

VitisOmics

(see contributors below)

October 14, 2015

Contents

1	Overview	2
1.1	Contributors	3
1.2	References	3
2	Data	3
2.1	URGI	4
2.2	NCBI	5
2.3	EBI	5
2.4	CRIBI	5
3	Results	5
3.1	Comparisons of original "assembly" files	6
3.2	Manipulations of files from URGI	7
3.2.1	Reformat sequence headers for VITVI_PN40024_8x_chroms_URGI	7
3.2.2	Reformat sequence headers for VITVI_PN40024_12x_v0_contigs_EMBL_r102	10
3.2.3	Reformat sequence headers for VITVI_PN40024_12x_v0_scaffolds_EMBL_r102	10
3.2.4	Reformat sequence headers for VITVI_PN40024_12x_v0_chroms_URGI	10
3.2.5	Reformat sequence headers for VITVI_PN40024_12x_v2_chroms_URGI	11
3.2.6	Format VITVI_PN40024_12x_v0_chroms_URGI for BLASTn	12
3.2.7	Index VITVI_PN40024_12x_v0_chroms_URGI for BWA	12
3.2.8	Index VITVI_PN40024_12x_v2_chroms_URGI for BWA	12
3.2.9	Prepare VITVI_PN40024_12x_v2_chroms_URGI for SAMtools and Picard	13
3.2.10	Index VITVI_PN40024_12x_v0_chroms_URGI for Bowtie2	13
3.2.11	Index VITVI_PN40024_12x_v2_chroms_URGI for Bowtie2	13
3.2.12	Index VITVI_PN40024_12x_v2_chroms_URGI for Bowtie2 compatible with Tassel	13
3.2.13	Translate CRIBI annotations from 12X.0 to 12X.2	14
3.3	Manipulations of files from NCBI	14

3.3.1	Reformat sequence headers for VITVI_PN40024_8x_chroms_NCBI	14
3.3.2	Reformat sequence headers for VITVI_PN40024_12x_v0_scaffolds_NCBI . . .	15
3.4	Creation of R/Bioconductor packages	15
3.4.1	BSgenome IGGP12Xv2 package	15
3.4.2	BSgenome IGGP12Xv0 package	16
3.4.3	TxDb IGGP12Xv0 package from NCBI annotations	17

1 Overview

This document describes the "VitisOmics" project. This project aims at handling "omics" data in the genus *Vitis* (e.g. grapevine) in an open and reproducible way. Nevertheless, it requires some basic knowledge and skills about bioinformatics on GNU/Linux computers.

A large amount of such "omics" data are already available, and several committes from the IGGP (International Grape Genome Program) strive at improving interoperability. However, several issues remain, among which:

- partially-overlapping data present at multiple locations (URGI, CRIBI, NCBI, EBI, etc);
- data downloadable as files in various formats not always easy to interchange (fasta, genbank, gff3, gtf, etc);
- data often available without meta-data inside the file;
- large files not always available in compressed form;
- main efforts dedicated to wet-labs grapevine biologists.

These have consequences in terms of ambiguity, inefficiency, potential mistakes, etc. Therefore, I hope that my attempt, via the usage of git and GitHub, could prove for the community to be a useful addition to the IGGP efforts.

The repository can be easily cloned from GitHub:

```
git clone https://github.com/timflutre/VitisOmics.git
```

The project directory is organized as advised by Noble (PLoS Computational Biology 2009). On any Unix-like system, it can be easily compressed and transferred (ignoring large data files):

```
cd ..; tar -czvf VitisOmics.tar.gz \
--exclude=VitisOmics/data --exclude=VitisOmics/results \
--exclude="*" --exclude="*" VitisOmics
```

In order to concretely promote collaborative editing in a distributed manner, the content of the "VitisOmics" repository should be based on plain text files. As a consequence, this document is written in the org format, and can thus be automatically exported, best by emacs, but also by pandoc, into the pdf and html formats for easy reading. A choice is also made to use as much as possible softwares widely available on any GNU/Linux computer, such as bash, awk, etc, but of course it may sometimes be much easier to use R (with Bioconductor), Python (with Biopython), etc.

Last but not least, feel free to contribute, by reporting issues or forking the repository!

1.1 Contributors

The person roles comply with R's guidelines (The R Journal Vol. 4/1, June 2012).

- Timothée Flutre (cre,aut)
- Gautier Sarah (ctb)
- ...

1.2 References

As an introduction, the NCBI has open web pages about genome assembly:

- Assembly information;
- NCBI Genome Assembly Model;
- AGP specification.

Then, the original article for grapevine is a must read:

- Jaillon, et al. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature 449, 463-467 (2007).

Also of interest about genome annotation:

- Vitulo et al. A deep survey of alternative splicing in grape reveals changes in the splicing machinery related to tissue, stress condition and genotype. BMC Plant Biology 14, 99+ (2014).

2 Data

```
mkdir -p data; cd data/
```

TODO: retrieve genome data from other cultivars than PN40024, e.g. Sultanina

2.1 URGI

- <https://urgi.versailles.inra.fr/Species/Vitis>

```
mkdir -p urgi; cd urgi/  
../../src/download_urgi.bash
```

Remarks:

- when needed, the script decompresses zip files and compress them again but with gzip instead;
- the AGP file for the PN40024 8x assembly is not available at URGI's web site.

At the bottom of the page, one can find the following assemblies summary.

12x.2 assembly:

- contigs: 14642
- scaffolds (> 2kb): 2059
- mapped scaffolds: 366 (coverage of the genome: 95%)

12X.0 assembly:

- contigs: 14642
- scaffolds (> 2kb): 2059
- mapped scaffolds: 211 (coverage of the genome: 91.2%)

8X assembly:

- contigs: 19577
- scaffolds: 3514
- mapped ultracontigs: 191 (coverage of the genome: 68.9%)

N. Choisne from URGI (personal communication, 13/10/2015):

- "mapped scaffolds": scaffolds anchored on the linkage groups (i.e. chromosomes) using the markers from the reference genetic map;
- unmapped scaffolds hence are unanchored, and gathered into chrUn;
- "supercontig" is a synonym of "scaffold";

- "ultracontig": one level above supercontigs; localized and orientated according to data from BAC libraries.

2.2 NCBI

- <http://www.ncbi.nlm.nih.gov/genome/401>
- ftp://ftp.ncbi.nlm.nih.gov/genomes/Vitis_vinifera/

```
mkdir -p ncbi; cd ncbi/
../../src/download_ncbi.bash
```

Remarks:

- the important file `scaffold_names` provides the correspondence between original scaffold names (i.e. from the sequencing center) and various NCBI identifiers (RefSeq, GenBank, etc);
- in `ARCHIVE/`, `BUILD.1.1/` corresponds to the 8x genome sequences of PN40024.

2.3 EBI

```
mkdir -p ebi; cd ebi/
../../src/download_ebi.bash
```

Remarks:

- a genome soft-masked by RepeatMasker is available.

2.4 CRIBI

- <http://genomes.cribi.unipd.it/grape/>

```
mkdir -p cribi; cd cribi/
../../src/download_cribi.bash
```

3 Results

```
mkdir -p results; cd results/
```

TODO: compress fasta files with `bgzip` instead of `gzip`

3.1 Comparisons of original "assembly" files

Files from URGI:

```
cd urgi/
zcat VV_8X_embl_98_WGS_contigs.fsa.gz | grep -c ">" # 19577
zcat VV_8X_embl_98_Scaffolds.fsa.gz | grep -c ">" # 3514
zcat VV_chr8x.fsa.gz | grep -c ">" # 35
zcat VV_12X_embl_102_WGS_contigs.fsa.gz | grep -c ">" # 14642
zcat VV_12X_embl_102_Scaffolds.fsa.gz | grep -c ">" # 2059
zcat VV_chr12x.fsa.gz | grep -c ">" # 33
cat 12x0_chr.agp | wc -l # 390
cat 12x0_scaffolds.lg | wc -l # 2059
cat 12x0_chr.lg | wc -l # 33
zcat 12Xv2_grapevine_genome_assembly.fa.gz | grep -c ">" # 20
```

Files from NCBI:

```
cd ncbi/
ls ARCHIVE/BUILD.1.1/CHRS/vvi_ref_chr*.fa.gz | grep -v "Pltd" | while read f; do
  zcat $f; done | grep -c ">" # 3514
ls ARCHIVE/BUILD.1.1/Assembled_chromosomes/vvi_ref_chr*.fa.gz | while read f; do
  zcat $f; done | grep -c ">" # 19
zcat ARCHIVE/BUILD.1.1/allcontig.agp.gz | grep -v "#" | cut -f 5 | sort | uniq -c #
F=1 N=16063 W=19577
cat ARCHIVE/BUILD.1.1/scaffold_names | sed 1d | wc -l # 3514
ls CHRS/vvi_ref_12X_chr*.fa.gz | grep -v -E "Pltd|MT" | while read f; do zcat $f;
done | grep -c ">" # 2059
ls Assembled_chromosomes/vvi_ref_12X_chr*.fa.gz | grep -v -E "Pltd|MT" | while read
f; do zcat $f; done | grep -c ">" # 19
cat scaffold_names | sed 1d | wc -l # 2061
```

See also the script `src/vitisomics.R` using R and Bioconductor. It confirms that the 12x scaffolds have the exact same sequence, whether they come from the URGI or the NCBI. Note however that the file from the NCBI allows to know easily on which chromosome a placed sequences is.

Remarks concerning PN40024 at URGI:

- the file `12x0_chr.agp.info` doesn't correspond to `12x0_chr.agp` (it doesn't even correspond to the description of a proper AGP file, as specified here);

- no mitochondrial nor chloroplastic data are available.

Remarks concerning PN40024 at NCBI:

- contig NC_007957.1 in ARCHIVE/BUILD.1.1/allcontig.agp.gz (with fragment_type=F for "finished") corresponds to the chloroplast;
- scaffold_names contains all 2059 scaffolds of nuclear DNA as well as the assembled genome of the mitochondria and the chloroplast.

For its build 1.1 (corresponding to the 8x sequences of the PN40024 variety), the NCBI has one file per assembled chromosome. However, all unlocalized and unplaced scaffolds are gathered in a single file chrUn. This is not the case at URGI which has unlocalized scaffolds in files as chr3_random and a chrUn_random file with all unplaced scaffolds (and only them). Unfortunately, the NCBI has the annotation of the 8x (in the GenBank format), but the URGI hasn't.

3.2 Manipulations of files from URGI

```
mkdir -p urgi; cd urgi/
```

3.2.1 Reformat sequence headers for VITVI_PN40024_8x_chroms_URGI

Launch script:

```
ln -s ../../data/urg/uv_chr8x.fsa.gz .
echo "../../src/reformat_uv_chr8x.bash" \
| qsub -cwd -j y -V -N reformat_uv_chr8x -q normal.q
```

Check:

```
zcat uv_chr8x.fsa.gz | wc -l # 8291865
zcat uv_chr8x.fsa.gz | grep -c ">" # 35
zcat VITVI_PN40024_8x_chroms_URGI.fa.gz | wc -l # 8291865
zcat VITVI_PN40024_8x_chroms_URGI.fa.gz | grep -c ">" # 35
diff <(zcat uv_chr8x.fsa.gz) <(zcat VITVI_PN40024_8x_chroms_URGI.fa.gz)
```

Only the headers differ, not the sequences, so everything is fine.

Basic stats:

```
zcat VITVI_PN40024_8x_chroms_URGI.fa.gz | md5sum # 4b6ea1cb4ff189ac587fa269077885b5
```

Length of each sequence:

```
zcat VITVI_PN40024_8x_chroms_URGI.fa.gz \  
  | awk 'BEGIN{RS=">"} {split($0,a,"\n");  
if(length(a)==0)next;  
sum=0; for(i=2;i<=length(a);++i){sum+=length(a[i])};  
print a[1]": "sum; sumTot+=sum} END{print sumTot}'
```


header	length (bp)
chr1 CU462738 Vitis vinifera PN40024 assembly8x chromosome1	15630816
chr10 CU462747 Vitis vinifera PN40024 assembly8x chromosome10	9647040
chr10 _{random}	2206354
chr11 CU462748 Vitis vinifera PN40024 assembly8x chromosome11	13936303
chr11 _{random}	1958407
chr12 CU462749 Vitis vinifera PN40024 assembly8x chromosome12	18540817
chr12 _{random}	2826407
chr13 CU462750 Vitis vinifera PN40024 assembly8x chromosome13	15191948
chr13 _{random}	1580403
chr14 CU462751 Vitis vinifera PN40024 assembly8x chromosome14	19480434
chr14 _{random}	5432426
chr15 CU462752 Vitis vinifera PN40024 assembly8x chromosome15	7693613
chr15 _{random}	4297576
chr16 CU462753 Vitis vinifera PN40024 assembly8x chromosome16	8158851
chr16 _{random}	4524411
chr17 CU462754 Vitis vinifera PN40024 assembly8x chromosome17	13059092
chr17 _{random}	1763011
chr18 CU462755 Vitis vinifera PN40024 assembly8x chromosome18	19691255
chr18 _{random}	5949186
chr19 CU462756 Vitis vinifera PN40024 assembly8x chromosome19	14071813
chr19 _{random}	1912523
chr1 _{random}	5496190
chr2 CU462739 Vitis vinifera PN40024 assembly8x chromosome2	17603400
chr2 _{random}	60809
chr3 CU462740 Vitis vinifera PN40024 assembly8x chromosome3	10186927
chr3 _{random}	1343266
chr4 CU462741 Vitis vinifera PN40024 assembly8x chromosome4	19293076
chr5 CU462742 Vitis vinifera PN40024 assembly8x chromosome5	23428299
chr6 CU462743 Vitis vinifera PN40024 assembly8x chromosome6	24148918
chr7 CU462744 Vitis vinifera PN40024 assembly8x chromosome7	15233747
chr7 _{random}	176143
chr8 CU462745 Vitis vinifera PN40024 assembly8x chromosome8	21557227
chr8 _{random}	12125
chr9 CU462746 Vitis vinifera PN40024 assembly8x chromosome9	16532244
chrUn _{random}	154883714
total	497508771

3.2.2 Reformat sequence headers for VITVI_PN40024_12x_v0_contigs_EMBL_r102

TODO

3.2.3 Reformat sequence headers for VITVI_PN40024_12x_v0_scaffolds_EMBL_r102

Launch script:

```
ln -s ../../data/urgi/VV_12X_embl_102_Scaffolds.fsa.gz .
echo "../../src/reformat_VV_12X_embl_102_Scaffolds.bash" \
| qsub -cwd -j y -V -N reformat_VV_12X_embl_102_Scaffolds -q normal.q
```

Check:

```
zcat VV_12X_embl_102_Scaffolds.fsa.gz | wc -l # 8091565
zcat VV_12X_embl_102_Scaffolds.fsa.gz | grep -c ">" # 2059
zcat VITVI_PN40024_12x_v0_scaffolds_EMBL_r102.fa.gz | wc -l # 8091565
zcat VITVI_PN40024_12x_v0_scaffolds_EMBL_r102.fa.gz | grep -c ">" # 2059
diff <(zcat VV_12X_embl_102_Scaffolds.fsa.gz) <(zcat
    VITVI_PN40024_12x_v0_scaffolds_EMBL_r102.fa.gz)
```

Only the headers differ, not the sequences, so everything is fine.

Basic stats:

```
zcat VITVI_PN40024_12x_v0_scaffolds_EMBL_r102.fa.gz | md5sum # 4
    fa2432d7a66c019c7cb41ee4d0cb7bc
zcat VITVI_PN40024_12x_v0_scaffolds_EMBL_r102.fa.gz | grep -v ">" | md5sum #
    df5cdb0c6f73cb133261905374cdf2f2
```

3.2.4 Reformat sequence headers for VITVI_PN40024_12x_v0_chroms_URGI

Launch script:

```
ln -s ../../data/urgi/VV_chr12x.fsa.gz .
echo "../../src/reformat_VV_chr12x.bash" \
| qsub -cwd -j y -V -N reformat_VV_chr12x -q normal.q
```

Check:

```

zcat VV_chr12x.fsa.gz | wc -l # 8240706
zcat VV_chr12x.fsa.gz | grep -c ">" # 33
zcat VITVI_PN40024_12x_v0_chroms_URGI.fa.gz | wc -l # 8240706
zcat VITVI_PN40024_12x_v0_chroms_URGI.fa.gz | grep -c ">" # 33
diff <(zcat VV_chr12x.fsa.gz) <(zcat VITVI_PN40024_12x_v0_chroms_URGI.fa.gz)

```

Only the headers differ, not the sequences, so everything is fine.

Basic stats:

```

zcat VITVI_PN40024_12x_v0_chroms_URGI.fa.gz | md5sum #
e487c28eaf19ef59b0b6128b73935af

```

3.2.5 Reformat sequence headers for VITVI_PN40024_12x_v2_chroms_URGI

Launch script:

```

ln -s ../../data/urgi/12Xv2_grapevine_genome_assembly.fa.gz .
echo "../../src/reformat_12Xv2_grapevine_genome_assembly.bash" \
| qsub -cwd -j y -V -N reformat_12Xv2_grapevine_genome_assembly -q normal.q

```

Check:

```

zcat 12Xv2_grapevine_genome_assembly.fa.gz | wc -l # 8103449
zcat 12Xv2_grapevine_genome_assembly.fa.gz | grep -c ">" # 20
zcat VITVI_PN40024_12x_v2_chroms_URGI.fa.gz | wc -l # 8103449
zcat VITVI_PN40024_12x_v2_chroms_URGI.fa.gz | grep -c ">" # 20
diff <(zcat 12Xv2_grapevine_genome_assembly.fa.gz) <(zcat
VITVI_PN40024_12x_v2_chroms_URGI.fa.gz)

```

Only the headers differ, not the sequences, so everything is fine.

Basic stats:

```

zcat VITVI_PN40024_12x_v2_chroms_URGI.fa.gz | md5sum # 4
e487c28eaf19ef59b0b6128b73935af

```

Length of each sequence:

```

zcat VITVI_PN40024_12x_v2_chroms_URGI.fa.gz \
| awk 'BEGIN{RS=">"} {split($0,a,"\n");
if(length(a)==0)next;
sum=0; for(i=2;i<=length(a);++i){sum+=length(a[i])};
print a[1]": "sum; sumTot+=sum} END{print sumTot}'

```

header	length (bp)
chr1 Vitis vinifera PN40024 assembly12x.2	24233538
chr2 Vitis vinifera PN40024 assembly12x.2	18891843
chr3 Vitis vinifera PN40024 assembly12x.2	20695524
chr4 Vitis vinifera PN40024 assembly12x.2	24711646
chr5 Vitis vinifera PN40024 assembly12x.2	25650743
chr6 Vitis vinifera PN40024 assembly12x.2	22645733
chr7 Vitis vinifera PN40024 assembly12x.2	27355740
chr8 Vitis vinifera PN40024 assembly12x.2	22550362
chr9 Vitis vinifera PN40024 assembly12x.2	23006712
chr10 Vitis vinifera PN40024 assembly12x.2	23503040
chr11 Vitis vinifera PN40024 assembly12x.2	20118820
chr12 Vitis vinifera PN40024 assembly12x.2	24269032
chr13 Vitis vinifera PN40024 assembly12x.2	29075116
chr14 Vitis vinifera PN40024 assembly12x.2	30274277
chr15 Vitis vinifera PN40024 assembly12x.2	20304914
chr16 Vitis vinifera PN40024 assembly12x.2	23572818
chr17 Vitis vinifera PN40024 assembly12x.2	18691847
chr18 Vitis vinifera PN40024 assembly12x.2	34568450
chr19 Vitis vinifera PN40024 assembly12x.2	24695667
chrUkn Vitis vinifera PN40024 assembly12x.2	27389308
total	486205130

3.2.6 Format VITVI_PN40024_12x_v0_chroms_URGI for BLASTn

TODO: change Vvin to VITVI

```
../../src/format_Vvin-PN40024-12x-chr_blastn.bash
```

3.2.7 Index VITVI_PN40024_12x_v0_chroms_URGI for BWA

Launch:

```
echo "../../src/bwa_index_VITVI_PN40024_12x_v0_chroms_URGI.bash" \
| qsub -cwd -j y -V -N bwa_index_VITVI_PN40024_12x_v0_chroms_URGI -q normal.q
```

3.2.8 Index VITVI_PN40024_12x_v2_chroms_URGI for BWA

Launch:

```
echo "../../src/bwa_index_VITVI_PN40024_12x_v2_chroms_URGI.bash" \  
| qsub -cwd -j y -V -N bwa_index_VITVI_PN40024_12x_v2_chroms_URGI -q normal.q
```

3.2.9 Prepare VITVI_PN40024_12x_v2_chroms_URGI for SAMtools and Picard

Make an index as well as a SAM header.

Launch:

```
echo "../../src/samtools-picard_prep_VITVI_PN40024_12x_v2_chroms_URGI.bash" \  
| qsub -cwd -j y -V -N samtools-picard_prep_VITVI_PN40024_12x_v2_chroms_URGI -q  
normal.q
```

3.2.10 Index VITVI_PN40024_12x_v0_chroms_URGI for Bowtie2

Launch:

```
echo "../../src/bowtie2_index_VITVI_PN40024_12x_v0_chroms_URGI.bash" \  
| qsub -cwd -j y -V -N bowtie2_build_VITVI_PN40024_12x_v0_chroms_URGI -q normal.q
```

3.2.11 Index VITVI_PN40024_12x_v2_chroms_URGI for Bowtie2

Launch:

```
echo "../../src/bowtie2_index_VITVI_PN40024_12x_v2_chroms_URGI.bash" \  
| qsub -cwd -j y -V -N bowtie2_build_VITVI_PN40024_12x_v2_chroms_URGI -q normal.q
```

3.2.12 Index VITVI_PN40024_12x_v2_chroms_URGI for Bowtie2 compatible with Tassel

Tassel requires numbers as chromosome identifiers.

Launch:

```
echo "../../src/bowtie2_index_VITVI_PN40024_12x_v2_chroms_URGI_for_Tassel.bash" \  
| qsub -cwd -j y -V -N bowtie2_build_VITVI_PN40024_12x_v2_chroms_URGI_for_Tassel -  
q normal.q
```

3.2.13 Translate CRIBI annotations from 12X.0 to 12X.2

Requirement: use or write a script taking as input the 12X.0 GFF3 file as well as the 12.0-12.2 AGP file, and returns as output the 12X.2 GFF3 file

The URGI provides the following AGP file: `golden_path_V2_111113_allChr.csv`. Unfortunately, after looking at the official specification of the AGP format, the URGI file doesn't seem to be valid, neither for version 1.1, nor 2.2. After contacting URGI, they told me they were working on it (October 2015).

Another script was developed by G. Sarah, but it suffers from several issues.

TODO: test CrossMap

3.3 Manipulations of files from NCBI

```
mkdir -p ncbi; cd ncbi/
```

3.3.1 Reformat sequence headers for VITVI_PN40024_8x_chroms_NCBI

TODO: concatenate unplaced scaffolds into a single chrUn ?

Launch script:

```
ln -s ../../data/ncbi/ARCHIVE/BUILD.1.1/Assembled_chromosomes/vvi_ref_chr*.fa.gz .
ln -s ../../data/ncbi/ARCHIVE/BUILD.1.1/CHRS/vvi_ref_chrUn.fa.gz
echo "../../src/reformat_chr_NCBI-build-1-1.bash" \
| qsub -cwd -j y -V -N reformat_chroms_NCBI-build-1-1 -q normal.q
```

Check:

```
\ls vvi_ref_chr* | while read f; do zcat $f; done | wc -l # 6499942
\ls vvi_ref_chr* | while read f; do zcat $f; done | grep -c ">" # 3343
zcat VITVI_PN40024_8x_chroms_NCBI.fa.gz | wc -l # 6499942
zcat VITVI_PN40024_8x_chroms_NCBI.fa.gz | grep -c ">" # 3343
diff <(\ls -v vvi_ref_chr* | while read f; do zcat $f; done) <(zcat
VITVI_PN40024_8x_chroms_NCBI.fa.gz)
```

Only the headers differ, not the sequences, so everything is fine.

Basic stats:

```
zcat VITVI_PN40024_8x_chroms_NCBI.fa.gz | md5sum # d8eeff80c824f1b5bd91b4274fddb696
```

3.3.2 Reformat sequence headers for VITVI_PN40024_12x_v0_scaffolds_NCBI

Launch script:

```
ls ../../data/ncbi/CHRS/vvi_ref_12X_chr*.fa.gz | grep -v -E "Pltd|MT" | while read f
; do ln -s $f .; done
echo "../../src/reformat_scaffs_NCBI-12x.bash" \
| qsub -cwd -j y -V -N reformat_scaffs_NCBI-12x -q normal.q
```

Check:

```
\ls vvi_ref_12X_chr* | while read f; do zcat $f; done | wc -l # 6934292
\ls vvi_ref_12X_chr* | while read f; do zcat $f; done | grep -c ">" # 2059
zcat VITVI_PN40024_12x_v0_scaffolds_NCBI.fa.gz | wc -l # 6934292
zcat VITVI_PN40024_12x_v0_scaffolds_NCBI.fa.gz | grep -c ">" # 2059
diff <(\ls -v vvi_ref_12X_chr* | while read f; do zcat $f; done) <(zcat
VITVI_PN40024_12x_v0_scaffolds_NCBI.fa.gz) | less
```

Only the headers differ, not the sequences, so everything is fine.

Basic stats:

```
zcat VITVI_PN40024_12x_v0_scaffolds_NCBI.fa.gz | md5sum # 20
fa822ed5679519a20fe768c422a701
zcat VITVI_PN40024_12x_v0_scaffolds_NCBI.fa.gz | grep -v ">" | md5sum # 9
ddb5761fe0e4356c7ef73410011ccb
```

3.4 Creation of R/Bioconductor packages

- <http://www.bioconductor.org/>
- Huber, W. et al. Orchestrating high-throughput genomic analysis with bioconductor. Nature Methods 12, 115-121 (2015). URL <http://dx.doi.org/10.1038/nmeth.3252>.

TODO: see AnnotationHub

3.4.1 BSgenome IGGP12Xv2 package

<http://bioconductor.org/packages/release/bioc/html/BSgenome.html>

Retrieve the sequence data from URGI:

```
cd results/
mkdir -p make_BSgenome_IGGP12Xv2
cd make_BSgenome_IGGP12Xv2/
ln -s ../../data/urg1/12Xv2_grapevine_genome_assembly.fa.gz .
```

Split into one chromosome per file (in the headers, discard everything after the first space):

```
zcat 12Xv2_grapevine_genome_assembly.fa.gz | awk 'BEGIN{RS=">"} {if(NF==0)next;
  split($0,a,"\n"); split(a[1],b," "); print b[1]; print ">"b[1] > b[1]".fa"; for(
    i=2;i<length(a);++i){print a[i] >> b[1]".fa"}}'
gzip chr*.fa
```

Prepare the seed file (IGGP12Xv2_seed.txt) by hand as indicated in the vignette as well as in the official R manual "Writing R extensions". Following this article, I chose the CC0 license (present in the R list of licenses in `share/licenses/license.db`).

Forge the target package from the seed file:

```
echo "date; echo \"library(BSgenome); forgeBSgenomeDataPkg(\\\\"IGGP12Xv2_seed.txt\\\\"
  ")\" | R --vanilla; date" | qsub -cwd -j y -V -N forge_BSgenome -q normal.q
```

Build the package and check it:

```
echo "date; R CMD build BSgenome.Vvinifera.URGI.IGGP12Xv2; date" | qsub -cwd -j y -V
  -N build_BSgenome -q normal.q
echo "date; R CMD check BSgenome.Vvinifera.URGI.IGGP12Xv2_0.1.tar.gz; date" | qsub -
  cwd -j y -V -N check_BSgenome -q normal.q
```

The target package is now ready to be installed:

```
R CMD INSTALL BSgenome.Vvinifera.URGI.IGGP12Xv2_0.1.tar.gz
```

A.-F. Adam-Blondon (part of IGGP) and other colleagues from INRA gave positive feedback. I hence sent the package to the Bioconductor team. I hope it will soon be available for download via `biocLite()`.

3.4.2 BSgenome IGGP12Xv0 package

<http://bioconductor.org/packages/release/bioc/html/BSgenome.html>

Retrieve the sequence data from URGI:


```
cd results/  
mkdir -p make_BSgenome_IGGP12Xv0  
cd make_BSgenome_IGGP12Xv0/  
ln -s ../../data/urgi/VV_chr12x.fsa.gz .
```

TODO: Prepare the seed file (IGGP12Xv0_seed.txt) by editing the one used for IGGP12Xv2.

3.4.3 TxDb IGGP12Xv0 package from NCBI annotations

<http://www.bioconductor.org/packages/release/bioc/html/GenomicFeatures.html>

TODO: use `makeTxDbFromGFF()`