Using several pair-wise informant sequences for de novo prediction of alternatively spliced transcripts

Paul Flicek* and Michael R Brent†

Addresses: *European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK. †Laboratory for Computational Genomics, Washington University, Saint Louis, MO 63130, USA.

Correspondence: Paul Flicek. Email: flicek@ebi.ac.uk

Published: 7 August 2006

Research

Background: Accurate prediction of protein-coding genes in mammals remains a challenging and active area of research [1]. In the past decade the most important advance in de novo gene prediction came with the initial availability of extensive human and mouse genomic sequences. Several gene prediction algorithms were introduced at that time that improved gene prediction by using the specific patterns of evolutionary conservation that are indicative of protein coding genes [2-4].

Dual-genome gene finding algorithms

All of the dual-genome (category 4) gene finders participating in EGASP rely on alignments to one or more informant genome sequences. For predicting human genes, dual-genome gene prediction algorithms most often use the mouse genome sequence as a source of evolutionary conservation information. This was originally a consequence of the early availability, with respect to other mammals, of the mouse genome sequence [5-8]. However, as additional genomes were sequenced, it became apparent that the...
evolutionarily divergence between human and mouse is near the point of optimal value for dual-genome gene prediction [9-11].

Twinscan is one of the most accurate de novo dual-genome gene prediction algorithms. It has proven effective for genome annotation in nematodes [12], plants [13], fungi [14], and mammals [6,15]. Recently, the gene-prediction program N-SCAN was introduced as a way to incorporate whole-genome multiple alignments into gene prediction [11]. Twinscan is a special case of the more general N-SCAN algorithm.

Both Twinscan and N-SCAN have focused on the prediction of the single mostly likely transcript in a given gene locus, although alternative splicing is now known to occur in a large majority of mammalian genes. In fact, Kan et al. [16] reported that nearly all genes with high expressed sequence tag (EST) coverage showed evidence of multiple splice forms. Even the well characterized human alpha globin cluster was recently shown to contain previously unknown, small, alternatively spliced exons [17]. Moreover, rare alternatively spliced transcripts can have important consequences in health and disease [18].

In an attempt both to address the problem of de novo prediction of alternatively spliced genes and to improve multi-genome de novo gene prediction, we developed the MARS (‘Multiple Informants: Alternative Splices’) extension to the Twinscan algorithm.

Almost all current methods for automatically annotating alternatively spliced transcripts rely on a rich EST database [19-21]. One of the few exceptions to an EST-based technique used a pair-hidden Markov model (pair-HMM) to successfully identify alternatively spliced exons conserved in human and mouse [22]. These conserved alternative splicing events are thought to be relatively rare [23]. MARS seeks to leverage the apparently more common situation that for some human genes only one splice variant appears to be conserved in another species [24,25]. One recently described example is the Tfam gene, which encodes a mitochondrial transcription factor and has a conserved alternative isoform in primates and rat, but not in mouse [26].

Description of the MARS algorithm
The MARS algorithm consists of two major steps. In the first step, transcript predictions are created from a number of different evolutionarily related informant sequences using Twinscan. For EGASP, MARS used the publicly available assemblies of the mouse (UCSC id mm5), rat (rn3), dog (canFam1), chicken (galGal1), frog (xenTro1), and opossum (monDom1) genomes as informant sources for Twinscan. The predictions described in this paper are based on the Twinscan algorithm.

The results for the updated MARS algorithm differ from those reported in the EGASP summary because of the updates to the MARS algorithm that are described above. Compared to the submitted predictions, those produced from the updated MARS algorithm are more sensitive compared to the GENCODE annotation, but less specific at both the transcript and exon levels. A summary of the accuracy of the EGASP submission version of the MARS algorithm and the updated version described in this paper is given in Table 1. The updated predictions also include approximately twice as many coding transcripts per gene as the predictions submitted to EGASP. Because we made very limited use of the 13 EGASP training regions, we have chosen to present results here based on all 44 regions. These
considerations result in a slight difference between the EGASP evaluation of the submitted results and those displayed in Table 1, but do not materially change the results or the interpretation of them.

**Transcript predictions from individual informant sources**

For the set of all 44 ENCODE regions, each individual informant results in a similar number of predicted transcripts (Table 2). Informant sources at greater evolutionary distances tend to result in fewer, longer transcripts than informants within the mammalian lineage. However, the summary information from the ENCODE regions presented in Table 2 only hints at the diversity of predicted transcripts from the various informant sources. For example, mouse and rat shared a common ancestor approximately 25 million years ago and align similar fractions of the human genome using our alignment procedure (see Materials and methods and Table 3), but using these two rodent genome sequences as informant sources leads to a significantly different set of transcripts. In fact, the total number of predicted transcripts made using the mouse genome as the informant sequence is similar to the total number of predicted transcripts using the rat genome as the informant sequence (486 and 476, respectively), but less than 50% (213) of these transcripts are predicted to have identical intron-exon structure. Similar results are seen on the human genome as a whole (data not shown).

The four mammalian informant sequences lead to more accurate predictions than either the frog or the chicken informant. Predictions based on the opossum informant sequence are slightly more accurate than those based on either mouse or rat (Figure 1). Compared to the rodent informant sequences the dog sequence aligns significantly more of the ENCODE regions, without additional alignment in the coding sequence. Conversely, the opossum aligns approximately one-third the total number of bases as the rodent sequences, while retaining alignment in 76% of the coding regions (Table 3).

**Informative value of the pair-wise alignments**

The alignment characteristics for each of the six informant sequences shown in Table 3 are primarily responsible for the characteristics of the pair-wise prediction sets shown in Table 2. To assess how the alignments affect the various
components of the Twinscan conservation model, we calculated the information gain of the alignments with respect to the training sequence annotations (see Figure 2 and Materials and methods). The difference in the number of exons per transcript is partially the effect of the amount of information available to the coding portion of the model and the translation initiation and termination signals (that is, the transcript ends). In cases such as mouse, rat, and opossum, where the information gain of the alignments with respect to the annotations is relatively high in both the coding regions and the transcript ends, the number of exons per transcript most closely resembles the annotation. When the information gain for the coding region portion of the conservation model is relatively high and the information gain for the transcript ends is relatively low, longer genes are predicted because the relative information gain of correct gene boundaries is low with respect to incorrect gene boundaries, thus the model is less inclined to end a transcript. In other words, for the case of the frog and the chicken informant sequence, it is more probable, under the model, for a gene to contain additional internal exons rather than boundary exons, which also contain the translation initiation or translation termination signals. This effect also leads to a greater number of exon predictions for the more distantly related informant species. For the case of the dog informant, in which the information gain in both the coding regions and the transcript ends of the model is relatively low, genes are predicted with fewer exons than the annotation. The number of exons per transcript from the dog informant-based predictions is more similar to \textit{ab initio} transcript predictions that do not use evolutionary conservation, such as those reported in group 2 of the EGASP experiment [1].

**MARS genes predicted from informant sets**

As MARS genes are created from an increasing number of informant sources, we see an increase in predictive sensitivity as the transcripts based on each additional informant sequence are added to the genes. At the same time, the gene specificity improves as addition of longer transcripts from non-mammalian informant sources leads to longer genes (Figure 3).

The predictive sensitivity of both the coding exons and complete coding transcripts also increases as the predictions based on each additional informant sequence are clustered together, but the specificity falls as the number of apparent false positive transcripts and CDS exons increases. The difference in the performance trend at the gene level and the transcript level is based on the definition of gene level accuracy, which rewards predicting at least one transcript correctly with no penalty for additional, incorrectly predicted transcripts.

Both the number of coding exons and the number of transcripts in each MARS gene increase with the size of the
informant set (Table 4). This increase corresponds partly to the usage of distantly related informant species in the informant set. For example, MARS genes predicted by the three species informant subset that includes mouse, rat, and dog average 8.07 exons per gene, while MARS genes from the frog, chicken, and opossum informant subset average 11.64 exons per gene.

We separately evaluated the subset of transcripts that are predicted based on at least two informant sequences. These transcripts are significantly more specific at all levels of the evaluation (Table 5), although the predictions are less sensitive (as expected). The set of transcripts common to only mammalian informant sources is more specific than the set common to all informant sources and only slightly less sensitive (Table 5).

**Prediction of alternatively spliced transcripts**

Using the informant set consisting of all six informant sources, MARS correctly predicts alternatively spliced transcripts for 11 of the 236 multi-exon GENCODE genes that are alternatively spliced in the coding region of their transcripts. For these 11 genes, a total of 24 (out of 59) correct transcripts are predicted (we observed that just 2 of these 11 genes accounted for 25 of the 59 coding transcripts: RP1-309K30.2 on ENr333 and RP4-696P19.3 on ENr334). Moreover, when compared to a set of 134 cassette (that is, skipped) coding exons from the GENCODE annotation, MARS predicted 85 of these exons correctly in at least one transcript, including 19 that are correctly predicted as cassette exons. MARS predicts a total of 247 cassette exons.

### Table 4

**Effect of increasing informant subset size**

| Informant set size | Two | Three | Four | Five | Six | Annotation |
|--------------------|-----|-------|------|------|-----|------------|
| Average transcripts per gene | 1.76 | 2.44 | 3.05 | 3.62 | 4.15 | 2.25 |
| Average exons per transcript | 9.31 | 9.67 | 9.94 | 10.16 | 10.35 | 8.64 |

The number of coding transcripts per gene and coding exons per transcripts increases with the cardinality of the informant set. The gene level accuracy also increases with informant set size (see Figure 3).

### Table 5

**Transcripts common to several informant sources**

| Prediction accuracy for transcripts common to several informants | ESn | ESp | TSn | TSp | GSn | GSp |
|-----------------------------------------------------------------|-----|-----|-----|-----|-----|-----|
| All transcripts | 74.4% | 45.1% | 19.4% | 10.4% | 40.6% | 33.0% |
| Common transcripts | 43.0% | 71.6% | 15.1% | 29.6% | 32.0% | 35.0% |
| Mammalian transcripts | 40.0% | 77.1% | 14.8% | 33.7% | 31.3% | 37.7% |

A comparison of the predictive accuracy for all MARS genes, with those having at least two transcripts predicted with identical structure from more than one informant source across the entire informant set, and with those having two transcripts with identical structures from at least two mammalian informant sources. Columns are defined as in Table 1.
When all six informant sources are used simultaneously, the predictive sensitivity is at its highest. MARS predicts about twice as many unique coding transcripts (1,873) as exist in the reference GENCODE annotation (975).

**Experimental verification**

An important part of the EGASP experiment is the attempt to experimentally validate a subset of the computational predictions outside of the reference GENCODE annotation. As part of EGASP, Guigo et al. [1] selected a total of 47 exon pairs predicted by MARS for experimental confirmation by RT-PCR. Of these, 7 (15%) were found to be expressed in at least one tissue. Interestingly, although a number of the other EGASP gene-prediction methods also predicted as many as 4 of these exon pairs, these 7 were the only ones that could be confirmed in the EGASP experiment.

**Discussion**

One of the goals of the ENCODE pilot project was to develop new high-throughput methods to identify the functional elements in the human genome [28,29]. To address the continued need for de novo gene discovery, we have introduced the MARS method for prediction of alternatively spliced transcripts without the use of any expressed sequence information. MARS genes are built by combining the predicted transcripts from a number of informant species and are significantly more likely to contain correctly predicted transcripts than any individual informant. MARS performed effectively when compared to other dual-genome de novo gene prediction systems in EGASP [1] and is unique among the EGASP methods in its ability to predict alternatively spliced transcripts using only patterns found in pair-wise alignments between a target sequence and a set of informant sequences.

We have updated the MARS algorithm between the EGASP workshop and submission of this paper. The updated version of MARS correctly predicts multiple alternatively spliced transcripts at one additional locus compared to the submitted version. Additionally, the updated algorithm is more sensitive for all measures, although this increased sensitivity comes at a cost of a significant reduction in specificity at the transcript and exon levels compared to the version submitted to the EGASP workshop. Regardless, we feel the update is justified on theoretical grounds because the original submission gives too much consideration to the mouse informant to the detriment of other informant sources. A second source of error comes from the addition of transcripts from the two non-mammalian informant sources, which appears to have enriched the prediction set for false positive transcripts.

A number of gene-finding algorithms create consensus genes by combining sets of gene predictions and other information [31]. One example in the EGASP experiment is JIGSAW [31], a program that uses ‘any information’ (EGASP category 1) to create gene structures. Much of the information used by JIGSAW is based on expressed sequences and is, therefore, not directly comparable to the EGASP dual-genome (category 4) predictions. Because MARS genes are created by overlapping transcript predictions from a number of sources, we were interested to see if these transcript predictions could be statistically combined to produce more accurate consensus gene structures. To directly address this question, we compared the MARS genes to consensus genes produced by GLEAN, a new gene-prediction algorithm that uses dynamic programming to discover gene structures that maximize the probability of several sources of evidence (A Mackey, personal communication). GLEAN was run using the transcript predictions from the six individual pair-wise sets (mouse, rat, chicken, dog, frog, and opossum) as its only input sources of evidence, although the transcript sets cannot be considered independent sources of information and thus represent a non-traditional use of the algorithm likely to reduce its statistical power (A Mackey, personal communication). The GLEAN consensus predictions at the gene and transcript levels were similar to predictions based on either the rat or dog informant only (that is, less sensitive and specific than the mouse or opossum informant, but more sensitive and specific than the chicken or frog informants). For coding exons, the GLEAN consensus predictions are more sensitive than any of the individual informants and less specific than predictions based on mammalian or marsupial informants.

Our analysis of the information in the pair-wise alignments shows that some characteristics of the transcript predictions are a consequence of the alignments themselves. Importantly, the concentration of alignments from the opossum in the coding sequences of the ENCODE regions and the pair-wise predictive accuracy of the opossum informant show that the draft genome sequence of Monodelphis domestica is already a valuable tool for dual-genome gene prediction. A more complete or even finished opossum assembly could prove especially powerful for annotating the functional regions in the human genome.

The MARS method is computationally tractable with computational requirements, growing essentially linearly with the number of informant sequences and it can take advantage of additional genome sequences as they become available without extensive reanalysis. Other methods for annotating alternatively spliced transcripts are generally based on information from expressed sequences; thus, the annotations produced are experimentally supported. MARS genes, in general, do not have such support and thus provide a potential pool for experimental validation of novel splice forms [32,33].

Recent reports indicate that alternatively spliced exons have specific sequence features associated with them, such as
exonic splicing enhancers (ESEs) and exonic splicing silencers (ESSs) [22]. Moreover, some alternatively spliced exons have conservation patterns unlike constitutively spliced exons [34]. Neither of these observations have been incorporated into the MARS model and doing so could lead to more accurate prediction of alternatively spliced transcripts that are not yet supported in EST databases.

Conclusions
The MARS algorithm is able to predict alternatively spliced transcripts without the use of expressed sequence information, although the number of loci in which multiple predicted transcripts match multiple alternatively spliced transcripts in the GENCODE annotation is relatively small. Based on the current GENCODE annotation, it seems unlikely the majority of alternatively spliced transcripts predicted by MARS are actually produced. However, the results of the EGASP experimental validation of novel predictions show that among the EGASP entries, more MARS predictions were confirmed than for any other method [1]. These results are consistent with the previous reannotation of chromosome 22 in light of additional data that resulted in a significant number of new annotations, including many alternatively spliced transcripts [35]. Finally, the large fraction of incomplete transcripts in the current GENCODE annotation suggests that we are still some distance from finished annotation.

We propose that the selection of other novel alternative transcripts for experimental confirmation may be guided by looking first to those transcripts predicted with identical structure using several informant sequences. In fact, the set of 449 complete transcripts that is common to more than one informant source is approximately three times more specific than the complete set of MARS transcripts.

Materials and methods
Sequences
All predictions were made on the ENCODE regions as mapped to NCBI Build 35 (UCSC id hg17) of the human genome [36] downloaded from the UCSC genome browser [37,38] on 3 June 2004. The human genome was masked for interspersed, but not low-complexity, repeats using RepeatMasker tables provided by UCSC.

Where possible, each ENCODE region was padded on each side with 750,000 base-pairs (bp) of genomic sequence from the corresponding chromosome to ensure that the predictions were made in true genomic context and because genes were expected to extend beyond the boundaries of the ENCODE regions. The size of the sequence context was chosen based on the memory usage of Twinscan. Restricting the input sequences to the exact boundaries of the ENCODE regions results in a small decrease in predictive accuracy of approximately 1% for all evaluation measures due to incorrectly truncated genes. Informant genome sequences were also downloaded from the UCSC Genome Browser. This set included NCBI Build 33 (UCSC id mm5) of the mouse genome sequence, the canFam1 assembly of the dog genome sequence, the monDom1 assembly of the opossum genome, the rn3 assembly of the rat genome, assembly galGal2 of the chicken genome, and assembly xenTro1 of the frog genome.

Twinscan version and training set
The results in this study use the TwinscanΦ executable [27], which is an updated version of Twinscan 1.1 [6]. Conservation parameters were trained separately for each of the six informant species on a set of 3,072 human RefSeq transcripts from 2,477 loci. Genes in the training set are spread across 112 one megabase fragments of the human genome and selected based on characteristics of the genes on the fragments, including gene density and gene length. These conservation parameters are optimized for accurate whole genome predictive accuracy. The training sequences and annotations are available at [39].

The 13 ENCODE training regions provided in advance of the EGASP submission were used only to determine the optimal size and members of the informant set.

Alignments
All alignments were done with WU-BLAST version 07-14-2004 [40] using a two-stage serial BLAST strategy [41]. First stage BLAST parameters were set at M=1 N=-1 Q=5 R=1 Z=3000000000 Y=1000000000 B=100000 V=100 W=11 X=30 S=30 gapS2=30 topcomboN=1. Second stage BLAST alignments used the following more stringent parameters: W=8 X=20 S=15 gapS2=30 topcomboN=3. For human-chicken and human-frog alignments, Z=1000000000 was used. The seg and dust filters were used for all alignments. BLAST databases were prepared as previously described [6].

Information gain calculation
We calculated the information gain for each of the informant sources using our training set by subtracting the 0th order conditional uncertainty of the annotation given the conservation parameters from the 1st order conditional uncertainty of the annotation given the conservation parameters and the previous conservation parameters. This gives the information gain over the conservation parameters.

\[
IG_1 = H(C_m) - H(C_m|A)
\]

where

\[
H(C_m) = -\Pr(C_m) \times \log_2 \Pr(C_m)
\]

and

\[
H(C_m|A) = -\Pr(M) (\Pr(C_m|M) \log_2 \Pr(C_m|M) + \Pr(n_m|M) \log_2 \Pr(n_m|M))
-\Pr(G) (\Pr(C_m|G) \log_2 \Pr(C_m|G) + \Pr(n_m|G) \log_2 \Pr(n_m|G))
-\Pr(U) (\Pr(C_m|U) \log_2 \Pr(C_m|U) + \Pr(n_m|U) \log_2 \Pr(n_m|U))
\]
Here \( C_m \) is defined as a random variable representing whether a given base in the genome should be classified as part of the given portion of the Twinscan conservation model \( c_{(m)} \) or as not a part of the given portion of the model \( n_{(m)} \). We use the maximum likelihood estimate of \( C_m \) from our training set. For the conditional uncertainty calculation, we condition the probability of \( C_m \) based on whether the corresponding conservation symbol from the given informant sequence is \( (M) \)atch, \( (G) \)ap/mismatch, or \( (U) \)unaligned. In this analysis here we use \( m \in \{ \text{coding, translation initiation and translation termination signal} \} \) portions of the Twinscan conservation model [2].

**Evaluation method**

All evaluations were performed as described [1] using the GENCODE annotations as a reference.

**Availability**

MARS source code is available on an open source license. All predictions, training materials, and source code are available at the MARS website [39].

**Acknowledgements**

The authors would like to thank the organizers of the EGASP workshop, Steve Searle for a helpful discussion about the right way to collapse overlapping transcripts, Aaron Mackey for creating the GLEAN annotations from the informant set transcripts, and Ewan Birney. MRB and the development of the Twinscan code base were supported in part by HG002778 from the National Human Genome Research Institute. Open access publication charges have been paid by the Wellcome Trust.

This article has been published as part of Genome Biology Volume 7, Supplement 1, 2006: EGASP ‘05. The full contents of the supplement are available online at http://genomébiology.com/supplements/7/S1.

**References**

1. Guigo R, Flicek P, Abril JF, Reymond A, Lagarde J, Denoeud F, Antonarakis S, Ashburner M, Bajic VB, Birney E, et al. EGASP: The human ENCODE Genome Annotation Assessment Project. Genome Biology 2006, 7(Suppl 1):S1140-S1148.
2. Korfl I, Flicek P, Duan D, Brent MR. Integrating genomic homology into gene structure prediction. Bioinformatics 2001, 17(Suppl 1):S140-S148.
3. Wiehe T, Gębauer-Jung S, Mitchell-Olds T, Guigo R. SGP-1: prediction and validation of homologous genes based on sequence alignments. Genome Res 2001, 11:1574-1583.
4. Batzoglou S, Pachter L, Mesirov J, Berger B, Lander E. Human and mouse gene structure: comparative analysis and application to exon prediction. Genome Res 2000, 10:950-958.
5. Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, Agarwal P, Agarwala R, Ainscough R, Alexandersson M, An P, et al. Initial sequencing and comparative analysis of the mouse genome. Nature 2002, 419:520-562.
6. Flicek P, Keibler E, Hu P, Korfl I, Brent MR. Leveraging the mouse genome for gene prediction in human: from whole-genome shotgun reads to a global synteny map. Genome Res 2003, 13:45-54.
7. Parra G, Agarwal P, Abril JF, Wiehe T, Fickett JW, Guigo R. Comparative gene prediction in human and mouse. Genome Res 2003, 13:108-117.
8. Alexandersson M, Cawley S, Pachter L. SLAM: across-species gene finding and alignment with a generalized pair hidden Markov model. Genome Res 2003, 13:496-502.
9. Wang M, Buhrer J, Brent M. The effects of evolutionary distance on TWINSAN, an algorithm for pair-wise comparative gene prediction. Cold Spring Harb Symp Quant Biol 2003, 68:125-130.
10. Zhang L, Pavlovic V, Cantor CR, Kasif S. Human-mouse gene identification by comparative evidence integration and evolutionary analysis. Genome Res 2003, 13:1190-1202.
11. Gross SS, Brent MR. Using multiple alignments to improve gene prediction. J Comput Biol 2006, 13:379-393.
12. Wei C, Lamesch P, Arumugam M, Rosenberg A, Higgs DR. C. elegans ORFeome by cloning TWINSAN predictions. Genome Res 2005, 15:577-582.
13. Allen JE, Pertea M, Salzberg SL. Computational gene prediction using multiple sources of evidence. Genome Res 2004, 14:1124-1148.
14. Tenney AE, Brown RH, Vaske C, Lodge J, Doering TL, Brent MR. Gene prediction and verification in a compact genome with numerous small introns. Genome Res 2004, 14:2330-2335.
15. Wu JQ, Shetye DB, Arumugam M, Gibbs RA, Brent MR. Identification of rat genes by TWINSAN gene prediction, RTPCR, and direct sequencing. Genome Res 2004, 14:665-671.
16. Kan Z, States D, Gish W. Selecting for functional alternative splices in ESTs. Genome Res 2002, 12:1837-1845.
17. Hughes JR, Cheng JF, Ventress N, Prabhakar S, Clark K, Anguita E, Godier MO, de Jong P, Rubin E, Higgs DR. Annotation of regulatory elements by identification, subclassification, and functional assessment of multispecies conserved sequences. Proc Natl Acad Sci USA 2005, 102:9830-9835.
18. Cáceres JF, Kornblith AR. Alternative splicing: multiple control mechanisms and involvement in human disease. Trends Genet 2002, 18:116-193.
19. Kan Z, Rouchka EC, Gish WR, States DJ. Gene structure prediction and alternative splicing analysis using genomically aligned ESTs. Genome Res 2001, 11:889-900.
20. Sugnet CW, Kent WJ, Ares M, Haussler D. Identification and amplification of rat genes by TWINSCAN gene prediction, RT-PCR, and direct sequencing. Genome Res 2002, 12:520-562.
21. Foissac S, Schiex T. Integrating alternative splicing detection into gene prediction. BMC Bioinformatics 2005, 6:265.
22. Ohler U, Shomron N, Burge CB. Recognition of unknown conserved alternatively spliced exons. PLoS Comput Biol 2005, 1:113-122.
23. Yeo GW, Nostrand EV, Holste D, Poggio T, Burge CB. Identification and analysis of alternative splicing events conserved in human and mouse. Proc Natl Acad Sci USA 2005, 102:2850-2855.
24. Valenzuela A, Talavera D, Orozco M, de la Cruz X. Alternative splicing mechanisms for the modulation of protein function: conservation between human and other species. J Mol Biol 2004, 335:495-502.
25. Pan Q, Bakowski MA, Morris Q, Zhang W, Frey BJ, Hughes TR, Blencowe BJ. Alternative splicing of conserved exons is frequently species-specific in human and mouse. Trends Genet 2005, 21:73-77.
26. D’Errico I, Dinardo MM, Capozzi O, Virgilio CD, Gadaleta G. History of the Tfam gene in primates. Gene 2005, 362:125-132.
27. Flicek P. Methods for improving gene prediction with evolutionary conservation. PhD thesis. Washington University, Department of Biomedical Engineering; 2004.
28. ENCODE Project Consortium. The ENCODE (ENCyclopedia Of DNA Elements) Project. Science 2004, 306:636-640.
29. The Encyclopedia of DNA Elements (ENCODE) Project. http://www.genome.gov/10005107
30. Pavlovic V, Garg A, Kasif S. A Bayesian framework for combining gene predictions. Bioinformatics 2004, 20:1216-1227.
31. Allen JE, Salzberg SL. JIGSAW: integration of multiple sources of evidence for gene prediction. Bioinformatics 2005, 21:3596-3603.
32. Guigo R, Dermitzakis ET, Agarwal P, Ponting CP, Parra G, Reymond A, Abril JF, Keibler E, Lyle R, Ucla C, et al. Comparison of mouse and human genomes: findings from experimental verification yields an estimated 1,019 additional genes. Proc Natl Acad Sci USA 2003, 100:1140-1145.
33. Eyars E, Reymond A, Castelo R, Bye JM, Camara F, Flicek P, Huckle BJ, Parra G, Sheteinberg DD, Wys C, et al. Gene finding in the chicken genome. BMC Bioinformatics 2005, 6:131.
34. Bejerano G, Pheasant M, Makunin I, Stephen S, Kent WJ, Mattick JS, Haussler D. Ultraconserved elements in the human genome. Science, 2004, 304:1321-1325.
35. Collins JF, Goward ME, Cole CG, Smink LJ, Huckle EJ, Knowles S, Bye JM, Beare DM, Dunham I. Reevaluating human gene anno-
36. International Human Genome Sequencing Consortium: Finishing the euchromatic sequence of the human genome. Nature 2004, 431:931-945.
37. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, Haussler D: The human genome browser at UCSC. Genome Res 2002, 12:996-1006.
38. The UCSC Genome Browser [http://genome.ucsc.edu]
39. Training Sequences and Annotations [http://www.ebi.ac.uk/~flicek/MARS/]
40. WU-BLAST [http://blast.wustl.edu]
41. Korf I: Serial BLAST searching. Bioinformatics 2003, 19:1492-1496.