Oncology Bioinformatics

PISCES: a package for quantitation and QC of big mRNA-seq datasets

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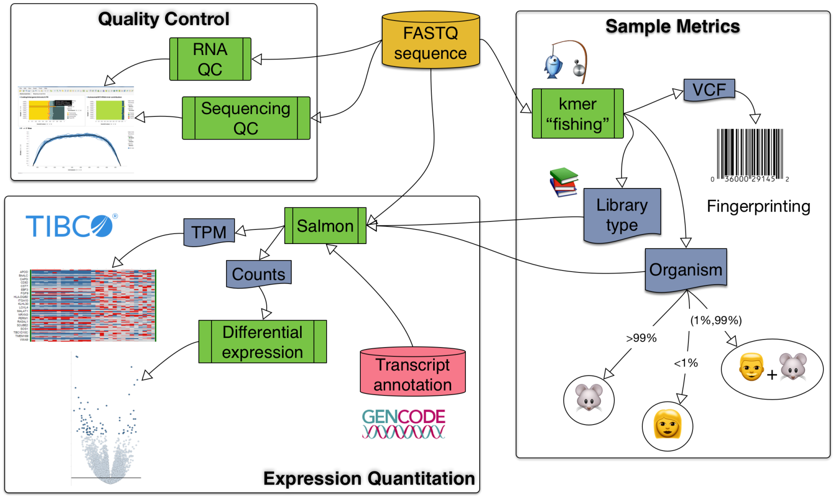
Why a new pipeline?

1. New tools are faster
2. Tooling around new tools is lacking
   - Expression QC
   - Genotyping/fingerprinting
3. Automation for reprocessing large datasets
4. Reproducibility

What is PISCES?

PISCES is a package that eases the burden of processing large numbers of mRNA-seq libraries, and subsequently reducing errors in parameter selection and QC validation and consisting of three analysis modules:



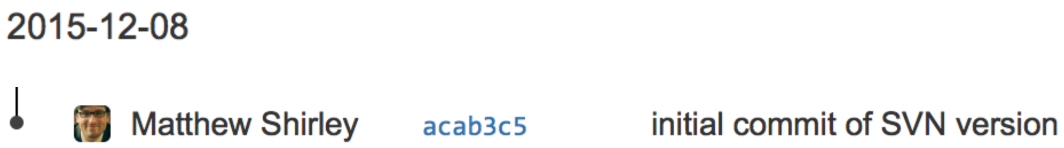
1. Single sample analysis of individual mRNA-seq libraries
   – species detection, SNP fingerprinting, library geometry detection, and quantitation using salmon
2. Multiple sample aggregation of analysis results
   – summarization, TMM normalization, and differential expression analysis of multiple libraries to produce data formats ready for visualization and further analysis
3. Multiple sample aggregation of quality control (QC) results
   – visualization of mRNA-seq library QC metrics

PISCES implementation details

1. PISCES is implemented as a python3 package
   - bundled with all necessary dependencies to enable reproducible analysis and easy deployment
2. Configuration files are specified to:
   - build transcriptome indices
   - supply sample metadata
   - define contrasts for differential expression analysis using DEseq2
   - define default program parameters
3. Development versions will be available on Bitbucket, with python packages installable using pip.

PISCES stats at Novartis Oncology (December 2016)

1. **2,894** RNAseq samples processed
   - ~30 CPU years for our previous cufflinks-based pipeline
   - ~2/3 CPU years for PISCES
   - We can reprocess TCGA, GTEx... When we need
2. **9,475** lines of code
   - 8757 python
   - 718 R
3. **Six** “stable” releases



2015-12-08

Matthew Shirley acab3c5 initial commit of SVN version

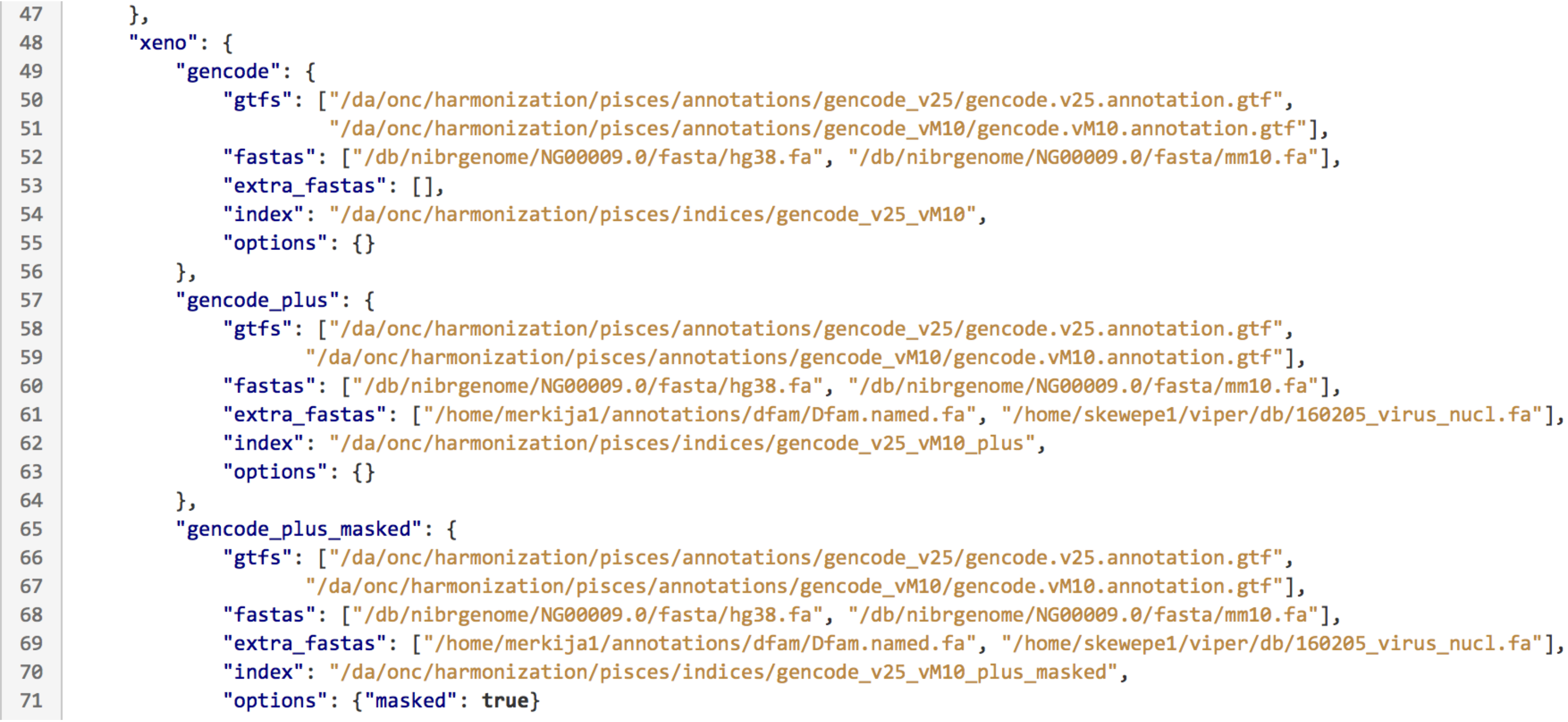
PISCES workflow

`pisces index`	Once
`pisces run`	Once each sample
`pisces qc`	Once each experiment
`pisces summarize`	Once each experiment

PISCES “index”

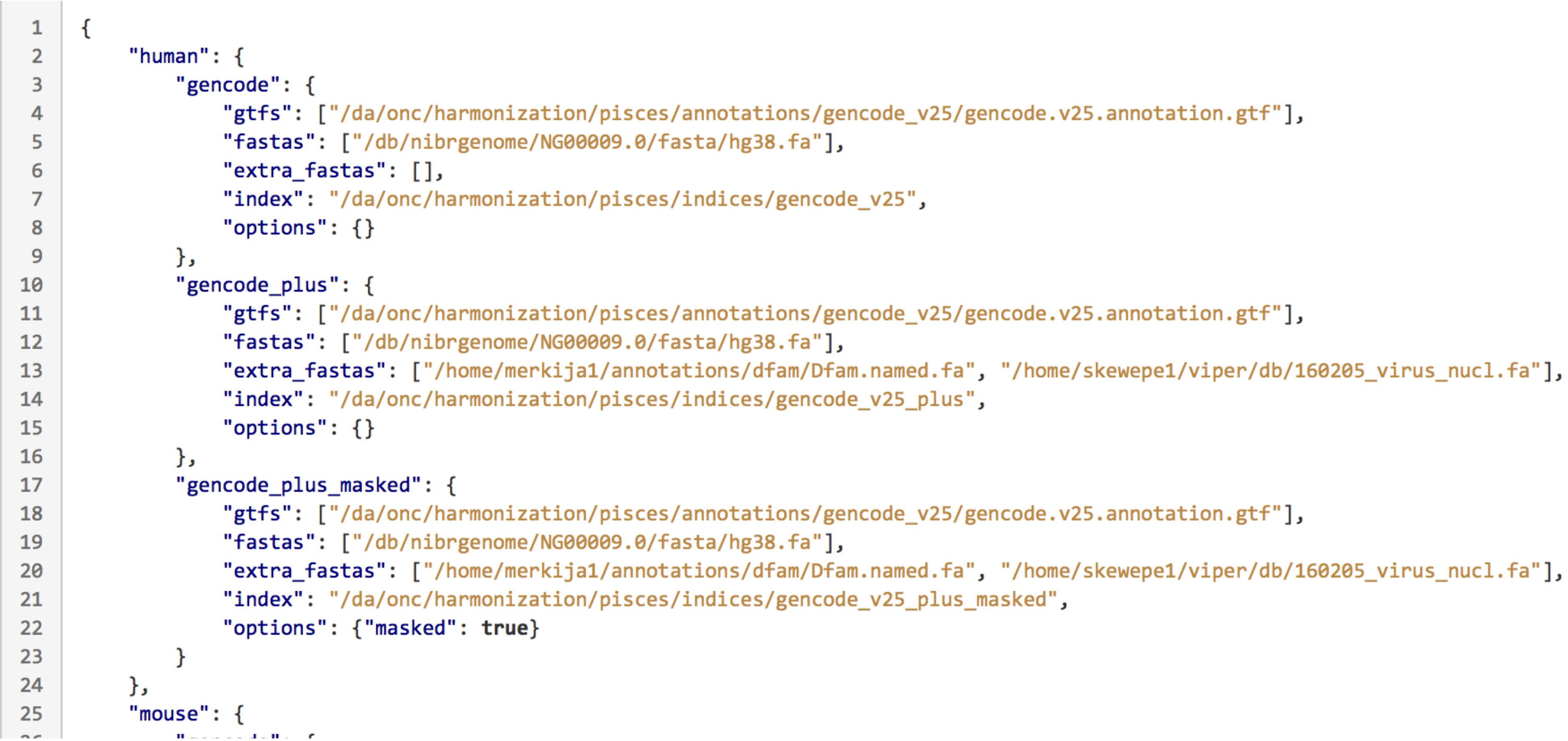
1. Creates transcriptome FASTA from input GTFs and genomic FASTAs
   - Optionally masks sequence – `ATCccccGTA → ATCNNNNGTA`
   - Add as many as you need: e.g. mouse/human xenograft
2. Incorporates “extra” user-defined FASTA files
   - e.g. **viral sequences**, repetitive elements
3. Generates salmon and bowtie2 index files
   - Bowtie2 indices are only used for QC metrics

Reproducible index builds



```
    },
    "xeno": {
        "gencode": {
            "gtfs": ["/da/onc/harmonization/pisces/annotations/gencode_v25/gencode.v25.annotation.gtf",
                     "/da/onc/harmonization/pisces/annotations/gencode_vM10/gencode.vM10.annotation.gtf"],
            "fastas": ["/db/nibrgenome/NG00009.0/fasta/hg38.fa", "/db/nibrgenome/NG00009.0/fasta/mm10.fa"],
            "extra_fastas": [],
            "index": "/da/onc/harmonization/pisces/indices/gencode_v25_vM10",
            "options": {}
        },
        "gencode_plus": {
            "gtfs": ["/da/onc/harmonization/pisces/annotations/gencode_v25/gencode.v25.annotation.gtf",
                   "/da/onc/harmonization/pisces/annotations/gencode_vM10/gencode.vM10.annotation.gtf"],
            "fastas": ["/db/nibrgenome/NG00009.0/fasta/hg38.fa", "/db/nibrgenome/NG00009.0/fasta/mm10.fa"],
            "extra_fastas": ["/home/merkija1/annotations/dfam/Dfam.named.fa", "/home/skewepe1/viper/db/160205_virus_nucl.fa"],
            "index": "/da/onc/harmonization/pisces/indices/gencode_v25_vM10_plus",
            "options": {}
        },
        "gencode_plus_masked": {
            "gtfs": ["/da/onc/harmonization/pisces/annotations/gencode_v25/gencode.v25.annotation.gtf",
                   "/da/onc/harmonization/pisces/annotations/gencode_vM10/gencode.vM10.annotation.gtf"],
            "fastas": ["/db/nibrgenome/NG00009.0/fasta/hg38.fa", "/db/nibrgenome/NG00009.0/fasta/mm10.fa"],
            "extra_fastas": ["/home/merkija1/annotations/dfam/Dfam.named.fa", "/home/skewepe1/viper/db/160205_virus_nucl.fa"],
            "index": "/da/onc/harmonization/pisces/indices/gencode_v25_vM10_plus_masked",
            "options": {"masked": true}
```

Reproducible index builds



```
{
    "human": {
        "gencode": {
            "gtfs": ["/da/onc/harmonization/pisces/annotations/gencode_v25/gencode.v25.annotation.gtf"],
            "fastas": ["/db/nibrgenome/NG00009.0/fasta/hg38.fa"],
            "extra_fastas": [],
            "index": "/da/onc/harmonization/pisces/indices/gencode_v25",
            "options": {}
        },
        "gencode_plus": {
            "gtfs": ["/da/onc/harmonization/pisces/annotations/gencode_v25/gencode.v25.annotation.gtf"],
            "fastas": ["/db/nibrgenome/NG00009.0/fasta/hg38.fa"],
            "extra_fastas": ["/home/merkija1/annotations/dfam/Dfam.named.fa", "/home/skewepe1/viper/db/160205_virus_nucl.fa"],
            "index": "/da/onc/harmonization/pisces/indices/gencode_v25_plus",
            "options": {}
        },
        "gencode_plus_masked": {
            "gtfs": ["/da/onc/harmonization/pisces/annotations/gencode_v25/gencode.v25.annotation.gtf"],
            "fastas": ["/db/nibrgenome/NG00009.0/fasta/hg38.fa"],
            "extra_fastas": ["/home/merkija1/annotations/dfam/Dfam.named.fa", "/home/skewepe1/viper/db/160205_virus_nucl.fa"],
            "index": "/da/onc/harmonization/pisces/indices/gencode_v25_plus_masked",
            "options": {"masked": true}
        }
    },
    "mouse": {
        "gencode": {
```

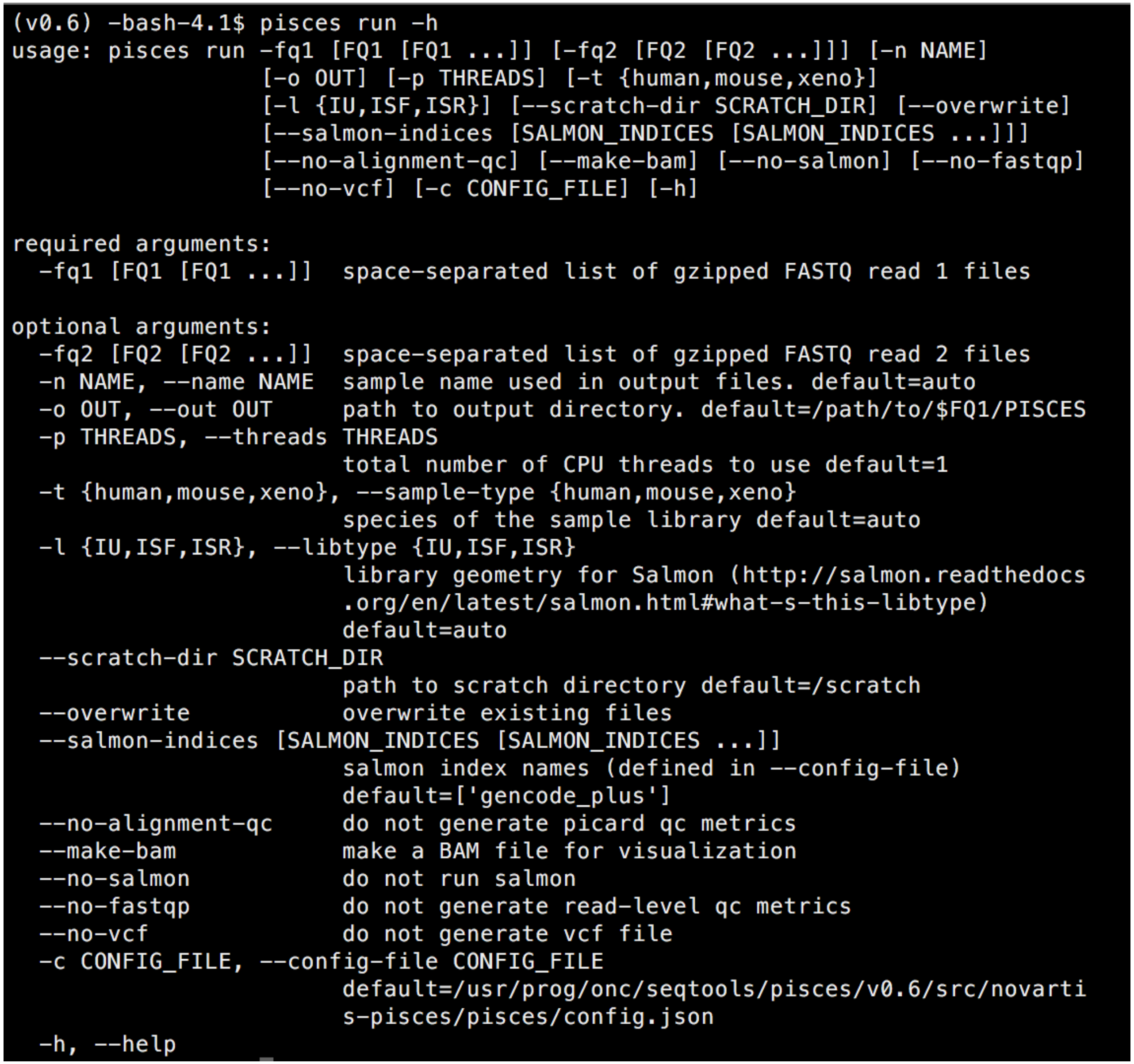
PISCES workflow

pisces index	Once
pisces run	Once each sample
pisces qc	Once each experiment
pisces summarize	Once each experiment

PISCES “run”

- Minimal examples
  - `pisces run –fq1 r1_1.fq.gz r1_2.fq  –fq2 r2_1.fq …`
  - `pisces run –fq1 r1.fq.gz`
  - `pisces run … --sample-type xeno ––salmon-indices gencode`
  - `pisces run … --threads 8 --name patient_10_liver`
  - `pisces run … --config user-config.json`
  - All parameters have defaults, or are inferred from the FASTQ files

PISCES “run”



```
(v0.6) -bash-4.1$ pisces run -h
usage: pisces run -fq1 [FQ1 [FQ1 ...]] [-fq2 [FQ2 [FQ2 ...]]] [-n NAME]
                  [-o OUT] [-p THREADS] [-t {human,mouse,xeno}]
                  [-l {IU,ISF,ISR}] [--scratch-dir SCRATCH_DIR] [--overwrite]
                  [--salmon-indices [SALMON_INDICES [SALMON_INDICES ...]]]
                  [--no-alignment-qc] [--make-bam] [--no-salmon] [--no-fastqp]
                  [--no-vcf] [-c CONFIG_FILE] [-h]

required arguments:
  -fq1 [FQ1 [FQ1 ...]]  space-separated list of gzipped FASTQ read 1 files

optional arguments:
  -fq2 [FQ2 [FQ2 ...]]  space-separated list of gzipped FASTQ read 2 files
  -n NAME, --name NAME  sample name used in output files. default=auto
  -o OUT, --out OUT     path to output directory. default=/path/to/$FQ1/PISCES
  -p THREADS, --threads THREADS
                        total number of CPU threads to use default=1
  -t {human,mouse,xeno}, --sample-type {human,mouse,xeno}
                        species of the sample library default=auto
  -l {IU,ISF,ISR}, --libtype {IU,ISF,ISR}
                        library geometry for Salmon (http://salmon.readthedocs
                        .org/en/latest/salmon.html#what-s-this-libtype)
                        default=auto
  --scratch-dir SCRATCH_DIR
                        path to scratch directory default=/scratch
  --overwrite           overwrite existing files
  --salmon-indices [SALMON_INDICES [SALMON_INDICES ...]]
                        salmon index names (defined in --config-file)
                        default=['gencode_plus']
  --no-alignment-qc     do not generate picard qc metrics
  --make-bam            make a BAM file for visualization
  --no-salmon           do not run salmon
  --no-fastqp           do not generate read-level qc metrics
  --no-vcf              do not generate vcf file
  -c CONFIG_FILE, --config-file CONFIG_FILE
                        default=/usr/prog/onc/seqtools/pisces/v0.6/src/novarti
                        s-pisces/pisces/config.json
  -h, --help
```

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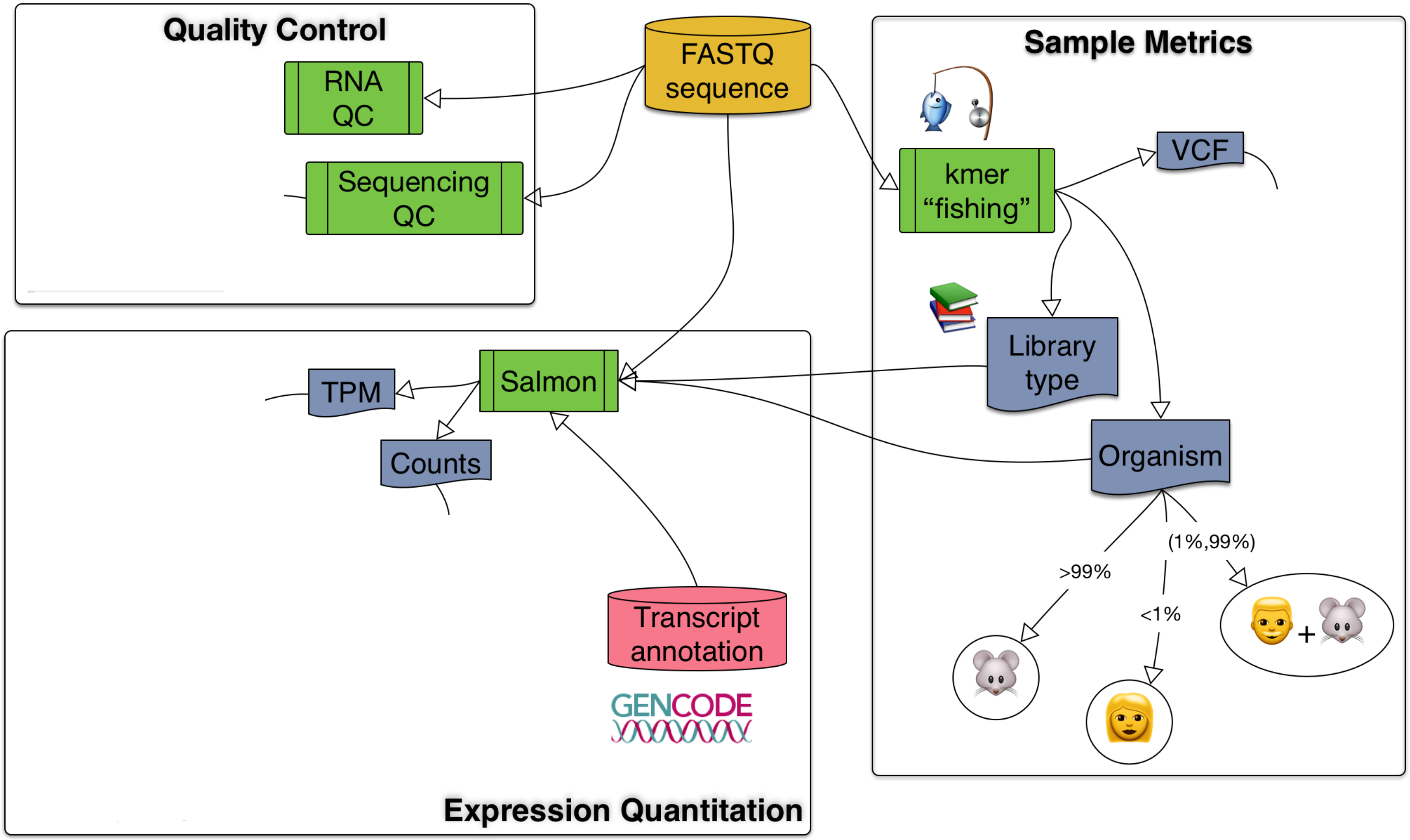
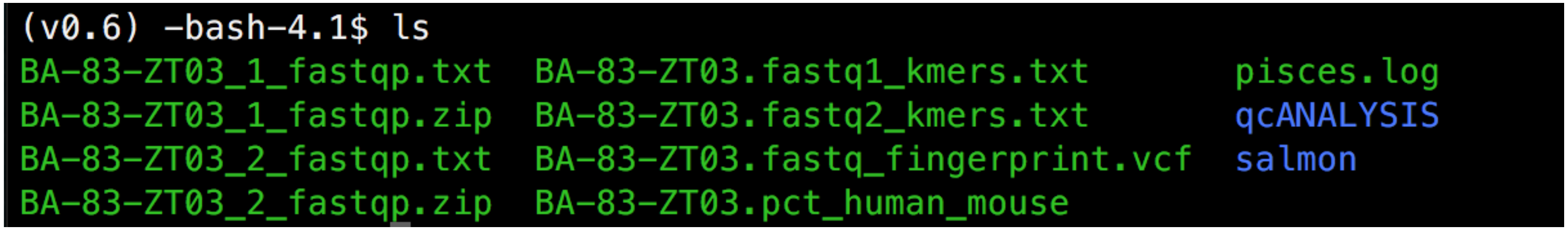
PISCES “run”


Quality Control
RNA QC
Sequencing QC
FASTQ sequence
Sample Metrics
kmer “fishing”
VCF
Library type
Organism
>99%
(1%,99%)
<1%
+
Salmon
TPM
Counts
Transcript annotation
GENCODE
Expression Quantitation

PISCES “run” outputs



```
(v0.6) -bash-4.1$ ls
BA-83-ZT03_1_fastqp.txt  BA-83-ZT03.fastq1_kmers.txt        pisces.log
BA-83-ZT03_1_fastqp.zip  BA-83-ZT03.fastq2_kmers.txt        qcANALYSIS
BA-83-ZT03_2_fastqp.txt  BA-83-ZT03.fastq_fingerprint.vcf   salmon
BA-83-ZT03_2_fastqp.zip  BA-83-ZT03.pct_human_mouse
```

- --name “BA-83-ZT03”
- **fastqp** Python clone of FastQC
  - https://github.com/mdshw5/fastqp
- **fastq_fingerprint.vcf*: genotypes derived from kmer counts
- **pct_human_mouse*: estimate of mouse/human percent derived from beta-actin kmers
- *qcANALYSIS*: picard metrics from **100,000 downsampled alignments** using bowtie2
- Salmon directory contains one or more salmon quant.sf files corresponding to –salmon-indices defined in --config

PISCES workflow

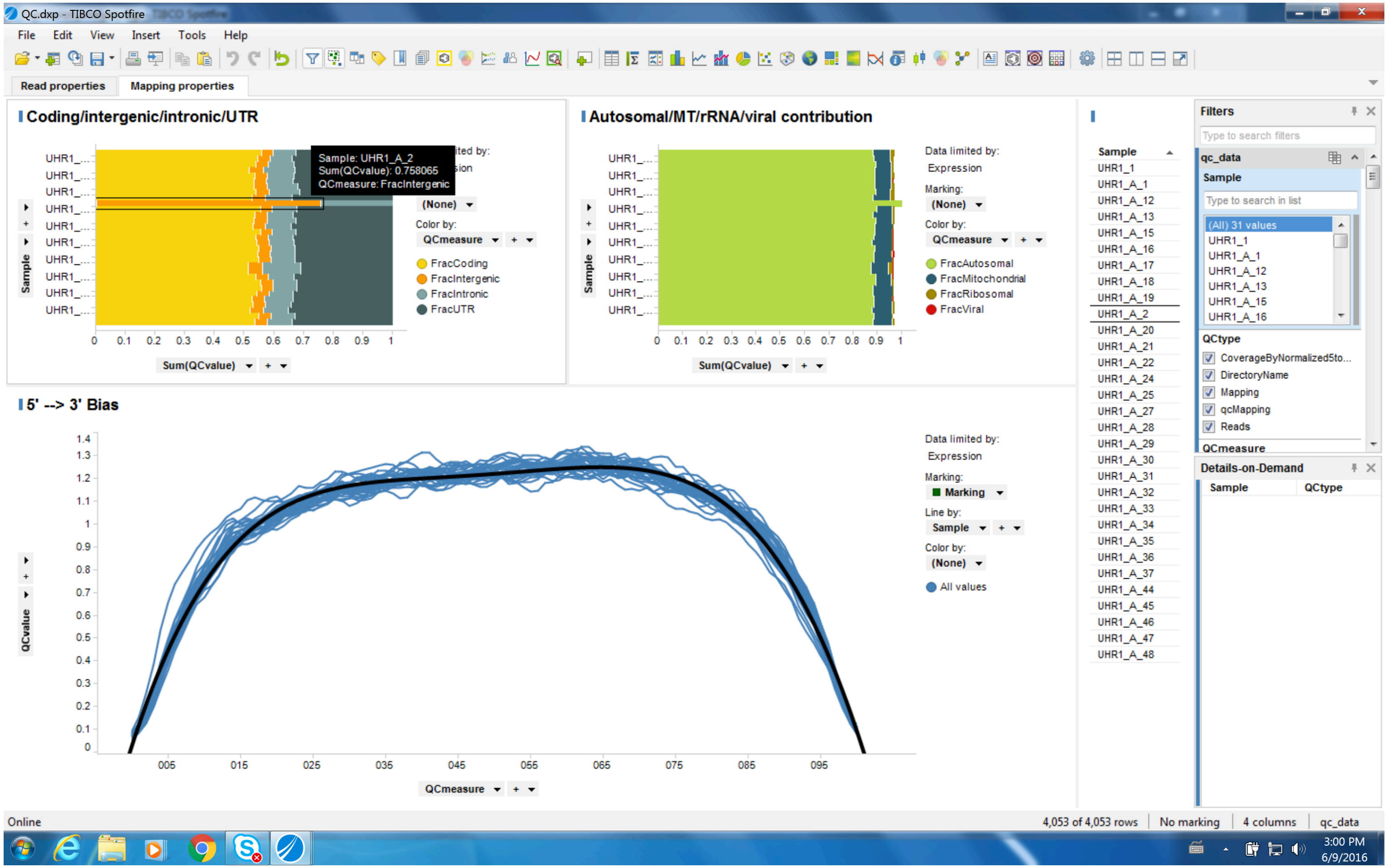
`pisces index`	Once
`pisces run`	Once each sample
`pisces qc`	Once each experiment
`pisces summarize`	Once each experiment

PISCES “qc”

- Minimal examples
  - `pisces qc –tab out.table –tall out.tall [dir1 [dir2...]]`
  - `pisces qc –metadata samples.csv`
  - `pisces qc –fingerprint [dir1 [dir2...]]`

- --tab output gathers statistics in a wide table
- --tall output is a tidy table used for visualization
- --fingerprint produces a table of sample identities and pairwise probabilities
  - Use this to find sample swaps

PISCES “qc” Spotfire vis



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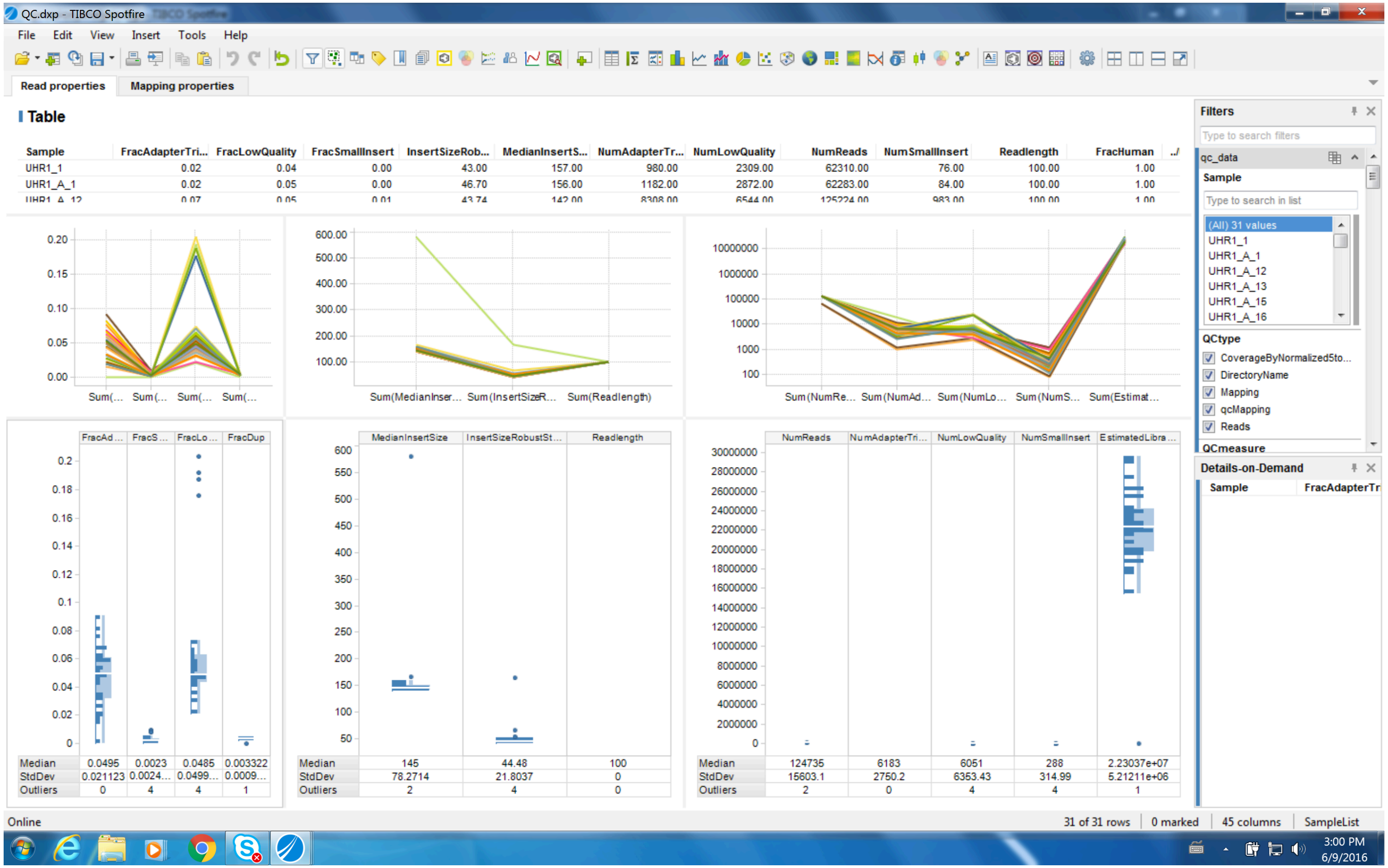
PISCES “qc” Spotfire vis



Sample	FracAdapterTri...	FracLowQuality	FracSmallInsert	InsertSizeRob...	MedianInsertS...	NumAdapterTr...	NumLowQuality	NumReads	NumSmallInsert	Readlength	FracHuman	../
UHR1_1	0.02	0.04	0.00	43.00	157.00	980.00	2309.00	62310.00	76.00	100.00	1.00	
UHR1_A_1	0.02	0.05	0.00	46.70	156.00	1182.00	2872.00	62283.00	84.00	100.00	1.00	
UHR1_A_12	0.07	0.05	0.01	43.74	142.00	8308.00	6544.00	125224.00	983.00	100.00	1.00	

	FracAd...	FracS...	FracLo...	FracDup
Median	0.0495	0.0023	0.0485	0.003322
StdDev	0.021123	0.0024...	0.0499...	0.0009...
Outliers	0	4	4	1

	MedianInsertSize	InsertSizeRobustSt...	Readlength
Median	145	44.48	100
StdDev	78.2714	21.8037	0
Outliers	2	4	0

	NumReads	NumAdapterTri...	NumLowQuality	NumSmallInsert	EstimatedLibra...
Median	124735	6183	6051	288	2.23037e+07
StdDev	15603.1	2750.2	6353.43	314.99	5.21211e+06
Outliers	2	0	4	4	1

PISCES “summarize”

- Minimal examples
  - pisces summarize [dir1 [dir2...]]
  - pisces summarize –metadata sample.csv
  - pisces summarize –metadata sample.csv –group-by cell_line –norm-by treatment –control-factor DMSO
  - pisces summarize –metadata sample.csv –deseq-contrasts contrasts.yaml –patsy ~treatment+cell_line

- Output files are prefixed by –name

PISCES “summarize”

- Minimal examples
  - `pisces summarize [dir1 [dir2...]]`
  - `pisces summarize –metadata sample.csv`
  - `pisces summarize –metadata sample.csv –group-by cell_line –norm-by treatment –control-factor DMSO`
  - `pisces summarize –metadata sample.csv –deseq-contrasts contrasts.yaml –patsy ~treatment+cell_line`

- Output files are prefixed by –name

PISCES “summarize”

- Output tables are genes/isoforms x samples (rows x columns)
- Read salmon files using tximport in DESeq2 package
- Annotation for gene-level summaries provided by https://github.com/stephenturner/annotables
- “Tidy” deseq table is 5 column: contrast, log2fc, log10p, basemean, stderr
- **Normalization**: TPM > remove mito/ribo genes > calculate TMM scaling on protein coding genes > TMM scale *all genes*

PISCES “summarize”

- Minimal examples
  - `pisces summarize –metadata sample.csv –group-by cell_line –norm-by treatment –control-factor DMSO`

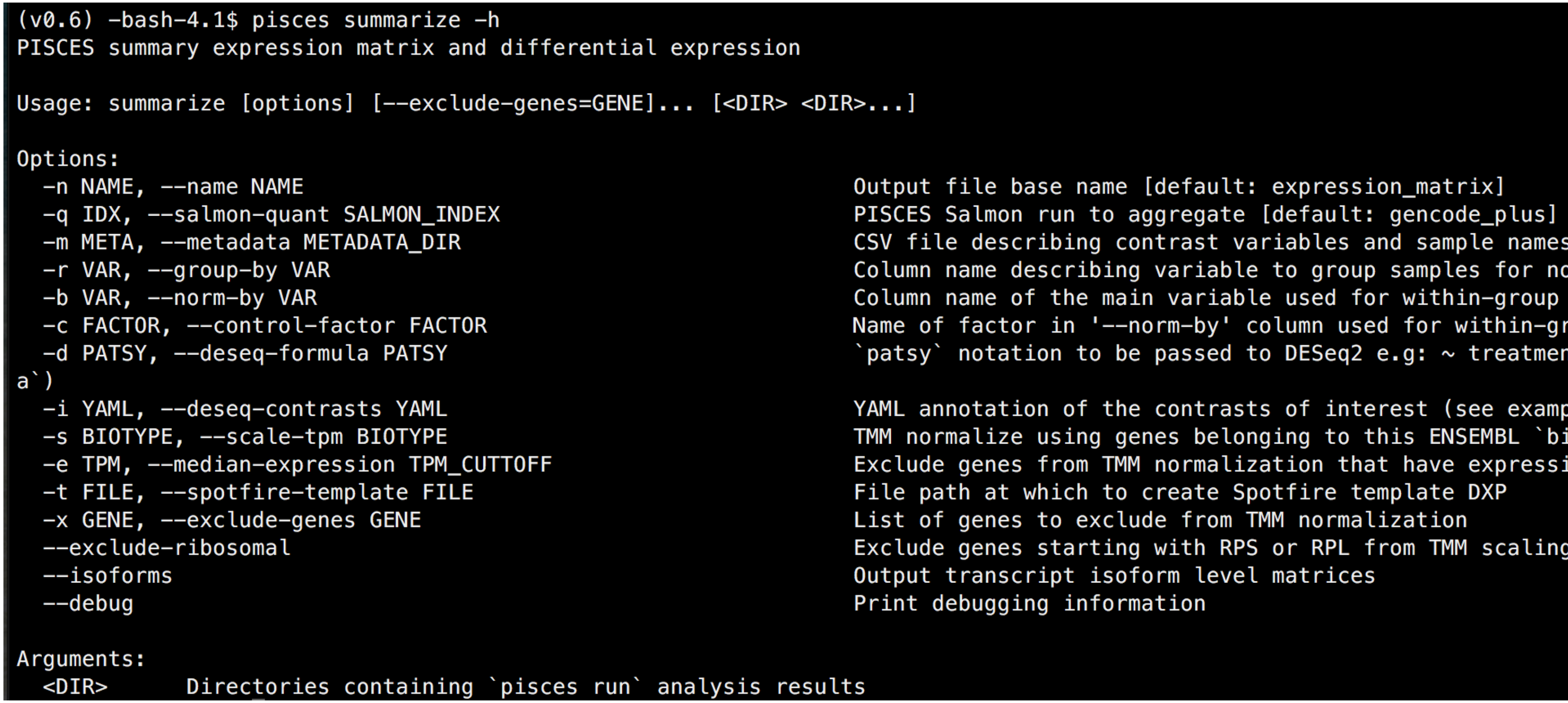
```
metadata.csv:

SampleID,UUID,CellLine,Treatment,Time,Directory,Groups
A3_DMSO_6hr_R1,CA-96-XXXX,A375,DMSO,6hr,../CA-96IY67/PISCES,A3_DMSO_6hr
A3_DMSO_6hr_R2,YA-97-XXXX,A375,DMSO,6hr,../YA-97-IB67/PISCES,A3_DMSO_6hr
A3_DMSO_24hr_R1,WA-95-XXXX,A375,DMSO,24hr,../WA-95-XA65/PISCES,A3_DMSO_24hr
A3_DMSO_24hr_R2,SA-95-XXXX,A375,DMSO,24hr,../SA-95-XE65/PISCES,A3_DMSO_24hr
```

PISCES “summarize”

- pisces summarize –metadata sample.csv –deseq-contrasts contrasts.yaml –patsy ~Treatment~CellLine

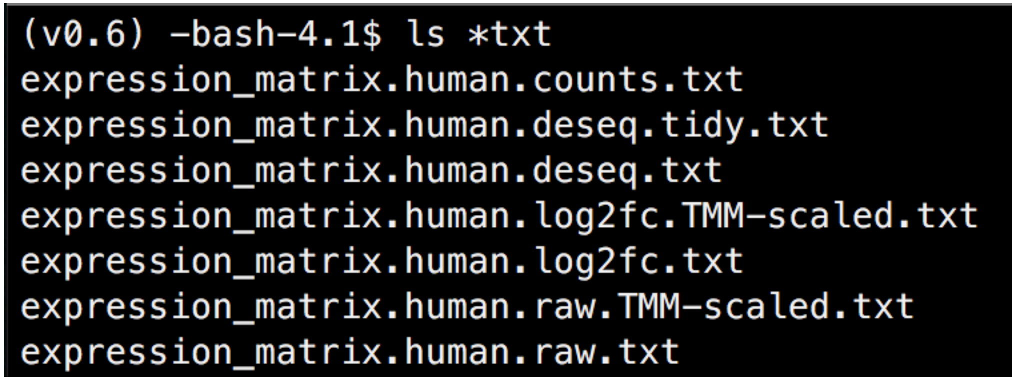
```
contrasts.yaml

Treatment:
  - [DrugA_1uM_6h, DMSO_0uM_6h]
  - [DrugA_5uM_6h, DMSO_0uM_6h]
  - [DrugA_1uM_16h, DMSO_0uM_16h]
  - [DrugA_5uM_16h, DMSO_0uM_16h]
  - [DrugB_1uM_6h, DMSO_0uM_6h]
  - [DrugB_5uM_6h, DMSO_0uM_6h]
  - [DrugB_1uM_16h, DMSO_0uM_16h]
  - [DrugB_5uM_16h, DMSO_0uM_16h]
  - [shRNA1_0uM_48h, Dox_0uM_48h]
  - [shRNA2_0uM_48h, Dox_0uM_48h]
  - [shRNA1_0uM_72h, Dox_0uM_72h]
  - [shRNA2_0uM_72h, Dox_0uM_72h]
```

PISCES “summarize”

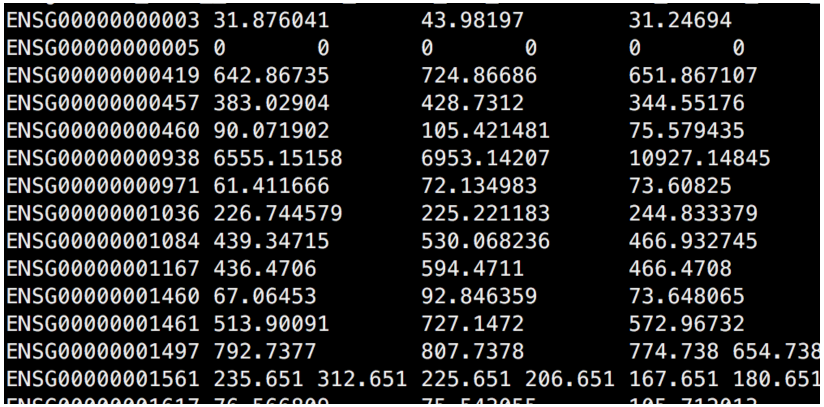


```
(v0.6) -bash-4.1$ pisces summarize -h
PISCES summary expression matrix and differential expression

Usage: summarize [options] [--exclude-genes=GENE]... [<DIR> <DIR>...]

Options:
  -n NAME, --name NAME                                Output file base name [default: expression_matrix]
  -q IDX, --salmon-quant SALMON_INDEX                 PISCES Salmon run to aggregate [default: gencode_plus]
  -m META, --metadata METADATA_DIR                    CSV file describing contrast variables and sample names
  -r VAR, --group-by VAR                              Column name describing variable to group samples for no
  -b VAR, --norm-by VAR                               Column name of the main variable used for within-group
  -c FACTOR, --control-factor FACTOR                  Name of factor in '--norm-by' column used for within-gr
  -d PATSY, --deseq-formula PATSY                     `patsy` notation to be passed to DESeq2 e.g: ~ treatmen
a`)
  -i YAML, --deseq-contrasts YAML                     YAML annotation of the contrasts of interest (see examp
  -s BIOTYPE, --scale-tpm BIOTYPE                     TMM normalize using genes belonging to this ENSEMBL `bi
  -e TPM, --median-expression TPM_CUTTOFF             Exclude genes from TMM normalization that have expressi
  -t FILE, --spotfire-template FILE                   File path at which to create Spotfire template DXP
  -x GENE, --exclude-genes GENE                       List of genes to exclude from TMM normalization
  --exclude-ribosomal                                 Exclude genes starting with RPS or RPL from TMM scaling
  --isoforms                                          Output transcript isoform level matrices
  --debug                                             Print debugging information

Arguments:
  <DIR>      Directories containing `pisces run` analysis results
```

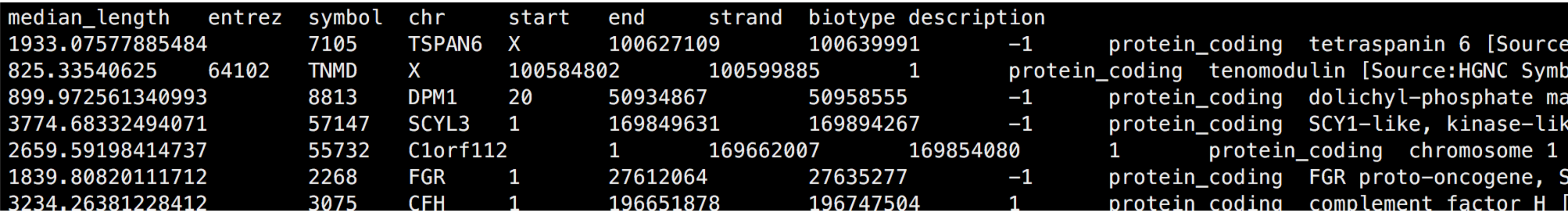
PISCES “summarize”



```
(v0.6) -bash-4.1$ ls *txt
expression_matrix.human.counts.txt
expression_matrix.human.deseq.tidy.txt
expression_matrix.human.deseq.txt
expression_matrix.human.log2fc.TMM-scaled.txt
expression_matrix.human.log2fc.txt
expression_matrix.human.raw.TMM-scaled.txt
expression_matrix.human.raw.txt
```

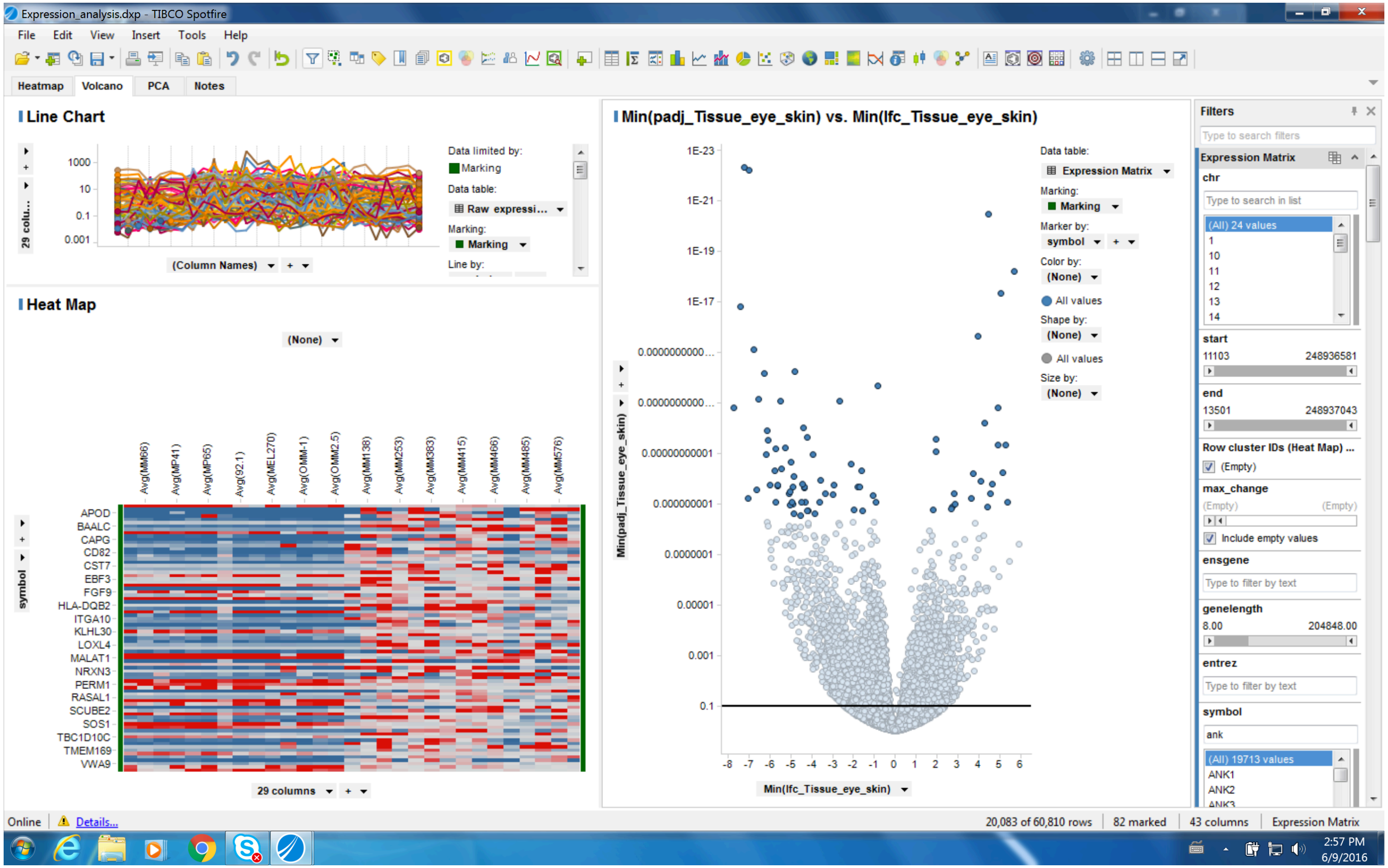


```
ENSG00000000003 31.876041       43.98197        31.24694
ENSG00000000005 0       0       0       0       0       0
ENSG00000000419 642.86735       724.86686       651.867107
ENSG00000000457 383.02904       428.7312        344.55176
ENSG00000000460 90.071902       105.421481      75.579435
ENSG00000000938 6555.15158      6953.14207      10927.14845
ENSG00000000971 61.411666       72.134983       73.60825
ENSG00000001036 226.744579      225.221183      244.833379
ENSG00000001084 439.34715       530.068236      466.932745
ENSG00000001167 436.4706        594.4711        466.4708
ENSG00000001460 67.06453        92.846359       73.648065
ENSG00000001461 513.90091       727.1472        572.96732
ENSG00000001497 792.7377        807.7378        774.738 654.738
ENSG00000001561 235.651 312.651 225.651 206.651 167.651 180.651
ENSG00000001617 76.566809       75.543055       105.712012
```



```
median_length   entrez  symbol  chr     start   end     strand  biotype description
1933.07577885484        7105    TSPAN6  X       100627109       100639991       -1      protein_coding  tetraspanin 6 [Source
825.33540625    64102   TNMD    X       100584802       100599885       1       protein_coding  tenomodulin [Source:HGNC Symb
899.972561340993        8813    DPM1    20      50934867        50958555        -1      protein_coding  dolichyl-phosphate ma
3774.68332494071        57147   SCYL3   1       169849631       169894267       -1      protein_coding  SCY1-like, kinase-lik
2659.59198414737        55732   C1orf112        1       169662007       169854080       1       protein_coding  chromosome 1
1839.80820111712        2268    FGR     1       27612064        27635277        -1      protein_coding  FGR proto-oncogene, S
3234.26381228412        3075    CFH     1       196651878       196747504       1       protein_coding  complement factor H [
```

PISCES “summarize” Spotfire vis



Data limited by: 🟩 Marking

Data table: Raw expressi...

Marking: 🟩 Marking

Line by:

Data table: Expression Matrix

Marking: 🟩 Marking

Marker by: symbol

Color by: (None)

● All values

Shape by: (None)

● All values

Size by: (None)

chr

(All) 24 values
1
10
11
12
13
14

start

11103 248936581

end

13501 248937043

Row cluster IDs (Heat Map) ...

☑ (Empty)

max_change

(Empty) (Empty)

☑ Include empty values

ensgene

genelength

8.00 204848.00

entrez

symbol

ank

(All) 19713 values
ANK1
ANK2
ANK3

20,083 of 60,810 rows | 82 marked | 43 columns | Expression Matrix

2:57 PM 6/9/2016

Near term development goals

1. Normalization efforts
   - Best practices for TMM normalization
   - Investigate *shoal* for improving abundance estimates during *pisces summarize*
     - https://github.com/COMBINE-lab/shoal
2. Automated re-identification of samples against a multi-sample VCF
3. Determine best practice for sequence masking
4. **Open source visualizations**
5. Publication

Takeaways

1. PISCES was developed to solve real-world issues:
   - Large number of datasets
   - Realize gains in efficiency using new “alignment-free” tools
   - Quick, routine QC of each sample, with fingerprinting identity
   - Identify sample/species swaps
   - Integrated tools to produce analysis or visualization-ready tables
   - Packaging of tool dependencies
   - Reproducibility of results
   - Standardization of RNAseq analysis within NIBR
2. PISCES builds on (mostly) open-source tools
3. I’ll be publishing the framework as a preprint Q1 2017

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