Practical 1: Getting started with FAN-C

(1) Start the VirtualBox machine for the course on your computer.

(2) FAN-C (Kruse et al., Genome Biology 2020) is a python3 module for the analysis of Hi-C data. It is pre-installed in the course virtual machine. To install it by yourself, we recommend to do so following the pip installation steps in: https://fan-c.readthedocs.io/en/latest/getting_started.html#installation

(3) In order to confirm that FAN-C is properly installed, launch a shell window inside the Linux virtual machine. Select the top search icon in the left-hand banner, and search for 'terminal'.

(4) Type the following command into the shell.

```
fanc --version
```

(5) You can always access the help section of FAN-C by typing:

```
fanc --help
```

(6) FAN-C is itself a toolkit consisting of multiple subcommands for the analysis of Chromatin Conformation Capture data. You can explore the available analyses in the help message that we obtained above. FAN-C provides a convenient subcommand that automates the generation of normalised Hi-C matrices from raw FASTQ files called 'fanc auto'. We can access each FAN-C subcommand by typing its name after the fanc command like:

```
fanc auto
```

(7) The code above should have given us an error due to missing arguments needed to run it. In this case, we needed to provide input FASTQ files, the name of the restriction enzyme used, the genome studied and an output folder. To know the arguments that are needed to run each analysis and get a little help on the subcommand of interest we can simply type: fanc –help. For example, we can check the help for fanc auto by typing:

```
fanc auto --help
```

(8) The help section includes a brief description of the subcommand purpose, which we can always extend a bit further by checking the fanc documentation in https://fan-c.readthedocs.io/en/latest/fanc.html

For fanc auto, the help section includes two types of arguments: positional and optional arguments. Some arguments are expected in a specific order after the subcommand and thus are called positional arguments. In addition to positional arguments, optional arguments allow for more control over the subcommand. In case of fanc auto important optional arguments include specifying the genome and restriction enzymes used.

Practical 2: Example Hi-C matrix generation with FAN-C

For this example, we are going to use the command fanc auto (see Generating Hi-C matrices with fanc) to construct a Hi-C map from a subset of a previously published adrenal tissue dataset (SRR4271982 of GSM2322539). For this course we have also downloaded K562 Hi-C data which will be analysed in practical 11 using what we learnt in this example. If the MiSeq sequencing for your samples has already finished by now, we can also analyse this data instead.

(1) Copy example files from shared folder into working folder

```
cd
scp /media/sf_VM_Shared_Folder/examples.zip .
# Change the path to the shared folder path in your system
# Or download the data from our Keeper repository like: # wget -O examples.zip "https://keeper.mpdl.mpg
unzip examples.zip
cd examples
```

(2) From the newly created examples folder, run:

```
fanc auto SRR4271982_chr18_19_1.fastq.gzip \
  SRR4271982_chr18_19_2.fastq.gzip output \
  -g hg19_chr18_19.fa -i hg19_chr18_19/hg19_chr18_19 \
  -n fanc_example -t 4 -r HindIII \
  --split-ligation-junction -q 30 --run-with test
```

The –run-with test argument causes fanc to only print the commands it would execute, but to exit before running any processing steps. Use this to review the pipeline and ensure you chose the right parameters and that there are no errors. When you remove the –run-with test argument, fanc will work through the pipeline. On a modern desktop computer with at least four computing cores the command should take less than 30 minutes to finish. It will generate several binned, bias-corrected Hi-C matrices from the FASTQ input.

(3) Run fanc on the example data by removing the –run-with test argument from the command above. (THIS STEP WILL TAKE ~30 MINUTES.) Leave this process running and open a new terminal to work with in the meantime. You can read details about fanc auto and all of its parameters in Generating Hi-C matrices with fanc. (4) fanc auto will generate the following folder structure in the output folder:

```
output
   fastq
   sam
   pairs
   hic
      binned
   plots
       stats
```

You can check it by exploring the output folder in a new terminal window:

```
cd ~/examples/
ls -R output/
```

The processed .bam, .pairs and .hic files will be added in these folders after completion of the pipeline.

(5) Check the output bam file as soon as it is ready by typing

```
     samtools view output/sam/SRR4271982_chr18_19_1.bam | head
```

Congratulations! You have generated your first Hi-C matrix.

Practical 3: Example visualisation with fancplot

(1) We can plot the newly generated Hi-C maps easily using the fancplot command. Simply execute:

```
fancplot chr18:63mb-70mb -p triangular -vmax 0.05 \
  output/hic/binned/fanc_example_100kb.hic
```

This will plot the region 63-70Mb of chromosome 18 in the familiar Hi-C plot. Note that this dataset is very small and hence the quality of the matrix not particularly great - but TADs are clearly visible.

(2) You can find details about the plotting executable fancplot in Basic usage. Or by typing

```
fancplot --help
```

Similar to the fanc command, we can access the help section for each plot type by adding the plot type before the help argument:

```
fancplot -p triangular --help
```

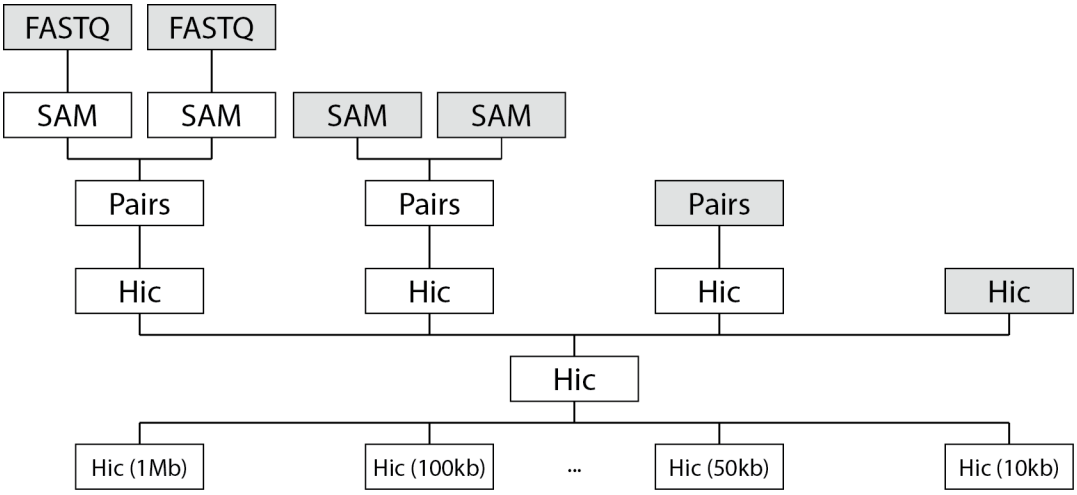
(3) Scroll around the genome and save a .png image using the interactive window.

(4) Alternatively, we can save the plot directly in pdf format by specifying an –output argument like:

```
fancplot chr18:63mb-70mb \
  --output output/plots/Figure1_test.pdf \
  -p triangular -vmax 0.05 \
  output/hic/binned/fanc_example_100kb.hic
```

Practical 4: Understanding individual steps of the fanc auto pipeline - mapping

The following schematic will give you an overview of what file types fanc auto can handle and how they are processed downstream.



fanc auto will map reads in FASTQ (or gzipped FASTQ) files to a reference genome, generating SAM/BAM files. SAM/BAM files with paired-end reads will be automatically sorted and mate pairs will be matched to generate Pairs files. Pairs files will be converted into fragment-level Hic objects. Multiple fragment-level Hic objects will be merged into a single Hi-C object. Finally, the fragment-level Hic object will be binned at various bin sizes.

Internally, fanc auto constructs its Hi-C processing pipeline from more specialised fanc commands. When describing the different pipeline steps and how you can control them below, we will also reference the specialised command that is used to build each step of the pipeline.

(1) Check again the individual steps run by fanc auto by running

```
fanc auto SRR4271982_chr18_19_1.fastq.gzip \
  SRR4271982_chr18_19_2.fastq.gzip output2 \
  -g hg19_chr18_19.fa -i hg19_chr18_19/hg19_chr18_19 \
  -n fanc_example -t 4 -r HindIII \
  --split-ligation-junction -q 30 --run-with test
```

Three major steps constitute the core of the pipeline: fanc map, fanc pairs and fanc hic. To (iteratively) map FASTQ files directly with FAN-C, use the fanc map command.

(2) Here is a minimal example: (DO NOT RUN)

```
fanc map SRR4271982_chr18_19_1.fastq.gzip \
   bwa-index/hg19_chr18_19.fa \
   SRR4271982_chr18_19_1.sam
```

fanc map will autodetect if you supply a BWA or Bowtie2 index, so the following command would use Bowtie2 as a mapper: (DO NOT RUN)

```
fanc map SRR4271982_chr18_19_1.fastq.gzip \
      hg19_chr18_19/hg19_chr18_19 \
      SRR4271982_chr18_19_1.sam
```

You can change the suffix of the output file to .bam and fanc map will automatically convert the mapping output to BAM format.

(3) Because of the chimeric nature of Hi-C fragments, fanc auto performs iterative mapping as a default: Reads are initially trimmed to 25bp (change this with the -m option) before mapping, and then iteratively expanded by 10bp (change the step size with the -s option) until a unique, high quality mapping location can be found. The associated quality cut-off is 3 for BWA and 30 for Bowtie2, but can be changed with the -q parameter. (DO NOT RUN)

```
# expand by 5bp every iteration and accept lower quality
fanc map SRR4271982_chr18_19_1.fastq.gzip \
      bwa-index/hg19_chr18_19.fa \
      SRR4271982_chr18_19_1.sam -m 20 -s 5 -q 10
```

(4) BWA will automatically split chimeric reads and return both mapping locations. This is especially useful for Hi-C data, as reads are often sequenced through a ligation junction, which BWA can often detect automatically. Nonetheless, mapping may be improved by splitting reads at predicted ligation junctions from the start. To enable this, use the -r parameter and supply the name of a restriction enzyme (e.g. HindIII or MboI). The name will be used to look up the enzyme's restriction pattern, predict the sequence of a ligation junction, and split reads at the predicted junction before mapping starts. Reads split in this manner will have an additional attribute in the SAM/BAM file ZL:i: where is an integer denoting the part of the split read. (DO NOT RUN)

```
# Split reads at HindIII ligation junction before mapping
fanc map SRR4271982_chr18_19_1.fastq.gzip \
      bwa-index/hg19_chr18_19.fa \
      SRR4271982_chr18_19_1.sam -t 16 -r HindIII
```

Final practical arguments to speed up the mapping. We can assign more threads to the mapping process using the -t parameter. If you are using Bowtie2, you can additionally use the –memory-map option, which will load the entire Bowtie2 index into memory to be shared across all Bowtie2 processes. Use this option if your system has a lot of memory available to speed up the mapping. Finally, if you are using the -tmp option, which causes fanc auto to perform most pipeline steps in a temporary directory, you may want to use the –split-fastq option to split the FASTQ files into smaller chunks before mapping, so you can save space on your tmp partition. In practice, most of these parameters have sensible defaults in fanc auto. You might want to enforce iterative mapping to increase the number of reads recovered and increase the number of threads to be used

Practical 5: fanc pairs: Generating and filtering read Pairs

The fanc pairs command handles the creation and modification of Pairs objects, which represent the mate pairs in a Hi-C library mapped to restriction fragments. Possible inputs are: two SAM/BAM files (paired-end reads, sorted by read name), a HiC-Pro valid pairs file, a 4D Nucleome pairs file, or an existing FAN-C Pairs object.

To process SAM/BAM files, no additional external software is required. However, we do recommend the

installation of Sambamba, which can greatly speed up the SAM sorting step required for merging mate pairs into the Pairs object.

(1) A minimal fanc auto command using SAM/BAM files could look like this: (DO NOT RUN)

```
fanc auto output/sam/SRR4271982_chr18_19_1.bam \
      output/sam/SRR4271982_chr18_19_2.bam \
      output2/ -g hg19_chr18_19_re_fragments.bed
```

(2) while minimal fanc pairs command using SAM/BAM files could look like this: (DO NOT RUN)

```
fanc pairs output/sam/SRR4271982_chr18_19_1_sort.bam \
      output/sam/SRR4271982_chr18_19_2_sort.bam \
      output/pairs/SRR4271982_chr18_19.pairs \
      -g hg19_chr18_19_re_fragments.bed
```

The -g or –genome parameter is mandatory, and is used to load (or construct) the restriction fragment regions necessary for building the fragment-level Hi-C object. You can either directly provide a region-based file with restriction fragments (most file formats are supported, including BED and GFF), or use a FASTA file with the genomic sequence in conjunction with the -r or –restriction-enzyme parameter. In the latter case, fanc auto will perform an in silico digestion of the genome and use the resulting restriction fragments from there.

Practical 6: Pair-level filtering

fanc pairs provides a lot of parameters for filtering read pairs according to different criteria. By default, if not specified otherwise, no filtering is performed on the read pairs (passthrough). Typically, however, you will at least want to filter out unmappable (-m) and multimapping reads (-u or -us). It is also a good idea to filter by alignment quality (-q). Good cutoffs for Bowtie2 and BWA are 30 and 3, respectively. If you suspect your Hi-C library to be contaminated by DNA from a different organism, you can align your original reads to a different genome and pass the resulting SAM/BAM file to the -c patameter (ensure no unmappable reads are in the file!). This will filter out all reads that have a valid alignment in the putative contaminants genome (by qname). All of the above filters operate on single reads, but will filter out the pair if either of the reads is found to be invalid due to a filtering criterion.

(1) while minimal fanc pairs command using SAM/BAM files could look like this: (DO NOT RUN)

```
fanc pairs output/sam/SRR4271982_chr18_19_1_sort.bam \
      output/sam/SRR4271982_chr18_19_2_sort.bam \
      output/pairs/SRR4271982_chr18_19.pairs \
      -g hg19_chr18_19_re_fragments.bed \
      -us \
      -q 3
```

(2) You can actually check how many pairs were filtered at this step by checking the statistics plots being generated in the output folder (See the plot on the left)

```
ls output/plots/stats/fanc_example.pairs.stats.pdf
```

(3) An additional set of filters operates on the properties of the read pair. You may want to filter out self-ligated fragments, which provide no spatial information with the -l parameter. As Hi-C experiments generally rely on PCR amplification, it is expected to find a lot of PCR duplicates in the library. You can filter those with the -p parameter, where denotes the distance between the start of two alignments that would still be considered a duplicate. Normally you would use 1 or 2, but you can use higher values to be more stringent with filtering. (DO NOT RUN)

```
fanc pairs output/pairs/SRR4271982_chr18_19.pairs \
      -l  # filter self-ligated fragments \
      -p 2  # filter PCR duplicates mapping within 2bp
```

(4) You can also check how many pairs were filtered at this step by checking the statistics plots being generated in the output folder (See the plot on the right)

(5) Depending on the experimental setup, it is sometimes expected to find valid Hi-C alignments near restriction sites. You can filter read pairs for their (cumulative) distance to the nearest restriction sites using the -d parameter. To determine that cutoff, or to detect any issues with the Hi-C library, you can first use the –re-dist-plot parameter. Note that this will only plot a sample of 10,000 read pairs for a quick assessment: (DO NOT RUN)

```
fanc pairs --re-dist-plot re-dist.png \
     output/pairs/SRR4271982_chr18_19.pairs
```

(6) You can find this plot already generated as part of the fanc auto pipeline in

```
ls output/plots/stats/fanc_example.pairs.re_dist.pdf
```

(7) Jin et al. (2013) have identified several errors that stem from incomplete digestion and which can be identified from different types of ligation products. You can filter these using the -i and -o parameters, for the inward and outward ligation errors, respectively. If you need help finding a good cut-off, you may use the –ligation-error-plot parameter. (DO NOT RUN)

```
fanc pairs --ligation-error-plot ligation-err.png \
     output/pairs/SRR4271982_chr18_19.pairs
```

(8) You can also find this plot already generated as part of the fanc auto pipeline in

```
ls output/plots/stats/fanc_example.pairs.ligation_error.pdf
```

Usually 1-10kb are often reasonable cut-offs. You can also let FAN-C attempt to find suitable cut-offs based on the over-representation of certain ligation products using the –filter-ligation-auto parameter, but this is not always 100% reliable.

Practical 7: fanc hic: Generating, binning, and filtering Hic objects

The fanc hic command is used to generate fragment-level and binned+filtered Hi-C matrices.

(1) You can use FAN-C Pairs files as input for fanc hic: (DO NOT RUN)

```
fanc hic \
      output/pairs/SRR4271982_chr18_19.pairs \
      output/hic/fragment_level.hic
```

Without additional parameters, this will generate a fragment-level Hic object and exit. Multiple Pairs files will be converted into fragment-level Hic objects which are then merged into a single object.

If you already have a fragment-level Hic file and you want to bin it, or perform filtering or matrix balancing, you can also use this as input:

(2) You can use FAN-C Pairs files as input for fanc hic: (DO NOT RUN)

```
fanc hic \
      output/hic/fragment_level.hic \
      output/hic/binned/example_1mb.hic -b 1mb
```

You can use the -b parameter to bin the fragment-level Hi-C matrix. You can either use integers (1000000) or common abbreviations (1Mb). The filtering steps outlined below only apply to binned Hic matrices.

Practical 8: Filtering and balancing

fanc hic provides a few filtering options. Most likely you want to apply a coverage filter using -l to specify a coverage threshold in absolute number of pairs per bin, or -r to apply a coverage threshold based on a fraction of the median number of pairs pair bin. -a is simply a pre-set for -r 0.1.

For some applications it might be useful to remove the prominent Hi-C diagonal. You can use the -d parameter to remove all pairs in pixels up to a distance of n from the diagonal.

You can balance your Hi-C matrices using the -n parameter. By default, this applies Knight-Ruiz (KR) balancing to each chromosome. You can opt for another normalisation method using –norm-method, for example –norm-method ice for ICE balancing, or –norm-method vc for vanilla coverage normalisation (which in this case is equivalent of a single ICE iteration). We typically recommend KR balancing for performance reasons. Each chromosome in the matrix is corrected independently, unless you specify the -w option.

Finally, you can output the filtering statistics to a file or plot using the -s and –statistics-plot parameters, respectively.

(1) You can find this plot already generated as part of the fanc auto pipeline in:

```
ls output/plots/stats/fanc_example_*.stats.pdf
```

Practical 9: Quality control: Cis/trans ratio

A useful metric to assess the quality of a Hi-C experiment is the ratio between Hi-C contacts happening between loci inside the same chromosome (cis) and contacs happening between fragments in different chromosomes (trans). Problems with suboptimal fixation would lead to a loss of cis interactions and a gain of trans interactions. Additionally, problems with and excess of ligations would lead to an artificial increase in trans contacts.

(1) FAN-C provides a tool to calculate the cis/trans metric and to run it you can just run:

```
fanc cis-trans output/hic/binned/fanc_example_1mb.hic
```

Please note that this is a normalised cis/(cis+trans) ratio, so it corresponds to the percentage of cis interactions. It ranges from 0 to 1. The closest to 1, the better.

For this toy example, we are only using read pairs for which either end maps to chr18 or chr19, so the cis/trans ratio is bound to be artificially high since we are missing many inter-chromosomal contacts.

Practical 10: Working with Cooler, Juicer, and text files

FAN-C is fully compatible with Hi-C files from Cooler (.cool and .mcool) and Juicer (.hic). There is no need to convert them to FAN-C format, you can use them directly in any fanc command. Text files, such as HiC-Pro output or 4D Nucleome pairs files must be converted to FAN-C format before they can be used in any commands. Please note that juicer's .hic format is not the same as FAN-C's .hic format.

(1) To convert our format to either cooler's multi-resolution .cool format or juicer's .hic format you can simply:

```
fanc to-cooler \ output/hic/binned/fanc_example_1mb.hic \ output/hic/binned/fanc_example_1mb.cool
```

(2) or:

```
fanc to-juicer \ output/hic/binned/fanc_example_1mb.hic \ output/hic/binned/fanc_example_1mb.juicer.hic
```

After doing that you will also be able to use all the tools associated to these formats. For example, look at the multiple resolution matrices stored in them:

```
cooler tree output/hic/binned/fanc_example_1mb.cool
cooler ls output/hic/binned/fanc_example_1mb.cool
```

Once we have transformed our data in cooler format, we can visualise it in HiGlass (Kerpedjiev et al., 2018), a tool able to visualise dynamically Hi-C data. For this course, the people at Wellcome Connecting Science have already kindly installed the higlass-manage tool for you, but if you need to install it in your own computer follow the instructions in https://docs.higlass.io/tutorial.html. If you need it in a server install higlass-server instead. There is a great tutorial to use HiGlass in https://hms-dbmi.github.io/hic-data-analysis-bootcamp/#24

(3) In order to visualise our matrix in HiGlass (and if Docker ir running) we need to first start HiGlass:

```
higlass-manage start
# navigate to http://localhost:8989/app in firefox
```

HiGlass already has available multiple processed cooler files, gene annotations and genome coordinates for easy use. We can add either of these by clicking on the plus button.

(4) As a test, load the Rao 2014 K562 Hi-C map analysed with the hg19 genome

(5) Add Gene annotations, genome coordinates and epilogos tracks by clicking on plus and typing hg19

(6) Enable toggle position search box and navigate to your favourite gene by typing the name of it in the navigation bar to the right of 'hg19'.

(7) Now, let's compare this with our analysed file by ingesting it using:

```
higlass-manage ingest \
      --assembly hg38 \
      output/hic/binned/fanc_example_1mb.cool
```

You can now load it using the plus sign in firefox.

Practical 11: K562 Hi-C data analysis. Setting up.

We will now analyse K562 Hi-C data generated by Moquin et al 2018. This data is stored in the Gene Expression Database (GEO) database under the accession number GSE98120.At this point we have three analysis options. Depending on how fast we were on the first 10 exercises we will run fanc auto either in A. the entire K562 dataset, B. in a subset of the data for chromosomes 18 and 19 or C. In the libraries that were generated during the course. We can also split in groups that do each and compare results at the end. Please, modify the commands below to point to the correct .fastq files.

Option A.

(1) Sequence Read Archives (SRA) files should be available in the shared folder under hic_k562/. These SRA files were downloaded using commands like: (DO NOT RUN)

```
wget https://sra-pub-run-odp.s3.amazonaws.com/sra/SRR5470534/SRR5470534
```

(2) We can convert the downloaded SRA files into FASTQ files using the sratoolkit like: (This will take ~15-20 minutes to run, pick one of the SRA files and follow this tutorial with it.)

```
fastq-dump --outdir ./ --gzip --split-3 SRR5470534
```

The fastq-dump command will split the SRA file into two .fastq files if the library is pair-ended or a single .fastq file if it is a single-end library. Additionally, it will save space by compressing them into .gzip format.

(3) As mentioned earlier in the course, it is always a good idea to run fastQC on any next-generation sequencing library to spot potential technical problems with adaptor contaminations and remove them beforehand:

```
mkdir fastqc
fastqc -o fastqc/ SRR5470534_1.fastq.gz
fastqc -o fastqc/ SRR5470534_2.fastq.gz
```

(4) Examine the fastQC output. How big are they? Are they any good? Should we run adaptor trimming on them?

Option B.

The subset data for this accession number can be found in our shared Keeper library:https://keeper.mpdl.mpg.de/d/9b1c1788f97642a188f3/

(1) If not available in the shared folder, please download each subset .fastq file into your computer by clicking on the sample that you will focus on. Then let's assess the quality of the .fastq files:

```
mkdir fastqc
fastqc -o fastqc/ SRR5470534_1.chr18_chr19.fastq.gz
fastqc -o fastqc/ SRR5470534_2.chr18_chr19.fastq.gz
```

(2) Examine the fastQC output. How big are they? Are they any good? Should we run adaptor trimming on them?

Option C.

Copy the .fastq files generated in the course to your home.

(3) As mentioned earlier in the course, it is always a good idea to run fastQC on any next-generation sequencing library to spot potential technical problems with adaptor contaminations and remove them beforehand:

```
mkdir fastqc
fastqc -o fastqc/ SRR5470534_1.chr18_chr19.fastq.gz
fastqc -o fastqc/ SRR5470534_2.chr18_chr19.fastq.gz
```

(4) Examine the fastQC output. How big are they? Are they any good? Should we run adaptor trimming on them?

Practical 12: Running FAN-C

As we did in practical number 2, we can run the entire FAN-C pipeline with the fanc auto command. We need to make a couple of adjustments to make sure that FAN-C runs smoothly. Option A. (1) This is code that we usually use to analyse new Hi-C files in our lab. Can you spot the 7 changes that we made to the example in practical number 2? (DO NOT RUN YET)

```
GENOME='/Genomes/UCSC/hg38/Sequence/hg38.negspy.fa'
GENOMEIDX='/Genomes/ UCSC/hg38/BWAIndex/hg38.negspy.fa'
mkdir hic_BWA
fanc auto \
      SRR5470534_1.fastq.gz \
      SRR5470534_2.fastq.gz \
      hic_BWA/SRR5470534/ \
      --genome $GENOME \
      --restriction-enzyme MboI \
```

```
      --genome-index $GENOMEIDX \
      --basename SRR5470534 \
      --threads 16 \
      --le-inward-cutoff 5000 \
      --le-outward-cutoff 5000 \
      -tmp --split-ligation-junction -f
```

Here is the fanc command from practical number 2 for comparison:

```
fanc auto SRR4271982_chr18_19_1.fastq.gzip \
      SRR4271982_chr18_19_2.fastq.gzip output \
      -g hg19_chr18_19.fa -i hg19_chr18_19/hg19_chr18_19 \
      -n fanc_example -t 4 -r HindIII \
      --split-ligation-junction -q 30
```

Answers

1. For this analysis, we switched to using the full hg38 genome instead of only two hg19 chromosomes. It's always a good idea to use the most recent version of the genome! Please note that we additionally changed the order of the chromosomes to match the negspy repository for compatibility down the line with HiGlass.
2. We switched to using BWA as a mapping algorithm. (In our hands this mapper recovers a higher proportion of aligned reads without iterative mapping.)
3. We changed the restriction enzyme since these libraries were generated using MboI instead of HindIII
4. We increased the number of cores that FAN-C was be able to use in parallel to increase efficiency. This made it possible to finish in a couple of hours. (And ran this in a computer cluster.) In a normal computer it would take a minimum of 4-6 h. 5-6. We manually specified the inward and outward thresholds for pair-level filtering. (If in doubt, thresholds between 5-10k are usually ok but it is important to confirm with the inward-outward plot.)
5. We changed the mapping quality (MAPQ) threshold needed for reads to be counted as valid alignments (-q). This is because BWA and Bowtie2 MAPQ scores have different ranges. In our experience, -q 3 works best for filtering multi-mapping reads when using BWA, while -q 30 works best for Bowtie2. (DO NOT RUN YET)

```
GENOME='/Genomes/Homo_sapiens/UCSC/hg38/Sequence/hg38.negspy.fa'
GENOMEIDX='/Genomes/Homo_sapiens/UCSC/hg38/BWAIndex/hg38.negspy.fa'
mkdir hic_BWA

fanc auto \
      SRR5470534_1.fastq.gz \
      SRR5470534_2.fastq.gz \
      hic_BWA/SRR5470534/ \
      --genome $GENOME \
      --restriction-enzyme MboI \
      --genome-index $GENOMEIDX \
      --basename SRR5470534 \
      --threads 16 \
      --le-inward-cutoff 5000 \
      --le-outward-cutoff 5000 \
      --split-ligation-junction -q 3 -f
```

As a note, binned .hic files and stat plots that result from running the above pipeline can be found in our shared Keeper library.

IMPORTANT: For people following option A, change the number of threads to 4, run the command above

and wait. (You can find the result files from running fanc in this mode already in our shared keeper library: https://keeper.mpdl.mpg.de/d/9b1c1788f97642a188f3/) For people following option B change the GENOME variable to the absolute path of the FASTA file (hg19_chr18_19.fa) and GENOMEIDX variable to the absolute path of the (bwa-index/hg19_chr18_19.fa) in the examples folder. Then change the paths to the subset fastq files you chose and change the number of threads to 4, run the command above and wait. For people running C. Change the paths to the subset fastq files you chose and change the number of threads to 4, run the command above and wait.

For the steps in the next tutorial, please use the results from this step to perform the rest of the analysis.

Practical 13: Quality control

(1) Calculate the inward/outward ligation error plot.

```
fanc pairs \
      --ligation-error-plot \
      SRR5470534.pairs.ligation_error.pdf \
      SRR5470534.pairs
```

Is it ok that we filter out any inward or outward interactions happening among DNA loci <5000 bp apart? Or should we have used smaller thresholds to retain more reads?

(2) Look at the read pair filter statistics plot generated as part of fanc auto. How does this library compare to the one used in the first example?

(3) Calculate cis-trans ratios on the 2mb (or 1mb) binned matrix.

```
fanc cis-trans SRR5470534_2mb.hic
```

Are these scores indicative of a good library or a bad one? Can you see a difference if you calculate the same score in higher resolution matrices?

```
fanc cis-trans SRR5470534_1mb.hic
```

Practical 14: Plotting

(1) Plot the same region that we looked at 100 kb resolution. Can you see a significant difference with the first matrix? Is this library sequenced to a higher sequencing depth?

```
fancplot chr18:63mb-70mb \
      --output Figure2_100kb.png \
      -p triangular -vmax 0.05 \
      SRR5470534_100kb.hic
```

(2) Plot the same region at 500 or 50 kb resolution. Is the data of enough quality to be visualised at these resolutions?

Disclaimer

This tutorial is heavily based on the FAN-C documentation available at:

https://fan-c.readthedocs.io/