Statistical Analysis of Coding Sequences

Set-up of an R package
statanacoseq
distributed on GitHub
to carry future implementations of
Codon Bias Indexes.

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for the master degree in
Bioinformatics and Computational Biology
by

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accepted on the faculty reunion:

Declaration

under Art. 28 Para. 2 RSL 05

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Matriculation number: 99-119-380

Programme: Bioinformatics and Computational Biology

Thesis title: Statistical Analysis of Coding Sequences

Thesis supervisor: Dr. Cannarozzi Gina

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person, except where due acknowledgement has been made in the text. In accordance with academic rules and ethical conduct, I have fully cited and referenced all material and results that are not original to this work. I am well aware of the fact that, on the basis of Article 36 Paragraph 1 Letter o of the University Law of 5 September 1996, the Senate is entitled to deny the title awarded on the basis of this work if proven otherwise. I grant inspection of my thesis.

Bern, 12.08.2016

University of Bern, 2016

But for a champion, the lasting memory
is always the loss in the race unfinished.
[…] My race is now unfinished.
— **Abebe Bikila**
from *Atletu* (2009)

To my friends. . .

Acknowledgements

First of all I would like to thank my supervisor Gina Cannarozzi to support my idea to not only transcribed the codon bias functions to a language we students are familiar with but to generate a R package that resembles a complete code. Thanks go to my Boss Zerihun Tadele for accepting a master student in his Teff group that is more interested in working on the bits that defines the teff genome than on the plants itself and more interested to run grasslands than to breed grasses. I thank the whole department to give me a nice introduction into plant science and to leave some food in the kitchen for me to survive the long coding evenings. I would like to thank all my friends who accepted my regression to go back to school and my attitude to no longer join them in drinking and eating out and all those that found other ways to entertain me. Special thanks go to my friends that offered me a bed to bring variation in my trening sessions to discover new running tracks and all sportsmen motivating me in the competitions. Almost superfluous is to thank all those contributors who worked in the past and parallely on open source codes to bring tools and innovation to the scientific community that is nowadays regarded as taken for granted but we only get the full awareness when we have to submit our work, showing our weakness and limits, for public usage.

Bern, August 12 2016 Fredy Siegrist

Preface

A preface is not mandatory. It would typically be written by some other person (eg your thesis director).

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Bern, August 12 2016 Gina Cannarozzi

Abstract

The basic idea of this project was to implement codon bias indicies that have been listed before [**?**] together with important indices that came after and improvements. The standard genetic code, as we know it for most of the species, has 61 sense codons translating into 20 amino acids. These codons are usually decoded by a much larger number of different tRNAs, that in addition can 'wobble', that is some tRNAs are used to read more than one codon. For example, yeast has 41 tRNAs to decode its 61 sense codons. The choice of synonymous codon is not at all random, some codons are used frequently and others are hardly used at all [**?**]. In this project, we implemented several function that compute indices of codon usage and applied them to coding sequences of the grass plant *Eragostis tef*. We set up an R project based on another package seqinr and transcribed the code for standard indicies that measure codon usage and bias such as the codon adaptation index (CAI), the tRNA pairing index (tPI) and others from a code-library called CodonIndices of the language Darwin.
First functions were written for text- and graphical-based visualization of analyses based on codon usage that can be asses by demonstrations of the package. Further work may assess the significance of groups with extreme values of these indices using Enrichment.

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Key words: R package; Codon Bias; Codon Usage; Statistical Analysis

Zusammenfassung

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Stichwörter: R Sammlung; Kodon-Vorliebe; Kodon-Gebrauch; Statistische Analyse

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Introduction

This document is written based on a Generic EPFL Thesis Template in LaTeX v. 5.57 and is SUBDUE to further changes as it should be included in the GitHub project and some of the Chapters should contain text elements that are used to build up a proper R vigniette of the package. Meaning to give examples for the usage of the different indices and functions for biological interpretation with the code for repeating the *in silico* experiments by applying the code lines step by step or by copy pasting the entire code for a sample experiment wich raw data can be found in a public database. . . .

0.1 Aim

We are aiming to set up a tool to automatically calculate and evaluate statistical properties of codon bias, especially implementing the calculation for different codon bias indices and for genes of organisms where its genome is sequenced for the most part. That means that we have at least a partially sequenced genome and the information on expressed sequences or protiens The most simpple calculation of codon bias is the GC content of the third base of synonymous codons of a single gene or coding DNA sequence (CDS). This codon bias reflects neutral mechanisms like mutational selection for a high or low GC content in an organism. . . . reference [**?**] . . .

Such mechanisms based on a single gene can be revised by incorporating species independent information of general translation efficiency and RNA folding. Moreover, instead of analysing a single nucleotide position alone (mononucleotides), information on cytosine (C)-methylation can be taken into account and calculation on dinucleotides (GC) for mammals or quadnucleotide (GATC) or pentnucleotides (CCWGG) wich harbours a degenerate nucleotide position that can be A or T. The succession of codons (bicodons or even tricodons) may of course also be of interest but may only refine codon bias indexes where information on the whole genome or even for species is taken into account.

When we aim for introducing a little bit more suffisticated indexes, they will depend on

additional information such as, the transcriptome, the genome, gene expression, tRNA availability

One aspect for some codon bias mesurements is the definition of the optimal codon, thus the one with the most desirable quality. This information is used to mesure the frequency of optimal codons (Fop) or the P1 index, a meaure of the influence of tRNA availability. Which of the codons is optimal can be manifoldly defined, such as predicted using the tRNA gene copy number as a indicator as tRNA levels correlate with gene copy number in general [**?**](Chaney and Clark 2015).

0.2 Teff genome

Eragostis tef is not only a grass but an important crop, that was neglected in sdcientific studies before the setup of the 'Teff Improvement Project" ten years ago at the University of Bern. Applied science generated improved plants that made it to the farmers in Ethiopia and research is ongoing to discover new break-through plants that show increased seed size, soil acidity, drought and herbizide tolerance along with the improved yield and to maintain lodging resistance without being semi-dwarf. A valuable resource for plants genetics is also the sequencing and annotation of the tetraploid genome of Teff and the evaluation of its properties [**?**]. Therefore, we used as sample organism here, sequences from this genome to examine what obstacles one encounters by working with a almost totally sequenced but not yet fully annotated genome. By learning from its codon usage, variations generated by technologies already used in the 1960 for screening of genetic variations of agricultural plants combined with DNA sequencing technologie may improve the selection criterias for optimal mutants used for back crossing.

0.3 Biological aspects of Codon usage

The ribosome is coordinating the translation from messenger RNA (mRNA) to proteins in the cells together with tranlsational factors and aminoacyl-transfer RNAs (tRNA) and guanosinetriphosphate as substrate. The efficiency of translation is thereby defined by the mRNA quantity and stability, but also by the composition (concinnity, order and the distribution of codons, the bioavailability of the mRNA and the translational factors and much more. The bias for using particular codons preferentially must be differentiated between a *codon bias*, which tends to use those codons that will lead to an optimal use of tRNA for having an optimal cost-benefit factor between a fast and a accurate protein synthesis (including aminoacid misincorporations and protein malfolding) and a *codon adaptation*, that is aiming to optimize the use of codons towards a enhanced

translational efficiency and this is subject to a exchange trend towards more optimal codons. For estimation of the codon bias we can compute codon usage bu measuring the bias to use adaptive codons for example with the Codon Adaptation Index (CAI) that is implemented in our code. The relative adaptiveness (RA) has therby be calculated form a seperate set of sequences, usually from the usage of highly expressed genes (codon fitness values). For the moment that is the users task, but may be decided on gene expression data in the future in our package. In contrast, the Number of effective Codons (NEC) in our package mesures only the degree of deviation from an uniform codon usage based on basic statistics, regardeless of which codons are overrepresented [**?**]. Morover the package could be expanded in the future to compute additional indices such as the S value, that measures the strength of selection for codon adaptation (S), to perform within-group correspondence analysis (WCA) of codon usage, give information on replication strand skew analysis, the GC skew index (GCSI), single statisitics (S) or PCA to estimate the relative selection strength within a genome.

0.3.1 Genetic Code

Here is shown a table with the standard genetic code (eg. *E. coli*), generated with the first of the following functions:

```
require(seqinr)
tablecode()
# to plot a codon table with ratios and frequencies with the overwriting function
  from statanacoseq
require(statanacoseq)
tablecode(mylist(whatout=1))
```

Genetic code 1 : standard							
T T T	Phe	T C T	Ser	T A T	Tyr	T G T	Cys
T T C	Phe	T C C	Ser	T A C	Tyr	T G C	Cys
T T A	Leu	T C A	Ser	T A A	Stp	T G A	Stp
T T G	Leu	T C G	Ser	T A G	Stp	T G G	Trp
C T T	Leu	C C T	Pro	C A T	His	C G T	Arg
C T C	Leu	C C C	Pro	C A C	His	C G C	Arg
C T A	Leu	C C A	Pro	C A A	Gln	C G A	Arg
C T G	Leu	C C G	Pro	C A G	Gln	C G G	Arg
A T T	Ile	A C T	Thr	A A T	Asn	A G T	Ser
A T C	Ile	A C C	Thr	A A C	Asn	A G C	Ser
A T A	Ile	A C A	Thr	A A A	Lys	A G A	Arg
A T G	Met	A C G	Thr	A A G	Lys	A G G	Arg
G T T	Val	G C T	Ala	G A T	Asp	G G T	Gly
G T C	Val	G C C	Ala	G A C	Asp	G G C	Gly
G T A	Val	G C A	Ala	G A A	Glu	G G A	Gly
G T G	Val	G C G	Ala	G A G	Glu	G G G	Gly

Figure 1: Table with standard genetic code

1 Setup of data and project

In this chapter we will discuss the setup of the project.

1.1 Getting familiar with the data

Before the project was set-up the initial task was to gather information for calculating one of the first defined and most basic codon bias index called frequency of optimal codons (Fop). The optimality of a codon can be estimated by the usage in the given gene, the transcriptome or by accessing the numbers of tRNA genes found in the genome. As we have already the data of tRNA genes for the teff genome available, this was the choice to work with and to import the information for closely related species for mays and millet.

1.1.1 read tRNA stats and decide on optimality of a codon

```
#03.01.2016 Master project read in stat files from view-source:http://gtrnadb.ucsc.edu/GtRNAdb2/genomes/eukaryota/

#reading the files
setwd("E:/R")
readstats <- function(fileno=2, chunksize=1) {
  statfile=dir(pattern=".stats$")[fileno]
  con <- file(statfile, "r", blocking = FALSE)
  for(i in seq(1,3500,chunksize)){
        d=scan(con,what="a",nlines=chunksize,sep="|", quote="",quiet=TRUE)
    if (length(d)>0 && d[1]==""){
      print(substr(d[2:(length(d)-1)], 2, 15) )
      reps <- as.numeric(gsub("[^0-9]","", d[2:(length(d)-1)]))
      tRNAs <- substr(d[2:(length(d)-1)], 2, 4)
      codons <- substr(d[2:(length(d)-1)], 6, 8)
        closeAllConnections()
      return(data.frame(tRNAs, codons, reps))
    }
```

```
  }
}
(Zmays <- readstats() )
eins <- function(x=c("Val","UUU","1")){optfactor <- as.numeric(x[3])/max(Zmays[
  tRNAs==x[1],3])}
optfact <- apply(Zmays, 1, eins)
isopt <- (optfact == 1)
Zmays.out <- data.frame(Zmays, optfact, isopt)
Zmays.out[Zmays.out$isopt==TRUE,-4]
```

```
tRNAscan-SE v.1.3 (March 2011) scan results (on host aero.cse.ucsc.edu)
Started: Thu Jul 26 22:25:36 PDT 2012

--------------------------------------------------------------
Search Mode:                 Eukaryotic
Searching with:              tRNAscan + EufindtRNA -> Cove
Covariance model:            TRNA2-euk.cm
tRNAscan parameters:         Strict
EufindtRNA parameters:       Relaxed (Int Cutoff= -32.1)

Reporting HMM/2' structure score breakdown
--------------------------------------------------------------

First-pass (tRNAscan/EufindtRNA) Stats:
---------------
Sequences read:         13
Seqs w/at least 1 hit:  13
Bases read:             2066432718 (x2 for both strands)
Bases in tRNAs:         2747160
tRNAs predicted:        28967
Av. tRNA length:        94
Script CPU time:        417.21 s
Scan CPU time:          1253.04 s
Scan speed:             3298.3 Kbp/sec

First pass search(es) ended: Thu Jul 26 22:54:15 PDT 2012

Cove Stats:
-----------
Candidate tRNAs read:     28967
Cove-confirmed tRNAs:     2249
Bases scanned by Cove:  3210632
% seq scanned by Cove:  0.1 %
Script CPU time:          117.99 s
Cove CPU time:            2336.55 s
Scan speed:               1374.1 bp/sec

Cove analysis of tRNAs ended: Fri Jul 27 00:06:48 PDT 2012

Summary
--------
Overall scan speed: 1001957.8 bp/sec

tRNAs decoding Standard 20 AA:              1455
Selenocysteine tRNAs (TCA):                 4
Possible suppressor tRNAs (CTA,TTA):        7
```

```
47 tRNAs with undetermined/unknown isotypes:   12
48 Predicted pseudogenes:                      771
49                                             -------
50 Total tRNAs:                                2249
51
52 tRNAs with introns:         54
53
54 | Thr-GGT: 1 | Val-AAC: 3 | Val-CAC: 1 | Val-TAC: 1 | Ser-GCT: 1 | Arg-TCT: 1 |
     Leu-TAA: 2 | Asn-ATT: 1 | Ile-AAT: 1 | Met-CAT: 24 | Tyr-ATA: 1 | Tyr-GTA: 16 |
     Cys-ACA: 1 |
55
56 Isotype / Anticodon Counts:
57
58 Ala    : 122    AGC: 79      GGC:         CGC: 28      TGC: 15
59 Gly    : 55     ACC:         GCC: 33      CCC: 11      TCC: 11
60 Pro    : 59     AGG: 15      GGG: 1       CGG: 9       TGG: 34
61 Thr    : 58     AGT: 21      GGT: 13      CGT: 7       TGT: 17
62 Val    : 61     AAC: 25      GAC: 15      CAC: 16      TAC: 5
63 Ser    : 68     AGA: 13      GGA: 23      CGA: 7       TGA: 11      ACT:         GCT
     : 14
64 Arg    : 90     ACG: 45      GCG:         CCG: 6       TCG: 7       CCT: 12      TCT
     : 20
65 Leu    : 79     AAG: 30      GAG:         CAG: 14      TAG: 9       CAA: 20      TAA
     : 6
66 Phe    : 27     AAA: 2       GAA: 25
67 Asn    : 53     ATT: 2       GTT: 51
68 Lys    : 350                              CTT: 206     TTT: 144
69 Asp    : 45     ATC: 1       GTC: 44
70 Glu    : 59                               CTC: 30      TTC: 29
71 His    : 33     ATG: 4       GTG: 29
72 Gln    : 39                               CTG: 14      TTG: 25
73 Ile    : 79     AAT: 73      GAT:                      TAT: 6
74 Met    : 90                               CAT: 90
75 Tyr    : 28     ATA: 2       GTA: 26
76 Supres: 7                                 CTA: 6       TTA: 1
77 Cys    : 30     ACA: 2       GCA: 28
78 Trp    : 30                               CCA: 30
79 SelCys: 4                                               TCA: 4
```

However this code had to be adapted because it is only reading in the tRNA genes with introns. Interestingly, by browsing some of the output data from tRNAscan for other species we discovered some that have a huge amount of the alternative usage of the opal stop codon "UGA" that is messing up with the display of the overview in a hierarchical plot for all the tRNA numbers even when standardized for each tRNA, we can see that some of the species show a much higher total number of tRNAs.

If we plot the log values of the tRNA gene count and normalize by species (column) then we can see that some species have very low numbers of certain codons that is usually compensated by using another block of codons that are rare in the other kingdoms. We also observe some species that have a high number of tRNAs where the isoaccepter

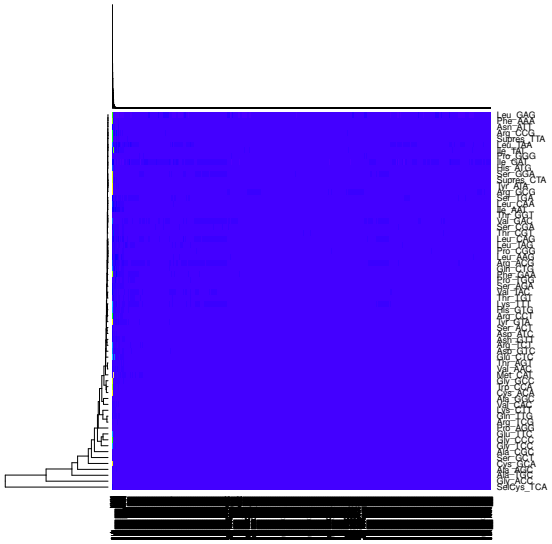

Figure 1.1: Heat map of tRNA gene counts normalized per row in all species

remains unknown (codon 65).

1.2 text chunk - tRNA database

The tRNA genome numbers for eukariotic, bacterial, archaea and one viral genome was extracted from tRNAscan-SE v.1.3 run statistics of the GtRNAdb 2.0 (available at Genomic tRNA Database 2.0, http://gtrnadb.ucsc.edu/GtRNAdb2/genomes/). [**?**][Chan PP et al. 2016]

To decide on optimal codons the number of genes for the given anticodon was divided by the maximal number of genes for a anticodon for the same aminoacid to give fraction to optimal codon. The codon was accepted as optimal if this fraction was 1. In a list for every genome with available run statistic data, a frame with aminoacid, codon, number of genes, fraction to optimal codon, fraction to all codons, and the decision if it is a optimal codon was stored for the four superkingdoms seperately.

```
The genomes with names containing # or * have to be treated as special
cases, as even the browser failed to fetch the files.
Therefore # was replaced with %23
```

However, in those run statistics, from vertebrates especially non-primate mammals, are littered with tRNA-derived repetitive elements with primary sequences very similar to

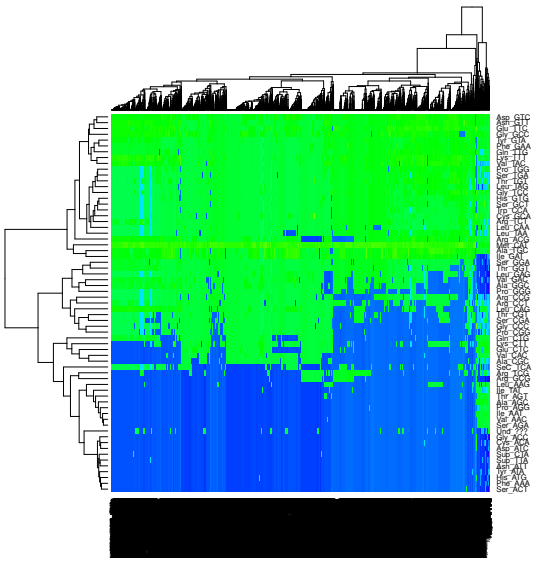

Figure 1.2: Heat map of logged(tRNA gene counts) normalized per column in all species

real tRNAs. So they apply a non-unveiled post filter after the tRNAscan-SE on those genomes before depositing the predictions to GtRNAdb. Therefore, and because they were not willing to share the database with the summary statistics, these had to be rebuild from scanning the headers of the fasta files. Therefore the fasta file name had to be scanned in the index.html file and the fasta files were renamed according to the directory name to facilitate automation.

The statistics is now enhanced by an 65th line containing undefined aminoacid all other anticodons with degenerated base information are counted to the undefined species (as they do in the summary page)

codon ramp (rare codons at the start Tuller et al.) "This “codon ramp” hypothesis should apply primarily in the context of strong translation, but we found that using rare codons at the N terminus increases expression regardless of translation strength." –ANECTODE– When analysing the moving average of the optionality factor of the codons for the tRNA genes, we don’t really detect a codon ramp at the 5’ terminus, first, but the first codon was always optimal. No wonder because there is only one codon for Methionine, the start codon. Therefore we should only analyse the aminoacids that have a choice of anti-codons to use.

For analyzing codon usage in E. teff Gina’s validated protein-transcript fasta files were used, however there were two issues in the database: first one of the proteins (Et_s4372-0.17-1) was truncated, didn’t startet with methionine and was not corresponding to the

truncated transcript, secondly the number of transcripts matched the number of proteins, but there was one orphan entry on each side (Et_s2692-0.26-1, Et_s14755-0.7-1), that had to be filled up with the corresponding entry of the other datafile.

1.2.1 text chunk - Project notes

implemented Foc retrieved tRNA database problems: normalization, anomolous values solutions: standardize data computed codon usage in tef problems: odd characters in data, mismatch, solution: preprocess data sequences in frame: solution: preprocess data is implementation of a suffix tree a possibility?

testing - use the sequences form the original papers and show that you get the same values

CRAN or Bioconductor? look at https://cran.r-project.org/doc/manuals/r-release/R-exts.html to write an R package bioconductor https://www.bioconductor.org/developers/how-to/buildingPackagesForBioc/

3 components code testing documentation

start with CAI look at implementatino in seqinR- what is available already in SeqinR? use as much of their stuff as possible but we want to keep the possibility to change base data if you want (tRNA mapping and frequencies) look at what the possibilities are and what parts of the exisiting implementation can be used? if not , reimplement in such a way that it is more flexible

1.2.2 Plot OCU demo

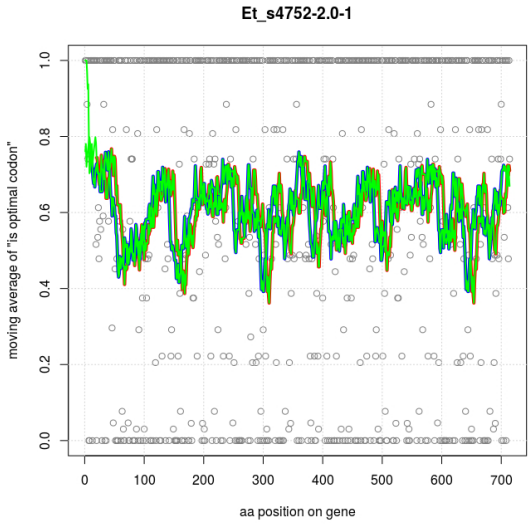

Figure 1.3: A sample figure (from plot OCU *demo(plot(OCU)*, of statanacoseq, got from https://github.com/fredysiegrist/statanacoseq).

2 R package setup and maintenance

In this chapter we will describe the generation of the R package structure, implementation in R Studio and hosting on GitHub.

2.1 Generation of R package

The first intention was to generate a package named "statanacoseq" in the linux version of R. The name is pretty long and has not been found to be used as R package name anywhere else, that is screened by google. Setting up of the R package has been done with the help of devtools, some few lines of code generate a backbone that then can be filled with the content and basically be packed after having put the first function in to an R file.

```
# Construction of the first package
# set the working directory to the shared partition
setwd("E:/R/")
# setwd("/windows/R/")
# install.packages("devtools")
library("devtools")
# devtools::install_github("klutometis/roxygen")
library(roxygen2)
# Creation of empty package
create("statanacoseq")
# save function as "E:/R/statanacoseq/R/countcodonfreq.R"
setwd("./statanacoseq")
document()
setwd("..")
install("statanacoseq")
sessionInfo()
detach("package:statanacoseq", unload=TRUE)
```

In this case a simple function with misleading name was countcodonfreq but was used to check the 'validated' fasta files by printing out the fraction of codons to aminoacids for the nucleotide and aminoacids with the same entry name.

```
countcodonfreq <- function(no=1, wdir="/windows/R/") {
    require(seqinr)
    list(myseq, myaa) <- mylist(wdir)
    print(length(myseq[[no]])/3/length(myaa[[attr(myseq[[no]], "name")]]))
}
```

The correct interpretation of the roxygen function documentation can be checked by adressing the help of the function in a internal window or as html style:

```
options(help_type = "text") # open help internally
options(help_type = "html") # open help in browser
?countcodonfreq
```

2.1.1 Maintenance with R Studio

To be more efficient in managing the project the project initiated with devtools was imported into R Studio, a software package that brings many functions for assisting with R code writing, documentation and communication with the version control system [**?**].

2.1.2 Publishing on GitHub - Description of versions

After the package got the first function to work independently on sample data from the package it was submitted to GitHub, a hosting service for software projects, for version control and to make it available for revision.
The origin is placed at https://github.com/fredysiegrist/statanacoseq.git [**?**]. Since no version has been generated where the indices that are not implemented in Darwin has been drawed up, the version remained at 0.0.0.900x, marking it as under development. At the moment of writing this text the version is 0.0.0.9001 and will be set to 0.0.0.9002 for the evaluation of this work and 0.0.0.9003 after the corrections. Any further version will mark improvements to the code made post thesis submission.

The version 0.0.1 should mark the mile stone of achieving better performance than the Darwin package, incuding the calculation of not-implemented functions in that code.

Version 0.1.0 should be the version that is distributed to the scientific community and following versions may be described in articles.

Version 1.0.0 will be defined after distribution to CRAN or bioconductor, if a bug free code that is consistant in it-self is created and will have some back-references from other

packages.

3 Transcription of Darwin Code

In this chapter we will describe the transcription of darwin codes and the adaptation of some function to the R enviorenment.

3.1 Transcription of Darwin functions

One of the tasks was to set up a system that can handle the functions implemented in Darwin in the source compilation *CodonIndices* and process genetic files given from local fasta files, sequence files from public databases or sample files from the R package if no custom sequence is entered. The remaining problem is to understand how the variable *Entries* is build up from the database *db* and to mirror that function as we built up the R package on the existing R project SeqInR *seqinr* that uses a distict different sequence database handling. Some other functions, where indices are calculated on the sequence information itself, where adaptable without dealing with the sequence database problem and were transcribed 1:1 to a function R code, however the loops are not acknoledging the functionality of the R environment.

3.2 R environement adaptation

To upvalue the code transcibed from Darwin, the function *ComputeNEC* was simplified to R language by eliminating the loops and introducing table and sapply function instead. The source code now looks pretty much like a R code that was setup in that language at first (by me). Below we list this code for demonstration, the original code in Darwin can be found in the appendix.

```
1 ComputeNEC <- function(cds) {
2   if(!(checkCDS(cds))) {stop("non valid CDS)", call.=FALSE)}
```

```
3     else {
4       cds <- tolower(cds)
5       cod <- rep(0, times=64)
6       names(cod) <- sapply(as.character(Tef$codons), reversecomplement)
7       cod <- cod[c(-59, -60, -64)]
8       aa <- rep(0, times=20)
9       names(aa) <- levels(Tef[,1])[c(-16,-18)]
10      cods <- count(s2c(cds), word = 3, by=3)
11      cod[names(cod)] <- as.vector(cods[tolower(names(cod))])
12      AA <- table(translate(s2c(cds)))
13      aa[names(aa)] <- AA[a(names(aa))]
14      NC <- rep(1, times=length(aa))
15      names(NC) <- names(aa)
16      Acods <- sapply(names(NC), function(i) sapply(as.character(Tef[,2]),
          reversecomplement)[Tef[,1]==i])
17      contributors <- names(aa)[sapply(Acods, length)>1&aa>1]
18      Nc <- sapply(contributors, function(i) {
19       n <- sum(cod[unlist(Acods[i])])
20       S <- sum(sapply(cod[unlist(Acods[i])], function(x) (x/n)^2))
21       F <- (n*S-1) / (n-1)
22       return(1/F)
23       }
24      )
25      NC[contributors] <- Nc[contributors]
26      return(sum(NC))
27    }
28  }
```

4 Vignette

This document is a vignette for using the R package statanacoseq:

4.1 statanacoseq

4.2 Demos

Fop Computes the Frequency of Optimal Codons in the Eragostis tef Sample Set
NEC Computes the Overall Number of Effective Codons in the Eragostis tef Sample Set
plotOCU Plots the Codon Optimality Score for Each Aminoacid in the Eragostis tef Sample Set with Moving Averages

4.3 Tests

checkCDNA
Fop
NEC
NucleotideContent
species

4.4 Functions

4.4.1 Indices

ComputeCAI Codon Adaptation Index
ComputeCAIVector Codon Adaptation Index Vector
ComputeCarboneRA Compute Carbone Relative Adaptiveness
ComputeFop Frequency of Optimal Codons
ComputeGC3syn GC content 3rd position of synonymous codons
ComputeNEC Effective number of codons
RelativeAdaptiveness Compute Relative Adaptiveness

Intermediator functions for indices

RSCU Relative Synonymous Codon Usage

Void indices

ComputeCBI Codon Bias Index
ScaledChiSquare Scaled Chi-Square
SilentSiteComposition Base Composition at Silent Sites

Database Function from Darwin left untranscribed

Entries Entries

4.4.2 Helperfunctions

anticodoncount Calculate Global Anticodon Usage
areopts Optimality Decision for Codons of Custom Genome
checkCDS Checks Integrity of CDS Sequence
CodonProb Codon Probability table
CodonProbabilities Codon Probabilities
CodonUsage Table for Codon Usage and Optimality of Codons

FindHighlyExpressedGenes Find Highly Expressed Genes
NucleotideContent Nucleotide Content
reversecomplement Reverse Complement Anticodon
SetupRA Relative Synonymous Codon Usage

Graphical functions

plotOCU Plot of Optimality Score for Codons in a Single Protein

3rd party functions

movingAverage Calculating a Moving Average

Obsolete functions

countcodonfreq Count Codon Frequency

Data Handling

mylist My List of Nucleotide-Sequences and Aminoacid-Sequences
readfasta Processed tRNA Containing Fasta File Headers to Count Anticodons
readintronic Read tRNA Intronic Gene Count Statistics
readstats Read tRNA Gene Count Statistics
readtRNAout Read tRNA Gene Count Statistics
searchfafile Search and Return a Fasta File Name from Partial Name

4.5 Sampledata

4.5.1 External Data

Etef.sample.protein.fasta Selectped Teff Protein sequences, matching selected CDS
Etef.sample.transcript.fasta Selected Teff CDS, closely related to rice sequences

seq30469.out tRNAscan-SE 2.0 online output file for teff tRNA sequences is used as example imput for readtRNAout

4.5.2 Datasets

aa_ac Aminoacids and Anticodons
GtRNAdb2species Viruses, Eukaryota, Archaea, Bacteria Species Names on GtRNAdb2
Tef Optimal Codon Table for Eragostis tef Decided on tRNAscan-SE count of tRNA genes in sequenced genome
veab Viruses, Eukaryota, Archaea, Bacteria Species File Names on GtRNAdb2
veabfa Viruses, Eukaryota, Archaea, Bacteria Species Fasta File Names on GtRNAdb2

5 Outlook

In this chapter we will give a brief overview on what Indices are meant to be implemented in the future, what results could be generated by applying them and what the future for this package looks like.

5.1 Codon Bias Indices

There are dozens of codon bias indices that yet have to be implemented in the package. The original Darwin code has alredy defined some of the most wanted indices yet to be implemented. Here is an overview on the implemented, the ones which have a void function backbone and the non-implemented functions tab:CodonBiasIndices. The indices are taken from a review [**?**] and can be found in other reviews of codon indices [**?**].

The table 5.1 is a floating table showing which indices have already been implemented in statanacoseq.

5.2 Biological meaning and statistics

The basic idea of a statistical analysis package for codon analysis is to combine the most frequently used codon indices in one software to evaluate which of them is best performing regarding to the availability (quality) and the biological properties for the given species. By putting it into the environement of the statistical open source program R following analysis steps as correlation to Gene Ontology (GO) tags can be done without having to deal with data handling to other software. The open structure of R codes also allows to use other implementation of algorithms already developed for R as we

here used the functions RSCU from seqinr package for example. That allows other scientist to work with there own interpretation of new indices or better implementation of algorithms to calculate them. Furthermore, it is designed to adapt collaborators or third party code in the package, because every function has its own file and comes with the description of the function. The basic biological problem to solve, as soon as all the major indices are implemented, is to find correlations of indices or clusters with other biological information such as gene expression (that is already part of the calculation of some indices), intracellular location, stress responses, enzymatic families, pathways, development stages and many more. One possible approach is to correlate the index properties to GO attributes and to find out wether there are indices that are significantly representing some of them, in order that a prediciton of the function or other properties of unknown proteins can be predicted.

5.3 Package development

The future development of the package is heavily dependent of the scientific interest in a package that computes all the codon bias indices and to find the best correlation to the phenotypic attribute to be investigated on.

Table 5.1: Measures of codon bias

name	implemented	backbone	missing
Relative codon frequencies			
g_c / g_{ac} global codon frequency	x*		
f_{ac} frequency of codon c encoding amino acid a	x*		
r_{ac} relative synonymous codon usage (RSCU) for codon c and amino acid a	x*		
w_{ac} relative adaptiveness	x		
Measures based on reference			
Fop Frequency of optimal codons	x		
CBI Codon Bias Index		x	
B / E Codon Usage Bias (expression)			x
CEC Codon-Enrichment Correlation			x
Measures based on the geometric mean			
P Codon Preference			x
CAI Codon Adaptation Index	x		
rCAI Relative Codon Adaptation Index			x
RCB Relative Codon Usage Bias			x
RCA Relative codon adaptation			x
FFT Autocorrelation			x
GCB Iterative approach to determining codon bias			x
tAI tRNA adaptation index			x
nTE Normalized translational efficiency			x
Measures based on deviation from an expected distribution			
CPB Codon-Preference Bias Measure			x
MCB Maximum-likelihood Codon Bias			x
B_a Bias of an individual amino acid			x
χ^2 Scaled **chi squared** statistic		x	
Ew Weighted sum of relative entropy			x
SCUO Synonymous codon usage order			x
Measures focusing on tRNA interaction			
P1 Influence of tRNA availability			x
P2 Bias for anticodon–codon interactions of intermediary strength			x
TPI tRNA-pairing index			x
Measures based on intrinsic properties of codon usage			
GC3s Base composition at silent sites	x*		
Nc Effective number of codons	x		
MILC Measure independent of length and composition			x
MELP MILC Expression Level Prediction			x
ICDI Intrinsic codon bias index			x
HK Multivariate statistical method by Hey and Kliman			x
S2str Strength of mRNA secondary structure			x
ER Evolutionary rate			x
v(c) Codon volatility			x
PLS Partial least squares regression			x
SUMBLE Synonymous codon usage bias maximum-likelihood estimation			x
SEMPPR Stochastic evolutionary model of protein production rate			x
Measures for total codon usage - genome wide			
tot. Codon using			x
D_{mean} **Mean** dissimilarity index			x
BC6 base composition of 6mers *(new)*			x
Indices measuring amino acid usage			
GRAVY Grand averages of hydropathy			x
AROMA Aromaticity score			x
k_s Rate of protein decay			x

* needs proper implementation

6 R package on GitHub

The project is hosted on GitHub and freely available for the scientific community interested in it: https://github.com/fredysiegrist/statanacoseq

To install the R package on a R version > 3.3.0 the following lines of codes are sufficient to load the package:

```
# install 3rd party software for communicating with GitHub
install.packages("devtools")
library(devtools)
# install software package of master thesis
install_github("fredysiegrist/statanacoseq")
library(statanacoseq)
```

To run a short demo on some of the functionalities one can now enter the following lines to test the package installation.

```
demo(plotOCU)
demo(Fop)
```

A Appendix - the Darwin code for codon indices

In this appendix we list the CodonIndices file of the Darwin software package that was used to transcribed most of the functions of statanacoseq package.
Data Analysis and Retrieval With Indexed Nucleotide/peptide sequences homepage

```
#
# Several indices for codon usage.
#
#                          Alexander Roth (2005-2007)


# Frequency of optimal codons (Ikemura 1981).  AR (April 2007)
ComputeFop := proc(d:string)
  cu:=CodonUsage();
  aviod:={op(AToCodon('$')),
          op(AToCodon('M')),
          op(AToCodon('W'))};
  xop:=0; xnon:=0;
  for i to length(d) by 3 do
    c := d[i..i+2];
    if not member(c,aviod) then
      if c=cu[CodonToInt(c),1,1] then
        xop:=xop+1;
      else
        xnon:=xnon+1;
      fi;
    fi;
  od;
  xop/(xop+xnon);
end:


# Effective number of codons* (Wright 1990, *Fuglsang 2004).  AR (April 2007)
ComputeNEC := proc(d:string)
  cod:=CreateArray(1..64);
  aa:=CreateArray(1..20);
```

```
32    aviod:={op(AToCodon('$'))};
33    count:=0;
34    for i to length(d) by 3 do
35      c := d[i..i+2];
36      if not member(c,aviod) then
37        ai:=CodonToInt(c);
38        ci:=CodonToCInt(c);
39        cod[ci]:=cod[ci]+1;
40        aa[ai]:=aa[ai]+1;
41        count:=count+1;
42      fi;
43    od;
44
45    Nc:=0;
46    for i to 20 do
47      Acods := IntToCInt(i);
48      k := length(Acods);
49      if k<2 then Nc := Nc + 1; next; fi;
50      n := sum([seq(cod[Acods[x]], x=1..k)]);
51      S := sum([seq((cod[Acods[x]]/n)^2, x=1..k)]);
52      F := (n*S-1) / (n-1);
53      Nc := Nc + 1/F;
54    od;
55    Nc;
56  end:
57
58  #one:=''; all:='';
59  #for x to 3 do
60  #for i to 20 do for j to length(IntToCodon(i)) do
61  #    all:=all.IntToCodon(i)[j];
62  #    one:=one.IntToCodon(i)[1];
63  #od od od;
64
65  # Nucleotide content.  AR (2006)
66  NucleotideContent := proc( ; tD:{string, Entry}, pos=[1,2,3]:list(posint)) -> list
      ;
67    o := CreateArray(1..4);
68    n:=0;
69    if not assigned(tD) then
70      for z to DB[TotEntries] do
71        d:=SearchTag(DNA, Entry(z));
72        for i1 to length(d)-max(pos) by 3 do for i2 in pos do
73            i:=i1+i2;
74            n:=n+1;
75            o[BToInt(d[i])] := o[BToInt(d[i])]+1
76        od od;
77      od;
78    else
79      if type(tD, Entry) then d:=SearchTag('DNA', tD)
80      else d:=tD fi;
81      for i1 to length(d)-max(pos) by 3 do for i2 in pos do
82          i:=i1+i2;
83          n:=n+1;
84          o[BToInt(d[i])] := o[BToInt(d[i])]+1
85      od od;
86    fi;
```

```
87    return(o/n);
88  end:
89
90
91  # G+C content 3rd position of synonymous codons.  AR (April 2007)
92  ComputeGC3syn:= proc(td:string)
93    if member(td[-3..-1], AToCodon('$')) then d:=td[1..-4] else d:=td fi; # remove
        stop codon
94    o := CreateArray(1..4);
95    n:=0;
96    for i to length(d) by 3 do
97      c:=d[i..i+2];
98      if length(IntToCInt(CodonToInt(c)))>1 then
99        n:=n+1;
100       oi:=BToInt(c[3]);
101       o[oi] := o[oi]+1
102     fi;
103   od;
104   o:=o/n;
105   return(o[2]+o[3]);
106 end:
107
108
109 # Base composition at silent sites.
110 SilentSiteComposition := proc(d:string)
111   # to be implemented
112 end:
113
114
115 # Scaled Chi-Square.
116 ScaledChiSquare := proc(d:string)
117   # to be implemented
118 end:
119
120
121 # Codon Bias Index (CBI) (Bennetzen and Hall 1982).
122 ComputeCBI :=  proc(d:string)
123   # to be implemented
124 end:
125
126
127 # Relative synonymous codon usage.  AR (2007)
128 RSCU := proc(;d:string)
129   if nargs>0 then
130     cc:=CodonCount(d);
131   else
132     cc := CodonCount();
133   fi;
134   rscu := CreateArray(1..64);
135   for i to 64 do
136     s:=0;
137     syn:=IntToCInt(CIntToInt(i));
138     l:=length(syn);
139     for j in syn do
140       s:=s+cc[j];
141     od;
```

```
    if s=0 then next fi;
    rscu[i]:=cc[i]/(s/l);
  od;
  rscu;
end:


# Compute CAI, the Codon Adaptation Index (Sharp and Li 1987).
# Markus Friberg and Alexander Roth (Dec 2005)
ComputeCAI := proc(DNA:{string, Entry})
  # check global variables and scan arguments
  if not assigned(RA) then
    error('Error in ComputeCAI: RA not assigned, use e.g. SetupRA(yeast);') fi;
  if type(DNA, Entry) then dna:=copy(SearchTag('DNA', DNA))
  else dna:=DNA fi;
  UseCodonProb := false;
  for i from 2 to nargs do
    if length(args[i]) = 2 and args[i, 1] = 'UseCodonProb' then
      UseCodonProb := args[i, 2]
    else
      error('Unknown argument ', args[i]);
    fi;
  od;
  # compute cai
  w := 0;
  n := length(dna)/3;
  for j to length(dna) by 3 do
    cint := CodonToCInt(dna[j..j+2]);
    codprob := If(UseCodonProb, CodonProb[cint], 1);
    if CIntToA(cint) <> '$' then    # don't consider stop codons
    w := w + ln(codprob * RA[cint]) fi;
  od;
  exp(1/n * w)
end:

ComputeCAIVector := proc(e:Entry)
  if not assigned(RA) then
    error('Error in ComputeCAI: RA not assigned, use e.g. SetupRA(yeast);') fi;
  dna := SearchTag('DNA', e);
  wa := CreateArray(1..20);
  na := CreateArray(1..20);
  for j to length(dna) by 3 do
    cint := CodonToCInt(dna[j..j+2]);
    a := CIntToInt(cint);
    if a <= 20 then
      wa[a] := wa[a] + ln(RA[cint]);
      na[a] := na[a]+1;
    fi;
  od;
  res := CreateArray(1..21);
  for i to 20 do
    res[i] := If(na[i]=0, 'NA', exp(1/na[i] * wa[i])) od;
  res[21] := exp(1/sum(na) * sum(wa));
  res
end:

```

```
198 SetupRA := proc(s:string)
199   global RA, CodonProb;
200   CodonProb := [0.9910, 0.9750, 0.9793, 0.9691, 0.9318, 0.9268, 0.8389, 0.9636,
201                 0.9622, 0.8830, 0.8633, 0.9223, 0.9179, 0.9598,      1, 0.9825,
202                 0.9720, 0.8660, 0.8883, 0.9223, 0.9530, 0.8176, 0.7371, 0.9253,
203                 0.5895, 0.5874, 0.4657, 0.8154, 0.9370, 0.7825, 0.8817, 0.9173,
204                 0.9832, 0.9475, 0.9284, 0.9727, 0.9341, 0.9249, 0.8082, 0.9614,
205                 0.8887, 0.8914, 0.8059, 0.9475, 0.9074, 0.9249, 0.9072, 0.9719,
206                      0, 0.9460,      0, 0.9436, 0.9328, 0.9347, 0.8408, 0.9737,
207                      0, 0.7542, 0.8870, 0.8534, 0.9722, 0.9748, 0.9819, 0.9703]:
208   if s = 'yeast' then  #based on the original CAI paper by Shart and Li
209     RA := [0.135,     1,     1, 0.053, 0.012,     1, 0.006, 0.921,
210               1, 0.031, 0.003, 0.021, 0.003,     1,     1, 0.823,
211               1,     1, 0.007, 0.245,     1, 0.009, 0.002, 0.047,
212            0.002, 0.002, 0.002, 0.137, 0.039, 0.003, 0.003, 0.006,
213               1,     1, 0.016, 0.554, 0.015, 0.316, 0.001,     1,
214            0.002, 0.020, 0.004,     1, 0.002, 0.831, 0.018,     1,
215               1,     1,     1, 0.071, 0.036, 0.693, 0.005,     1,
216               1, 0.077,     1,     1, 0.117,     1,     1, 0.113]:
217   elif s = 'yeast2perc' then
218     RA := [0.1277, 1, 1, 0.08078603, 0.02564103, 0.9501, 0.01, 1, 1, 0.0313253,
219            0.00159109, 0.03253012, 0.00383632, 1, 1, 0.8325, 1, 1, 0.00572082,
220            0.2407, 1,  0.00333704, 0.01, 0.08676307, 0.01, 0.01, 0.01, 0.1687,
221            0.04752971, 0.01, 0.00125078, 0.00562852, 1, 1, 0.01611863, 0.6806,
222            0.00955593, 0.3007, 0.00337268, 1, 0.00286369, 0.01890034, 0.00229095,
                 1,
223            0.00169635, 0.7625, 0.01526718, 1, 1, 1, 1, 0.08910891, 0.02409639,
                 0.6892,
224            0.00120482, 1, 1, 0.05555556, 1, 1, 0.1451, 1, 1, 0.1765]:
225   elif s = 'yeast1perc' then
226     RA := [0.07619048, 1, 1, 0.04887218, 0.01160093, 1, 0.01, 0.9722, 1,
227            0.02690583, 0.01, 0.02690583, 0.00233645, 1, 1, 0.7664, 1, 1, 0.01,
228            0.2192, 1, 0.00190114, 0.01, 0.07984791, 0.01, 0.01,
229            0.01, 0.1402, 0.03326180, 0.01, 0.00107296, 0.00536481, 1, 1,
230            0.01156069, 0.6220, 0.00392542, 0.2826, 0.00098135, 1, 0.01,
                 0.01452282,
231            0.00207469, 1, 0.01, 0.8253, 0.02028081, 1, 1, 1, 1, 0.06722689,
232            0.01345291, 0.7691, 0.01, 1, 1, 0.08333333, 1, 1, 0.1159, 1, 1,
                 0.1405]:
233   elif s = 'yeast05perc' then
234     RA := [0.06239168, 1, 1, 0.025, 0.004329, 1, 0.01, 0.8095, 1,
235            0.01877934, 0.01, 0.00938967, 0.00452489, 1, 1, 0.7285, 1, 1, 0.01,
236            0.1574, 1, 0.01, 0.01, 0.04961832, 0.01, 0.01,
237            0.01, 0.08362369, 0.02471910, 0.01, 0.01, 0.00449438, 1, 1,
238            0.01518987, 0.5362, 0.01, 0.2367, 0.00189394, 1, 0.01, 0.00852878,
239            0.00426439, 1, 0.01, 0.8644, 0.01261830, 1, 1, 1, 1, 0.05263158,
240            0.00938967, 0.7793, 0.01, 1, 1, 0.1132, 1, 1, 0.07865169, 1, 1,
241            0.08415842]:
242   elif s = 'yeasttop24protexpr' then
243     RA := [0.3403, 1, 1, 0.3230, 0.1646, 0.6951, 0.04268293, 1, 1, 0.09117647,
244            0.04307692, 0.1059, 0.07407407, 0.6931, 1, 1, 1, 1, 0.1014, 0.7321, 1,
245            0.03353659, 0.00914634, 0.1982, 0.01, 0.00923077, 0.01, 0.2308,
246            0.1548, 0.01092896, 0.04007286, 0.07103825, 1, 0.9475, 0.1169, 1,
                 0.1366,
247            0.3707, 0.02764228, 1, 0.03382353, 0.06470588, 0.02647059, 1,
                 0.07712766,
```

```
248             0.7048, 0.09574468, 1, 1, 1, 1, 0.4009, 0.1324, 0.6029, 0.02941176, 1,
                  1,
249             0.07865169, 1, 1, 0.357, 1, 1, 0.3927]:
250     elif s = 'yeasttop24mrnaexpr' then
251       RA := [0.1286, 1, 1, 0.08292683, 0.04761905, 1, 0.04761905, 0.9864, 1,
252             0.03783784, 0.00904977, 0.02702703, 0.00621118, 1, 1, 0.677, 1, 1,
253             0.00625, 0.17, 1, 0.00485437, 0.01941748, 0.09223301, 0.01, 0.00452489,
254             0.01, 0.2081, 0.04885057, 0.00862069, 0.01, 0.01436782, 1, 1,
255             0.00914634, 0.6516, 0.01081081, 0.327, 0.01, 1, 0.0080429, 0.02144772,
256             0.00268097, 1, 0.01754386, 0.8816, 0.01754386, 1, 1, 1, 1, 0.08163265,
257             0.07027027, 0.6757, 0.00540541, 1, 1, 0.125, 1, 1, 0.1782, 1, 1,
                  0.1019]:
258     elif s = 'carbone' then
259       RA := ComputeCarboneRA();
260     else
261       error('Error in SetupRA: not yet implemented for that organism')
262     fi;
263   end:
264
265
266   ComputeCarboneRA := proc( ; t=0.01:nonnegative, initfrac=1:nonnegative, iterfrac
        =0.5:nonnegative, mode:string)
267     global RA;
268     if not assigned(DB) then error('DB must be assigned') fi;
269     x := 1;  # fraction of the sequences used to compute RA in this iteration
270     AllGenes := [seq(i, i=1..DB[TotEntries])]:
271     genes := Shuffle(AllGenes)[1..round(initfrac * DB[TotEntries])]:
272     bestCorr := 0;
273     cai := CreateArray(1..DB[TotEntries]):
274     while length(genes) / DB[TotEntries] > t do
275       RA := RelativeAdaptiveness(genes);
276       for i to DB[TotEntries] do
277         dna:=SearchTag('DNA',Entry(i));
278         if SearchString('X', dna)<>-1 then next fi;
279         cai[i] := ComputeCAI(dna) od;
280       x := x * iterfrac;
281       res := transpose([AllGenes, cai]):
282       if mode='reverse' then
283         res := transpose(sort(res, res -> res[2])):
284       else
285         res := transpose(sort(res, res -> -res[2])):
286       fi;
287       genes := res[1][1..round(x * DB[TotEntries])]:
288     od;
289     RA
290   end:
291
292
293   RelativeAdaptiveness := proc(entries:list(posint))
294     CodonCounts := CreateArray(1..64);
295     for i in entries do
296       dna := SearchTag('DNA', Entry(i));
297       for j to length(dna) by 3 do
298         cod := CodonToCInt(dna[j..j+2]);
299         if cod=0 then next fi;   # to avoid XXX
300         CodonCounts[cod] := CodonCounts[cod]+1;
```

```
    od;
  od;
  RA := CreateArray(1..64);
  aa := 1;
  for aa to 20 do
    codons := IntToCInt(aa);
    counts := [seq(CodonCounts[i], i=codons)];
    freqs := counts / sum(counts);
    for i to length(codons) do
      cod := codons[i];
      RA[cod] := freqs[i] / max(freqs);
    od;
  od;
  for i to length(RA) do       # set minimum RA value to 0.01
    if RA[i] = 0 then
      RA[i] := 0.01 fi od;
  for i in AToCInt('$') do     # set RA value of stop codons to 1
    RA[i] := 1; od;
  RA
end:

# for each codon, compute the probability that it occurs at least once in a gene
CodonProbabilities := proc()
  res := CreateArray(1..64);
  for e in Entries() do
    occurs := CreateArray(1..64);
    dna := SearchTag('DNA', e);
    for c to length(dna) by 3 do
      cint := CodonToCInt(dna[c..c+2]);
      occurs[cint] := 1;
    od;
    res := res + occurs;
  od;
  res / DB[TotEntries]
end:


FindHighlyExpressedGenes := proc( ; n=100:integer, tag='PROTEXPR':string)
  # tags: 'PROTEXPR' 'MRNAEXPR'
  expr := CreateArray(1..DB[TotEntries]);
  for i to DB[TotEntries] do
    ex := sscanf(SearchTag(tag, Entry(i)), '%f');
    if ex <> [] then
      expr[i] := op(ex) fi;
  od;
  sorted := sort(expr);
  limit := sorted[length(sorted)-n+1];
  genes := [];
  for i to DB[TotEntries] do
    if expr[i] >= limit then
      genes := append(genes, i) fi od;
  genes
end:
```

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Objective: Master in Bioinformatics and Computational Biology

EDUCATION

2016 (ongoing), Universities of Bern and Fribourg, Switzerland, Master in Bioinformatics, English.

2011, Apr 13, University of Basel, Switzerland, PhD Cell Biology, English.

2003, Oct 24, University of Bern, Switzerland, Master Biochemisty, German.

1999, Jun 17, Gymnasium Bern-Kirchenfeld, Switzerland, Matura type C, German.

ACTIVITIES

Editorial Acitivities and Teaching

Served as referee for: BMC Research Notes (BioMed Central), International Journal of Interferon, Cytokine and Mediator Research (Dove Press).

Modular course in Clinical Medicine for bachelor students (University of Basel) Tracking down genes modern genetics in clinical research

Administration

Administrative Board Member, Moosbad Immobilien AG, Emmenmatt, Switzerland

Publication Record

h index / Sum of the Times Cited (Web of science)
5 / 317

Computer Experience

Programming Langugaes (OO): R, PHP5, Python, Java. Programming Langugages (procedural, script): Unix-Bourne-Shell / bash, DOS-Batch, GW-BASIC. Operating Systems: Windows, Ubuntu, MAC-OS, MS-DOS. Software: MS Office / LibreOffice, Oracle VM Virtual Box CMS: OpenText, Joomla, Adobe Experience Manager, LSF / SGE Networks: Windows Server, Novell NetWare Databases: SQL / MySQL, MS Access

Work Experience

Publisher (CMS), Webeditor, IT-Support, Brewery Assistant.

EMPLOYMENT

Publisher, Federal Food Safety and Veterinary Office, Liebefeld, Switzerland 2016, Feb - Mai

Scientific Associate, Clinic of Nephrology, Bern University Hospital, Switzerland 2014, Feb - Jul

Post-doc, Laboratory of Virology, University Hospitals of Geneva, Switzerland 2011, Mar - 2013, Feb

PhD, Molecular Medicine and Toxicology, Roche, Basel, Switzerland 2006, Mar - 2010, Sep

Trainee, Functional Genomics, Novartis, Basel, Switzerland 2004, Apr - 2005, Mar

SCIENTIFIC AFFILIATIONS

2016 International Society for Computational Biology
2008 - 2014 International Society for Interferon and Cytokine Research

PROJECT SUMMARIES

Short-term scientific associate, University Hospital of Bern *Department of Nephrology, Hypertension and clinical Pharmacology; Prof B. Frey Department of medical Oncology; Dr M. Zweifel* Genome-mutations in the androgen-receptor ligand-binding domain in mamma- and ovarian carcinomas
Mutations in the androgen-receptor ligand-binding domain is addressed by next-generation sequencing in mamma-, ovarian- and prostate-cancer samples. Driver mutations will be analysed in-vitro for sensitivity to dihydrotestosteron derivatives in reporter assays. *Department of Nephrology; Prof U. Huynh-Do* Genomic analyses of chronic hypoxia exposed fetal kidneys
Kidney from mice embryos hold in a hypoxia chamber were analysed by microarrays and significant genes are verified by qPCR, in-situ hybridization and immunohistochemistry to validate expression. Epigenetic DNA status (MetC and hMetC) of candidate genes will be assessed by MeDIP-qPCR.

Post-doctoral position, University Hospitals of Geneva *Laboratory of Virology; Prof L. Kaiser and Dr C. Tapparel* Small RNA sequencing of rhinovirus infections
Deep sequencing data from small RNA Illumina libraries were analysed in rhinovirus infected cell culture samples, small viral RNAs are detected and human miRNA quantified. HeLa cells were infected with different rhinovirus types. Viral RNA fragments and human miRNAs were analysed with northern blots, primer extension and rapid amplification of cDNA ends assays. Impact of RNA fragments on viral replication and translation was addressed with quantitative PCR, Luciferase and immuno-fluorescence.

PhD Thesis, F. Hoffmann-La Roche AG *Molecular Medicine Laboratories; Prof U. Certa* SOCS proteins in IFN silencing
Function of SOCS Proteins in IFN signalling was studied by gene expression analysis with semi quantitative RT- qPCR. Selected candidates were cloned in a mammalian expression vector and fusion proteins with SNAP-tag (Covalys) were cloned and analysed with fluorescence microscopy. Stable SOCS expressing cell lines were generated and characterized for their interferon response by gene and miRNA expression microarrays and statistical analysis with R/Bioconductor. Bimolecular fluorescence complementation with eYFP and STAT1/2 fusion proteins was insensitive to STAT activation by IFN, but localization of STAT fusion proteins as for untagged dimers.
Cell-to-cell transfer of a proliferation control protein (IFITM3)
The transfer of IFITM3 proteins from a generator cell to a recipient cell was assessed by fluorescence microscopy, eYFP marked cell lines were sorted by FACS and analysed for protein transfer by immunoblotting. Protein transfer and proliferation assay (metabolite calorimetry, BrdU ELISA, FACS) excluded transfer of cytostatic effect. Phylogenetic analyses demonstrated recent IFITM gene development.

Traineeship, Novartis Pharma AG, NIBR, Basel *Functional Genomics Group; Dr F. Natt* siRNA stability in biological fluids
Obstacles for siRNA therapeutics are siRNA delivery and the short half-life of non-modified oligonucleotides. I have established a method for rapid analysis of degradation and demonstrated benefit of novel siRNA modifications (especially at their 3 overhang) for serum stability. I synthesized siRNA derivatives together with hydrolysis-stable MOE-RNA standard marker and analysed degradation of differently modified siRNA with HPLC, CE and Gel- electrophoresis. Furthermore, I have specified mechanism of degradation using LC-MS.

Diploma Thesis, University of Bern *Department of Chemistry and Biochemistry; Prof R. Haener* Construction and analysis of a cis-acting Ribonucleasemimic
Ribozyme mimics are generally linked to a nucleic-acid-backbone for specific recognition of the targeted gene transcripts. Efficient substitutes for the big catalytic domain are metal complexes like Cutrpy for example. I have demonstrated self-cleavage of a RNA-Cutrpy-based ribozyme mimic at a specific phosphordiester bond. Main work involved the RNA backbone design (single bulge cleaving site), RNA modification (functional and radioactive / fluorescence labelling) and purification. Finally, triggering of cleavage and analysis of RNA degradation (PAGE).

PUBLICATIONS

Original articles published or accepted in peer reviewed journals (IF 2012)

Scott R, Siegrist F, Foser S, Certa U. Interferon-alpha induces reversible DNA demethylation of the IFITM3 core promoter in human melanoma cells. J Interferon Cytokine Res 2011 Aug 11; 31(8):601-8. IF: 3.3

Cortzar D, Kunz C, Selfridge J, Lettieri T, Saito Y, MacDougall E, Wirz A, Schuermann D, Jacobs A, Siegrist F, Steinacher R, Jiricny J, Bird A, Schr P. Embryonic Lethal Phenotype Reveals a Function of TDG in Maintaining Epigenetic Stability. Nature 2011 Feb 17; 470 (7334): 419-423. IF: 38.6

Siegrist F, Singer T, Certa U. MicroRNA Expression Profiling by Bead Array Technology in Human Tumor Cell Lines Treated with Interferon-Alpha-2a. Biol Proced Online 2009 Dec; 11 (1): 113-29. IF: 1.0

Hallen LC, Burki Y, Ebeling M, Broger C, Siegrist F, Oroszlan-Szovik K, Bohrmann B, Certa U, Foser S. Antiproliferative Activity of the Human IFN-alpha-Inducible Protein IFI44. J Interferon Cytokine Res 2007 Aug; 27 (8): 675-80. IF: 3.3

Reviews published or accepted in peer reviewed journals (IF 2012)

Tapparel C, Siegrist F, Petty TJ, Kaiser L. Picornavirus and enterovirus diversity with associated human diseases. Infect Genet Evol. 2013; 14: 282-293. IF: 2.8

Siegrist F, Ebeling M, Certa U. The small interferon induced transmembrane genes and proteins. J Interferon Cytokine Res 2011 Jan; 31 (1): 183-97. IF: 3.3

Original articles, reviews, editorials, letters, published or accepted in non-peer reviewed journals

Siegrist F, Certa U. Micro RNA Induction by Interferon Alpha and a Potential Role to Interfere with SOCS. In 7th Joint Conference Montral, Qubec, Canada, October 12-16, 2008 Editor: John Hiscott. Medimont International Proceedings 2008: 93-97.

Thesis

Siegrist F. Transcriptional responses of tumor cell lines to interferon-alpha. PhD Thesis, cell biology, University of Basel 2011.

Siegrist F. Auf der Spur eines artifiziellen, selbstspaltenden RNA-Molekls. Diplomathesis, Master of biochemistry, University of Bern 2003.

Abstracts presented at international and national meetings

Siegrist F, Otten-Hernandez P, Thomas Y, Farinelli L, Kaiser L and Tapparel C. Viral genome sequencing and small RNA detection by next generation sequencing. Cytokines 2012 Sep; 59(3): 565.

Siegrist F, Otten P, Thomas Y, Farinelli L, Kaiser L and Tapparel C. Viral genome sequencing and small RNA detection by next generation sequencing. 3rd Swiss Funtamental Virology Workshop 2011 Aug.

Urfer PM, Siegrist F, Noreen F, Weis S, Certa U, Truninger K, Schr P. Integrating transcriptome and epigenome analyses to identify DNA methylation changes associated with colorectal carcinogenesis. BioValley Science Day 2010 Sep.

Cortazar D, Kunz C, Selfridge J, Lettieri T, Wirz A, Schrmann D, Jacobs A, Siegrist F, Jiricny J, Bird A, Schr P. Embryonic lethality of TDG-deficient mice reveals a function of TDG in the maintenance of epigenetic stability. BioValley Science Day 2010 Sep.

Siegrist F, Certa U. Suppression of interferon alpha mediated gene expression by SOCS1 and SOCS3. FEBS-Special Meeting: Jak-Stat Signalling: from Basics to Disease 2010 Feb.

Siegrist F, Ebeling M, Certa U. Phylogenetic analysis of interferon inducible transmembrane gene family and functional aspects of IFITM3. Cytokine 2009 Oct-Nov; 48 (1-2): 87.

Siegrist F, Certa U. Micro RNA induction by interferon alpha and their potential role to interfere in the negative feedback pathway. Cytokine, 2008 Sep; 43 (3): 284-285.

Scott RW, Siegrist F, Burki Y, Foser S, Certa U. Methylation Status Influence On Interferon-alpha Sensitivity In Human Melanoma Cells. 3rd Swiss Meeting on Genome Stability DNA Dynamics and Epigenetics. 2007 Oct.

PUBLISHED DATASETS 39

GSE16421, GSE20693, GSE21158, GSE22801, GSE37872, GSE37873, CY079542.2-CY079549.