The Biological Repository (BioR) and BioRTools User Guide v2.2.x

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BioR is an annotation engine. Inside Mayo, it's primary use is to annotate human variation, but it is not limited to that – it is a general purpose genomic data integration tool that enables coordinate based searches and joins based on strings. BioR is like programming using lego blocks, each block may not be exactly what you want, but you can put the blocks together to create programs extremely rapidly. The component 'blocks' include all existing UNIX commands, stand alone tools (e.g. bedtools), and the bior_toolkit. This user guide will help get you up to speed in how to use BioR in one document. Please note that BioR is a complex system, and you should have some experience with UNIX (especially pipes) before using BioR. BioR consists of two parts 1) the BioR toolkit which depends on Java (some commands also depend on SNPEFF and VEP) and 2) the BioR catalogs which are the data files used by the system. A BioR catalog is basically a BED-JSON hybrid file that is indexed using Tabix for coordinate based search and BioR's own indexing system for string matching based searches.

1. Installation:

Installing on a Stand-Alone Server or Workstation (or on a server on the cloud)

This includes Java JDK 1.7, Tabix and BGZIP. Most BioR functions will still work if you don't install SNPEFF/VEP and all of their dependencies, but bior_annotate will have limited functionality and bior_vep and bior_snpeff will not work. The environment variable, `$BIOR_LITE_HOME` represents the location where BioR is installed.

Installing the BioR software

System Requirements

Linux/Unix Distribution: preferably Ubuntu 12.04.
Memory: 4gb (8gb prefered)
Architecture: 64 bit system
Disk Space: 30Gb+ (Toolkit requires 1GB, catalogs can very, please see the BioR download website for more details)

Prerequisites:

BioR is written in Java, so in principle it will work on any machine, but it depends on some command line tools (e.g. SNPEFF, VEP) that are not so friendly. The development team has BioR working on both Macintosh and Linux. To install, first make sure first that Java 1.6+ is installed and on your path (Java 1.7 is preferred). Then download the BioR executable and place it in your path.

Java Installation:

We prefer to use the official version of Java directly from Oracle (Sun). Use uname -a on your Linux machine to determine the version of Java that you should be running on your server. Sign up for an Oracle account (if needed) and download the correct version. Copy it to your server (i.e. ~ - your home directory for this guide). You can run java -version to see if it is already installed on your server to skip this step.

Download Link: http://www.oracle.com/technetwork/java/javase/downloads/index.html

Installation Instructions:

Check the version of your Linux OS by typing 'uname -a' at the command line. if it says x86_64, you have a 64bit system (most

systems). Based on the version of your OS, download Java directly from Oracle. We recommend JDK 1.7. (I usually download it to my home computer and then scp it up to the server using winscp or scp from the command prompt on a mac. This will result in a file in your home directory called jdk-7u45-linux-i586.tar.gz. Unzip Java and set your JAVA_HOME.

Once you have java downloaded to your PC/Mac, you will need to copy it up to your server. If you have a mac, you can use scp from the command line, on Windows, you can use WinSCP or similar program. For example to upload from my Mac I use the following scp command (after downloading from Oracle):

```
$ scp -i biorloginkey.pem ~/Downloads/jdk-7u45-linux-x64.tar.gz
ubuntu@10.148.2.10:~/jdk-7u45-linux-x64.tar.gz
```

Yours will differ because your cloud server will be on a different IP and you will have a different credential file (the .pem).

This will place a copy of Java in my home directory (~ = /home/ubuntu) Extract it.

```
ubuntu@biorinstall2:~$ pwd
/home/ubuntu
ubuntu@biorinstall2:~$ tar -zxvf jdk-7u45-linux-x64.tar.gz
```

Then you will need to put it in your path:

```
ubuntu@biorinstall2:~$ ls
bior_2.2.0  bior_2.2.0.tar.gz  jdk1.7.0_45  jdk-7u45-linux-x64.tar.gz
ubuntu@biorinstall2:~$ JAVA_HOME=/home/ubuntu/jdk1.7.0_45
ubuntu@biorinstall2:~$ PATH=$PATH:$HOME/bin:$JAVA_HOME/bin
ubuntu@biorinstall2:~$ export $JAVA_HOME
-bash: export: `/home/ubuntu/jdk1.7.0_45': not a valid identifier
ubuntu@biorinstall2:~$ export JAVA_HOME
ubuntu@biorinstall2:~$ export PATH
ubuntu@biorinstall2:~$ javac
(usage information should be here, if not it is not correctly installed)
```

I put these commands in ~/.bash_profile so that they are in place next time I log in.

Tabix / BGZIP:

Install:

Make sure you have a version of bgzip2 installed:

```
$ sudo apt-get install bzip2
```

You may also be able to install tabix in the same way:

```
$ sudo apt-get install tabix
```

Tabix Manual install:

If the apt-get command fails, you can download and install it yourself:

Download Link: http://sourceforge.net/projects/samtools/files/tabix/

In this case, we chose the 0.2.6 version.

Download from the link above and unzip:

```
$ bunzip2 tabix-0.2.6.tar.bz2
$ tar -xvf tabix-0.2.6.tar
$ cd tabix-0.2.6/
```

Compile Tabix:

```
$ make
```

Add Tabix and BGZIP to your path:

```
$ PATH=~/tabix-0.2.6:$PATH
$ which tabix
/home/dquest/tabix-0.2.6/tabix
```

You may want this command also to be in your .bashrc file so that tabix, bgzip work properly.

SNPEFF+VEP:

SNPEFF+VEP have complex setups, please see below in the user guide on how to set them up and get them working with the toolkit. Note, you will not need these tools for most of the BioR commands or the quickstart.

BioR Toolkit Installation:

Download Link:

You can download BIOR and Catalog datasources from http://bioinformaticstools.mayo.edu/research/bior/.

Installation Instructions:

1) Download the toolkit: (e.g.)
$ wget https://s3-us-west-2.amazonaws.com/mayo-bic-tools/bior/bior_2.2.0.tar.gz

2) Unzip the bior_version zip file you downloaded. (unzip bior_version.zip -d target directory) e.g.:
$ tar -xzvf bior_2.2.0.tar.gz

3) Make sure all your files in bior_pipeline project are executable:
$ cd bior_2.2.0/
$ chmod -R +x bin/

4) Now you need to setup the environment variables and add to the path.
$ export BIOR_LITE_HOME=YOUR BIOR_ FOLDER
$ export PATH=$BIOR_LITE_HOME/bin:$PATH
There is a quick script that comes with BioR that can help with the setup: setupEnv.sh. Just source the file:
$ source setupEnv.sh

You will need to setup your paths each time you login so it might make sense to add this command into your .bash_profile/.bashrc.

5) Now try bior_ and press tab key twice on terminal. Now you should see all bior commands displayed. If they are not being displayed, look inside the setupEnv.sh and change the paths so that they work with your envorinment. (BioR is using BASH). Change it as needed or ask a system administrator for help. Make sure you can type bior_(tab tab) and get all of the commands back before moving on to the next step.

6) Verify that it is installed correctly by typing bior_pretty_print -h. You should see a help message, if you see an error like “java: not found” then you need to install java correctly.

Now you have successfully installed the toolkit. The quickstart guide is a good place to go to check if your toolkit is functioning properly and to run some biologically motivated examples contributed from our bioinformaticians (they even use versions of these in production!).

One of the hardest to set up commands is bior_annotate. Bior_annotate is a kitchen sink command and requires the catalogs, command line tools and all dependencies be installed properly on your system. The next three sections will go over how to install all of the catalogs it needs, and how to install SNPEFF and VEP. For now, it is important to highlight the bior.properties file in $BIOR_LITE_HOME/conf. For example:

```
$ pwd
/home/ubuntu/bior_2.2.0/conf
ubuntu@biorinstall2:~/bior_2.2.0/conf$ ls
allCatalogs.columns.properties  allCatalogs.columns.tsv  bior.properties  cli
log4j.properties  tools
```

Edit the configuration file so that all tools, catalogs, and paths are consistent with your install locations (see the next section).

Installing the Biological Repository Catalogs

Catalogs can be found at $BIOR_CATALOG ($bior in this documentation) If you are doing a stand alone server, download the catalog flat files and place them locally on your server in a similar directory structure. BioR Tools does not make any assumptions about the location of catalogs relative to each other, but it does assume that tabix indexes are in the same directory as the compressed catalog and that ID indices are in a folder called index in the same directory as the catalog. However, bior_annotate does have a configuration file that will make that command not work if you don't change the configuration file or place the data repositories in the same location as we do at Mayo (or provide a symbolic link). We put the data here: $BIOR_CATALOG=/data5/bsi/catalogs/bior/v1. More details for installing the catalog structure properly on a stand alone server can be found in the next section "Installing on a Stand-Alone Server or Workstation" (next).

1) use wget to get all of the BioR catalogs and place them in /data. There are two scripts:
$BIOR_LITE_HOME/scripts/downloadFullCatalogs.sh
and
$BIOR_LITE_HOME/scripts/downloadSmallCatalogs.sh
that can be used to easily download the production catalogs and example catalogs respectively. For example do this to download the full catalogs:

```
$ cd $BIOR_LITE_HOME
$ cd scripts
$ ./downloadFullCatalogs.sh /data/
```

This will download and extract the downloaded catalogs into the /data directory.

3) Now, for bior_annotate to work, you will need to set the properties (for all of the rest of BioR, you are good to go).

You will find a file named *bior.properties* under the folder conf in your bior_version directory (See the section above on installing the toolkit). This is the file where you need to set the tools path and home path of catalogs directory. Tool commands like bior_vep and bior_snpeff and as well as bior_annotate make use of this properties file. My file is here:

```
$ pwd
/home/ubuntu/bior_2.2.0/conf
$ ls
allCatalogs.columns.properties  allCatalogs.columns.tsv  bior.properties  cli
log4j.properties  tools
```

The rest of the setup guide will assume that your bior.properties file is set up as follows (note fileBase = /data/ and it MUST end in a slash), the paths you may need to change are in bold:

```
$ cat bior.properties
###============================================================================================
### NOTE: These keys are defined in BiorProperties.java and must match the keys in the enum there
###============================================================================================

###SNPEFF ==========================================
SnpEffJar=/data/snpEff_2_0_5d/snpEff.jar
SnpEffConfig=/data/snpEff_2_0_5d/snpEff.config
SnpEffMaxHeap=4g

###VEP ==========================================
BiorVepPerl=/usr/bin/perl
BiorVep=/data/variant_effect_predictor/variant_effect_predictor.pl
BiorVepCache=/data/variant_effect_predictor/cache/


###BIOR_TREAT ======================================
### AnnotateMaxLinesInFlight:
### NOTE: Min = 2.  Default = 10.  Max = 50 (could do more, but not recommended)
### WARNING: Do not increase it to much more than 50 or you may encounter a hang state, especially
with a high number of fanouts, as the process buffers will overflow!
AnnotateMaxLinesInFlight=10
### NOTE: make sure the "fileBase" path ends with a slash!
fileBase=/data/
genesFile=NCBIGene/GRCh37_p10/genes.tsv.bgz
bgiFile=BGI/hg19/LuCAMP_200exomeFinal.maf_GRCh37.tsv.bgz
espFile=ESP/build37/ESP6500SI_GRCh37.tsv.bgz
hapMapFile=hapmap/2010-08_phaseII+III/allele_freqs_GRCh37.tsv.bgz
dbsnpFile=dbSNP/137/00-All_GRCh37.tsv.bgz
dbsnpClinvarFile=dbSNP/137/clinvar_20130226_GRCh37.tsv.bgz
cosmicFile=cosmic/v63/CosmicCompleteExport_GRCh37.tsv.bgz
kGenomeFile=1000_genomes/20110521/ALL.wgs.phase1_release_v3.20101123.snps_indels_sv.sites_GRCh37.tsv.b
gz
blacklistedFile=ucsc/hg19/wgEncodeDacMapabilityConsensusExcludable_GRCh37.tsv.bgz
repeatFile=ucsc/hg19/rmsk_GRCh37.tsv.bgz
regulationFile=ucsc/hg19/oreganno_GRCh37.tsv.bgz
uniqueFile=ucsc/hg19/wgEncodeDukeMapabilityRegionsExcludable_GRCh37.tsv.bgz
tssFile=ucsc/hg19/switchDbTss_GRCh37.tsv.bgz
tfbsFile=ucsc/hg19/tfbsConsSites_GRCh37.tsv.bgz
enhancerFile=ucsc/hg19/vistaEnhancers_GRCh37.tsv.bgz
### conservationFile=ucsc/hg19/phastConsElements46wayPrimates_GRCh37.tsv.bgz
conservationFile=ucsc/hg19/phastConsElements46way_GRCh37.tsv.bgz
hgncFile=hgnc/2012_08_12/hgnc_GRCh37.tsv.bgz
hgncIndexFile=hgnc/2012_08_12/index/hgnc_GRCh37.Entrez_Gene_ID.idx.h2.db
hgncEnsemblGeneIndexFile=hgnc/2012_08_12/index/hgnc_GRCh37.Ensembl_Gene_ID.idx.h2.db
omimFile=omim/2013_02_27/genemap_GRCh37.tsv.bgz
omimIndexFile=omim/2013_02_27/index/genemap_GRCh37.MIM_Number.idx.h2.db
mirBaseFile=mirbase/release19/hsa_GRCh37.p5.tsv.bgz
```

Now in the file you need to set fileBase="catalogs directory" value to your catalogs directory.

If you chose to download the chr17-only catalogs, make sure to update the paths in the bior.properties file as appropriate.

Some users notice a problem with Sage (an error in the bior.log file when running with the --log flag) not

being able to make a connection. If you get this error, make sure the SAGE_ENVIRONMENT is set as follows: SAGE_ENVIRONMENT=null, (or =prod)
in the Global.properties file (found in: /home/ubuntu/bior_2.2.0/conf/cli on the example system, or $BIOR_LITE_HOME/conf/cli)

Example : fileBase=/data/
Next step is tools installation.

Dependant Tools Installation and Setup

We have integrated two tools SNPEff and Variant Effect Predictor (VEP) into our toolkit.

Variant Effect Predictor (VEP):

The Version of VEP we support is 2.7.
http://useast.ensembl.org/info/docs/tools/vep/script/vep_download.html#versions

NOTE: There is a breaking change in the latest VEP tools that replaces the --hgnc flag with --symbol. Make sure to use version 2.7 to avoid this!

You can follow the installation instructions in the above page. Here is how we install it step by step:

1) Download VEP from their website:

```
$ cd /data
$ curl -o variant_effect_predictor.v69.tar.gz
"http://cvs.sanger.ac.uk/cgi-bin/viewvc.cgi/ensembl-tools/scripts/variant_effect_predictor
.tar.gz?view=tar&root=ensembl&pathrev=branch-ensembl-69"
```

NOTE: The quotes in the command above can cause problems when copy-and-pasting from this document into your terminal windows. If that is the case, try deleting the quotes at the command line and adding them back in by typing them manually.

2) Extract

```
$ tar -xzvf variant_effect_predictor.tar.gz
```

3) Make sure you have Perl version 14 on your computer:

```
$ perl -v
```

4) If the perl is the correct version, and you have admin rights, you can now install the Perl libraries needed by VEP:

```
$ sudo apt-get install libwww-perl
$ sudo perl -MCPAN -e'force install "LWP::Simple"'
$ sudo apt-get install libdbi-perl
$ sudo apt-get install libdbd-mysql-perl
```

You can also download the Perl libraries separately and point your PERL5LIB environment variable to them. For more information, see:
http://linuxgazette.net/139/okopnik.html

5) Then run the VEP installer:

```
cd variant_effect_predictor

perl INSTALL.pl [options]
```

6) Make sure to download the needed cache files or correct the install (in my case the download script failed)
It may ask you to install cache files into your ~/.vep directory. If you choose to do this, and it succeeds, make sure to update the bior.properties file outlined in a previous step to point to this directory. Choose the two options for homo_sapiens by specifying them both, separated by a space:

```
25 : homo_sapiens_refseq_vep_73.tar.gz
26 : homo_sapiens_vep_73.tar.gz
```

You may follow instructions at http://www.ensembl.org/info/docs/api/api_installation.html which provides alternate options for the API installation. Example download of the cache files.

```
 $ nohup wget
ftp://ftp.ensembl.org/pub/release-69/variation/VEP/homo_sapiens_vep_69.tar.gz &
 $ nohup wget
ftp://ftp.ensembl.org/pub/release-69/variation/VEP/homo_sapiens_vep_69_sift_poly
phen.tar.gz &
```

7) unzip the alignment files to a directory called "cache" inside the same directory as VEP.

```
cd /data/variant_effect_predictor/
mkdir cache
cd cache
tar -zxvf ../homo_sapiens_vep_69_sift_polyphen.tar.gz
tar -zxvf ../homo_sapiens_vep_69.tar.gz
```

8) Change the bior.properties file to point at the version of vep that you just installed.

```
###VEP ============================================
BiorVepPerl=/usr/bin/perl
BiorVep=/data/variant_effect_predictor/variant_effect_predictor.pl
BiorVepCache=/data/variant_effect_predictor/cache/
```

9) Test that VEP works stand-alone on a VCF file to ensure it is installed correctly. Here is an example command (note there is an example.vcf is in $BIOR_LITE_HOME/examples/quickstart2/example.vcf but any properly formatted vcf will work):

```
perl /data/variant_effect_predictor/variant_effect_predictor.pl -i example.vcf -o
STDOUT -dir /data/variant_effect_predictor/cache/ -vcf -polyphen b -sift b --offline
```

10) Test that VEP works inside the BioR wrapper:

```
cat example.vcf | bior_vep > vepAnnotated.tjson
```

NOTE: If VEP fails here, try it again with the --log flag, then cat the bior.log file. If it shows an error similar to "Unable to locate file on classpath: /coreutils-8.21/linux-i686/stdbuf", then you are most likely trying to run it on a 32-bit OS. This is currently not supported. Please try on a 64-bit OS for now.

SNPEff:

Currently we support SNPEff verison 2.0.5d.This was recommended by GATK for worst pick logic. Installation files and instructions can be found at
http://snpeff.sourceforge.net/download.html
If you using linux or Mac you can just use wget command to download the files in steps 2 and 3. After doing so, make sure to change SNPEFF config file snpEff.config to include the path to the database you downloaded.

1) Make sure you have unzip installed so you can extract the zip file (on ubuntu linux, use apt-get. Use yum or whatever package manager to install unzip on other boxes):

```
$ sudo apt-get install unzip
```

2) Extract SNPEFF

```
$ cd /data
$ wget http://sourceforge.net/projects/snpeff/files/snpEff_v2_0_5d_core.zip
$ unzip snpEff_v2_0_5d_core.zip
```

3) By default, SNPEFF expects that you place the data in a directory called 'data' residing in the same directory as snpEff.jar (SNPEFF_HOME). Extract the data for SNPEFF to SNPEFF_HOME/data.

```
$ cd snpEff_2_0_5d
$ wget
http://sourceforge.net/projects/snpeff/files/databases/v2_0_5/snpEff_v2_0_5_GRCh37.64.zip
$ unzip snpEff_v2_0_5_GRCh37.64.zip
###(This will create the /data/snpEff_2_0_5d/data/ directory)
```

4) After you have installed SNPEff, set the paths in bior.properties file located in conf folder under your bior_pipeline directory.
Example:
###SNPEFF ==========
SnpEffJar=/data/snpEff_2_0_5d/snpEff.jar
SnpEffConfig=/data/snpEff_2_0_5d/snpEff.config

5) check that SNPEFF works as a stand alone tool. This will take at least a minute to load the database before it starts processing vcf lines (note there is an example.vcf is in $BIOR_LITE_HOME/examples/quickstart2/example.vcf but any properly formatted vcf will work).

```
cat example.vcf | java -Xmx4g -jar /data/snpEff_2_0_5d/snpEff.jar eff -c
/data/snpEff_2_0_5d/snpEff.config -v -o vcf -noLog -noStats GRCh37.64
```

NOTE: If you are running on a system with less than 4GB of memory, this will throw an exception. Run again with the --log flag, then check the bior.log file for an error similar to "AbnormalExitException". Please change the -Xmx4g option to a smaller value to fit your hardware limitations (such as -Xmx2g). Likewise, if you have more memory than that, or get an exception because SnpEff runs out of memory, you can up the memory as well.

6) Run the BioR wrapper for SNPEFF:
cat example.vcf | bior_snpeff > annotated.tjson

NOTE: As before, the memory allocated to SnpEff might be a problem. For the bior_snpeff command, you can specify the max heap size by altering this field in the bior.properties file:

```
SnpEffMaxHeap=4g
```

Instructions for Mayo Users.

There is no need to install anything, just use the mayobiotools command on the RCF. Please read the drupal documentation here: http://bsiweb.mayo.edu/bior-20-installing-command-line-client

Installing BioR Tools from Source

Source installation requires that you have both Java 1.7 and Maven installed and on your path. It also requires that you have access to the Mayo NEXUS servers or you place several libraries in your ~/.m2 directory.

If you have troubles installing BioR or compiling it, please contact the BioR Team (dlrstitbiorall@mayo.edu) so we can update the documentation and make the process easier.

Java Heap Size

On some machines, the default JVM size is 2GB. This is very large for BioR. By default the BioR toolkit is capped at 128M. To change this setting, change the Maven bior_pipeline/pom.xml (e.g. <jvmOpts>-Xmx128m</jvmOpts>).

2. Overview

Introduction

BioR uses a Pipe-And-Filter architecture. Data to be annotated by BioR is streamed through a pipeline, a sequence of one or more pipes. Pipes is based on Flow Based Programming by J.P. Morrison. DataFlow-Article, Flow-Based-Programing.

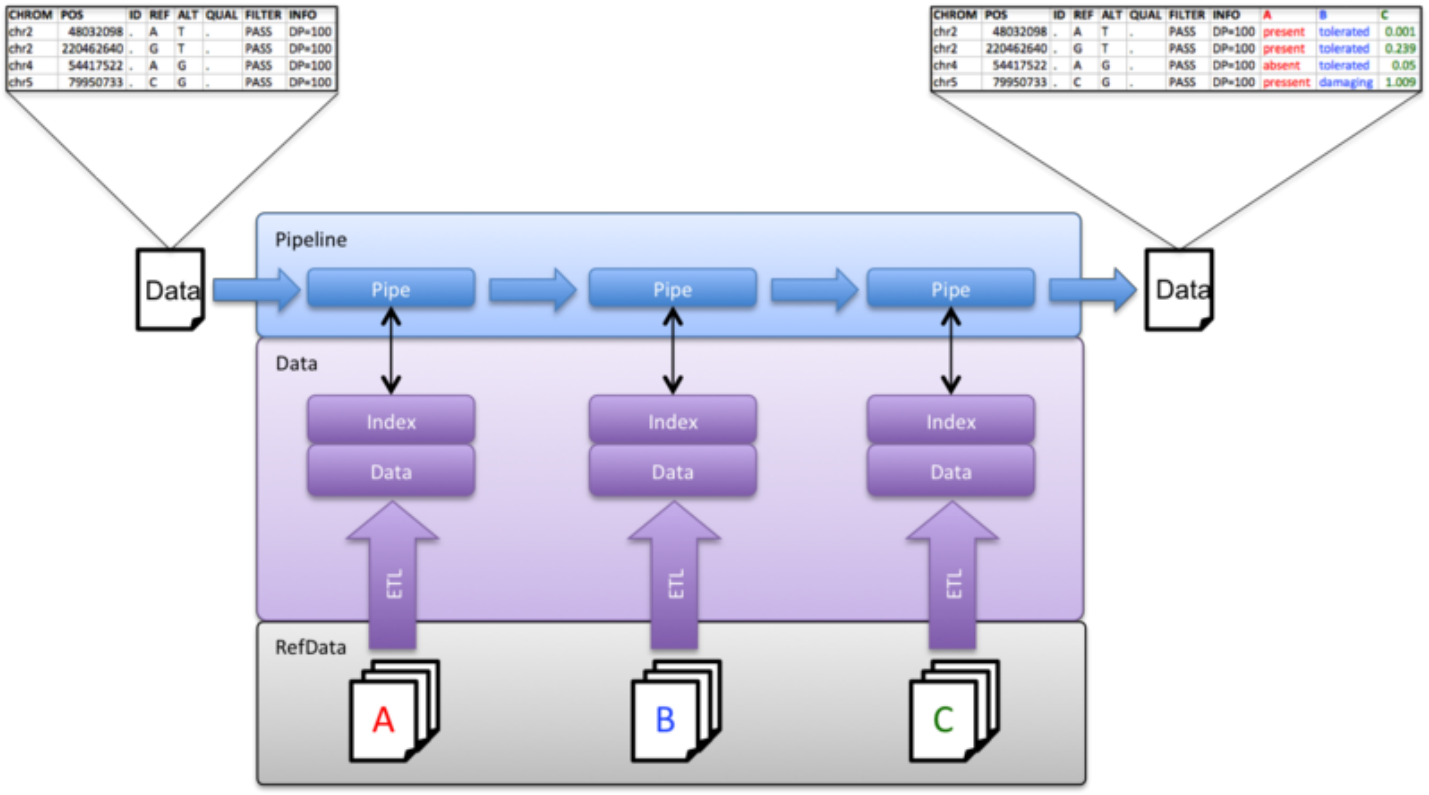
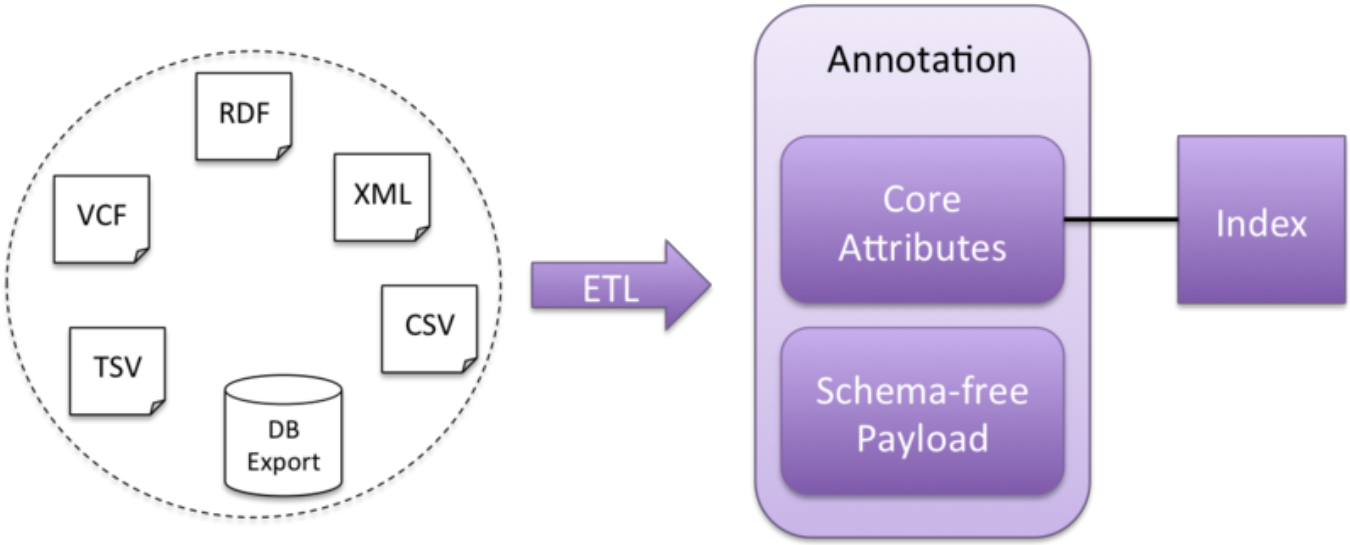


Figure 1: BioRTools works by adding annotation to the right on the original file.

BioR leverages UNIX pipes to flow data from program to program. As BioR programs work on the data, they place annotation to the right (the red, blue and green columns in Figure 1).

Data Modeling

BioR has adopted a lightweight approach to modeling annotation data. Only **core** annotation fields are modeled to enable supported search capabilities (e.g. coordinate search, accession ID search). Anything not classified as **core** is modeled into a "schema-free" data structure.



BioR Catalog Shortcut

BioR commands commonly use long paths to files. One of the first things you will want to do when using BioR is to make an alias to the location of the BioR catalogs. For example if the BioR catalogs are located in `$bior`
Then, on bash, execute the following command at the command line:

```
$ export bior=/data/path/
```

You may want to put this command in your .bashrc or .bash_profile so that the $bior environment variable shows up next time you log in.

Finding out what is in a Catalog

Each data source is 'published' into a BioR catalog file for use by the BioR scripts. A Catalog is a collection of files (both data and indexes) that is understood by the BioR Pipes infrastructure. BioR's reference data consists of the raw files downloaded/updated and made available to BioR users. These files ARE NOT catalogs. Catalogs are transformed into the BioR standard catalog structure so that pipes can work on the content. BioR catalogs are bgziped files[1] that contain 4 columns (_landmark, _minBP, _maxBP, and JSON). A more comprehensive description of the BioR catalog format is in Chapter 3.

To see what is in a catalog, use the `zcat` command (`gzcat` on a mac) followed by the catalog filename, followed by `less`:

[1] http://samtools.sourceforge.net/tabix.shtml

```
$ zcat $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz | less
1       10954   11507
{"_type":"gene","_landmark":"1","_strand":"+","_minBP":10954,"_maxBP":11507,"gene":"LOC100506145","
te":"Derived by automated computational analysis using gene prediction method: GNOMON. Supporting
evidence includes similarity to: 1 Protein","pseudo":"","GeneID":"100506145"}
...
```

Unix `less` is a good-low-memory command to look at data. Type `q <enter>` to quit `less`. Type `man less` at the command line to see how to use the less command. You can use up and down arrows to scroll through the data a line at a time or 'f' and 'b' to scroll a page at a time.

Showing the Commands in BioR Toolkit

All BioR commands start with `bior_` so once BioRTools is installed and on your path you can type `bior_` followed by the tab key (twice) and it will show you all of the current commands in the toolkit:

```
$ bior_
bior_annotate                       bior_create_catalog_props       bior_lookup
bior_snpeff                         bior_vep                        bior_bed_to_tjson
bior_create_config_for_tab_to_tjson bior_overlap                    bior_tab_to_tjson
bior_compress                       bior_drill                      bior_pretty_print
bior_tjson_to_vcf                   bior_create_catalog             bior_index_catalog
bior_same_variant                   bior_vcf_to_tjson
```

Table 1 has a more complete description of these commands.
Commands in the toolkit operate on tab delimited data with a VCF style header (starting with "#"). Commands in the toolkit insert additional annotation to the right. Raw annotation is obtained by comparing JSON objects in columns to JSON objects in catalogs. Table 1.0 shows the format of columns <in,out> of each BioR function. For example bior_vcf_to_tjson takes as an input VCF columns (and the header) and outputs VCF + JSON in the last column.

Command	Input, Output	Description
Transform Functions		
bior_overlap	TJSON, TJSON	Extract annotations from a catalog based on genomic location overlap. The overlap is computed from the Start and End genomics position of a variant.
bior_same_variant	TJSON,	Extract annotations from a catalog based on

	TJSON	variant position, reference and alternate allele definition.
bior_lookup	TJSON, TJSON	Extract annotations from a catalog based on matching values of an identifier.
bior_snpeff	TJSON, TJSON	Use SNPEffect[1] to annotate variants. Chromosome ID, Start and Stop genomics position, reference and alternate allele of the variant is required .
bior_vep	TJSON, TJSON	Use VEP[2] to annotate variants. Chromosome ID, Start and Stop genomics position, reference and alternate allele of the variant is required.
bior_drill	TJSON, TJSON	Extract an element from nested JSON string.
bior_compress	TJSON, TJSON	Compress entries from provided set of identifiers into a single entry with each value separated by a delimiter.
Utility Functions		
bior_index_catalog	identifier, index	Index the specified identifier in a catalog. Indices a stored in a separate index file.
bior_create_catalog	TJSON, catalog	Convert a text tabulated file into a catalog. Chromosome ID, Start and End genomics position fields have to be explicitly named.
bior_ create_catalog_props	catalog, property	Create property files from the metadata extracted from a catalog. Property files are needs for proper metadata handling.
bior_create_config_for_tab_to_tjson	TSV,config	Create a configuration file that describes column description. This file is needed when uploading a tab delimited file.
Input/Output Functions		
bior_vcf_to_tjson	VCF, TJSON	Load a VCF file and convert to TJSON format.
bior_tjson_to_vcf	TJSON, VCF	Convert TJSON to VCF format for file output.
bior_bed_to_tjson	BED, TJSON	Load a BED file and convert to TJSON format.
bior_tab_to_tjson	TSV, TJSON	Load a tab-delimited file and convert to TJSON format.

bior_pretty_print	TJSON, STDOUT	Convert TJSON in a readable format for screen or file output.
Miscellaneous Functions		
bior_annotate	VCF, TJSON	Append to the VCF 'info' field a set of commonly used annotations.

Table 1: List of commands available in the BioR Toolkit. Detailed description and example is displayed when executing the command with the –h flag.

[1]Cingolani, P. et al. (2012) A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin). 6(2) :p. 80-92.

[2]McLaren W et al. (2010) Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor.BMC Bioinformatics 26(16):2069-70

Most every one of these commands supports the –h (help) flag to get information about how to use the command. To get help on bior_vcf_to_tjson type:

```
$ bior_vcf_to_tjson -h

NAME
bior_vcf_to_tjson -- converts VCF data into JSON as an additional column

SYNOPSIS
bior_vcf_to_tjson [--log] [--help]
...
```

Several of the above functions use 'Golden Identifiers' to match records across catalogs. Table 2 shows the current golden identifiers used in the codebase and what function(s) use them.

'Golden Identifier'	Functions	Definition
`landmark`	`bior_overlap`, `bior same variant`	Chromosome, or sequence ID that the interval is located on
`minBP`	`bior_overlap`, `bior same variant`	Minimum 1-based position (e.g. NCBI coordinates) on the landmark sequence
`maxBP`	`bior_overlap`, `bior same variant`	Maximum 1-based position on the landmark sequence
`_refAllele`	`bior same variant`	REF as in VCF standard
`_altAlleles`	`bior same variant`	ALT as in VCF standard

Pretty Print

Data in the 4th column of a catalog is stored as JSON. JSON can be deeply nested and hard to read if it is all smashed into one line. BioR has a command `bior_pretty_print` that can make reading JSON text easier. Take the earlier example and replace `less` with `bior_pretty_print`:

```
$ zcat $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz | bior_pretty_print
#  COLUMN NAME  COLUMN VALUE
-  -----------  ------------
1  UNKNOWN_1    1
2  #UNKNOWN_2   10954
3  #UNKNOWN_3   11507
4  #UNKNOWN_4   {
                 "_type": "gene",
                 "_landmark": "1",
                 "_strand": "+",
                 "_minBP": 10954,
                 "_maxBP": 11507,
                 "gene": "LOC100506145",
                 "note": "Derived by automated computational analysis using gene prediction method:
GNOMON. Supporting evidence includes similarity to: 1 Protein",
                 "pseudo": "",
                 "GeneID": "100506145"
               }
$
```

Use –r to specify the row to pretty print. This is very useful when handling sparse data, where the values for columns you are interested in do not appear on every line. In JSON if there is no value for a given key, the key is not shown (instead of reporting NULL), so you may need to hunt around in the dataset a bit to find keys of interest.

Get all Variants in a Gene

Lets do something useful -- say we wanted all genetic variants in VCF format that overlap the BRCA1 gene from dbSNP. This section will illustrate how to use BioR to rapidly build a program that does just that. BioR is executed at the Linux/UNIX command line, so any command that is available at the command line can be used with BioR (grep, cut, sed, awk, perl, …). Lets start with the echo command to find BRCA1 in the gene catalog.

```
$ echo "BRCA1" | bior_lookup -p gene -d $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz | bior_pretty_print
#  COLUMN NAME  COLUMN VALUE
-  -----------  ------------
1  UNKNOWN_1    BRCA1
2  LookupPipe   {
                 "_type": "gene",
                 "_landmark": "17",
                 "_strand": "-",
```

```
                 "_minBP": 41196312,
                 "_maxBP": 41277500,
                 "gene": "BRCA1",
                 "gene_synonym": "BRCAI; BRCC1; BROVCA1; IRIS; PNCA4; PPP1R53; PSCP; RNF53",
                 "note": "breast cancer 1, early onset; Derived by automated computational analysis
using gene prediction method: BestRefseq.",
                 "GeneID": "672",
                 "HGNC": "1100",
                 "HPRD": "00218",
                 "MIM": "113705"
               }
$
```

The UNIX pipe ('|') allows you to stream the output of one command to the next. In this example, echo prints BRCA1 to the screen. `bior_lookup` uses this ID to find the entry in the gene catalog with the key gene and value 'BRCA1'. Now we have the genomic coordinates for BRCA1. Lets use these positions to find all catalog entries in dbSNP that are between 41196312 and 41277500 on chromosome 17.

```
$  echo "BRCA1" | bior_lookup -p gene -d $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz | bior_overlap -d
$bior/dbSNP/137/00-All_GRCh37.tsv.bgz | bior_pretty_print
#  COLUMN NAME  COLUMN VALUE
-  -----------  ------------
1  UNKNOWN_1    BRCA1
2  LookupPipe   {
                 "_type": "gene",
                 "_landmark": "17",
                 "_strand": "-",
                 "_minBP": 41196312,
                 "_maxBP": 41277500,
                 "gene": "BRCA1",
                 "gene_synonym": "BRCAI; BRCC1; BROVCA1; IRIS; PNCA4; PPP1R53; PSCP; RNF53",
                 "note": "breast cancer 1, early onset; Derived by automated computational analysis
using gene prediction method: BestRefseq.",
                 "GeneID": "672",
                 "HGNC": "1100",
                 "HPRD": "00218",
                 "MIM": "113705"
               }
3  OverlapPipe  {
                 "CHROM": "17",
                 "POS": "41196363",
                 "ID": "rs8176320",
                 "REF": "C",
                 "ALT": "T",
                 "QUAL": ".",
                 "FILTER": ".",
```

```
                "INFO": {
                  "RSPOS": 41196363,
                  "RV": true,
                  "GMAF": 0.0050,
                  "dbSNPBuildID": 117,
                  "SSR": 0,
                  "SAO": 0,
                  "VP": "050000800201040517000100",
                  "GENEINFO": "BRCA1:672",
                  "WGT": 1,
                  "VC": "SNV",
                  "REF": true,
                  "U3": true,
                  "VLD": true,
                  "HD": true,
                  "GNO": true,
                  "KGPhase1": true,
                  "KGPROD": true,
                  "OTHERKG": true,
                  "PH3": true
                },
                "_id": "rs8176320",
                "_type": "variant",
                "_landmark": "17",
                "_refAllele": "C",
                "_altAlleles": [
                  "T"
                ],
                "_minBP": 41196363,
                "_maxBP": 41196363
              }
$
```

This command shows the first match in dbSNP that overlaps the BRCA1 gene according to the NCBI annotation. The version of dbSNP used to publish the catalog was a VCF file, therefore many fields from the VCF standard are represented in the JSON. A combination of the UNIX `cut` command and `bior_drill` can quickly extract a VCF file. When trying this example, decompose the commands and use them one at a time to understand what each command is doing.

```
$ echo "BRCA1" | bior_lookup -p gene -d $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz | bior_overlap -d
$bior/dbSNP/137/00-All_GRCh37.tsv.bgz |  bior_drill -p CHROM -p POS  | cut -f 1,3,4 | head -10

##BIOR=<ID="bior.gene37p10",Operation="bior_lookup",DataType="JSON",ShortUniqueName="gene37p10",Sou
e="NCBIGene",Description="NCBI's Gene Annotation directly from the gbs
file",Version="37p10",Build="GRCh37.p10",Path="/data5/bsi/catalogs/bior/v1/NCBIGene/GRCh37_p10/gene
tsv.bgz">
##BIOR=<ID="bior.dbSNP137",Operation="bior_overlap",DataType="JSON",ShortUniqueName="dbSNP137",Sour
```

```
="dbSNP",Description="NCBI's dbSNP Variant
Database",Version="137",Build="GRCh37.p5",Path="/data5/bsi/catalogs/bior/v1/dbSNP/137/00-All_GRCh37
sv.bgz">
##BIOR=<ID="bior.dbSNP137.CHROM",Operation="bior_drill",Field="CHROM",DataType="String",Number="1",
eldDescription="Chromosome. (VCF
field)",ShortUniqueName="dbSNP137",Source="dbSNP",Description="NCBI's dbSNP Variant
Database",Version="137",Build="GRCh37.p5",Path="/data5/bsi/catalogs/bior/v1/dbSNP/137/00-All_GRCh37
sv.bgz">
##BIOR=<ID="bior.dbSNP137.POS",Operation="bior_drill",Field="POS",DataType="Integer",Number="1",Fie
Description="The reference position, with the 1st base having position 1. (VCF
field)",ShortUniqueName="dbSNP137",Source="dbSNP",Description="NCBI's dbSNP Variant
Database",Version="137",Build="GRCh37.p5",Path="/data5/bsi/catalogs/bior/v1/dbSNP/137/00-All_GRCh37
sv.bgz">
#UNKNOWN_1    bior.dbSNP137.CHROM    bior.dbSNP137.POS
BRCA1    17    41196363
BRCA1    17    41196368
BRCA1    17    41196372
BRCA1    17    41196403
BRCA1    17    41196408
```

The result: a simple VCF-like file constructed for all variants in the BRCA1 gene! There are a few small fixes that will need to be made to make it truly VCF-compliant, and this quickstart glosses over many features such as the metadata and headers. These and many other issues will be covered in more detail in the following sections.

3. BioR Catalogs

The BioR Catalog Format

BioR enables users to rapidly transform tabular, hierarchical (e.g. XML) relational, and flat files into catalogs that can be indexed and searched. Catalogs are read-only snapshots of annotation data. In production, we snapshot data sets from outside groups and run an automated 'publishing' process that keeps all of the BioR catalogs up to date with reference data sources. Data in catalogs is organized as a BED-JSON hybrid (a subset of TJSON, or tab-delimited JSON). Columns 1-3 are identical to the required fields in BED files[2,3] and thus allow many existing tools such as Tabix to work directly on BioR catalogs. Column 4 is a JSON string encoded object representing the entire contents of the original file. BioRTools depends on *golden identifiers* (identifiers that start with an underscore) to enable search. *Golden identifiers* are semantically-consistent tightly-controlled fields that are used by the toolkit to enable filtering and search (e.g. _minBP/_maxBP corresponds to one-based fully-closed genomic min/max base-pairs).

Catalog Creation Details

As an illustration, we will take a single gene BRCA1 and show it in the original annotation file and in BioR Catalog structure.

ORIGINAL
The gene BRCA1 is shown below from the original Genbank formatted file:

hs_ref_GRCh37.p10_chr17.gbs.gz:

```
  gene            complement(41196312..41277500)
                      /gene="BRCA1"
                      /gene_synonym="BRCAI; BRCC1; BROVCA1; IRIS; PNCA4;
                      PPP1R53; PSCP; RNF53"
                      /note="breast cancer 1, early onset; Derived by automated
                      computational analysis using gene prediction method:
                      BestRefseq."
                      /db_xref="GeneID:672"
                      /db_xref="HGNC:1100"
                      /db_xref="HPRD:00218"
                      /db_xref="MIM:113705"
```

CATALOG
Below is the corresponding Catalog structure for the final column of gene BRCA1.

```
  {
       "gene": "BRCA1",
       "gene_synonym": "BRCAI; BRCC1; BROVCA1; IRIS; PNCA4; PPP1R53; PSCP; RNF53",
       "note": "breast cancer 1, early onset; Derived by automated computational analysis using ger
prediction method: BestRefseq.",
       "GeneID": "672",
       "HGNC": "1100",
       "HPRD": "00218",
       "MIM": "113705",
       "_type": "gene",
       "_landmark": "17",
       "_strand": "-",
       "_minBP": 41196312,
       "_maxBP": 41277500
  }
```

The catalog format is simple, easy to read, and can be readily processed by third party JSON libraries. The format is also incredibly flexible, and has allowed us to ingest deeply nested XML structures and complex relational schemas into BioR. Construction of catalogs can be done with whatever programming language the user is familiar with. Once the raw data is formatted, bgzip and tabix can be used to compress and then index the catalog for genomic coordinate-based queries.

Catalogs Available In BioR

The BioR team has created more than 8,000 catalogs relevant to variant annotation from the following sources.

Data sources currently available in BioR

Datasource	URL	Version
1000Genomes	http://www.1000genomes.org/category/ftp	20110521
BGI	http://soap.genomics.org.cn/soapsnp.html	hg19

COSMIC	**http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/**	**V63**
dbSNP	**http://www.ncbi.nlm.nih.gov/snp/**	**137**
ESP6500	**https://esp.gs.washington.edu/drupal/**	**build37**
HapMap	**http://hapmap.ncbi.nlm.nih.gov**	**2010-08_phaseII+III**
HGNC	**http://www.genenames.org**	**2012_08_12**
miRBase	**http://www.mirbase.org**	**8_12_12**
NCBIGene	**http://www.ncbi.nlm.nih.gov/gene**	**GRCh37_p10**
OMIM	**http://www.omim.org**	**2013_02_27**
PharmGKB	**http://www.pharmgkb.org/downloads/**	**June 2013**
DrugBank	**http://www.drugbank.ca/downloads**	**3.0**
Therapeutic Target Database	**http://bidd.nus.edu.sg/group/cjttd/TTD_Download.asp**	**4.3.02**
UCSC	**http://hgdownload.cse.ucsc.edu/goldenPath/hg19/database/ (note catalogs were created for each UCSC track)**	**hg19**

Table S3: list of data sources from which BioR catalogs are derived. A description of the catalog is available at http://bioinformaticstools.mayo.edu

Creating a Catalog in Seconds

Many users have data in an arbitrary format (e.g. an Excel file from a paper) or another source of annotation such as BioMART. BioR allows users to integrate additional sources of information into the system as catalogs extremely rapidly. Unlike most other tools, BioR does not require a specific bioinformatics format, all you need to be able to do is convert the files into JSON, and BioR has many utilities to do that for you!

As an example, lets integrate dbSNFP2.1 into BioR (availible from here: https://sites.google.com/site/jpopgen/dbNSFP).

First Genes:

```
$ head -n 1 dbNSFP2.1_gene | tr "(" "_" | tr ")" "_" |
bior_create_config_for_tab_to_tjson > gene.config
$ vim gene.config  (to identify the _landmark golden identifier)
$ cat dbNSFP2.1_gene | bior_tab_to_tjson -c gene.config > dbNSFP2.1_gene.tjson
$ bior_create_catalog -c -1 -i dbNSFP2.1_gene.tjson -o dbNSFP2.1_gene
```

Then Variants:

```
$ cat dbNSFP2.1_variant* | grep -v "^#" > dbNSFP2.1_variant
$ head -n 1  dbNSFP2.1_variant.chr1|bior_create_config_for_tab_to_tjson >
variant.config
$ vim variant.config  (to columns for _landmark, _minBP, _maxBP, _refAllele, and
```

```
_altAllele)
$ cat dbNSFP2.1_gene | bior_tab_to_tjson -c gene.config > dbNSFP2.1_gene.tjson
$ bior_create_catalog -c -1 variant.tjson -o dbNSFP2.1_variant
```

It is really that simple, now dbNSFP is integrated into BioR! To use it, make sure to index as needed using bior_index_catalog command.

4. Examples Matching Genomic Features

Positional Matches Using Tabix

BioR uses the same technology for compression (BGZIP) and coordinate based indexing as Tabix[2]. This means that coordinate-based queries can use the traditional Tabix commands. For example, to show all genes in a BioR catalog on Chromosome 17 in the range 41196312 - 41277500:

```
$ which tabix
/usr/bin/tabix

$ which bgzip
/usr/bin/bgzip

$ tabix $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz 17:41196312-41277500
17  41196312  41277500
{"_type":"gene","_landmark":"17","_strand":"-","_minBP":41196312,"_maxBP":41277500,"gene":"BRCA1","
ne_synonym":"BRCAI; BRCC1; BROVCA1; IRIS; PNCA4; PPP1R53; PSCP; RNF53","note":"breast cancer 1, ear
onset; Derived by automated computational analysis using gene prediction method:
BestRefseq.","GeneID":"672","HGNC":"1100","HPRD":"00218","MIM":"113705"}
174123127841231833{"_type":"gene","_landmark":"17","_strand":"+","_minBP":41231278,"_maxBP":4123183
"gene":"RPL21P4","gene_synonym":"RPL21_58_1548","note":"ribosomal protein L21 pseudogene 4; Derived
by automated computational analysis using gene prediction method: Curated
Genomic.","pseudo":"","GeneID":"140660","HGNC":"17959"}
```

On the Mayo RCF servers, tabix is located at: /projects/bsi/bictools/apps/alignment/tabix/0.2.5/tabix. You may need to type something like /usr/bin/tabix instead of just tabix if it is not in your path (/usr/bin is usually is your path). To put it in your path edit your $PATH environment variable. In bash this is done by typing export PATH=$PATH:/usr/bin

Annotating Variants with Genes that Overlap

A common and simple use of BioR is to ask what genes overlap variants of interest. NCBI Generates an annotation of genes that they store here: ftp.ncbi.nih.gov/genomes/Homo_sapiens

This set of files is one of the authoritative sources for storing both the IDs for genes and the genomic coordinates. Unfortunately the gbs file is hard to use without the use of libraries. BioR allows you to do many quick and dirty analyses based on the position of genes. The following example assumes a VCF-like file with only 8 columns e.g. (note there is an example.vcf is in $BIOR_LITE_HOME/examples/quickstart2/example.vcf but any properly formatted vcf will work):

[2] http://bioinformatics.oxfordjournals.org/content/27/5/718.abstract

```
$ head example.vcf
##fileformat=VCFv4.0
#CHROM  POS  ID  REF  ALT  QUAL  FILTER  INFO
12  1584  8808  rs116645811  G  A  ...
21  2696  5148  rs1135638    G  A  ...
21  2696  5172  rs010576     T  C  ...
21  2696  5205  rs1057885    T  C  ...
21  2697  6144  rs116331755  A  G  ...
21  2697  6222  rs7278168    C  T  ...
21  2697  6237  rs7278284    C  T  ...
21  2697  8790  rs75377686   T  C  ...
```

Now, lets annotate these variants based on the genes they overlap:

```
$ cat example.vcf | bior_vcf_to_tjson | bior_overlap -d $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz |
bior_drill -p GeneID -p gene | cut -f 9 --complement > example.vcf.genes
$ head example.vcf.genes
##fileformat=VCFv4.0
#CHROMPOSIDREFALTQUALFILTERINFOGeneIDgene
1215848808rs116645811GA...7399USH2A
2126965148rs1135638GA...54148MRPL39
2126965172rs010576TC...54148MRPL39
2126965205rs1057885TC...54148MRPL39
2126976144rs116331755AG...54148MRPL39
2126976222rs7278168CT...54148MRPL39
2126976237rs7278284CT...54148MRPL39
2126978790rs75377686TC...54148MRPL39
```

Feel free to use bior_pretty_print instead of bior_drill to explore the data. Try drilling out other columns. In-fact, if anything is unclear, break the command apart and run parts of the command to get a better understanding of what steps are doing (e.g. run `cat`, then `cat | bior_vcf_to_tjson | bior_pretty_print`, then `cat | bior_vcf_to_tjson | bior_overlap | bior_pretty_print`, and so on to understand the transformations done in the pipeline).

This is a simple script based on the above technique to show the genes that contain variants in your VCF file:

```
$ head example.vcf
##fileformat=VCFv4.0
#CHROMPOSIDREFALTQUALFILTERINFO
1215848808rs116645811GA...
2126965148rs1135638GA...
2126965172rs010576TC...
2126965205rs1057885TC...
2126976144rs116331755AG...
2126976222rs7278168CT...
2126976237rs7278284CT...
2126978790rs75377686TC...
$
```

In many examples, more than one gene may overlap a variant. By default, BioR will 'fan-out' the rows replicating each input row for each result in the result set.

Here is an example of a quick script to look for rsIDs in an entire exome sequencing run (followed by variant calling formatted as VCF) where we annotate the rsID-gene relationships:

```
$ cat /data2/bsi/staff_analysis/m088341/BioR/exome_test/s_P68.variants.final.vcf | cut -f 3 | grep
"\." | bior_lookup  -p ID  -d $bior/dbSNP/137/00-All_GRCh37.tsv.bgz | grep -v "##" | grep -v "^ID"|
bior_overlap -d $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz | bior_drill -p gene | cut -f 2 --complemen
| head
#UNKNOWN_1gene
rs146405013LINC00115
rs3115849LINC00115
rs61768173LINC00115
rs4970461LOC100130417
rs4372192SAMD11
rs6605066SAMD11
rs6672356SAMD11
rs6605067SAMD11
rs6605067NOC2L
```

This is one way to get the variants that overlap more than one gene:

```
$ cat /data2/bsi/staff_analysis/m088341/BioR/exome_test/s_P68.variants.final.vcf | cut -f 3 | grep
"\." | bior_lookup  -p ID  -d $BIOR_CATALOG/dbSNP/137/00-All_GRCh37.tsv.bgz | grep -v "##" | grep -
"^ID"| bior_overlap -d $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz | bior_drill -p gene | cut -f 2
--complement | grep -v "#UNKNOWN" | grep -v "\." | cut -f 1 | uniq -c | grep -v "1 rs"
```

```
      2 rs6605067
      2 rs2839
      2 rs262688
      2 rs1043703
      2 rs17692
      2 rs2294532
      2 rs1043683
      2 rs1043681
      2 rs10523
      2 rs649639
...
```

In this case, the variants are sorted, so `uniq` can be used directly, but in other cases, consider the unix `sort` command (right before `uniq`). How many variants overlap at least two genes in this exome sample?

```
$ $ wc -l  moreThan1.rsID
3778 moreThan1.rsID
```

Compressing output to enforce 1-1 semantics

Lets say we want to enforce 1-in/1-out semantics (no duplicated variants), BioR has a utility (`bior_compress`) that can help with that. Here we will start directly with the rare variants. A simple sed command replaces the counts and gets us back to rsIDs.

```
$ sed 's/     .* //' < moreThan1.rsID
rs6605067
rs2839
rs262688
rs1043703
rs17692
rs2294532
rs1043683
rs1043681
rs10523
rs649639
...
```

Now we can annotate them in much the same way as before: (or we could modify the above pipeline – probably want to do that when we want to keep all the input data, but this gives us example variants that overlap two genes quickly). Run this example without `bior_compress` to see the default behavior when there is more than one result for a row.

```
$ sed 's/     .* //' < moreThan1.rsID | bior_lookup  -p ID  -d
```

```
$BIOR_CATALOG/dbSNP/137/00-All_GRCh37.tsv.bgz | bior_overlap -d
$bior/NCBIGene/GRCh37_p10/genes.tsv.bgz | bior_drill -p gene | cut -f 1,3 | bior_compress 2 | head
#UNKNOWN_1gene
rs6605067SAMD11|NOC2L
rs2839SAMD11|NOC2L
rs262688PRKCZ|LOC100506504
rs1043703THAP3|DNAJC11
rs17692THAP3|DNAJC11
rs2294532THAP3|DNAJC11
rs1043683THAP3|DNAJC11
rs1043681THAP3|DNAJC11
rs10523THAP3|DNAJC11
$
```

5. Expanded Genes (Xrefs)

The HUGO/HGNC table has database cross-references for gene ids and names. The `bior_lookup` command allows us to 'walk' these cross references. Here is an example:

```
$ bior_vcf_to_tjson < example.vcf | bior_overlap -d $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz |
bior_drill -p GeneID -p gene | cut -f 9 --complement | bior_lookup -d
$bior/hgnc/2012_08_12/hgnc_GRCh37.tsv.bgz -p Approved_Symbol | bior_drill -p Approved_Symbol -p
Entrez_Gene_ID -p Ensembl_Gene_ID -p UniProt_ID
##fileformat=VCFv4.0
#CHROMPOSIDREFALTQUALFILTERINFOGeneIDgeneApproved_SymbolEntrez_Gene_IDEnsembl_Gene_IDUniProt_ID
1215848808rs116645811GA...7399USH2AUSH2A7399ENSG00000042781O75445
2126965148rs1135638GA...54148MRPL39MRPL3954148ENSG00000154719Q9NYK5
2126965172rs010576TC...54148MRPL39MRPL3954148ENSG00000154719Q9NYK5
2126965205rs1057885TC...54148MRPL39MRPL3954148ENSG00000154719Q9NYK5
2126976144rs116331755AG...54148MRPL39MRPL3954148ENSG00000154719Q9NYK5
...
```

Lookup requires that the referenced column (last by default change it with the –c flag) is an ID that has been indexed in the source catalog. ID based indexes are stored in a directory called 'index' at the same level in the filesystem as the catalog. For example, here are all of the indexes for the HGNC catalog:

Indexing Catalogs

```
$ ls $bior/hgnc/2012_08_12/index/
hgnc_GRCh37.Approved_Symbol.idx.h2.db  hgnc_GRCh37.Entrez_Gene_ID.idx.h2.db
hgnc_GRCh37.UniProt_ID.idx.h2.db
hgnc_GRCh37.Ensembl_Gene_ID.idx.h2.db  hgnc_GRCh37.HGNC_ID.idx.h2.db
```

On the RCF, the administrators are very restrictive about space, so additional indexes must be placed in user/project space. Stand-alone installs can easily place all indexes in the index directory directly under the directory the catalog is in. BioR allows users to make additional indexes through the bior_index_catalog command. The help documentation contains:

```
1) bior_index  -d $BIOR_CATALOG/NCBIGene/GRCh37_p10/genes.tsv.bgz  -p HGNC

OR

2) bior_index  -d $BIOR_CATALOG/NCBIGene/GRCh37_p10/genes.tsv.bgz  -p HGNC  -i
/data/myindexes/genes.HGNC.idx.h2.db
```

Option 1, used by the BioR team to create indexes, will create the index file in the index folder in the same directory as the catalog (as shown in the example for hgnc above). Option 2, most often used by BioR end users, creates the index in any directory. When using an index created via the second method, you need to adjust the lookup command appropriately. This will be covered more comprehensively in the section on creating custom catalogs.

To make an index, use bior_pretty_print to show the contents of the catalog, and then run the index command.

Looking Up Information about a Gene

Say we wanted to find "Approved_Symbol", "Entrez_Gene_ID", "Ensembl_Gene_ID", "UniProt_ID", and other common alternative symbols for every gene we have in a list. We can use the BioR lookup command:

First, we don't know the catalog Structure of HGNC, here is a way to look at the structure of a catalog:

```
$ zcat $bior/hgnc/2012_08_12/hgnc_GRCh37.tsv.bgz | bior_pretty_print
#  COLUMN NAME  COLUMN VALUE
-  -----------  ------------
1  UNKNOWN_1    .
2  #UNKNOWN_2   0
3  #UNKNOWN_3   0
4  #UNKNOWN_4   {
                  "HGNC_ID": "HGNC:5",
                  "Approved_Symbol": "A1BG",
                  "Approved_Name": "alpha-1-B glycoprotein",
                  "Status": "Approved",
                  "Locus_Type": "gene with protein product",
                  "Locus_Group": "protein-coding gene",
                  "Previous_Symbols": [],
                  "Previous_Names": [],
                  "Synonyms": [],
                  "Name_Synonyms": [],
                  "Chromosome": "19q",
```

```
                  "Date_Approved": "1989-06-30",
                  "Date_Modified": "2010-07-08",
                  "Accession_Numbers": [],
                  "Enzyme_IDs": [],
                  "Entrez_Gene_ID": "1",
                  "Ensembl_Gene_ID": "ENSG00000121410",
                  …
                 "Pubmed_IDs": [
                    "2591067"
                  ],
                  "RefSeq_IDs": [
                    "NM_130786"
                  ],
                  "Record_Type": "Standard",
                  "Primary_IDs": [],
                  "Secondary_IDs": [],
                  "CCDS_IDs": [
                    "CCDS12976.1"
                  ],
                  "VEGA_IDs": [],
                  "mapped_GDB_ID": "GDB:119638",
                  "mapped_Entrez_Gene_ID": "1",
                  "mapped_OMIM_ID": "138670",
                  "mapped_RefSeq": "NM_130786",
                  "UniProt_ID": "P04217",
                  "mapped_Ensembl_ID": "ENSG00000121410",
                  "UCSC_ID": "uc002qsd.4",
                  "mapped_Mouse_Genome_Database_ID": "MGI:2152878",
                  "mapped_Rat_Genome_Database_ID": "RGD:69417"
                }
$
```

To join the information in this catalog, to the information that we have collected in the gene table, we need to tell bior what field in the HGNC table matches the LAST column in our sample data + annotation. In this case, we will join on approved symbol (note: if you ever get an error with doing a lookup, you may need an index file - look into the bior_index_catalog command documentation, using –h for help, or contact the bior team for help – running bior commands).

```
[m102417@crick4 ~]$ cat mygenes.txt
MRPL39
PANX2
BRCA1
[m102417@crick4 ~]$ cat mygenes.txt | bior_lookup -d $bior/hgnc/2012_08_12/hgnc_GRCh37.tsv.bgz -p
Approved_Symbol
#UNKNOWN_1LookupPipe
MRPL39{"HGNC_ID":"HGNC:14027","Approved_Symbol":"MRPL39","Approved_Name":"mitochondrial ribosomal
```

```
protein L39","Status":"Approved","Locus_Type":"gene with protein
product","Locus_Group":"protein-coding
gene","Previous_Symbols":[],"Previous_Names":[],"Synonyms":["RPML5","MRP-L5","MGC104174","PRED66","
ED22","C21orf92","L39mt","MSTP003","MGC3400","FLJ20451"],"Name_Synonyms":[],"Chromosome":"21q11.2-q
","Date_Approved":"2001-02-28","Date_Modified":"2012-09-13","Accession_Numbers":["AB051346"],"Enzym
IDs":[],"Entrez_Gene_ID":"54148","Ensembl_Gene_ID":"ENSG00000154719","Mouse_Genome_Database_ID":"MG
1351620","Specialist_Database_Links":"<!--,--> <!--,--> <!--,--> <!--,--> <!--,--> <!--,--> <!--,--
<!--,--> <!--,--> <!--,--> <a
href=\"http://www.sanger.ac.uk/perl/genetics/CGP/cosmic?action=gene&amp;ln=MRPL39\">COSMIC</a><!--,
> <!--,--> <!--,--> <!--,--> <!--,--> <!--,-->
","Specialist_Database_IDs":["","","","","","","","","","","MRPL39","","","","","","",""],"Pubmed_IDs"
"11543634"],"RefSeq_IDs":["NM_017446"],"Gene_Family_Tag":"MRPL","Gene_family_description":"\"Mitoch
drial ribosomal proteins / large
subunits\"","Record_Type":"Standard","Primary_IDs":[],"Secondary_IDs":[],"CCDS_IDs":["CCDS13573.1",
CDS33522.1"],"VEGA_IDs":["OTTHUMG00000078371"],"mapped_GDB_ID":"GDB:11503068","mapped_Entrez_Gene_I
:"54148","mapped_OMIM_ID":"611845","mapped_RefSeq":"NM_017446","UniProt_ID":"Q9NYK5","mapped_Ensemb
ID":"ENSG00000154719","UCSC_ID":"uc002yln.3","mapped_Mouse_Genome_Database_ID":"MGI:1351620"}
PANX2...
...
$
```

Now lets extract Entrez_Gene_ID, Ensembl_Gene_ID, and UniProt_ID from the catalog:

```
$ cat mygenes.txt | bior_lookup -d /data5/bsi/catalogs/bior/v1/hgnc/2012_08_12/hgnc_GRCh37.tsv.bgz
Approved_Symbol | bior_drill -p Entrez_Gene_ID -p Ensembl_Gene_ID -p UniProt_ID
#UNKNOWN_1Entrez_Gene_IDEnsembl_Gene_IDUniProt_ID
MRPL3954148ENSG00000154719Q9NYK5
PANX256666ENSG00000073150Q96RD6
BRCA1672ENSG00000012048P38398
$
```

Example of Walking Cross References

The HGNC table does not contain information about the disease/condition, only the ID in OMIM. Lets say you would like to also find this information for a select set of genes. In this case, we can use two catalogs, (1) the HGNC catalog and (2) the genemap directly from OMIM. The figure below shows the contents of the genemap catalog currently in BioR:

```
$ zcat $bior/omim/2013_02_27/genemap_GRCh37.tsv.bgz | bior_pretty_print
#  COLUMN NAME  COLUMN VALUE
-  -----------  ------------
1  UNKNOWN_1    .
2  #UNKNOWN_2   .
3  #UNKNOWN_3   .
4  #UNKNOWN_4   {
```

```
                  "Chromosome.Map_Entry_Number": 1.1,
                  "MonthEntered": 9,
                  "Day": 11,
                  "Year": 95,
                  "Cytogenetic_location": "1pter-p36.13",
                  "GeneSymbols": "CCV",
                  "Gene_Status": "P",
                  "Title": "Cataract, congenital, Volkmann type",
                  "Title_cont": "",
                  "MIM_Number": 115665,
                  "Method": "Fd",
                  "Comments": "",
                  "Disorders": "Cataract, congenital, Volkmann type (2)",
                  "Disorders_cont": " "
                }
$
```

In this catalog, "MIM_Number" represents the OMIM id for the “Disorder” free text field describing the disease. Given a list of genes, if we want the value of the “Disorder” field in OMIM we can cross-walk from the gene list through the HGNC catalog to find the MIM number and then again to genemap catalog to produce a Gene-OMIM_ID-Disorder file:

```
$ cat mygenes.txt
MRPL39
PANX2
BRCA1
$ cat mygenes.txt | bior_lookup -d $bior/hgnc/2012_08_12/hgnc_GRCh37.tsv.bgz -p Approved_Symbol |
bior_drill -p mapped_OMIM_ID | bior_lookup -d $bior/omim/2013_02_27/genemap_GRCh37.tsv.bgz -p
MIM_Number | bior_drill -p Disorders
#UNKNOWN_1mapped_OMIM_IDDisorders
MRPL39611845
PANX2608421.
BRCA1113705{Breast-ovarian cancer, familial, 1}, 604370 (3); {Pancreatic cancer,
$
```

Note: period '.' always means the value was not in the dataset. So in this case, some genes are not associated with disorders in OMIM.

Generating an OMIM Disorder Report for a Set of rsIDs

```
Want OMIM

cat example.vcf | bior_vcf_to_tjson | bior_overlap --d $catalogs/NCBIGene/GRCh37_p10/ genes.tsv.bgz
bior_drill --p GeneID --p gene --p MIM | cut --f9 -- --complement | bior_lookup --d
$catalogs/omim/2013_02_27/ genemap_GRCh37.tsv.bgz --p MIM_Number | bior_drill --p Disorders >
```

```
example.w_omim.
```

Use lookup to also find any disease/condition information in OMIM. First, the gene catalog just happens to have the OMIM id ("MIM"), so alter the command to drill that out:

```
Want OMIM

cat example.vcf | bior_vcf_to_tjson | bior_overlap --d $catalogs/NCBIGene/GRCh37_p10/ genes.tsv.bgz
bior_drill --p GeneID --p gene --p MIM | cut --f9 -- --complement | bior_lookup --d
$catalogs/omim/2013_02_27/ genemap_GRCh37.tsv.bgz --p MIM_Number | bior_drill --p Disorders >
example.w_omim.
```

```
$ cat example.vcf | bior_vcf_to_tjson | bior_overlap -d $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz |
bior_drill -p GeneID -p gene  | cut -f 9 --complement | bior_lookup -d
$bior/hgnc/2012_08_12/hgnc_GRCh37.tsv.bgz -p Approved_Symbol

 $ cat example.vcf | bior_vcf_to_tjson | bior_overlap -d $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz |
bior_drill -p GeneID -p gene -p MIM | cut -f 9 --complement
##fileformat=VCFv4.0
#CHROMPOSIDREFALTQUALFILTERINFOGeneIDgeneMIM
1215848808rs116645811GA...7399USH2A608400
1215848808rs116645811GT...7399USH2A608400
…
 $
```

```
$  cat example.vcf | bior_vcf_to_tjson | bior_overlap -d $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz |
bior_drill -p GeneID -p gene -p MIM | cut -f 9 --complement | bior_lookup -d
$bior/omim/2013_02_27/genemap_GRCh37.tsv.bgz -p MIM_Number | bior_pretty_print
#   COLUMN NAME  COLUMN VALUE
-   -----------  ------------
1   CHROM        1
2   POS          215848808
3   ID           rs116645811
4   REF          G
5   ALT          A
6   QUAL         .
7   FILTER       .
8   INFO         .
9   GeneID       7399
10  gene         USH2A
11  MIM          608400
```

```
12  LookupPipe   {
                   "Chromosome.Map_Entry_Number": 1.1272,
                   "MonthEntered": 1,
                   "Day": 27,
                   "Year": 4,
                   "Cytogenetic_location": "1q41",
                   "GeneSymbols": "USH2A, RP39",
                   "Gene_Status": "C",
                   "Title": "Usherin",
                   "Title_cont": "",
                   "MIM_Number": 608400,
                   "Method": "Fd",
                   "Comments": "",
                   "Disorders": "Usher syndrome, type 2A, 276901 (3); Retinitis pigmentosa 39,
613809",
                   "Disorders_cont": " ",
                   "Mouse_correlate": "1(Ush2a)"
                 }
 $
```

Looks like we want the column "Disorders":

```
$  cat example.vcf | bior_vcf_to_tjson | bior_overlap -d $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz |
bior_drill -p GeneID -p gene -p MIM | cut -f 9 --complement | bior_lookup -d
$bior/omim/2013_02_27/genemap_GRCh37.tsv.bgz -p MIM_Number | bior_drill -p Disorders
##fileformat=VCFv4.0
#CHROMPOSIDREFALTQUALFILTERINFOGeneIDgeneMIMDisorders
1215848808rs116645811GA...7399USH2A608400Usher syndrome, type 2A, 276901 (3); Retinitis pigmentosa
39, 613809
...
2250616806rs5771206AG...56666PANX2608421.
 $
```

OK, lets go and get some information from some variant catalogs that are not Allele frequencies:

First, dbSNP has all kinds of useful information including
"INFO.dbSNPBuildID":
"INFO.SSR": SSR 1 Integer 247,783 0.49% SNP Suspect Reason Code SNP Suspect Reason Code, 0 - unspecified, 1 - Paralog, 2 - byEST, 3 - Para_EST, 4 - oldAlign, 5 - other. Count in column D is non-zero
Sequence Annotation Flags
"INFO.SCS": Integer 12,533 0.02% SNP Clinical Significance SNP Suspect Reason Code, 0 -

unspecified, 1 - Paralog, 2 - byEST, 3 - Para_EST, 4 - oldAlign, 5 - other. Count in column D is non-zero
"INFO.CLN": CLN 0 Flag 31,524 0.06% SNP is Clinical Includes LSDB,OMIM,TPA,Diagnostic
"INFO.SAO": SAO 1 Integer 14,908 0.03% SNP Allele Origin SNP Allele Origin: 0 - unspecified, 1 - Germline, 2 - Somatic, 3 - Both. Count in column D is non-zero
"_id": The rs_id, a (near)universal identifier for the Variant.
(to see a compiled list of what is in this, go to the bsi documentation: http://bsiweb.mayo.edu/dbsnp)
This text file is a good guide (downloaded from dbSNP: ftp://ftp.ncbi.nih.gov/snp/organisms/human_9606/VCF/00-snp_info_tags.txt)

```
$ cat example.vcf | bior_vcf_to_tjson | bior_same_variant -d $bior/dbSNP/137/00-All_GRCh37.tsv.bgz
bior_pretty_print -r 17
#   COLUMN NAME      COLUMN VALUE
-   -----------      ------------
1   CHROM            21
2   POS              26965148
3   ID               rs1135638
4   REF              G
5   ALT              A
6   QUAL             .
7   FILTER           .
8   INFO             .
9   VCF2VariantPipe  {
                       "CHROM": "21",
...
                     }
10  SameVariantPipe  {
                       "CHROM": "21",
                       "POS": "26965148",
                       "ID": "rs1135638",
                       "REF": "G",
                       "ALT": "A",
                       "QUAL": ".",
                       "FILTER": ".",
                       "INFO": {
                         "RSPOS": 26965148,
                         "RV": true,
                         "GMAF": 0.2395,
                         "dbSNPBuildID": 86,
                         "SSR": 0,
                         "SAO": 0,
                         "VP": "05030000030507051f000100",
                         "GENEINFO": "MRPL39:54148",
                         "WGT": 1,
                         "VC": "SNV",
                         "S3D": true,
                         "SLO": true,
                         "REF": true,
                         "SYN": true,
```

```
                        "ASP": true,
                        "VLD": true,
                        "G5A": true,
                        "G5": true,
                        "HD": true,
                        "GNO": true,
                        "KGPhase1": true,
                        "KGPilot123": true,
                        "KGPROD": true,
                        "OTHERKG": true,
                        "PH3": true
                      },
                      "_id": "rs1135638",
                      "_type": "variant",
                      "_landmark": "21",
                      "_refAllele": "G",
                      "_altAlleles": [
                        "A"
                      ],
                      "_minBP": 26965148,
                      "_maxBP": 26965148
                    }
$
```

To match variants, use same_variant:

Now build a table with: rs_id, dbSNPBuildID, SSR, SCS, CLN, SAO, and CLN, do this:

```
$ cat example.vcf | bior_vcf_to_tjson | bior_same_variant -d $bior/dbSNP/137/00-All_GRCh37.tsv.bgz
bior_drill -p _id -p dbSNPBuildID -p INFO.SSR -p INFO.SCS -p INFO.CLN -p INFO.SAO -p  INFO.CLN | cu
-f 9 --complement
```

unfortunately, the variants in this example file, did not have any results, as these annotations are rather sparse. Finding variants with these properties can be a trick. Here is a trick that I use to cat all variants from a specific gene:

```
$ zcat $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz | grep "\"gene\":\"BRCA1\""
174119631241277500{"_type":"gene","_landmark":"17","_strand":"-","_minBP":41196312,"_maxBP":4127750
"gene":"BRCA1","gene_synonym":"BRCAI; BRCC1; BROVCA1; IRIS; PNCA4; PPP1R53; PSCP;
RNF53","note":"breast cancer 1, early onset; Derived by automated computational analysis using gene
prediction method: BestRefseq.","GeneID":"672","HGNC":"1100","HPRD":"00218","MIM":"113705"}
 $
```

Then to find a variant in dbSNP with an SAO annotation:

```
$ zcat $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz | grep "\"gene\":\"BRCA1\"" | bior_overlap -d
$bior/dbSNP/137/00-All_GRCh37.tsv.bgz | grep SAO | bior_pretty_print
#  COLUMN NAME  COLUMN VALUE
-  -----------  ------------
1  UNKNOWN_1    17
2  #UNKNOWN_2   41196312
3  #UNKNOWN_3   41277500
4  #UNKNOWN_4   {
                  "_type": "gene",
                  "_landmark": "17",
                  "_strand": "-",
                  "_minBP": 41196312,
                  "_maxBP": 41277500,
                  "gene": "BRCA1",
                  "gene_synonym": "BRCAI; BRCC1; BROVCA1; IRIS; PNCA4; PPP1R53; PSCP; RNF53",
                  "note": "breast cancer 1, early onset; Derived by automated computational analysi
using gene prediction method: BestRefseq.",
                  "GeneID": "672",
                  "HGNC": "1100",
                  "HPRD": "00218",
                  "MIM": "113705"
                }
5  #UNKNOWN_5   {
                  "CHROM": "17",
                  "POS": "41196363",
                  "ID": "rs8176320",
                  "REF": "C",
                  "ALT": "T",
                  "QUAL": ".",
                  "FILTER": ".",
                  "INFO": {
                    "RSPOS": 41196363,
                    "RV": true,
                    "GMAF": 0.0050,
                    "dbSNPBuildID": 117,
                    "SSR": 0,
                    "SAO": 0,
                    "VP": "050000800201040517000100",
                    "GENEINFO": "BRCA1:672",
                    "WGT": 1,
                    "VC": "SNV",
                    "REF": true,
                    "U3": true,
                    "VLD": true,
                    "HD": true,
                    "GNO": true,
                    "KGPhase1": true,
                    "KGPROD": true,
```

```
                    "OTHERKG": true,
                    "PH3": true
                  },
                  "_id": "rs8176320",
                  "_type": "variant",
                  "_landmark": "17",
                  "_refAllele": "C",
                  "_altAlleles": [
                    "T"
                  ],
                  "_minBP": 41196363,
                  "_maxBP": 41196363
                }
 $
```

COSMIC:

```
$ cat example.vcf | bior_vcf_to_tjson | bior_same_variant -d
$bior/cosmic/v63/CosmicCompleteExport_GRCh37.tsv.bgz | bior_pretty_print -r 40
#   COLUMN NAME      COLUMN VALUE
-   -----------      ------------
1   CHROM            21
2   POS              40190405
3   ID               rs115908228
4   REF              G
5   ALT              A
6   QUAL             .
7   FILTER           .
8   INFO             .
9   VCF2VariantPipe  {
                       "CHROM": "21",
...
 }
10  SameVariantPipe  {
                       "Gene_name": "ETS2",
                       "Accession_Number": "ENST00000360214",
                       "HGNC_ID": "3489",
                       "Sample_name": "107702",
                       "ID_sample": "1520464",
                       "ID_tumour": "1442839",
                       "Primary_site": "breast",
                       "Site_subtype": "NS",
                       "Primary_histology": "carcinoma",
                       "Histology_subtype": "HER-positive_carcinoma",
                       "Genome-wide_screen": "n",
                       "Mutation_ID": "94254",
```

```
                      "Mutation_CDS": "c.646G\u003eA",
                      "Mutation_AA": "p.G216S",
                      "Mutation_Description": "Substitution - Missense",
                      "Mutation_GRCh37_genome_position": "21:40190405-40190405",
                      "Mutation_GRCh37_strand": "+",
                      "Mutation_somatic_status": "Confirmed somatic variant",
                      "Pubmed_PMID": "20668451",
                      "Sample_source": "NS",
                      "Tumour_origin": "primary",
                      "_type": "variant",
                      "_landmark": "21",
                      "_refAllele": "G",
                      "_altAlleles": [
                        "A"
                      ],
                      "_minBP": 40190405,
                      "_maxBP": 40190405,
                      "_id": "."
                    }
 $
```


```
$ cat example.vcf | bior_vcf_to_tjson | bior_same_variant -d
$bior/cosmic/v63/CosmicCompleteExport_GRCh37.tsv.bgz | bior_drill -p Mutation_ID -p Mutation_CDS -p
Mutation_AA -p Mutation_GRCh37_strand | cut -f 9 --complement
##fileformat=VCFv4.0
#CHROMPOSIDREFALTQUALFILTERINFOMutation_IDMutation_CDSMutation_AAMutation_GRCh37_strand
1215848808rs116645811GA.......
1215848808rs116645811GT.......
...
2140190405rs115908228GA...94254c.646G>Ap.G216S+
...
2230857373rs2240345AC...330401c.1005T>Gp.D335E-
...
2239621797rs35978693GT...39683c.657C>Ap.P219P-
 $
```

Want UCSC Tracks (blacklisted)cat example.vcf | bior_vcf_to_tjson | bior_overlap --d $catalogs/ucsc/hg19/ wgEncodeDacMapabilityConsensusExcludable_GR

Ch37.tsv.bgz | bior_drill --p score | complement > example.w_ucsc.vcf

UCSC:
The UCSC catalogs related to TREAT are the following:

```
export ucsc=$bior/ucsc/ ;
export blacklistedFile=$ucsc/hg19/wgEncodeDacMapabilityConsensusExcludable_GRCh37.tsv.bgz ;
export repeatFile=$ucsc/hg19/rmsk_GRCh37.tsv.bgz ;
export regulationFile=$ucsc/hg19/oreganno_GRCh37.tsv.bgz ;
export uniqueFile=$ucsc/hg19/wgEncodeDukeMapabilityRegionsExcludable_GRCh37.tsv.bgz ;
export tssFile=$ucsc/hg19/switchDbTss_GRCh37.tsv.bgz ;
export tfbsFile=$ucsc/hg19/tfbsConsSites_GRCh37.tsv.bgz ;
export enhancerFile=$ucsc/hg19/vistaEnhancers_GRCh37.tsv.bgz ;
export conservationFile=$ucsc/hg19/phastConsElements46wayPrimates_GRCh37.tsv.bgz ;
```

To annotate with any of these files, do something like this:

```
$ cat example.vcf | bior_vcf_to_tjson | bior_overlap -d $blacklistedFile | bior_drill -p score | cu
-f 9 --complement
##fileformat=VCFv4.0
#CHROMPOSIDREFALTQUALFILTERINFOscore
1215848808rs116645811GA....
1215848808rs116645811GT....
1215848808rs116645811GG....
1215848808rs116645811GC....
...
```

unfortunately, our example file does not overlap many of these rare features. Another way to think about this is "what genes of interest overlap some UCSC genomic feature".

```
$ zcat $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz | bior_overlap -d $blacklistedFile | grep -v "{}" |
bior_drill -c -2 -p gene | cut -f 5
gene
MTND1P23
MTND2P28
TTC34
RNU1-1
RSPO1
HFM1
AMY2A
NOTCH2NL
NBPF17P
PMF1
PMF1-BGLAP
PCNXL2
RYR2
MTND2P27
...
```

This list of genes could then be used in a lookup query later, or you could cut the JSON instead of the gene name and use that to overlap the data in your VCF file in a filtering process.

A similar technique can be use to pair down the variants based on those variants that you do NOT want because overlapping some genomic feature would indicate it is unlikely to be significant.

Putting it all Together – Making a Genomic Feature Annotation Program

Below is a simple example of an annotation program using the simple scripts.

```
$ cat treatGF.bior
bior_vcf_to_tjson < /dev/stdin \
| bior_overlap -d $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz  \
| bior_drill -p gene -p GeneID -p MIM \
| bior_lookup -d $bior/hgnc/2012_08_12/hgnc_GRCh37.tsv.bgz -p Approved_Symbol -c -3 \
| bior_drill -p Approved_Symbol -p Entrez_Gene_ID -p Ensembl_Gene_ID -p UniProt_ID \
| bior_lookup -d $bior/omim/2013_02_27/genemap_GRCh37.tsv.bgz -p MIM_Number -c -5 \
| bior_drill -p Disorders \
| bior_overlap -d $bior/mirbase/release19/hsa_GRCh37.p5.tsv.bgz -c -9 \
| bior_drill -p ID \
| bior_overlap -d $bior/ucsc/hg19/wgEncodeDacMapabilityConsensusExcludable_GRCh37.tsv.bgz -c -10 \
| bior_drill -p score \
| bior_overlap -d $bior/ucsc/hg19/phastConsElements46way_GRCh37.tsv.bgz -c -11 \
| bior_drill -p score \
| bior_overlap -d $bior/ucsc/hg19/oreganno_GRCh37.tsv.bgz -c -12 \
| bior_drill -p score \
| bior_overlap -d $bior/ucsc/hg19/tfbsConsSites_GRCh37.tsv.bgz -c -13 \
| bior_drill -p score \
| bior_overlap -d $bior/ucsc/hg19/switchDbTss_GRCh37.tsv.bgz -c -14 \
| bior_drill -p score \
| bior_overlap -d $bior/ucsc/hg19/vistaEnhancers_GRCh37.tsv.bgz -c -15 \
| bior_drill -p score \
| bior_overlap -d $bior/ucsc/hg19/wgEncodeDukeMapabilityRegionsExcludable_GRCh37.tsv.bgz -c -16 \
| bior_drill -p score \
| bior_overlap -d $bior/ucsc/hg19/rmsk_GRCh37.tsv.bgz -c -17 \
| bior_drill -p score \
| bior_overlap -d $bior/ucsc/hg19/wgEncodeDukeMapabilityRegionsExcludable_GRCh37.tsv.bgz -c -18 \
| bior_drill -p score \
| ./removeJSON.pl

$
```

6. Examples Matching Alleles (bior_same_variant)

Allele Frequencies:

on the RCF:

BGI:

```
$ cat example.vcf | bior_vcf_to_tjson | bior_same_variant -d $bior/BGI/hg19/LuCAMP_200exomeFinal.maf_GRCh37.tsv.bgz |
bior_pretty_print -r 17
#   COLUMN NAME     COLUMN VALUE
-  -----------     ------------
1  CHROM          21
2  POS            26965148
3  ID             rs1135638
4  REF            G
5  ALT            A
6  QUAL           .
7  FILTER         .
8  INFO           .
9  VCF2VariantPipe  {
                  "CHROM": "21",
                  "POS": "26965148",
                  "ID": "rs1135638",
                  "REF": "G",
                  "ALT": "A",
                  "QUAL": ".",
                  "FILTER": ".",
                  "INFO": {
                    ".": true
                  },
                  "_id": "rs1135638",
                  "_type": "variant",
                  "_landmark": "21",
                  "_refAllele": "G",
                  "_altAlleles": [
                    "A"
                  ],
                  "_minBP": 26965148,
                  "_maxBP": 26965148
                }
10  SameVariantPipe  {
                  "chromosome_id": "chr21",
                  "genomic_position": 25887019,
                  "index_of_major_allele": 0,
                  "major_allele": "A",
                  "index_of_minor_allele": 2,
                  "minor_allele": "G",
                  "number_A": 710,
                  "number_C": 1,
                  "number_G": 428,
```

```
            "number_T": 2,
            "estimated_minor_allele_freq": 0.278705,
            "estimated_major_allele_freq": 0.721295,
            "is_in_dbSNP": 1,
            "_landmark": "21",
            "_refAllele": "G",
            "_altAlleles": [
              "A"
            ],
            "_minBP": 26965148,
            "_maxBP": 26965148,
            "_type": "variant",
            "_id": "."
          }
$
```

```
$ cat example.vcf | bior_vcf_to_tjson | bior_same_variant -d
$bior/BGI/hg19/LuCAMP_200exomeFinal.maf_GRCh37.tsv.bgz | bior_drill -p estimated_major_allele_freq
estimated_minor_allele_freq | cut --complement -f 9
…
2230823196rs5753130TC...0.5765180.423482
2230856121rs35764129GA...0.9573590.042641
2230857373rs2240345AC...0.6109330.389067
2230857448rs5749104AG...0.5872320.412768
2230857645rs114917409CG.....
2230858149rs115111929AC.....
2230860830rs2269961CT...0.8081760.191824
…
```

dbSNP:

```
$ cat example.vcf | bior_vcf_to_tjson | bior_same_variant -d $bior/dbSNP/137/00-All_GRCh37.tsv.bgz | bior_pretty_print -r 17
#   COLUMN NAME     COLUMN VALUE
-  -----------     ------------
1  CHROM         21
2  POS           26965148
3  ID           rs1135638
4  REF           G
5  ALT           A
6  QUAL          .
7  FILTER        .
```

```
8  INFO          .
9  VCF2VariantPipe  {
                "CHROM": "21",
                "POS": "26965148",
                "ID": "rs1135638",
                "REF": "G",
                "ALT": "A",
                "QUAL": ".",
                "FILTER": ".",
                "INFO": {
                  ".": true
                },
                "_id": "rs1135638",
                "_type": "variant",
                "_landmark": "21",
                "_refAllele": "G",
                "_altAlleles": [
                  "A"
                ],
                "_minBP": 26965148,
                "_maxBP": 26965148
              }
10  SameVariantPipe  {
                "CHROM": "21",
                "POS": "26965148",
                "ID": "rs1135638",
                "REF": "G",
                "ALT": "A",
                "QUAL": ".",
                "FILTER": ".",
                "INFO": {
                  "RSPOS": 26965148,
                  "RV": true,
                  "GMAF": 0.2395,
                  "dbSNPBuildID": 86,
                  "SSR": 0,
                  "SAO": 0,
                  "VP": "05030000030507051f000100",
                  "GENEINFO": "MRPL39:54148",
                  "WGT": 1,
                  "VC": "SNV",
                  "S3D": true,
                  "SLO": true,
                  "REF": true,
                  "SYN": true,
                  "ASP": true,
                  "VLD": true,
                  "G5A": true,
                  "G5": true,
```

```
                "HD": true,
                "GNO": true,
                "KGPhase1": true,
                "KGPilot123": true,
                "KGPROD": true,
                "OTHERKG": true,
                "PH3": true
              },
              "_id": "rs1135638",
              "_type": "variant",
              "_landmark": "21",
              "_refAllele": "G",
              "_altAlleles": [
                "A"
              ],
              "_minBP": 26965148,
              "_maxBP": 26965148
            }
$
```

```
$ cat example.vcf | bior_vcf_to_tjson | bior_same_variant -d $bior/dbSNP/137/00-All_GRCh37.tsv.bgz | bior_pretty_print -r 17
#   COLUMN NAME     COLUMN VALUE
-  -----------     ------------
1  CHROM           21
2  POS             26965148
3  ID              rs1135638
4  REF             G
5  ALT             A
6  QUAL            .
7  FILTER          .
8  INFO            .
9  VCF2VariantPipe  {
              "CHROM": "21",
              "POS": "26965148",
              "ID": "rs1135638",
              "REF": "G",
              "ALT": "A",
              "QUAL": ".",
              "FILTER": ".",
              "INFO": {
                ".": true
              },
              "_id": "rs1135638",
              "_type": "variant",
              "_landmark": "21",
              "_refAllele": "G",
```

```
            "_altAlleles": [
              "A"
            ],
            "_minBP": 26965148,
            "_maxBP": 26965148
          }
10 SameVariantPipe {
            "CHROM": "21",
            "POS": "26965148",
            "ID": "rs1135638",
            "REF": "G",
            "ALT": "A",
            "QUAL": ".",
            "FILTER": ".",
            "INFO": {
              "RSPOS": 26965148,
              "RV": true,
              "GMAF": 0.2395,
              "dbSNPBuildID": 86,
              "SSR": 0,
              "SAO": 0,
              "VP": "05030000030507051f000100",
              "GENEINFO": "MRPL39:54148",
              "WGT": 1,
              "VC": "SNV",
              "S3D": true,
              "SLO": true,
              "REF": true,
              "SYN": true,
              "ASP": true,
              "VLD": true,
              "G5A": true,
              "G5": true,
              "HD": true,
              "GNO": true,
              "KGPhase1": true,
              "KGPilot123": true,
              "KGPROD": true,
              "OTHERKG": true,
              "PH3": true
            },
            "_id": "rs1135638",
            "_type": "variant",
            "_landmark": "21",
            "_refAllele": "G",
            "_altAlleles": [
              "A"
            ],
            "_minBP": 26965148,
```

```
          "_maxBP": 26965148
        }
$
```

dbSNP:

```
##fileformat=VCFv4.0
#CHROMPOSIDREFALTQUALFILTERINFOINFO.dbSNPBuildIDINFO.SSRINFO.SCSINFO.CLNINFO.SAO_id
1215848808rs116645811GA.........
1215848808rs116645811GT.........
1215848808rs116645811GG.........
1215848808rs116645811GC.........
1215848808rs116645811CA.........
...
 $
```

ESP:

```
$ cat example.vcf | bior_vcf_to_tjson | bior_same_variant -d
$bior/ESP/build37/ESP6500SI_GRCh37.tsv.bgz | bior_pretty_print -r 17
#   COLUMN NAME      COLUMN VALUE
-   -----------      ------------
1   CHROM            21
2   POS              26965148
3   ID               rs1135638
4   REF              G
5   ALT              A
6   QUAL             .
7   FILTER           .
8   INFO             .
9   VCF2VariantPipe  {
                       "CHROM": "21",
                       "POS": "26965148",
                       "ID": "rs1135638",
                       "REF": "G",
                       "ALT": "A",
                       "QUAL": ".",
                       "FILTER": ".",
                       "INFO": {
                         ".": true
                       },
                       "_id": "rs1135638",
```

```
                      "_type": "variant",
                      "_landmark": "21",
                      "_refAllele": "G",
                      "_altAlleles": [
                        "A"
                      ],
                      "_minBP": 26965148,
                      "_maxBP": 26965148
                    }
10  SameVariantPipe  {
                      "CHROM": "21",
                      "POS": "26965148",
                      "ID": "rs1135638",
                      "REF": "G",
                      "ALT": "A",
                      "QUAL": ".",
                      "FILTER": "PASS",
                      "INFO": {
                        "DBSNP": [
                          "dbSNP_86"
                        ],
                        "EA_AC": [
                          "7111",
                          "1489"
                        ],
                        "AA_AC": [
                          "3307",
                          "1099"
                        ],
                        "TAC": [
                          "10418",
                          "2588"
                        ],
                        "MAF": [
                          "17.314",
                          "24.9433",
                          "19.8985"
                        ],
                        "GTS": [
                          "AA",
                          "AG",
                          "GG"
                        ],
                        "EA_GTC": [
                          "2954",
                          "1203",
                          "143"
                        ],
                        "AA_GTC": [
```

```
      "1229",
      "849",
      "125"
    ],
    "GTC": [
      "4183",
      "2052",
      "268"
    ],
    "DP": 75,
    "GL": [
      "MRPL39"
    ],
    "CP": 1.0,
    "CG": 3.0,
    "AA": "A",
    "CA": [
      "."
    ],
    "EXOME_CHIP": [
      "no"
    ],
    "GWAS_PUBMED": [
      "."
    ],
    "GM": [
      "NM_017446.3",
      "NM_080794.3"
    ],
    "FG": [
      "coding-synonymous",
      "coding-synonymous"
    ],
    "AAC": [
      ".",
      "."
    ],
    "PP": [
      "299/339",
      "299/354"
    ],
    "CDP": [
      "897",
      "897"
    ],
    "GS": [
      ".",
      "."
    ],
```

```
                        "PH": [
                          ".",
                          "."
                        ]
                      },
                      "_id": "rs1135638",
                      "_type": "variant",
                      "_landmark": "21",
                      "_refAllele": "G",
                      "_altAlleles": [
                        "A"
                      ],
                      "_minBP": 26965148,
                      "_maxBP": 26965148
                    }
 $
```

HapMap:

```
$ cat example.vcf | bior_vcf_to_tjson | bior_same_variant -d
$bior/hapmap/2010-08_phaseII+III/allele_freqs_GRCh37.tsv.bgz | bior_pretty_print -r 17
#   COLUMN NAME      COLUMN VALUE
-   -----------      ------------
1   CHROM            21
2   POS              26965148
3   ID               rs1135638
4   REF              G
5   ALT              A
6   QUAL             .
7   FILTER           .
8   INFO             .
9   VCF2VariantPipe  {
                       "CHROM": "21",
                       "POS": "26965148",
                       "ID": "rs1135638",
                       "REF": "G",
                       "ALT": "A",
                       "QUAL": ".",
                       "FILTER": ".",
                       "INFO": {
                         ".": true
                       },
                       "_id": "rs1135638",
                       "_type": "variant",
                       "_landmark": "21",
```

```
                      "_refAllele": "G",
                      "_altAlleles": [
                        "A"
                      ],
                      "_minBP": 26965148,
                      "_maxBP": 26965148
                    }
10  SameVariantPipe  {
                      "rsNumber": "rs1135638",
                      "chrom": "chr21",
                      "pos": 25887019,
                      "strand": "+",
                      "build": "ncbi_b36",
                      "refallele": "G",
                      "otherallele": "A",
                      "_type": "variant",
                      "_landmark": "21",
                      "_minBP": 26965148,
                      "_maxBP": 26965148,
                      "_strand": "+",
                      "_refAllele": "G",
                      "_altAlleles": [
                        "A"
                      ],
                      "_id": "rs1135638",
                      "CEU": {
                        "center": "sanger",
                        "protLSID": "urn:LSID:illumina.hapmap.org:Protocol:Human_1M_BeadChip:3",
                        "assayLSID": "urn:LSID:sanger.hapmap.org:Assay:H1Mrs1135638:3",
                        "panelLSID": "urn:lsid:dcc.hapmap.org:Panel:CEPH-60-trios:4",
                        "QC_code": "QC+",

...
```

```
$ cat example.vcf | bior_vcf_to_tjson | bior_same_variant -d
$bior/hapmap/2010-08_phaseII+III/allele_freqs_GRCh37.tsv.bgz | bior_pretty_print -r 17
#   COLUMN NAME      COLUMN VALUE
-   -----------      ------------
1   CHROM            21
2   POS              26965148
3   ID               rs1135638
4   REF              G
5   ALT              A
6   QUAL             .
7   FILTER           .
8   INFO             .
9   VCF2VariantPipe  {
```

```
                      "CHROM": "21",
                      "POS": "26965148",
                      "ID": "rs1135638",
                      "REF": "G",
                      "ALT": "A",
                      "QUAL": ".",
                      "FILTER": ".",
                      "INFO": {
                        ".": true
                      },
                      "_id": "rs1135638",
                      "_type": "variant",
                      "_landmark": "21",
                      "_refAllele": "G",
                      "_altAlleles": [
                        "A"
                      ],
                      "_minBP": 26965148,
                      "_maxBP": 26965148
                    }
10  SameVariantPipe  {
                      "rsNumber": "rs1135638",
                      "chrom": "chr21",
                      "pos": 25887019,
                      "strand": "+",
                      "build": "ncbi_b36",
                      "refallele": "G",
                      "otherallele": "A",
                      "_type": "variant",
                      "_landmark": "21",
                      "_minBP": 26965148,
                      "_maxBP": 26965148,
                      "_strand": "+",
                      "_refAllele": "G",
                      "_altAlleles": [
                        "A"
                      ],
                      "_id": "rs1135638",
                      "CEU": {
                        "center": "sanger",
                        "protLSID": "urn:LSID:illumina.hapmap.org:Protocol:Human_1M_BeadChip:3",
                        "assayLSID": "urn:LSID:sanger.hapmap.org:Assay:H1Mrs1135638:3",
                        "panelLSID": "urn:lsid:dcc.hapmap.org:Panel:CEPH-60-trios:4",
                        "QC_code": "QC+",
                        "refallele_freq": 0.177,
                        "refallele_count": 40,
                        "otherallele_freq": 0.823,
                        "otherallele_count": 186,
                        "totalcount": 226
```

```
. . .
```

```
"center": "sanger",
                "protLSID": "urn:LSID:illumina.hapmap.org:Protocol:Human_1M_BeadChip:3",
                "assayLSID": "urn:LSID:sanger.hapmap.org:Assay:H1Mrs1135638:3",
                "panelLSID": "urn:lsid:dcc.hapmap.org:Panel:US_African-30-trios:4",
                "QC_code": "QC+",
                "refallele_freq": 0.277,
                "refallele_count": 31,
                "otherallele_freq": 0.723,
                "otherallele_count": 81,
                "totalcount": 112
              },
              "CHD": {
                "center": "sanger",
                "protLSID": "urn:LSID:illumina.hapmap.org:Protocol:Human_1M_BeadChip:3",
                "assayLSID": "urn:LSID:sanger.hapmap.org:Assay:H1Mrs1135638:3",
                "panelLSID": "urn:lsid:dcc.hapmap.org:Panel:US_Chinese:4",
                "QC_code": "QC+",
                "refallele_freq": 0.289,
                "refallele_count": 63,
                "otherallele_freq": 0.711,
                "otherallele_count": 155,
                "totalcount": 218
              },
              "GIH": {
                "center": "sanger",
                "protLSID": "urn:LSID:illumina.hapmap.org:Protocol:Human_1M_BeadChip:3",
                "assayLSID": "urn:LSID:sanger.hapmap.org:Assay:H1Mrs1135638:3",
                "panelLSID": "urn:lsid:dcc.hapmap.org:Panel:US_Gujarati:4",
                "QC_code": "QC+",
                "refallele_freq": 0.49,
                "refallele_count": 97,
                "otherallele_freq": 0.51,
                "otherallele_count": 101,
                "totalcount": 198
              },
              "MEX": {
                "center": "sanger",
                "protLSID": "urn:LSID:illumina.hapmap.org:Protocol:Human_1M_BeadChip:3",
                "assayLSID": "urn:LSID:sanger.hapmap.org:Assay:H1Mrs1135638:3",
                "panelLSID": "urn:lsid:dcc.hapmap.org:Panel:US_Mexican-30-trios:4",
                "QC_code": "QC+",
```

```
            "refallele_freq": 0.237,
            "refallele_count": 27,
            "otherallele_freq": 0.763,
            "otherallele_count": 87,
            "totalcount": 114
          },
          "YRI": {
            "center": "sanger",
            "protLSID": "urn:LSID:illumina.hapmap.org:Protocol:Human_1M_BeadChip:3",
            "assayLSID": "urn:LSID:sanger.hapmap.org:Assay:H1Mrs1135638:3",
            "panelLSID": "urn:lsid:dcc.hapmap.org:Panel:Yoruba-60-trios:4",
            "QC_code": "QC+",
            "refallele_freq": 0.269,
            "refallele_count": 79,
            "otherallele_freq": 0.731,
            "otherallele_count": 215,
            "totalcount": 294
          }
        }
$
```

```
"CHB": {
                        "center": "sanger",
                        "protLSID": "urn:LSID:illumina.hapmap.org:Protocol:Human_1M_BeadChip:3",
                        "assayLSID": "urn:LSID:sanger.hapmap.org:Assay:H1Mrs1135638:3",
                        "panelLSID": "urn:lsid:dcc.hapmap.org:Panel:Han_Chinese:4",
                        "QC_code": "QC+",
                        "refallele_freq": 0.278,
                        "refallele_count": 74,
                        "otherallele_freq": 0.722,
                        "otherallele_count": 192,
                        "totalcount": 266
                      },
                      "TSI": {
                        "center": "sanger",
                        "protLSID": "urn:LSID:illumina.hapmap.org:Protocol:Human_1M_BeadChip:3",
                        "assayLSID": "urn:LSID:sanger.hapmap.org:Assay:H1Mrs1135638:3",
                        "panelLSID": "urn:lsid:dcc.hapmap.org:Panel:Italian:4",
                        "QC_code": "QC+",
                        "refallele_freq": 0.201,
                        "refallele_count": 41,
                        "otherallele_freq": 0.799,
                        "otherallele_count": 163,
                        "totalcount": 204
                      },
                      "JPT": {
```

```
                        "center": "sanger",
                        "protLSID": "urn:LSID:illumina.hapmap.org:Protocol:Human_1M_BeadChip:3",
                        "assayLSID": "urn:LSID:sanger.hapmap.org:Assay:H1Mrs1135638:3",
                        "panelLSID": "urn:lsid:dcc.hapmap.org:Panel:Japanese:4",
                        "QC_code": "QC+",
                        "refallele_freq": 0.339,
                        "refallele_count": 76,
                        "otherallele_freq": 0.661,
                        "otherallele_count": 148,
                        "totalcount": 224
                      },
                      "LWK": {
                        "center": "sanger",
                        "protLSID": "urn:LSID:illumina.hapmap.org:Protocol:Human_1M_BeadChip:3",
                        "assayLSID": "urn:LSID:sanger.hapmap.org:Assay:H1Mrs1135638:3",
                        "panelLSID": "urn:lsid:dcc.hapmap.org:Panel:Luhya_Kenyan:4",
                        "QC_code": "QC+",
                        "refallele_freq": 0.323,
                        "refallele_count": 71,
                        "otherallele_freq": 0.677,
                        "otherallele_count": 149,
                        "totalcount": 220
                      },
                      "MKK": {
                        "center": "sanger",
                        "protLSID": "urn:LSID:illumina.hapmap.org:Protocol:Human_1M_BeadChip:3",
                        "assayLSID": "urn:LSID:sanger.hapmap.org:Assay:H1Mrs1135638:3",
                        "panelLSID": "urn:lsid:dcc.hapmap.org:Panel:Maasai_Kenyan-60-trios:4",
                        "QC_code": "QC+",
                        "refallele_freq": 0.163,
                        "refallele_count": 51,
                        "otherallele_freq": 0.837,
                        "otherallele_count": 261,
                        "totalcount": 312
                      },
                      "ASW": {

...
```


```
$ cat example.vcf | bior_vcf_to_tjson | bior_same_variant -d
$bior/hapmap/2010-08_phaseII+III/allele_freqs_GRCh37.tsv.bgz | bior_drill -p CEU.refallele_freq -p
CEU.otherallele_freq -p YRI.refallele_freq -p YRI.otherallele_freq -p JPT.refallele_count -p
JPT.otherallele_count -p JPT.totalcount -p CHB.refallele_count -p CHB.otherallele_count -p
CHB.totalcount | cut --complement -f 9
##fileformat=VCFv4.0
#CHROMPOSIDREFALTQUALFILTERINFOCEU.refallele_freqCEU.otherallele_freqYRI.refallele_freqYRI.otherall
e_freqJPT.refallele_countJPT.otherallele_countJPT.totalcountCHB.refallele_countCHB.otherallele_coun
```

```
HB.totalcount
1215848808rs116645811GA.............
1215848808rs116645811GT.............
1215848808rs116645811GG.............
1215848808rs116645811GC.............
1215848808rs116645811CA.............
1215848808rs116645811CT.............
1215848808rs116645811CG.............
1215848808rs116645811CC.............
1215848808rs116645811AA.............
1215848808rs116645811AT.............
1215848808rs116645811AG.............
1215848808rs116645811AC.............
1215848808rs116645811TA.............
1215848808rs116645811TT.............
1215848808rs116645811TG.............
1215848808rs116645811TC.............
2126965148rs1135638GA...0.1770.8230.2690.7317614822474192266
2126965172rs010576TC.............
2126965205rs1057885TC...0.1540.8460.2380.762305686265884
2126976144rs116331755AG.............
2126976222rs7278168CT...1.000.7390.261761086791190
2126976237rs7278284CT.............
2126978790rs75377686TC.............
2126978950rs3989369AG...0.0350.9650.2650.735222422610264274
...
```

1000 Genomes:

```
$ cat example.vcf | bior_vcf_to_tjson | bior_same_variant -d
$bior/1000_genomes/20110521/ALL.wgs.phase1_release_v3.20101123.snps_indels_sv.sites_GRCh37.tsv.gz |
bior_pretty_print -r 17
#   COLUMN NAME      COLUMN VALUE
-   -----------      ------------
1   CHROM            21
2   POS              26965148
3   ID               rs1135638
4   REF              G
5   ALT              A
6   QUAL             .
7   FILTER           .
8   INFO             .
9   VCF2VariantPipe  {
```

```
                      "CHROM": "21",
                      "POS": "26965148",
                      "ID": "rs1135638",
                      "REF": "G",
                      "ALT": "A",
                      "QUAL": ".",
                      "FILTER": ".",
                      "INFO": {
                        ".": true
                      },
                      "_id": "rs1135638",
                      "_type": "variant",
                      "_landmark": "21",
                      "_refAllele": "G",
                      "_altAlleles": [
                        "A"
                      ],
                      "_minBP": 26965148,
                      "_maxBP": 26965148
                    }
10  SameVariantPipe  {
                      "CHROM": "21",
                      "POS": "26965148",
                      "ID": "rs1135638",
                      "REF": "G",
                      "ALT": "A",
                      "QUAL": "100",
                      "FILTER": "PASS",
                      "INFO": {
                        "AVGPOST": 1.0,
                        "RSQ": 0.9999,
                        "SNPSOURCE": [
                          "LOWCOV",
                          "EXOME"
                        ],
                        "AN": 2184,
                        "LDAF": 0.7609,
                        "VT": "SNP",
                        "AA": "A",
                        "AC": [
                          1661
                        ],
                        "ERATE": 2.0E-4,
                        "THETA": 3.0E-4,
                        "AF": 0.76,
                        "ASN_AF": 0.71,
                        "AMR_AF": 0.8,
                        "AFR_AF": 0.72,
                        "EUR_AF": 0.8
```

```
                      },
                      "_id": "rs1135638",
                      "_type": "variant",
                      "_landmark": "21",
                      "_refAllele": "G",
                      "_altAlleles": [
                        "A"
                      ],
                      "_minBP": 26965148,
                      "_maxBP": 26965148
                    }
 $
```

```
$ cat example.vcf | bior_vcf_to_tjson | bior_same_variant -d
$bior/1000_genomes/20110521/ALL.wgs.phase1_release_v3.20101123.snps_indels_sv.sites_GRCh37.tsv.gz |
bior_drill -p INFO.AF -p INFO.EUR_AF -p INFO.ASN_AF -p INFO.AFR_AF -p INFO.AMR_AF | cut -f 9
--complement
##fileformat=VCFv4.0
#CHROMPOSIDREFALTQUALFILTERINFOINFO.AFINFO.EUR_AFINFO.ASN_AFINFO.AFR_AFINFO.AMR_AF
1215848808rs116645811GA........

...
1215848808rs116645811TC........
2126965148rs1135638GA...0.760.80.710.720.8
2126965172rs010576TC...0.01..0.040.01
2126965205rs1057885TC...0.760.80.710.720.8
2126976144rs116331755AG...9.0E-4..0.0041.
2126976222rs7278168CT...0.110.00260.140.240.14
2126976237rs7278284CT...0.120.00260.140.270.14
2126978790rs75377686TC...0.01..0.040.01
2126978950rs3989369AG...0.910.960.970.750.94
...
$
```

Putting it All Together Building an AF Pipeline

```
TREAT]$ cat treatAF.bior
export bior=$bior/
cat /dev/stdin | bior_vcf_to_tjson \
| bior_same_variant -d $bior/dbSNP/137/00-All_GRCh37.tsv.bgz \
| bior_drill -p _id -p INFO.dbSNPBuildID -p INFO.SSR -p INFO.SCS -p INFO.CLN -p INFO.SAO  \
| bior_same_variant -c -7  -d $bior/cosmic/v63/CosmicCompleteExport_GRCh37.tsv.bgz  \
| bior_drill -p Mutation_ID -p Mutation_CDS -p Mutation_AA -p Mutation_GRCh37_strand \
```

```
| bior_same_variant -c -11  -d
$bior/1000_genomes/20110521/ALL.wgs.phase1_release_v3.20101123.snps_indels_sv.sites_GRCh37.tsv.gz
| bior_drill -p INFO.ASN_AF  -p INFO.AMR_AF -p INFO.AFR_AF -p INFO.EUR_AF  \
| bior_same_variant -c -15 -d $bior/BGI/hg19/LuCAMP_200exomeFinal.maf_GRCh37.tsv.bgz   \
| bior_drill -p estimated_minor_allele_freq \
| bior_same_variant -c -16 -d $bior/ESP/build37/ESP6500SI_GRCh37.tsv.bgz \
| bior_drill -p INFO.MAF[0] -p INFO.MAF[1] -p INFO.MAF[2] \
| bior_same_variant -c -19 -d $bior/hapmap/2010-08_phaseII+III/allele_freqs_GRCh37.tsv.bgz \
| bior_drill -p CEU.refallele_freq -p CEU.otherallele_freq \
| ./removeJSON.pl
 TREAT]$
```

7. Extracting Data with JSONPaths (bior_drill)

To extract data that is embedded in a JSON document as an array you can use drill.path[1] to get the first element in the array, drill.path[1].field to get a field in a json array or drill.path[*] to get all elements in the array.

8. Command Line Tools

Want SNPeff

```
cat example.vcf | bior_snpeff | bior_drill -p Effect -p Effect_impact -p Functional_class -p
Amino_acid_change | cut --f 9 -- --complement > example.w_genes.vcf
```

Want SIFT & PolyPhen

```
cat example.vcf | bior_vep | bior_drill -p Consequence -p SIFT -p PolyPhen -p SIFT_Score -p
PolyPhen_Score | cut --f 9 -- --complement > example.w_genes.vcf

 TREAT]$ cat treatTOOLS.bior
bior_vep < /dev/stdin \
| bior_drill -p Allele -p Gene -p Feature -p Feature_type -p Consequence -p cDNA_position -p
CDS_position -p Protein_position -p Amino_acids -p Codons -p HGNC -p SIFT_TERM -p SIFT_Score -p
PolyPhen_TERM -p PolyPhen_Score \
| bior_snpeff \
| bior_drill -p Effect -p Effect_impact -p Functional_class -p Codon_change -p Amino_acid_change -p
Gene_name -p Gene_bioType -p Coding -p Transcript -p Exon
 TREAT]$
```

9. Mixing In Scripts and Languages

To find all overlapping genes that are not the same gene:

```
zcat $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz | bior_overlap -d
$bior/v1/NCBIGene/GRCh37_p10/genes.tsv.bgz | perl -e 'while (<>) {chomp; @a=split(/\t/,$_); if($a[3
ne $a[4]){print $a[3]."\t".$a[4]."\n";} }' | bior_drill -c -2 -p gene | bior_drill -c -2 -p gene |
 less
```

10. Common Problems

Handling VCF Files with VERY large headers

All BioR commands store the header in memory. This is done because commands like bior_vcf_to_tjson use the header to understand the structure of the data lines and parse the lines into JSON more intelligently (e.g. identify numbers instead of strings, identify arrays, ect.). In production, we have noticed that some headers are extreamly large (multiple megabytes). When a user runs BioR, the header is expanded into objects in memory for each BioR command. This can lead to BioR slowing to a crawl when the ram on the machine is exceeded. Internally what happens is that the header is chopped off and stored in memory, then each row streams through the system as an array of strings. The data rows are not that large, but the metadata in the header may get copied many times in memory as transformations are done on the data. The best workaround for this problem is to use grep to cut off all excess header lines (e.g. lines that are not descriptive) then push the BioR output on to the file. Recombine the header if needed.

e.g.

zcat example.vcf.gz | head -n 10000 | grep -v "##" > mylongheader.vcf

zcat example.vcf.gz | bior_vcf_to_tjson | bior_mycommands >> mylongheader.vcf

Large Memory Requirements

Sometimes users complain about large memory requrirements from BioR – especially SNPEff. SNPEff, when run in production requires 4Gb of Ram. BioR will align large insertions and deletions prior to sending them to SNPEff using the same exact method used in SNPEff. When processing these large variants, both BioR and SNPEff can crash. The current work-around for dealing with large variants is to pre-screen them and filter them out to another file prior to annotating with SNPEff. Hopefully the BioR team will be able to collect better statistics and not align large variants in the future.

BioR exits with some error I don't understand

Rerun the same exact command with logging enabled (-l) and submit both the input file, and the results of the log to the BioR team. We will try to help you ASAP.

11. Creating Catalogs

Indexing your Samples

Lets say you want to get variants in your sample that overlap a gene. One way to do this is to stream the variants e.g:

```
> cat example.vcf | head
##fileformat=VCFv4.0
#CHROM POS ID REF ALT QUAL FILTER INFO
21 26960070 rs116645811 G A . . .
21 26965148 rs1135638 G A . . .
21 26965172 rs010576 T C . . .
21 26965205 rs1057885 T C . . .
21 26976144 rs116331755 A G . . .
21 26976222 rs7278168 C T . . .
21 26976237 rs7278284 C T . . .
21 26978790 rs75377686 T C . . .
>cat example.vcf | bior_vcf_to_tjson | bior_overlap -d $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz | gr
"\"gene\":\"PANX2\""
22 50616005 rs35195493 C G . . .
{"CHROM":"22","POS":"50616005","ID":"rs35195493","REF":"C","ALT":"G","QUAL":".","FILTER":".","INFO"
".":true},"_id":"rs35195493","_type":"variant","_landmark":"22","_refAllele":"C","_altAlleles":["G"
"_minBP":50616005,"_maxBP":50616005}
{"_type":"gene","_landmark":"22","_strand":"+","_minBP":50609160,"_maxBP":50618724,"gene":"PANX2","
ne_synonym":"hPANX2; PX2","note":"pannexin 2; Derived by automated computational analysis using gen
prediction method: BestRefseq.","GeneID":"56666","HGNC":"8600","HPRD":"09760","MIM":"608421"}
22 50616806 rs5771206 A G . . .
{"CHROM":"22","POS":"50616806","ID":"rs5771206","REF":"A","ALT":"G","QUAL":".","FILTER":".","INFO":
.":true},"_id":"rs5771206","_type":"variant","_landmark":"22","_refAllele":"A","_altAlleles":["G"],
minBP":50616806,"_maxBP":50616806}
{"_type":"gene","_landmark":"22","_strand":"+","_minBP":50609160,"_maxBP":50618724,"gene":"PANX2","
ne_synonym":"hPANX2; PX2","note":"pannexin 2; Derived by automated computational analysis using gen
prediction method: BestRefseq.","GeneID":"56666","HGNC":"8600","HPRD":"09760","MIM":"608421"}
$
```

If you just want variants that overlap any gene, you can always do something like:

```
>zcat $bior/NCBIGene/
GRCh37_p10/genes.tsv.bgz | bior_overlap -d ./example.tsv.gz |
grep -v "{}" | less
```

That works fine for a single gene, but what if you are starting with a list of genes? e.g.

```
>cat mygenes.txt
MRPL39
PANX2
```

```
BRCA1
...
```

In this case you may want to use an index on your data. To create the index, do something like:

```
>cat example.vcf | bior_vcf_to_tjson | grep "^#" | cut -f 1,2,9 |

 bior_drill -k -p _maxBP > example.tsv

>sort -k1,1 -k2,2n example.tsv
>bgzip example.tsv
>tabix example.tsv.gz
>tabix -s 1 -b 2 -e 3 example.tsv.gz
```

Now use lookup to get the gene locations, and overlap to overlap those locations with your data:

```
>cat mygenes.txt | bior_lookup -p gene
-d $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz |
bior_overlap -d ./example.tsv.gz | bior_pretty_print
```

You can now use bior_same_variant to annotate variants that overlap your genes.

Creating Custom Catalogs

One of the most powerful things about BioR is that users can publish their own catalogs and integrate new data into the system. They can also share these catalogs with others making the system extensible and much more powerful than a system where the catalogs must all be maintained by a single annotation team.

The Publication Process

Publishing a catalog requires (1) a parser that understands arbitrarily formatted file formats, and (2) indexing tools. Parsers convert arbitrary data representations into JSON with a set of 'golden identifiers' the BioR system understands. Example 'golden identifiers' include _landmark, _minBP, and _maxBP. 'Golden identifiers' are always prefixed with an underscore ('_') and must be absolutely consistent at both in terms of syntax and semantics. For example, _minBP uses the standard 1-based coordnate system (e.g. NCBI/Blast) not interbase coordinates (http://gmod.org/wiki/Introduction_to_Chado#Interbase_Coordinates), and _strand is represented as '+', '-', or '.' and NOT 'complement' as in the gbs files from NCBI. One of the functions of a parser, is to convert from arbitrary file formats into JSON, the other is to extract the 'golden identifiers' and place them in the JSON. 'Golden identifiers' are created so that BioR programs (e.g. bior_overlap.sh) can work on the information regardless of the source file format (e.g. VCF, GFF, GBS, XML, RelationalDB, Tab-Delimited, ...).
As they become availible, parsers, will be exposed to users as command line tools. For example,

bior_vcf_to_variants.sh is a parser that converts vcf to BioR JSON.

In summary, to make a custom catalog, you need:

1. Columns 1-3 bed-like (chr start stop) [1-based]
2. The 4th column is a series of key-value pairs enclosed by quotes and brackets
3. The 4 column contains "Golden identifiers" [_landmark, _minBP, and _maxBP]

Once this is created, use bgzip & tabix to compress and index it for genomic search. For those samples that do NOT have a genomic position, use the following values (`bior_create_catalog` will do this for you).

Golden Identifier	Default Value
`_landmark`	UNKNOWN (a period '.' is also ok)
`_minBP`	0
`_maxBP`	0

Zero is important because it has to be an integer and must be greater than zero. The JSON does not have to have the golden attribute if you won't search on it.

Parsing and Converting the Data

If a parser for the file format is available (e.g. `bior_vcf_to_tjson`, `bior_bed_to_tjson`, ect.) publishing a custom catalog is extremely easy. Using the standard BioR tools, a publication pipeline can be constructed rapidly. For example:

```
zcat 00-All.vcf.gz | bior_vcf_to_tjson.sh | cut -f 9 | bior_drill.sh -k -p _landmark -p _minBP -p
_maxBP > dbSNP.tsv
```

This pipeline streams the original VCF file past the parser (bior_vcf_to_tjson), removes the content of the original VCF (cut -f 9) - this is ok, as all of this information is duplicated in the JSON format, drill out the key attributes (bior_drill.sh) so that they can be indexed, and then output to a raw data file (dbSNP.tsv). The raw output file should look like this:

```
$ head dbSNP.tsv
1    10144    10145
 {"CHROM":"1","POS":"10144","ID":"rs144773400","REF":"TA","ALT":"T","QUAL":".","FILTER":".","INFO":
RSPOS":10145,"dbSNPBuildID":134,"SSR":0,"SAO":0,"VP":"0500000000050000002000200","WGT":1,"VC":"DIV",
SP":true,"OTHERKG":true},"_id":"rs144773400","_type":"variant","_landmark":"1","_refAllele":"TA","_
tAlleles":["T"],"_minBP":10144,"_maxBP":10145}
1    10177    10177
 {"CHROM":"1","POS":"10177","ID":"rs201752861","REF":"A","ALT":"C","QUAL":".","FILTER":".","INFO":{
SPOS":10177,"dbSNPBuildID":137,"SSR":0,"SAO":0,"VP":"0500000000050000002000100","WGT":1,"VC":"SNV","
P":true,"OTHERKG":true},"_id":"rs201752861","_type":"variant","_landmark":"1","_refAllele":"A","_al
lleles":["C"],"_minBP":10177,"_maxBP":10177}
...
```

Indexing the Data for Coordinate Based Search

For positional search, BioR supports indexing using Tabix. Tabix/bgzip should be installed in the RCF environment. First, compress the raw input. Assuming it is sorted:

```
$ bgzip dbSNP.tsv
```

Then run the tabix command:

```
$ tabix -s 1 -b 2 -e 3 dbSNP.tsv.gz &
```

That's it! you can now use your custom catalog as a database in BioR commands (e.g. bior_overlap.sh -d /path/to/your/database.tsv.gz).

Hints on Creating Indexes on Custom Catalogs

In addition to coordinate based search, users may also want to search a custom catalog based on IDs. The process is exactly the same as in indexing a catalog described earlier in this document, but there are some gotcha's that users need to be aware of.

1. The catalog structure will not automatically join data. This can be frustrating as the data provider may not give the data to you in a desirable form (e.g. you may want to know everything the data provider knows about a gene, but they may have their data organized by variant or drug) so you will have to 'flip' the data around so that all information about a gene can be provided to users of your catalog. The BioR team has done this many times, and for Java programmers, there is a robust library (BioR-Catalog) and examples to help in the publication of new-complex catalogs.
2. The BioR indexer command currently does not tolerate duplicate keys, so while duplicate keys can be in the data itself, you can't index on those keys. Running bior_index_catalog with logging enabled will help to ensure the keys you would like to index on are valid. To index multiple ways simultaneously, multiple catalogs need to be created
3. Regardless of what tools are used to construct the JSON column, it must validate as proper JSON. Use jslint to validate: http://jsonlint.com/
4. JSON should not contain fields that are empty. While adding period "." As the value for a given key will work, it wastes space and consumes additional CPU resources so is not recommended.

Use BioR to map SNP on rsID and find overlapping genes.

Say we obtained a simple tab-delimited file that is not in VCF format, but we still want to obtain an annotation. The following file's header for this is: rsid without the "rs", chrom, position, and 0/1 representing presence or absence in our study. There are over 5 million in this file. The goal is to show how the first 100 or 1000 of these map to various genes

```
$ zcat b132_SNPChrPosOnRef_37_1.bcp.gz | more
3       13      32446841        0
4       13      32447221        0
5       7       91839109        1
6       7       91747130        1
7       7       91779556        1
8       7       92408328        0
9       7       92373453        0
10      7       92383887        0
11      7       11364200        0
12      7       11337163        0
13      7       11387690        0
14      7       11380841        0
15      7       11602931        1
16      7       11602898        1
17      7       11583798        1
18      7       11597474        1
19      7       11597155        1
20      7       11597104        1
21      7       11596933        1
22      7       11596501        1
…
```

Try playing around with something like this to get started: (it may not be exactly what you want but we can work on that)

NCBIGene:

```
$ cat example.vcf | bior_vcf_to_tjson | bior_overlap -d $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz |
bior_pretty_print
#   COLUMN NAME      COLUMN VALUE
-   -----------      ------------
1   CHROM            1
2   POS              215848808
3   ID               rs116645811
4   REF              G
5   ALT              A
6   QUAL             .
7   FILTER           .
8   INFO             .
9   VCF2VariantPipe  {
                       "CHROM": "1",
                       "POS": "215848808",
                       "ID": "rs116645811",
                       "REF": "G",
                       "ALT": "A",
                       "QUAL": ".",
```

```
                      "FILTER": ".",
                      "INFO": {
                        ".": true
                      },
                      "_id": "rs116645811",
                      "_type": "variant",
                      "_landmark": "1",
                      "_refAllele": "G",
                      "_altAlleles": [
                        "A"
                      ],
                      "_minBP": 215848808,
                      "_maxBP": 215848808
                    }
10  OverlapPipe       {
                      "_type": "gene",
                      "_landmark": "1",
                      "_strand": "-",
                      "_minBP": 215796236,
                      "_maxBP": 216596738,
                      "gene": "USH2A",
                      "gene_synonym": "dJ1111A8.1; RP39; US2; USH2",
                      "note": "Usher syndrome 2A (autosomal recessive, mild); Derived by automated
computational analysis using gene prediction method: BestRefseq.",
                      "GeneID": "7399",
                      "HGNC": "12601",
                      "HPRD": "02042",
                      "MIM": "608400"
                    }
 $
```

```
$ cat example.vcf | bior_vcf_to_tjson | bior_overlap -d $bior/NCBIGene/GRCh37_p10/genes.tsv.bgz |
bior_drill -p GeneID -p gene | cut -f 9 --complement
##fileformat=VCFv4.0
#CHROM              POS         ID            REF         ALT         QUAL     FILTER   INFO
gene       GeneID
1              215848808           rs116645811       G             A             .
.               .              USH2A   7399
...
21           26965148             rs1135638            G             A             .
.               .              MRPL39               54148
21           26965172             rs010576             T             C             .
.               .              MRPL39               54148
```

```
21          26965205            rs1057885           T            C           .
.              .              MRPL39              54148
21          26976144            rs116331755      A           G           .
.              .              MRPL39              54148
21          26976222            rs7278168           C            T           .
.              .              MRPL39              54148
21          26976237            rs7278284           C            T           .
.              .              MRPL39              54148
21          26978790            rs75377686          T            C           .
.              .              MRPL39              54148
21          26978950            rs3989369           A            G           .
.              .              MRPL39              54148
21          26979752            rs61735760          C            T           .
.              .              MRPL39              54148
21          34022588            rs115683257      C           A           .
.              .              SYNJ1     8867
21          34029195            rs114053718      A           G           .
.              .              SYNJ1     8867
21          34058146            rs114942253      C           T           .
.              .              SYNJ1     8867
21          34059352            rs2254562           T            C           .
.              .              SYNJ1     8867
...
 $
```

Now, we want to find "Approved_Symbol", "Entrez_Gene_ID", "Ensembl_Gene_ID", "UniProt_ID", ...
We can use the BioR lookup command:

First, we don't know the catalog Structure of HGNC, here is a way to look at the structure of a catalog:

Case Study: Creating a Report that Maps rsIDs to Genes.

```
$ zcat $bior/hgnc/2012_08_12/hgnc_GRCh37.tsv.bgz | bior_pretty_print
#  COLUMN NAME  COLUMN VALUE
-  -----------  ------------
1  UNKNOWN_1    .
2  #UNKNOWN_2   0
3  #UNKNOWN_3   0
4  #UNKNOWN_4   {
                  "HGNC_ID": "HGNC:5",
                  "Approved_Symbol": "A1BG",
                  "Approved_Name": "alpha-1-B glycoprotein",
                  "Status": "Approved",
                  "Locus_Type": "gene with protein product",
                  "Locus_Group": "protein-coding gene",
                  "Previous_Symbols": [],
                  "Previous_Names": [],
                  "Synonyms": [],
                  "Name_Synonyms": [],
                  "Chromosome": "19q",
                  "Date_Approved": "1989-06-30",
                  "Date_Modified": "2010-07-08",
                  "Accession_Numbers": [],
                  "Enzyme_IDs": [],
                  "Entrez_Gene_ID": "1",
                  "Ensembl_Gene_ID": "ENSG00000121410",
                  "Specialist_Database_Links": "\u003c!--,--\u003e \u003c!--,--\u003e
\u003c!--,--\u003e \u003c!--,--\u003e \u003c!--,--\u003e \u003c!--,--\u003e \u003c!--,--\u003e
\u003c!--,--\u003e \u003c!--,--\u003e \u003ca
href\u003d\""\u003eMEROPS\u003c/a\u003e\u003c!--,--\u003e \u003ca
href\u003d\""\u003eCOSMIC\u003c/a\u003e\u003c!--,--\u003e \u003c!--,--\u003e \u003c!--,--\u003e
\u003c!--,--\u003e \u003c!--,--\u003e \u003c!--,--\u003e ",
                  "Specialist_Database_IDs": [
                    "",
                    "",
                    "",
                    "",
                    "",
                    "",
```

```
                    "",
                    "",
                    "",
                    "I43.950",
                    "A1BG",
                    "",
                    "",
                    "",
                    "",
                    "",
                    ""
```

```
],
                  "Pubmed_IDs": [
                    "2591067"
                  ],
                  "RefSeq_IDs": [
                    "NM_130786"
                  ],
                  "Record_Type": "Standard",
                  "Primary_IDs": [],
                  "Secondary_IDs": [],
                  "CCDS_IDs": [
                    "CCDS12976.1"
                  ],
                  "VEGA_IDs": [],
                  "mapped_GDB_ID": "GDB:119638",
                  "mapped_Entrez_Gene_ID": "1",
                  "mapped_OMIM_ID": "138670",
                  "mapped_RefSeq": "NM_130786",
                  "UniProt_ID": "P04217",
                  "mapped_Ensembl_ID": "ENSG00000121410",
                  "UCSC_ID": "uc002qsd.4",
                  "mapped_Mouse_Genome_Database_ID": "MGI:2152878",
                  "mapped_Rat_Genome_Database_ID": "RGD:69417"
                }
 $
```

To join the information in this catalog, to the information that we have collected in the gene table, we need to tell bior what field in the HGNC table matches the LAST column in our sample data +

annotation. In this case, we will join on approved symbol (note: if you ever get an error with doing a lookup, you may need an index file - look into the bior_index_catalog command or contact the bior team for help).

```
grep "^22.*rs3721" gene_snp.db132.gene.coding.dat | more
22 7332 UBE2L3 rs372150 29047
22 150223 YDJC rs372150 23030
22 164592 CCDC116 rs372150 15754
22 23753 SDF2L1 rs372150 8782
22 23753 SDF2L1 rs372108 45008
22 23759 PPIL2 rs372150 -12903
22 23759 PPIL2 rs372108 0
22 29799 YPEL1 rs372150 -44455
22 29799 YPEL1 rs372108 -8229
22 83746 L3MBTL2 rs3721 0
22 150356 CHADL rs3721 0
22 5905 RANGAP1 rs3721 -14542
```

12. Sun Grid Engine

This section gives tips on how to configure a Sun Grid Engine (SGE) job to request the right amount of resources to successfully execute one or more BioR toolkit commands.

Enable SGE at Mayo

At Mayo, for example, you can log onto an RCF system, such as crick7, then run "mayobiotools" and choose "69. ogs", then select the available option, choose "0" to save and exit, then log out and back in again. You should now be able to run SGE commands such as "qsub".

Multiple Cores

By default, an SGE job will run on a single core. It's possible to run a job on multiple cores is specified via the `qsub` command's parallel environment option "`-pe`".

```
-pe parallel_environment n[-[m]]|[-]m,...
```

To get a list of available parallel environments setup by your SGE admin:

```
> qconf -spl

fluent_pe
make
mpich2_141_hydra
mpich2_mpd
namd2
openmpi
pvm
pvm-tight
threaded
```

Here is an example of requesting 4 cores for a job:

```
> qsub -pe threaded 4
```

The following table gives recommend core values for toolkit commands.

Command	**Cores**	**Notes**
Arbitrary UNIX commands	0	examples: /bin/cat, /bin/grep, /bin/cut
bior_vcf_to_tjson	1	
bior_overlap	1	
bior_same_variant	1	
bior_lookup	1	
bior_drill	1	

bior_compress	1	
bior_vep	2	Warning: Variant Effect Predictor is implemented using PERL. The virtual memory for the PERL process grows linearly with more variants.
bior_snpeff	2	SnpEff loads data into memory for performance
bior_annotate	29	Annotate performs many commands in parallel
bior_pretty_print	1	

Virtual Memory

Virtual memory is specified via the `qsub` command's resource request list option "`-l`".

```
-l resource=value,...
```

NOTE: Resources specified with this option are **per-core**. If your job uses 2 cores, you will need to divide the resource value by 2.

For virtual memory, the resource name to use is `h_vmem`. Here is an example of requesting 10MB of virtual memory for a job running on 1 core:

```
> qsub -l h_vmem=10M
```

The following table gives recommend virtual memory values for toolkit commands.

Command	**Virtual Memory**	**Notes**
Arbitrary UNIX commands	100M	examples: /bin/cat, /bin/grep, /bin/cut
bior_vcf_to_tjson	600M	
bior_overlap	600M	
bior_same_variant	600M	
bior_lookup	600M	
bior_drill	600M	
bior_compress	600M	
bior_vep	1200M*	Warning: Variant Effect Predictor is implemented using PERL. The virtual memory for the PERL process grows

		linearly with more variants.
bior_snpeff	5100M	SnpEff loads data into memory for performance
bior_annotate	24000M	
bior_pretty_print	225M	

Resources for a Toolkit Pipeline

This section describes how to request the right resources for a multi-command Toolkit pipeline. Here is an example script that will be submitted to SGE:

```
> cat example.sh

#!/bin/sh

# dbSNP 137 catalog
DBSNP_CATALOG=/path/to/catalogs/dbSNP/137/00-All_GRCh37.tsv.bgz

# run toolkit pipeline to annotate my variants with dbSNP rsIDs
cat data.vcf | bior_vcf_to_tjson | bior_same_variant -d $DBSNP_CATALOG | bior_drill -p INFO.ID
```

The number of cores needed to run this script's processes in parallel can be calculated by referencing the table in the Multiple Cores section. The example script will require 3 cores to run optimally.

Command	**Cores**
example.sh	0
/bin/cat	0
bior_vcf_to_tjson	1
bior_same_variant	1
bior_drill	1

The virtual memory needed to run this script can be calculated by referencing the table in the Virtual Memory section. The example script will require 2000M of virtual memory (100 + 100 + 600 + 600 + 600).

Command	**Virtual Memory**
example.sh	100M
/bin/cat	100M
bior_vcf_to_tjson	600M
bior_same_variant	600M

bior_drill	600M

The virtual memory setting `h_vmem` is specified on a **per-core** basis. Since example.sh will be using 3 cores and 2000MB of virtual memory total, h_vem is 2000/3 or roughly 670.

Find an Open Queue

```
> qconf -sql
```

Then choose a queue from the list to run your script under. For example, we'll assume there is a queue in the list called "MY_QUEUE" which we'll use in the final command.

Here is the final `qsub` command with the correct resource requirements:

```
> qsub -m bae -M myemail@company.com  -q 1-day -l h_vmem=5000M -pe threaded 3 -V -cwd  example.sh
```

-cwd The -cwd param will specify output to go into the current directory (execute job from current dir).
-wd You can specify a target directory for output using -wd <pathToDir>.
-V Using -V param will export ALL or your environment variables.
-M Send email
-m Notify me by mail (with -M flag) when certain conditions occur

Status of your Command

Find the status of your command - here it is waiting in the queue, but has not yet started processing:

```
> qstat
job-ID  prior   name       user         state submit/start at     queue         slots ja-task-ID
-----------------------------------------------------------------------------------------------
 119477 0.00000 example.sh m054457      qw     11/22/2013 09:28:11                    3
```

After kicking off the process, it looks like:

```
$ qstat
job-ID  prior   name         user          state submit/start at       queue                slots
ja-task-ID
------------------------------------------------------------------------------------------------
 119477 0.51062 example.sh   m054457       r     11/22/2013 10:47:47   1-day@dnode91.mayo.edu   3
```

Get Command Results

The grid will output several files that begin with the script name you executed, and end with the queue jobId.

```
> ls -la example.sh.*
-rw-r--r--   1 m054457 biostat    0 Nov 22 09:38 example.sh.pe119477
-rw-r--r--   1 m054457 biostat    0 Nov 22 09:38 example.sh.pe119477
-rw-r--r--   1 m054457 biostat    0 Nov 22 09:38 example.sh.o119477
-rw-r--r--   1 m054457 biostat 283 Nov 22 09:38 example.sh.e119477
```