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1 Introduction

(DRAFT: Okay like this?)

Hi-C is a method commonly used for getting 3D-information of genomes. Such technologies tend to suffer from technical (e.g. sequencing, mapping) [1] and biological factors (e.g. distinct chromatin states) [2], making them inherently inaccurate.

However, a basic assumption about the structure of the genome can be made, which is that every location has the same amount of interactions (with other locations) as every other location. The data does not show this however, which is probably due to PCR artifacts [3].

Now our approach is to just take this assumption, and "normalize" the data we have iteratively. Eigenvector decomposition of the normalized data can then give us new insights on local chromatin states or global patterns of chromosomal interaction [4].

We will not do the Eigenvector decomposition, but the iterative correction ("normalization") before that.

1.1 Core setup

(DRAFT: Okay like this?)

This work is part of the HiCExplorer (Section 3.1) tool from the Deeptools (Section 3.2) framework. HiCExplorer is mainly written in Python, with this implementation needing too much working memory (RAM) for the iterative correction. For some time, the goal was to reduce memory usage by not copying a huge matrix a couple of times. From the Rust side, it would have been possible to read and write to the matrix in-memory, but we decided against doing so due to concerns regarding correctness. Still, the focus is to investigate which version requires less resources (CPU Time/RAM/...).

This is going to be an interesting comparison, since, the (before) default Python-implementation was only using one core. Rust code written for single-core applications can easily be turned to multicore code. During the work of this project, an implementation of a different algorithm (Section **??**) written in C++ got added, so We'll compare with this one now as well.

(EXTEND: maybe add more details)

1.2 Algorithm

(TODO: describe the algorithm)

(TODO: Reference description from additional notes from the 2012-paper and) **(EXTEND: the description of the algorithm to be easily understandable, include code (probably pseudocode, not python or rust))** Our fundamental assumption is that every location in our Matrix has in total as many interactions (with other locations) as every other location. Taking this in mind, the algorithm itself is pretty straightforward.

1.3 Operation

(DRAFT: Okay like this?)

(DRAFT: Change Name!) smb can be run on any Unix-based operating system (tested using ubuntu-18.04) with Python, Rust and common development packages installed (e.g. libopenssl-dev, python3-dev, build-essential, ...). For best performance, the size of the matrix should correlate with the number of available cores and the amount of available RAM **(EXTEND: Give rough factors!)**.

1.4 Motivation

(DRAFT: Okay like this?)

There is several reasons for using Rust, including faster development than C++ and better tooling, while still having the same if not better performance. A killer feature here is the easiness of adding parallelism, and the modularity of Rust code. Libraries in Rust do not do weird things, and the compiler will complain if the memory is not handled the way it should be (in C++ it is common practise to misunderstand which part should care about what memory, easily resulting in Segfaults and other hard to debug issues). The benefits to using Rust instead of Python are only apparent if Performance or Safety (of execution, at runtime) is needed - which is the case here.

(TODO: Add motivation for this whole Hi-C part)

2 Background

Explain the math and notation.

(TODO: Add section about DNA?)

2.1 Chromosome Conformation Capture

Chromosome Conformation Technologies describe several similiar methods to compare genomic loci. They all start by:

- creating chromatin crosslinks
- digesting the crosslinked chromatin and
- ligating the ends

to get a reversed crosslink.

2.1.1 Starting Conditions

Before the creation of chromatin crosslinks, let us specify what chromatin crosslinks are, and what is needed to make them happen.

We start with intact tissue from plants, animals or human samples, or cultured cells.

Chromatin is packaged into three-dimensional structures, that retain a relationship between genomic and physical distance. Sequences that are closer on the same chromosome, are also closer in physical space. Our method exploits this relationship between linkage and proximity to enable whole chromosomes scaffolding and phasing of genomes.

The DNA in the sample is cross-linked in-vivo to fix DNA sequences present inside the same cell. Cross-linking trap sequence interactions across the entire genome and between different chromosomes.

Cross-Linked DNA is fragmented with endonucleases. Fragmented loci are then biotin elated and ligated creating chimeric junctions between adjecent sequences. This process is called proximity ligation.

The more often two sequences are joined together, the closer these two sequences are in genomic space.

Biotinylated junctions are purified and subjected to paired-end sequencing. The proximity-ligation-reads are then mapped onto a draft assembly.

Proximity information is used to assign context to chromosomes, and order and orient them along chromosome scale scaffolds.

This results in fully scaffolded chromosomes of virtually any size. This process also detects structural variation and corrects assembly miss-joins as well as maps the three-dimensional conformation of chromatin within a population of cells.

See Figure 1.

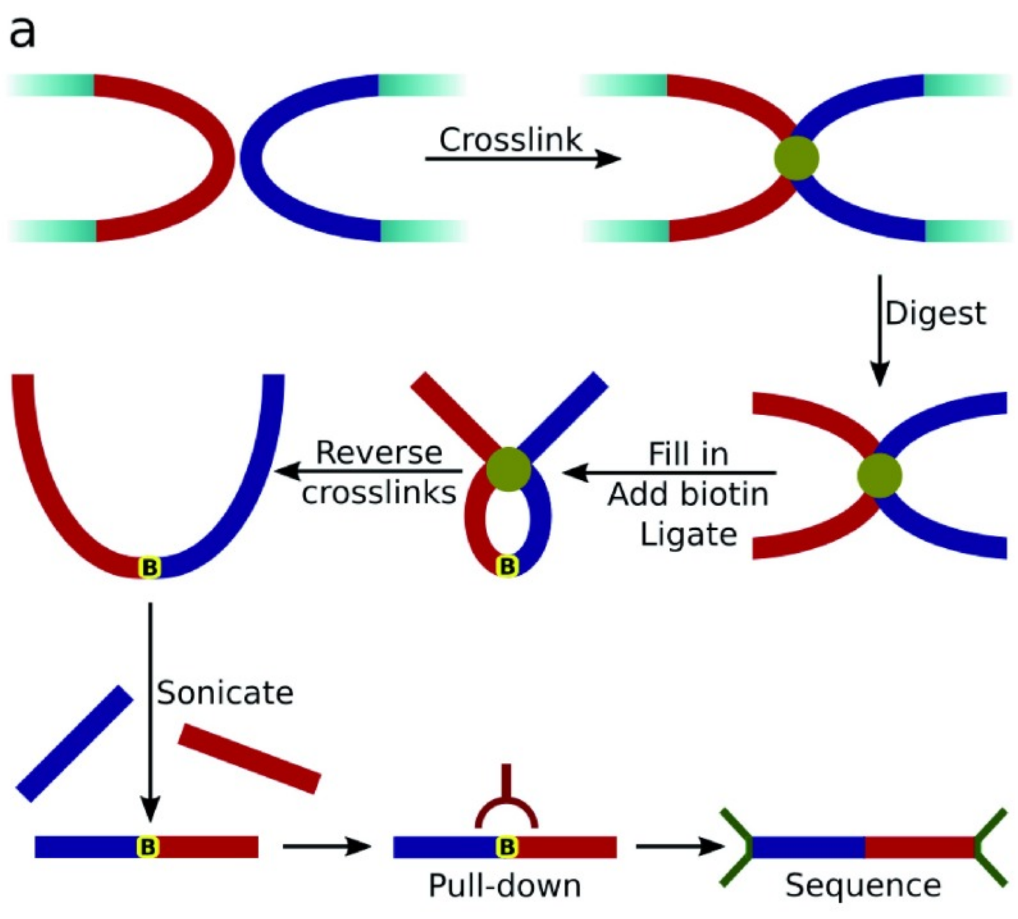
2.1.2 Chromatin Crosslinks

2.2 Hi-C

(TODO: Cite/introduce/... the given papers, and introduce the required concepts)

Required concepts:

- Biology:
- Chromosome Conformation Capture
- Hi-C + pipeline
- The iterative algorithm (again ?)
- analysis that can be done further
- outlook. Meaning: What can be done, when having the 3C-Data?



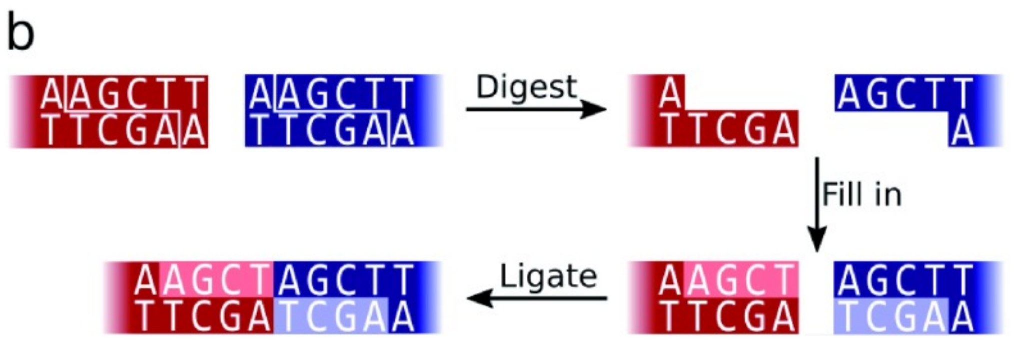


Figure 1: **a)** Diagram summarising the Hi-C experimental protocol. The red and blue rectangles represent cross-linked restriction fragments while the yellow marker shows the position of biotin incorporation. **b)** Generation of the Hi-C ligation junction sequence by successive digestion (with HindIII in this example), fill in and blunt-ended ligation steps. The modified restriction site sequence is not found in the original genomic sequence. Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4706059/?report=reader (HiCUP-paper)

3 Related Work

(TODO: introduce original implementation in python and KR-algorithm in C++)

3.1 HiCExplorer

(TODO: introduce hicexplorer and)

3.2 Deeptools

(TODO: introduce the whole deeptools framework)

(TODO: is that really everything related?)

4 Approach

The approach usually starts with the problem definition and continues with what you have done. Try to give an intuition first and describe everything with words and then be more formal like 'Let g be ...'.

4.1 Choosing the right API to call Rust from Python

There are three main ways to execute Rust code from Python. In the following, common techniques are investigated.

One common way is rust-cpython. This library requires Rust 1.25 or higher (current versions are 1.33/34/35 for stable/beta/nightly respectively). Rust-cpython grants access to the python gil (global interpreter lock) with which Python code can be evaluated and Python objects modified. The resulting library (directly from compiled rust) can easily be imported into Python (but needs to be renamed). Native Rust code requires some wrapping first, as shown here:

```
#[macro_use] extern crate cpython;
use cpython::{PyResult, Python};
// add bindings to the generated python module
// N.B: names: "librust2py" must be the name of the `.so` or `.pyd` file
py_module_initializer!(librust2py, initlibrust2py, PyInit_librust2py, |py, m|
    m.add(py, "__doc__", "This␣module␣is␣implemented␣in␣Rust.")?;
    m.add(py, "sum_as_string", py_fn!(py, sum_as_string_py(a: i64, b:i64))
    Ok(())
});
// logic implemented as a normal rust function
fn sum_as_string(a:i64, b:i64) -> String {
    format!("{}", a + b).to_string()
}
// rust-cpython aware function. All of our python interface could be
// declared in a separate module.
// Note that the py_fn!() macro automatically converts the arguments from
// Python objects to Rust values; and the Rust return value back into a P
fn sum_as_string_py(_: Python, a:i64, b:i64) -> PyResult<String> {
    let out = sum_as_string(a, b);
    Ok(out)
}
```

This kind of wrapping, though quite common and based on the Python C-API makes it hard to write idiomatic Code in Rust. Also, since Python is directly affected, the interactions with Python need to be considered while writing Rust-Code. In computer science one does usually not intentionally strive for complexity.

Another common approach is using the pyO3-library, which started off as a fork of rust-cpython, but has since seen quite drastic changes. For example, its using requires at least Rust version '1.30.0-nightly 2018-08-18'. This has been updated to '1.34.0-nightly 2019-02-06' with the most recently update. This is due to the usage of several unstable features, most of which have recently been able to be promoted to stable. Still missing is Specialisation though, which has at the time of writing still a long way to go. The library would also result in an easily importable (needs to be renamed first, still) cdylib (same as rust-cpython). The still intermingled way of writing the interface (certainly better but not by much compared to rust-cpython) as well as the dependency on unstable nightly rust versions led to the decision of not using it either.

The third way, that is actually been promoted in the official Rust docs, is to generate a dylib and import that in python. No renaming necessary, but the communication between Rust and Python is a bit more low-level. The main wrapper is on the side of Python, transforming Arguments to Pointers and C-Representations, whilst the Rust part needs to conform to C-practices, which includes receiving a list by getting a pointer and the length of it. Other than that, the Rust code has some additional `\#[no_mangle]` and `\#[repr(C)]` (procedural) macros, which result in these parts actually accessible from e.g. Python or C. Since like this neither language depends on something only internal (or combinatorial), and both just depend upon the 'common, unchanging' C-interface, this seems to be the preferred way.

(TODO: Introduce main approach, problems I came across and more)

5 Experiments

times (precomputed correction factors):

```
542.32user 447.26system 16:26.02elapsed 100\%CPU (0avgtext+0avgdata 116107220maxresident)
14528inputs+8outputs (20major+159388812minor)pagefaults 0swaps
```

(TODO: The time-resource-measures done)

6 Conclusion

7 Acknowledgments

First and foremost, I would like to thank...

- advisers
- examiner
- person1 for the dataset
- person2 for the great suggestion
- proofreaders

ToDo Counters

To Dos: 11; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11

Parts to extend: 3; 1, 2, 3

Draft parts: 5; 1, 2, 3, 4, 5

Bibliography

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