

# Statistical Analysis of Coding Sequences

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

---

Master Thesis 2016  
presented on August 12 2016  
at the Faculty of Science  
Institute of Plant Sciences (IPS)  
in the master program of Bioinformatics  
University of Bern and University of Fribourg  
for the master degree in  
Bioinformatics and Computational Biology  
by

Fredy Siegrist

accepted on the faculty reunion:

## Declaration

under Art. 28 Para. 2 RSL 05

Last, first name: Siegrist Fredy

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

*u<sup>b</sup>*

---

b  
**UNIVERSITÄT  
BERN**

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).*

Lorem ipsum dolor sit amet, consectetur adipiscing elit. Ut purus elit, vestibulum ut, placerat ac, adipiscing vitae, felis. Curabitur dictum gravida mauris. Nam arcu libero, nonummy eget, consectetur id, vulputate a, magna. Donec vehicula augue eu neque. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Mauris ut leo. Cras viverra metus rhoncus sem. Nulla et lectus vestibulum urna fringilla ultrices. Phasellus eu tellus sit amet tortor gravida placerat. Integer sapien est, iaculis in, pretium quis, viverra ac, nunc. Praesent eget sem vel leo ultrices bibendum. Aenean faucibus. Morbi dolor nulla, malesuada eu, pulvinar at, mollis ac, nulla. Curabitur auctor semper nulla. Donec varius orci eget risus. Duis nibh mi, congue eu, accumsan eleifend, sagittis quis, diam. Duis eget orci sit amet orci dignissim rutrum.

Nam dui ligula, fringilla a, euismod sodales, sollicitudin vel, wisi. Morbi auctor lorem non justo. Nam lacus libero, pretium at, lobortis vitae, ultricies et, tellus. Donec aliquet, tortor sed accumsan bibendum, erat ligula aliquet magna, vitae ornare odio metus a mi. Morbi ac orci et nisl hendrerit mollis. Suspendisse ut massa. Cras nec ante. Pellentesque a nulla. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus. Aliquam tincidunt urna. Nulla ullamcorper vestibulum turpis. Pellentesque cursus luctus mauris.

Nulla malesuada porttitor diam. Donec felis erat, congue non, volutpat at, tincidunt tristique, libero. Vivamus viverra fermentum felis. Donec nonummy pellentesque ante. Phasellus adipiscing semper elit. Proin fermentum massa ac quam. Sed diam turpis, molestie vitae, placerat a, molestie nec, leo. Maecenas lacinia. Nam ipsum ligula, eleifend at, accumsan nec, suscipit a, ipsum. Morbi blandit ligula feugiat magna. Nunc eleifend consequat lorem. Sed lacinia nulla vitae enim. Pellentesque tincidunt purus vel magna. Integer non enim. Praesent euismod nunc eu purus. Donec bibendum quam in tellus. Nullam cursus pulvinar lectus. Donec et mi. Nam vulputate metus eu enim. Vestibulum pellentesque felis eu massa.

*Bern, August 12 2016*

Gina Cannarozzi





# Abstract

Lorem ipsum dolor sit amet, consectetur adipiscing elit. Ut purus elit, vestibulum ut, placerat ac, adipiscing vitae, felis. Curabitur dictum gravida mauris. Nam arcu libero, nonummy eget, consectetur id, vulputate a, magna. Donec vehicula augue eu neque. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Mauris ut leo. Cras viverra metus rhoncus sem. Nulla et lectus vestibulum urna fringilla ultrices. Phasellus eu tellus sit amet tortor gravida placerat. Integer sapien est, iaculis in, pretium quis, viverra ac, nunc. Praesent eget sem vel leo ultrices bibendum. Aenean faucibus. Morbi dolor nulla, malesuada eu, pulvinar at, mollis ac, nulla. Curabitur auctor semper nulla. Donec varius orci eget risus. Duis nibh mi, congue eu, accumsan eleifend, sagittis quis, diam. Duis eget orci sit amet orci dignissim rutrum. Nam dui ligula, fringilla a, euismod sodales, sollicitudin vel, wisi. Morbi auctor lorem non justo. Nam lacus libero, pretium at, lobortis vitae, ultricies et, tellus. Donec aliquet, tortor sed accumsan bibendum, erat ligula aliquet magna, vitae ornare odio metus a mi. Morbi ac orci et nisl hendrerit mollis. Suspendisse ut massa. Cras nec ante. Pellentesque a nulla. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus. Aliquam tincidunt urna. Nulla ullamcorper vestibulum turpis. Pellentesque cursus luctus mauris.

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



# Zusammenfassung

Lorem ipsum dolor sit amet, consectetur adipiscing elit. Ut purus elit, vestibulum ut, placerat ac, adipiscing vitae, felis. Curabitur dictum gravida mauris. Nam arcu libero, nonummy eget, consectetur id, vulputate a, magna. Donec vehicula augue eu neque. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Mauris ut leo. Cras viverra metus rhoncus sem. Nulla et lectus vestibulum urna fringilla ultrices. Phasellus eu tellus sit amet tortor gravida placerat. Integer sapien est, iaculis in, pretium quis, viverra ac, nunc. Praesent eget sem vel leo ultrices bibendum. Aenean faucibus. Morbi dolor nulla, malesuada eu, pulvinar at, mollis ac, nulla. Curabitur auctor semper nulla. Donec varius orci eget risus. Duis nibh mi, congue eu, accumsan eleifend, sagittis quis, diam. Duis eget orci sit amet orci dignissim rutrum. Nam dui ligula, fringilla a, euismod sodales, sollicitudin vel, wisi. Morbi auctor lorem non justo. Nam lacus libero, pretium at, lobortis vitae, ultricies et, tellus. Donec aliquet, tortor sed accumsan bibendum, erat ligula aliquet magna, vitae ornare odio metus a mi. Morbi ac orci et nisl hendrerit mollis. Suspendisse ut massa. Cras nec ante. Pellentesque a nulla. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus. Aliquam tincidunt urna. Nulla ullamcorper vestibulum turpis. Pellentesque cursus luctus mauris.

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



# Contents

<b>Acknowledgements</b>	<b>i</b>
<b>Preface</b>	<b>iii</b>
<b>Abstract (English / Deutsch)</b>	<b>v</b>
<b>List of figures</b>	<b>xi</b>
<b>List of tables</b>	<b>xiii</b>
<b>Introduction</b>	<b>1</b>
0.1 Aim . . . . .	1
0.2 Teff genome . . . . .	2
0.3 Biological aspects of Codon usage . . . . .	2
<b>1 Setup of data and project</b>	<b>5</b>
1.1 Getting familiar with the data . . . . .	5
1.1.1 read tRNA stats and decide on optimality of a codon . . . . .	5
1.2 text chunk - tRNA database . . . . .	7
1.2.1 text chunk - Project . . . . .	9
1.2.2 Plot OCU demo . . . . .	10
<b>2 R package setup and maintenance</b>	<b>13</b>
2.1 Generation of R package . . . . .	13
2.1.1 Maintenance with R Studio . . . . .	14
2.1.2 Publishing on GitHub - Description of versions . . . . .	14
<b>3 Transcription of Darwin Code</b>	<b>15</b>
3.1 Transcription of Darwin functions . . . . .	15
3.2 R environnement adaptation . . . . .	15
	ix

## Contents

---

<b>4</b>	<b>Vignette</b>	<b>17</b>
4.1	statanacoseq . . . . .	17
4.2	Demos . . . . .	17
4.3	Tests . . . . .	17
4.4	Functions . . . . .	18
4.4.1	Indices . . . . .	18
4.4.2	Helperfunctions . . . . .	18
4.5	Sampledata . . . . .	19
4.5.1	External Data . . . . .	19
4.5.2	Datasets . . . . .	20
<b>5</b>	<b>Outlook</b>	<b>21</b>
5.1	Codon Bias Indices . . . . .	21
5.2	Biological meaning and statistics . . . . .	22
5.3	Package development . . . . .	22
<b>6</b>	<b>R package on GitHub</b>	<b>25</b>
<b>A</b>	<b>Appendix - the Darwin code for codon indices</b>	<b>27</b>
	<b>Bibliography</b>	<b>35</b>
	<b>Curriculum Vitae</b>	<b>35</b>



## List of Figures

1.1	Heat map for tRNA counts . . . . .	8
1.2	A sample figure from demo plotOCU . . . . .	11







# List of Tables

5.1	Codon Bias Indices . . . . .	23
-----	------------------------------	----



# 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 vignette 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

## List of Tables

---

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

One aspect for some codon bias measurements is the definition of the optimal codon, thus the one with the most desirable quality. This information is used to measure the frequency of optimal codons (Fop) or the P1 index, a measure 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 an 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 scientific 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 herbicide tolerance along with the improved yield and to maintain lodging resistance without being semi-dwarf. A valuable resource for plant 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 an almost totally sequenced but not yet fully annotated genome. By learning from its codon usage, variations generated by technologies already used in the 1960s for screening of genetic variations of agricultural plants combined with DNA sequencing technologies may improve the selection criteria 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 translational 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 an accurate protein synthesis (including amino acid misincorporations and protein misfolding) and a *codon adaptation*, that is aiming to optimize the use of codons towards an enhanced

### 0.3. Biological aspects of Codon usage

---

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 by 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 thereby be calculated from a separate 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 measures only the degree of deviation from an uniform codon usage based on basic statistics, regardless of which codons are overrepresented [?]. Moreover 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 statistics (S) or PCA to estimate the relative selection strength within a genome.



# 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

```
1  #03.01.2016 Master project read in stat files from view-source:http://gtrnadb.ucsc
   .edu/GtRNAdb2/genomes/eukaryota/
2
3  #reading the files
4  setwd("E:/R")
5  readstats <- function(fileno=2, chunksize=1) {
6    statfile=dir(pattern=".stats$")[fileno]
7    con <- file(statfile, "r", blocking = FALSE)
8    for(i in seq(1,3500,chunksize)){
9      d=scan(con,what="a",nlines=chunksize,sep="|", quote="",quiet=TRUE)
10     if (length(d)>0 && d[1]==""){
11       print(substr(d[2:(length(d)-1)], 2, 15) )
12       reps <- as.numeric(gsub("[~0-9]", "", d[2:(length(d)-1)]))
13       tRNAs <- substr(d[2:(length(d)-1)], 2, 4)
14       codons <- substr(d[2:(length(d)-1)], 6, 8)
15       closeAllConnections()
16       return(data.frame(tRNAs, codons, reps))
17     }
```

## Chapter 1. Setup of data and project

---

```
18   }
19 }
20 (Zmays <- readstats() )
21 eins <- function(x=c("Val","UUU","1")){optfactor <- as.numeric(x[3])/max(Zmays[
    tRNAs==x[1],3])}
22 optfact <- apply(Zmays, 1, eins)
23 isopt <- (optfact == 1)
24 Zmays.out <- data.frame(Zmays, optfact, isopt)
25 Zmays.out[Zmays.out$isopt==TRUE,-4]

1 tRNAscan-SE v.1.3 (March 2011) scan results (on host aero.cse.ucsc.edu)
2 Started: Thu Jul 26 22:25:36 PDT 2012
3
4 -----
5 Search Mode:                Eukaryotic
6 Searching with:             tRNAscan + EufindtRNA -> Cove
7 Covariance model:          TRNA2-euk.cm
8 tRNAscan parameters:       Strict
9 EufindtRNA parameters:     Relaxed (Int Cutoff= -32.1)
10
11 Reporting HMM/2' structure score breakdown
12 -----
13
14 First-pass (tRNAscan/EufindtRNA) Stats:
15 -----
16 Sequences read:             13
17 Seqs w/at least 1 hit:     13
18 Bases read:                 2066432718 (x2 for both strands)
19 Bases in tRNAs:             2747160
20 tRNAs predicted:           28967
21 Av. tRNA length:           94
22 Script CPU time:            417.21 s
23 Scan CPU time:              1253.04 s
24 Scan speed:                 3298.3 Kbp/sec
25
26 First pass search(es) ended: Thu Jul 26 22:54:15 PDT 2012
27
28 Cove Stats:
29 -----
30 Candidate tRNAs read:       28967
31 Cove-confirmed tRNAs:       2249
32 Bases scanned by Cove:      3210632
33 % seq scanned by Cove:      0.1 %
34 Script CPU time:            117.99 s
35 Cove CPU time:              2336.55 s
36 Scan speed:                 1374.1 bp/sec
37
38 Cove analysis of tRNAs ended: Fri Jul 27 00:06:48 PDT 2012
39
40 Summary
41 -----
42 Overall scan speed: 1001957.8 bp/sec
43
44 tRNAs decoding Standard 20 AA:           1455
45 Selenocysteine tRNAs (TCA):              4
46 Possible suppressor tRNAs (CTA,TTA):      7
```



## 1.2. text chunk - tRNA database

```
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.

## 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://gttrnadb.ucsc.edu/GtRNAdb2/genomes/>). [?][Chan

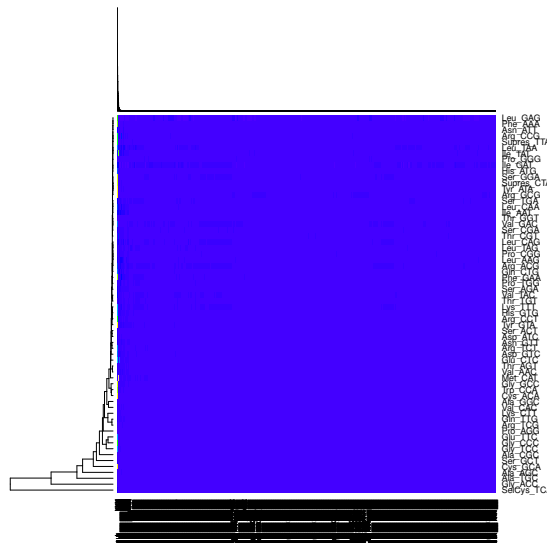


Figure 1.1: Heat map of tRNA gene counts normalized per row in all species

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 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 start 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

The standard genetic code has 61 sense codons that code for 20 amino acids and that are decoded by tRNAs that 'wobble', that is some tRNAs can read more than one codon. For example, yeast has 41 tRNAs to decode the 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. For this project, you will code several indices of codon usage and apply them to the coding sequences of plants in the grass families.

For this project you will:

code standard indices that measure codon usage and bias such as the codon adaptation index (CAI), the tRNA pairing index (tPI) and others

write code for text-based (or graphical-based) visualization of analyses based on codon usage

assess the significance of groups with extreme values of these indices using Enrichment

Analysis

References: A Role for Codon Order in Translation Dynamics. Cannarozzi, Gina et al. Cell, Volume 141 , Issue 2 , 355 - 367

## Chapter 1. Setup of data and project

---

Suggested Languages: R, PERL Idea : implement indices in book chapter plus what came after

\*\*\*\*\*

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 from 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

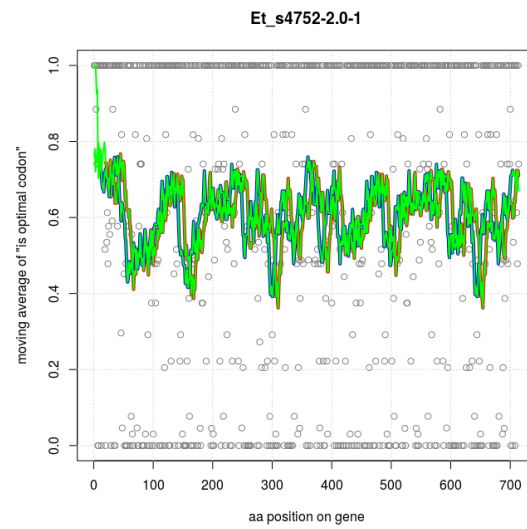


Figure 1.2: A sample figure (from plot OCU *demo(plot(OCU))*, of *statanacoseq*, got from <https://github.com/fredysiegrist/statanacoseq>).



## 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.

```
1 # Construction of the first package
2 # set the working directory to the shared partition
3 setwd("E:/R/")
4 # setwd("/windows/R/")
5 # install.packages("devtools")
6 library("devtools")
7 # devtools::install_github("klutometis/roxygen")
8 library(roxygen2)
9 # Creation of empty package
10 create("statanacoseq")
11 # save function as "E:/R/statanacoseq/R/countcodonfreq.R"
12 setwd("./statanacoseq")
13 document()
14 setwd("../")
15 install("statanacoseq")
16 sessionInfo()
17 detach("package:statanacoseq", unload=TRUE)
```

## Chapter 2. R package setup and maintenance

---

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.

```
1 countcodonfreq <- function(no=1, wdir="/windows/R/") {  
2   require(sequinr)  
3   list(myseq, myaa) <- mylist(wdir)  
4   print(length(myseq[[no]])/3/length(myaa[[attr(myseq[[no]], "name")]]))  
5 }
```

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

```
1 options(help_type = "text") # open help internally  
2 options(help_type = "html") # open help in browser  
3 ?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/statana> [?]. Since no version has been generated where the indices that are not implemented in Darwin has been drawn 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, including 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 environment.

### 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 distinct 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 acknowledging the functionality of the R environment.

### 3.2 R environment adaptation

To upvalue the code transcribed from Darwin, the function *ComputeNEC* was simplified to R language by eliminating the loops and introducing table and supply 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)}
```

### Chapter 3. Transcription of Darwin Code

---

```
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]),
17      reversecomplement)[Tef[,1]==i])
18    contributors <- names(aa)[sapply(Acods, length)>1&aa>1]
19    Nc <- sapply(contributors, function(i) {
20      n <- sum(cod[unlist(Acods[i])])
21      S <- sum(sapply(cod[unlist(Acods[i])], function(x) (x/n)^2))
22      F <- (n*S-1) / (n-1)
23      return(1/F)
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 Selected 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 input 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 already 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 [?].

Indices to be Implemented: Use from `SeqinR`

Implement Indices from the book chapter

Intrinsic Codon Bias HK measure Strength of mRNA secondary structure codon Volatility Evolutionary Rate (ER) stochastic evolutionary model of protein production rate synonymous codon usage bias maximum-likelihood estimation Genome wide usage Mean dissimilarity index (Dmean)

Not in the book chapter base composition of 6mers

review of codon indices <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC153734/>

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 environment 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.



### 5.3. Package development

Table 5.1: Measures of codon bias

name	implemented	backbone	missing
<b>Relative codon frequencies</b>			
$g_c / g_{ac}$ 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		
<b>Measures based on reference</b>			
Fop Frequency of optimal codons	x		
<b>CBI</b> Codon Bias Index		x	
<b>B / E</b> Codon Usage Bias (expression)			x
<b>CEC</b> Codon-Enrichment Correlation			x
<b>Measures based on the geometric mean</b>			
<b>P</b> Codon Preference			x
<b>CAI</b> Codon Adaptation Index	x		
<b>rCAI</b> Relative Codon Adaptation Index			x
<b>RCB</b> Relative Codon Usage Bias			x
RCA Relative codon adaptation			x
<b>FFT</b> Autocorrelation			x
<b>GCB</b> Iterative approach to determining codon bias			x
<b>tAI</b> tRNA adaptation index			x
nTE Normalized translational efficiency			x
<b>Measures based on deviation from an expected distribution</b>			
<b>CPB</b> Codon-Preference Bias Measure			x
<b>MCB</b> Maximum-likelihood Codon Bias			x
$B_a$ Bias of an individual amino acid			x
$\chi^2$ Scaled <b>chi squared</b> statistic		x	
<b>Ew</b> Weighted sum of relative entropy			x
<b>SCUO</b> Synonymous codon usage order			x
<b>Measures focusing on tRNA interaction</b>			
<b>P1</b> Influence of tRNA availability			x
P2 Bias for anticodon-codon interactions of intermediary strength			x
<b>TPI</b> tRNA-pairing index			x
<b>Measures based on intrinsic properties of codon usage</b>			
<b>GC3s</b> Base composition at silent sites	x*		
<b>Nc</b> Effective number of codons	x		
<b>MILC</b> Measure independent of length and composition			x
<b>MELP</b> MILC Expression Level Prediction			x
<b>ICDI</b> Intrinsic codon bias index			x
<b>HK</b> Multivariate statistical method by Hey and Kliman			x
<b>S2str</b> Strength of mRNA secondary structure			x
<b>ER</b> Evolutionary rate			x
v(c) Codon volatility			x
PLS Partial least squares regression			x
<b>SUMBLE</b> Synonymous codon usage bias maximum-likelihood estimation			x
<b>SEMPPR</b> Stochastic evolutionary model of protein production rate			x
<b>Measures for total codon usage in genomes</b>			
tot. Codon using			x
$D_{mean}$ <b>Mean</b> dissimilarity index			x
<b>BC6</b> base composition of 6mers			x
<b>Indices measuring amino acid usage</b>		23	
GRAVY Grand averages of hydrophathy			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:

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

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

```
1 demo(plotOCU)
2 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

```
1 #
2 # Several indices for codon usage.
3 #
4 # Alexander Roth (2005-2007)
5
6
7 # Frequency of optimal codons (Ikemura 1981). AR (April 2007)
8 ComputeFop := proc(d:string)
9   cu:=CodonUsage();
10   aviod:={op(AToCodon('$')),
11           op(AToCodon('M')),
12           op(AToCodon('W'))};
13   xop:=0; xnon:=0;
14   for i to length(d) by 3 do
15     c := d[i..i+2];
16     if not member(c,aviod) then
17       if c=cu[CodonToInt(c),1,1] then
18         xop:=xop+1;
19       else
20         xnon:=xnon+1;
21       fi;
22     fi;
23   od;
24   xop/(xop+xnon);
25 end;
26
27
28 # Effective number of codons* (Wright 1990, *Fuglsang 2004). AR (April 2007)
29 ComputeNEC := proc(d:string)
30   cod:=CreateArray(1..64);
31   aa:=CreateArray(1..20);
```

## Appendix A. Appendix - the Darwin code for codon indices

---

```
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 ;
68   o := CreateArray(1..4);
69   n:=0;
70   if not assigned(tD) then
71     for z to DB[TotEntries] do
72       d:=SearchTag(DNA, Entry(z));
73       for i1 to length(d)-max(pos) by 3 do for i2 in pos do
74         i:=i1+i2;
75         n:=n+1;
76         o[BToInt(d[i])] := o[BToInt(d[i]])+1
77       od 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;

```

## Appendix A. Appendix - the Darwin code for codon indices

---

```
142     if s=0 then next fi;
143     rscu[i]:=cc[i]/(s/l);
144   od;
145   rscu;
146 end:
147
148
149 # Compute CAI, the Codon Adaptation Index (Sharp and Li 1987).
150 # Markus Friberg and Alexander Roth (Dec 2005)
151 ComputeCAI := proc(DNA:{string, Entry})
152   # check global variables and scan arguments
153   if not assigned(RA) then
154     error('Error in ComputeCAI: RA not assigned, use e.g. SetupRA(yeast);') fi;
155   if type(DNA, Entry) then dna:=copy(SearchTag('DNA', DNA))
156   else dna:=DNA fi;
157   UseCodonProb := false;
158   for i from 2 to nargs do
159     if length(args[i]) = 2 and args[i, 1] = 'UseCodonProb' then
160       UseCodonProb := args[i, 2]
161     else
162       error('Unknown argument ', args[i]);
163     fi;
164   od;
165   # compute cai
166   w := 0;
167   n := length(dna)/3;
168   for j to length(dna) by 3 do
169     cint := CodonToCInt(dna[j..j+2]);
170     codprob := If(UseCodonProb, CodonProb[cint], 1);
171     if CIntToA(cint) <> '$' then # don't consider stop codons
172       w := w + ln(codprob * RA[cint]) fi;
173   od;
174   exp(1/n * w)
175 end:
176
177 ComputeCAIVector := proc(e:Entry)
178   if not assigned(RA) then
179     error('Error in ComputeCAI: RA not assigned, use e.g. SetupRA(yeast);') fi;
180   dna := SearchTag('DNA', e);
181   wa := CreateArray(1..20);
182   na := CreateArray(1..20);
183   for j to length(dna) by 3 do
184     cint := CodonToCInt(dna[j..j+2]);
185     a := CIntToInt(cint);
186     if a <= 20 then
187       wa[a] := wa[a] + ln(RA[cint]);
188       na[a] := na[a]+1;
189     fi;
190   od;
191   res := CreateArray(1..21);
192   for i to 20 do
193     res[i] := If(na[i]=0, 'NA', exp(1/na[i] * wa[i])) od;
194   res[21] := exp(1/sum(na) * sum(wa));
195   res
196 end:
197
```



---

```

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,
223            1,
224            0.00169635, 0.7625, 0.01526718, 1, 1, 1, 1, 0.08910891, 0.02409639,
225            0.6892,
226            0.00120482, 1, 1, 0.05555556, 1, 1, 0.1451, 1, 1, 0.1765]:
227   elif s = 'yeast1perc' then
228     RA := [0.07619048, 1, 1, 0.04887218, 0.01160093, 1, 0.01, 0.9722, 1,
229            0.02690583, 0.01, 0.02690583, 0.00233645, 1, 1, 0.7664, 1, 1, 0.01,
230            0.2192, 1, 0.00190114, 0.01, 0.07984791, 0.01, 0.01,
231            0.01, 0.1402, 0.03326180, 0.01, 0.00107296, 0.00536481, 1, 1,
232            0.01156069, 0.6220, 0.00392542, 0.2826, 0.00098135, 1, 0.01,
233            0.01452282,
234            0.00207469, 1, 0.01, 0.8253, 0.02028081, 1, 1, 1, 1, 0.06722689,
235            0.01345291, 0.7691, 0.01, 1, 1, 0.08333333, 1, 1, 0.1159, 1, 1,
236            0.1405]:
237   elif s = 'yeast05perc' then
238     RA := [0.06239168, 1, 1, 0.025, 0.004329, 1, 0.01, 0.8095, 1,
239            0.01877934, 0.01, 0.00938967, 0.00452489, 1, 1, 0.7285, 1, 1, 0.01,
240            0.1574, 1, 0.01, 0.01, 0.04961832, 0.01, 0.01,
241            0.01, 0.08362369, 0.02471910, 0.01, 0.01, 0.00449438, 1, 1,
242            0.01518987, 0.5362, 0.01, 0.2367, 0.00189394, 1, 0.01, 0.00852878,
243            0.00426439, 1, 0.01, 0.8644, 0.01261830, 1, 1, 1, 1, 0.05263158,
244            0.00938967, 0.7793, 0.01, 1, 1, 0.1132, 1, 1, 0.07865169, 1, 1,
245            0.08415842]:
246   elif s = 'yeasttop24protexpr' then
247     RA := [0.3403, 1, 1, 0.3230, 0.1646, 0.6951, 0.04268293, 1, 1, 0.09117647,
248            0.04307692, 0.1059, 0.07407407, 0.6931, 1, 1, 1, 1, 0.1014, 0.7321, 1,
249            0.03353659, 0.00914634, 0.1982, 0.01, 0.00923077, 0.01, 0.2308,
250            0.1548, 0.01092896, 0.04007286, 0.07103825, 1, 0.9475, 0.1169, 1,
251            0.1366,
252            0.3707, 0.02764228, 1, 0.03382353, 0.06470588, 0.02647059, 1,
253            0.07712766,

```

## Appendix A. Appendix - the Darwin code for codon indices

---

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

---

```

301     od;
302 od;
303 RA := CreateArray(1..64);
304 aa := 1;
305 for aa to 20 do
306     codons := IntToCInt(aa);
307     counts := [seq(CodonCounts[i], i=codons)];
308     freqs := counts / sum(counts);
309     for i to length(codons) do
310         cod := codons[i];
311         RA[cod] := freqs[i] / max(freqs);
312     od;
313 od;
314 for i to length(RA) do          # set minimum RA value to 0.01
315     if RA[i] = 0 then
316         RA[i] := 0.01 fi od;
317 for i in AToCInt('$') do      # set RA value of stop codons to 1
318     RA[i] := 1; od;
319 RA
320 end:
321
322 # for each codon, compute the probability that it occurs at least once in a gene
323 CodonProbabilities := proc()
324     res := CreateArray(1..64);
325     for e in Entries() do
326         occurs := CreateArray(1..64);
327         dna := SearchTag('DNA', e);
328         for c to length(dna) by 3 do
329             cint := CodonToCInt(dna[c..c+2]);
330             occurs[cint] := 1;
331         od;
332         res := res + occurs;
333     od;
334     res / DB[TotEntries]
335 end:
336
337
338 FindHighlyExpressedGenes := proc( ; n=100:integer, tag='PROTEXPR':string)
339     # tags: 'PROTEXPR' 'MRNAEXPR'
340     expr := CreateArray(1..DB[TotEntries]);
341     for i to DB[TotEntries] do
342         ex := sscanf(SearchTag(tag, Entry(i)), '%f');
343         if ex <> [] then
344             expr[i] := op(ex) fi;
345     od;
346     sorted := sort(expr);
347     limit := sorted[length(sorted)-n+1];
348     genes := [];
349     for i to DB[TotEntries] do
350         if expr[i] >= limit then
351             genes := append(genes, i) fi od;
352     genes
353 end:

```



## **Fredy SIEGRIST**

Schnbergweg 11  
CH-3006 Bern  
+41 31 511 0512

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

### **ACTIVITIES**

#### **Editorial Activities 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 Languages (OO): R, PHP5, Python, Java. Programming Languages (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 dihydrotestosterone 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. Bi-molecular 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 phosphodiester 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 Fundamental 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

37

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