Orangutan       TCTCAGCCTGTATTTCCATATTTAAATCCTAGAAAATGTAGAGTCCCCATGACTCTGTGC
Gibbon          TCTCAGCCTGTATTTCCATATTTAAATCCTAGAAAATGTACAGTCCCCATGACTCTGTGC
Rhes_cDNA       TCTAAGCCTGTATTTCCATATTTAAATCCCAGAAAATGTACAGTCCCCATGACTCTGTGC
Baboon          TCTAAGCCTGTATTTCCATATTTAAATCCCAGAAAATGTACAGTCCCCATGACTCTGTGC
AGM             TCTAAGCCTGTATTTCCATATTTAAATCCCAGAAAATGTACAGTCCCCATGACTCTGTGC
AGM_cDNA        TCTAAGCCTGTATTTCCATATTTAAATCCCAGAAAATGTACAGTCCCCATGACTCTGTGC
Tant_cDNA       TCTAAGCCTGTATTTCCATATTTAAATCCCAGAAAATGTACAGTCCCCATGACTCTGTGC
Patas           TCTAAGCCTGTATTTCCATATTTAAATCCCAGAAAATGTACAGTCCCCATGACTCTGTGC
Colobus         TCTAAGCCTGTATTTCCATATTTAAATCCTAGAAAATGTACAGTCCCCATGACTCTGTGC
DLangur         TCTAAGCCTGTATTTCCATATTTAAATCCTAGAAAATGTACAGTCCCCATGACTCTGTGC
PMarmoset       TCTTATCCTGTATTTCCATATTTCAGTCCTACGACATGTGAATTACCCATGACTCTGTGC
Tamarin         TCTTATCCTGTATTTCCATATTTCAGTCCTATGACATGTGAATTACCCATGACTCTGTGT
Squirrel        TCTTATCCTGTATTTCCATATTTCAATCCTATGACGTGTGAATTACCCATGACTCTGTGC
Owl             TGTTATCCTGTATTTCCATATTTCAGTCCTATGACATGTGAATTACCCATGACTCTGTGC
Titi            TCTTATCCTGTATTTCCATATTTCAGTCCTATGACATGTGAATTACCCATGACTCTGTGC
Saki            TCTGATTCTGTATTTCCATATTTCAGTCCTATGACATGTGAATTACCCATGACTCTGTGC
Howler          TCTTATCCTGTATTTCCATATTTCAGTCCTATGACATGTGAATTACCCATGACTCTGTGC
Spider          TCTTATCCTGTATTTCCATATTTCAGTCCTATGATATGTAAATTACCCATGACTCTGTGC
Woolly          TCTTGTCCTGTATTTCCATATTTCAGTCCTATGACATGTAAATTACCCATGACTCTGTGC

Human           TCACCAAGCTCTTGA(493)
Chimp           TCACCAAGCTCTTGA
Gorilla         TCGCCAAGCTCTTGA
Orangutan       TCACCAAGCTCTTGA
Gibbon          TCACCAAGCTCTTGA
Rhes_cDNA       TCACCAAGCTCTTGA
Baboon          TCACCAAGCTCTTGA
AGM             TCACCAAGCTCTTGA
AGM_cDNA        TCACCAAGCTCTTGA
Tant_cDNA       TCACCAAGCTCTTGA
Patas           TCACCAAGCTCTTGA
Colobus         TCACCAAGCTCTTGA
DLangur         TCACCAAGCTCTTGA
PMarmoset       TCACCAAGCTCTTGA
Tamarin         TCACCAAGCTCTTGA
Squirrel        TCACCAAGGTCTTGA
Owl             TCACCAAGCTCTTGA
Titi            TCACCAAGGTCTTGA
Saki            TCACCAAGATCTTGA
Howler          TCACCAAGCTCTTGA
Spider          TCACCAAGCTCTTGA
Woolly          TCACCAAGCTCTTGA
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Supporting Information

Files in this Data Supplement:

Supporting Table 2
Supporting Appendix 1
Supporting Data Set 1
Supporting Figure 4

Supporting Data Set 1

Data Set 1. Alignment of TRIM5α sequences from primates analyzed in this study. Protein domains are indicated by boxes on the human sequence. Numbers at the end of each line (in parentheses) indicate the human codon number. Vertical lines denote exon breakpoints. PAML-highlighted codons (codons evolving under positive selection) are denoted with blue (highlighted in analysis of full data set) or yellow (highlighted in hominid+OWM data set) highlighting. Purple boxed human sequence and purple translated residues above the alignment indicate the SPRY "patch." Blocks of sequence in orange type contain gaps and were omitted from the PAML analysis of the full data set. The small section blocked with orange lines (and orange type) cannot be unambiguously aligned to the rest of the alignment. Two AGM sequences are present in the alignment. "AGM-cDNA" is cDNA read no. AY625003 from GenBank. This sequence was not used in the final analysis, and instead the read titled "AGM" (our sequence from Cos-7 DNA) was used. The owl monkey sequence in this alignment (gift from J. Luban, Columbia University, New York) also was not used in the analysis, because it has not been shown that TRIM5α is transcribed as well as TRIM-Cyp.

Supporting Figure 4

Fig. 4. Positive selection of the SPRY domain. dN is plotted vs. dS for all pairwise combinations of primate sequences. These values are calculated by using the Nei–Gojobori method by PAML software [Yang, Z. (1997) *Comput. Appl. Biosci.* **13,** 555–556]. Pairwise combinations of hominids (hom),

Old World monkeys (owm), and New World monkeys (nwm) are plotted with different symbols as shown in the legend. Points that are making comparisons between human and another primate are in a black box. In the first graph, the entire gene length is analyzed, and in the second and third graphs, the gene has been divided into two at the beginning of the SPRY (as defined in the alignment shown). The SPRY domain is predominantly evolving under positive selection, whereas the remainder of the gene shows a net signature of purifying selection.

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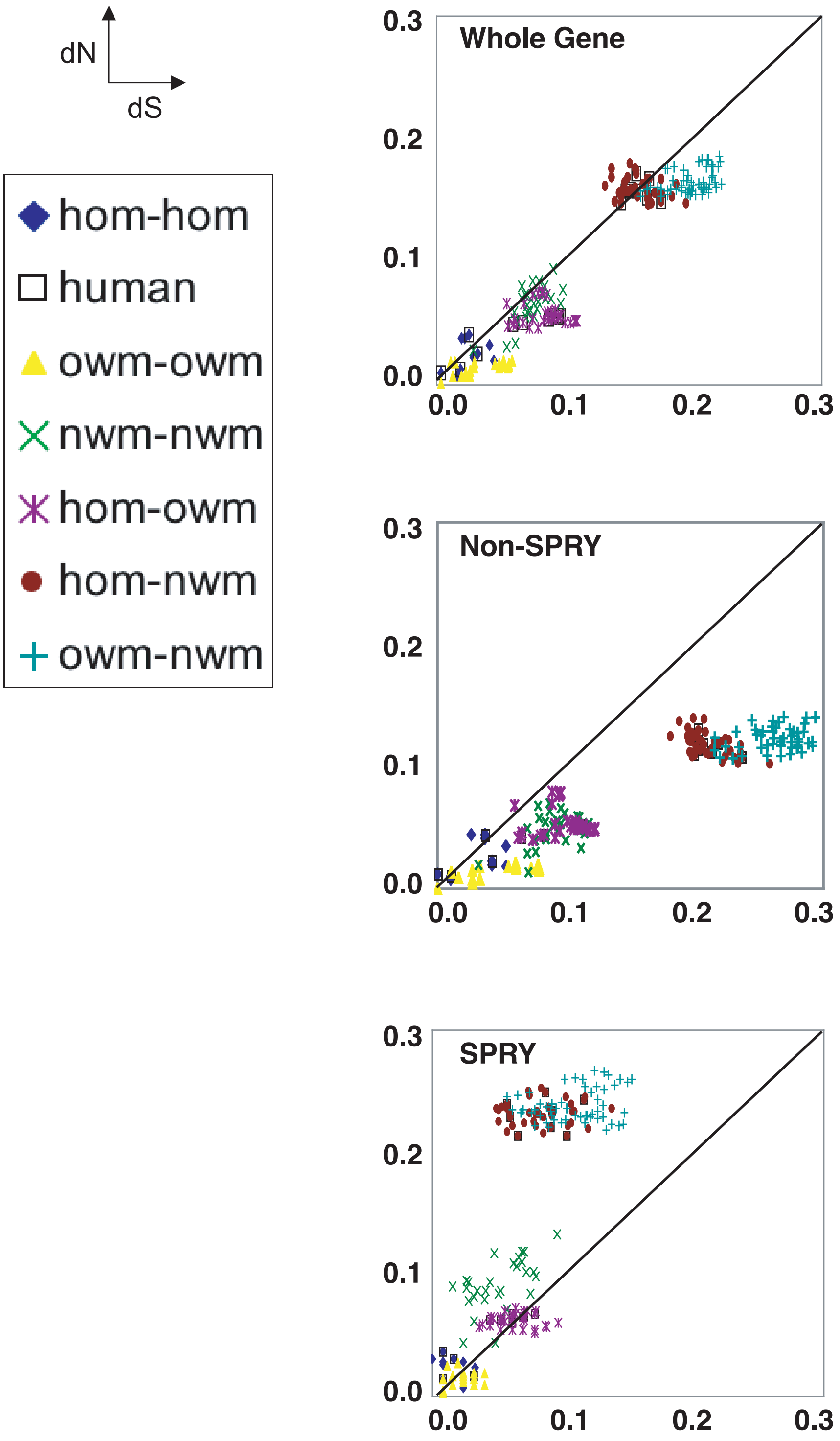
dN
dS
◆ hom-hom
☐ human
▲ owm-owm
× nwm-nwm
⋇ hom-owm
● hom-nwm
+ owm-nwm
Whole Gene
Non-SPRY
SPRY
0.3
0.2
0.1
0.0
0.0
0.1
0.2
0.3