Swift_T_Variant_Calling Documentation

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Contents

Welcome to this documentation site for a complete Variant Calling pipeline written in Swift/T. This guide leads you through the workflow in terms of what it does, and how to up and running in using it.

The pipeline has been implemented according to the GATK's best practices for germline variant calling in Whole Genome and Whole Exome Next Generation Sequencing datasets, given a single sample or a cohort of samples, paired- or single-end reads with flexibility in choosing analysis stages, software tools and their versions, and their individual parameters for the specific analysis scenario.

CHAPTER 1

Pipeline architecture and function

This pipeline implements the GATK's best practices for germline variant calling in Whole Genome and Whole Exome Next Generation Sequencing datasets, given a cohort of samples.

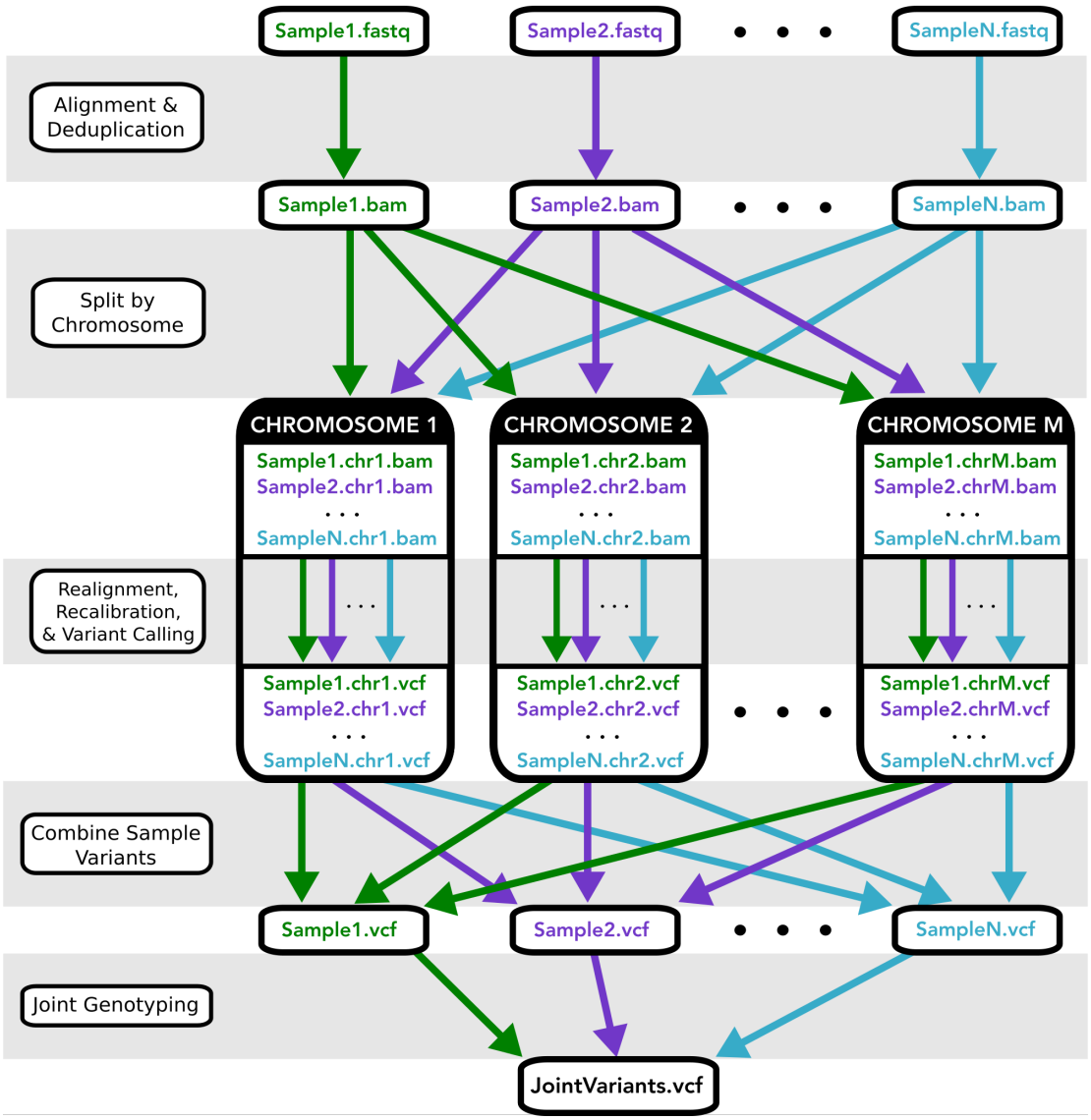
This pipeline was disigned for GATK 3.X, which include the following stages:

1. Map to the reference genome
2. Mark duplicates
3. Perform indel realignment and/or base recalibration (BQSR)*
4. Call variants on each sample
5. Perform joint genotyping

* The indel realignment step was recommended in GATK best practices <3.6).

Additionally, this workflow provides the option to split the aligned reads by chromosome before calling variants, which often speeds up performance when analyzing WGS data.

An overview of the Workflow architecture is depicted in Figure 1 below


Sample1.fastq
Sample2.fastq
SampleN.fastq
Alignment & Deduplication
Sample1.bam
Sample2.bam
SampleN.bam
Split by Chromosome
CHROMOSOME 1
Sample1.chr1.bam
Sample2.chr1.bam
SampleN.chr1.bam
CHROMOSOME 2
Sample1.chr2.bam
Sample2.chr2.bam
SampleN.chr2.bam
CHROMOSOME M
Sample1.chrM.bam
Sample2.chrM.bam
SampleN.chrM.bam
Realignment, Recalibration, & Variant Calling
Sample1.chr1.vcf
Sample2.chr1.vcf
SampleN.chr1.vcf
Sample1.chr2.vcf
Sample2.chr2.vcf
SampleN.chr2.vcf
Sample1.chrM.vcf
Sample2.chrM.vcf
SampleN.chrM.vcf
Combine Sample Variants
Sample1.vcf
Sample2.vcf
SampleN.vcf
Joint Genotyping
JointVariants.vcf

CHAPTER 2

Installation

2.1 Dependencies

First, you need Swift/T installed in your system. Depending on your system, the instructions below will guide you through the process:

http://swift-lang.github.io/swift-t/guide.html#_installation

Next, depending on the analysis step you like, you also need the installation path of the following tools in your system:

Step	Tool options
Alignment	Bwa mem or Novoalign
Sorting	Novosort
Marking Duplicates	Samblaster,
	Novosort, or
	Picard
IndelRealignment	GATK
BaseRecalibration	
Variant Calling	
Joint Genotyping	
Miscellaneous	Samtools, and
	Novosort

2.2 Workflow Installation

Simply, clone the repository:

```
git clone https://github.com/ncsa/Swift-T-Variant-Calling/
```

Additionally, you may need `R` installed along with the following packages `shiny`, `lubridate`, `tidyverse` and `forcats`. Detailed instructions are on the Logging functionality section of the *User Guide*

CHAPTER 3

User Guide

For maximum flexibility, the workflow is controlled by modifying the variables contained within a runfile.

A template.runfile is packaged within the source repo, and one can simply change the respective variables according to analysis needs. The coming sections explain the possible options in details.

3.1 Runfile Options

Different options are available by setting the variables below. Ordering is, of course, irrelevant in this context, but the workflow is sensitive to spelling, so variable names should be identical.

Variable	Effect and meaning
`SAMPLEINFORMATION`	**The file that contains the paths to each sample's** reads, where each sample is on its own line in the form: `SampleName /path/to/read1.fq /path/to/read2.fq` **Alternatively, if analyzing single-end reads, the format** is simply: `SampleName /path/to/read1.fq` *It is necessary that no empty line is inserted at the end of this file*
`OUTPUTDIR`	**The path that will serve as the root of all of the output files** generated from the pipeline (See *Output directories and files generated from a typical run of the pipeline*)

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Table 1 – continued from previous page

Variable	Effect and meaning
TMPDIR	The path to where temporary files will be stored (See *Output directories and files generated from a typical run of the pipeline*)
REALIGN	YES if one wants to realign before recalibration, NO if not.
SPLIT	YES if one wants to split-by-chromosome before calling variants, NO if not.
PROGRAMS_PER_NODE	**Sometimes it is more efficent to double (or even triple) up runs of an** application on the same nodes using half of the available threads than letting one run of the application use all of them. This is because many applications only scale well up to a certain number of threads, and often this is less than the total number of cores available on a node. Under the hood, this variable simply controls how many threads each tool gets. If CORES_PER_NODE is set to 20 but PROGRAMS_PER_NODE is set to 2, each tool will use up to 10 threads. **IMPORTANT NOTE** **It is up to the user at runtime** to be sure that the right number of processes are requested per node when calling Swift-T itself (See *Running the Pipeline*), as this is what actually controls how processes are distributed.
CORES_PER_NODE	**Number of cores within nodes to be used in the analysis. For** multi-threaded tools: $NumberOfThreads = \frac{CoresPerNode}{ProgramsPerNode}$
EXIT_ON_ERROR	**If this is set to YES, the workflow will quit after a sample fails** quality control. **If set to NO, the workflow will let samples fail, and continue** processing all of those that did not. The workflow will only stop if none of the samples remain after the failed ones are filtered out. This option is provided because for large sample sets one may expect a few of the input samples to fail quality control, and it may be acceptable to keep going if a few fail. However, exercise caution and monitor the Failures.log generated in the DELIVERYFOLDER/docs folder to gauge how many of the samples are failing.
ALIGN_DEDUP_STAGE CHR_SPLIT_STAGE	**These variables control whether each stage is ran or skipped (only** stages that were successfully run previously can be skipped, as the "skipped" option simply looks for the output files that were generated from a previous run.) **Each of these stage variables can be set to Yes, In addition,** all but the last stage can be set to End, which will stop the pipeline after that stage has been executed (think of the End setting as shorthand for "End after this stage") See *Pipeline Interruptions and Continuations* for more details.

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Table 1 – continued from previous page

Variable	Effect and meaning
VC_STAGE	
COMBINE_VARIANT_STAGE	
JOINT_GENOTYPING_STAGE	
PAIRED	0 if reads are single-ended only; 1 if they are paired-end reads
ALIGNERTOOL	Tool for the alignment stage. either: BWAMEM or NOVOALIGN
MARKDUPLICATESTOOL	Tool for marking duplicates. either: SAMBLASTER, PICARD, or NOVOSORT
BWAINDEX	**Depending on the tool being used, one of these variables specify**
NOVOALIGNINDEX	the location of the index file
BWAMEMPARAMS; NOVOALIGNPARAMS	**This string is passed directly as arguments to the corresponding tool** as (an) argument(s). For example: BWAMEMPARAMS=-k 32 -I 300,30 **Note:** There is no space between the '=' character and your parameters **Note:** Do not set the thread count or paired/single-ended flags, as they are taken care of by the workflow itself
CHRNAMES	List of chromosome/contig names separated by a :. For example: chr1:chr2:chr3 or 1:2:3 *Note:* **chromosome names must match those found in the files located i** the directory that INDELDIR points to, as well as those in the reference fasta files
NOVOSORT_MEMLIMIT	**Novosort is a tool that used a lot of RAM. If doubling up novosort runs** on the same node, this may need to be reduced to avoid an OutOfMemory Error. Otherwise, just set it to most of the RAM on a node. *You need to set this value regardless of you analysis scenario* **This is set in bytes, so if you want to limit novosort to using 30 GB,** one would set it to NOVOSORT_MEMLIMIT=30000000000
MAP_CUTOFF	The minimum percentage of reads that were successfully mapped in a successful alignment
DUP_CUTOFF	The maximum percentage of reads that are marked as duplicates in a successful sample
REFGENOME	Full path to the reference genome /path/to/example.fa. **It is assumeed reference has .dict and .fai (index) files in the same** directory
DBSNP	Full path to the dbsnp vcf file (GATK assumes that this file is indexed)

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Table 1 – continued from previous page

Variable	Effect and meaning
INDELDIR	**Full path to the directory that contains the standard indel variant files** used in the realignment/recalibration step **Within the directory, the vcf files should be named with only the** chromosome name in front and nothing else. **For example, if the chromosome is `chr12` or `12`, name the vcf** files `chr12.vcf` or `12.vcf`, respectively. **If not splitting by chromosome, the workflow will look for all of the** vcf files in the directory.
JAVAEXE	Full path of the appropriate executable file
BWAEXE	
SAMBLASTEREXE	
SAMTOOLSEXE	
NOVOALIGNEXE	
NOVOSORTEXE	
PICARDJAR	Full path of the appropriate jar file
GATKJAR	
JAVA_MAX_HEAP_SIZE	**Memory area to store all java objects. This should be tuned in relevanc** the speed and frequency at which garbage collection should occur. With larger input size, larger heap is needed.

3.2 Running the Pipeline

3.2.1 Requesting Resources from the Job Scheduler

Swift-T works by opening up multiple "slots", called processes, where applications can run. There are two types of processes this workflow allocates:

1. SERVERS - Control the execution of Swift-T itself; all Swift-T applications must have at least one of these.
2. WORKERS - Run the actual work of each application in the workflow; these will make up the vast majority of processes

Controlling various aspects of the job submission is achieved by setting environment variables to the desired values. For example, the user can fine control the total number of processes needed by setting PROCS=<Number of MPI processes>, and/or the number of workers via TURBINE_WORKERS and the number of servers via ADLB_SERVERS. Similarly, one can specify QUEUE, WALLTIME and PROJECT specifications. More coverage of these is provided in the Swift/T sites guide.

Other options allow control of logging options. Especially for users unfamiliar with Swift/T, we recommend always setting the environment variable ADLB_DEBUG_RANKS=1 and checking the beginning of the Swift/T log to be sure processes are being allocated as the user expects.

Often when we use a cluster we set the PPN variable to the number of cores on each node. Swift/T will allocate PPN processes on each node. Normally, we set PPN to the number of cores for maximal concurrency, although the PPN setting can be used to over- or under-subscribe processes. For example, an application that is short on memory might set a lower PPN, where an I/O intensive application might set a higher PPN.

For convenience, we recommend setting all such environment variables in a file, and then adding it to the Swift/T command. This is shown in the sections below for different schedulers (*PBS Torque (general)*, *Cray System (Like Blue Waters at UIUC)*, *SLURM based Systems (Like Biocluster2 at UIUC, and Stampede1/Stampede2 on XSEDE)*, *Systems without a resource manager:*).

3.2.2 Executing the Swift-T Application

If using multiple nodes, one should set the `SWIFT_TMP` to another location besides the default `/tmp`, that is shared by all of the nodes

For example, `export SWIFT_TMP=/path/to/home/directory/tmp`

The type of job scheduler dictates how one calls Swift-T will be seen in the sections below.

PBS Torque (general)

Usually, one can use swift-t's built-in job launcher for PBS Torque schedulers (calling swift-t with `-m pbs`)

```
$ cat settings.sh       # Conveniently, we save environment variables in settings.sh
export PPN=<PROGRAMS_PER_NODE>
export NODES=<#samples/PROGRAMS_PER_NODE + (1 or more)>
export PROCS=$(($PPN * $NODES))
export WALLTIME=<HH:MM::SS>
export PROJECT=<Project ID>
export QUEUE=<queue>
export SWIFT_TMP=/path/to/directory/temp

# (Optional variables to set)
export TURBINE_LOG=1
export ADBL_DEBUG_RANKS=1
export TURBINE_OUTPUT=/path/to/output_log_location

$ swift-t -m pbs -O3 -s settings.sh \
  -o /path/to/where/compiled/should/be/saved/compiled.tic \
  -I /path/to/Swift-T-Variant-Calling/src/ \
  -r /path/to/Swift-T-Variant-Calling/src/bioapps \
  /path/to/Swift-T-Variant-Calling/src/VariantCalling.swift \
  -runfile=/path/to/your.runfile
```

This command will compile and run the pipeline all in one command, and the flags used in this call do the following:

- `-O3` Conduct full optimizations of the Swift-T code during compilation (Even with full optimizations, compilation of the code takes only around 3 seconds)
- `-m pbs` The job scheduler type, pbs torque in this case
- `-s settings.sh` The file with environment variables' settings for the scheduler
- `-o` The path to the compiled swift-t file (has a .tic extension); on the first run, this file will be created.
- `-I` This includes some source files that are imported during compilation
- `-r` This includes some tcl package files needed during compilation
- `-n` The number of processes (ranks) Swift-T will open for this run of the workflow **(this overrides the PROCS specification above, so I'm not sure we should use both – ask/advise)**
- `-runfile` The path to the runfile with all of the configuration variables for the workflow

PBS Torque (alternative)

If you need to import a module to use Swift/T (as is the case on iForge at UIUC), one cannot simply use the swift-t launcher as outlined above, since the module load command is not part of the qsub file that Swift-t generates and submits.

This command must be included (along with any exported environment variables and module load commands) in a job submission script and not called directly on a head/login node.

```
swift-t -O3 -o </path/to/compiled_output_file.tic> \
-I /path/to/Swift-T-Variant-Calling/src \
-r /path/to/Swift-T-Variant-Calling/src/bioapps \
-n < Node# * PROGRAMS_PER_NODE + 1 or more > \
/path/to/Swift-T-Variant-Calling/src/VariantCalling.swift \
-runfile=/path/to/example.runfile
```

It is important to note that (at least for PBS Torque schedulers) when submitting a qsub script, the `ppn` option should be set, not to the number of cores on each compute node, but to the number of `WORKERS` Swift-T needs to open up on that node.

Example

If one is wanting to run a 4 sample job with `PROGRAMS_PER_NODE` set to 2 in the runfile (meaning that two BWA runs can be executing simultaneously on a given node, for example), one would set the PBS flag to `-l nodes=2:ppn=2` and the `-n` flag when calling the workflow to 5 (nodes*ppn + 1)

Cray System (Like Blue Waters at UIUC)

Configuring the workflow to work in this environment requires a little more effort.

Create and run the automated qsub builder

To get the right number of processes on each node to make the `PROGRAMS_PER_NODE` work correctly, one must set `PPN= PROGRAMS_PER_NODE` and `NODES` to `#samples/PROGRAMS_PER_NODE + (1 or more)`, because at least one process must be a Swift-T SERVER. If one wanted to try running 4 samples on 2 nodes but with `PPN=3` to make room for the processes that need to be SERVER types, one of the nodes may end up with 3 of your WORKER processes running simultaneously, which may lead to memory problems when Novosort is called.

(The exception to this would be when using a single node. In that case, just set `PPN=#PROGRAMS_PER_NODE + 1`)

So, with that understanding, call swift-t in the following way:

```
$ cat settings.sh
export PPN=<PROGRAMS_PER_NODE>
export NODES=<#samples/PROGRAMS_PER_NODE + (1 or more)>
export PROCS=$(($PPN * $NODES))
export WALLTIME=<HH:MM:SS>
export PROJECT=<Project ID>
export QUEUE=<Queue>
export SWIFT_TMP=/path/to/directory/temp

# CRAY specific settings:
export CRAY_PPN=true

# (Optional variables to set)
export TURBINE_LOG=1    # This produces verbose logging info; great for debugging
export ADLB_DEBUG_RANKS=1   # Displays layout of ranks and nodes
```

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```
export TURBINE_OUTPUT=/path/to/log/directory    # This specifies where the log info␣
↪will be stored; defaults to one's home directory

$ swift-t -m cray -O3 -n $PROCS -o /path/to/where/compiled/should/be/saved/compiled.
↪tic \
-I /path/to/Swift-T-Variant-Calling/src/ -r /path/to/Swift-T-Variant-Calling/src/
↪bioapps \
/path/to/Swift-T-Variant-Calling/src/VariantCalling.swift -runfile=/path/to/your.
↪runfile
```

Kill, fix, and rerun the generated qsub file

Swift-T will create and run the qsub command for you, however, this one will fail if running on two or more nodes, so immediately kill it. Now we must edit the qsub script swift produced

To fix this, we need to add a few variables to the submission file that was just created.

The file will be located in the `$SWIFT_TMP` directory and will be called `turbine-cray.sh`

Add the following items to the file:

`#PBS -V`

Note: Make sure this directory is created before running the workflow, and make sure it is not just '/tmp'

```
export SWIFT_TMP=/path/to/tmp_dir
export TMPDIR=/path/to/tmp_dir
export TMP=/path/to/tmp_dir
```

Now, if you submit the turbine-cray.sh script with qsub, it should work.

SLURM based Systems (Like Biocluster2 at UIUC, and Stampede1/Stampede2 on XSEDE)

As in the case with the pbs-based clusters, it is sufficient to only specify the scheduler using `-m slurm`, and then proceed as above. Additionaly, the same `settings.sh` file can be used, except that the user can also instruct the scheduler to send email notifications as well. The example below clarifies these:

```
$ cat settings.sh
export PPN=<PROGRAMS_PER_NODE>
export NODES=<#samples/PROGRAMS_PER_NODE + (1 or more)>
export PROCS=$(($PPN * $NODES))
export WALLTIME=<HH:MM:SS>
export PROJECT=<Project ID>
export QUEUE=<Queue>
export SWIFT_TMP=/path/to/directory/temp

# SLURM specific settings
export  MAIL_ENABLED=1
export  MAIL_ADDRESS=<the desired email address for sending notifications- on job␣
↪start, fail and finish >
export TURBINE_SBATCH_ARGS=<Other optional arguments passed to sbatch, like --
↪exclusive and --constraint=.. etc>

# (Optional variables to set)
export TURBINE_LOG=1    # This produces verbose logging info; great for debugging
export ADLB_DEBUG_RANKS=1   # Displays layout of ranks and nodes
export TURBINE_OUTPUT=/path/to/log/directory    # This specifies where the log info␣
↪will be stored; defaults to one's home directory
```

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```
$ swift-t -m slurm -O3 -n $PROCS -o /path/to/where/compiled/should/be/saved/compiled.
↪tic \
-I /path/to/Swift-T-Variant-Calling/src/ -r /path/to/Swift-T-Variant-Calling/src/
↪bioapps \
/path/to/Swift-T-Variant-Calling/src/VariantCalling.swift -runfile=/path/to/your.
↪runfile
```

Systems without a resource manager:

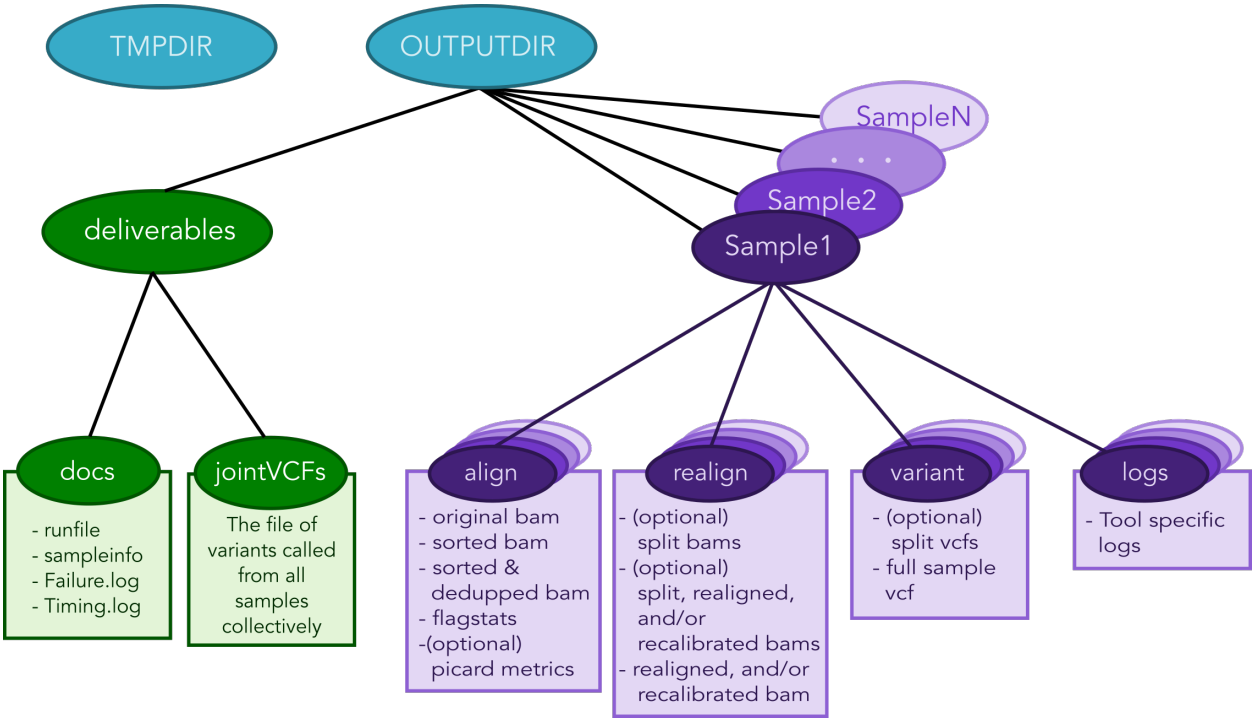
For these system, specifying the `settings.sh` file as above doesn't really populate the options to turbine when using `Swift/T version 1.2`. The workaround in such cases would be to export the settings directly to the environment, and `nohup` or `screen` the script launching the swift/t pipeline. Below is a good example:

```
$ cat runpipeline.sh
#!/bin/bash
export PROCS=$( PROGRAMS_PER_NODE * (#samples/PROGRAMS_PER_NODE + (1 or more)))
export SWIFT_TMP=/path/to/directory/temp

# (Optional variables to set)
export TURBINE_LOG=1    # This produces verbose logging info; great for debugging
export ADLB_DEBUG_RANKS=1   # Displays layout of ranks and nodes
export TURBINE_OUTPUT=/path/to/log/directory    # This specifies where the log info␣
↪will be stored; defaults to one's home directory

$ swift-t -O3 -l -u -o /path/to/where/compiled/should/be/saved/compiled.tic \
-I /path/to/Swift-T-Variant-Calling/src/ -r /path/to/Swift-T-Variant-Calling/src/
↪bioapps \
/path/to/Swift-T-Variant-Calling/src/VariantCalling.swift -runfile=/path/to/your.
↪runfile

echo -e "Swift-T pipeline run on $HOSTNAME has concluded successfully!" | mail -s
↪"swift_t_pipeline" "your_email"

$
$ nohup ./runpipeline.sh &> log.runpipeline.swift.t.nohup &
```

3.3 Output Structure

The figure below shows the Directory structure of various Output directories and files generated from a typical run of the pipeline

Fig. 1: Output directories and files generated from a typical run of the pipeline



3.4 Logging functionality

3.4.1 Swift/T logging options

While the outputs generated by all the tools of the workflow itself will be logged in the log folders within the `OUTDIR` structure, Swift-T generates a log itself that may help debug if problems occur.

Setting the environment variable `TURBINE_LOG=1` will make the log quite verbose

Setting `ADLB_DEBUG_RANKS=1` will allow one to be sure the processes are being allocated to the nodes in the way one expects

3.4.2 Workflow logging options

The provided scripts allow you to check out the trace of a successful run of the pipeline. To invoke it, and for the time being, you need R installed in your environment along with the `shiny` package.

To do so, proceed as follows:

1. Go to the R-project webpage, and follow the instructions based on your system
2. Once the step above is completed and R is installed, open a terminal window, type `R`, then proceed as follows:

```
if (!require(shiny)) {
    install.packages('shiny')
    library(shiny)
}
runGitHub(repo = "ncsa/Swift-T-Variant-Calling", ref = "master",
          subdir = "src/plotting_app" )
```

The first time you run these commands in your system it will also install some libraries for you in case you don't have them already, namely: `lubridate, tidyverse and forcats`.

Once all is done, a webpage should open up for you to actually take a look at your trace files. For a taste of how things look, you may take a look at the sample `Timing.log` file provided in the repo

To take a look at your own analysis trace, you need to have a copy of this branch first, Run it on you samples, and then find your own `Timing.log` file within `<OUTPUTDIR>/delivery/docs`, where `OUTPUTDIR` is specified as per the *runfile*. Simply upload this file, and start using the app.

3.4.3 Important Notes

- To investigate a partial pipeline run, you may `cat` the contents of all the small files in your `TMPDIR` (See *runfile* options). In the example below, the contents of thid directory are catted to the `partial_run_timing.log`, which is then uploaded to the logging webpage.

```
$ cd <TMPDIR> #TMPDIR is what has been specified in the runfile
$ find . -name '*.txt' -exec cat {} \; > partial_run_timing.log
```

- The overall summary tab of the logging webpage is handy in summarizing which samples, and which chromosomes have run successfully. It is easier to look at it when in doubt.
- Running this pipeline in its current form is expected to be more expensive than normal, due to the manual logging involved. The alternative is to use the native `MPE` library (or equivalent), which requires re-compiling the Swift/T source. This approach is **currently limited at the moment**, but some discussions with the Swift/T team on this is found here

3.5 Data preparation

For this pipeline to work, a number of standard files for calling variants are needed (besides the raw reads files which can be fastq/fq/fastq.gz/fq.gz), namely these are the reference sequence and database of known variants (Please see this link).

For working with human data, one can download most of the needed files from the GATK's resource bundle. Missing from the bundle are the index files for the aligner, which are specific to the tool that would be used for alignment (i.e., bwa or novoalign in this pipeline)

Generally, for the preparation of the reference sequence, the following link is a good start the GATK's guidelines.

If splitting by chromosome for the realignment/recalibration/variant-calling stages, the pipeline needs a separate vcf file of known variants for each chromosome/contig, and each should be named as: `*${chr_name}.vcf` . Further, all these files need to be in the `INDELDIR` which should be within the `REFGENOMEDIR` directory as per the *runfile*.

3.6 Resource Requirements

The table below describes the number of nodes each stage needs to achieve the maximum level of parallelism. One can request fewer resources if necessary, but at the cost of having some portions running in series.

Analysis Stage	Resource Requirements
Alignment and Deduplication	$Nodes = \frac{Samples}{PROGRAMS\_PER\_NODE}$
Spliting by Chromosome/Contig	$Nodes = Chromosomes * \frac{Samples}{PROGRAMS\_PER\_NODE}$
Realignment, Recalibration, and Variant Calling (w/o splitting by chr)	$Nodes = \frac{Samples}{PROGRAMS\_PER\_NODE}$
Realignment, Recalibration, and Variant Calling (w/ splitting by chr)	$Nodes = Chromosomes * \frac{Samples}{PROGRAMS\_PER\_NODE}$
Combine Sample Variants	$Nodes = \frac{Samples}{PROGRAMS\_PER\_NODE}$
Joint Genotyping	$Nodes = 1$

Notes:

- *PROGRAMS_PER_NODE* is a variable set in the runfile. Running 10

processes using 20 threads in series may actually be slower than running the 10 processes in pairs utilizing 10 threads each

- The call to GATK's GenotypeGVCFs must be done on a single node. It

is best to separate out this stage into its own job submission, so as not to waste unused resources.

3.7 Pipeline Interruptions and Continuations

3.7.1 Background

Because of the varying resource requirements at various stages of the pipeline, the workflow allows one to stop the pipeline at many stages and jump back in without having to recompute.

This feature is controlled by the `*_STAGE` variables of the runfile. At each stage, the variable can be set to `Y` if it should be computed, and `N` if that stage was completed on a previous execution of the workflow. If `N` is selected, the program will simply gather the output that should have been generated from a previous run and pass it to the next stage.

In addition, one can set each stage but the final one to `End`, which will stop the pipeline after that stage has been executed. Think of `End` as a shorthand for "End after this stage".

3.7.2 Examples

If splitting by chromosome, it may make sense to request different resources at different times.

One may want to execute only the first two stages of the workflow with `# Nodes = # Samples`. For this step, one would use these settings:

```
ALIGN_STAGE=Y
DEDUP_SORT_STAGE=Y
CHR_SPLIT_STAGE=End          # This will be the last stage that is executed
VC_STAGE=N
COMBINE_VARIANT_STAGE=N
JOINT_GENOTYPING_STAGE=N
```

Then for the variant calling step, where the optimal resource requirements may be something like # Nodes = (# Samples * # Chromosomes), one could alter the job submission script to request more resources, then use these settings:

```
ALIGN_STAGE=N
DEDUP_SORT_STAGE=N
CHR_SPLIT_STAGE=N
VC_STAGE=End                # Only this stage will be executed
COMBINE_VARIANT_STAGE=N
JOINT_GENOTYPING_STAGE=N
```

Finally, for the last two stages, where it makes sense to set # Nodes = # Samples again, one could alter the submission script again and use these settings:

```
ALIGN_STAGE=N
DEDUP_SORT_STAGE=N
CHR_SPLIT_STAGE=N
VC_STAGE=N
COMBINE_VARIANT_STAGE=Y
JOINT_GENOTYPING_STAGE=Y
```

This feature was designed to allow a more efficient use of computational resources.

CHAPTER 4

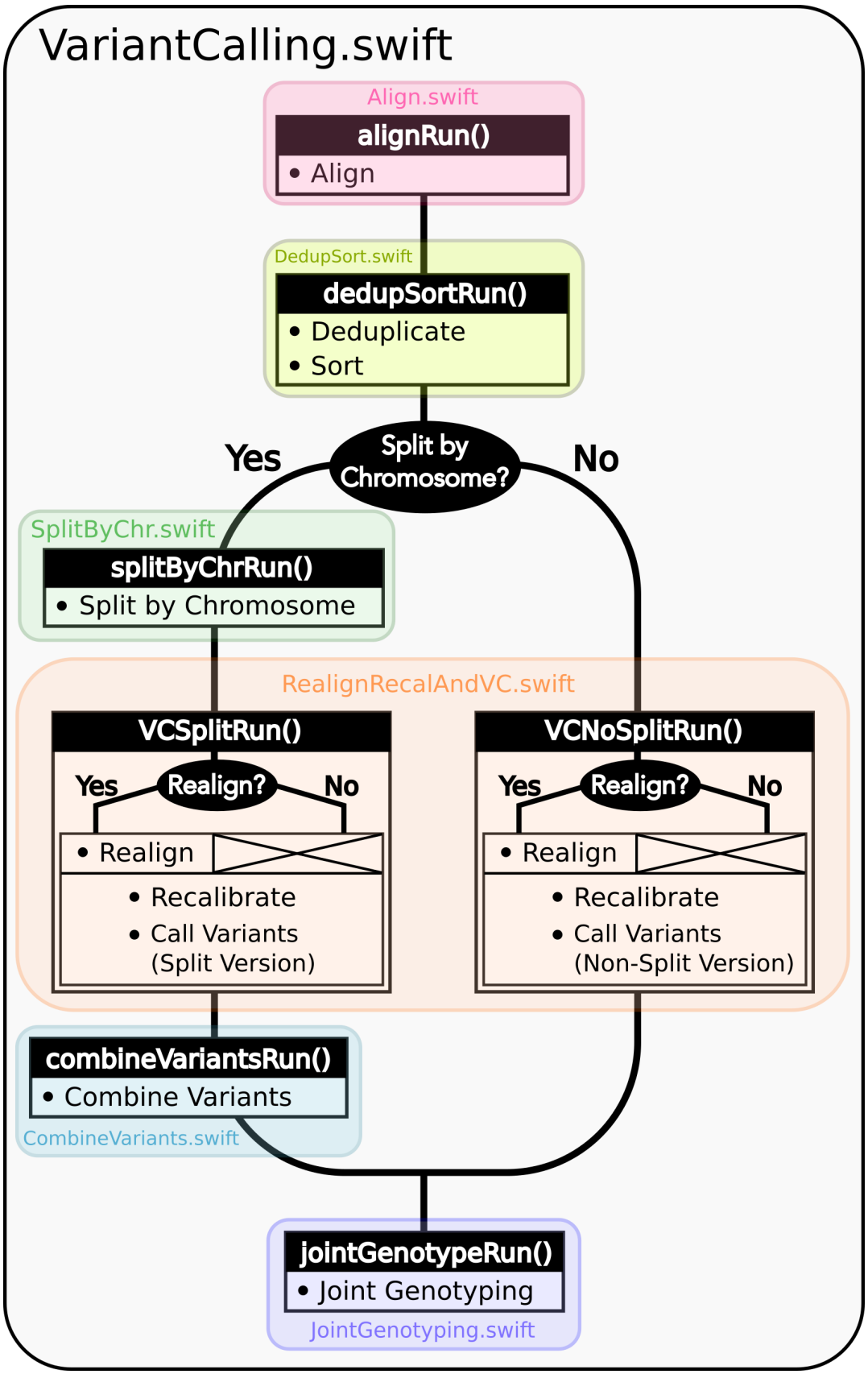
Under The Hood

Each Run function has two paths it can use to produce its output:

1. One path actually performs the computations of this stage of the pipeline

2. The other skips the computations and just gathers the output of a prior execution of this stage.

The later is useful when one wants to jump into different sections of the pipeline, and also allows Swift/T's dependency driven execution to correctly string the stages together into one workflow.


VariantCalling.swift
Align.swift
alignRun()
• Align
DedupSort.swift
dedupSortRun()
• Deduplicate
• Sort
Split by Chromosome?
Yes
No
SplitByChr.swift
splitByChrRun()
• Split by Chromosome
RealignRecalAndVC.swift
VCSplitRun()
Yes
Realign?
No
• Realign
• Recalibrate
• Call Variants (Split Version)
VCNoSplitRun()
Yes
Realign?
No
• Realign
• Recalibrate
• Call Variants (Non-Split Version)
combineVariantsRun()
• Combine Variants
CombineVariants.swift
jointGenotypeRun()
• Joint Genotyping
JointGenotyping.swift

CHAPTER 5

Troubleshooting

5.1 General Troubleshooting Tips

Regardless of the platform, one can use the following environmental variables to better debug the workflow:

- `ADLB_DEBUG_RANKS=1` One can see if the processes are spread across the nodes correctly
- `TURBINE_LOG=1` Makes the Swift-T log output very verbose
- `TURBINE_LOG_FILE=<filePath>` Changes the Swift-T log output from

StdOut to the file of choice

More debug info can be found here

5.2 FAQs

- The pipeline seems to be running, but then prematurely stops at one of the tools?
  - Solution: make sure that all tools are specified in your runfile up to the executable itself (or the jar file if applicable)
- The realignment/recalibration stage produces a lot of errors or strange results?
  - Solution: make sure you are preparing your reference and extra files (dbsnp, 1000G,...etc) according to the guidelines in the *Data Preparation* section
- Things that should be running in parallel appear to be running sequencially
  - Solution: make sure you are setting the `-n` flag to a value at least one more than `PROGRAMS_PER_NODE * NODES`, as this allocates processes for Swift/T itself to run on
- **The job is killed as soon as BWA is called?**
  - Solution: make sure there is no space in front of `BWAMEMPARAMS`
  - DO-THIS: `BWAMEMPARAMS=-k 32 -I 300,30`

  - NOT-THIS: `BWAMEMPARAMS= -k 32 -I 300,30`

- I'm not sure how to run on a cluster that uses torque as a resource manager?
  - Clusters are typically configured to kill head node jobs that run longer than a few minutes, to prevent users from hogging the head node. Therefore, you may qsub the initial job, the swift-t command with its set variables, and it will qsub everybody else from its compute node.
- I'm having difficulty running the plotting app. I get an error regarding plotly
  - The logging app depends on many R packages, including `plotly` and `tidyverse`. Some of these packages however require some OS specific packages. Fore deb systems (Debian, Ubuntu, ..etc), you may need to install `libssl-dev`, `libcurl4-openssl-dev` and `libxml2-dev` with your favourite package manager for `tidyverse` and `plotly` packages to work.

CHAPTER 6

Developer Guide

Files in this repo are organized as follows:

Folder	Content
`docs`	The files for this companion site
`media`	Various figures used in the documentation
`src`	The source code of the pipeline, written in Swift/T. See the section Under The Hood for how it is designed
`test`	Files for testing the pipeline on different platforms: XSEDE, Biocluster, Blue Waters _, iForge, and stand alone server

CHAPTER 7

Citation and Licensing

If you would like to cite the code of this workflow, please use this doi: *doi_number* <*doi_link*>. If you would like a specific code version however, please use the doi associated with that version (in the release notes).

Alternatively, you may refer to these works:

- Mainzer LS, Ahmed AE, et al. "Comparative Analysis of Genomic Sequencing Workflow Management Systems". Poster presentation at the Intelligent Systems for Molecular Biology (ISMB) 2018 conference | Chicago, USA 6-10 July 2018 `[pdf]`
- Heldenbrand J*, Ahmed AE*, Rodriguez E, et. al. "Modular genomic variant calling workflow in Swift/T". Poster presentation at the 15th Rocky Mountain Bioinformatics Conference | Aspen/Snowmass, Colorado, USA 7–9 Dec 2017 `[pdf]`