Bachelor Thesis

von

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Programmierung einer Auswertsoftware für RNA-seq Daten

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1 Einleitung

1.1 Zusammenfassung

Die Vorliegende Arbeit beschreibt die Architektur und die Anwendungsmöglichkeiten einer neuen Software zur Analyse von RNA-seq Daten.

Bei der RNA-seq - Methode, werden komplementäre DNAs (cDNAs), die aus den zu untersuchenden RNAs hergestellt wurden, mit neuartigen (eng. next-generation) Technologien sequenziert. [NWS10] Die Einführung dieser neuartiger Technologien der Gen-Sequenzierung ist ein wichtiger Meilenstein auf dem Weg zu einem besseren und tieferen Verständnis der Funktionsweise der Zellen. Bei der Durchführung von RNA-seq Experimenten fallen grosse Mengen von Daten in Form von relativ kurzen Sequenzabschnitten (< 200 Basen) an. Die Sequenzdaten werden dann an die NCBI und andere zentrale Sequenzarchive (SRA - Sequence Read Archive) übermittelt. Zum heutigen Tag sind bereits grosse Mengen an Daten gesammelt worden, die nun öffentlich online verfügbar sind.

Diese Daten werden mit der bereits bekannten Genomsequenz verglichen um die Bereiche des Genoms zu finden, die transkribiert werden. Transkription wird nicht für alle Genombereiche erwartet, sondern nur dort, wo sich proteinkodierende Abschnitte bzw. Gene für RNA-Moleküle befinden. Die Analyse der RNA-Seq Daten wird daher durch die Visualisierung entscheidend unterstützt. Darüber hinaus kann die Annotation eines Genoms durch die Verfügbarkeit der Transkriptdaten verbessert werden.

Die in dieser Diplomarbeit programmierte Web-Anwendung wurde erstellt, um die von RNA-seq Experimenten generierten Daten in einer bereits existierenden, populären, Visualisierungsanwendung, dem *UCSC Archaea Browser* [Sch06], anzuzeigen. Durch die Verwendung der von dieser Anwendung unterstützten, bzw. definierten, Protokolle zur Formatierung und Darstellung der Daten, stehen dem Anwender viele Funktionen zur Verfügung, die sich mit einer gekapselten Desktop-Anwendung nur unter grossem Aufwand realisieren liessen. So können die Daten im *UCSC Archaea Browser* beispielsweise immer im Kontext mit den *aktuellen* RefSeq Gen-Annotationen betrachtet werden.

1.2 Summary

RNA-seq is a Method, where complementary DNAs (cDNAs), produced from the RNAs to be studied, are sequenced with next-generation technology. The advent of these new technologies are an important milestone in the quest to gain a better and deeper understanding of the cells. RNA-seq experiments produce large amounts of short sequences (< 200 bases). This data is transmitted to the NCBI and other Sequence Read Archives (SRA). To this date, large amounts of data has been collected which is now publicly available online.

This data is being compared to known gene sequences to find the regions that have been transcribed. Transcription is not expected for all regions of the genome,

but only for those containing protein-coding sequences or sequences of RNA-molecules. The visualization of RNA-seq data therefore greatly facilitates its analysis. In addition to that, the annotation of the genome can be improved through the availability of the transcript data.

The web-application that has been programmed in this diploma thesis was created to display the data generated by RNA-seq experiments in an already existing popular visualization-software, the UCSC Archaea Browser. By fully supporting the display-protocols and data-exchange-formats used, and in some cases defined, by that application, many features that could'nt be implemented in a desktop-application are now at the users disposal. One example is the possibility to always display the current RefSeq annotations together with the data.

1.3 Motivation / Ziel

Der Fokus vieler RNA-seq Studien liegt in der Analyse von eukaryontischen Transkriptomen. Die generierten Sequenzdaten sind z.B. sehr hilfreich, um die unterschiedlichen Transkripte eines Gens zu erfassen.

Für die prinzipiell wesentlich einfachere Auswertung von RNA-seq Daten von Prokaryonten stehen bisher keine geeigneten Auswerteprogramme zur Verfügung.

Die Programmierung einer solchen Software im Rahmen einer Bachelor-Thesis bietet sich daher an. Zum einen erhalte ich als Student der Biomedizinischen Informatik einen Einblick in die die Welt der Genforschung. Zum andern kann ich hoffentlich mit meiner Arbeit einen kleinen Beitrag zur Verbesserung der Zugänglichkeit zu der beeindruckenden Menge an RNA-Sequenzdaten leisten.

1.4 Theoretischer Hintergrund

Seit einiger Zeit bieten eine Handvoll Produzenten Gensequenzierungsanlagen an, bei denen die Kosten pro sequenziertem Basenpaar drastisch verringert werden konnten. Diese neuartigen Sequenzierungs-Plattformen werden dazu verwendet, sowohl das Genom wie auch das Transkriptom, also die gesamte RNA-Substanz die in einer Zelle zu einem definierten Zeitpunkt vorhanden ist, zu sequenzieren.

Gegenüber der klassischen Transkritomanalyse mit Mikroarrays, bietet RNA-seq den gewichtigen Vorteil, dass damit *alle* Abschnitte des Genoms auf Transkription geprüft werden können.

Mit RNA-seq können darum - und das ist auch der Teil für den die vorliegende Software eingesetzt werden soll - auch *neue Gene* gefunden bzw. untranskribierte Bereiche erkannt werden, wo bisher ein Gen vermutet wurde. [CT10]

Ein weiterer Vorteil von RNA-seq Technologien, ist die Möglichkeit, das Expressionsniveau jeder einzelnen Base exakt messen zu können. Indem die Anzahl der Reads bezüglich Ihrer Länge und der Länge des Gens auf dem sie liegen normalisiert wird, werden dadurch verschiedene Studien auch sehr gut miteinander vergleichbar. [CT10; WGS09]

2 Technologie

2.1 Betriebssystem

UNIX ist aus Entwicklersicht aus verschiedenen Gründen eine optimale Betriebssystemplatform, um Datenintensive Berechnungen, wie sie bei der Verarbeitung und Aufbereitung von RNA-seq Daten anfallen durchzuführen. Die Architektur des Systems hat sich bewährt und ist weitherum bekannt für Ihre Robustheit, Performanz.

So sind denn auch die meisten Programme, die in jüngster Vergangenheit entwickelt wurden, um RNA-seq Daten zu verarbeiten, auf UNIX Umgebungen programmiert worden und darum dort auch ohne Probleme lauffähig.

Für die Softwareentwicklung sind auf der UNIX Platform eine Vielzahl verschiedener Programmbibliotheken und Sprachumgebungen Verfügbar. Die Qualität variiert, doch der Wettbewerb selektiert schlussendlich immer die guten.

Es wurde also ein UNIX Betriebssystem und zwar Debian GNU/Linux 6.0.4 (codename "squeeze") verwendet.

2.2 Programmiersprache

Die Programmiersprache *Python*, für die im Jahre 1991 ein erster Interpreter publiziert wurde und die in den letzten Jahren sehr populär geworden ist, war schon gesetzt. Sie eignet sich besonders gut für schnelle Entwicklung, da der Quellcode nicht in einem separaten Schritt kompiliert werden muss und darum geänderter Code viel schneller getestet werden kann.

2.3 Testing

Das Testen einzelner Softwarekomponenten wird mit dem *unittest* Modul aus der *Python Standard Library* orchestriert. Das *nosetest* Modul erweitert die Funktionalität des Standard Moduls noch. Es erzeugt in Zusammenarbeit mit dem *coverage* Programmpaket auf Befehl hin einen *Test Coverage Report*. Die Test-Coverage lässt sich als Prozentzahl angeben. Sie gibt Auskunft darüber, wie gross der Anteil der getesteten Code-Statements im Vergleich zur Gesamtzahl von Code-Statements ist.

2.4 weitere Bausteine

Bei der Auswahl der Technologien zur Implementation der vorliegende Software, habe ich versucht möglichst solche Bausteine und Bibliotheken zu verwenden, die gut dokumentiert sind, eine möglichst grosse Anwenderbasis haben und sich bereits möglichst lange im Wettbewerb behaupten konnten.

SQLite	Die SQLite Datenbank Engine (RDBMS) unterscheidet sich dadurch von allen anderen bekannten relationalen Datenbanken, wie MySQL, PostgreSQL, etc., dass hier die Daten nicht mit einem Server Prozess über ein Netzwerk-Socket zur Verfügung gestellt werden, sondern die Engine in einer meist dynamisch verlinkten System-Library, implementiert ist. Die Daten werden daher auch nicht vom Server verwaltet, sondern befinden sich in einer ganz gewöhnlichen Datei, die an einem Beliebigen Ort abgelegt werden kann.
SQLAlchemy	Das SQLAlchemy Projekt bietet einen sogenannten Object Relational Mapper für Datenobjekte an. Die Software ist sehr gut durchdacht und bietet ein umfassendes Feature-Paket.
Pyramid	Das Pyramid Web Application Framework wurde unter anderem von ehemaligen Zope-Entwicklern programmiert und ist ebenfalls sehr gut durchdacht und dokumentiert.
Paste	Ein bereits etwas älteres Tool-Paket von Ian Bicking, das u.a. von Pyramid verwendet wird und ein sehr nützlichen Deployment Tool bietet, mit dem sich auf einfache weise ein UNIX Daemon Prozess realisieren lässt. Dieses Tool findet im Worker Daemon Verwendung.
Buildbot	Um zu gewährleisten, dass nach grösseren Änderungen an der Software (immer noch) alles funktioniert wie geplant, setzt man in der Software-Entwicklung immer mehr auf sogenannte Continuous- Integration. Die u.U. von mehreren Entwicklern geschriebenen Code-Teile werden vom CI-System, z.B. Buildbot, laufend getestet und Test-Failures oder Errors werden gut sichtbar angezeigt, damit das Problem so schnell wie möglich behoben werden kann. Für die Buildbot Software wurde ein kleines Trac Plugin programmiert, welches den Build-Status laufend auf der Projekt-Homepage anzeigt.
Sphinx	Das Sphinx Dokumenten-Verarbeitungs-System kommt unter anderem bei der Python Software Foundation zum Einsatz. Es generiert dort die API-Dokumentations-Seiten. Das tut es auch in diesem Projekt und zwar werden die Dokument-Inhalte mit dem autodoc Plugin direkt aus den Quellcode-Docstrings extrahiert.
Bash	Die Bash-Shell ist ein sehr nützliches Tool, um einfache "Kommandolisten" in Scripts oder auch Funktionen zu kapseln. Bash Scripts wurden u.a. eingesetzt, um stündlich den Inhalt des lokalen Quellcode-Repositories mit einem zweiten Server, dessen Festplatteninhalt automatisch gesichert wird, abzugleichen.

Git	Das Git Quellcodeverwaltungssystem basiert auf einer genialen Daten-Architektur. Es bietet schier unbegrenzte Möglichkeiten, zur Dokumentation des Entwicklunsprozesses und ermöglicht so die zeilengenaue Nachvollziehbarkeit, der Entstehung des Software-Quellcodes. Das System wurde unter anderem eingesetzt um im umfangreichen Quellcodearchiv die Übersicht über die Autorenschaft jeder einzelnen Zeile zu behalten ('git commit --author=...' / 'git blame').
Apache / mod_wsgi	Der Apache HTTP Server ist ein weit verbreiteter Web-Server. Er läuft zuverlässig und ist einfach konfigurierbar. Er wurde eingesetzt, um in Verbindung mit mod_wsgi, die Applikations- Webseite zu generieren. WSGI steht für Web Server Gateway Interface. Ein Standard, der die Schnittstelle zwischen Python Programmen und dem Apache Webserver definiert.
jQuery	Das von John Resig ins leben gerufene jQuery Projekt erfreut sich bereits seit einigen Jahren grosser Beliebtheit. Es ist eine Javascript Library, die dem Entwickler vor allem den Zugang zum DOM, dem Objektmodell einer Webseite im Browser, erleichtert. Die Library wurde für diverse Zwecke direkt eingesetzt und wird zudem von vielen anderen eingesetzten Libraries als Abhängigkeit vorausgesetzt.
Backbone.js	Dies ist eine Javascript Library, mit deren Hilfe Software- Objekte auf Client-Seite sauber modelliert und vor allem sehr bequem, über ein REST-Interface, automatisch vom Server aktualisiert werden können. Durch die Verwendung des Observer Patterns werden dann die Views laufend aktualisiert.
plupload	Der Upload von Datein mit einem Browser zum Server Lässt sich im Grunde genommen leicht realiesieren. Um die User Expierience allerdings möglichst angenehm zu gestalten, ist jedoch beispielsweise die Anzeige des Upload-Status fast Pflicht. Bei der Realisierung solcher Details findet man sich schnell in einem grossen Haufen kleiner zwar lösbarer, aber Zeitraubender Details. Diese Detailarbeit wurde vom plupload Projekt erledigt und die Software steht frei zur Verfügung. Wenn die Software genügend Sponsoren findet, sollte das Problem damit für die nächsten paar Jahre gelöst sein.
Python logging	Wird verwendet um Diagnosemessages zu Protokollieren.
Docopt	Ein geniales neuartiges Tool zur Programmierung von Kommandozeileninterfaces.

Twitter Bootstrap	Ein solides Grundgerüst für alle Arten von Webseiten.
Zope Page-Templates	Ein gut funktionierendes HTML-Templating System.
RESTfull API	Transport von JSON Daten via HTTP GET, POST, PUT und DELETE requests.
TopHat	Führt Bowtie aus und generiert aus einem FASTQ und einem FASTA input File einen fertigen BAM Track.
Bowtie2	Ordner die Short Reads der entsprechenden stelle im Genom zu.
samtools	Wandelt SAM in BAM, und umgekehrt um. Wird von TopHat und pysam benötigt.
pysam	Bietet eine Python API zur Verarbeitung von SAM/BAM Daten.
Biopython	Bietet Python API Bridges zu den NCBI eutilities und für den Umgang mit Genbank, Fasta und anderen bioinformatischen Datenformaten.
"Kent tree"	Bietet neben dem nicht lokal verwendeten Genome Browser einige Tools zur Datenumwandlung, insbesondere wigToBigWig u.a.
sra_sdk	Der Offizielle Toolkit für die Umwandlung von .sra in .fastq Daten.
bcbb	Ein Code Tree, der unter anderem ein bam_to_wiggle.py Skript beinhaltet, mit dem der Coverage Track generiert wird.
trac	Wird verwendet für die Koordination der Entwicklung. Bietet ein gut funktionierendes Wiki System.
transterm_hp	Sucht eine Basensequenz nach potentiellen Hairpin-Terminatoren ab.
s3cmd	Ist ein Python Programm und Library, die verwendet werden kann um Daten in die Amazon S3 Cloud zu transferieren.
nosetests	Eine Erweiterung des Unittest Paketes.
Debian Gnu/Linux	Eine bekannte stabile UNIX Distribution.

PyGit	Bietet eine API zu Git Source Code Repositories. Wird u.a. dazu verwendet, die Autorenschaft der einzelnen Files im Source-Code Tree zu dokumentieren.
Galaxy	Ein Projekt der Penn-State Univerity zur online RNA-seq Datenverarbeitung.

3 Anhang

Inhalt

3.1 Abbildungen

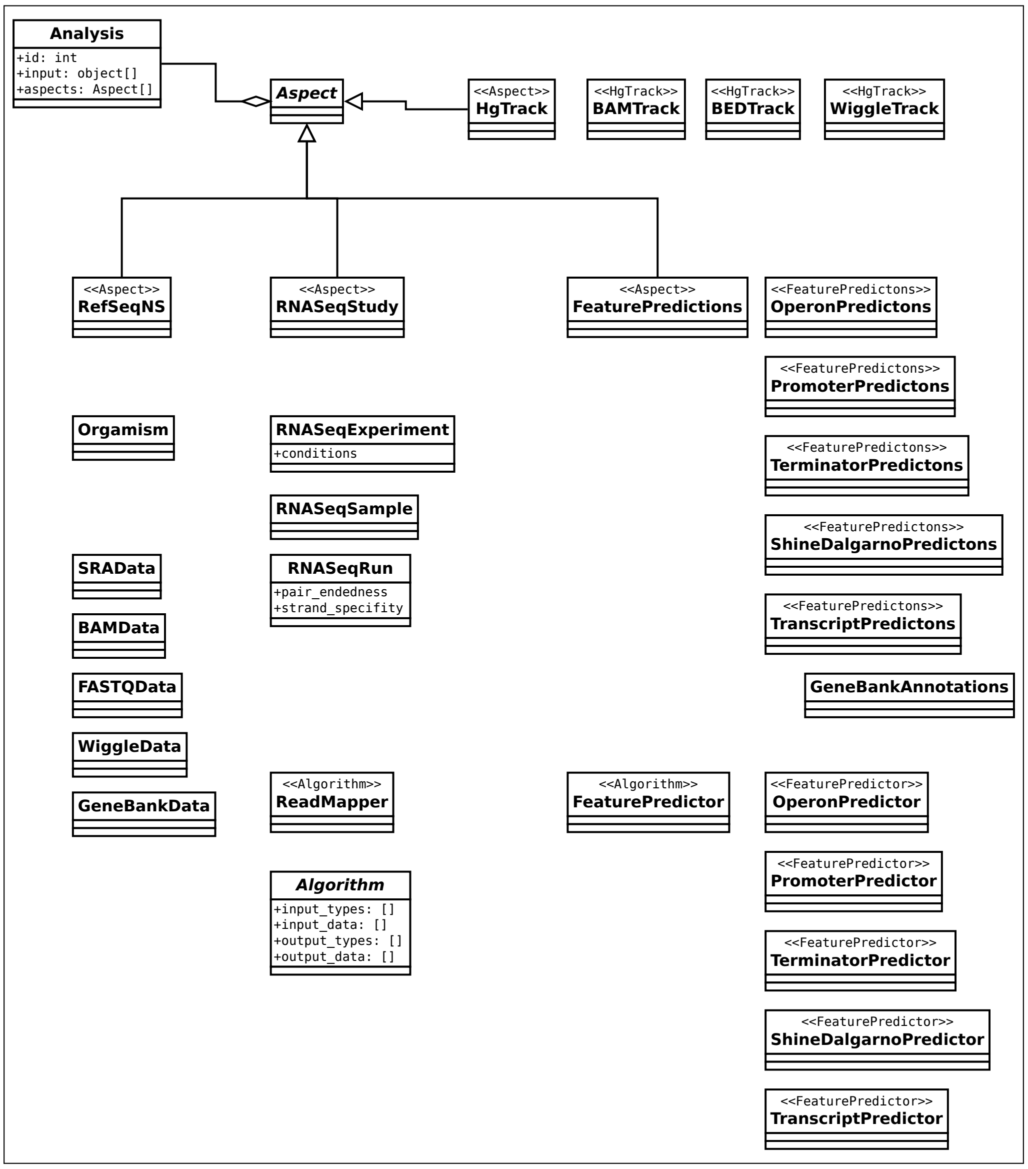


Abb. 1: Das geplante Domain-Modell. Die Feature-Predictions/-Predictors wurden ohne Persistente Datenbankobjekte implementiert und bestehen nur aus Daten- bzw. Programm-Files.

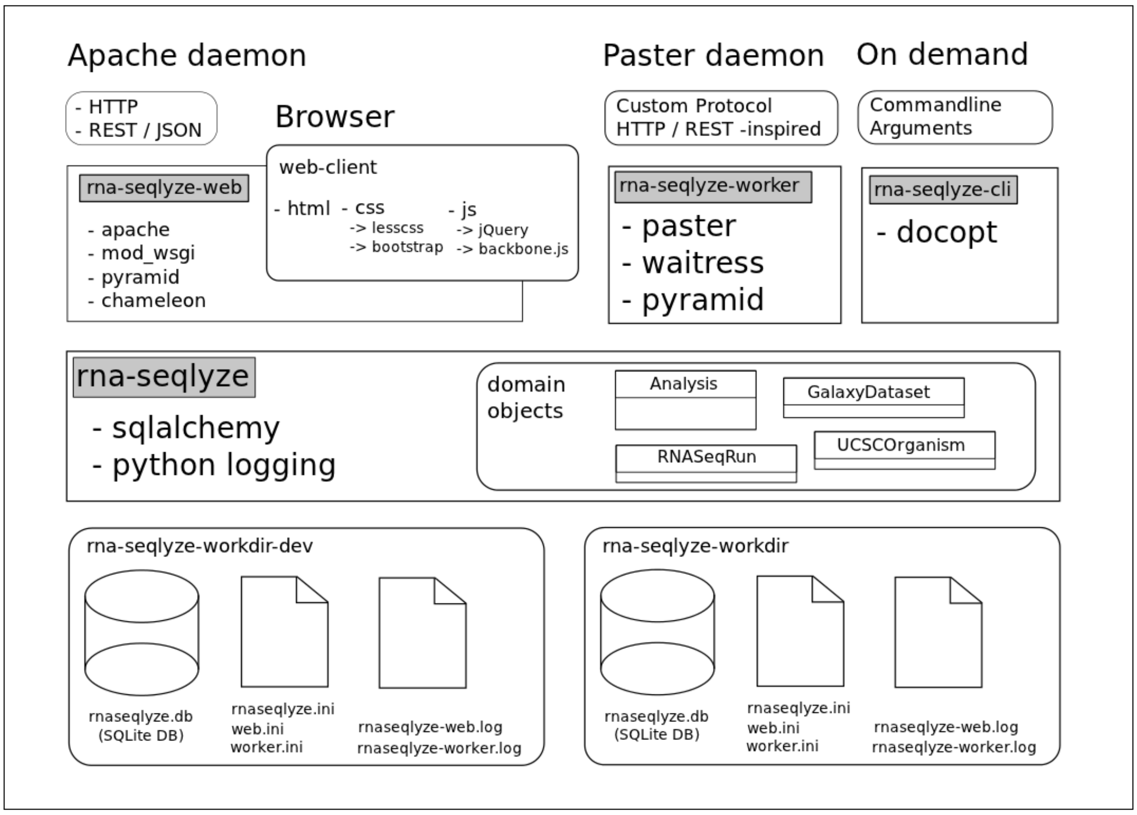
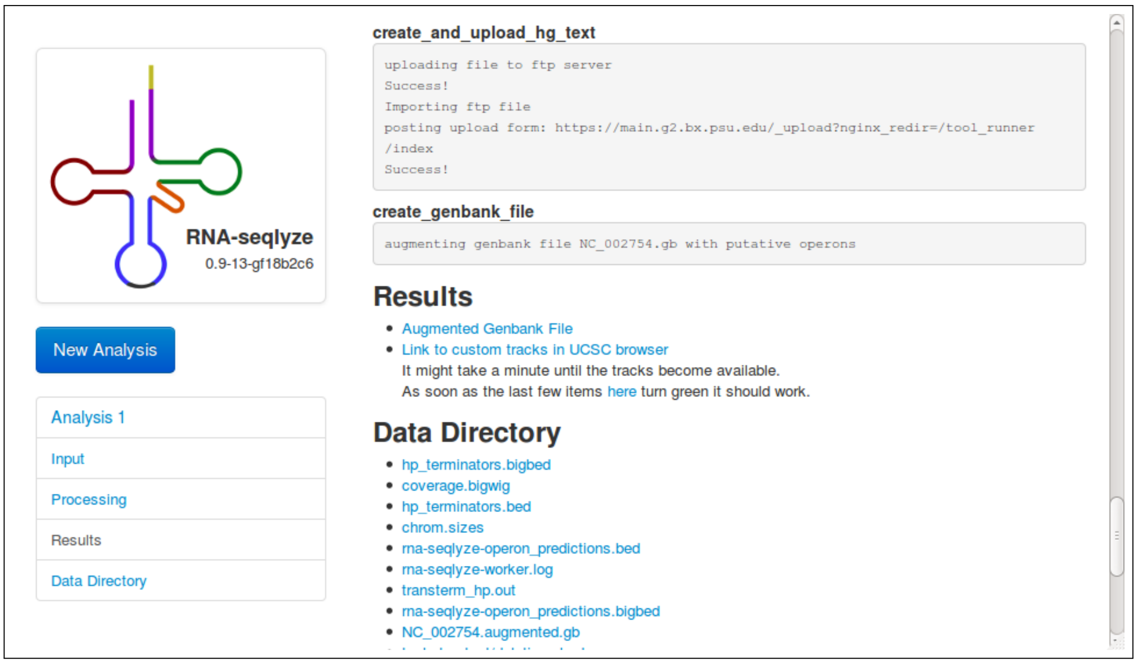


Abb. 2: Übersicht der Architektur der RNA-Seqlyze Applikation. Zuunterst die Konfigurations- und Datenbank- Dateien. In der Mitte die Kernfunktionalität, mit SQLAlchemy als 3.4Object-Relational Mapper. Darüber die einzelnen Schnittstellen. Dann die von den Schnittstellen verwendeten Protokolle. Zuoberst die den Code ausführenden Prozesse.



create_and_upload_hg_text

```
uploading file to ftp server
Success!
Importing ftp file
posting upload form: https://main.g2.bx.psu.edu/_upload?nginx_redir=/tool_runner
/index
Success!
```

create_genbank_file

```
augmenting genbank file NC_002754.gb with putative operons
```

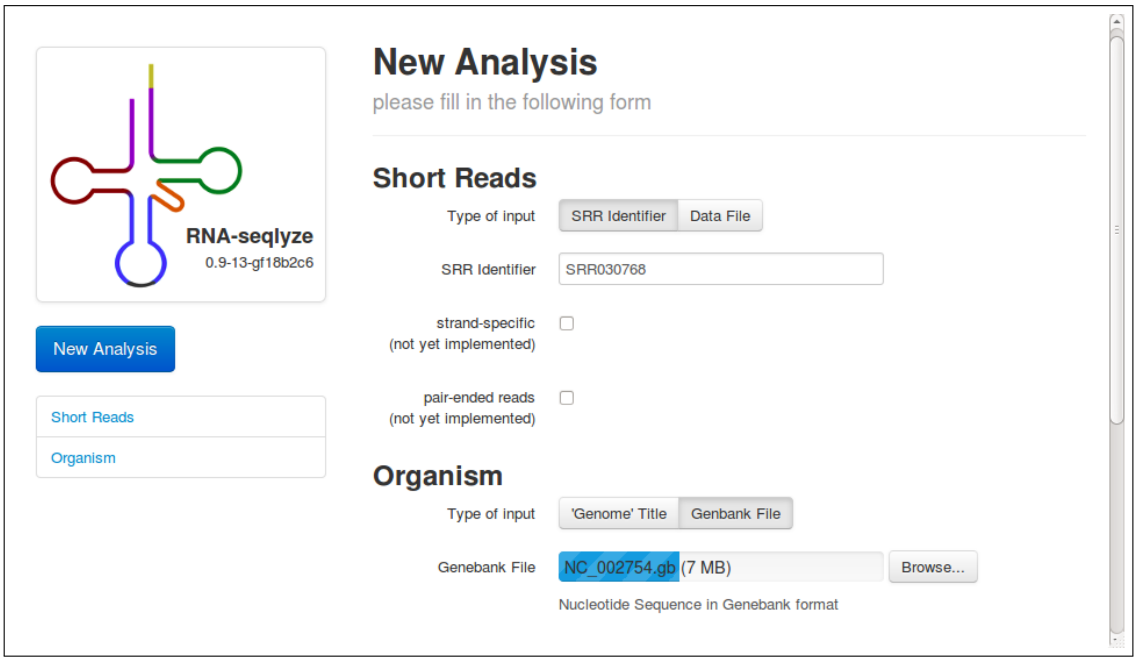
Results

- Augmented Genbank File
- Link to custom tracks in UCSC browser
  It might take a minute until the tracks become available.
  As soon as the last few items here turn green it should work.

Data Directory

- hp_terminators.bigbed
- coverage.bigwig
- hp_terminators.bed
- chrom.sizes
- rna-seqlyze-operon_predictions.bed
- rna-seqlyze-worker.log
- transterm_hp.out
- rna-seqlyze-operon_predictions.bigbed
- NC_002754.augmented.gb

Abb. 3: Darstellung einer bereits beendeten Analyse. Der graue Hintergrund der Log-Ausgaben des letzten Analyseschrittes zeigt an, dass die Analyse beendet ist. Unter der Überschrift "Results" finden sich ein Link zum UCSC Genom Browser, wo die Resultate angezeigt werden können und ein Link zu einer Genbank Datei, in der die gefundenen Operons annotiert wurden.



New Analysis
please fill in the following form

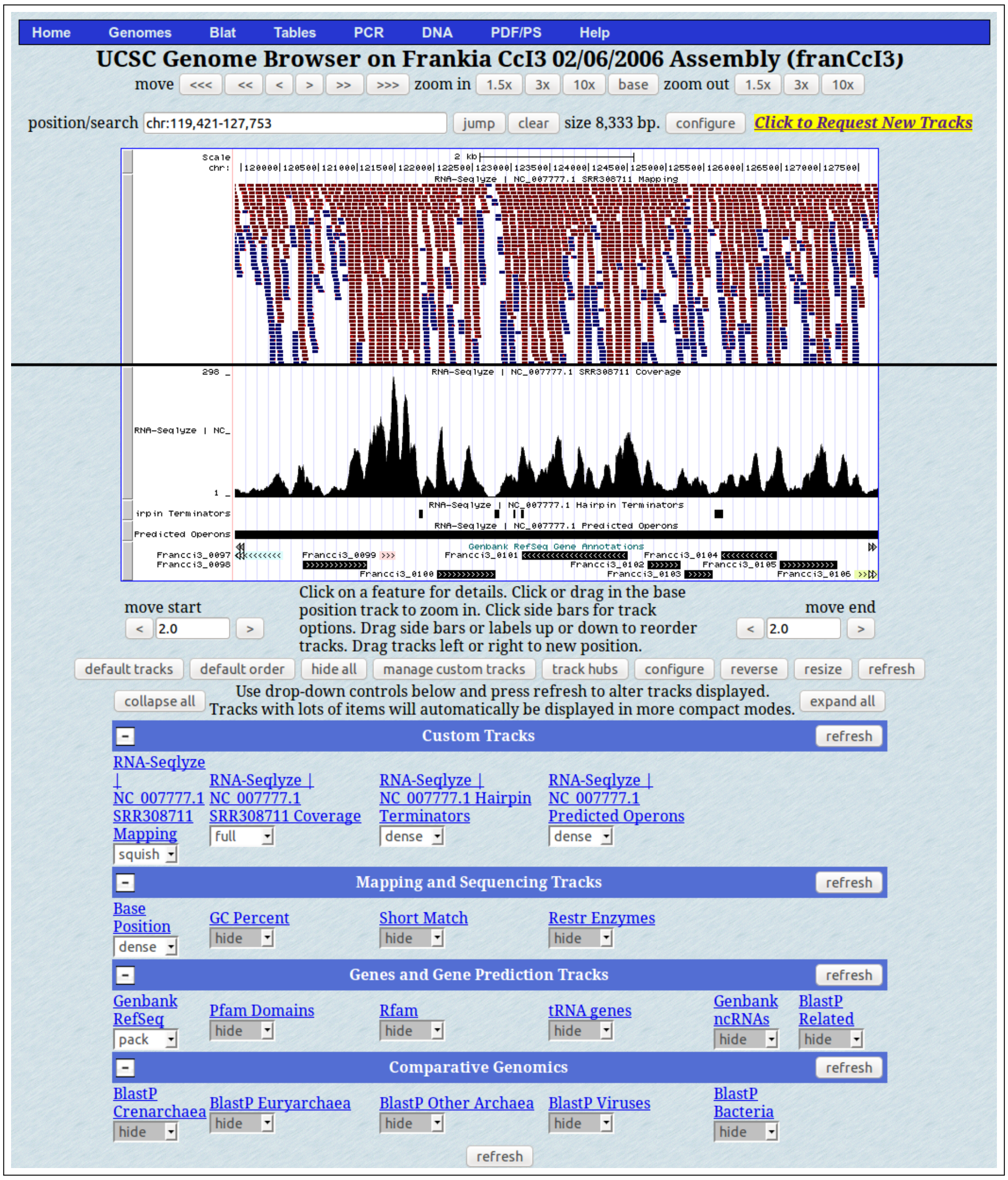
Short Reads

Type of input	SRR Identifier / Data File
SRR Identifier	SRR030768
strand-specific (not yet implemented)	☐
pair-ended reads (not yet implemented)	☐

Organism

Type of input	'Genome' Title / Genbank File
Genebank File	NC_002754.gb (7 MB)
	Nucleotide Sequence in Genebank format

Abb. 4: Starten einer neuen Analyse. Benötigt werden Short Read Daten und die Referenzsequenz. Für beide Inputs kann entweder die NCBI Identifikationsnummer angegeben oder eine Datei direkt hochgeladen werden werden.



UCSC Genome Browser on Frankia CcI3 02/06/2006 Assembly (franCcI3)

position/search chr:119,421-127,753 size 8,333 bp.

Click on a feature for details. Click or drag in the base position track to zoom in. Click side bars for track options. Drag side bars or labels up or down to reorder tracks. Drag tracks left or right to new position.

move start 2.0

move end 2.0

Use drop-down controls below and press refresh to alter tracks displayed.
Tracks with lots of items will automatically be displayed in more compact modes.

Custom Tracks

RNA-Seqlyze | NC_007777.1 SRR308711 Mapping: squish	RNA-Seqlyze | NC_007777.1 SRR308711 Coverage: full	RNA-Seqlyze | NC_007777.1 Hairpin Terminators: dense	RNA-Seqlyze | NC_007777.1 Predicted Operons: dense

Mapping and Sequencing Tracks

Base Position: dense	GC Percent: hide	Short Match: hide	Restr Enzymes: hide

Genes and Gene Prediction Tracks

Genbank RefSeq: pack	Pfam Domains: hide	Rfam: hide	tRNA genes: hide	Genbank ncRNAs: hide	BlastP Related: hide

Comparative Genomics

BlastP Crenarchaea: hide	BlastP Euryarchaea: hide	BlastP Other Archaea: hide	BlastP Viruses: hide	BlastP Bacteria: hide

Abb. 5: UCSC Browser.

3.2 Quellcode

3.2.1 Package `rnaseqlyze`

Modul `rnaseqlyze`

```python
"""
Top level package module

Importing this package configures the python "logging"
module in a way that messages of any level go to sys.stderr.
"""

#: the project base-name
#: the -cli, -web and -worker project names
#: are constructed by appending the part name to this one
project_name = "rna-seqlyze"

import pkg_resources
#: The __version__ property is set automatically set to the value of
#: pkg_resources.get_distribution(project_name).version on module import time.
try:
    __version__ = pkg_resources.get_distribution(project_name).version
except:
    # When the packe is initially installed, this module is imported by setup.py
    # and the the `project_name` attribute defined above is used to set the
    # project name, in order to make that information non-redundant.
    #
    # The version, however, is determined at install/build time by running
    # `git --describe` - in setup.py. So it doesn't matter if it is not set at
    # that time.  Later on, because `python setup.py install/develop` writes it
    # to rna-seqlyze.egg-info/PKG-INFO, where pkg_resources picks it up,
    # it will be available whenever the package is imported.
    pass
del pkg_resources

import logging
logging.basicConfig(level=0, format="%(levelname)-5.5s [%(name)s] %(message)s")
del logging

def configure(_workdir):
    """
    Calling this function

        - sets rnaseqlyze.workdir to <workdir>

        - sets rnaseqlyze.<setting> attributes for all
          settings under [rnaseqlyze] in '<workdir>/rnaseqlyze.ini'.

        - imports Bio.Entrez and sets Bio.Entrez.email to rnaseqlyze.admin_email
    """

    global workdir
    workdir = _workdir
    from os.path import join
    from ConfigParser import ConfigParser
    config = ConfigParser(dict(here=workdir))
    config.read(join(workdir, 'rnaseqlyze.ini'))

    for name, value in config.items("rnaseqlyze"):
        globals()[name] = value

    import Bio.Entrez
    Bio.Entrez.email = admin_email
```

Modul `rnaseqlyze.build`

```
"""
The rna-seqlyze software consisty of several parts. The majority of those parts
have been developped independently of this project and have been released under
a permissive license that allows them to be used in other (permissive licenced)
projects like this one.

This file defines a simple system and stores the commands necessary to build and
install those third-party components.
"""

from __future__ import print_function

import os, sys, shutil
from os import environ as env
from types import MethodType
import subprocess, multiprocessing

# a bit of infrastructure
#########################

class PartType(type):

    def __init__(cls, *ign):
        """
        auto-create and stock instances
        upon creation of "Part" (sub)classes

        appends a new instance of the
        created class to the "parts" list, if it exists
        """
        try:
            parts.append(cls())
        except NameError:
            pass

class Part(object):

    __metaclass__ = PartType

    def __init__(self):
        self.name = self.__class__.__name__
        try:
            self.subdir = "src/" + self.srcdir
        except AttributeError:
            self.subdir = "src/" + self.name

    def execute(self, phase):
        cmds = getattr(self, phase, None)
        if cmds == None: return
        print("#" * 80)
        print("# executing %s '%s' phase" % (self.name, phase))
        print("#")
        dev_null = file("/dev/null")
        logdir = "report/buildlogs"
        if not os.path.isdir(logdir): os.mkdir(logdir)
        logpath = logdir + "/%s-%s.log" % (self.name, phase)
        T = subprocess.Popen(["tee", logpath], stdin=subprocess.PIPE)
        try:
            import time
            log = lambda msg="": print(msg, file=T.stdin)
            log(time.asctime())
            log()
            log("\n".join("%s=%s" % nv
                for nv in filter(lambda i: i[0] in (
                    "TOPDIR", "PREFIX", "BINDIR", "LIBDIR",
                    "MACHTYPE", "NCPUS_ONLN"), env.iteritems())))
            log()
            if type(cmds) not in (list, tuple):
                cmds = cmds, # make it a 1-tuple
            for cmd in cmds:
                log("$ cd " + self.subdir)
                log("$ " + "\n  ".join(str(cmd).split("\n")))
                log()
                if type(cmd) == str:
                    ret = subprocess.call(cmd, shell=True, cwd=self.subdir,
                                stdin=dev_null, stdout=T.stdin, stderr=T.stdin)
                elif type(cmd) == MethodType:
                    def tgt():
                        sys.stdin = dev_null
                        sys.stdout = sys.stderr = T.stdin
                        os.chdir(self.subdir)
                        return cmd()
                    sp = multiprocessing.Process(target=tgt)
                    sp.start()
                    sp.join()
                    ret = sp.exitcode
                else:
                    raise Exception("Invalid '%s' phase command: %s" % (
                                              phase,              repr(cmd)))
                log()
            log(time.asctime())
            if ret != 0:
                raise Exception("%s '%s' phase failed -- exit code %d" % (
                                    self.name, phase, ret))
        finally:
            T.stdin.close()
            T.wait()

# parts & phases
################

parts = []
phases = 'build', 'test', 'install'

class bcbb(Part):
    srcdir = "bcbb/nextgen"
    build = "python setup.py build"
    # save some time
    #test = "nosetests"
    install = "python setup.py install --prefix=$PREFIX"

class biopython(Part):
    build = "python setup.py build"
    # save some time
    #test = "python setup.py test"
    install = "python setup.py install --prefix=$PREFIX"

class bowtie2(Part):
    build = "make -j$NCPUS_ONLN"
    def install(self):
        """
        the bowtie2 install function
        exists because there is no 'install' target in
        the makefile, so the binaries need to be installed manually
        """
        import shutil
        for f in ("bowtie2" + x for x in ("", "-align", "-build", "-inspect")):
            shutil.copy(f, env["BINDIR"])
            os.chmod(env["BINDIR"] + "/" + f, 0775)

class ncurses(Part):
    build = "./configure --prefix $HOME/.local && make"
    install = "make install"

class samtools(Part):
    depends = ncurses
    build = (
        'make -j$NCPUS_ONLN -C bcftools',
        'make -j2 SUBDIRS=.'
            ' LIBPATH=-L$PREFIX/lib LIBCURSES=-lncurses'
            ' CFLAGS="$(echo -I$PREFIX/include{,/ncurses})"'
    )
    install = "cp samtools $PREFIX/bin"

class cufflinks(Part):
    depends = samtools
    build = "./configure --prefix=$PREFIX"        \
                " --with-eigen=$TOPDIR/src/eigen" \
                " --with-bam=$TOPDIR/src/samtools && make"
```

```python
    install = "make install"

class kent(Part):
    build = "make -C src/lib"
    def install(self):
        """
        the kent install function was created, because rna-seqlyze need only
        a small subset of the included ulities and the easiest way to build
        those is to run "make" with custom arguments for each one of them
        """
        for util in "wigToBigWig bedToBigBed".split(" "):
            if subprocess.call("make -C src/utils/" + util, shell=True) != 0:
                raise Exception("kent.install(): couldn't install '%s'" % util)

class pysam(Part):
    build = "python setup.py build"
    # tests failing...
    #test = "cd tests; nosetests --exe"
    #test = "cd tests; ./pysam_test.py"
    install = "python setup.py install --prefix=$PREFIX"


class rna_seqlyze_cli(Part):
    srcdir = "rna-seqlyze-cli"
    build = "python setup.py build"
    test = "python setup.py test"
    install = "python setup.py develop --prefix=$PREFIX"

class rna_seqlyze_web(Part):
    srcdir = "rna-seqlyze-web"
    build = "python setup.py build"
    test = "python setup.py test"
    install = "python setup.py develop --prefix=$PREFIX"

class rna_seqlyze_worker(Part):
    srcdir = "rna-seqlyze-worker"
    build = "python setup.py build"
    test = "python setup.py test"
    install = "python setup.py develop --prefix=$PREFIX"

class sra_sdk(Part):
    # To get this to compile, I
    # 1) created a symlink src/sra_sdk/libxml2.so
    #    pointing to /usr/lib/libxml2.so.2 and added
    #    LDFLAGS=-L$PWD to avoid having to install libxml2-dev
    # 2) replaced the content of src/sra_sdk/libs/ext/Makefile
    #    with "all:" to skip unnesessary downloading of zlib and libbz2
    build = "LD_RUN_PATH=$LIBDIR make STATIC= STATICSYSLIBS= LDFLAGS=-L$PWD"
    install = (
        "cp -a linux/pub/gcc/$ARCH/bin/* $BINDIR",
        "cp -a linux/pub/gcc/$ARCH/lib/* $LIBDIR",
        "cp -a linux/pub/gcc/$ARCH/mod $LIBDIR/ncbi",
        "cp -a linux/pub/gcc/$ARCH/wmod $LIBDIR/ncbi",
    )
```

```
class tophat(Part):
    build = "./configure --prefix=$PREFIX" \
                      " --with-bam=$TOPDIR/src/samtools && make"
    install = "make install"

class trac(Part):
    build = "python setup.py build"
    # save some time
    #test = "python setup.py test"
    install = "python setup.py install --prefix=$PREFIX"

class trac_env(Part):
    def install(self):
        # need to discuss server
        # configuration with admin
        #destdir = "%(PREFIX)s/var/trac_env" % env
        #basedir = os.path.dirname(destdir)
        #if not os.path.isdir(basedir):
        #    os.mkdir(basedir)
        #shutil.copytree(".", destdir, symlinks=True)
        #print("Copied %s to %s\n" % (os.getcwd(), destdir))
        print("\n".join((
"""\
The following still needs to be done manually:
 1) Set up a database
 2) Restore the backup:
    $ cd """ + os.getcwd() + """
    $ mysql -uUSERNAME -pPASSWORD DATABASE < mysql-db-backup.sql
 4) Adjust the 'database' variable in the [trac] section in 'conf/trac.ini':
    database = mysql://USERNAME:PASSWORD@localhost/DATABSE
"""
        )))

class transterm_hp(Part):
    build = "make"
    def install(self):
        prog = "transterm"
        data = "expterm.dat"
        shutil.copy(prog, env["BINDIR"])
        os.chmod(env["BINDIR"] + "/" + prog, 0775)
        shutil.copy(data, env["LIBDIR"])

class s3cmd(Part):
    install = "python setup.py install --prefix=$PREFIX"

class docopt(Part):
    install = "python setup.py install --prefix=$PREFIX"
```

Modul rnaseqlyze.efetch

```
from Bio import Entrez

import rnaseqlyze
```

```
nc_db = "nuccore"
gb_type = "gb"
gb_mode = "text"

def get_nc_id(accession):
    handle = Entrez.esearch(db=nc_db, term=accession + "[Accession]")
    id_list = Entrez.read(handle)["IdList"]
    if len(id_list) != 1:
        raise Exception("unexpected reply from Entrez: id_list: %s" % id_list)
    return id_list[0]

def fetch_nc_gb(gb_id, out_file):
    handle = Entrez.efetch(db=nc_db, id=gb_id, rettype=gb_type, retmode=gb_mode)
    from shutil import copyfileobj as copy
    copy(handle, out_file)
```

Modul `rnaseqlyze.galaxy`

```
"""
RNA-Seqlyze Galaxy Module

Shamelessly piggy-back onto Penn-State University's "Galaxy" Project.

RNA-Seqlyze needs a some publicly available Web-Space, which PSU provides
plenty of for bioinformatics reseach data (250.0 Gb per user as of 4 Jul 2012).

Thanks go to Penn-State University!

http://www.psu.edu/

"""

# FIXME: the whole code here needs heavy refactoring

import logging
log = logging.getLogger(__name__)

import os, json, time, ftplib, \
       urllib, urllib2, cookielib
from time import time
from threading import local
from datetime import datetime, timedelta

import lxml.html

import rnaseqlyze
from rnaseqlyze import multipart

email = 'ucgxccgr@mailinator.com'
password = 'brtbhcdg'
api_key = 'dddb2c53c96c0c4d263e6c74b507d203'
hostname = 'main.g2.bx.psu.edu'

```

```
default_history = '16f9a8e916e0e908'

default_history_url = 'https://main.g2.bx.psu.edu/u/dcgdftvcdv/h/rna-seqlyze'

history_path_template = '/api/histories/{history}/contents'
ucsc_bam_track_template = \
        '/display_application/{dataset}/ucsc_bam/archaea/None/param/track'

ucsc_bam_path_template = \
        '/display_application/{dataset}/' \
        'ucsc_bam/archaea/None/data/galaxy_{dataset}.bam'

dataset_info_url_template = "/api/histories/{history}/contents/{dataset}"

dataset_display_url_template = "/datasets/{dataset}/display"

rq_headers = {}

class Session(local):
    cookies = None
    created = None

session = Session()

def api_call(path):
    url = "https://" + hostname + path
    return urllib2.urlopen(url + "?key=" + api_key).read()

def login():
    cookie_jar = cookielib.CookieJar()
    urllib2.install_opener(urllib2.build_opener(
                urllib2.HTTPCookieProcessor(cookie_jar)))
    log.info("Loggin in to galaxy server %s ..." % hostname)
    login = "https://" + hostname + "/user/login"
    rq = urllib2.Request(login, headers=rq_headers)
    request = urllib2.urlopen(rq)
    doc = lxml.html.parse(request).getroot()
    form = doc.forms[0]
    form.fields["email"] = email
    form.fields["password"] = password
    submit = "login_button", form.fields["login_button"]
    data = urllib.urlencode(form.form_values() + [submit])
    log.debug("posting login form: %s" % form.action)
    rq = urllib2.Request(form.action, headers=rq_headers)
    request = urllib2.urlopen(rq, data)
    doc = lxml.html.parse(request).getroot()
    log.info("Success!")
    return cookie_jar

def import_upload(filename):
    if not (session.created
            and session.created > (datetime.now() - timedelta(minutes=30))):
        session.cookies = login()
        session.created = datetime.now()

    urllib2.install_opener(urllib2.build_opener(
                    urllib2.HTTPCookieProcessor(session.cookies)))
    log.info("Importing ftp file")
    tool = "https://" + hostname + "/tool_runner?tool_id=upload1"
    rq = urllib2.Request(tool, headers=rq_headers)
    request = urllib2.urlopen(rq)
    doc = lxml.html.parse(request).getroot()
    found = False
    form = doc.forms[0]
    inp = form.inputs["files_0|ftp_files"]
    if isinstance(inp, lxml.html.InputElement):
        if inp.attrib['value'] == filename:
            found = inp.checked = True
    elif isinstance(inp, lxml.html.CheckboxGroup):
        for box in inp:
            if box.attrib['value'] == filename:
                found = box.checked = True
    else:
        raise Exception("unexpected html element: %s" % inp)
    if not found:
        raise Exception("file not available for import: %s" % filename)
    submit = "runtool_btn", form.fields["runtool_btn"]
    data = multipart.urlencode(form.form_values() + [submit])
    log.debug("posting upload form: %s" % form.action)
    rq = urllib2.Request(form.action, data, headers=rq_headers)
    request = multipart.urlopen(rq)
    doc = lxml.html.parse(request).getroot()
    log.info("Success!")

def ftpupload(fileobj, filename):
    """
    upload a file object to galaxy
    based on http://love-python.blogspot.com/2008/02/ftp-file-upload.html
    """
    log.info("uploading file to ftp server")
    ftp = ftplib.FTP(hostname, email, password)

    try:
        total = os.stat(fileobj.name).st_size
    except:
        total = 0

    class cbc(object):
        def __init__(self, total):
            self.total = total
            self.sent = 0
            self.then = time()
        def __call__(self, buf):
            self.sent += len(buf)
            now = time()
            if self.then < now - 15:
                self.then = now
                log.info("%d kb left" % ((self.total - self.sent) / 1024))
    log.info("sending %d kb of data..." % ((total / 1024 or -1)))
    ftp.storbinary('STOR ' + filename, fileobj, callback=cbc(total))
    log.info("Success!")
    ftp.quit()

def upload(fileobj, filename):

    # can't initialize this at module import time
    # because rnaseqlyze.xxx properties not initialized
    global rq_headers
    try:
        mail = rnaseqlyze.admin_email
    except:
        # rnaseqlyze not .configure()d
        mail = os.getenv("USER") + "@" + os.uname()[1]
    rq_headers = {
        'User-Agent': "%s (version:%s / admin:%s)" % (
            rnaseqlyze.project_name, rnaseqlyze.__version__, mail),
    #    'User-Agent': "Mozilla/5.0 (X11; Ubuntu; Linux x86_64; rv:13.0)" \
    #                  " Gecko/20100101 Firefox/13.0.1",
    }

    ftpupload(fileobj, filename)
    import_upload(filename)
    datasets = json.loads(api_call(
        history_path_template.format(history=default_history)))
    # assume objects are ordered chronologically...
    for dataset in reversed(datasets):
        if dataset['name'] == filename:
            return dataset['id']
    raise Exception("Couldn't find id of uploaded file in dataset")
```

Modul `rnaseqlyze.gb2ptt`

```
import logging
log = logging.getLogger(__name__)

import sys

from Bio import SeqIO
from Bio.SeqFeature import ExactPosition

def gb2ptt(in_file, out_file):

    debug = False

    seq = SeqIO.parse(in_file, "genbank").next()

    # expected input:
    #
    # CDS 249..857
    #     /locus_tag="SSO0001"
    #     /note="Predicted membrane protein, conserved in archaea"
    #     /codon_start=1
    #     /transl_table=11
    #     /product="hypothetical protein"
    #     /protein_id="NP_341578.1"
    #     /db_xref="GI:15896973"
    #     /db_xref="GeneID:1455258"
    #     /translation="MITEFLLKKKLEEHLSHVKEENTIYVTDLVRCPRRVRYESEYKE
    #     LAISQVYAPSAILGDILHLGLESVLKGNFNAETEVETLREINVGGKVYKIKGRADAII
    #     RNDNGKSIVIEIKTSRSDKGLPLIHHKMQLQIYLWLFSAEKGILVYITPDRIAEYEIN
    #     EPLDEATIVRLAEDTIMLQNSPRFNWECKYCIFSVICPAKLT"

    # intended output:
    #
    # Sulfolobus solfataricus P2 chromosome, complete genome - 1..2992245
    # 2978 proteins
    # Location        Strand  Length  PID
    # 249..857        +       202     15896973

    #        Gene    Synonym Code    COG     Product
    #        -       SSO0001 -       COG1468L        hypothetical protein

    import csv
    writer = csv.writer(out_file, delimiter='\t', lineterminator='\n')
    writer.writerow((seq.description,))
    writer.writerow(())
    writer.writerow(('Location', 'Strand', 'Length', 'PID',
                     'Gene', 'Synonym', 'Code', 'COG', 'Product'))

    n=0
    for f in seq.features:

        n+=1
        if debug and n > 10:
            break

        if f.type != 'CDS':
            continue

        if type(f.location.start) != ExactPosition \
           or type(f.location.end) != ExactPosition:
            log.info("skipping non-exact location '%s' in '%s'" % (
                                                   f.location, f.type))
            continue

        _len = f.location.end.position - f.location.start.position
        if _len < 0:
            _len = len(seq.seq) \
                    - f.location.start.position + f.location.end.position
        if _len % 3:
            log.info("implausible feature length (%d) in '%s'" % (_len, f.type))
        _len //= 3 # integer division
        _len -= 1 # omit stop codon

        xrefs = dict(map(lambda s: s.split(':'), f.qualifiers['db_xref']))
```

```
        for r in 'GI', 'GeneID':
            if r not in xrefs:
                xrefs[r] = '-'

        for q in 'gene', 'product', 'locus_tag':
            if q not in f.qualifiers:
                f.qualifiers[q] = '-'

        # need to convert between biopython (0-based, incl:excl)
        # and genbank (1-based, incl:incl) position boundary notation here
        writer.writerow((
            "%d..%d" % (f.location.start.position+1, f.location.end.position),
            ['-', '+'][(f.strand + 1) / 2],
            _len,
            xrefs['GI'],
            f.qualifiers['gene'][0],
            f.qualifiers['locus_tag'][0],
            '-',
            '-',
            f.qualifiers['product'][0]
        ))

    log.info("wrote %d rows" % n)
```

Modul rnaseqlyze.install

```
"""
RNA-Seqlyze Install

This command builds and installs all software components
included with and required by the RNA-Seqlyze web application.

Usage:
    rnas-install
    rnas-install --prefix <dir>
    rnas-install -h|--help

Note:
    The command has must run from the top level RNA-Seqlyze source directory.

Options:
    --prefix <dir>
                        The option is passed on to the ./configure and install
                        scripts of the various prorgams that this command installs.
                        The effect is, that all produced executables will be
                        installed under the that directory.

                        If not specified, defaults to ``$HOME/.local``

.. important::
                        The `PREFIX` variable in the "/etc/init.d/rnaseqlyze.sh"
                        worker daemon startup script and the `prefix` variable
                        in the "/var/www/../rna-seqlyze.wsgi" script must both
                        be set to the directory specified here!
```

```
"""

import os, re
from os import environ as env
from os.path import join, exists

from rnaseqlyze.build import parts, phases

def main():
    import docopt
    opts = docopt.docopt(__doc__)

    assert exists("src/rna-seqlyze/rnaseqlyze/__init__.py"), \
    "This command must be run from the top level RNA-Seqlyze source directory!"

    topdir = os.getcwd()
    prefix = opts['--prefix'] \
             or join(os.getenv("HOME"), ".local")

    env["TOPDIR"] = topdir
    env["PREFIX"] = prefix
    env["BINDIR"] = prefix + "/bin"
    env["LIBDIR"] = prefix + "/lib"
    env["MACHTYPE"] = os.uname()[4]
    env["ARCH"] = re.sub('i.86', 'i386', env["MACHTYPE"])
    env["NCPUS_ONLN"] = str(os.sysconf("SC_NPROCESSORS_ONLN"))

    for part in parts:
        for phase in phases:
            part.execute(phase)

    print "RNA-Seqlyze sucessfully installed."
    print
    print "   PREFIX=%s" % prefix
    print
```

Modul rnaseqlyze.multipart

```
"""
Multipart form-data handling
based on http://code.activestate.com/recipes/146306/
"""
import uuid, urllib2, mimetypes

def urlopen(url, data=None):
    if isinstance(url, basestring):
        rq = urllib2.Request(url, data)
    elif isinstance(url, urllib2.Request):
        rq = url
        data = rq.data
    else:
        raise Exception("'url' parameter must be a string or urllib2.Request")

    try:
        boundary = data[2:data.index("\r")]
    except ValueError, e:
        raise Exception("couldn't find boundary string in data", e)
    rq.add_header('Content-Type', 'multipart/form-data; boundary=%s' % boundary)
    return urllib2.urlopen(rq)

def urlencode(fields, files=None):
    """
    :param asd:
      is a sequence of ``(name, value)`` elements for regular form fields.

    :param files:
      is a sequence of ``(name, filename, value)``
      elements for data to be uploaded as files

    :returns:
      ``str`` of **multipart/form-data** encoded fields + files
    """
    boundary = str(uuid.uuid4())
    data = []
    for (key, value) in fields:
        data.append('--' + boundary)
        data.append('Content-Disposition: form-data; name="%s"' % key)
        data.append('')
        data.append(value)
    if files:
        for (key, filename, value) in files:
            data.append('--' + boundary)
            data.append('Content-Disposition: form-data' \
                        '; name="%s"; filename="%s"' % (key, filename))
            data.append('Content-Type: %s' % get_content_type(filename))
            data.append('')
            data.append(value)
    data.append('--' + boundary + '--')
    data.append('')

    return '\r\n'.join(data)

def get_content_type(filename):
    return mimetypes.guess_type(filename)[0] or 'application/octet-stream'
```

Modul rnaseqlyze.org_cache

```
"""
RNA-Seqlyze keeps a cache of organisms available
in the UCSC Browser. In addition to that, for each of
those organisms, the matching refseq accession is cached.
"""
import logging
log = logging.getLogger(__name__)

import csv
import difflib
```

```python
from pkg_resources import resource_stream

from Bio import Entrez

from rnaseqlyze import ucscbrowser
from rnaseqlyze.core.entities import UCSCOrganism

prokaryotes_tsv = "refseq-data/prokaryotes.txt"

def refresh(db_session):
    """
    Refresh the organism cache.

    The cache is initialized from the list of organisms available in the
    UCSC genome browser. A list of rnaseqlyze.orm.UCSCOrganism's is
    retrieved by calling rnaseqlyze.ucscbrowser.get_org_list().

    The retrieved list is not ready to be .add()ed to the :param:db_session
    however, because the objects' primary keys, the refseq accession,
    are still missing.

    Those are determined by parsing the list of gomplete genomes available in
    the ncbi "genome" database, which is stored in

        rnaseqlyze/refseq-data/prokaryotes.txt

    The file was retrieved from

        ftp://ftp.ncbi.nih.gov/genomes/GENOME_REPORTS/prokaryotes.txt

    on Mon, 02 Jul 2012.

    Once found, the rnaseqlyze.orm.UCSCOrganism objects are updated with the
    refseq accessions and .add()ed to the passed :param:db_session.
    """

    organisms = ucscbrowser.get_org_list()
    accessions = get_accessions()

    for org in organisms:
        ot = org.title
        for gt, acc in accessions:
            if ot == gt:
                org.acc = acc
                db_session.add(org)

    not_found = set(organisms) - \
                set(db_session.query(UCSCOrganism).all())

    # try fuzzy-matching the title
    # of those organisms that were not found
    for org in not_found:
        ot = org.title
        best_ratio = 0
        best_match = None
        for gt, acc in accessions:
            ratio = difflib.SequenceMatcher(None, ot, gt).ratio()
            if ratio > best_ratio:
                best_ratio = ratio
                best_match = acc
                best_match_t = gt

        if best_ratio > 0.8:
            if db_session.query(UCSCOrganism).get(best_match):
                log.debug(("NOT using '{match}' for '{org}'"
                           " despite match ratio of: {ratio}").format(
                               match=best_match_t, org=ot, ratio=best_ratio))
            else:
                log.info(("using '{match}' for '{org}'"
                          " match ratio: {ratio}").format(
                               match=best_match_t, org=ot, ratio=best_ratio))
                org.acc = best_match
                db_session.add(org)
        else:
            log.warn(("'{org}' not found in NCBI 'genome' database"
                      " (best match ratio only {ratio})").format(
                          org=ot, ratio=best_ratio))

    # make sure that that regular expression in views.post() that translates
    # the 'org_accession' from 'title (db/accession)', as generated in
    # rnaseqlyze.create.js, back to 'accession' doesn't fail
    for org in db_session.query(UCSCOrganism).all():
        if any(needle in heystack
                    for needle in '()'
                    for heystack in (org.db, org.acc, org.title)):
            log.warn("Droping organism with parentesis"
                     " to avoid problems in parsing auto"
                     "completed form input in views.post()")
            db_session.expunge(org)

def get_accessions():

    data_file = resource_stream(__name__, prokaryotes_tsv)
    reader = csv.reader(data_file, delimiter='\t')
    headings = reader.next()
    colnums = dict(zip(headings, map(headings.index, headings)))
    # -> { '#Organism/Name': 0, ..., 'Chromosomes/RefSeq': 7 }
    ret = []
    for cols in reader:
        if cols[colnums['Chromosomes/RefSeq']] == '-':
            continue
        ret.append((cols[colnums['#Organism/Name']],
                    cols[colnums['Chromosomes/RefSeq']]))
    return ret
```

Modul `rnaseqlyze.s3`

```python
"""
Upload files to Amazon S* using the s3cmd tools

The access credentials must be configured in ~/.s3cfg .
The s3cmd creates this file interactively with the --configure option.
"""

#: The s3 bucket name in "S3Uri" format
base_uri = "s3://biocalc/"

import os

# import this to fix a circular import dependency problem in s3cmd ...
import S3.Exceptions

from S3.S3 import S3
from S3.Config import Config
from S3.S3Uri import S3Uri
from S3.SortedDict import SortedDict

# same "interface" like rnaseqlyze.galaxy
def upload(fileobj, filename):

    cfg = Config()

    cfg.read_config_file(os.path.join(os.getenv("HOME"), ".s3cfg"))
    cfg.progress_meter = False
    cfg.acl_public = True

    s3 = S3(cfg)

    headers = SortedDict(ignore_case = True)
    headers["x-amz-acl"] = "public-read"
    headers["x-amz-storage-class"] = "REDUCED_REDUNDANCY"

    remote_uri = S3Uri(base_uri + filename)

    fileobj.seek(0,2) # seek to end
    size = fileobj.tell()
    fileobj.seek(0) # seek to start

    response = s3.send_file_multipart(fileobj, headers, remote_uri, size)

    assert response['status'] == 200

    return remote_uri.public_url()
```

Modul `rnaseqlyze.transterm`

```python
"""
A module to run transterm_hp
"""

import os, subprocess
```

```
from subprocess import PIPE

def run(args, out=None, err=None):
    """
    Run transterm_hp with the given arguments plus "-p expterm.dat"
    """
    def findit():
        for path in os.getenv("PATH").split(os.path.pathsep):
            for name in os.listdir(os.path.join(path, "../lib")):
                if name == 'expterm.dat':
                    return os.path.join(path, "../lib", name)
    expterm_dat = findit()
    if not expterm_dat:
        raise Exception("'expterm.dat' not found")
    cmd = ('transterm', '-p', expterm_dat) + tuple(args)
    proc = subprocess.Popen(cmd, stdout=out, stderr=err)
    proc.wait()
    if proc.returncode != 0:
        raise Exception(str(cmd) + " failed")

def tt2bed(tt_output, bed_file):
    for id, begin, end, strand, confidence in iterator(tt_output):
        # let color vary from 0 (black) to 100 (gray)
        rgb_color = ','.join((str(100 - int(confidence)),)*3)
        print >> bed_file, '\t'.join((
            'chr', begin, end, 'TERM_' + id,
            str(confidence), strand, begin, end, rgb_color
        ))

def iterator(tt_output):
    for line in tt_output:
        if not line.startswith("  TERM"):
            continue
        TERM, id, begin, dash, end, \
            strand, position, confidence, rest = line.split(None, 8)
        # switch begin & end on reverse strand
        if strand == '-':
            begin, end = end, begin
        yield id, begin, end, strand, int(confidence)
```

Modul rnaseqlyze.ucscbrowser

```
"""
Tools to deal with the UCSC genome browser at http://archaea.ucsc.edu/
"""
import logging
log = logging.getLogger(__name__)

from json import load
from urllib2 import urlopen
from urlparse import urljoin
from StringIO import StringIO
from shutil import copyfileobj
from os import listdir, makedirs
from os.path import join, dirname, isdir

from lxml.html import parse
from lxml.etree import dump

import rnaseqlyze
from rnaseqlyze.core import security
# delay import because of a
# circular import dependency ...
#from rnaseqlyze.core.entities import UCSCOrganism

cart_reset_url = "http://archaea.ucsc.edu/cgi-bin/cartReset"
custom_track_url = "http://archaea.ucsc.edu/cgi-bin/hgTracks"
custom_track_params = "?db={org_db}&hgt.customText={track_url}"

class BigDataTrack(str):
    """
    A UCSC "Big Data Track"

    You should pass a 'name' and a 'url'
    keyword argument to the constructor.
    """

    template = 'track type="{type}" name="{name}" bigDataUrl="{url}"'

    __new__ = lambda cls, **kwargs: cls.template.format(type=cls.type, **kwargs)

class BAMTrack(BigDataTrack): type = "bam"
class BigWigTrack(BigDataTrack): type = "bigWig"
class BigBedTrack(BigDataTrack): type = "bigBed"

# FIXME:
#    The org_list_default_dir = dirname(__file__)
#    hack will not work if the distribution is installed
#    as a zipped .egg. pkg_resources.resource_stream or
#    pkg_resources.resource_string should be used instead.
org_list_base_url = "http://archaea.ucsc.edu/wp-content/data/"
org_list_default_dir = join(dirname(__file__), "ucscbrowser-data")
json_links_file_name = "ucsc-wp-data.html"

def get_org_list():
    global UCSCOrganism
    from rnaseqlyze.core.entities import UCSCOrganism

    global org_list_cache_dir
    if not hasattr(rnaseqlyze, 'ucsc_org_list_cache_dir'):
        raise Exception("rnaseqlyze.configure(workdir) "
                        "must be called before calling this function")
    org_list_cache_dir = rnaseqlyze.ucsc_org_list_cache_dir

    if not isdir(org_list_cache_dir):
        makedirs(org_list_cache_dir)

    orgs = []
    for org in get_organisms(get_json_files()):
        for existing in orgs:
            if existing.title == org.title:
                log.warn("'%s' already present (db: %s/%s)" % \
                                    (org.title, org.db, existing.db))
                break
        else:
            orgs.append(org)
    return orgs

def get_json_files():

    json_links_file = None
    json_files = None

    for get_json_links_file in (get_json_links_file_web,
                                 get_json_links_file_cache,
                                 get_json_links_file_default,):
        try:
            log.debug("trying %s" % get_json_links_file.func_name)
            json_links_file = get_json_links_file()
        except Exception, e:
            log.warn("%s failed: %r" % (get_json_links_file.func_name, e))
            continue

        for get_json_files in (get_get_json_files_web(json_links_file),
                                get_json_files_cache,
                                get_json_files_default,):

            try:
                log.debug("trying %s" % get_json_files.func_name)
                return get_json_files()
            except Exception, e:
                log.warn("%s failed: %r" % (get_json_files.func_name, e))

    raise Exception("Couldn't get json organism lists")

# getting the links file

def get_json_links_file_web():
    json_links_file = StringIO()
    copyfileobj(urlopen(org_list_base_url), json_links_file)
    json_links_file.seek(0)
    return json_links_file

def get_json_links_file_cache():
    return open(join(org_list_cache_dir, json_links_file_name))

def get_json_links_file_default():
    return open(join(org_list_default_dir, json_links_file_name))


# getting json files

```

```
def get_get_json_files_web(json_links_file):

    def get_json_files_web():
        for e in parse(json_links_file).getroot().iter("a"):
            link = e.attrib['href']
            if link.endswith(".json"):
                security.check_valid_filename(link)
                # FIXME: The json files should also be returned as StringIO
                #        buffers only and the cache files shouldn't be
                #        overwritten until it is certain that the newly
                #        downloaded files contain the expected data
                json_file = open(join(org_list_cache_dir, link), "w+")
                copyfileobj(urlopen(urljoin(
                        org_list_base_url, link)), json_file)
                json_file.seek(0)
                yield json_file

        try:
            json_links_file.fileno()
            # json_links_file was defaults or cached
        except:
            # json_links_file was a memory buffer - save it because
            # if this code is is reached, it means there was no error,
            # in the code above, so the buffer likely contains good links
            json_links_file.seek(0)
            copyfileobj(json_links_file, open(join(
                    org_list_cache_dir, json_links_file_name), "w"))

    return get_json_files_web

def get_json_files_cache():
    for json in listdir(org_list_cache_dir):
        if json.endswith(".json"):
            yield open(join(org_list_cache_dir, json))

def get_json_files_default():
    for json in listdir(org_list_default_dir):
        if json.endswith(".json"):
            yield open(join(org_list_default_dir, json))


# creating UCSCOrganism objects from json files

def get_organisms(json_files):
    for json_file in json_files:
        for object in load(json_file):
            for child in object['children']:
                for grandchild in child['children']:
                    if grandchild['attr']['rel'] == 'genome':
                        yield UCSCOrganism(db=grandchild['attr']['id'],
                                           title=grandchild['data']['title'])
```

3.2.2 Package rnaseqlyze.core

Modul rnaseqlyze.core

```
"""
Core functionality
"""
```

Modul rnaseqlyze.core.analysis

```
"""
Property getters and methods for Analysis instances
"""

import logging
log = logging.getLogger(__name__)

import os
from os.path import join, exists
import datetime
from urllib import quote

from sqlalchemy import ForeignKey
from sqlalchemy import Table, Column
from sqlalchemy import Boolean, Integer, String, Text, DateTime
from sqlalchemy.orm import relationship, backref, validates
from sqlalchemy.orm.properties import RelationshipProperty
from sqlalchemy.ext.declarative import declared_attr, declarative_base

import rnaseqlyze
from rnaseqlyze import galaxy
from rnaseqlyze import ucscbrowser
from rnaseqlyze.core import security

class Methods(object):
    """
    Here the various analysis configurations are handled
    as transparently as possible. The properties should be
    seasy to deal with so the worker.core code doesn't get too hairy.

    .. note::

        Weather the input is an SRR identifier or a sra/fastq file is
        distinguished by checking "self.inputfile_name == None" in at least
            - the Worker
            - the analysis.pt template
            - in this file

    .. note::

        Weather the organism input is a NCBI RefSeq accession or a genbank file
        is distinguished by checking "self.genbankfile_name == None" in at least
            - the Worker
            - the analysis.pt template
            - in this file
    """

    def __init__(self, **kwargs):
        super(Methods, self).__init__(**kwargs)
        self.creation_date = datetime.datetime.utcnow()

    def create_data_dir(self):
        if not os.path.isdir(self.data_dir):
            os.makedirs(self.data_dir)

    # org_db and hg_url (which depends upon org_db) are not set
    # as a db attribute, so old analyses where the organism was not
    # known at creation time automatically get the right url set if the
    # organism later on becomes available in the UCSC Browser

    def get_hg_url(self, org_db):
        if not self.galaxy_hg_text:
            return
        hg_url = ucscbrowser.custom_track_url + \
                    ucscbrowser.custom_track_params.format(
                        org_db=org_db, track_url=quote(self.hg_url))
        return hg_url

    def get_galaxy_id(self, name):
        for ds in self.galaxy_datasets:
            if ds.name == name:
                return ds

class Properties(object):

    """
    .. note::

        - `input` means `short reads data`
        - `genbank` means `"genome" database nucleotide sequence`
    """

# data uploaded or id specified ?
# -------------------------------

    @property
    def inputfile_uploaded(self):
        return self._inputfile_name and True

    @property
    def genbankfile_uploaded(self):
        return self._genbankfile_name and True

# directories
# -----------

    @property
    def data_dir(self):
        return join(rnaseqlyze.analyses_path, str(self.id))

    @property
    def input_data_dir(self):
        if self.inputfile_uploaded:
            return self.data_dir
        else:
            return self.rnaseq_run.data_dir

    @property
    def genbank_data_dir(self):
        if self.genbankfile_uploaded:
            return self.data_dir
        else:
            return join(rnaseqlyze.shared_data_path, self.org_accession)

# short reads files
# -----------------

    @property
    def inputfile_name(self):
        return self._inputfile_name \
                or self.rnaseq_run and self.rnaseq_run.srr + ".sra"

    @inputfile_name.setter
    def inputfile_name(self, value):
        self._inputfile_name = value

    @property
    def inputfile_path(self):
        return join(self.input_data_dir, self.inputfile_name)

    @property
    def inputfile_base_name(self):
        return self.inputfile_name.rsplit(".", 1)[0]

    @property
    def inputfile_fq_name(self):
        return self.inputfile_base_name + ".fastq"

    @property
    def inputfile_fq_path(self):
        return join(self.input_data_dir, self.inputfile_fq_name)

    @property
    def inputfile_header(self):
        if not exists(self.inputfile_fq_path):
            return
        fq_file = open(self.inputfile_fq_path)
        lines = [fq_file.readline() for i in range(4)]
        log.info("Header: %s" % lines[0])
        fq_file.close()
        return "".join(lines)

# organism files
```

```
    # --------------

        @property
        def genbankfile_name(self):
            return self._genbankfile_name \
                    or self.org_accession and self.org_accession + ".gb"

        @genbankfile_name.setter
        def genbankfile_name(self, value):
            self._genbankfile_name = value

        @property
        def genbankfile_path(self):
            return join(self.genbank_data_dir, self.genbankfile_name)

        @property
        def genbankfile_base_name(self):
            return self.genbankfile_name.rsplit(".", 1)[0]

        @property
        def genbankfile_fa_name(self):
            return self.genbankfile_base_name + ".fa"

        @property
        def genbankfile_fa_path(self):
            return join(self.genbank_data_dir, self.genbankfile_fa_name)

        @property
        def xgenbankfile_name(self):
            return self.genbankfile_base_name + ".augmented.gb"

        @property
        def xgenbankfile_path(self):
            return join(self.data_dir, self.xgenbankfile_name)

    # magic galaxy_xxx attributes
    # ---------------------------

        galaxy_stuff = "hg_text bam coverage hp_terms pr_operons".split()

        for x in galaxy_stuff: exec """if True: # just to enable indentation ...
            @declared_attr
            def galaxy_%s(self):
                return relationship("GalaxyDataset",
                                    uselist=False, primaryjoin="%s")""" % (x,

                    "and_(GalaxyDataset.type == '%s', "
                         "Analysis.id == GalaxyDataset.analysis_id)" % x)
        del x
        @validates(*("galaxy_" + x for x in galaxy_stuff))
        def _set_galaxy_(self, attr, dataset):
            dataset.type=attr[7:]
            return dataset
```

```
# other things
# ------------

    @property
    def hg_url(self):
        if not self.galaxy_hg_text:
            return
        return "https://" + galaxy.hostname \
                     + galaxy.dataset_display_url_template \
                         .format(dataset=self.galaxy_hg_text.id)

    @property
    def data_dir_state(self):
        return hash(tuple((x,tuple(y),tuple(z))
                             for x, y, z in os.walk(self.data_dir)))

    @property
    def stage_logs_state(self):
        return hash(tuple(log.text for log in self.stage_logs))

class Validators(object):
    @validates('org_accession')
    def validate_org_accession(self, attr, acc):
        security.check_valid_filename(acc)
        return acc.upper()

    @validates('strandspecific', 'pairended')
    def validate_boolean(self, attr, val):
        return val and True or False

    @validates('inputfile_name', 'genbankfile_name')
    def validate_x_file_name(self, attr, name):
        if '\\' in name:
            name = name.rsplit('\\', 1)[1]
        security.check_valid_filename(name)
        if name.find('.') < 0:
            raise Exception("Please make sure your input file has a"
                            " (meaningful) extension, like .fastq or .sra")
        return name

class Mixins(Methods, Properties, Validators):
    pass
```

Modul `rnaseqlyze.core.entities`

```
"""
SQLAlchemy Database Entities

A nice tutorial showing how everything works is `here
    <http://docs.sqlalchemy.org/en/latest/orm/tutorial.html>`_.
"""

from rnaseqlyze.core.orm import *
```

```
class Analysis(AnalysisMixins, Entity):

    # The order of superclasses matters!
    # AnalysisMethods.__init__ calls Entity.__init__

    """
    The central entity.
    Represents an analysis by a researcher.
    The whole rnaseqlyze project basically revolves around this entity.
    """

    id                    = Column(Integer, primary_key=True)

    org_gid               = Column(Integer)  # Organisms Genebank/Entrez gid
    org_accession         = Column(String)   # Organisms Genebank accession number

    _inputfile_name       = Column("inputfile_name", String)
    inputfile_type        = Column(String)
    _genbankfile_name     = Column("genbankfile_name", String)

    strandspecific        = Column(Boolean)
    pairended             = Column(Boolean)
    pairendlen            = Column(Integer)

    owner                 = relationship("User", backref=backref("analyses"))
    owner_name            = Column(String, ForeignKey('user.name'))

    creation_date         = Column(DateTime)
    started               = Column(Boolean)
    finished              = Column(Boolean)
    stage                 = Column(String)
    error                 = Column(String)

    rnaseq_run            = relationship("RNASeqRun", backref=backref("analyses"))
    rnaseq_run_srr        = Column(String, ForeignKey('rnaseqrun.srr'))

    # ft_predictions      = `backref` from FeaturePredictions
    # hg_tracks           = `backref` from HgTrack
    # galaxy_datasets     = `backref` from GalaxyDataset

class UploadSession(Entity):
    """
    Is created when somebody uploads a file
    """
    id                = Column(Integer, primary_key=True)
    analysis_id       = Column(Integer, ForeignKey(Analysis.id))
    analysis          = relationship(Analysis, uselist=False)

class User(Entity):
    """
    Constitutes a user of this service
    """
    name              = Column(String, primary_key=True)
    # analyses        = `backref` from Analysis
    def __init__(self, name):
        self.name = name

# SRA analogons

class RNASeqStudy(Entity): # stub
    """
    Constitues an SRA "SRP" == SRA Study
    """
    srp             = Column(String, primary_key=True)
    # analyses      = `backref` from Analysis
    # experiments   = `backref` from RNASeqExperiment

class RNASeqExperiment(Entity): # stub
    """
    Constitutes an SRA "SRX" == SRA Experiment
    """
    srx         = Column(String, primary_key=True)
    srp_srp     = Column(Integer, ForeignKey(RNASeqStudy.srp))
    srp         = relationship(RNASeqStudy, backref=backref("experiments"))
    # runs      = `backref` from RNASeqRun

class RNASeqRun(RNASeqRunMixins, Entity):
    """
    Constitutes an SRA "SRR" == SRA Run
    """
    srr         = Column(String, primary_key=True)
    srx_srx     = Column(Integer, ForeignKey('rnaseqexperiment.srx'))
    srx         = relationship(RNASeqExperiment, backref=backref("runs"))

class UCSCOrganism(Entity):
    """
    Holds information about the mapping of UCSC browser "db" names to
    "gene id 'title'"s, and RefSeq Accessions.
    """
    acc         = Column(String, primary_key=True)
    db          = Column(String, unique=True)
    title       = Column(String, unique=True)

class GalaxyDataset(Entity):
    """
    Holds a mapping from an analysis to a galaxy dataset id
    """
    id          = Column(String, primary_key=True)
    analysis_id = Column(Integer, ForeignKey(Analysis.id), primary_key=True)
    analysis    = relationship(Analysis, backref=backref("galaxy_datasets"))
    type        = Column(String)
    name        = Column(String)

class StageLog(Entity):
    """
    Holds the log output of one processing stage
    """
    # the primary key could be stage/analysis_id
    # but using an id automatically adds ordering
    # which comes handy, because how to order the stages otherwise ?
    id          = Column(Integer, primary_key=True)
    stage       = Column(String)
    analysis_id = Column(Integer, ForeignKey(Analysis.id))
    analysis    = relationship(Analysis, backref=backref("stage_logs"))
    text        = Column(Text)
```

Modul rnaseqlyze.core.orm

```
"""
This module declares and imports everyting needed to
define the database entitiy classes in :mod:`.entities`.
"""

from sqlalchemy import (
        ForeignKey,
        Table, Column,
        Boolean, Integer,
        String, Text, DateTime
)
from sqlalchemy.orm import relationship, backref
from sqlalchemy.ext.declarative import declared_attr

from rnaseqlyze.core.analysis import Mixins as AnalysisMixins
from rnaseqlyze.core.srr import Mixins as RNASeqRunMixins

class _Entity(object):

    @declared_attr
    def __tablename__(cls):
        return cls.__name__.lower()

    def __setattr__(self, name, value):

        # raise an exception when setting
        # attributes that are not db columns
        if not (name.startswith('_') or hasattr(type(self), name)):
            raise Exception("'%s' is not a declared attribute" % name)

        super(_Entity, self).__setattr__(name, value)

from sqlalchemy.ext.declarative import declarative_base
Entity = declarative_base(cls=_Entity)
del declarative_base
```

Modul rnaseqlyze.core.security

```
"""
A collection of security related functions.

In case a check fails, an exception is raised, otherwise None is returned.
"""
```

```
def check_valid_filename(name):
    """
    Assert that the passed name doesn't contain
    any "funny" characters (e.g. ../../../../sensitive.txt)
    """

    max_len = 128
    assert len(name) < max_len, "Filename too long"

    import string
    assert set(name) < set(string.digits + string.letters + '._'), \
            "Only digits, letters, point and underscore allowed in filenames"
```

Modul rnaseqlyze.core.service

```
import logging
log = logging.getLogger(__name__)

import os
import urllib2

import rnaseqlyze
from rnaseqlyze.core import security
from rnaseqlyze.core.entities import Analysis, User, RNASeqRun, UploadSession
def get_upload_session(db_session):
    sess = UploadSession()
    db_session.add(sess)
    db_session.flush()
    return sess

def get_uploadfile(db_session, session, name, type):
    # This doesn't look right, but it works. The database needs to
    # be locked here to make sure that the first upload request that
    # comes in creates the analysis and the second uses the same analysis.
    # We need to lock the whole database and this seemingly useless statement
    # does just that. With SQLite. I have been asking on irc #sqlalchemy about
    # how to do it the right way, but I didn't get any useful reply. I have
    # checked the SQLAlchemy as well as the SQLite docs and tried various things
    # like DBSession.execute("BEGIN") and such things - nothing seems to work
    # - this is the only solution I have found.
    #
    # EDT: it could be as simple as increasing the sqlalchemy debug level, check
    #      what sql statements are executed and then DBSession.execute() those
    #
    session.analysis = session.analysis
    #
    if not session.analysis:
        session.analysis = Analysis()
        db_session.add(session.analysis) # needed ?
        db_session.flush() # sets analysis.id
        session.analysis.create_data_dir()

    assert type in ('inputfile', 'genbankfile')
    typename = type + '_name'

    # inputfile_name -> Short Reads in SRA or FASTQ format
    # genabnkfile_name -> Organism genbank file
    #=if session.analysis.inputfile_name:
    if getattr(session.analysis, type + '_uploaded'):
        # you land here if a user uploads
        # more than one file per type
        # this is not intended, BUT
        # these are the interwebs!
        pass # FIXE: remove old

    #=session.analysis.inputfile_name = name
    setattr(session.analysis, typename, name)

    log.debug("creating upload file '%s' for analysis #%d" % (
                                    name,            session.analysis.id))

    # this would be the place to throw in a wrapper
    # to track upload progress the old way, i.e.
    # with server callbacks...

    #inputfile_path is a @property
    return open(getattr(session.analysis, type + '_path'), "w+b")

def get_analysis(db_session, attributes):

    # owner handling
    if 'owner' not in attributes:
        owner = db_session.query(User).get("anonymous")
        if not owner:
            owner = User("anonymous")
            db_session.add(owner)
        attributes['owner'] = owner

    # srr handling
    rnaseq_run = None
    if 'rnaseq_run' in attributes \
       and 'inputfile_name' not in attributes:
        log.debug("rnaseq_run: %s" % attributes['rnaseq_run'])
        rnaseq_run = db_session.query(RNASeqRun).get(attributes['rnaseq_run'])
        if rnaseq_run:
            attributes['rnaseq_run'] = rnaseq_run
        else:
            try:
                log.debug("creating new RNASeqRun")
                rnaseq_run = RNASeqRun(srr=attributes['rnaseq_run'])
                attributes['rnaseq_run'] = rnaseq_run
                rnaseq_run.create_directories()
                db_session.add(rnaseq_run)
            except Exception, e:
                # The RNASeqRun constructor checks the SRRnnnnnn argument
                # and raises an exception unless it passes the checks
                # e.g. if field was left blank/at default value
                # TODO: decide/document what to do
                log.debug("failed: %r" % e)
        del attributes['rnaseq_run']

    upload_session = db_session.query(UploadSession) \
                        .get(attributes['upload_session'])
    del attributes['upload_session']
    if not upload_session:
        raise Exception('this session has expired -'
                        ' reload the "New Analysis" page to start a new one')

    # the analysis exist already if the user uploaded something
    if upload_session.analysis:
        analysis = upload_session.analysis
        for attr, value in attributes.items():
            setattr(analysis, attr, value)

    else:
        # create db object
        log.debug("creating new analysis: %s" % attributes)
        analysis = Analysis(**attributes)
        db_session.add(analysis)
        db_session.flush() # sets analysis.id
        analysis.create_data_dir()

    # allow no more uploads to this analysis
    db_session.delete(upload_session)

    # if no input file has been uploaded
    if not analysis.inputfile_name:
        # an SRR identifier is needed
        if rnaseq_run:
            analysis.rnaseq_run = rnaseq_run
        else:
            raise Exception("Please upload an input file or specify an SRR id")

    return analysis

def start_analysis(analysis):
    url = "http://127.0.0.1:6543/analyses/%d"
    rq = RNASWorkerSTARTRequest(url % analysis.id)
    opener = urllib2.build_opener(HTTRNASWorkerHandler())
    rsp = opener.open(rq)
    body = rsp.read()
    rsp.close()

class RNASWorkerSTARTRequest(urllib2.Request):
    def get_method(self):
        return 'START'

class HTTRNASWorkerHandler(urllib2.HTTPHandler):
    def http_error(self, req, fp, code, msg, hdrs):
        raise WorkerException(fp.read())
    http_error_400 = http_error
    http_error_500 = http_error

class WorkerException(Exception):
    def __init__(self, exc_body):
        self.exc_body = exc_body
    def __repr__(self):
        return "WorkerException()"
    def __str__(self):
        return self.exc_body
```

Modul rnaseqlyze.core.sra

Modul rnaseqlyze.core.srr

```
"""
Sequence Run Archive interaction
"""

import logging
log = logging.getLogger(__name__)

import os
from os import path
from time import time
from urllib2 import urlopen
from urlparse import urlparse
from httplib import HTTPConnection
from socket import timeout

from sqlalchemy.orm import validates

import rnaseqlyze

url_template = "http://ftp-private.ncbi.nlm.nih.gov" \
        "/sra/sra-instant/reads/ByRun/sra/{srr:.3}/{srr:.6}/{srr}/{srr}.sra"
# e.g.  "/sra/sra-instant/reads/ByRun/sra/SRR/SRR000/SRR000001/SRR000001.sra"

class Methods(object):

    def download(self):
        srr_url = url_template.format(srr=self.srr)
        log.info("fetching %s" % srr_url)
        url_parts = urlparse(srr_url)

        resp = None
        max_tries = 3

        for tries_left in reversed(range(max_tries)):
            try:
                conn = HTTPConnection(url_parts.netloc, strict=True, timeout=60)

                msg = "connecting to server"
                log.debug(msg)
                conn.connect()

                msg = "sending GET request"
                log.debug(msg)
                conn.request("GET", url_parts.path)

                msg = "waiting for response from server"
                log.debug(msg)
                resp = conn.getresponse()

                break
            except timeout:
                log.warn("timeout " + msg)
                if tries_left > 0:
                    log.info("trying %d more time%s" %
                                    (tries_left, tries_left > 1 and 's' or ''))
                else:
                    log.error("tried %d times - giving up", max_tries)
                    raise

        # Impossible really, but...
        assert resp != None

        if resp.status != 200:
            problem = "Bad response from server: %d - %s" % (
                                        resp.status, resp.msg.status)
            log.error(problem)
            raise Exception(problem)

        try:
            local = open(self.sra_path, "w")
            total = resp.length
            read = 0
            then = time()
            log.info("transfering %d kb data..." % (total / 1024
                                                   if total else -1))
            while True:
                buf = resp.read(16*1024)
                read += len(buf)
                now = time()
                if then < now - 15:
                    then = now
                    log.info("%d kb left" % (((total or 0) - read) / 1024))
                if not buf:
                    break
                local.write(buf)
        except Exception, e:
            log.error("Error downloading SRR: %r" % e)
            os.unlink(self.sra_path)
            raise
        finally:
            # note:
            #  in case of an error, unlinking wil precede closing
            #   -- no problem on unix
            local.close()

        log.debug("Success!")

class Properties(object):
    @property
    def data_dir(self):
        return path.join(rnaseqlyze.shared_data_path, self.srr)

    @property
    def sra_path(self):
        return path.join(self.data_dir, self.sra_name)

    @property
    def sra_name(self):
        return self.srr + ".sra"

    def create_directories(self):
        if not os.path.isdir(self.data_dir):
            os.makedirs(self.data_dir)

class Validators(object):
    @validates('srr')
    def check_srr(self, key, srr):
        import string
        assert len(srr) == 9
        assert srr[:3] == 'SRR'
        assert set(srr[3:]) < set(string.digits)
        # ... what a powerful language python is! :-)
        # http://docs.python.org/library/stdtypes.html#set
        # http://docs.python.org/library/string.html#string-constants
        return srr.upper()

class Mixins(Methods, Properties, Validators):
    pass
```

3.2.3 Package `rnaseqlyze.cli`

Modul `rnaseqlyze.cli`

```
"""\
Programs runnable from the command line.

For each module contained in this package, a wrapper script
called `rnas-<module name>` will be installed in `<prefix>/bin`.
"""

from .. import project_name
project_name += "-cli"
```

Modul `rnaseqlyze.cli.apidoc`

```
"""\
RNA-Seqlyze ApiDoc Generator

Usage:
    rnas-apidoc -h|--help
    rnas-apidoc [-s|--source] <path> ...

Generates one <package>.rst sphinx apidoc source file,
in the current directory, for each package found in <path>.

Options:
    -s --source    Use `literalinclude` in addition to `automodule`.
                   When using this option, the generated output will be
                   optimized for processing with the spinx latexpdf module that
                   generates a pdf document. Even without this option, when
                   using the html output module, the modules source code will
                   still be available in the generated Website, but not on the
                   same pages as the rest of the modules documentation
                   (controlled by the "html_show_sourcelink" option in
                   apidoc/conf.py).

"""
import os, sys
from pkgutil import walk_packages

def main():
    import docopt
    opts = docopt.docopt(__doc__)

    global pkg_tpl
    global mod_tpl
    if opts['--source']:
        pkg_tpl, mod_tpl = pkg_src_tpl, mod_src_tpl

    #: implicit args to write():
    #:  pkg.outfile, pgkpath, name, filename
    def write(tpl, **kwargs):
        pkg.outfile.write(tpl.format(
            name=name, path=pkgpath + os.sep + filename,
            equals="=" * len(name), dashes="-" * len(name), **kwargs))

    packages = {}
    Package = type('', (), {})

    # requirement/assumption:
    #  parent packages will come before their children
    for loader, name, is_pkg in walk_packages(opts['<path>']):

        pkgname = name.rsplit('.', 1)[0]
        pkgpath = os.path.relpath(loader.path, ".")

        if is_pkg:
            # note: 'pkgname' is actually the
            #       _parent_ package name in this case

            # associate non-root-packages with their parents
            if '.' in name:
                packages[pkgname].subpackages.append(name)

            # create & init a 'Package' object,
            # open <fully-qualified-package-name>.rst
            # and set filename to <package-name>/__init__.py
            pkg = Package()
            pkg.subpackages = []
            print "creating %s.rst" % name
            pkg.outfile = open(name + '.rst', 'w')
            filename = name.split('.')[-1] + os.sep + '__init__.py'

            # add the package to the list
            # and write the packages .rst file heading
            packages[name] = pkg
            write(pkg_tpl)

        else:
            # skip modules that are not part of any package, like setup.py
            if pkgname not in packages:
                continue

            # find the containing package and set filename to <module>.py
            pkg = packages[pkgname]
            filename = name[len(pkgname)+1:] + '.py'

            # append the doc entry for this module to the package .rst file
            write(mod_tpl)

    if not opts['--source']:
        for pkg in packages.values():
            if pkg.subpackages:
                write(sub_pkg_tpl, names="\n\t".join(pkg.subpackages))

pkg_tpl = """\
:mod:`{name}`
{equals}=======

.. automodule:: {name}

"""

pkg_src_tpl = """\
:mod:`{name}`
{equals}=======

:mod:`{name}`
{dashes}-------

.. automodule:: {name}

Source Code:
```

```
.. literalinclude:: {path}

"""

mod_tpl = """\
:mod:`{name}`
{dashes}-------

.. automodule:: {name}

"""

mod_src_tpl = """\
:mod:`{name}`
{dashes}-------

.. automodule:: {name}

Source Code:

.. literalinclude:: {path}

"""

sub_pkg_tpl = """\
Subpackages
-----------

.. toctree::
        :titlesonly:

        {names}

"""
```

Modul rnaseqlyze.cli.galaxy_upload

```
"""\
RNA-Seqlyze Galaxy-Upload

Usage:
    rnas-galaxy-upload <local_file>
"""
import sys, os
from rnaseqlyze import galaxy

def main():

    if len(sys.argv) < 2 or sys.argv[1] in ('-h', '--help'):
        print __doc__
        return

    print galaxy.upload(open(sys.argv[1]), os.path.basename(sys.argv[1]))
```

Modul `rnaseqlyze.cli.gb2fasta`

```python
"""\
RNA-Seqlyze gb2fasta

Convert a genbank file to fasta format

Usage:
    rnas-gb2fasta <input.gb> <output.fa>

If <input.gb> is '-', use 'sys.stdin, if <output.fa> is '-', use 'sys.stdout'.
"""
import sys
import Bio.SeqIO

def main():

    if len(sys.argv) < 2 or sys.argv[1] in ('-h', '--help'):
        print __doc__
        return

    inputfile = sys.argv[1] == '-' and sys.stdin or open(sys.argv[1])
    outputfile = sys.argv[2] == '-' and sys.stdout or open(sys.argv[2], "w")
    Bio.SeqIO.write(Bio.SeqIO.parse(inputfile, "genbank"), outputfile, "fasta")
```

Modul `rnaseqlyze.cli.gb2ptt`

```python
"""\
RNA-Seqlyze gb2ptt

Convert a genbank file to ptt (protein table) format

Usage:
    rnas-gb2ptt <input.gb> <output.ptt>

If <input.gb> is '-', use 'sys.stdin, if <output.ptt> is '-', use 'sys.stdout'.
"""
import sys, logging
from rnaseqlyze.gb2ptt import gb2ptt

def main():

    if len(sys.argv) < 2 or sys.argv[1] in ('-h', '--help'):
        print __doc__
        return

    inputfile = sys.argv[1] == '-' and sys.stdin or open(sys.argv[1])
    outputfile = sys.argv[2] == '-' and sys.stdout or open(sys.argv[2], "w")

    loggin.basicConfig(level=logging.NOTSET) # logs to stderr

    gb2ptt(inputfile, outputfile)
```

Modul `rnaseqlyze.cli.init`

```
"""\
RNA-Seqlyze Init

(Re-)initialize an rnaseqlyze 'workdir'.

Usage:
    rnas-init <workdir>
    rnas-init --recreatedb <workdir>
    rnas-init --development <workdir>
    rnas-init -h|--help

Options:
    --recreatedb
                    Remove and re-initialize the database if it exists.

    --development
                    Use the development versions of the config file templates.

Arguments:
    <workdir>
                    The filesystem path to the directory to be initialized.
                    If the directory already exists, by default, existing
                    files inside that directory are not overwritten.

.. important::
                    The `WORKDIR` variable in the "/etc/init.d/rnaseqlyze.sh"
                    worker daemon startup script and the `workdir` variable
                    in the "/var/www/../rna-seqlyze.wsgi" script must both
                    be set to the directory specified here!

Documentation:

    The 'workdir' holds

        - configuration files        (`*.ini`)
        - the application database   (`rnaseqlyze.db`)
        - log files                  (`*.log`)
        - shared data                (`shared_data/`)
        - individual analysis data   (`analyses/`)

    The rnas-init command

        - creates the <workdir> if it does not already exist
        - copies default configuration files 'rnaseqlyze.ini', 'web.ini'
          and 'worker.ini' into the <workdir> if they do not already exist.
        - initializes the database that is configured in the 'rnaseqlyze.ini'
          config file if it doesn't already exist and --recreatedb is not given.

    The database to be initialized is configured with "db_url =" in the
    "[rnaseqlyze]" section in 'rnaseqlyze.ini'.  It is expected to be an sqlite
    database.  If the command creates the sqlite database file, it changes it's
    unix access mode to (octal) 0664 and the group membership is changed to
    <group>.  <group> can be confgured in 'rnaseqlyze.ini'.  If the command
    creates the <workdir>, it changes it's unix access mode to (octal) 0775 and
    the group membership is also changed to <group>.  The command changes the
    unix access mode and group membership of all .log files inside the workdir
    to (octal) 0664 and <group>.
"""

import logging
log = logging.getLogger(__name__)

import os, sys, grp, shutil

import pkg_resources
from sqlalchemy import create_engine
from sqlalchemy.orm import sessionmaker

import rnaseqlyze
import rnaseqlyze.web
import rnaseqlyze.worker
from rnaseqlyze.core.entities import Entity

Session = sessionmaker()


def main():

    import docopt
    opts = docopt.docopt(__doc__)

    workdir = os.path.abspath(opts['<workdir>'])

    # create the workdir if it does not exist
    wd_created = False
    if not os.path.isdir(workdir):
        if os.path.exists(workdir):
            log.error("not a directory: '%s'" % workdir)
            sys.exit(1)
        log.info("creating workdir '%s'" % workdir)
        os.makedirs(workdir)
        wd_created = True

    # create each config file that does not exist
    for pkg in rnaseqlyze, rnaseqlyze.web, rnaseqlyze.worker:

        # determine the destination file name
        conf_name = pkg.__name__.split('.')[-1] + ".ini"
        conf_path = os.path.join(workdir, conf_name)
        if os.path.exists(conf_path):
            continue

        # determine the source file name
        if pkg.__name__ == "rnaseqlyze":
            # for the core package there is currently
            # only one config file template
            ini = 'rnaseqlyze.ini'
        else:
            # for the non-core packages, take the desired version
            ini = opts['--development'] and "development.ini" or "production.ini"
        # get the file as a resource stream, which works even
        # if the distribution if installed as a zipped .egg
        req = pkg_resources.Requirement.parse(pkg.project_name)
        res = pkg_resources.resource_stream(req, ini)
        log.info("creating config file '%s'" % conf_name)
        shutil.copyfileobj(res, open(conf_path, "w"))

    # init rnaseqlyze configuration -- creates all .log files
    rnaseqlyze.configure(workdir)

    # set proper permissions on the log files
    for name in os.listdir(workdir):
        if name.endswith('.log'):
            path = os.path.join(workdir, name)
            log.info("adjusting permissions on '%s'" % name)
            os.chmod(path, 0664)
            os.chown(path, -1, grp.getgrnam(rnaseqlyze.group).gr_gid)

    # delayed because 'rnaseqlyze.group' was
    # not known before calling rnaseqlyze.configure() above
    if wd_created:
        log.info("adjusting permissions on '%s'" % workdir)
        # change permission bits
        os.chmod(workdir, 0775)
        # change group membership
        os.chown(workdir, -1, grp.getgrnam(rnaseqlyze.group).gr_gid)

    # get the database file path
    db_path = rnaseqlyze.db_url.split(":", 1)[1]

    # remove the databse file
    # if it exists and --recreatedb is given
    if os.path.exists(db_path) and opts['--recreatedb']:
        log.info("removing existing database file '%s'" %
                                                db_path.split('/')[-1])
        os.unlink(db_path)

    # create the database if it doesn't exist
    if not os.path.exists(db_path):

        log.info("recreating database '%s'" % rnaseqlyze.db_url)

        # create sqlalchemy db engine
        engine = create_engine(rnaseqlyze.db_url)

        # create the file and initialize the schema
        with engine.begin() as conn:
            Entity.metadata.create_all(conn)

        log.info("adjusting permissions on database file")

        # change permission bits
        os.chmod(db_path, 0664)
        # change group membership
        os.chown(db_path, -1, grp.getgrnam(rnaseqlyze.group).gr_gid)

        log.info("initializing organism cache")

        # initialize UCSC Browser list of organisms
        from rnaseqlyze import org_cache
        with engine.begin() as conn:
            session = Session(bind=conn)
            org_cache.refresh(session)
            session.commit()

    log.info("workdir initialized")
```

Modul rnaseqlyze.cli.transterm

```
"""\
RNA-Seqlyze transterm

Calls the transterm program
with an additional "-p <path to>/expterm.dat" argument.

Usage:
    rnas-transterm [--] <transterm arguments> ...
    rnas-transterm -h|--help
"""
import sys
from rnaseqlyze.transterm import run

def main():

    if len(sys.argv) > 1 and sys.argv[1] == '--':
        sys.argv.pop(1)
    elif len(sys.argv) < 2 or sys.argv[1] in ('-h', '--help'):
        print __doc__
        return

    run(sys.argv[1:])
```

Modul rnaseqlyze.cli.xmltool

```
"""\
RNA-Seqlyze xmltool

XML version of of `python -m json.tool`.

Takes an xml file or stream as input and pretty-prints it.

Usage:
    rnas-xmltool <input.xml> <output.xml>

If <input.xml> is '-', use 'sys.stdin, if <output.xml> is '-', use 'sys.stdout'.
"""
```

```
import sys
from lxml import etree

def main():

    if len(sys.argv) < 2 or sys.argv[1] in ('-h', '--help'):
        print __doc__
        return

    inputfile = sys.argv[1] == '-' and sys.stdin or open(sys.argv[1])
    outputfile = sys.argv[2] == '-' and sys.stdout or open(sys.argv[2], "w")

    tree = etree.parse(inputfile,
                       etree.XMLParser(remove_blank_text=True))
    print >> outputfile, etree.tostring(tree.getroot(), pretty_print=True)
```

3.2.4 Package rnaseqlyze.web

Modul rnaseqlyze.web

```
"""
**pyramid.web** is a Pyramid Web Framework Application.

To learn more about the applications architecture, head over to the wonderful
world of the pyramid web framework at http://www.pylonsproject.org/.

This application has been created using the ``pcreate`` command with the ``-s
alchemy`` option to create and sqlalchemy scaffold. There is plenty of `very
good documentation <http://docs.pylonsproject.org/projects/pyramid/en/latest/\
narr/project.html#scaffolds-included-with-pyramid>`_ available on how to do it.

In case you have trouble with anything pyramid-related, use the `source code on
github <https://github.com/Pylons/pyramid>`_ or ask 'mcdonc' on freenode irc
`#pyramid <http://webchat.freenode.net/?channels=#pyramid>`_.
"""

import logging
log = logging.getLogger(__name__)

from sqlalchemy import create_engine
from sqlalchemy.orm import sessionmaker, scoped_session
from pyramid.config import Configurator
#: the zope transaction extension
from zope.sqlalchemy import ZopeTransactionExtension

import rnaseqlyze
from rnaseqlyze.web.jsonx import jsonx
project_name = rnaseqlyze.project_name + "-web"

#: a session managed by
#: ZopeTransactionExtension
#:
#: - http://stackoverflow.com/a/6044925
```

```python
#: - pyramid_tm (transaction manager) is configured
DBSession = scoped_session(sessionmaker(extension=ZopeTransactionExtension()))

#: an unmanaged session
#:
#: used by :meth:`rnaseqlyze.web.views.post`
#: because the session needs to be commited early there
DBSession_unmanaged = scoped_session(sessionmaker())

def main(global_config, **settings):
    """
    Create and return a Pyramid WSGI application.
    """
    log.debug("rnaseqlyze.web version %s : main()" % rnaseqlyze.__version__)

    # make sure to be able to delete files created by webapp
    # as user/group www-data/www-data from the command line
    # (as user/group johndoe/www-data)
    import os
    os.umask(0002)

    engine = create_engine(rnaseqlyze.db_url)
    DBSession.configure(bind=engine)
    DBSession_unmanaged.configure(bind=engine)

    config = Configurator(settings=settings)

    config.add_renderer('jsonx', jsonx)

    config.scan()

    config.add_route('home', '/')
    config.add_route('upload', '/upload')
    config.add_route('analyses', '/analyses')
    config.add_route('analysis', '/analyses/{id}')
    config.add_route('analysis_files', '/analyses/{id}/files*subpath')

    config.add_route('analysis_rest', '/rest/analyses/{id}')
    config.add_route('analysis_logs_rest', '/rest/analyses/{id}/logs')
    config.add_route('analysis_files_rest', '/rest/analyses/{id}/files')

    config.add_route('organisms_rest', '/rest/organisms')

    for path in 'less', 'css', 'img', 'js':
        config.add_static_view(path, path)

    return config.make_wsgi_app()

from pyramid.events import subscriber
from pyramid.events import BeforeRender
from pyramid.renderers import get_renderer

@subscriber(BeforeRender)
def before_render(event):
    """
    This function is called by Pyramid after the view callable has returned
    and before the renderer (json / chameleon) is called. We inject some
    convienience functions and objects that are used in the `zope page
    templates <http://pagetemplates.org/docs/latest/reference.html>`_
    (.pt files) which `the chameleon template engine
    <http://pagetemplates.org/>`_ then renders.
    """

    base = get_renderer('templates/base.pt').implementation()

    rq = event['request']
    path = lambda sub: rq.route_path('home') + sub
    relpath = lambda sub: rq.current_route_path() + '/' + sub

    event.update({
        'base': base,
        'path': path,
        'relpath': relpath,
        'version': rnaseqlyze.__version__,
        'debug': log.getEffectiveLevel() <= logging.DEBUG,
    })
```

Modul rnaseqlyze.web.errors

```
"""
Pyramid Application Custom Error Views
"""

import logging
log = logging.getLogger(__name__)

from string import Template

from pyramid.view import view_config
from pyramid.response import Response, FileResponse
from pyramid.httpexceptions import (
        HTTPFound, HTTPError, HTTPServiceUnavailable, HTTPInternalServerError
)

import transaction
from sqlalchemy.exc import DBAPIError

import rnaseqlyze
from rnaseqlyze.web import DBSession, DBSession_unmanaged
from rnaseqlyze.core import service
from rnaseqlyze.core.entities import Analysis

@view_config(context=Exception)
def error(request):
    """
    **Exception view**

    This is a catch-all view that serves up any errors
    that have occured while processing the a request.

    The view just creates and returns a custom error response object.
    """
    return HTTPRNASeqError(request.exc_info)

class HTTPRNASeqError(HTTPError):
    """
    Custom HTTP Error class.

    This is a custom HTTP error class that extends
    :class:`pyramid.httpexceptions.HTTPError`, which extends
    :class:`pyramid.httpexceptions.WSGIHTTPException`. Have a look at the
    `source code <http://git.io/CqrfOg#L157>`_ to see how it works.

    It's ``code`` is 500, which generaly means "Internal Server Error".  If the
    application is in debugging mode -- i.e. the log level is DEBUG or less, a
    stack trace is added to the generated page as well as the log file.
    Otherwise, an informational message is displayed and only one line,
    containing the type of the error is logged.
    """
    code = 500
    title = "RNA-Seqlyze Web Application Error"
    explanation = "An Exception was raised in rnaseqlyze.web"
    html_template_obj = Template(Template('\n'.join(map(lambda s: s[8:], """\
        <html>
        <head>
        <title>${title}</title>
        </head>
        <body style="margin: 20px;">
        <h1>${title}</h1>
        ${body}
        </body>
        </html>
        """.split('\n')))).safe_substitute(title=title))
    def __init__(self, exc_info):
        e = exc_info[1]
        log.error(repr(e))
        body_template = "<b>${explanation}</b>\n<hr/>\n"
        cls = e.__class__.__name__
        if not e.args:
            self.explanation = "%s" % cls
        else:
            self.explanation = "%s: %s" % (cls, e.args[0])

        if log.getEffectiveLevel() > logging.DEBUG:     # no debug
            detail = production_error_msg % \
                        rnaseqlyze.admin_email
            body_template += "${detail}"
        else:                                          # debug
            detail = ''
            if isinstance(e, DBAPIError):
                detail += dberror_msg
            import traceback
            detail += '%s\n\nStack trace:\n' % e
            detail += ''.join(traceback.format_tb(exc_info[2]))

            log.debug(detail)
            body_template += "<pre>\n${detail}</pre>"

        HTTPError.__init__(self, detail, body_template=body_template)

dberror_msg = """\
This is a database related eror.

If it is not yet initialized or the schema has changed,
just run the "rnas-dbinit" script to (re-)initialize it.

Afterwards, restart the Pyramid application, i.e. send a
SIG_INT to the apache mod_wsgi daemon processes, and try again.
"""

production_error_msg = """\
If you think that this is a bug, please contact the application administrator,
%s, and inform him/her of the time the error occurred, and anything you might
have done that may have caused the error.
Thank You!
"""
# 'You' is intentionally capitalized! :-) Rule 84: http://goo.gl/BLBwX
```

Modul rnaseqlyze.web.jsonx

```
"""
Pyramid JSON renderer that serializes arbitrary objects

Copies the object's __dict__, looks up all attrs
in all base classes's __dict__'s on the object
and then strips any unknown attribute types.
"""

#import logging
#log = logging.getLogger(__name__)

import json

#: a custom json renderer
jsonx = lambda info: render_json

def render_json(value, system):
    """
    custom json renderer implementation

    based on http://git.io/a6BFGQ#L169
    """

    request = system.get('request')
    if request is not None:
        response = request.response
        response.content_type = 'application/json'
    return json.dumps(value, default=render_object, indent=4)

def render_object(obj):
    """
    "default" function for json.dumps()
    """
    attrs = dict((attr, getattr(obj, attr))
                    for base in obj.__class__.__mro__
                    for attr in base.__dict__
                    if attr[0] != '_')

    attrs.update(obj.__dict__)
#    log.debug(attrs)
    return dict(filter(filter_attributes, attrs.iteritems()))

none_type = type(None)
def filter_attributes(kv):
    """
    Helper function for render_object
    """
    if kv[0][0] != '_' and \
       type(kv[1]) in (none_type, bool, int, long, float, str, unicode, list):
        return True
    return False
```

Modul rnaseqlyze.web.rest

```
"""
Pyramid REST Views
"""
import logging
log = logging.getLogger(__name__)

import os

from pyramid.view import view_config


import rnaseqlyze
from rnaseqlyze.web import DBSession, DBSession_unmanaged
from rnaseqlyze.core import service
from rnaseqlyze.core.entities import Analysis, StageLog, UCSCOrganism


@view_config(route_name='analysis_rest', renderer='jsonx')
def display(request):
    """
    **REST Analysis View**
    """
    analysis = DBSession.query(Analysis).get(int(request.matchdict["id"]))
    first = DBSession.query(UCSCOrganism) \
                .filter(UCSCOrganism.acc.like(
                    analysis.org_accession + '%')).first()
    org_db = first and first.db
    analysis.__dict__.update({
        'org_db': org_db,
        'hg_url': analysis.get_hg_url(org_db)})
    return analysis

@view_config(route_name='analysis_logs_rest', renderer='jsonx')
def analysis_stage_logs(request):
    """
    ***REST Stage Logs View***
    """
    criterion = StageLog.analysis_id == int(request.matchdict["id"])
    logs = DBSession.query(StageLog).filter(criterion).all()
    return sorted(logs, key=lambda log: log.id)

@view_config(route_name='analysis_files_rest', renderer='jsonx')
def analysis_files(request):
    """
    **REST Files View**

    This view provides a (minimalistic, only GET is
    implemented) REST interface to '/analysis/{id}/files'.
    """
    files = []
    analysis = DBSession.query(Analysis).get(int(request.matchdict["id"]))
    os.chdir(analysis.data_dir)
    for dirpath, dirnames, filenames in os.walk("."):
        dir = dirpath[2:]
        for fn in filenames:
            files.append({'path': os.path.join(dir, fn)})
    return files

@view_config(route_name='organisms_rest', renderer='jsonx')
def organisms(request):
    """
    ***REST Organisms View***

    Displays the list of organism titles
    along with their UCSC db and NCBI RefSeq accession identifiers
    """
    return DBSession.query(UCSCOrganism).all()
```

Modul `rnaseqlyze.web.upload`

```
"""
Pyramid Application Upload View

This module handles the upload of analysis files.

The upload interface consists of `plupload`
(from http://www.plupload.com/) on the client
and this hack on the server side. Combining the
two and creating a working solution was not trivial.

```

```python
Documentation and inspiration to create this
was, amongst others, taken from the following documents:

 - http://www.plupload.com/documentation.php
 - http://hg.python.org/cpython/file/2.7/Lib/cgi.py#l353
 - https://raw.github.com/moxiecode/plupload/master/examples/upload.php
 - https://github.com/hcwebdev/plupload/blob/master/examples/server.py
 - https://github.com/Pylons/webob/blob/master/webob/request.py#L102
 - https://hg.gawel.org/gp.fileupload/file/default/gp/fileupload/storage.py#l97
"""

import logging
log = logging.getLogger(__name__)

import transaction
from pyramid.view import view_config

from rnaseqlyze.web import DBSession
from rnaseqlyze.core import service
from rnaseqlyze.core.entities import UploadSession

@view_config(route_name='upload', request_method='POST', renderer="json")
def upload(request):
    log.debug("upload(): content-type '%s'" % request.content_type)
    fs = FieldStoragx(fp=request.environ['wsgi.input'], environ=request.environ)
    return dict(jsonrpc="2.0", result=None, id=None)

import cgi
class FieldStoragx(cgi.FieldStorage):
    def __init__(self, fp=None, headers=None, outerboundary="",
                 environ=None, keep_blank_values=0, strict_parsing=0):
        self.environ = environ
        cgi.FieldStorage.__init__(self, fp, headers, outerboundary,
                                  environ, keep_blank_values, strict_parsing)
        if self.filename:
            return
        assert len(self.value) < 1000
        if self.name == 'session':
            environ['rnaseqlyse.upload_session'] = \
                   DBSession.query(UploadSession).get(int(self.value))
        elif self.name in ('name', 'type'):
            environ['rnaseqlyse.upload_' + self.name] = self.value
        else:
            return
        log.debug("FieldStoragx(%s -> %s)" % (self.name, self.value))

    def make_file(self, binary=None):

        assert self.filename
        log.debug("FieldStoragx.make_file(%s)" % self.filename)

        args = {}
        for kw in 'session', 'name', 'type':
            args[kw] = self.environ['rnaseqlyse.upload_' + kw]
        fd = service.get_uploadfile(DBSession, **args)
        # commit the (managed) session early here, so later
        # requests can re-use the Analysis object that the
        # first one has implicitly created by calling
        # service.get_uploadfile
        import transaction
        transaction.commit()

        return fd
```

Modul rnaseqlyze.web.views

```
"""
Pyramid Application User Views
"""

import logging
log = logging.getLogger(__name__)

import re
from os.path import join

from pyramid.view import view_config
from pyramid.response import FileResponse
from pyramid.httpexceptions import HTTPFound

import transaction
from sqlalchemy.exc import DBAPIError

import rnaseqlyze
from rnaseqlyze import galaxy
from rnaseqlyze.web import DBSession, DBSession_unmanaged
from rnaseqlyze.core import service
from rnaseqlyze.core.entities import Analysis, UCSCOrganism

autocomplete_re = re.compile(r"^[^(]+\([^/]+/([^)]+)\).*$")

@view_config(route_name='home', renderer='templates/home.pt')
def home(request):
    """
    **Home Page**

    This is the main entry point to the application. I.e. the landing page,
    the page that users see first.
    """
    return {}

@view_config(route_name='analyses', renderer='templates/create.pt')
def create(request):
    """
    **Create Page**

    This page is shown when the "New Analysis" button is clicked.
    """
    sess = service.get_upload_session(DBSession)
    return { 'upload_session': sess.id }

@view_config(route_name='analysis', renderer='templates/analysis.pt')
def display(request):
    """
    **Analysis Page**

    This page is displayed after the the anaysis has been created.
    When the user clicks "Submit" on the 'create' page, after
    the files are uploaded and the form information is submitted
    to the :func:`~post` view, the browser is redirected here.

    The page can also be viewed any time later on, no matter
    weather the analysis has already been completed or not.

    In case it is not yet completed, the page is constantly
    updated via XMLHTTPRequests to reflect the current status.
    """

    id = int(request.matchdict["id"])
    return {
        'analysis': DBSession.query(Analysis).get(id),
        'galaxy_history_url': galaxy.default_history_url,
    }

@view_config(route_name='analysis_files')
def analysis_files(request):
    """
    **Files View**

    This view serves up the files associated with
    an analysis on 'http://<rnaseqlyze>/analysis/{id}/files'.
    """
    return FileResponse(join(rnaseqlyze.analyses_path,
                request.matchdict['id'], *request.subpath))

import mimetypes
#mimetypes.add_type("text/plain", ".")
#mimetypes.add_type("text/plain", ".")
#mimetypes.add_type("text/plain", ".")
#mimetypes.add_type("text/plain", ".")
mimetypes.add_type("text/plain", ".gb")
mimetypes.add_type("text/plain", ".log")
mimetypes.add_type("text/plain", ".log0")
mimetypes.add_type("text/plain", ".info")
# FileResponse automatically sets the Content-Type header based on this

@view_config(route_name='analyses', request_method='POST')
def post(request):
    """
    **Create-Form Action**

    This view just redirects the client to the created analysis page.
    Before it is actually called, the files to be analyzed, are uploaded
    using the :func:`~.upload.upload` view callable.
    """
    # for documentation on the documentation reference syntax, see
    # http://sphinx.pocoo.org/domains.html#cross-referencing-python-objects

    # TODO: csrf security checks
    #       see "shootout" pyramid demo app

    # note:
    #  when using the "DBSession" (managed), the
    #  try:/except: rollback construct is not needed
    #  because the session is automatically rolled back
    #  otoh, if the _unmanaged session is used, it _has_ to
    #  be manually commited or rolled back if objects are modified

    if 'org_accession' in request.POST:
        request.POST['org_accession'] = \
                autocomplete_re.sub(r"\1", request.POST['org_accession'])
    try:
        analysis = service.get_analysis(
                    DBSession_unmanaged, attributes=request.POST)

        # the analysis must exist in the database
        # so the worker can find it and start working
        DBSession_unmanaged.commit()

        service.start_analysis(analysis)
        log.debug("started analysis #%d by '%s'" % (
                        analysis.id, analysis.owner.name))

        return HTTPFound(request.route_path('analysis', id=analysis.id))
    except:
        log.info("abort")
        DBSession_unmanaged.rollback()
        log.debug("rollback complete")
        raise
```

Modul `rnaseqlyze.web.wsgi`

```
"""
RNA-Seqlyze WSGI Apllication

Provides the get_app(workdir) function,
which returns a wsgi application callable.
"""

def get_app(workdir):
    """
    Basically returns wrapper around paster.get_app
    that strips the ".wsgi" extension from SCRIPT_NAME
    """
    # configure the core package
    import rnaseqlyze
    rnaseqlyze.configure(workdir)

    # default configuraion file name
    import os.path
    web_ini = os.path.join(workdir, 'web.ini')

    # configure logging
    import logging.config
    logging.config.fileConfig(web_ini, dict(here=workdir))

    # create the pyramid wsgi app
    import pyramid.paster
    pyramid_app = pyramid.paster.get_app(web_ini, 'main')

    # return a wrapper that adjusts SCRIPT_NAME
    def app(environ, start_request):
        environ['SCRIPT_NAME'] = \
                environ['SCRIPT_NAME'][:-5]
        return pyramid_app(environ, start_request)

    return app
```

3.2.5 Package `rnaseqlyze.worker`

Modul `rnaseqlyze.worker`

```
"""
**pyramid.worker** is a Pyramid Web Framework Application.

The framework is used here to keep things simple. Even thought not many of the
frameworks features are used, building this "-worker" part of the project as a
Pyramid Web Framework Application, just like the "-web" part, hopefullly makes
it easy to understand for anybody already understanding the "-web" part.

The key features from then Pyramid Web Framework used here, are

 1) The "pserve" command, which makes running the application as a unix daemon
    process very simple. It's direct use has actually been depreciated during
    the development and a custom command, "rnas-worker", has been created,
    which uses the same python functions and modules like "pserve".

 2) The pyramid.config.Configurator class, that is used to define the
    applications "routes" and "view callables". These "views" provide the
    applications interface.  They are served on a tcp port bound to localhost
    (127.0.0.1) and are therefore only available to processes running on the
    same host.

The "-worker" applications interface has "HTTP-like" semantics.

The following commands are accepted:

  - ``GET /analyses/{id}``:      Show the current status.
  - ``START /analyses/{id}``:    Start processing an analysis.

Only for development purposes, one additional command exists:

  - ``RESTART /analyses/{id}``:  Restart an analysis
                                 that has already been started.

The commands are executed by the "-web" part of the application by subclassing
HTTPRequest and coverriding the get_method() function. They can also be executed
from the command line however, using the popular "curl" binary with the "-X"
option, e.g.

  - ``curl -X GET localhost:/analyses/3``
  - ``curl -X START localhost:/analyses/3``
  - ``curl -X RESTART localhost:/analyses/3``

"""

import logging
log = logging.getLogger(__name__)

from pyramid.view import view_config
from pyramid.view import view_defaults
from pyramid.config import Configurator
from pyramid.response import Response
from pyramid.httpexceptions import (
        HTTPError,
        HTTPBadRequest,
        HTTPInternalServerError,
)

from sqlalchemy import create_engine
from sqlalchemy.orm import sessionmaker, scoped_session
from zope.sqlalchemy import ZopeTransactionExtension

import rnaseqlyze
project_name = rnaseqlyze.project_name + "-worker"

from rnaseqlyze.core.entities import Analysis
from rnaseqlyze.worker.core import (
        Manager,
        ManagerBusyException,
        AnalysisAlreadyStartedException,
)

DBSession = scoped_session(sessionmaker(extension=ZopeTransactionExtension()))

def main(global_config, **settings):
    """
    Return a Pyramid(!) WSGI application.
    """

    # make sure to be able to delete files created by webapp
```

```python
    # as user/group www-data/www-data from the command line
    # (as user/group johndoe/www-data)
    import os
    os.umask(0002)

    engine = create_engine(rnaseqlyze.db_url)
    DBSession.configure(bind=engine)

    Waitress.manager = Manager()

    config = Configurator(settings=settings)
    config.add_route('analyses', '/analyses/{id}')
    config.scan()
    return config.make_wsgi_app()


@view_defaults(route_name='analyses', renderer='string')
class Waitress(object):

    def __init__(self, request):
        id = int(request.matchdict['id'])
        self.analysis = DBSession.query(Analysis).get(id)

    @view_config(request_method='GET')
    def status(self):
        import pprint
        return pprint.pformat({
            'context': self, # Waitress
            'manager': self.manager, # Manager
            'analysis': self.analysis, # Analysis
        })

    @view_config(request_method='START')
    def start(self):
        self.manager.analysis_requested(self.analysis)
        return "started analysis #%d" % self.analysis.id

    @view_config(request_method='RESTART')
    def restart(self):
        self.manager.analysis_requested(self.analysis, True)
        return "restarted analysis #%d" % self.analysis.id


@view_config(context=Exception)
def error_view(error, request):
    errdict = {
        AnalysisAlreadyStartedException:    HTTPBadRequest,
        ManagerBusyException:               HTTPInternalServerError,
    }
    if isinstance(error, HTTPError):
        return error
    elif type(error) in errdict:
        return errdict[type(error)](error)
    else:
        return HTTPInternalServerError(error)


# monkey-patch some HTTPException classes to get simpler error messages

from pyramid.response import Response
def _WHE_init(self, arg=None):
    Exception.__init__(self, arg)
    if isinstance(arg, Exception):
        if False: # production
            e, t = arg, type(arg)
            arg = "%s %s" % (t.__name__, e.args)
        else: # debug
            import traceback
            arg = traceback.format_exc(999)
    Response.__init__(self,
        '%s %s\n\n%s' % (self.code, self.title, arg),
        content_type='text/plain', status='%s %s' % (self.code, self.title))

from pyramid.httpexceptions import WSGIHTTPException
WSGIHTTPException.__init__ = _WHE_init
del WSGIHTTPException.__call__
del WSGIHTTPException.prepare
```

Modul `rnaseqlyze.worker.core`

```
"""
RNA-Seqlyze Worker Daemon Core

Worker parent class with basic infrastructure to run the
various analysis steps defined in :class:`~.WorkerStages`.
"""

import logging
log = logging.getLogger(__name__)
root_logger = logging.getLogger()

from threading import Thread
from logging import Formatter
from logging import StreamHandler
from StringIO import StringIO
from contextlib import contextmanager

from sqlalchemy import create_engine
from sqlalchemy.orm import sessionmaker

import rnaseqlyze
from rnaseqlyze import efetch
from rnaseqlyze.core.entities import Analysis, StageLog
from rnaseqlyze.worker.stages import WorkerStages

DBSession = sessionmaker()
```

```python
log_format = "%(levelname)-5.5s [%(name)s] %(message)s"

class Manager(object):
    def __init__(self):
        self.worker = Thread()

    def analysis_requested(self, analysis, re=False):
        if analysis.started and not re:
            raise AnalysisAlreadyStartedException
        if self.worker.is_alive():
            raise ManagerBusyException
        self.worker = Worker(analysis)
        self.worker.start()

class StageLogStream(object):
    def __init__(self, analysis, stage, session):
        self.stage_log = StageLog(analysis=analysis, stage=stage, text="")
        self.session = session
        session.add(self.stage_log)
        session.commit()
    def write(self, data):
        self.stage_log.text += data
        self.session.commit()

class AnalysisAlreadyStartedException(Exception):
    pass

class ManagerBusyException(Exception):
    pass

class Worker(Thread, WorkerStages):
    """
    The Worker
    """

    def __init__(self, analysis):
        Thread.__init__(self)
        self.analysis_id = analysis.id

    def _thread_init(self):
        from os import path
        self.session = DBSession(bind=create_engine(rnaseqlyze.db_url))
        self.analysis = self.session.query(Analysis).get(self.analysis_id)

        self.logfile = open(path.join(
                self.analysis.data_dir, "rna-seqlyze-worker.log"), "w")
        self.log_handler = StreamHandler(self.logfile)
        self.log_handler.setFormatter(Formatter(log_format))
        root_logger.addHandler(self.log_handler)
        log.info("starting work on analysis #%d" % self.analysis_id)
        self.analysis.finished = False
        self.analysis.started = True
        self.analysis.error = None
        self.session.commit()
    @contextmanager
    def _stage_log_manager(self, stage):
        handler = StreamHandler(
                    StageLogStream(self.analysis, stage, self.session))
        root_logger.addHandler(handler)
        try:
            yield
        finally:
            root_logger.removeHandler(handler)

    def run(self):

        # TODO: invent a way to avoid calling stages that won't do anything
        #       maybe @stages -> @stages(condition) something ...

        self._thread_init()
        try:
            for stage in self.stages:
                if not stage.should_run(self):
                    continue
                log.info("=== %s ===" % stage.func_name)
                with self._stage_log_manager(stage.func_name):
                    self.analysis.stage = stage.func_name
                    self.session.commit()
                    stage(self)
        except Exception, e:
            self.analysis.error = repr(e)
            raise
        finally:
            if self.analysis.error:
                log.error(self.analysis.error)
            else:
                log.info("analysis finished")

            self.analysis.finished = True
            self.session.commit()
            root_logger.removeHandler(self.log_handler)
            self.logfile.close()
```

Modul rnaseqlyze.worker.daemon

```
"""
RNA-Seqlyze Worker

Start, stop or restart the worker daemon
or run it in the foreground, in development mode.

Usage:
    rnas-worker <workdir> (start|stop|restart)
    rnas-worker <workdir> --development
    rnas-worker -h|--help

Arguments:
    <workdir> The path to the workers 'workdir'.
              The 'workdir' is where the configuration, the
              application database and all analysis data are stored.

    start|stop|restart

              If one of those arguments is given, the daemon is
              run in the background. It will write it's PID to the
              file <workdir>/worker-daemon.pid and its output will be logged
              to <workdir>/worker-daemon.log. This is not the "log file"
              however. The "log file" path can be configured
              in <workdir>/worker.ini.

    --development

              If this argument is present, the worker daemon is run in
              development mode, which means that it will no fork to the
              background. If any source files (.py) are changed when the
              daemon is running in development mode, it will be
              automatically restarted.

"""

from os.path import abspath, join

from paste.script import serve
import docopt

import rnaseqlyze

def main():
    opts = docopt.docopt(__doc__)

    for command in "start|stop|restart".split('|'):
        if opts[command]:
            mode = "production"
            args = [command, "--daemon"]
            break
    else:
        mode = "development"
        args = ["--reload"]

    workdir = abspath(opts['<workdir>'])
    rnaseqlyze.configure(workdir)

    if mode == 'production':
        args.extend([
            "--user=" + rnaseqlyze.worker_user,
            "--group=" + rnaseqlyze.group,
            "--log-file=" + join(workdir, 'worker-daemon.log'),
            "--pid-file=" + join(workdir, 'worker-daemon.pid'),
        ])

    conf_file = join(workdir, 'worker.ini')
    serve.ServeCommand("serve").run([conf_file] + args)
```

Modul rnaseqlyze.worker.stages

```
"""
RNA-Seqlyze Worker Stages

    -- **this** is where things are actually getting done! :-)
"""
import logging
log = logging.getLogger(__name__)

import os
from os.path import join, exists, isdir, relpath
from subprocess import Popen, PIPE
from StringIO import StringIO
from threading import Thread
from urllib import quote

import pysam

from Bio import SeqIO
from Bio.SeqFeature import \
        SeqFeature, FeatureLocation, ExactPosition

from psutil import cpu_percent

from rnaseqlyze import efetch
from rnaseqlyze import galaxy
from rnaseqlyze import ucscbrowser
from rnaseqlyze import transterm, gb2ptt
from rnaseqlyze.ucscbrowser import BAMTrack, BigWigTrack, BigBedTrack
from rnaseqlyze.core.entities import GalaxyDataset


class Operon(object):
    def __init__(self, **kwargs):
        self.__dict__.update(kwargs)

_stages = []
_stage_conds = {}
def stage(method):
    """
    Just a small helper to collect the stages in the order defined.

    To add a new stage, simply add a method to :class:`~WorkerStages`.
    It will be automatically executed for all new analyses.
    """
    if method.func_name in _stage_conds:
        method.should_run = _stage_conds[method.func_name]
        del _stage_conds[method.func_name]
    else:
        method.should_run = lambda self: True
    _stages.append(method)
    return method

def stage_cond(method):
    """
    Stage Condition

     - must be declared before @stage
     - must return true for the @stage of the same name to run
    """
    _stage_conds[method.func_name] = method

class WorkerStages(object):
    """
    Available attributes:

        - self.analysis
        - self.session = DBSession(bind=create_engine(rnaseqlyze.db_url))

    .. note::
        After changing one of the attributes of the self.analysis object,
        **allways** **immediately** call self.session.commit(). Otherwise
        the database will stay locked and the web frontend can't update the ui.

    """

    ############################################################################
    # Utility Methods & Properties


    def log_cmd(self, *cmd):
        # can't wait() on subprocess with a timeout, alas start up
        # a 2nd thread to do it and join() on that one with a timeout
        log.info("forking subprocess: $ %s" % ' '.join(map(repr, cmd)))
        proc = Popen(cmd, stdout=self.logfile, stderr=self.logfile)
        waiter = Thread(target=proc.wait)
        cpu_percent(0, True)
        waiter.start()
        waiter.join(15)
        while waiter.is_alive():
            log.info("subprocess still running - system load: " +
                     " / ".join(("%d%%" % p for p in cpu_percent(0, True))))
            waiter.join(15)
        if proc.returncode != 0:
            raise Exception("%s failed" % (cmd,))

    @property
    def srr_name(self):
        return self.analysis.inputfile_base_name

    @property
    def bam_name(self):
        return "%s %s Mapping" % (self.genbank_record.id, self.srr_name)
    @property
    def coverage_name(self):
        return "%s %s Coverage" % (self.genbank_record.id, self.srr_name)

    @property
    def hp_terms_name(self):
        return "%s Hairpin Terminators" % (self.genbank_record.id,)

    @property
    def pr_operons_name(self):
        return "%s Predicted Operons" % (self.genbank_record.id,)

    #
    ################################################################################
    # Stages

    @stage_cond
    def determine_inputfile_type(self):
        return self.analysis.inputfile_uploaded
    @stage
    def determine_inputfile_type(self):
        _8bytes = open(self.analysis.inputfile_path).read(8)
        log.info("first 8 bytes of input data: %r" % _8bytes)
        self.analysis.inputfile_type = (
                      'fastq' if _8bytes[0] == '@'
                 else 'sra'   if _8bytes    == 'NCBI.sra' else None)
        self.session.commit()
        if not self.analysis.inputfile_type:
            raise Exception("Unknown input data type")

    @stage_cond
    def fetch_srr(self):
        # don't download if private
        # file uploaded or srr already in cache
        return not self.analysis.inputfile_uploaded \
               and not os.path.exists(self.analysis.rnaseq_run.sra_path)
    @stage
    def fetch_srr(self):
        self.analysis.rnaseq_run.download()

    @stage_cond
    def convert_input_file(self):
        return not exists(self.analysis.inputfile_fq_path)
    @stage
    def convert_input_file(self):
        os.chdir(self.analysis.input_data_dir)
        self.log_cmd("fastq-dump", "-B", self.analysis.inputfile_name)
        log.debug("created %s" % self.analysis.inputfile_fq_path)

    @stage_cond
    def fetch_genbank_file(self):
        return not exists(self.analysis.genbankfile_path)
    @stage
    def fetch_genbank_file(self):
        if not exists(self.analysis.genbank_data_dir):
            os.makedirs(self.analysis.genbank_data_dir)
        log.info("Fetching '%s' from entrez..." %
                                    self.analysis.org_accession)
        gb_id = efetch.get_nc_id(self.analysis.org_accession)
        efetch.fetch_nc_gb(gb_id, open(self.analysis.genbankfile_path, "w"))
        log.info("...done")

    @stage
    def read_genbank_file(self):
        self.genbank_record = SeqIO.parse(open(self.analysis \
                                .genbankfile_path), "genbank").next()
        ngenes = sum(1 for f in self.genbank_record.features
                        if f.type == 'gene')
        log.info("genbank file lists %d genes" % ngenes)

    @stage_cond
    def genbank_to_fasta(self):
        return not exists(self.analysis.genbankfile_fa_path)
    @stage
    def genbank_to_fasta(self):
        log.info("Converting '%s' to fasta format" %
                                          self.analysis.genbankfile_name)
        record = self.genbank_record
        saved_id = record.id
        record.id = "chr" # make ucsc browser custom tracks work
        SeqIO.write(record, open(
            self.analysis.genbankfile_fa_path, "w"), "fasta")
        record.id = saved_id

    @stage_cond
    def bowtie_build(self):
        return not exists(join(self.analysis.genbank_data_dir,
                               self.analysis.genbankfile_base_name + ".1.bt2"))
    @stage
    def bowtie_build(self):
        os.chdir(self.analysis.genbank_data_dir)
        self.log_cmd("bowtie2-build", self.analysis.genbankfile_fa_name,
                                      self.analysis.genbankfile_base_name)

    @stage_cond
    def tophat(self):
        return not exists(join(self.analysis.data_dir,
                               "tophat-output", "accepted_hits.bam"))
    @stage
    def tophat(self):
        os.chdir(self.analysis.data_dir)
        n_cpus = os.sysconf("SC_NPROCESSORS_ONLN")
        fq = relpath(self.analysis.inputfile_fq_path)
        gb = relpath(join(self.analysis.genbank_data_dir,
                          self.analysis.genbankfile_base_name))
        self.log_cmd("tophat",
                        "-p", str(n_cpus),
                        "-o", "tophat-output",
                        "--segment-length", "999999999",
                        "--no-coverage-search", "--no-novel-juncs", gb, fq)

    @stage_cond
    def create_coverage_track(self):
        return not exists(join(self.analysis.data_dir, "coverage.bigwig"))
    @stage
    def create_coverage_track(self):
        os.chdir(self.analysis.data_dir)
        # the script automatically converts it's
        # output to bigwig if it finds kent's wigToBigWig
        self.log_cmd("bam_to_wiggle.py", "-o", "coverage.bigwig",
                            "tophat-output/accepted_hits.bam")

    @stage_cond
    def genbank_to_ptt(self):
        return not exists(join(self.analysis.genbank_data_dir,
                            self.genbank_record.id + ".ptt"))
    @stage
    def genbank_to_ptt(self):
        ptt_name = self.genbank_record.id + ".ptt"
        ptt_path = join(self.analysis.genbank_data_dir, ptt_name)
        os.symlink(ptt_name, join(self.analysis.genbank_data_dir, "chr.ptt"))
        log.debug("converting %s to ptt" % self.analysis.genbankfile_name)
        ptt_file = open(ptt_path, "w")
        gb_file = open(self.analysis.genbankfile_path)
        gb2ptt.gb2ptt(gb_file, ptt_file)
        ptt_file.close()
        gb_file.close()

    @stage_cond
    def transterm_hp(self):
        return not exists(join(self.analysis.data_dir,
                            "hp_terminators.bigbed"))
    @stage
    def transterm_hp(self):
        os.chdir(self.analysis.data_dir)
        log.debug("running transterm")

        tt_out = open("transterm_hp.out", "w+")
        # --min-conf=n n is the cut-off confidence value,
        #               between 0 and 100, the default is 76
        tt_args = ("--min-conf=47",
                   self.analysis.genbankfile_fa_path,
                   relpath(join(self.analysis.genbank_data_dir, "chr.ptt")))
        transterm.run(tt_args, out=tt_out, err=self.logfile)
        tt_out.seek(0)
        # keep a copy in memory
        self.hp_terminators = list(transterm.iterator(tt_out))
        tt_out.seek(0)
        log.info("found {0} possible hairpin terminators"
                          .format(len(self.hp_terminators)))
        # create a bed track
        bed_file = open("hp_terminators.bed", "w")
        transterm.tt2bed(tt_out, bed_file)
        bed_file.close()
        tt_out.close()

        log.debug("running bedToBigBed")

        # convert it to bigBed
        chrs = open("chrom.sizes", "w")
        chrs.write("chr %d" % len(self.genbank_record.seq))
        chrs.close()
        self.log_cmd("bedToBigBed", "hp_terminators.bed",
                     "chrom.sizes", "hp_terminators.bigbed")

    @stage
    def predict_operons(self):

        # extract the coverage data from the bam track created by tophat
        bam_path = join(join(self.analysis.data_dir,
                        "tophat-output", "accepted_hits.bam"))
        if not exists(bam_path + ".bai"):
            pysam.index(bam_file)

        self.max = 0
        self.covered = 0
        self.coverage = [0] * len(self.genbank_record.seq)

        sam_reader = pysam.Samfile(bam_path, "rb")
        chrom, length = sam_reader.references[0], sam_reader.lengths[0]

        assert chrom == "chr" and length == len(self.genbank_record.seq), (
                "Something went badly wrong"
                " -- the bam track or genbank file cold be corrupted...")

        for base in sam_reader.pileup(chrom, 0, length):
            self.covered += 1
            if base.n > self.max:
                self.max = base.n
            self.coverage[base.pos] = base.n

        if not self.covered:
            raise Exception("Not a valid bam file")

        log.debug("maximum coverage: %d" % self.max)
        log.debug("number of bases covered by short reads: %d/%d" % (
                                    self.covered, len(self.genbank_record.seq)))

        # available objects at this point
        # --------------------------------
        #
        # - self.genbank_record: Biopython SeqIO.parse()d genbank file
        #
        # - self.coverage: [n,n,n,n,...] / len = len(self.genbank_record.seq)
        #
        # - self.max: max(n)
        #
        # - self.hpterminators: ((id, begin, end, strand, confidence), ...)
        #                         str, str, str, str (1/-), int
        #

        # FIXME: this is just a dummy implementation, that predicts an
        #        operon for every transcribed (i.e. coverage > 1) region

        self.operons = []

        operon = None
        for i, n in enumerate(self.coverage):
            if n > 0:
                if not operon:
                    operon = Operon(begin=i + 1, strand=1, confidence=1)
            else:
                if operon:
                    operon.end = i
                    self.operons.append(operon)
                    operon = None

        # create a bed track
        track_name = "rna-seqlyze-operon_predictions"
        os.chdir(self.analysis.data_dir)
        bed_file = open(track_name + ".bed", "w")
        for i, o in enumerate(self.operons):
            begin, end = str(o.begin), str(o.end)
            rgb_color = ','.join((str(100 - int(o.confidence)),)*3)
            print >> bed_file, '\t'.join((
                'chr', begin, end,
                'OPERON_%d' % i, str(o.confidence),
                '+' if o.strand > 0 else '-', begin, end, rgb_color
            ))
        bed_file.close()

        # convert it to bigBed
        # chrom_sizes already generated during "transterm_hp"
        self.log_cmd("bedToBigBed", track_name + ".bed",
                     "chrom.sizes", track_name + ".bigbed")

    @stage
    def upload_track_data(self):

        # FIXME: names are not unique on galaxy:
        # is "%s_%s" % (srr_name, self.analysis.org_accession) good enough ?

        if not self.analysis.galaxy_bam:
            bam_path = join(self.analysis.data_dir,
                            "tophat-output", "accepted_hits.bam")
            log.info("uploading accepted_hits.bam to galaxy")
            self.analysis.galaxy_bam = GalaxyDataset(
                id=galaxy.upload(open(bam_path), self.bam_name))
            log.info("...done - id: %s" % self.analysis.galaxy_bam.id)
            self.session.commit()
        if not self.analysis.galaxy_coverage:
            coverage_path = join(self.analysis.data_dir, "coverage.bigwig")
            log.info("uploading coverage.bigwig to galaxy")
            self.analysis.galaxy_coverage = GalaxyDataset(
                id=galaxy.upload(open(coverage_path), self.coverage_name))
            log.info("...done - id: %s" % self.analysis.galaxy_coverage.id)
            self.session.commit()

        if not self.analysis.galaxy_hp_terms:
            hp_terms_path = join(self.analysis.data_dir,
                                 "hp_terminators.bigbed")
            log.info("uploading hp_terminators.bigbed to galaxy")
            self.analysis.galaxy_hp_terms = GalaxyDataset(
                id=galaxy.upload(open(hp_terms_path), self.hp_terms_name))
            log.info("...done - id: %s" % self.analysis.galaxy_hp_terms.id)
            self.session.commit()

        if not self.analysis.galaxy_pr_operons:
            track_filename = "rna-seqlyze-operon_predictions.bigbed"
            pr_operons_path = join(self.analysis.data_dir, track_filename)
            log.info("uploading %s to galaxy" % track_filename)
            self.analysis.galaxy_pr_operons = GalaxyDataset(
                id=galaxy.upload(open(pr_operons_path), self.pr_operons_name))
            log.info("...done - id: %s" %
                                    self.analysis.galaxy_pr_operons.id)
            self.session.commit()

    @stage
    def create_and_upload_hg_text(self):
        """
        ``hgt.customText`` is a paremeter of the UCSC
        "hgTracks" genome browser application that makes it
        possible to share "cutom tracks" via a url.

        The value of the ``hgt.customText`` parameter is itself
        an URL. The shareable "custom tracks url" is therefore an
        URL that containes another URL. The other url must be "escaped"
        for this to work. That actually happens in
        :meth:`~rnaseqlyze.core.analysis.AnalysisMixins.hg_url`.

        The details are explained here:
        http://genome.ucsc.edu/goldenPath/help/customTrack.html#SHARE
        """

        if self.analysis.galaxy_hg_text:
            return

        tracks = []

        # FIXME: this cries for refactoring -- with logging!

        # bam track (mapping)
        bam_url = "https://" + galaxy.hostname \
                    + galaxy.ucsc_bam_path_template \
                    .format(dataset=self.analysis.galaxy_bam.id)
        tracks.append(BAMTrack(url=bam_url,
                               name="RNA-Seqlyze | %s" % self.bam_name))

        # bigwig track (coverage)
        coverage_url = "https://" + galaxy.hostname \
                        + galaxy.dataset_display_url_template \
                            .format(dataset=self.analysis.galaxy_coverage.id)
        tracks.append(BigWigTrack(url=coverage_url,
                                  name="RNA-Seqlyze | %s" % self.coverage_name))

        # bigbed track (terminators)
        hp_terms_url = "https://" + galaxy.hostname \
                        + galaxy.dataset_display_url_template \
                            .format(dataset=self.analysis.galaxy_hp_terms.id)
        tracks.append(BigBedTrack(url=hp_terms_url,
                                  name="RNA-Seqlyze | %s" % self.hp_terms_name))

        # bigbed track (predicted operons)
        pr_operons_url = "https://" + galaxy.hostname \
                        + galaxy.dataset_display_url_template \
                            .format(dataset=self.analysis.galaxy_pr_operons.id)
        tracks.append(BigBedTrack(url=pr_operons_url,
                                  name="RNA-Seqlyze | %s" %
                                                    self.pr_operons_name))

        track_file = StringIO()
        track_file.write('\n'.join(tracks))
        track_file.seek(0)
        self.analysis.galaxy_hg_text = GalaxyDataset(
                    id=galaxy.upload(track_file,
                                    "UCSC Tracks Analysis%d.txt" %
                                                        self.analysis.id))
        self.session.commit()

    @stage
    def create_genbank_file(self):
        """
        Greate a genbank file containing

        For more documentation on how to create new features, visit

         - http://biopython.org/\\
                DIST/docs/api/Bio.SeqRecord.SeqRecord-class.html#__getitem__
         - http://biopython.org/\\
                DIST/docs/api/Bio.SeqFeature.SeqFeature-class.html

         - http://www.ebi.ac.uk/\\
                embl/Documentation/FT_definitions/feature_table.html
        """

        log.info("augmenting genbank file %s with putative operons" %
                                    self.analysis.genbankfile_name)
        for i, o in enumerate(self.operons):
            location = FeatureLocation(ExactPosition(o.begin),
                                       ExactPosition(o.end))
            self.genbank_record.features.append(
                SeqFeature(location,
                    type='mRNA',
                    strand=o.strand,
                    qualifiers=dict(
                        note='putative, confidence %d%%' % o.confidence,
                        operon='rnas-%d' % i)))

        self.genbank_record.features.sort(
                key=lambda f: f.location.start.position)

        xgb_file = open(self.analysis.xgenbankfile_path, "w")

        SeqIO.write(self.genbank_record, xgb_file, "genbank")
    #
    ###########################################################################

assert not _stage_conds, "@stage_cond's must be declared before @stage's"
WorkerStages.stages = _stages
del _stages, _stage_conds
```

3.2.6 Javascript Code

rnaseqlyze.js

```
/*
 * RNA-seqlyze javascript routines
 */

$(function() {

    // global variables and helpers
    // ----------------------------

    // ___ el ___
    //  from http://joestelmach.github.com/laconic/
    window.el = $.el;

    // log.info() and log.debug()
    window.log = {
        'info': function() {
            console.log.apply(console, arguments);
        },
        'debug': function() {}
    }
    if (rnaseqlyze_debug)
        window.log.debug = window.log.info;

    // page initializaion
    // ------------------
    // use bootstrap's
    // "scrollspy" plugin
    //  -- patched version - see https://github.com/twitter/bootstrap/pull/3829
    $(window).scrollspy({
//            offset: 200,
            wrap: $('#wrap')[0],
    });


    // Generally useful stuff
    // ----------------------

    // based on http://stackoverflow.com/a/4673436

    String.prototype.format = function() {
        var i = 0; args = arguments;
        return this.replace(/{}/g, function() {
            return args[i++];
        });
    };

});

// http://stackoverflow.com/a/7531350
jQuery.fn.extend({
    scrollToBottom: function () {
        var top = $(this).offset().top;
        var offtop = top - 250 + $(this).height();
        jQuery('html,body').animate({scrollTop: offtop}, 100);
    },
});
```

rnaseqlyze-create.js

```
/*
 * RNA-seqlyze javascript routines
 *
 *     for the "create" page
 */

$(document).ready(function() {

    /*
     * Toggle input type
     */

    $('#input_type_radio').click(function(event) {
        if ($(event.target).hasClass("srr")) {
            // user chose the "Data File" option
            $('#sra-controls').hide();
            $('#srr-controls').show();
        } else if ($(event.target).hasClass("sra")) {
            // user chose the "SRR Identifier" option
            $('#srr-controls').hide();
            $('#sra-controls').show();
        } // else
            // what the...
    });
    $('#input_type_radio .srr').click();

    /*
     * discretionary #pairendlenControls
     */

    function maybe_show_pairendlen_controls() {
        if ($('#pairendedInput').attr('checked'))
            $('#pairendlenControls').show();
        else
            $('#pairendlenControls').hide();
    }

    maybe_show_pairendlen_controls();
    $('#pairendedInput').change(maybe_show_pairendlen_controls);


    /*
     * Toggle organism input type
     */

    $('#org_type_radio').click(function(event) {
        if ($(event.target).hasClass("title")) {
            // user chose "Title"
            $('#genbankfile-controls').hide();
            $('#org_title-controls').show();
        } else if ($(event.target).hasClass("file")) {
            // user chose "Genbank File"
            $('#org_title-controls').hide();
            $('#genbankfile-controls').show();
        } // else
            // what the ...
    });
    $('#org_type_radio .title').click();

    /*
     * Organism input autocompletion
     */

    var organisms = new Array();

    $.ajax({
        url: "rest/organisms",
        dataType: "json",
        success: function(data) {
            // No idea yet what to do with those that have multiple
            // accessions listed in 'genome' -- filter them out here for now
            //
            // see ftp://ftp.ncbi.nih.gov/genomes/GENOME_REPORTS/prokaryotes.txt
            //
            _(data).each(function(org) {
                if (org.acc.indexOf(",") >= 0)
                    return;
                organisms.push("{} ({}/{})".format(
                                org.title, org.db, org.acc)); }); },
    });

    $('#organismInput').typeahead({
        source: organisms,
    });

    /*
     * plupload -- from src/plupload/examples/custom.html
     */

    var uploads = {
        'inputfile': {},
        'genbankfile': {}
    };

    var context = function(name) {

        var self = this;

        var options = {
            url:      'upload',
            runtimes:      'html5,gears,flash,silverlight,browserplus,html4',
            browse_button:    name + '_browse',
            drop_element:       name + '_progress',
            multipart_params:     { 'type': name,
                                    'session': upload_session },
            flash_swf_url:          path_js + '/plupload.flash.swf',
            silverlight_xap_url:      path_js + '/plupload.silverlight.xap',
        };

        var events = {
            // 'Init': function(up, params) {
            //     $('#' + name + '_progress .filestatus').text(
            //                         "Current runtime: " + params.runtime);
            // },
            'FilesAdded': function(up, up_files) {
                // remove all other files already present
                // plupload features multiple files in one widget
                // we have two widgets and one name per widget
                up.splice();
                self.active = true;
                log.debug("FilesAdded", up.files, up_files);
                $('#' + name + '_progress .filestatus').text(
                    up_files[0].name +
                        ' (' + plupload.formatSize(up_files[0].size) + ')');
            },
            'UploadComplete': function(up, up_files) {
                self.complete = true;
                for (nam in uploads)
                    if (uploads[nam].active)
                        if(!uploads[nam].complete)
                            return;
                log.debug("UploadComplete", "go");
                $('#create_form').submit();
            },
            'UploadProgress': function(up, up_file) {
                $('#' + name + '_progress .bar').css(
                            "width", up_file.percent + '%');
                // $('#' + name + '_progress .filestatus').text(
                //                                 up_file.percent + '%');
            },
        };

        this.active = false;
        this.complete = false;

        var up = this.up = new plupload.Uploader(options);

        $('#' + name + '_progress').click(function() {
            $('#' + name + '_browse').click();
        });

        $('#create_form_submit').click(function() {
            for (nam in uploads)
                if (uploads[nam].active)
                    { up.start(); return false; }
        });

        for (x in events)
            up.bind(x, events[x]);

        log.debug("debug");

        up.init();
    };

    for (name in uploads)
        uploads[name] = new context(name);
});

// vim: et:sw=4
```

rnaseqlyze-analysis.js

```
/*
 * RNA-seqlyze 'analysis' view javascript
 */

// backbone.js Models
// ------------------
//
```

```
// -> http://backbonejs.org/#Model

// The Analysis
window.Analysis = Backbone.Model.extend({
    urlRoot: "../rest/analyses",
    initialize: function () {
        this.files = new DataDirListing();
        this.files.analysis = this;

        this.stage_logs = new StageLogList();
        this.stage_logs.analysis = this;

        // "cascade": update files list
        this.bind("change:data_dir_state", function (self) {
            self.files.fetch({add: true});
        });
        // "cascade": update stage_logs
        this.bind("change:stage_logs_state", function (self) {
            var len = self.stage_logs.size();
            self.stage_logs.fetch({
                add: true,
                // The last stage_log is the current one and updates frequently.
                // It's id stays the same though and which causes backbone.js
                // to regard it as a duplicate and drop it. But a copy of the
                // ajax response is passed to the success callback. So we pick
                // the changed log text from there and fire a "change" event
                // by set()ting the 'text' attribute of the affected model.
                success: function (stage_logs, rsp) {
                    if (len)
                        stage_logs.at(len-1).set('text', rsp[len-1].text);
                },
            });
        });
    }
});
// log output of one stage
window.StageLog = Backbone.Model.extend({
    defaults: {
        stage: null,
        text: null,
    },
    idAttribute: "id",
});
// log output of all stages
window.StageLogList = Backbone.Collection.extend({
    model: StageLog,
    url: function () {
        return this.analysis.url() + "/logs";
    },
});
// a model for the files
window.DataDirFile = Backbone.Model.extend({
    defaults: {
        path: null,
    },
    idAttribute: "path",
});
// and for a collection of files
window.DataDirListing = Backbone.Collection.extend({
    model: DataDirFile,
    url: function () {
        return this.analysis.url() + "/files";
    },
});

// note1:
//
//  Concerning the above code:
//  It might have been simpler to work the files list right into the
//  analysis model on the server and stick to one model here.
//  But then again, there is no harm in doing it like this, because
//  now the files list is more independent and could for
//  example also be displayed on a page of its own.


/* note2:
 *
 *  In the code below,
 *
 *  "el"           is defined in rnaseqlyze.js as "$.el", which is defined
 *                 in laconic.js - see  http://joestelmach.github.com/laconic/
 *
 *  "this.$el"
 *  "this.el"      are the view's (jQuery wrapped) DOM element in the
 *                 backbone.js architecture - see http://backbonejs.org/#View-el
 *
 *  "render().el"  is also the view's "el" and works because we always
 *                 "return this;" from render() - see http://backbonejs.org/#View-render
 */

// Two Views showing different details about the analysis
// -------------------------------------------------------

// These render the "Processing" and "Results" section on the
// analysis page. The Processing view is displayed above the Results view.
//
// -> http://backbonejs.org/#View

// The "Processing" section
window.ProcessingView = Backbone.View.extend({

    initialize: function () {
        this.model.bind("change", this.change, this);
        this.stage_logs = (
            new StageLogListView({model: this.model.stage_logs}).render().el);
    },
    change: function (model, value, options) {

        // just re-render the whole thing for now
        this.$el.empty();
        this.render();

        // remove the busy indicator when finished
        if (model.get('finished'))
            $('#spinner').remove();

        // make scrollspy refresh it's coordinates
        // because the page size has likely changed
        $(window).scrollspy('refresh');
    },
    render: function () {
        // toJSON doesn't really do much besides turning
        // the model.attributes into a useable object
        // see http://backbonejs.org/#Model-toJSON
        var analysis = this.model.toJSON();

        this.$el.append(
            el.h2("Processing")
        );

        this.$el.append(el.div(
            el.h3("Input Check")
        ,
            analysis.inputfile_uploaded ?
                el.p("Type of input: ",
                     analysis.inputfile_type ?
                        el.strong(analysis.inputfile_type) :
                        el.span("not detected"))
                :
                null
        ,
            analysis.inputfile_header ?
                el.p("First read in input data: ",
                     el.pre(analysis.inputfile_header))
                :
                null

        ));

        this.$el.append(el.div(
            el.h3("Stage Logs")
        ,
            this.stage_logs
        ));

        if (analysis.error)
            this.$el.append(
                el.div({class: "alert alert-error"},
                       el.h4({class: "alert-heading"},
                             "An error occured while analyzing the data"),
                       analysis.error));
        return this;
    },
});

// The monospaced stage log blocks
window.StageLogListView = Backbone.View.extend({
    initialize: function () {
        this.model.bind("add", this.add, this);
        if (!this.model.analysis.get('finished')) {
            this.model.analysis.bind("change:finished",
                                     this.analysis_change, this);
        }
    },
    analysis_change: function (model, value, options) {
        if (value) {
            this.$el.contents().find("pre")
                    .last().css('background-color', '');
        }
    },
    add: function (model) {
        this.$el.append(
            new StageLogView({model: model}).render().el
        );
        if (!this.model.analysis.get('finished')) {
            this.$el.contents().find("pre")
                .not(':last').css('background-color', '')
                .prevObject.last().css('background-color', '#ddf');
            this.$el.contents().last().scrollToBottom();
        }
        $(window).scrollspy('refresh');
    },
});

// _One_ monospaced stage log block
window.StageLogView = Backbone.View.extend({
    initialize: function () {
        this.model.bind("change", this.change, this);
    },
    change: function (model, options) {
        this.$el.children("pre").text(model.get('text'));
        this.$el.scrollToBottom();
        $(window).scrollspy('refresh');
    },
    render: function () {
        var log = this.model.toJSON();
        this.$el.attr('id', log.stage);
        this.$el.append(el.h4(log.stage));
        this.$el.append(el.pre(log.text));
        return this;
    },
});

// The "Results" section
window.ResultsView = Backbone.View.extend({
    initialize: function () {
        this.model.bind("change", this.change, this);
        this.model.files.bind("add", this.fileadd, this);
    },
    change: function (model, value, options) {
        this.$el.empty();
        this.render();
        $(window).scrollspy('refresh');
    },
    fileadd: function () {
        var augmented_gb = this.model.files.find(function (file) {
            return file.get('path').match(/augmented\.gb$/);
        });
        if (!this.augmented_gb) {
            this.augmented_gb = augmented_gb;
            this.$el.empty();
            this.render();
        }
    },
    render: function () {
        var analysis = this.model.toJSON();
        if (analysis.hg_url || this.augmented_gb) {
            this.$el.append(el.h2("Results"));
            var $ul = $(el.ul())
            this.$el.append($ul[0]);
            if (this.augmented_gb) {
                var href = _id + '/files/' + this.augmented_gb.get('path');
                $ul.append(el.li(
                    el.a({href: href},
                         "Augmented Genbank File")));
            }
            if (analysis.hg_url) {
                $ul.append(el.li(
                    el.a({href: analysis.hg_url},
                         "Link to custom tracks in UCSC browser"),
                    el.p("It might take a minute until the tracks become " +
                         "available.", el.br(),
                         "As soon as the last few items ",
                         el.a({href: galaxy_history_url}, "here"),
                         " turn green it should work.")));
            }
        }
        return this;
    },
});

// A View displaying the list of files associated with
// this analysis available on the server (log files, mostly).
// This is currently rendered inside the "Processing" section above.
window.DataDirView = Backbone.View.extend({
    initialize: function () {
        this.model.bind("reset", this.reset, this);
        this.model.bind("add", this.add, this);
    },
    reset: function (model, value, options) {
        this.$el.empty();
        this.render();
    },
    render: function () {
        this.$el.append(el.h2("Data Directory"));
        var ul = el.ul();
        this.$ul = $(ul);
        this.$el.append(ul);
        $(window).scrollspy('refresh');
        return this;
    },
    add: function(model) {
        this.$ul.append(
            new DataDirFileView({model: model}).render().el
        );
        $(window).scrollspy('refresh');
    },
});
// An View, that renders one file
window.DataDirFileView = Backbone.View.extend({
    el: "<li>",
    render: function (model) {
        var file = this.model.toJSON();
        var href = _id + '/files/' + file.path;
        this.$el.html(el.a({href: href}, file.path));
        return this;
    }
});


// Initialization
// --------------

$(document).ready(function () {

    // the id of the displayed analysis
    _id = _(window.location.pathname.split('/')).last();

    // create a backbone.js Model
    // with an associated Collection
    analysis = new Analysis({
        id: _id,
    });

    // create two backbone.js views for the
    // analysis, render and insert them into the DOM
    $('#processing').html(
        new ProcessingView({model: analysis}).render().el
    );
    $('#results').html(
        new ResultsView({model: analysis}).render().el
    );
    $('#datadir').html(
        new DataDirView({model: analysis.files}).render().el
    );

/*
    // uncomment this to see what's going on in backbone.js
    if (rnaseqlyze_debug) {
        analysis.bind("all", function (event) {
            log.debug("analysis", arguments);
        });
        analysis.files.bind("all", function (event) {
            log.debug("analysis.files", arguments);
        });
        analysis.stage_logs.bind("all", function (event) {
            log.debug("analysis.stage_logs", arguments);
        });
    }
 */

    // update the models until the analysis is finished
    var update = function () {
        // check at the beginning and not at the end
        // because the fetch() calls are asynchronous
        if (analysis.attributes.finished)
            return;
        analysis.fetch();
//        log.debug("analysis.fetch()");
        window.setTimeout(update, 7000); // re-update in 7 seconds
    }
    update();
});
```

3.3 Software Requirement Specification

RNA-Seqlyze

Software Requirement Specification

Professor:
Prof. Dr. Georg Lipps

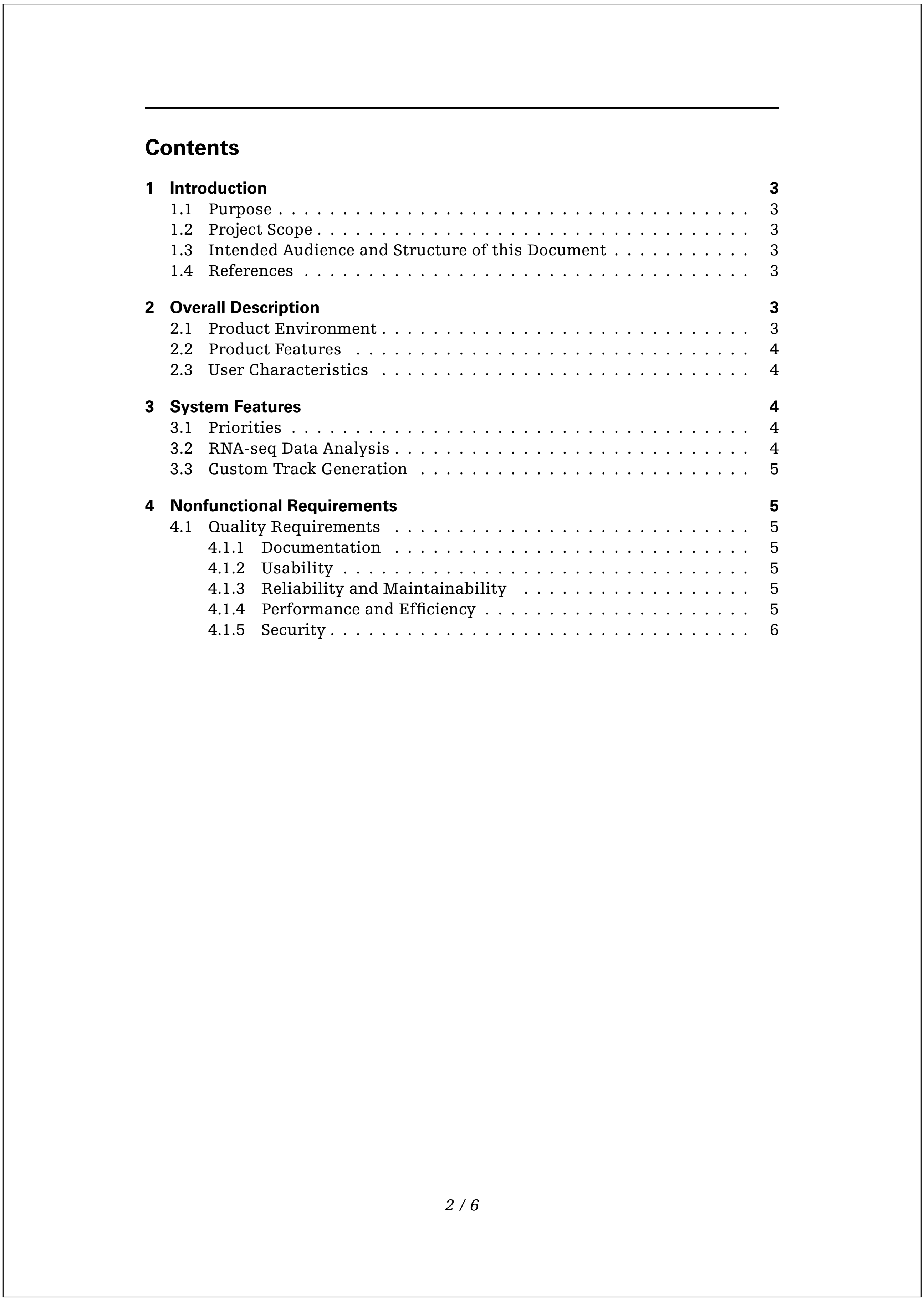
Student:
Patrick Pfeifer

Bachelor Thesis

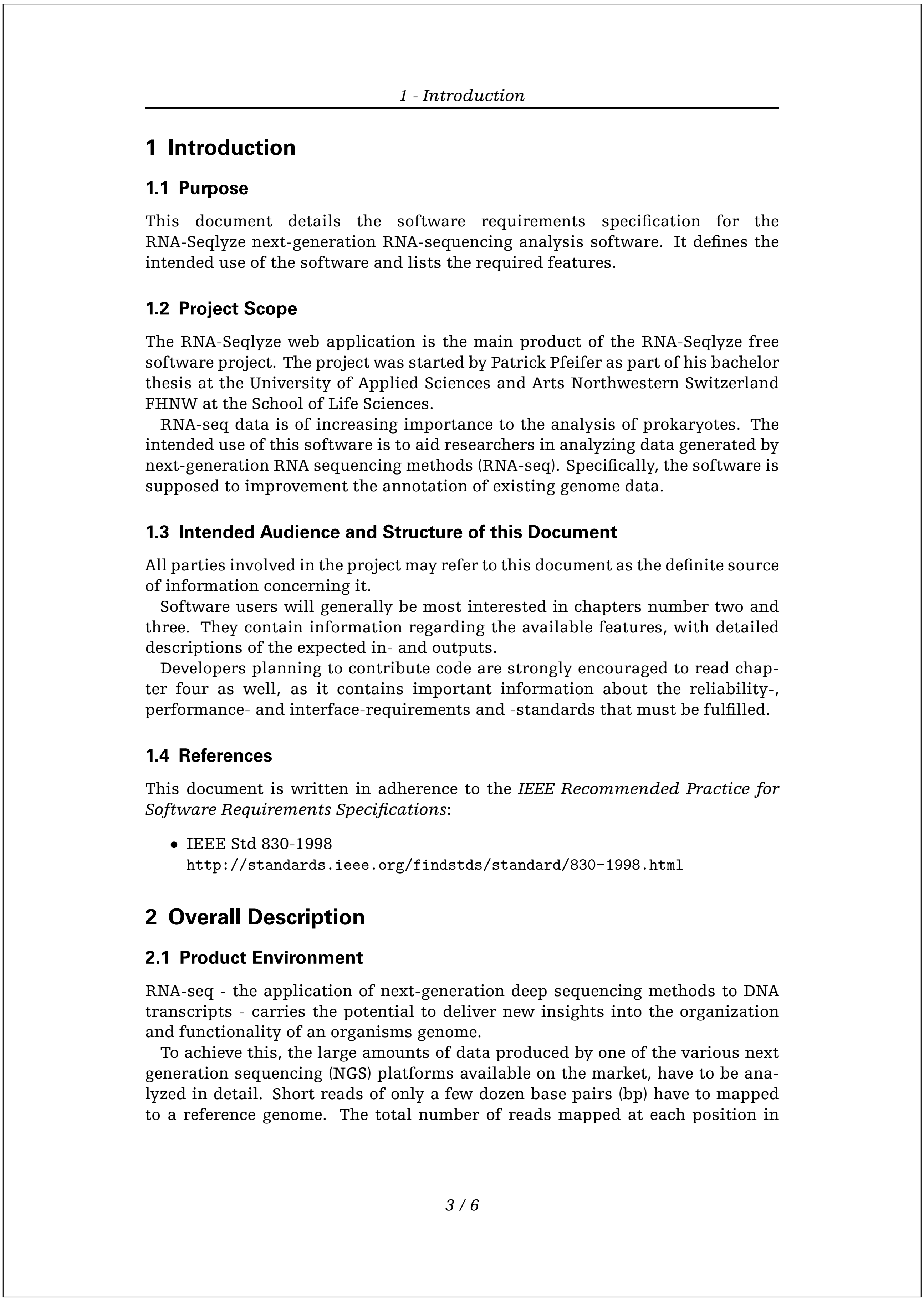
Appendix II

Version	Author	Comment	Date
0.1	Patrick Pfeifer	Document created	25. Mai 2012
0.2	Patrick Pfeifer	fix some wordings	30. Mai 2012
0.3	Patrick Pfeifer	Remove Glossary	13. Juli 2012

n|w University of Applied Sciences and Arts Northwestern Switzerland
School of Life Sciences



Contents



1 Introduction

1.1 Purpose

This document details the software requirements specification for the RNA-Seqlyze next-generation RNA-sequencing analysis software. It defines the intended use of the software and lists the required features.

1.2 Project Scope

The RNA-Seqlyze web application is the main product of the RNA-Seqlyze free software project. The project was started by Patrick Pfeifer as part of his bachelor thesis at the University of Applied Sciences and Arts Northwestern Switzerland FHNW at the School of Life Sciences.

RNA-seq data is of increasing importance to the analysis of prokaryotes. The intended use of this software is to aid researchers in analyzing data generated by next-generation RNA sequencing methods (RNA-seq). Specifically, the software is supposed to improvement the annotation of existing genome data.

1.3 Intended Audience and Structure of this Document

All parties involved in the project may refer to this document as the definite source of information concerning it.

Software users will generally be most interested in chapters number two and three. They contain information regarding the available features, with detailed descriptions of the expected in- and outputs.

Developers planning to contribute code are strongly encouraged to read chapter four as well, as it contains important information about the reliability-, performance- and interface-requirements and -standards that must be fulfilled.

1.4 References

This document is written in adherence to the *IEEE Recommended Practice for Software Requirements Specifications*:

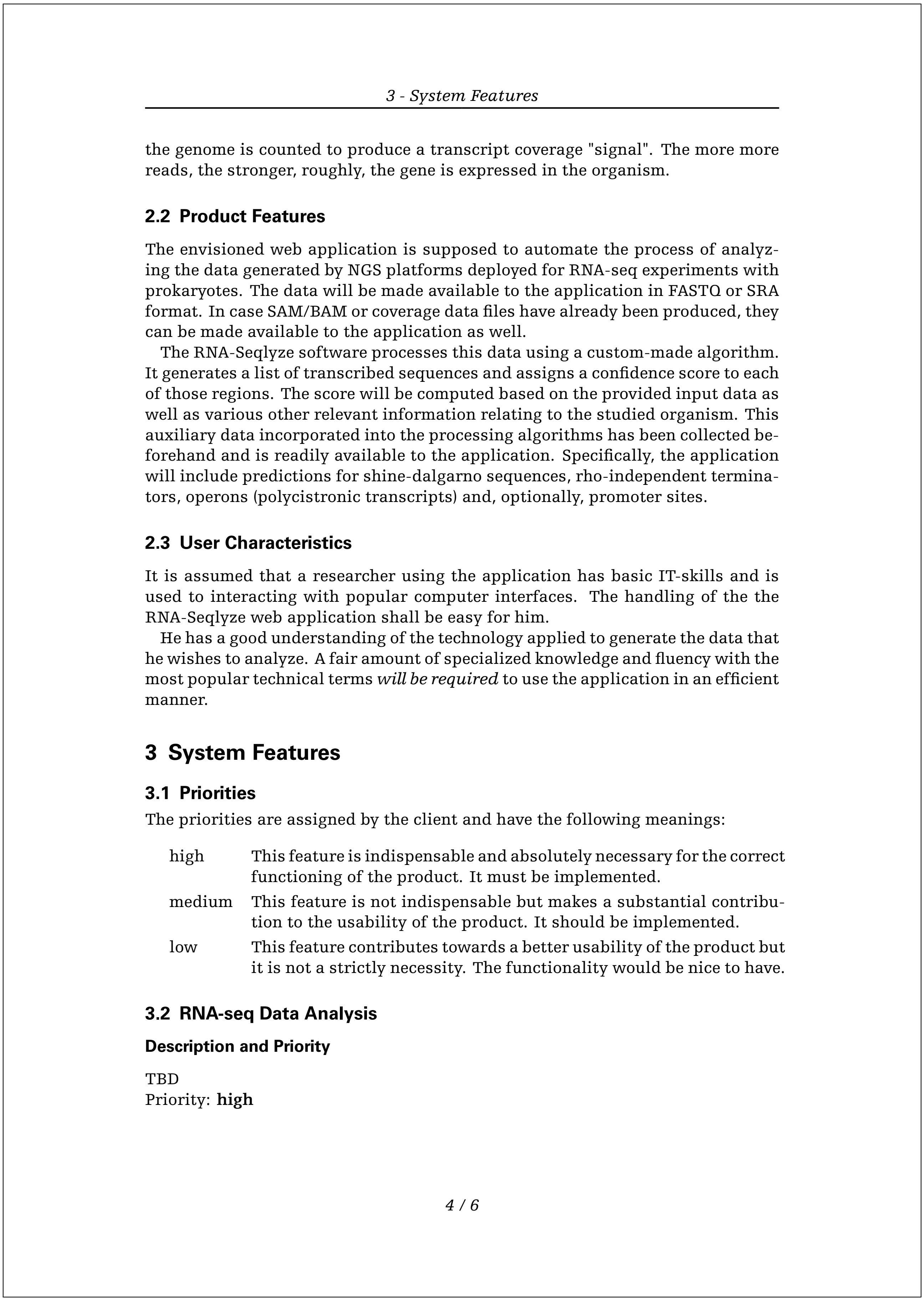
- IEEE Std 830-1998
  `http://standards.ieee.org/findstds/standard/830-1998.html`

2 Overall Description

2.1 Product Environment

RNA-seq - the application of next-generation deep sequencing methods to DNA transcripts - carries the potential to deliver new insights into the organization and functionality of an organisms genome.

To achieve this, the large amounts of data produced by one of the various next generation sequencing (NGS) platforms available on the market, have to be analyzed in detail. Short reads of only a few dozen base pairs (bp) have to mapped to a reference genome. The total number of reads mapped at each position in



the genome is counted to produce a transcript coverage "signal". The more more reads, the stronger, roughly, the gene is expressed in the organism.

2.2 Product Features

The envisioned web application is supposed to automate the process of analyzing the data generated by NGS platforms deployed for RNA-seq experiments with prokaryotes. The data will be made available to the application in FASTQ or SRA format. In case SAM/BAM or coverage data files have already been produced, they can be made available to the application as well.

The RNA-Seqlyze software processes this data using a custom-made algorithm. It generates a list of transcribed sequences and assigns a confidence score to each of those regions. The score will be computed based on the provided input data as well as various other relevant information relating to the studied organism. This auxiliary data incorporated into the processing algorithms has been collected beforehand and is readily available to the application. Specifically, the application will include predictions for shine-dalgarno sequences, rho-independent terminators, operons (polycistronic transcripts) and, optionally, promoter sites.

2.3 User Characteristics

It is assumed that a researcher using the application has basic IT-skills and is used to interacting with popular computer interfaces. The handling of the the RNA-Seqlyze web application shall be easy for him.

He has a good understanding of the technology applied to generate the data that he wishes to analyze. A fair amount of specialized knowledge and fluency with the most popular technical terms *will be required* to use the application in an efficient manner.

3 System Features

3.1 Priorities

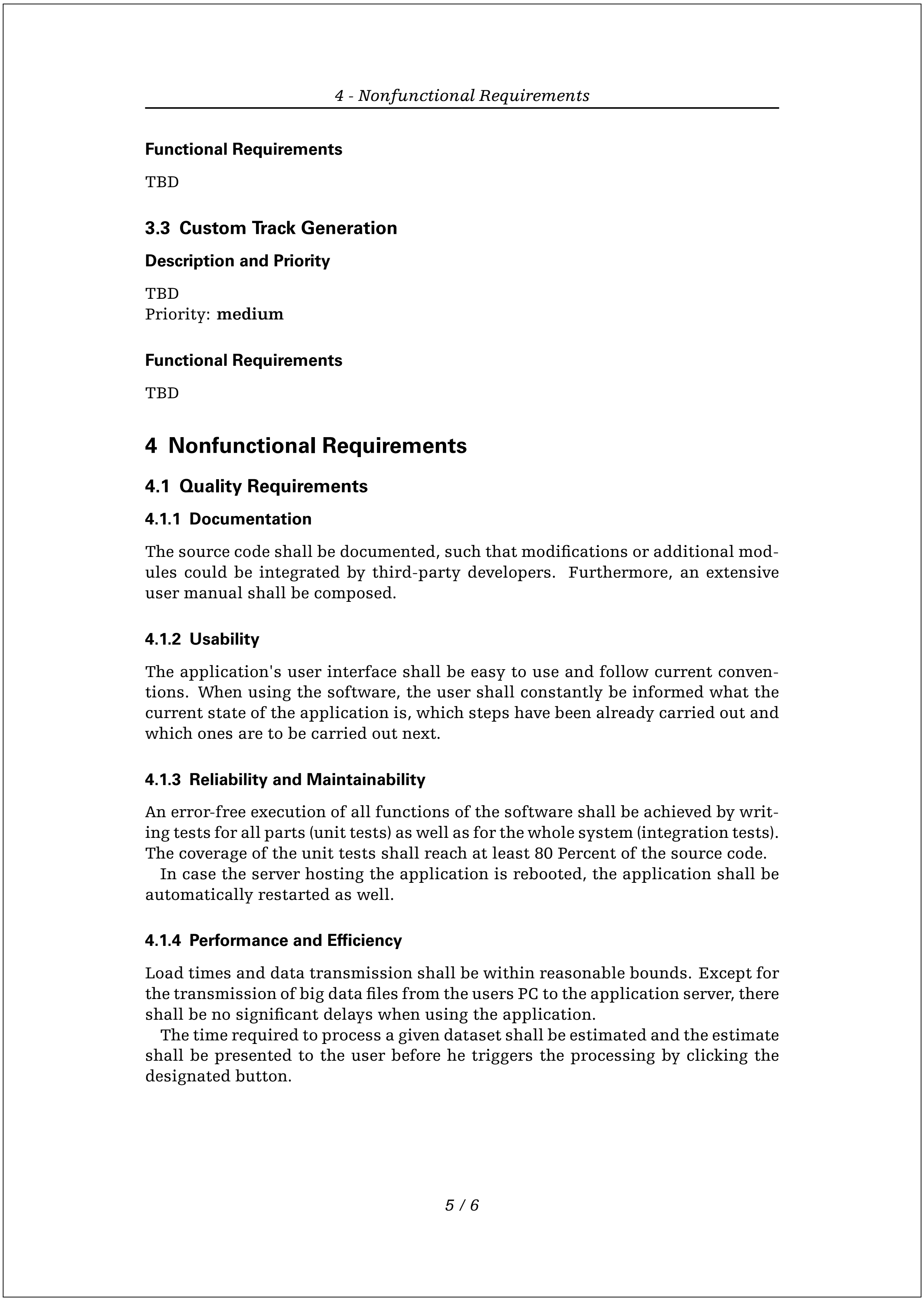
The priorities are assigned by the client and have the following meanings:

high	This feature is indispensable and absolutely necessary for the correct functioning of the product. It must be implemented.
medium	This feature is not indispensable but makes a substantial contribution to the usability of the product. It should be implemented.
low	This feature contributes towards a better usability of the product but it is not a strictly necessity. The functionality would be nice to have.

3.2 RNA-seq Data Analysis

Description and Priority

TBD
Priority: **high**



Functional Requirements

TBD

3.3 Custom Track Generation

Description and Priority

TBD
Priority: **medium**

Functional Requirements

TBD

4 Nonfunctional Requirements

4.1 Quality Requirements

4.1.1 Documentation

The source code shall be documented, such that modifications or additional modules could be integrated by third-party developers. Furthermore, an extensive user manual shall be composed.

4.1.2 Usability

The application's user interface shall be easy to use and follow current conventions. When using the software, the user shall constantly be informed what the current state of the application is, which steps have been already carried out and which ones are to be carried out next.

4.1.3 Reliability and Maintainability

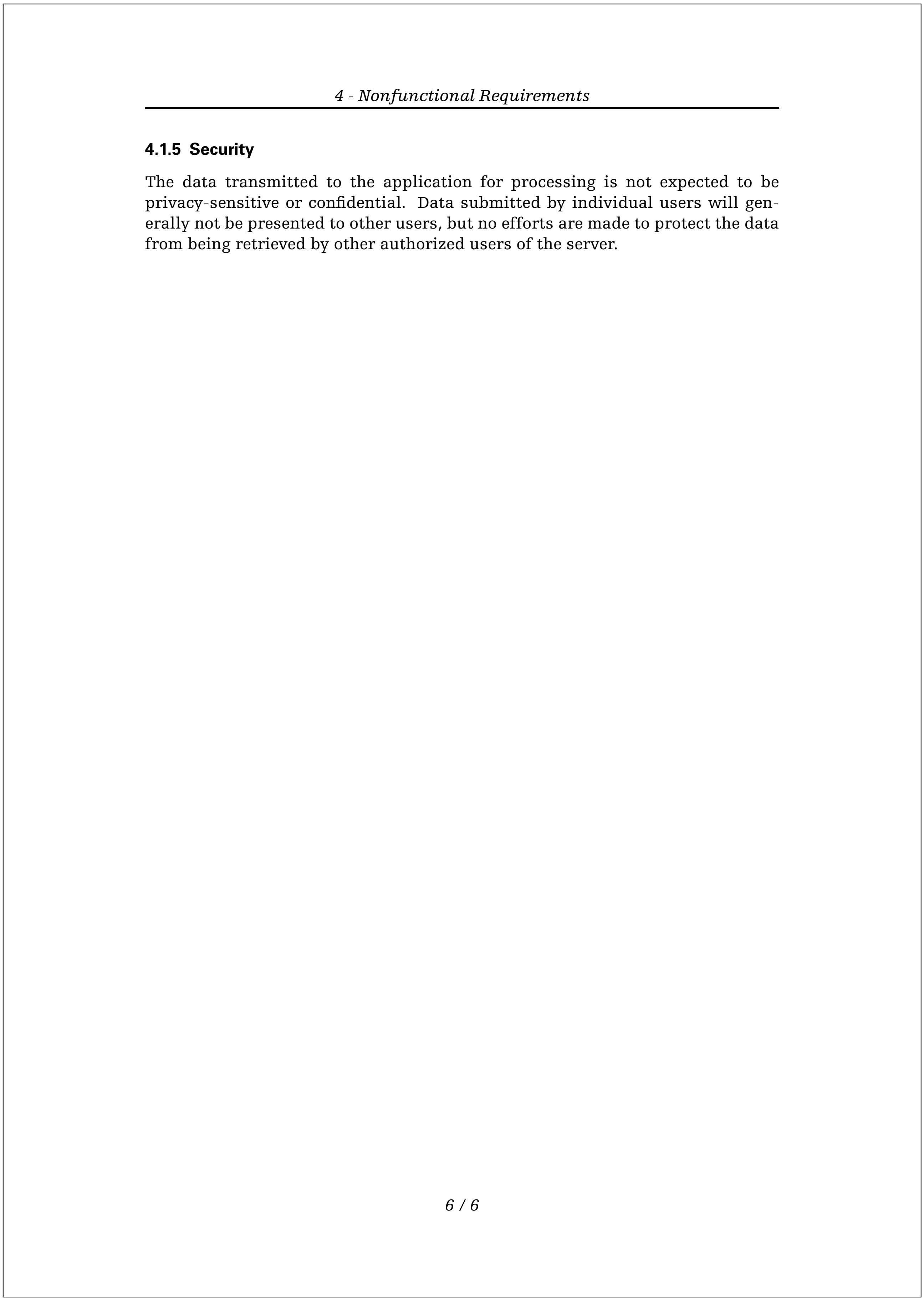
An error-free execution of all functions of the software shall be achieved by writing tests for all parts (unit tests) as well as for the whole system (integration tests). The coverage of the unit tests shall reach at least 80 Percent of the source code.

In case the server hosting the application is rebooted, the application shall be automatically restarted as well.

4.1.4 Performance and Efficiency

Load times and data transmission shall be within reasonable bounds. Except for the transmission of big data files from the users PC to the application server, there shall be no significant delays when using the application.

The time required to process a given dataset shall be estimated and the estimate shall be presented to the user before he triggers the processing by clicking the designated button.



4.1.5 Security

The data transmitted to the application for processing is not expected to be privacy-sensitive or confidential. Data submitted by individual users will generally not be presented to other users, but no efforts are made to protect the data from being retrieved by other authorized users of the server.

3.4 Glossar

RNA-seq	Next-generation Sequenzierungs-Technologe angewendet auf das Profiling kompletter Transkriptome
Object Relational Mapper	Ein Programmbibliothek, die automatisch die von einer Software verwendeten *Business-* bzw. *Domain-* Objekte in einer Datenbank persistiert.

3.5 Quellenverzeichnis

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