Check for updates

Chapter 10

Coding Exon-Structure Aware Realigner (CESAR): Utilizing Genome Alignments for Comparative Gene Annotation

Virag Sharma and Michael Hiller

Abstract

Alignment-based gene identification methods utilize sequence conservation between orthologous protein-coding genes to annotate genes in newly sequenced genomes. CESAR is an approach that makes use of existing genome alignments to transfer genes from one genome to other aligned genomes, and thus generates comparative gene annotations. To accurately detect conserved exons that exhibit an intact reading frame and consensus splice sites, CESAR produces a new alignment between orthologous exons, taking information about the exon's reading frame and splice site positions into account. Furthermore, CESAR is able to detect most evolutionary splice site shifts, which helps to annotate exon boundaries at high precision. Here, we describe how to apply CESAR to generate comparative gene annotations for one or many species, and discuss the strengths and limitations of this approach. CESAR is available at https://github.com/hillerlab/CESAR2.0.

Key words Comparative gene annotation, Genome alignment, CESAR, Splice site shift

1 Introduction

Identifying coding genes in genomic sequences is an important step in annotating a genome. Several different approaches exist for this task [1]. Transcriptome-based methods align entire or parts of sequenced mRNAs to the genome to infer exons and introns. Ab initio gene prediction methods detect genes solely based on characteristic sequence patterns. Homology-based approaches utilize the fact that homologous genes often have conserved sequences and use information about genes in a related species to search for similar sequences in the given genome.

One type of homology-based approaches makes use of alignments between entire genomes to project (or map) an existing gene annotation of a "reference" species to an aligned "query" species that lacks a gene annotation [2]. These projection approaches assume that exons of the reference species that align well to the query species are likely homologous exons. Thus, the coordinates

Martin Kollmar (ed.), *Gene Prediction: Methods and Protocols*, Methods in Molecular Biology, vol. 1962, https://doi.org/10.1007/978-1-4939-9173-0_10, © Springer Science+Business Media, LLC, part of Springer Nature 2019

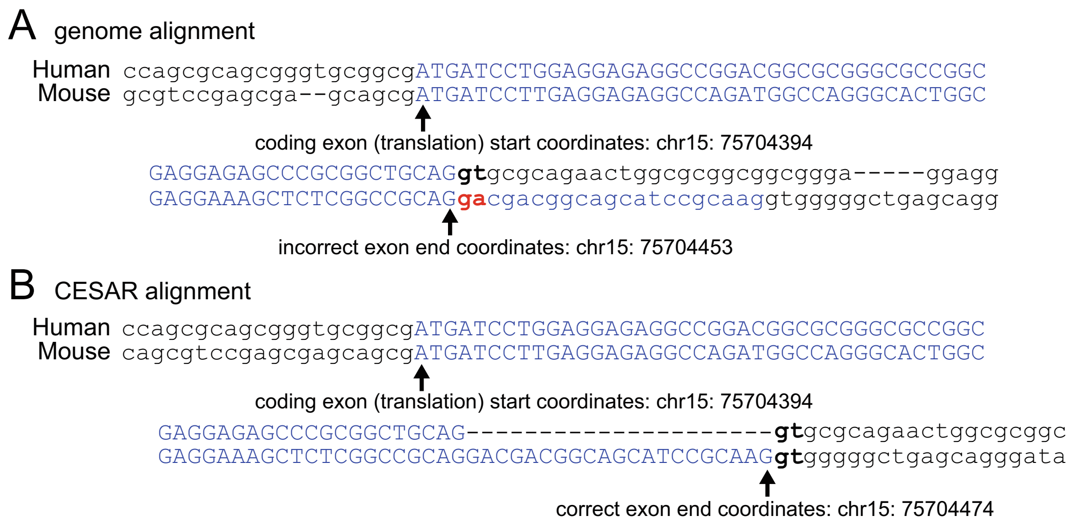

A genome alignment
Human ccagcgcagcgggtgcggcgATGATCCTGGAGGAGAGGCCGGACGGCGCGGGCGCCGGC
Mouse gcgtccgagcga--gcagcgATGATCCTTGAGGAGAGGCCAGATGGCCAGGGCACTGGC
coding exon (translation) start coordinates: chr15: 75704394
GAGGAGAGCCCGCGGCTGCAGgtgcgcagaactggcgcggcggcggga-----ggagg
GAGGAAAGCTCTCGGCCGCAGgacgacggcagcatccgcaaggtgggggctgagcagg
incorrect exon end coordinates: chr15: 75704453
B CESAR alignment
Human ccagcgcagcgggtgcggcgATGATCCTGGAGGAGAGGCCGGACGGCGCGGGCGCCGGC
Mouse cagcgtccgagcgagcagcgATGATCCTTGAGGAGAGGCCAGATGGCCAGGGCACTGGC
coding exon (translation) start coordinates: chr15: 75704394
GAGGAGAGCCCGCGGCTGCAG---------------------gtgcgcagaactggcgcggc
GAGGAAAGCTCTCGGCCGCAGGACGACGGCAGCATCCGCAAGgtgggggctgagcagggata
correct exon end coordinates: chr15: 75704474

Fig. 1 Coordinates of aligned exon boundaries do not always correspond to real exon coordinates in another genome. (**a**) Part of the genome alignment between human and mouse that covers the first coding exon of *RHPN1* (blue font). The human consensus donor dinucleotide ("gt", bold) aligns to a non-consensus donor site (red font) in the mouse, indicating that the exon end coordinates may not correspond to the respective mouse exon end. (**b**) CESAR re-aligns this sequence and detects a consensus donor site that is shifted 21 nt downstream. These exon end coordinates precisely correspond to the exon end in mouse. Please note that the CESAR alignment shows a 21 nt insertion in mouse. These additional 21 exonic bases are translatable in the same reading frame

of aligned exon boundaries in the query genome reveal the location of likely homologous exons (Fig. 1a).

Utilizing genome alignments for projecting gene annotations has several advantages. First, genome alignments do not only align exons but also the surrounding genomic context, which is helpful to distinguish orthologs from paralogs or processed pseudogenes as the latter are often located in a different syntenic context. Second, many protein-coding exons are conserved over large phylogenetic distances. If sensitive alignment parameters are used, genome alignments capture the majority of human coding exons in other mammals and even other vertebrates [3]. Third, by making use of existing multiple genome alignments, gene annotations can be projected to numerous query species, as we recently demonstrated by projecting human genes to 143 other vertebrates [3].

Despite their utility for projecting gene annotations, genome alignments have two serious limitations. First, genome alignment programs are not aware of the reading frame and splice sites of the reference exon. Consequently, alignments between conserved exons may incorrectly exhibit frameshifts or non-consensus splice sites due to alignment ambiguities. Since one aims at projecting only truly conserved exons that exhibit an intact reading frame and consensus splice sites in the query species, such alignment ambiguities cause conserved exons to be missed in the resulting gene annotation. Second, the position of splice sites of truly conserved exons can shift during evolution [4]. Since genome alignments do not aim at generating an exon alignment with consensus splice sites, the position of the projected exon boundaries in the query genome may be incorrect for such exons.

CESAR is a method to resolve these two limitations [4, 5]. For a given exon, CESAR uses the query sequence provided by the genome alignment and then re-aligns this putative exonic sequence, incorporating both information about the reading frame and the splice sites of the reference exon. For the given exonic sequence, CESAR aims at finding an alignment that (1) has consensus splice sites and (2) preserves the reading frame and thus lacks inactivating mutations such as frameshifts and in-frame stop codons. As a result, CESAR correctly infers exon conservation for more than 5300 exons that had a broken reading frame or non-consensus splice sites in the genome alignment between human and mouse, and it is able to correctly detect >90% of evolutionary splice site shifts [4] (Fig. 1b). This leads to an accurate comparative gene annotation, exemplified by our observation that 99.1% of the human exons that CESAR projects to the mouse genome overlap annotated mouse exons, and for 96.8% of the projected exons both boundaries are correct. An example illustrating a gene annotation produced by CESAR is shown in Fig. 2.

We recently re-implemented CESAR in C (CESAR 2.0), which drastically reduces runtime and memory consumption [5]. In contrast to the original implementation that only allowed to re-align a single coding exon (referred to as "single-exon mode" in the following), CESAR 2.0 also provides a "multi-exon mode" that allows to re-align entire multi-exon genes at once against a locus in the query genome. In multi-exon mode, CESAR 2.0 can detect exons that do not align in the genome alignment (Fig. 3a), and it can recognize intron deletion events that result in a larger composite exon in the query species. Furthermore, CESAR 2.0 improves the ability to detect distal evolutionary splice site shifts, which further enhances the precise identification of exon boundaries [5]. In the following, we describe how to use this new implementation (simply referred to CESAR in the following) to obtain comparative gene annotations.

2 Materials

2.1 Availability

CESAR's source code, pre-compiled binaries, and other tools required to annotate exons in a query genome are available from the github repository https://github.com/hillerlab/CESAR2.0. Open a terminal in your Linux-like environment and do the following:

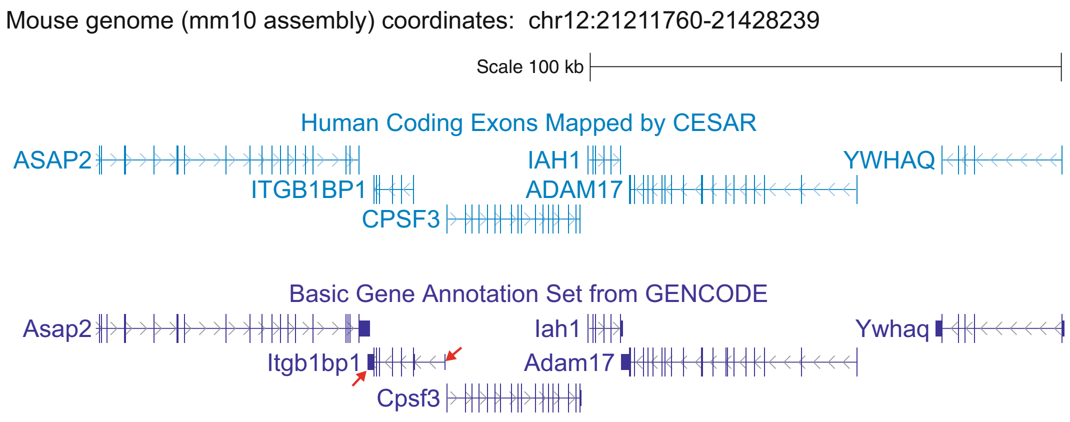

Mouse genome (mm10 assembly) coordinates: chr12:21211760-21428239
Scale 100 kb
Human Coding Exons Mapped by CESAR
ASAP2
ITGB1BP1
CPSF3
IAH1
ADAM17
YWHAQ
Basic Gene Annotation Set from GENCODE
Asap2
Itgb1bp1
Cpsf3
Iah1
Adam17
Ywhaq

Fig. 2 UCSC genome browser screenshot showing CESAR's gene annotation in the mouse genome. CESAR was applied to project human exons to mouse, resulting in a gene annotation that matches the mouse Gencode annotation. Please note that CESAR only considers coding exons. Thus, UTR exons are not projected, as indicated by red arrows for *Itgb1bp1*

```
git clone https://github.com/hillerlab/CESAR2.0/
cd CESAR2.0/
```

2.2 Installation

Compiling CESAR's source code (written in C) requires the gcc compiler:

```
make
```

Alternatively, a CESAR binary, pre-compiled under Linux 64 bit is present in the precompiledBinary_x86_64 subdirectory.

To automate task of annotating exons in a query genome, it is necessary to add the "tools" subdirectory to the $PATH variable in your environment and to set the $profilePath variable. This allows to call the tools located in this directory without specifying the full path.

If you are using a bash shell, do

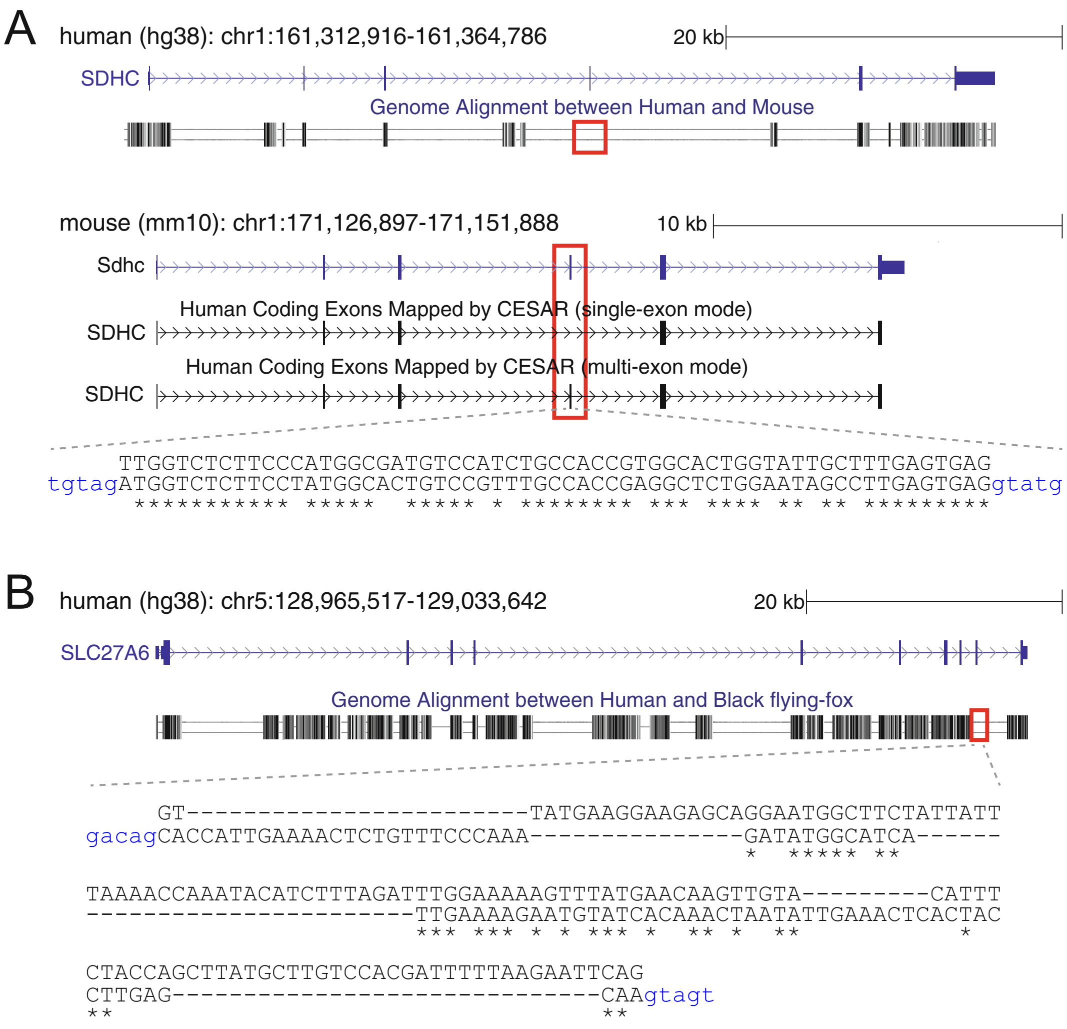
```
export PATH=$PATH:‘pwd‘/tools
export profilePath=‘pwd‘
```

If you are using C-shell, do

```
setenv PATH ${PATH}:‘pwd‘/tools
setenv profilePath ‘pwd‘
```

2.3 Computational Requirements

CESAR has been tested on different distributions of Linux (CentOS, Ubuntu, SUSE). The simplest way to get CESAR running is to use the precompiled binary. For users working with a Windows


A
human (hg38): chr1:161,312,916-161,364,786
20 kb
SDHC
Genome Alignment between Human and Mouse
mouse (mm10): chr1:171,126,897-171,151,888
10 kb
Sdhc
Human Coding Exons Mapped by CESAR (single-exon mode)
SDHC
Human Coding Exons Mapped by CESAR (multi-exon mode)
SDHC
B
human (hg38): chr5:128,965,517-129,033,642
20 kb
SLC27A6
Genome Alignment between Human and Black flying-fox

```
     TTGGTCTCTTCCCATGGCGATGTCCATCTGCCACCGTGGCACTGGTATTGCTTTGAGTGAG
tgtagATGGTCTCTTCCTATGGCACTGTCCGTTTGCCACCGAGGCTCTGGAATAGCCTTGAGTGAGgtatg
      *********** *****  ***** * ******** *** **** ** ** *********
```

```
     GT------------------------TATGAAGGAAGAGCAGGAATGGCTTCTATTATT
gacagCACCATTGAAAACTCTGTTTCCCAAA---------------GATATGGCATCA------
                                              *  ***** **

TAAAACCAAATACATCTTTAGATTTGGAAAAAGTTTATGAACAAGTTGTA---------CATTT
-----------------------TTGAAAAGAATGTATCACAAACTAATATTGAAACTCACTAC
                       *** *** * * *** *  ** *  **           *

CTACCAGCTTATGCTTGTCCACGATTTTTAAGAATTCAG
CTTGAG------------------------------CAAgtagt
**                                  **
```

Fig. 3 Strength and weakness of CESAR's multi-exon mode. (**a**) Multi-exon mode recovers an exon that does not align in the genome alignment. Top: UCSC genome browser screenshot showing the human *SDHC* gene and the genome alignment to mouse. The fourth exon does not align (red box). Bottom: UCSC genome browser showing the orthologous mouse *Sdhc* gene and CESAR's gene annotation obtained in single- and multi-exon mode. In contrast to the single-exon mode, multi-exon mode detects exon 4 (red box) with its precise splice sites, as shown by the sequence alignment underneath. (**b**) Multi-exon mode detects a false exon that is truly absent. The ninth coding exon (red box) of human *SLC27A6* does not align to the black flying-fox genome and this coding exon is truly deleted [9]. Other exons exhibit numerous frameshifting and stop codon mutations, showing that this gene is inactivated in the black flying-fox [9]. CESAR's multi-exon mode nevertheless annotates the ninth coding exon in the black flying-fox; however, the sequence alignment reveals several large insertions and deletions and a low sequence identity. Thus, a post-processing step can filter out such poorly aligning exons that are unlikely to be real

Table 1
Memory requirements for CESAR for short, typical, and very long exons or genes in both single-exon and multi-exon mode

Reference length (bp)	Query length (bp)	Memory (GB)	Mode
100	152	0.001	Single-exon
1,005	1,170	0.01	Single-exon
5,001	4,664	0.18	Single-exon
10,227	10,038	0.77	Single-exon
984	5,484	0.04	Multi-exon
5,004	137,114	5.72	Multi-exon
9,510	135,903	10.03	Multi-exon
17,673	19,225	2.55	Multi-exon

Multi-exon mode refers to aligning all exons of the reference to the entire query locus that contains the entire orthologous gene

machine, a virtual machine (VMware or VirtualBox) running Linux should be able to support CESAR, though this has not been tested.

Memory requirement is proportional to the length of the reference and query sequence. As shown in Table 1, a desktop machine with 32 GB of RAM is sufficient to run CESAR in single-exon mode on all human genes using the human-mouse genome alignment. The memory requirements for CESAR's multi-exon mode are more demanding as intronic sequences can be large. Still, 32 GB of RAM is sufficient to re-align 99.6% of the human genes in their entirety to the respective mouse genomic locus. Importantly, before allocating memory, CESAR pre-computes an upper bound of the required memory and exits with a warning if more memory is needed than specified by the user with the "-maxMemory" parameter (set to 16 GB by default).

2.4 Input

CESAR's gene-annotation workflow requires the following data as input:

1. The genomes of the reference and all query species.
2. Transcripts annotated in the reference genome.
3. A genome alignment between the reference and one or more query genomes.

How to obtain each input data is described below in Subheadings 3.1–3.3.

3 Annotating Genes from a Genome Alignment

3.1 Preparing the Genome Assembly Input Data

Obtain the genome sequence of both the reference and all query species. To this end, one can download the genome as a single file in fasta format from NCBI (https://www.ncbi.nlm.nih.gov/assembly), from Ensembl (https://www.ensembl.org/downloads.html) or from the UCSC genome browser (http://hgdownload.soe.ucsc.edu/downloads.html). Each fasta file must be converted into a 2bit file format by using faToTwoBit from the UCSC source code [6]. For example, if the fasta file for mouse genome is called "mm10.fa," the following command converts it to a 2bit file:

```
faToTwoBit mm10.fa mm10.2bit
```

Afterward, create a "2bitDir" directory. In this directory, each species must have a subdirectory that is identical to the assembly name (e.g. hg38 for human, mm10 for mouse, oryAfe1 for aardvark). An example is provided with CESAR's source code:

```
find extra/miniExample/2bitDir
```

which lists the following files:

```
extra/miniExample/2bitDir
extra/miniExample/2bitDir/hg38
extra/miniExample/2bitDir/hg38/chrom.sizes
extra/miniExample/2bitDir/hg38/hg38.2bit
extra/miniExample/2bitDir/oryAfe1
extra/miniExample/2bitDir/oryAfe1/oryAfe1.2bit
extra/miniExample/2bitDir/oryAfe1/chrom.sizes
```

In addition, create a file called "chrom.sizes" that contains the size of all scaffolds for each genome by using twoBitInfo from the UCSC source code:

```
for file in `find 2bitDir -name "*.2bit"` ; do
 d=`dirname $file`;
 f=`basename $file`;
 twoBitInfo $d/$f $d/chrom.sizes;
done
```

3.2 Preparing the Reference Gene Annotation Input Data

The second step in the CESAR gene-annotation workflow is obtaining the set of the reference species' transcripts of which you wish to annotate their orthologs in the query genome(s). For example, if the reference species is human, the human Ensembl gene annotation can be used [7]. Ensembl transcripts can be downloaded from Ensembl ftp site (https://www.ensembl.org/info/

data/ftp/index.html) by clicking on the "GTF" link under "Gene sets" for Human. At the time of writing, Ensembl v93 genes are available for the human GRCh38 assembly. Clicking on "Homo_-sapiens.GRCh38.93.gtf.gz" would save the human gene set file to the disk. Alternatively, the UCSC genome browser provides gene annotations, which can be downloaded in gtf format from the Table browser (http://genome.ucsc.edu/cgi-bin/hgTables) [6].

After download, transcripts in gtf format need to be converted to genePred format using gtfToGenePred from the UCSC source code:

```
# go to the directory that contains the downloaded transcripts, e.g.
cd ~/Downloads
# unzip the file, in case it is compressed
gzip -d Homo_sapiens.GRCh38.93.gtf.gz
# this produces a file called Homo_sapiens.GRCh38.93.gtf.
# Convert to genePred format
gtfToGenePred Homo_sapiens.GRCh38.93.gtf Homo_sapiens.
GRCh38.93.gp
```

Ensure that the generated genePred file has the right format (*see* **Note 1**).

Next, we filter the transcripts to retain only protein-coding transcripts. Additionally, this filtering step also discards the following problematic transcripts: (1) transcripts with a CDS length that is not a multiple of 3 (e.g. genes that utilize programmed ribosomal frameshifts or exhibit a polymorphism in the reference), and (2) transcripts with micro-introns smaller than 30 bp as such introns often occur in incorrectly annotated transcripts.

```
# At this stage, it is useful to specify the input file as a variable
# (here in Bash notation)
export inputGenes=Homo_sapiens.GRCh38.93.gp
formatGenePred.pl ${inputGenes} ${inputGenes}.CESAR ${input-
Genes}.ignore
```

Instead of considering all available coding transcripts of a gene, one can run the gene-annotation workflow also with the longest transcript only. In this case, add the "-longest" flag:

```
formatGenePred.pl ${inputGenes} ${inputGenes}.CESAR ${input-
Genes}.ignore -longest
```

3.3 Preparing the Genome Alignment

CESAR requires as input a genome alignment between the selected reference and one or more query genomes in maf format (https://genome.ucsc.edu/FAQ/FAQformat.html#format5). CESAR can handle both a pairwise or a multiple genome alignment stored in this format. Genome alignments can be downloaded from the

UCSC genome browser (http://hgdownload.soe.ucsc.edu/downloads.html). Alternatively, genome alignments can be created with the chaining and netting pipeline [8]. The entire process of creating a pairwise genome alignment in maf format can be automated by the UCSC script doBlastzChainNet.pl, as described in http://genomewiki.ucsc.edu/index.php/Whole_genome_alignment_howto.

CESAR's workflow requires that the genome alignment is indexed by the provided mafIndex tool, which uses the chrom.size file of the reference genome:

```
mafIndex ali.maf ali.bb -chromSizes=extra/miniExample/2bit-
Dir/hg38/chrom.sizes
```

3.4 Preparing and Executing the CESAR Gene Annotation Jobs

After preparing the three types of input (genome sequences, transcript information and the genome alignment), the different variables that are used as inputs to the CESAR gene-annotation workflow need to be defined.

```
export reference=...     # the assembly name of the reference
(e.g. hg38)
export twoBitDir=...     # the directory containing the genomes
and chrom.size
                         # files (e.g.
extra/miniExample/2bitDir)
export alignment=...     # the indexed alignment file (ali.bb
above)
export querySpecies=... # a comma-separated list of the query
species that you
                         # want to annotate. Each query species
must be contained
                         # in ${alignment}.
export outputDir=...     # name of the output directory that
will contain exon
                         # coordinates (in subdirectories). The
directory will be
                         # created, if it does not exist.
export resultsDir=...    # name of the directory that will
contain the final gene
                         # annotation (one gene annotation file
per query species)
export maxMemory=...     # maximum amount of memory in GB that
CESAR is allowed
                         # to allocate
export profilePath=...   # path to the directory that contains
the 'extra'
                         # subdirectory containing CESAR's
profiles and matrices
```

Next, we generate the gene-annotation workflow commands for all filtered transcripts:

```
for transcript in `cut -f1 ${inputGenes}.forCESAR`; do
  echo "annotateGenesViaCESAR.pl ${transcript} ${alignment}
${inputGenes}.forCESAR ${reference} ${querySpecies} ${output-
Dir} ${twoBitDir} ${profilePath} -maxMemory ${maxMemory}"
done > jobList
```

The result is a file called "jobList" in which each line consists of a single job that re-aligns a single transcript to all query species. Each job is completely independent of any other job. Hence, each job can be run in parallel on a compute cluster. In the absence of a compute cluster, the jobs can be run sequentially:

```
chmod +x jobList
./jobList
```

Using CESAR to project 196,259 human coding exons to mouse takes approximately 7 h on a desktop machine using a single core. The memory requirement will vary on the size of the input gene (*see* **Note 2** and Table 1).

3.5 Merging CESAR's Output Into a Single Gene Annotation File per Species

In this step, we collect the results obtained in the previous step (after each job has successfully finished) in a single genePred file for each query species.

```
for species in `echo $querySpecies | sed 's/,/ /g'`; do
 echo "bed2GenePred.pl $species $outputDir /dev/stdout | awk
'{if ($4 != $5) print $0}' > $resultsDir/$species.gp"
done > jobListGenePred
chmod +x jobListGenePred
./jobListGenePred
```

This step takes only a few minutes. The final results are in $resultsDir (specified as a variable above) as a single genePred-formatted file per query species. GenePred files can be converted to gtf format using genePredToGtf from the UCSC source code:

```
genePredToGtf file mm10.gp mm10.gtf
```

The $outputDir directory that is used to store temporary results may be deleted afterward.

3.6 Visualizing the Gene Annotations

This step is optional. An obtained genePred file can be visualized in the UCSC genome browser of the query genome, as shown in Fig. 2. This can be done by converting the genePred file into gtf format, as described above, and then uploading this file to the UCSC genome browser via their "Custom Track" feature.

3.7 Running CESAR in Multi-exon Mode

To run CESAR in multi-exon mode, all the steps described above are exactly the same (Subheadings 3.1–3.3 and 3.5) except Subheading 3.4. After specifying the variables, the following command will generate the jobs that run CESAR in multi-exon mode:

```
for transcript in `cut -f1 ${inputGenes}.forCESAR`; do
  echo  "annotateGenesViaCESAR_multi_exon.pl  ${transcript}
${alignment}  ${inputGenes}.forCESAR  ${reference}  ${querySpe-
cies}  ${outputDir}  ${twoBitDir}  ${profilePath}  -maxMemory
${maxMemory}"
done > jobList
```

The jobs listed in "jobList" can be executed on a compute cluster or run sequentially.

4 Notes

1. In case of problems with the transcript file, one can use UCSC's genePredCheck tool to check if the converted genePred has a valid format.
2. A limitation of CESAR is that its memory requirement is proportional to the lengths of the input sequences. By default, CESAR stops with a warning if it estimates that more than 16 GB of memory may be required:

   ```
   CRITICAL src/Cesar.c:117 main(): The memory consumption is
   limited to 16.0000 GB by default. Your attempt requires
   30.1539 GB. You can change the limit via --max-memory.
   ```

   If your computer provides more memory, set the maxMemory above to a higher value. For example, 32 GB of RAM are sufficient to align all human exons in single-exon mode.

   In multi-exon mode, CESAR may require even more memory in case the transcript has many exons or introns in the query genome are large. For such genes, CESAR can be run in single-exon mode.

5 Special Cases

1. In case exons are truly deleted or overlap an assembly gap in the query, CESAR's multi-exon mode has a tendency to align random intronic sequence to such reference exons, instead of producing an alignment where these exons are entirely deleted. Such exon alignments are characterized by large insertions and

deletions and a low sequence identity (Fig. 3b). A subsequent filtering step can be used to remove those exons from the resulting gene annotation that are poorly aligning and thus unlikely to be real exons.

2. CESAR's multi-exon mode requires that all aligning exons of a gene are located on a single locus in the query genome (same scaffold and same strand in a co-linear order). It is therefore recommended to use the single-exon mode for query assemblies with a high degree of fragmentation, where many genes will partially align to different scaffolds. Alternatively, CESAR can be run in both single- and multi-exon mode, and the resulting annotations can be combined.
3. CESAR's source code provides splice site profiles obtained for human. These profiles are used in the re-alignment process to locate orthologous or shifted splice sites. Splice site profiles will be similar for closely related species such as mammals; however, they may differ if species distantly related to human are used as the reference. In this case, it is recommended to obtain new splice site profiles for the reference species, which can be done as follows:

```
# obtain a file that contains the longest transcript per gene
for the reference
formatGenePred.pl ${inputGenes} ${inputGenes}.CESAR
${inputGenes}.ignore -longest
# define the following variables
export input=${inputGenes}.CESAR
export ref_2bit=... # the path to the two bit file of
reference species
# extract the sequences around the splice sites from all
transcripts
extract_splice_sites.pl $input acc_seqs.txt donor_seqs.txt
$ref_2bit
# extract the sequence upstream of the first exon from the
genes
get_start_context.pl $input start_seqs.txt $ref_2bit
# Lastly, convert these sequences to profiles:
create_profiles.pl acc_seqs.txt acc_profile.txt
create_profiles.pl donor_seqs.txt do_profile.txt
create_profiles.pl start_seqs.txt firstCodon_profile.txt
# clean-up
rm acc_seqs.txt donor_seqs.txt start_seqs.txt
# Move these files to the relevant clade so that CESAR can
read these profiles
export clade=... # name of the new clade, for example chicken
mkdir -p CESAR2.0/extra/tables/$clade
```

```
mv acc_profile.txt CESAR2.0/extra/tables/$clade
mv do_profile.txt CESAR2.0/extra/tables/$clade
mv firstCodon_profile.txt CESAR2.0/extra/tables/$clade
# copy the original stop codon profile and the codon
substitution matrix
cp CESAR2.0/extra/tables/human/lastCodon_profile.txt
CESAR2.0/extra/tables/$clade
cp CESAR2.0/extra/tables/human/eth_codon_sub.txt
CESAR2.0/extra/tables/$clade
```

Acknowledgment

This work was supported by the Max Planck Society and the German Research Foundation (HI 1423/3-1).

References

1. Picardi E, Pesole G (2010) Computational methods for ab initio and comparative gene finding. Methods Mol Biol 609:269–284. https://doi.org/10.1007/978-1-60327-241-4_16
2. Zhu J, Sanborn JZ, Diekhans M, Lowe CB, Pringle TH, Haussler D (2007) Comparative genomics search for losses of long-established genes on the human lineage. PLoS Comput Biol 3(12):e247. https://doi.org/10.1371/journal.pcbi.0030247
3. Sharma V, Hiller M (2017) Increased alignment sensitivity improves the usage of genome alignments for comparative gene annotation. Nucleic Acids Res 45(14):8369–8377. https://doi.org/10.1093/nar/gkx554
4. Sharma V, Elghafari A, Hiller M (2016) Coding exon-structure aware realigner (CESAR) utilizes genome alignments for accurate comparative gene annotation. Nucleic Acids Res 44(11): e103. https://doi.org/10.1093/nar/gkw210
5. Sharma V, Schwede P, Hiller M (2017) CESAR 2.0 substantially improves speed and accuracy of comparative gene annotation. Bioinformatics 33 (24):3985–3987. https://doi.org/10.1093/bioinformatics/btx527
6. Casper J, Zweig AS, Villarreal C, Tyner C, Speir ML, Rosenbloom KR, Raney BJ, Lee CM, Lee BT, Karolchik D, Hinrichs AS, Haeussler M, Guruvadoo L, Navarro Gonzalez J, Gibson D, Fiddes IT, Eisenhart C, Diekhans M, Clawson H, Barber GP, Armstrong J, Haussler D, Kuhn RM, Kent WJ (2018) The UCSC Genome Browser database: 2018 update. Nucleic Acids Res 46(D1):D762–D769. https://doi.org/10.1093/nar/gkx1020
7. Zerbino DR, Achuthan P, Akanni W, Amode MR, Barrell D, Bhai J, Billis K, Cummins C, Gall A, Giron CG, Gil L, Gordon L, Haggerty L, Haskell E, Hourlier T, Izuogu OG, Janacek SH, Juettemann T, To JK, Laird MR, Lavidas I, Liu Z, Loveland JE, Maurel T, McLaren W, Moore B, Mudge J, Murphy DN, Newman V, Nuhn M, Ogeh D, Ong CK, Parker A, Patricio M, Riat HS, Schuilenburg H, Sheppard D, Sparrow H, Taylor K, Thormann A, Vullo A, Walts B, Zadissa A, Frankish A, Hunt SE, Kostadima M, Langridge N, Martin FJ, Muffato M, Perry E, Ruffier M, Staines DM, Trevanion SJ, Aken BL, Cunningham F, Yates A, Flicek P (2018) Ensembl 2018. Nucleic Acids Res 46(D1): D754–D761. https://doi.org/10.1093/nar/gkx1098
8. Kent WJ, Baertsch R, Hinrichs A, Miller W, Haussler D (2003) Evolution's cauldron: duplication, deletion, and rearrangement in the mouse and human genomes. Proc Natl Acad Sci U S A 100(20):11484–11489. https://doi.org/10.1073/pnas.1932072100
9. Sharma V, Hecker N, Roscito JG, Foerster L, Langer BE, Hiller M (2018) A genomics approach reveals insights into the importance of gene losses for mammalian adaptations. Nat Commun 9(1):1215. https://doi.org/10.1038/s41467-018-03667-1