The following Perl scripts were created and executed to generate the results in the article titled "Dual coding in alternative reading frames correlates with intrinsic protein disorder" by Erika Kovacs, Peter Tompa, Karoly Liliom, Lajos Kalmar. Note that several other simple steps were used to filter out the required datasets and lists ('grep' and 'awk' Linux commands).

Several other software should be also installed locally to execute the Perl scripts, like IUPred (http://iupred.enzim.hu), VSL2 (http://www.ist.temple.edu/disprot/Predictors.html), BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and some Perl modules (http://www.cpan.org/).

The initial dataset can be downloaded from the NCBI ftp site:
ftp://ftp.ncbi.nih.gov/gene/DATA/gene2accession.gz

Content of this document

```
#!/usr/bin/perl

################################################################################
#                                                                              #
# Copyright, 2009, Lajos Kalmar, Erika Kovacs                                  #
# Institute of Enzymology of the Hungarian Academy of Sciences                 #
#                                                                              #
# iupevol_get.pl: retrieve mRNA sequences from NCBI                            #
#                                                                              #
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#                                                                              #
################################################################################

use LWP::Simple;
$|++;

$fn = shift(@ARGV); # as an argument, you have to use a list of NCBI Gene IDs

open(LI, $fn);
$cnt = 0;
while (<LI>){
        chomp;
        $res =
get("http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&qty=1&c_start=1&list_uids=".$_.
"&uids=&dopt=gb&dispmax=5&sendto=t&fmt_mask=0&from=begin&to=end&extrafeatpresent=1&ef_STS=64&e
f_Exon=512&ef_CDD=8&ef_MGC=16&ef_HPRD=32&ef_tRNA=128&ef_microRNA=256");
        @sorok = split(/\n/, $res);
        $cdspres = 0;
        $passseq = 0;
        undef @exons;
        undef $seq;
        foreach $line (@sorok){
                if ($line =~ /^\/\//){
                        $passseq = 0;
                }
                if ($passseq == 1){
                        $seq .= $line;
                }
                if ($line =~ /^VERSION/){
                        @fields = split(' ', $line);
                        $def = ">".$fields[1]."\t".$fields[2];
                }
                if ($line =~ /^     CDS/){
                        $cdspres = 1;
                        @fields = split(' ', $line);
                        $def .= "\tCDS:".$fields[1];
                }
                if ($line =~ /^     exon/){
                        @fields = split(' ', $line);
                        push(@exons, $fields[1]);
                }
                if ($line =~ /^ORIGIN/){
                        $passseq = 1;
                }
```

```
        }
        if ($cdspres == 1){
                print $def."\texons:";
                print "@exons ";
                $seq =~ s/[\s\d]//g;
                $seq = uc($seq);
                print "\n".$seq."\n";
                $cnt++;
                print STDERR "\r".$cnt;
        }
}
```

```
#!/usr/bin/perl

################################################################################
#                                                                              #
# Copyright, 2009, Lajos Kalmar, Erika Kovacs                                  #
# Institute of Enzymology of the Hungarian Academy of Sciences                 #
#                                                                              #
# iupevol_transl.pl: translate RefSeq in 3 different frames                    #
#                                                                              #
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#                                                                              #
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#                                                                              #
################################################################################

open(FA, shift(@ARGV)); # as an argument provide the mRNA fasta dataset generated by
iupevol_get.pl
while (<FA>){
        if (/^>/){
                @fields = split(/\t/, $_);
                $fields[2] =~ s/CDS://g;
                ($begin, $end) = split(/\.\./, $fields[2]);
        }
        else {
                chomp;
                $dna_seq = substr($_, $begin-1, $end-$begin+1);
                for (0..2){
                        $prot_seq = dna2protHUM($dna_seq, $_);
                        print $fields[0]." frame".$_."\n";
                        print $prot_seq."\n";
                }
        }
}

sub dna2protHUM {
        my $dna_seq = shift(@_);
        my $frame = shift(@_);
        my %data = ('TTT' => 'F', 'TTC' => 'F', 'TTA' => 'L', 'TTG' => 'L', 'TCT' => 'S', 'TCC'
=> 'S', 'TCA' => 'S', 'TCG' => 'S', 'TAT' => 'Y', 'TAC' => 'Y', 'TAA' => 'X', 'TAG' => 'X',
'TGT' => 'C', 'TGC' => 'C', 'TGA' => 'X', 'TGG' => 'W', 'CTT' => 'L', 'CTC' => 'L', 'CTA' =>
'L', 'CTG' => 'L', 'CCT' => 'P', 'CCC' => 'P', 'CCA' => 'P', 'CCG' => 'P', 'CAT' => 'H', 'CAC'
=> 'H', 'CAA' => 'Q', 'CAG' => 'Q', 'CGT' => 'R', 'CGC' => 'R', 'CGA' => 'R', 'CGG' => 'R',
'ATT' => 'I', 'ATC' => 'I', 'ATA' => 'I', 'ATG' => 'M', 'ACT' => 'T', 'ACC' => 'T', 'ACA' =>
'T', 'ACG' => 'T', 'AAT' => 'N', 'AAC' => 'N', 'AAA' => 'K', 'AAG' => 'K', 'AGT' => 'S', 'AGC'
=> 'S', 'AGA' => 'R', 'AGG' => 'R', 'GTT' => 'V', 'GTC' => 'V', 'GTA' => 'V', 'GTG' => 'V',
'GCT' => 'A', 'GCC' => 'A', 'GCA' => 'A', 'GCG' => 'A', 'GAT' => 'D', 'GAC' => 'D', 'GAA' =>
'E', 'GAG' => 'E', 'GGT' => 'G', 'GGC' => 'G', 'GGA' => 'G', 'GGG' => 'G');
        my $prot_seq = '';
        for (my $i = $frame; $i < length($dna_seq); $i+=3){
                $prot_seq .= $data{substr($dna_seq, $i, 3)};
        }
        return $prot_seq;
}
```

```
#!/usr/bin/perl

################################################################################
#                                                                              #
# Copyright, 2009, Lajos Kalmar, Erika Kovacs                                  #
# Institute of Enzymology of the Hungarian Academy of Sciences                 #
#                                                                              #
# iupevol_eval.pl: extract relevant information from BLAST result (XML)        #
#                                                                              #
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#                                                                              #
################################################################################

open (XML, shift(@ARGV)); # first argument is the path of the XML BLAST result
$tresh = shift(@ARGV); # second argument is the length threshold of the dual-coding region (25
were used in the article)
$g2a_path = '' # define the path of the NCBI gene2accession file here

open (G, $g2a_path);
while (<G>){
        @fields = split(' ', $_);
        if ($fields[2] eq 'REVIEWED'){
                $geneAC{$fields[3]} = $fields[1];
        }
}

while (<XML>){
        if (/^      <Iteration_query-def>/){
                $query = getFromTag($_);
                ($queryID, $frame) = split(' ', $query);
        }
        if (/^      <Iteration_query-len>/){
                $len = getFromTag($_);
        }
        if (/^          <Hit_id>/){
                $hit = getFromTag($_);
                $hit =~ s/^lcl\|//;
        }
        if (/^          <Hit_len>/){
                $hlen = getFromTag($_);
        }
        if (/^              <Hsp_query-from>/){
                $hspqfrom = getFromTag($_);
        }
        if (/^              <Hsp_query-to>/){
                $hspqto = getFromTag($_);
        }
        if (/^              <Hsp_hit-from>/){
                $hsphfrom = getFromTag($_);
        }
        if (/^              <Hsp_hit-to>/){
                $hsphto = getFromTag($_);
        }
        if (/^              <Hsp_identity>/){
```

```
                $hspid = getFromTag($_);
        }
        if (/^              <Hsp_midline>/){
                $midline = getFromTag($_);
        }


        if (/^            <\/Hsp>/){
                if (($hspqto - $hspqfrom + 1) > $tresh){
                        if ($geneAC{$queryID} eq $geneAC{$hit}){
                                @cf = contFrag($midline);
                                if ($cf[0] > 25){
                                        print
$geneAC{$queryID}."\t".$query."\t".$len."\t".($hspqfrom + $cf[1])."-".($hspqfrom + $cf[1] +
$cf[0] - 1) ."\t".$hit."\t".($hlen - 1)."\t".($hsphfrom + $cf[1])."-".($hsphfrom + $cf[1] +
$cf[0] - 1)."\t".$cf[0]."\n";
                                }
                        }
                }
        }
}

sub getFromTag {
        (my $trash, my $temp) = split(/>/, $_[0]);
        ($temp , $trash) = split(/</, $temp);
        return $temp;
}

sub contFrag {
        @str = split(/|/, shift(@_));
        my $temp = 0;
        my $max = 0;
        my $cnt = 0;
        foreach (@str){
                $cnt++;
                if ($_ =~ /[A-Z]/){
                        $temp++;
                        if ($temp == 1){
                                $tempstart = $cnt;
                        }
                }
                else {
                        if ($temp > $max){
                                $max = $temp;
                                $start = $tempstart;
                        }
                        $temp = 0;
                }
        }
        if ($temp > $max){
                $max = $temp;
                $start = $tempstart;
        }
        return ($max, $start);
}
```

```
#!/usr/bin/perl

################################################################################
#                                                                              #
# Copyright, 2009, Lajos Kalmar, Erika Kovacs                                  #
# Institute of Enzymology of the Hungarian Academy of Sciences                 #
#                                                                              #
# iupevol_iupanal_pairs.pl: disorder prediction for dual-coding regions        #
#                                                                              #
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#                                                                              #
################################################################################

use Data::Dumper;
use Digest::MD5 qw/md5_hex/;

open (FH2, shift(@ARGV)); # first argument is the path of the tranlated protein dataset in
normal frame (0 frame)
while (<FH2>) {
        chomp;
        if (/^>/) {
                @fields = split(' ',$_);
                $fields[0] =~ s/>//g;
        }
        else {
                $_ =~ s/X//g;
                $seq{$fields[0]} = $_;
        }
}
close (FH2);

open (FH3, shift(@ARGV)); # second argument is the path of the file containing isoform pairs
with ARF, created by iupevol_eval.pl
print
"GeneID\tOrigVar\tshifttype\tOriglen\tRegrange\tregIUPredS\tregVSL2S\tNovelVar\tNovellen\tRegr
ange\tregIUPredS\tregVSL2S\n";
while (<FH3>) {
        @fields = split(' ',$_);
        ($beg,$end) = split(/\-/,$fields[4]);
        ($beg2,$end2) = split(/\-/,$fields[7]);
        $iupres = iupredAn($seq{$fields[1]}, 'long');
        $iupres2 = iupredAn($seq{$fields[5]}, 'long');
        $vsl2res = vsl2An($seq{$fields[1]});
        $vsl2res2 = vsl2An($seq{$fields[5]});
        $vsl2data = vsl2Avg($vsl2res, $fields[3], $beg, $end, 0.5);
        $vsl2data2 = vsl2Avg($vsl2res2, $fields[6], $beg2, $end2, 0.5);
        $iupdata = vsl2Avg($iupres, $fields[3], $beg, $end, 0.4);
        $iupdata2 = vsl2Avg($iupres2, $fields[6], $beg2, $end2, 0.4);
        print
$fields[0]."\t".$fields[1]."\t".$fields[2]."\t".$fields[3]."\t".$fields[4]."\t".($iupdata-
>{'reg'})*100 ."\t".($vsl2data->{'reg'})*100
."\t".$fields[5]."\t".$fields[6]."\t".$fields[7]."\t".($iupdata2->{'reg'})*100
."\t".($vsl2data2->{'reg'})*100 ."\n";
}
```

```
#subs
sub iupredAn {
	$ENV{'IUPred_PATH'}=''; # path of the iupred source directory
	my $iupred_path = ''; # path of the iupred binary
	my $seq = shift(@_);
	my $opt = shift(@_);
	my $resref = undef;
	open (TMP, '>/tmp/iutmp') || die "Couldn't write to tmp directory";
	print TMP ">iupquery\n";
	print TMP $seq;
	my $result=`$iupred_path /tmp/iutmp $opt`;
	my @lines = split(/\n/, $result);
	foreach $line (@lines){
		if ($line !~ /^#/){
			my @fields = split(' ', $line);
			$resref->{$fields[0]} = $fields[2];
		}
	}
	close (TMP);
	unlink ('/tmp/iutmp');
	return $resref;
}

sub vsl2An {
	my $vsl2_path = ''; # path of the VSL2.jar file
	my $resref = undef;
	my $seq = shift(@_);
	my @array;
	open (TMP, '>/tmp/vsl2tmp') || die "Couldn't write to tmp directory";
	print TMP $seq;
	my @result=split(/\n/, `java -jar $vsl2_path -s:/tmp/vsl2tmp`);
	close (TMP);
	unlink ('/tmp/vsl2tmp');
	push(@array, 'VSL2');
	my $pass = 0;
	foreach $line (@result){
		if ($line =~ /^\-\-\-\-\-/){
			$pass = 1;
		}
		if ($line =~ /\d/ && $pass == 1){
			my @fields = split(' ', $line);
			push (@array, $fields[2]);
		}
	}
	for ($i=1; $i <= $#array+1; $i++) {
		$resref->{$i} = $array[$i];
	}
	return $resref;
}

sub iupredAvg {
	my $sumiup = 0;
	my $score = 0;
	my $ref = undef;
	$ref = shift(@_);
	my $offset = shift(@_);
	my $length = shift(@_);
	for (my $i=$offset; $i < ($offset+$length); $i++){
		$sumiup += $ref->{$i};
	}
	my $avg = $sumiup / $length;
	return sprintf("%.4f", $avg);
}

sub iupredPerc {
	my $ref = shift(@_);
```

```
        my $offset = shift(@_);
        my $length = shift(@_);
        my $tresh = shift(@_);
        my $sumiup = undef;
        for (my $i=$offset; $i < ($offset+$length); $i++){
                if ($ref->{$i} > $tresh) {
                        $sumiup++;
                }
        }
        return sprintf("%.4f", $sumiup / $length);
}

sub vsl2Avg {
        my $data = undef;
        my $redata;
        my $ref = shift(@_);
        my $flength = shift(@_);
        my $beg = shift(@_);
        my $end = shift(@_);
        my $tresh = shift(@_);
        for ($i=1; $i <= $flength; $i++){
                $data->{'full'} += $ref->{$i};
                if ($i >= $beg && $i <= $end && $ref->{$i} > $tresh){
                        $data->{'reg'}++;
                }
        }
        $redata->{'full'} = sprintf("%.4f", ($data->{'full'} / $flength));
        $redata->{'reg'} = sprintf("%.4f", $data->{'reg'} / ($end - $beg + 1));
        if (($flength - ($end - $beg + 1)) != 0){
                $redata->{'regless'} = sprintf("%.4f", $data->{'regless'} / ($flength - ($end -
$beg + 1)));
        }
        else {
                $redata->{'regless'} = 0;
        }
        return $redata;
}
```

```
#!/usr/bin/perl

##############################################################################
#                                                                            #
# Copyright, 2009, Lajos Kalmar, Erika Kovacs                                #
# Institute of Enzymology of the Hungarian Academy of Sciences               #
#                                                                            #
# iupevol_pdb_disprot.pl: disorder prediction on PDB and DisProt datasets    #
#                                                                            #
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#                                                                            #
##############################################################################

use Data::Dumper;
$|++;

=pod #uncomment for PDB disorder prediction
open(LI, ''); # path to file with pdb_select IDs
while (<LI>){
        chomp;
        if (substr($_, 4, 1) eq '_'){
                $id = substr($_, 0, 4) . "_A";
        }
        else {
                $id = substr($_, 0, 4) . "_" . substr($_, 4, 1);
        }
        #print $id."\n";
        $sel{$id} = 1;
}
open(P, ''); # path to file pdb_seqres.txt (downloaded from PDB)
while (<P>){
        chomp;
        if (/^>/){
                $pass = 0;
                ($tid, $trash) = split(' ', $_);
                $tid =~ s/>//g;
                if ($sel{uc($tid)} == 1){
                        $pass = 1;
                }
        }
        else {
                if ($pass == 1 && !/X/ && !/HHHHHH/){
                        $cnt++;
                        print "\r".$cnt;
                        $iupred = iupredAn($_, 'long');
                        $vsl2 = vsl2An($_, 'hashref');
                        push(@iupreds, iupredPerc($iupred, 0, length($_), 0.4));
                        push(@vsl2s, iupredPerc($vsl2, 0, length($_), 0.5));
                }
        }
}
print "\n";
print mean(@iupreds)."\n".mean(@vsl2s)."\n";
=cut
```

```
# for disprot disorder prediction (if uncomment the PDB prediction, comment out this section)
open(F1, ''); # path to disprot sequence fasta file
while (<F1>){
	chomp;
	if (/^>/){
		@fields = split(' ', $_);
	}
	else{
		$counter++;
		print STDERR "\r".$counter;
		@ell = undef;
		for (1..length($_)){
			$ell[$_] = 0;
		}
		$dis = 0;
		for (@fields){
			if (/^\#/){
				$dis = 1;
				$_ =~ s/\#//g;
				($from, $to) = split(/\-/, $_);
				for ($from..$to){
					$ell[$_] = 1;
				}
			}
		}
		if ($dis == 1 && !/[BZ]/){
			$iupres = iupredAn($_, 'long');
			$vsl2res = vsl2An($_, 'hashref');
			$cnt = $iupcnt = $vsl2cnt = 0;
			for ($i=1; $i<=$#ell;$i++){
				if ($ell[$i] == 1){
					$cnt++;
					if ($iupres->{$i} > 0.4){
						$iupcnt++;
					}
					if ($vsl2res->{$i} > 0.5){
						$vsl2cnt++;
					}
				}
			}
			push(@vsl2s, ($vsl2cnt / $cnt));
			push(@iupreds, ($iupcnt / $cnt));
		}
	}

}
print mean(@iupreds)."\n".mean(@vsl2s)."\n";


sub mean {
	my $result;
	foreach (@_) { $result += $_ }
	return $result / ($#_ + 1) ;
}

sub rangeIUP {
	(my $seq, my $range) = @_;
	my $iupred = iupredAn($seq, 'long');
	my $vsl2 = vsl2An($seq, 'hashref');
	my $iupred_perc = iupredPerc($iupred, 100, 100+$range-1, 0.4);
	my $vsl2_perc = iupredPerc($vsl2, 100, 100+$range-1, 0.5);
	return ($iupred_perc + $vsl2_perc) / 2;
}

sub dna2protHUM {
	my $dna_seq = shift(@_);
```

```
	my $frame = shift(@_);
	my %data = ('TTT' => 'F', 'TTC' => 'F', 'TTA' => 'L', 'TTG' => 'L', 'TCT' => 'S', 'TCC'
=> 'S', 'TCA' => 'S', 'TCG' => 'S', 'TAT' => 'Y', 'TAC' => 'Y', 'TAA' => 'X', 'TAG' => 'X',
'TGT' => 'C', 'TGC' => 'C', 'TGA' => 'X', 'TGG' => 'W', 'CTT' => 'L', 'CTC' => 'L', 'CTA' =>
'L', 'CTG' => 'L', 'CCT' => 'P', 'CCC' => 'P', 'CCA' => 'P', 'CCG' => 'P', 'CAT' => 'H', 'CAC'
=> 'H', 'CAA' => 'Q', 'CAG' => 'Q', 'CGT' => 'R', 'CGC' => 'R', 'CGA' => 'R', 'CGG' => 'R',
'ATT' => 'I', 'ATC' => 'I', 'ATA' => 'I', 'ATG' => 'M', 'ACT' => 'T', 'ACC' => 'T', 'ACA' =>
'T', 'ACG' => 'T', 'AAT' => 'N', 'AAC' => 'N', 'AAA' => 'K', 'AAG' => 'K', 'AGT' => 'S', 'AGC'
=> 'S', 'AGA' => 'R', 'AGG' => 'R', 'GTT' => 'V', 'GTC' => 'V', 'GTA' => 'V', 'GTG' => 'V',
'GCT' => 'A', 'GCC' => 'A', 'GCA' => 'A', 'GCG' => 'A', 'GAT' => 'D', 'GAC' => 'D', 'GAA' =>
'E', 'GAG' => 'E', 'GGT' => 'G', 'GGC' => 'G', 'GGA' => 'G', 'GGG' => 'G');
	my $prot_seq = '';
	for (my $i = $frame; $i < length($dna_seq); $i+=3){
		$prot_seq .= $data{substr($dna_seq, $i, 3)};
	}
	return $prot_seq;
}


sub iupredAn {
	$ENV{'IUPred_PATH'}=''; # path of the iupred source directory
	my $iupred_path = ''; # path of the iupred binary
	my $seq = shift(@_);
	my $opt = shift(@_);
	my $resref = undef;
	open (TMP, '>/tmp/iutmp') || die "Couldn't write to tmp directory";
	print TMP ">iupquery\n";
	print TMP $seq;
	my $result=`$iupred_path /tmp/iutmp $opt`;
	my @lines = split(/\n/, $result);
	foreach $line (@lines){
		if ($line !~ /^#/){
			my @fields = split(' ', $line);
			$resref->{$fields[0]} = $fields[2];
		}
	}
	close (TMP);
	unlink ('/tmp/iutmp');
	return $resref;
}

sub vsl2An {
	my $vsl2_path = ''; # path of the VSL2.jar file
	my $resref = undef;
	my $seq = shift(@_);
	my @array;
	open (TMP, '>/tmp/vsl2tmp') || die "Couldn't write to tmp directory";
	print TMP $seq;
	my @result=split(/\n/, `java -jar $vsl2_path -s:/tmp/vsl2tmp`);
	close (TMP);
	unlink ('/tmp/vsl2tmp');
	push(@array, 'VSL2');
	my $pass = 0;
	foreach $line (@result){
		if ($line =~ /^\-\-\-\-\-/){
			$pass = 1;
		}
		if ($line =~ /\d/ && $pass == 1){
			my @fields = split(' ', $line);
			push (@array, $fields[2]);
		}
	}
	for ($i=1; $i <= $#array+1; $i++) {
		$resref->{$i} = $array[$i];
	}
	return $resref;
}
```

```
sub iupredPerc {
	my $ref = shift(@_);
	my $from = shift(@_);
	my $to = shift(@_);
	my $tresh = shift(@_);
	my $sumiup = undef;
	for ($from..$to){
		if ($ref->{$_} > $tresh) {
			$sumiup++;
		}
	}
	return sprintf("%.4f", $sumiup / ($to - $from + 1));
}
```

```
#!/usr/bin/perl

##############################################################################
#                                                                            #
# Copyright, 2009, Lajos Kalmar, Erika Kovacs                                #
# Institute of Enzymology of the Hungarian Academy of Sciences               #
#                                                                            #
# aacont1.pl: calculate amino acid composition of the translated datasets    #
#                                                                            #
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#                                                                            #
##############################################################################

@aaArr =
('W','F','Y','I','M','L','V','N','C','T','A','G','R','D','H','Q','K','S','E','P','X');

open(L,shift(@ARGV));
while(<L>){
        chomp;
        $ids{$_}=1;
}
close(L);
open (FA, ''); # path to translated protein dataset in normal frame
while (<FA>){
        chomp;
        if (/^>/){
                @fields = split(' ', $_);
        }
        else {
                if ($ids{$fields[0]} == 1){
                        $rseq .= $_;
                }
        }
}
$rseq =~ s/X//g;
close(FA);
open (FA1, ''); # path to translated protein dataset in shifted frames
while (<FA1>){
        chomp;
        if (/^>/){
                @fields = split(' ', $_);
        }
        else {
                if ($ids{$fields[0]} == 1){
                        if ($fields[1] eq 'frame1'){
                                $pseq .= $_;
                        }
                        if ($fields[1] eq 'frame2'){
                                $nseq .= $_;
                        }
                }
        }
}
close(FA1);
```

```
for (1..60000){
	$pos = int(rand(length($rseq)));
	$r_hash{substr($rseq, $pos, 1)}++;
	$p_hash{substr($pseq, $pos, 1)}++;
	$n_hash{substr($nseq, $pos, 1)}++;
}
foreach $aa (@aaArr){
	print $aa."\t".$r_hash{$aa}."\t".$p_hash{$aa}."\t".$n_hash{$aa}."\n";
}
```

```
#!/usr/bin/perl

################################################################################
#                                                                              #
# Copyright, 2009, Lajos Kalmar, Erika Kovacs                                  #
# Institute of Enzymology of the Hungarian Academy of Sciences                 #
#                                                                              #
# aacont2.pl: calculate amino acid composition in dual-coding regions          #
#                                                                              #
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#                                                                              #
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#                                                                              #
################################################################################

open (FA, ''); # path to translated protein dataset in normal frame
while (<FA>){
        chomp;
        if (/^>/){
                @fields = split(' ', $_);
                $fields[0] =~ s/>//g;
        }
        else{
                $seq{$fields[0]} = $_;
        }
}
close(FA);
open(L,''); # path to dataset created by iupevol_iupanal_pairs.pl
while(<L>){
        chomp;
        @fields = split(/\t/, $_);
        $data{$fields[7]}{'frame'} = $fields[2];
        $data{$fields[7]}{'type'} = 'new';
        ($data{$fields[7]}{'from'}, $data{$fields[7]}{'to'}) = split(/\-/, $fields[9]);
        $data{$fields[7]}{'AF'}{'AC'} = $fields[1];
        ($data{$fields[7]}{'AF'}{'from'}, $data{$fields[7]}{'AF'}{'to'}) = split(/\-/,
$fields[4]);
}
close(L);
open(LI, shift(@ARGV)); # list of transcript IDs to analyze
while(<LI>){
        chomp;
        $fullseq .= substr($seq{$data{$_}{'AF'}{'AC'}}, $data{$_}{'AF'}{'from'} - 1,
($data{$_}{'AF'}{'to'} - $data{$_}{'AF'}{'from'} +1));
}
close(LI);
$nn_ref = aaCont2($fullseq);
foreach $aa (@aaArr){
        print $aa."\t".$nn_ref->{$aa}."\n";
}

sub aaCont2 {
        my @matches;
        my $data;
        my $seq = uc(shift(@_));
```

```
	my @aaArr = ('W', 'C', 'F', 'I', 'Y', 'V', 'L', 'H', 'M', 'A', 'T', 'R', 'G', 'Q', 'S',
'N', 'P', 'D', 'E', 'K');
	foreach $aa (@aaArr){
		@matches = ();
		@matches = $seq =~ m/$aa/g;
		$data->{$aa} = ($#matches + 1);
	}
	return $data;
}
```

```
#!/usr/bin/perl

################################################################################
#                                                                              #
# Copyright, 2009, Lajos Kalmar, Erika Kovacs                                  #
# Institute of Enzymology of the Hungarian Academy of Sciences                 #
#                                                                              #
# iupevol_randiup.pl: calculate disorder in artificial frameshifts             #
#                                                                              #
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#                                                                              #
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#                                                                              #
################################################################################

use Data::Dumper;
$|++;

my %data = ('TTT' => 'F', 'TTC' => 'F', 'TTA' => 'L', 'TTG' => 'L', 'TCT' => 'S', 'TCC' =>
'S', 'TCA' => 'S', 'TCG' => 'S', 'TAT' => 'Y', 'TAC' => 'Y', 'TGT' => 'C', 'TGC' => 'C', 'TGG'
=> 'W', 'CTT' => 'L', 'CTC' => 'L', 'CTA' => 'L', 'CTG' => 'L', 'CCT' => 'P', 'CCC' => 'P',
'CCA' => 'P', 'CCG' => 'P', 'CAT' => 'H', 'CAC' => 'H', 'CAA' => 'Q', 'CAG' => 'Q', 'CGT' =>
'R', 'CGC' => 'R', 'CGA' => 'R', 'CGG' => 'R', 'ATT' => 'I', 'ATC' => 'I', 'ATA' => 'I', 'ATG'
=> 'M', 'ACT' => 'T', 'ACC' => 'T', 'ACA' => 'T', 'ACG' => 'T', 'AAT' => 'N', 'AAC' => 'N',
'AAA' => 'K', 'AAG' => 'K', 'AGT' => 'S', 'AGC' => 'S', 'AGA' => 'R', 'AGG' => 'R', 'GTT' =>
'V', 'GTC' => 'V', 'GTA' => 'V', 'GTG' => 'V', 'GCT' => 'A', 'GCC' => 'A', 'GCA' => 'A', 'GCG'
=> 'A', 'GAT' => 'D', 'GAC' => 'D', 'GAA' => 'E', 'GAG' => 'E', 'GGT' => 'G', 'GGC' => 'G',
'GGA' => 'G', 'GGG' => 'G');
@csere = values %data;

open(F1, ''); # path to dataset created by iupevol_iupanal_pairs.pl
while(<F1>){
        @fields = split(/\t/, $_);
        if ($fields[4] =~ /\-/){
                ($from, $to) = split(/\-/, $fields[4]);
                push(@regsizes, ($to - $from + 1));
        }
}
close(F1);
open(F2, ''); # path to human RefSeq fasta file
while (<F2>){
        if (/^>/){
                @fields = split(/\t/, $_);
                (undef, $temp) = split(/\:/, $fields[2]);
                ($from, $to) = split(/\.\./, $temp);
        }
        else {
                chomp;
                if (($to - $from + 1) > 900){
                        push(@seq, substr($_, ($from - 1), ($to - $from + 1)));
                }
        }
}
close(F2);
```

```
for (1..100){

	$aktseq = $seq[int(rand($#seq + 1))];
	$refprot = dna2protHUM($aktseq, 0);
	$refprot =~ s/X//g;
	$plusseq = substr($aktseq, 0, 300).substr($aktseq, 301);
	$minusseq = substr($aktseq, 0, 300)."A".substr($aktseq, 300);
	@plustemp = split(/|/, dna2protHUM($plusseq, 0));
	$plusprot = '';
	for (@plustemp){
		if ($_ eq 'X'){
			$_ = $csere[int(rand($#csere + 1))];
		}
		$plusprot .= $_;
	}
	@minustemp = split(/|/, dna2protHUM($minusseq, 0));
	$minusprot = '';
	for (@minustemp){
		if ($_ eq 'X'){
			$_ = $csere[int(rand($#csere + 1))];
		}
		$minusprot .= $_;
	}
	$range = $regsizes[int(rand($#regsizes + 1))];
	$temp1 = rangeIUP($refprot, $range);
	push (@refpercs, $temp1);
	if ($temp1 > 0.5){
		$refmost++;
	}
	$temp2 = rangeIUP($plusprot, $range);
	push (@pluspercs, $temp2);
	if ($temp2 > 0.5){
		$plusmost++;
	}
	$temp3 = rangeIUP($minusprot, $range);
	push (@minuspercs, $temp3);
	if ($temp3 > 0.5){
		$minusmost++;
	}
	print $_."\t".$range."\t".$temp1."\t".$temp2."\t".$temp3."\n";
}
print "\n";
print "RefSeq (iupavg): ".mean(@refpercs)." mostly disordered perc.: ".($refmost/1000)."\n";
print "RefSeq+1 (iupavg): ".mean(@pluspercs)." mostly disordered perc.:
".($plusmost/1000)."\n";
print "RefSeq-1 (iupavg): ".mean(@minuspercs)." mostly disordered perc.:
".($minusmost/1000)."\n";

sub mean {
	my $result;
	foreach (@_) { $result += $_ }
	return $result / ($#_ + 1) ;
}

sub rangeIUP {
	(my $seq, my $range) = @_;
	my $iupred = iupredAn($seq, 'long');
	my $vsl2 = vsl2An($seq);
	my $iupred_perc = iupredPerc($iupred, 100, 100+$range-1, 0.4);
	my $vsl2_perc = iupredPerc($vsl2, 100, 100+$range-1, 0.5);
	return ($iupred_perc + $vsl2_perc) / 2;
}

sub dna2protHUM {
	my $dna_seq = shift(@_);
	my $frame = shift(@_);
```

```
	my %data = ('TTT' => 'F', 'TTC' => 'F', 'TTA' => 'L', 'TTG' => 'L', 'TCT' => 'S', 'TCC'
=> 'S', 'TCA' => 'S', 'TCG' => 'S', 'TAT' => 'Y', 'TAC' => 'Y', 'TAA' => 'X', 'TAG' => 'X',
'TGT' => 'C', 'TGC' => 'C', 'TGA' => 'X', 'TGG' => 'W', 'CTT' => 'L', 'CTC' => 'L', 'CTA' =>
'L', 'CTG' => 'L', 'CCT' => 'P', 'CCC' => 'P', 'CCA' => 'P', 'CCG' => 'P', 'CAT' => 'H', 'CAC'
=> 'H', 'CAA' => 'Q', 'CAG' => 'Q', 'CGT' => 'R', 'CGC' => 'R', 'CGA' => 'R', 'CGG' => 'R',
'ATT' => 'I', 'ATC' => 'I', 'ATA' => 'I', 'ATG' => 'M', 'ACT' => 'T', 'ACC' => 'T', 'ACA' =>
'T', 'ACG' => 'T', 'AAT' => 'N', 'AAC' => 'N', 'AAA' => 'K', 'AAG' => 'K', 'AGT' => 'S', 'AGC'
=> 'S', 'AGA' => 'R', 'AGG' => 'R', 'GTT' => 'V', 'GTC' => 'V', 'GTA' => 'V', 'GTG' => 'V',
'GCT' => 'A', 'GCC' => 'A', 'GCA' => 'A', 'GCG' => 'A', 'GAT' => 'D', 'GAC' => 'D', 'GAA' =>
'E', 'GAG' => 'E', 'GGT' => 'G', 'GGC' => 'G', 'GGA' => 'G', 'GGG' => 'G');
	my $prot_seq = '';
	for (my $i = $frame; $i < length($dna_seq); $i+=3){
		$prot_seq .= $data{substr($dna_seq, $i, 3)};
	}
	return $prot_seq;
}


sub iupredAn {
	$ENV{'IUPred_PATH'}=''; # path of the iupred source directory
	my $iupred_path = ''; # path of the iupred binary
	my $seq = shift(@_);
	my $opt = shift(@_);
	my $resref = undef;
	open (TMP, '>/tmp/iutmp') || die "Couldn't write to tmp directory";
	print TMP ">iupquery\n";
	print TMP $seq;
	my $result=`$iupred_path /tmp/iutmp $opt`;
	my @lines = split(/\n/, $result);
	foreach $line (@lines){
		if ($line !~ /^#/){
			my @fields = split(' ', $line);
			$resref->{$fields[0]} = $fields[2];
		}
	}
	close (TMP);
	unlink ('/tmp/iutmp');
	return $resref;
}

sub vsl2An {
	my $vsl2_path = ''; # path of the VSL2.jar file
	my $resref = undef;
	my $seq = shift(@_);
	my @array;
	open (TMP, '>/tmp/vsl2tmp') || die "Couldn't write to tmp directory";
	print TMP $seq;
	my @result=split(/\n/, `java -jar $vsl2_path -s:/tmp/vsl2tmp`);
	close (TMP);
	unlink ('/tmp/vsl2tmp');
	push(@array, 'VSL2');
	my $pass = 0;
	foreach $line (@result){
		if ($line =~ /^\-\-\-\-\-/){
			$pass = 1;
		}
		if ($line =~ /\d/ && $pass == 1){
			my @fields = split(' ', $line);
			push (@array, $fields[2]);
		}
	}
	for ($i=1; $i <= $#array+1; $i++) {
		$resref->{$i} = $array[$i];
	}
	return $resref;
}
```

```
sub iupredPerc {
	my $ref = shift(@_);
	my $from = shift(@_);
	my $to = shift(@_);
	my $tresh = shift(@_);
	my $sumiup = undef;
	for ($from..$to){
		if ($ref->{$_} > $tresh) {
			$sumiup++;
		}
	}
	return sprintf("%.4f", $sumiup / ($to - $from + 1));
}
```

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    written offer, valid for at least three years and valid for as
    long as you offer spare parts or customer support for that product
    model, to give anyone who possesses the object code either (1) a
    copy of the Corresponding Source for all the software in the
    product that is covered by this License, on a durable physical
    medium customarily used for software interchange, for a price no
    more than your reasonable cost of physically performing this
    conveying of source, or (2) access to copy the

Corresponding Source from a network server at no charge.

c) Convey individual copies of the object code with a copy of the written offer to provide the Corresponding Source. This alternative is allowed only occasionally and noncommercially, and only if you received the object code with such an offer, in accord with subsection 6b.

d) Convey the object code by offering access from a designated place (gratis or for a charge), and offer equivalent access to the Corresponding Source in the same way through the same place at no further charge. You need not require recipients to copy the Corresponding Source along with the object code. If the place to copy the object code is a network server, the Corresponding Source may be on a different server (operated by you or a third party) that supports equivalent copying facilities, provided you maintain clear directions next to the object code saying where to find the Corresponding Source. Regardless of what server hosts the Corresponding Source, you remain obligated to ensure that it is available for as long as needed to satisfy these requirements.

e) Convey the object code using peer-to-peer transmission, provided you inform other peers where the object code and Corresponding Source of the work are being offered to the general public at no charge under subsection 6d.

A separable portion of the object code, whose source code is excluded from the Corresponding Source as a System Library, need not be included in conveying the object code work.

A "User Product" is either (1) a "consumer product", which means any tangible personal property which is normally used for personal, family, or household purposes, or (2) anything designed or sold for incorporation into a dwelling. In determining whether a product is a consumer product, doubtful cases shall be resolved in favor of coverage. For a particular product received by a particular user, "normally used" refers to a typical or common use of that class of product, regardless of the status of the particular user or of the way in which the particular user actually uses, or expects or is expected to use, the product. A product is a consumer product regardless of whether the product has substantial commercial, industrial or non-consumer uses, unless such uses represent the only significant mode of use of the product.

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