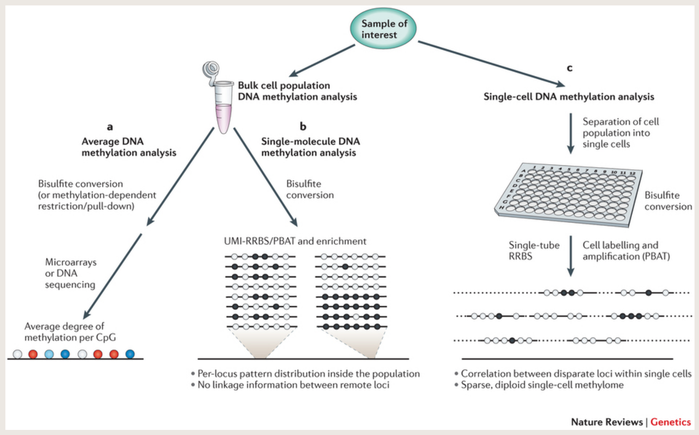
Screw: tools for building reproducible single-cell epigenomics workflows

Kieran O'Neill, Chelsey Fang, Benjamin Decato, Azhar Khandekar, Alexander Goncearenco, Ben Busby, Aly Karsan

Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, Canada

Background: Single-cell DNA Methylation Sequencing

DNA methylation is a heritable epigenetic mark that shows a strong correlation with transcriptional activity. The gold standard for detecting DNA methylation is whole genome bisulfite sequencing (WGBS). Recently, WGBS has been performed successfully on single cells (SC-WGBS) [2]. The resulting data represents a fundamental shift in the capacity to measure and interpret DNA methylation, especially in rare cell types and contexts where subtle cell-to-cell heterogeneity is crucial, such as in stem cells or cancer.



DNA Methylation, both bulk and single cell Schwartzman et al (2015) Nature Reviews in Genetics (used by permission)

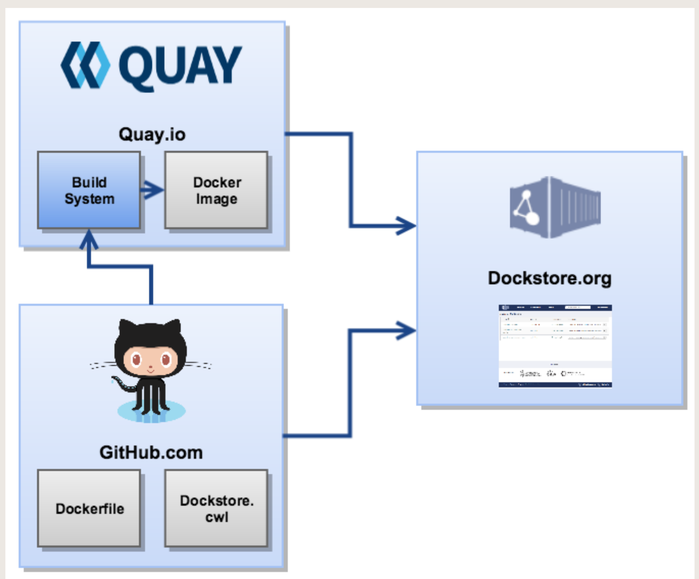
Reproducible Research with CWL and Docker

Reproducible research means completely reproducing a given bioinformatic analysis This requires having the exact **data**, **code** and **software** that was used.

- Open data in bioinformatics is a fairly solved problem.
- Code is getting there with RMarkdown/Jupyter, but could be better.
- Software versions (and accompanying OS/ecosystem) are a big problem.

CWL aims to provide a uniform and fully reproducible way of representing bioinformatics workflows. Docker aims to provide the exact environment in which an analysis was run. Together, they promise to help bioinformaticians to publish fully reproducible research.

As a side effect, Dockstore enables easy sharing of workflow components to help build new workflows.



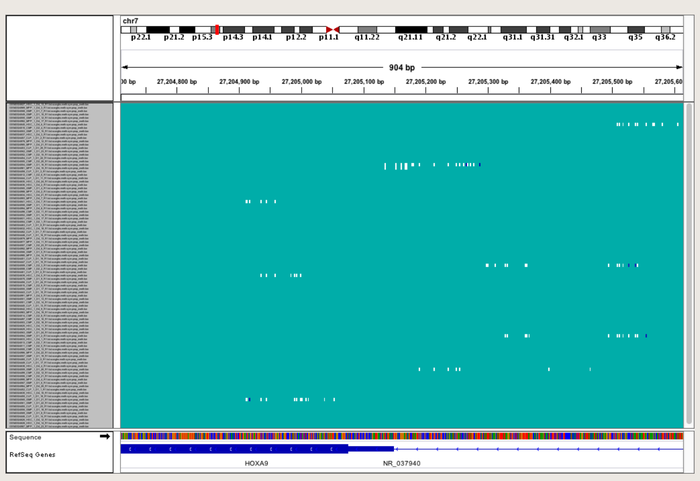
Docker, CWL and Dockstore. CWL workflows (and Docker build files) are kept on GitHub. Quay.io automatically builds Docker images from these. Dockstore enables sharing of CWL-specified tools and workflows, along with a Docker image containing exact software.

Screw: Single Cell Reproducible Epigenomics Workflow

Screw aims to provide a series of CWL+Dockerised mini-workflows and workflow components for creating fully reproducible single-cell DNA methylation analyses.

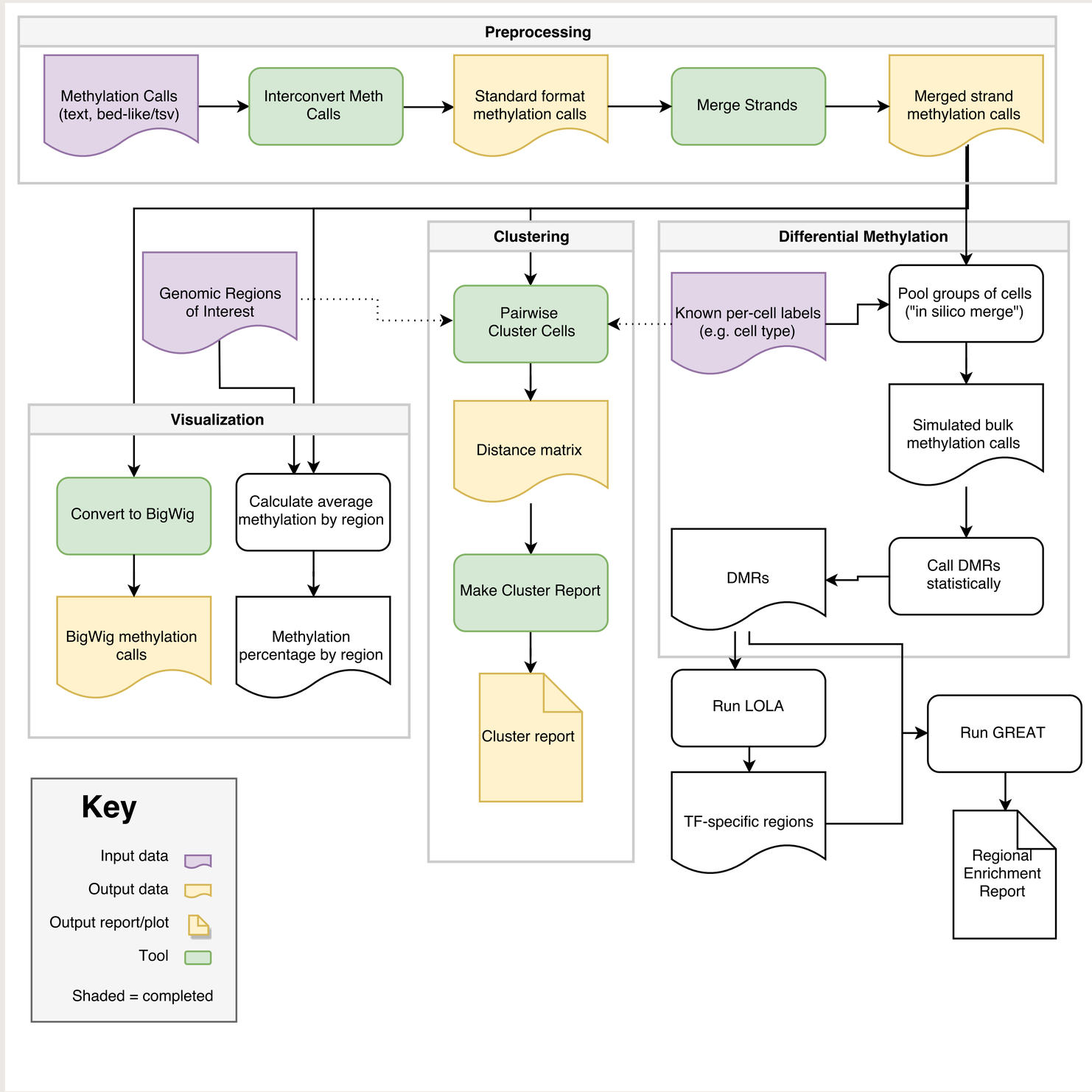
What SC-WGBS Data Looks Like

Lots of cells, but lots of dropout too.



DNA methylation around the *HOXA9* TSS, from 100 single cells. Blue blocks indicate methylated CpGs, white unmethylated. Teal indicates missing data. DNA methylation data is very sparse – only a handful of libraries have any coverage at all within a given window. Data from [1]; BigWIGS generated by Screw.

Screw Workflow



The Screw workflow, showing completed and short-term planned functionality.

Preprocessing: Format Interconversion

One of the challenges in DNA methylation is the lack of a standard data format. BED files are poorly suited, since multiple data columns are needed. Also, CpG sites are all point locations, so the end column is unncessary. WIG/BigWIG can help, but don't support enough data columns. The consequence is that every data set has its own custom BED-like format. We are working on providing

Farlik 2013

```
chr19   89480   -       1       0       CG      CGT
chr19   93048   +       0       1       CG      CGT
chr19   109711  -       1       0       CG      CGG
chr19   109828  -       1       0       CG      CGT
```

Farlik 2016

```
chr10   48037   1       1
chr10   48132   0       1
chr10   48140   0       1
chr10   48143   0       1
```

NovoMethyl

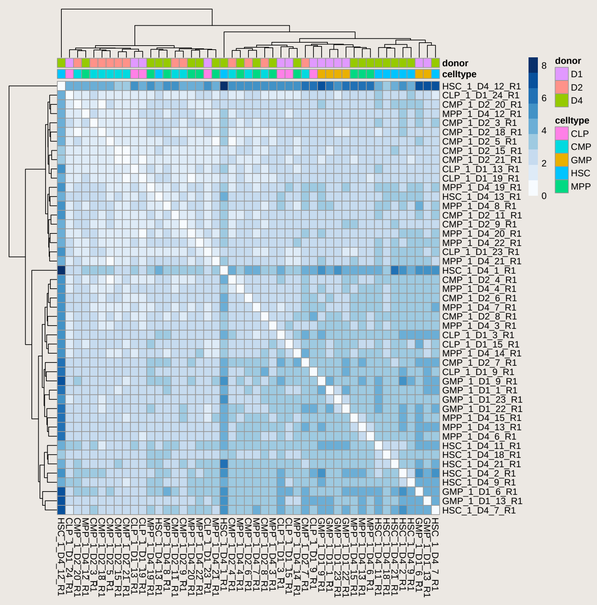
```
chr1    3113716 3113718 100     1       1       1
chr1    3327525 3327527 0       0       1       1
chr1    3327549 3327551 100     1       1       1
chr1    3642497 3642499 100     1       1       1
```

Screw

```
chr6 149393 * CpG 0.5 2
chr6 149422 * CpG 0.5 2
chr6 190375 * CpG 0.5 2
chr6 190632 * CpG 0.5 2
```

Diverse DNA methylation formats. Formats from two published papers ([**Farlik2013**] and [1]), NovoMethyl output format (unpublished), and Screw's standard internal format.

Clustering



Clustering of data from [1]. The data was subset to only include enhancer sites from Enhancer Atlas found in CD34+ bone marrow cells. Only 47/100 cells had coverage in those regions.

CWL+Docker Stumbling Blocks

- CWL does not have a good way of processing a whole directory full of files, then passing them on to a single tool.
- Quay.io maps tags to GitHub branches, but Docker treats tags like Git graph nodes, leading to problems refreshing branches while developing.
- Our hyper-secure clinical IT environment won't let us use Docker, but will install Udocker, which CWL runner does not support by default.

Future Plans

Many methods are currently being developed, and some have debuted in papers without (yet) an accompanying methods paper. In future we plan to include functionality for:

- tSNE plotting
- Epiphylogenomics
- DeepCpG
- Other machine learning pipelines (e.g. from [1])

Powered by hackathons and interns

Screw was initially created during the NCBI Genomics Hackathon in March 2017, organised by Ben Busby. Ben organises awesome hackathons that drive a lot of new open source tool development. Since then, Chelsey Fang, a summer intern, has been working on the framework.

The Screw team. From left to right, Azhar Khandekar, Benjamin Decato, , Kieran O'Neill, Alexander Goncearenco; separate photo: Chelsey Fang.

References

[1] M Farlik et al. "DNA Methylation Dynamics of Human Hematopoietic Stem Cell Differentiation". In: *Cell Stem Cell* 19.6 (2016), pp. 808–822.

[2] O Schwartzman and A Tanay. "Single-cell epigenomics: techniques and emerging applications". In: *Nature Reviews Genetics* 16.12 (2015), pp. 716–26.

Contact/GitHub

Code/examples: `https://github.com/Epigenomics-Screw`

Kieran O'Neill: koneill@bcgsc.ca