VitisOmics

(see contributors below)

May 6, 2016

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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 committee 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

The NCBI has web pages about genome assembly:

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

The original article for grapevine (PN40024) is a must read:

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

About genome annotations:

- Grimplet et al. Comparative analysis of grapevine whole-genome gene predictions, functional annotation, categorization and integration of the predicted gene sequences. BMC Research Notes 5, 213+ (2012).
- Grimplet et al. The grapevine gene nomenclature system. BMC Genomics 15, 1077+ (2014).
- 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 this 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.
- N. Choisne from URGI on the 18K SNP chip (personal communication, 09/02/2016): let's take an example
 - identifier chr1_27655_C_T;
 - probe sequence TTGTCTACGAAGTTTGACAATTTCTATTTTTCATAAGTTTACACAAATTA[T/C]TGAACAGTGAGTTAGTGAC
 - SNPs on the 18K Illumina Infinium microarray are named so that the first allele (here, C) corresponds to the reference (PN40024 12x.0) and the second allele (here, T) to the variant (almost sure for the "species" SNPs and should also be the case for the "vinifera" SNPs);
 - in the sequence, the fact that the T comes before the C only reflects the fact that the chip is an Infinium II, with 2 beads, only allowing loci A/G, A/C, T/G, T/C, whereas the Infinium I only allows A/T et C/G.

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
```

2.5 Genoscope

- http://www.genoscope.cns.fr/spip/Vitis-vinifera-whole-genome.html
- http://www.genoscope.cns.fr/externe/Download/Projets/Projet_ML/data/

```
mkdir -p genoscope; cd genoscope/
../../src/download_genoscope.bash
```

3 Results

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

TODO: compress fasta files with bgzip instead of gzip

3.1 Comparisons of original "assembly" files

3.1.1 URGI vs NCBI

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.

3.1.2 URGI vs CRIBI

File from CRIBI:

```
tar -tzvf Genome12X.tar.gz | wc -l # 33
```

See also the script src/vitisomics.R using R and Bioconductor. It confirms that each of the 12x.0 chromosomes have the exact same sequence at URGI and CRIBI, the only differences being the headers.

3.2 Comparisons of annotations

3.2.1 By Genoscope and from Genoscope (8x and 12x.0)

The Genoscope annotated the 8x and 12x.v0 assemblies with the Gaze software:

```
zcat 12X/annotation/Vitis_vinifera_annotation.gff.gz | md5sum # 2
    d568ed155422060dd2ca42eaf14bb3b
zcat 8X/annotation/Vitis_vinifera_annotation_v1.gff.gz | cut -f2 | sort | uniq -c #
    234890 Gaze_filter
zcat 12X/annotation/Vitis_vinifera_annotation.gff.gz | cut -f2 | sort | uniq -c #
    245272 Gaze
```

Both GFF files contain several types of annotations:

zcat 8X/annotation/Vitis_vinifera_annotation_v1.gff.gz | cut -f3 | sort | uniq -c
zcat 12X/annotation/Vitis_vinifera_annotation.gff.gz | cut -f3 | sort | uniq -c

| assembly | gene | mRNA | UTR | CDS |
|----------|-------|-------|-------|--------|
| 8x | 30434 | 30434 | 24671 | 149351 |
| 12x.0 | 26346 | 26346 | 35815 | 156765 |

For both 8x and 12x.0 assemblies, gene identifiers all start with GSVIVG, and by GSVIVT for mRNA:

```
zcat 8X/annotation/Vitis_vinifera_annotation_v1.gff.gz | awk -F"\t" '{if($3=="gene")
    {split($9,a," "); print substr(a[2],1,6)}}' | sort | uniq -c
zcat 8X/annotation/Vitis_vinifera_annotation_v1.gff.gz | awk -F"\t" '{if($3=="mRNA")
    {split($9,a," "); print substr(a[2],1,6)}}' | sort | uniq -c
zcat 12X/annotation/Vitis_vinifera_annotation.gff.gz | awk -F"\t" '{if($3=="gene"){
        split($9,a," "); print substr(a[2],1,6)}}' | sort | uniq -c
zcat 12X/annotation/Vitis_vinifera_annotation.gff.gz | awk -F"\t" '{if($3=="mRNA"){
        split($9,a," "); print substr(a[2],1,6)}}' | sort | uniq -c
```

3.2.2 By Genoscope but from URGI (8x and 12x.0)

The annotations made by Genoscope for the 12x.0 are also available for download at URGI (exact same file):

```
cd data/urgi/12x_annotation_Genoscope_V0/
zcat Vitis_vinifera_annotation.gff.gz | wc -1 # 245272
zcat Vitis_vinifera_annotation.gff.gz | md5sum # 2d568ed155422060dd2ca42eaf14bb3b
```

The URGI also provides more specific files, e.g. mRNA-only, peptide-only, as well as repeat annotations by RepeatMasker and TRF.

3.2.3 By CRIBI but from URGI (12x.0)

TODO

3.2.4 By CRIBI and from CRIBI

The CRIBI makes available many versions of its annotations (0, 1, 2, 2.1) and several README files give some details about the differences.

```
tar -xzOf data/cribi/GFF/VO.tar.gz VO/all.GAZE | wc -1 # 245276
tar -xzOf ../../data/cribi/GFF/V1.tar.gz | wc -1 # 244596
zcat data/cribi/GFF/V1_phase.gff3.gz | wc -1 # 392377
zcat data/cribi/V2/V2/V2.gff3.gz | wc -1 # 821809
zcat data/cribi/V2/V2.1/V2.1.gff3.gz | wc -1 # 820944
```

Look at the latest (2.1), which seems to be a correct GFF3 file, even though it has no meta-data:

| type | count |
|--------------------|--------|
| gene | 31845 |
| mRNA | 55564 |
| exon | 321050 |
| $five_{primeutr}$ | 58389 |
| CDS | 297312 |
| $three_{primeutr}$ | 56784 |

3.2.5 By NCBI (8x and 12x.0)

The NCBI annotated the 8x and 12x.0 assemblies with the GNOMON software.

TODO: convert NCBI annotation files from gbs into gff3 (see work in progress below)

3.3 Manipulations of files from URGI

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

3.3.1 Reformat sequence headers for VITVI_PN40024_8x_chroms_URGI

Launch script:

Check:

```
zcat VV_chr8x.fsa.gz | wc -1 # 8291865
zcat VV_chr8x.fsa.gz | grep -c ">" # 35
zcat VITVI_PN40024_8x_chroms_URGI.fa.gz | wc -1 # 8291865
zcat VITVI_PN40024_8x_chroms_URGI.fa.gz | grep -c ">" # 35
diff <(zcat VV_chr8x.fsa.gz) <(zcat VITVI_PN40024_8x_chroms_URGI.fa.gz)</pre>
```

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:

| header | length (bp) |
|--|-------------|
| $chr1\ CU462738 Vitis\ vinifera PN40024 assembly 8x chromosome_1$ | 15630816 |
| $chr 10\ CU 462747 Vitis\ vinifera PN 40024 assembly 8x chromosome_{10}$ | 9647040 |
| $ m chr 10_{random}$ | 2206354 |
| $chr 11\ CU 462748 Vitis\ vinifera PN 40024 assembly 8x chromosome_{11}$ | 13936303 |
| $ m chr11_{random}$ | 1958407 |
| $chr 12\ CU 462749 Vitis\ vinifera PN 40024 assembly 8x chromosome_{12}$ | 18540817 |
| $ m chr12_{random}$ | 2826407 |
| $chr 13\ CU 462750 Vitis\ vinifera PN 40024 assembly 8x chromosome_{13}$ | 15191948 |
| $ m chr 13_{random}$ | 1580403 |
| $chr 14\ CU 462751 Vitis\ vinifera PN 40024 assembly 8x chromosome_{14}$ | 19480434 |
| $ m chr14_{random}$ | 5432426 |
| $chr 15\ CU 462752 Vitis\ vinifera PN 40024 assembly 8x chromosome_{15}$ | 7693613 |
| $ m chr15_{random}$ | 4297576 |
| $chr 16\ CU 462753 Vitis\ vinifera PN 40024 assembly 8x chromosome_{16}$ | 8158851 |
| $ m chr16_{random}$ | 4524411 |
| $chr17\ CU462754 Vitis\ vinifera PN40024 assembly 8x chromosome_{17}$ | 13059092 |
| $ m chr17_{random}$ | 1763011 |
| $chr 18\ CU 462755 Vitis\ vinifera PN 40024 assembly 8x chromosome_{18}$ | 19691255 |
| $ m chr18_{random}$ | 5949186 |
| $chr 19\ CU 462756 Vitis\ vinifera PN 40024 assembly 8x chromosome_{19}$ | 14071813 |
| $ m chr19_{random}$ | 1912523 |
| $ m chr1_{random}$ | 5496190 |
| $chr 2\ CU 462739 Vitis\ vinifera PN 40024 assembly 8x chromosome_2$ | 17603400 |
| $ m chr2_{random}$ | 60809 |
| chr3 CU462740 Vitis vinifera PN40024 assembly8x chromosome3 | 10186927 |
| $ m chr3_{random}$ | 1343266 |
| chr4 CU462741 Vitis vinifera PN40024 assembly8x chromosome ₄ | 19293076 |
| $chr 5\ CU 462742 Vitis\ vinifera PN 40024 assembly 8x chromosome_5$ | 23428299 |
| $chr 6\ CU 462743 Vitis\ vinifera PN 40024 assembly 8x chromosome_6$ | 24148918 |
| chr7 CU462744 Vitis vinifera PN40024 assembly8x chromosome ₇ | 15233747 |
| $ m chr7_{random}$ | 176143 |
| chr8 CU462745 Vitis vinifera PN40024 assembly8x chromosome8 | 21557227 |
| $\mathrm{chr8_{random}}$ | 12125 |
| $chr9\ CU462746 Vitis\ vinifera PN40024 assembly 8x chromosome_9$ | 16532244 |
| $\mathrm{chrUn_{random}}$ | 154883714 |
| total | 497508771 |

3.3.2 Reformat sequence headers for VITVI_PN40024_12x_v0_scaffolds_EMBL_r102

Launch script:

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:

3.3.3 Reformat sequence headers for VITVI_PN40024_12x_v0_chroms_URGI

Launch script:

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)</pre>
```

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

Basic stats:

```
zcat VITVI_PN40024_12x_v0_chroms_URGI.fa.gz | md5sum #
   eff315994fafe35333462b9595e10ce5
```

3.3.4 Reformat sequence headers for VITVI_PN40024_12x_v2_chroms_URGI

Launch script:

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)</pre>
```

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

Basic stats:

Length of each sequence:

| 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 |

3.3.5 Format VITVI_PN40024_8x_chroms_URGI for BLAST

Launch:

3.3.6 Format VITVI_PN40024_12x_v0_chroms_URGI for BLAST

Launch:

3.3.7 Format VITVI_PN40024_12x_v2_chroms_URGI for BLAST

Launch:

3.3.8 Index VITVI_PN40024_12x_v0_chroms_URGI for BWA

Launch:

3.3.9 Index VITVI_PN40024_12x_v2_chroms_URGI for BWA

Launch:

3.3.10 Prepare VITVI_PN40024_12x_v2_chroms_URGI for SAMtools and Picard

Make an index as well as a SAM header.

Launch:

3.3.11 Index VITVI_PN40024_12x_v0_chroms_URGI for Bowtie2

Launch:

3.3.12 Index VITVI_PN40024_12x_v2_chroms_URGI for Bowtie2

Launch:

3.3.13 Index VITVI_PN40024_12x_v2_chroms_URGI for Bowtie2 compatible with Tassel

Tassel requires numbers as chromosome identifiers.

Launch:

3.3.14 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).

TODO: look at the annotations from CRIBI on 12x.0 transposed to 12x.2 by URGI

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

TODO: test CrossMap

3.3.15 Convert SNP data of the 18K Illumina array from xls to txt.gz

On the command-line, working with tabulated files is much easier, and they should be compressed (e.g. with gzip). The 18071 probe sequences also need to be saved in two fasta files, one for the 13562 "vinifera" SNPs and one for the 4509 "species" SNPs.

See the corresponding task in the script src/vitisomics.R.

Note that, as of January 2016, even though at least two articles were published which used this genotyping array, the data are not (yet?) part of dbSNP nor EVA, unfortunately. Therefore, no unambiguous SNP identifiers exist which can be used across studies and genome assemblies.

3.3.16 Align Illumina probes on PN40024 assemblies

Requires the VITVI_PN40024_12x_v0_chroms_URGI.fa.gz bank to be formatted for BLAST (see above).

```
echo "zcat GrapeReSeq_Illumina_18K_SNP_vinifera_probes.fa.gz | blastn -query - -task
    megablast -db VITVI_PN40024_12x_v0_chroms_URGI -out /dev/stdout -outfmt 6 |
    gzip > Ill18Kprobes-vinifera_12x0-chroms_megablast.txt.gz" | qsub -cwd -j y -V -
    q normal.q -N blastn-megablast_Ill18Kprobes-vinifera_12x0-chroms
```

The alignments are analyzed in the corresponding task in the script src/vitisomics.R.

Among the 13562 "vinifera" probes, 33 are not aligned on the 12x0 assembly of the PN40024 genome, 24 are aligned on different chromosomes than indicated (mostly plastid genomes), and all the others look fine.

3.4 Manipulations of files from NCBI

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

3.4.1 Reformat sequence headers for VITVI PN40024 8x scaffolds NCBI

Launch script:

Check:

```
\ls vvi_ref_chr* | while read f; do zcat $f; done | wc -1 # 6963886
\ls vvi_ref_chr* | while read f; do zcat $f; done | grep -c ">" # 3514
zcat VITVI_PN40024_8x_scaffolds_NCBI.fa.gz | wc -1 # 6963886
zcat VITVI_PN40024_8x_scaffolds_NCBI.fa.gz | grep -c ">" # 3514
diff <(\ls -v vvi_ref_chr* | while read f; do zcat $f; done) <(zcat
    VITVI_PN40024_8x_scaffolds_NCBI.fa.gz)</pre>
```

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

Basic stats:

```
zcat VITVI_PN40024_8x_scaffolds_NCBI.fa.gz | md5sum #
    a66f86ab2d89eb582935454ae3b7a49d
```

3.4.2 Reformat sequence headers for VITVI_PN40024_12x_v0_scaffolds_NCBI

Launch script:

Check:

```
\ls vvi_ref_12X_chr* | while read f; do zcat $f; done | wc -1 # 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 -1 # 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</pre>
```

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

Basic stats:

3.4.3 Format VITVI_PN40024_8x_scaffolds_NCBI for BLAST

Launch:

3.4.4 Format VITVI_PN40024_12x_v0_scaffolds_NCBI for BLAST

Launch:

3.4.5 Convert gbs files to GFF3

Check that there is one LOCUS entry per scaffold:

```
ls ../../data/ncbi/ARCHIVE/BUILD.1.1/CHRS/vvi_ref_chr*.gbs.gz | grep -v "Pltd" |
while read f; do zcat $f; done | grep -c "LOCUS" # 3514
```

Use the bp_{genbank2gff3.pl} script from BioPerl:

Use the convert_{genbanktogff3.py} script from biocode:

```
zcat ../../data/ncbi/ARCHIVE/BUILD.1.1/CHRS/vvi_ref_chr1.gbs.gz > vvi_ref_8x_chr1.
    gbs
convert_genbank_to_gff3.py -i vvi_ref_8x_chr1.gbs -o vvi_ref_8x_chr1.gff3 --no_fasta
# File "convert_genbank_to_gff3.py", line 196, in <module> main()
# File "convert_genbank_to_gff3.py", line 95, in main
# locus_tag = feat.qualifiers['locus_tag'][0]
# KeyError: 'locus_tag'
```

Additional remarks:

- it is written in Python;
- it uses Biopython, but also custom libraries;
- it is on GitHub;

- it doesn't handle gzipped file as input;
- it skips features not from type gene, mRNA, tRNA, rRNA and CDS.

Use the GFF library from BCBio (not yet integrated into Biopython) as explained here:

```
zcat ../../data/ncbi/ARCHIVE/BUILD.1.1/CHRS/vvi_ref_chr1.gbs.gz > vvi_ref_8x_chr1.
    gbs
genbank_to_gff.py vvi_ref_8x_chr1.gbs
```

Remarks:

- the sequence-region are interspersed in the output file;
- what does the first data line correspond to, with source annotation?
- the source is present in the output as a feature;
- why is =feature = added in the 2nd field?
- why is it written db_xref instead of Dbxref (from official specification)?
- same for note instead of Note?
- exons seem to have 2nd field as feature mRNA

TODO: Use gffutils (doc, code)

TODO: Use a custom script based on Biopython only:

```
genbank2gff3.py -i ../../data/ncbi/ARCHIVE/BUILD.1.1/CHRS/vvi_ref_chr1.gbs.gz -o
    vvi_ref_8x_chr1.gff.gz -t 29760 -g "NCBI 1.1" -s Genbank
```

TODO: check for "pseudo" but empty

3.5 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.
- http://bioconductor.org/packages/release/BiocViews.html#___Vitis_vinifera

TODO: see AnnotationHub

3.5.1 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/urgi/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
```

Using the latest version of Bioconductor and its BSgenome package, 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). Following suggestions from Hervé Pagès (Bioconductor staff):

- the common name field can be Grape;
- the organism_biocview field has to be Vitis_vinifera (see this link).

Forge the target package from the seed file:

```
echo "date; echo \"library(BSgenome); forgeBSgenomeDataPkg(\\"IGGP12Xv2_seed.txt\\\
"); sessionInfo()\" | 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 (INRA, member of IGGP) and other colleagues also from INRA gave positive feedback. I hence sent the package to the Bioconductor team (Hervé Pagès, maintainer of the BSgenome generic package). The 12Xv2 package is now available here, and it also appears in this list.

3.5.2 BSgenome IGGP12Xv0 package

Similarly as for the 12Xv2 package, 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 .
```

Split into one chromosome per file (headers as chr1, chr1_random, etc):

```
zcat VV_chr12x.fsa.gz | awk 'BEGIN{RS=">"} {if(NF==0)next; split($0,a,"\n"); split(a
    [1],b," "); print b[length(b)]; print ">"b[length(b)] > b[length(b)]".fa"; for(i
    =2;i<length(a);++i){print a[i] >> b[length(b)]".fa"}}'
gzip chr*.fa
```

Replace chrUn by chrUkn to be compatible with the 12Xv2:

```
zcat chrUn.fa.gz | sed 's/chrUn/chrUkn/' | gzip > chrUkn.fa.gz
diff <(zcat chrUn.fa.gz) <(zcat chrUkn.fa.gz) # check
rm chrUn.fa.gz</pre>
```

Prepare the seed file (IGGP12Xv0_seed.txt) using the one for IGGP12Xv2 as a template.

Forge the target package from the seed file:

```
echo "date; echo \"library(BSgenome); forgeBSgenomeDataPkg(\\\"IGGP12XvO_seed.txt\\\
"); sessionInfo()\" | 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.IGGP12Xv0; date" | qsub -cwd -j y -V -N build_BSgenome -q normal.q
echo "date; R CMD check BSgenome.Vvinifera.URGI.IGGP12Xv0_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.IGGP12Xv0_0.1.tar.gz
```

The 12Xv0 package is now available here.

3.5.3 BSgenome IGGP8x package

Similarly as for the 12Xv2 and 12Xv0 package, retrieve the sequence data from URGI:

```
cd results/
mkdir -p make_BSgenome_IGGP8X
cd make_BSgenome_IGGP8X/
ln -s ../../data/urgi/VV_chr8x.fsa.gz .
```

Split into one chromosome per file (headers as chr1, chr1_random, etc):

```
zcat VV_chr8x.fsa.gz | awk 'BEGIN{RS=">"} {if(NF==0)next; split($0,a,"\n"); split(a
    [1],b," "); print b[length(b)]; print ">"b[length(b)] > b[length(b)]".fa"; for(i
    =2;i<length(a);++i){print a[i] >> b[length(b)]".fa"}}'
gzip chr*.fa
```

Prepare the seed file (IGGP8X_seed.txt) using the one for IGGP12Xv0 as a template:

```
cp ../make_BSgenome_IGGP12Xv0/IGGP12Xv0_seed.txt IGGP8X_seed.txt
```

Forge the target package from the seed file:

```
echo "date; echo \"library(BSgenome); forgeBSgenomeDataPkg(\\\"IGGP8X_seed.txt\\\");
    sessionInfo()\" | 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.IGGP8X; date" | qsub -cwd -j y -V -N build_BSgenome -q normal.q
echo "date; R CMD check BSgenome.Vvinifera.URGI.IGGP8X_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.IGGP8X_0.1.tar.gz
```

The 8X package is now available here (Bioc 3.3).

3.5.4 TxDb IGGP12Xv0 package from CRIBI annotations (V2.1)

See the "Making and Utilizing TxDb Objects" vignette from the R/Bioconductor package GenomicFeature.

Set up the directory:

```
cd results/
mkdir -p make_TxDb_12Xv0_CRIBI
cd make_TxDb_12Xv0_CRIBI/
```

Make official GFF3 header:

```
echo -e "##gff-version 3" > V2.1_updated.gff3
tar -xzOf ../../data/cribi/Genome12X.tar.gz | awk 'BEGIN{RS=">"} {split($0,a,"\n");
   if(length(a)==0) next; seqlen=0; for(i=2;i<=length(a);++i){seqlen += length(a[i
])}; printf "##sequence-region "a[1]" 1 "seqlen"\n"}' | sort -k2,2V >> V2.1
   _updated.gff3
```

Concatenate the annotations below, using chrukn as was done for the BSgenome packages:

```
zcat ../../data/cribi/V2/V2.1/V2.1.gff3.gz \
   | sed 's/chrUn/chrUkn/g' >> V2.1_updated.gff3
gzip V2.1_updated.gff3
```

Make the package for Bioconductor:

```
echo "date; Rscript -e 'library(GenomicFeatures); library(BSgenome.Vvinifera.URGI.
    IGGP12Xv0); txdb <- makeTxDbFromGFF(file=\"V2.1_updated.gff3.gz\", format=\"auto
\", dataSource=\"CRIBI (http://genomes.cribi.unipd.it/DATA/V2/V2.1/V2.1.gff3;
    meta-data added; chrUn renamed into chrUkn)\", organism=\"Vitis vinifera\",
    taxonomyId=29760, chrominfo=seqinfo(BSgenome.Vvinifera.URGI.IGGP12Xv0));
    makeTxDbPackage(txdb, version=\"0.1\", maintainer=\"Timothee Flutre <timothee.
    flutre@supagro.inra.fr>\", author=\"Timothee Flutre <timothee.flutre@supagro.
    inra.fr>\", license=\"CCO\", pkgname=\"TxDb.Vvinifera.CRIBIV2v1.IGGP12Xv0\");
    sessionInfo()'; date" | qsub -cwd -j y -V -N stdout_make_TxDb -q normal.q
```

A few edits are made to the automatically-generated DESCRIPTION and man/package.Rd files (see https://support.bioconductor.org/p/82278/). A dot is also added at the end of the description.

Build the package and check it:

```
echo "date; R CMD build TxDb.Vvinifera.CRIBIV2v1.IGGP12Xv0; date" | qsub -cwd -j y - V -N build_TxDb -q normal.q
```

```
echo "date; R CMD check TxDb.Vvinifera.CRIBIV2v1.IGGP12Xv0_0.1.tar.gz; date" | qsub -cwd -j y -V -N check_TxDb -q normal.q
```

The target package is now ready to be installed:

```
R CMD INSTALL TxDb.Vvinifera.CRIBIV2v1.IGGP12Xv0_0.1.tar.gz
```

Check that information in the TxDb pkg is identical to information in the GFF3 file: see src/vitisomics.R.

The package was sent to Bioc (valerie.obenchain@roswellpark.org, as advised by Hervé Pagès).

TODO: wait for the pkg to appear on Bioc's website

3.5.5 TxDb IGGP12Xv0 package from Genoscope annotations

Set up the directory:

```
cd results/
mkdir -p make_TxDb_12Xv0_Genoscope
cd make_TxDb_12Xv0_Genoscope/
```

Convert the input GFF2 file into GFF3:

```
ln -s ../../data/urgi/12x_annotation_Genoscope_V0/Vitis_vinifera_annotation.gff.gz .
ln -s ../urgi/VITVI_PN40024_12x_v0_chroms_URGI.fa.gz .
echo "../../src/genoscope_gff2_to_gff3.py --gff2 Vitis_vinifera_annotation.gff.gz --
    gff3 Vitis_vinifera_annotation.gff3.gz" \
    | qsub -cwd -j y -V -N stdout_genoscope_gff2_to_gff3 -q normal.q
```

Use chrukn as was done for the BSgenome packages:

```
zcat Vitis_vinifera_annotation.gff3.gz \
    | sed 's/chrUn/chrUkn/g' | gzip > Vitis_vinifera_annotation_updated.gff3.gz
```

Make the package for Bioconductor:

```
echo "date; Rscript -e 'library(GenomicFeatures); library(BSgenome.Vvinifera.URGI.
    IGGP12Xv0); txdb <- makeTxDbFromGFF(file=\"Vitis_vinifera_annotation_updated.
    gff3.gz\", format=\"auto\", dataSource=\"Genoscope (from URGI at https://urgi.
    versailles.inra.fr/content/download/2157/19376/file/Vitis_vinifera_annotation.
    gff.gz; meta-data added; GFF2 converted into GFF3; chrUn renamed into chrUkn)\",
    organism=\"Vitis vinifera\", taxonomyId=29760, chrominfo=seqinfo(BSgenome.
    Vvinifera.URGI.IGGP12Xv0)); makeTxDbPackage(txdb, version=\"0.1\", maintainer=\"</pre>
```

```
Timothee Flutre <timothee.flutre@supagro.inra.fr>\", author=\"Timothee Flutre < timothee.flutre@supagro.inra.fr>\", license=\"CCO\", pkgname=\"TxDb.Vvinifera.

Genoscope.IGGP12XvO\"); sessionInfo()'; date" | qsub -cwd -j y -V -N stdout_make_TxDb -q normal.q
```

A few edits are made to the automatically-generated DESCRIPTION and man/package.Rd files (see https://support.bioconductor.org/p/82278/). The provider version is set at "0" to follow Grimplet et al (2012). A dot is also added at the end of the description.

Build the package and check it:

```
echo "date; R CMD build TxDb.Vvinifera.Genoscope.IGGP12Xv0; date" | qsub -cwd -j y - V -N build_TxDb -q normal.q
echo "date; R CMD check TxDb.Vvinifera.Genoscope.IGGP12Xv0_0.1.tar.gz; date" | qsub -cwd -j y -V -N check_TxDb -q normal.q
```

The target package is now ready to be installed:

```
R CMD INSTALL TxDb.Vvinifera.Genoscope.IGGP12Xv0_0.1.tar.gz
```

Check that information in the TxDb pkg is identical to information in the GFF3 file: see src/vitisomics.R.

TODO: submit the package to Bioc (valerie.obenchain@roswellpark.org, as advised by Hervé Pagès)

3.5.6 TxDb IGGP8X package from Genoscope annotations

Set up the directory:

```
cd results/
mkdir -p make_TxDb_8X_Genoscope
cd make_TxDb_8X_Genoscope/
```

TODO

3.5.7 TxDb IGGP12Xv0 package from NCBI annotations

TODO: see the "Making New Organism Packages" vignette from the R/Bioconductor package "AnnotationForge"

See also the new genbank package from Bioc http://www.bioconductor.org/packages/release/bioc/html/genbankr.html