Arioc User Guide

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Arioc User Guide

Introduction

Arioc is a set of computer programs that carry out the alignment of short DNA sequences to a comparatively large reference sequence or genome.

Arioc is interesting because it was constructed from the ground up to use general-purpose graphics processing unit (GPU) hardware to maximize its speed without sacrificing accuracy. In the field of biological sequence alignment, there have been a number of attempts to adapt successful CPU-based algorithms and implementations to GPUs. A decade of such efforts has demonstrated that this goal is not easy to reach! We certainly learned a great deal about how (and how not) to build Arioc by looking at and testing previous GPU implementations.

It's not easy to build a fast GPU-based read aligner

Probably the hardest obstacle to overcome in creating a fast GPU-based read aligner is that GPU programming requires a great deal of software engineering that isn't needed for writing programs that run exclusively on a CPU. GPU hardware can run tens of thousands of threads of execution concurrently, but speed improvements in GPU software are not obtained simply by replicating source code that runs efficiently on one or two dozen CPU threads.

There are two fundamental reasons for this. One has to do with the way in which GPUs manage threads. In NVidia's CUDA environment (for which Arioc is implemented), threads execute in fixed groups of 32, each of which executes the same instruction on different data simultaneously. This SIMD (single instruction, multiple data) model impacts the way that source code is structured and, ultimately, the way in which successful GPU-aware algorithms are designed. Algorithms that contain a significant amount of branching logic must be carefully re-implemented so as to accommodate SIMD parallelism.

But the second, and probably more important, reason why GPU application speeds don't correspond simply to the number of available threads is that there isn't enough memory bandwidth to keep up with all of those threads. GPU programming involves writing code that conforms to a variety of memory-layout and memory-addressing constraints that can fundamentally change assumptions that are taken for granted in algorithms that run efficiently on CPUs. Of course, memory optimization techniques are important in CPU software engineering as well, but the idiosyncrasies of GPU memory management tend to dominate other considerations in building a successful GPU application.

This all means that, in practice, GPU programmers are satisfied when they see a 10-fold speedup in a GPU-based application compared to a CPU-based application that performs the same computational task. (A hundred-fold GPU-versus-CPU speedup would be extraordinary and make one wonder whether the corresponding CPU application was properly optimized!) As far as Arioc is concerned, we did not release the software until we reached this 10x threshold.

What's interesting about Arioc

The central problem in read alignment is basically that of quantifying the similarity between two strings of symbols. This problem has attracted a great deal of attention for over 30 years, and a number of

successful algorithms have been developed in response. In particular, two algorithmic approaches — one based on dynamic programming [Smith and Waterman, 1981] and the other using Levenshtein edit distance [Ukkonen, 1983] — are by far the most commonly used algorithms in modern read aligners.

Unfortunately, string similarity computations are expensive. With a reference sequence the size of the human genome, it is far from practical to align a read by computing similarity at every possible reference-sequence location. For this reason, read aligners perform a great deal of preliminary work so as to narrow down the number of reference-sequence locations at which they must compute alignments. This work cannot easily be reduced to a single algorithmic description. Instead, read aligners rely on heuristics and on software-engineering considerations to limit the number of reference-sequence locations they examine for each read.

It is here that we can recognize an opportunity to apply GPU-based parallelism to improve performance. Arioc implements a well-known technique known as "seed and extend" [Altschul, 1992] in a way that the basic operations of table lookup and prioritization of potential reference-sequence locations are represented as sorting and adjacent-neighbor comparisons on long, one-dimensional arrays of integers. Such operations are well suited to SIMD implementation on GPU hardware. Arioc's speed and accuracy stem from GPU acceleration of this aspect of the read-alignment process.

What Arioc is (and isn't)

Arioc is a read aligner that uses GPU acceleration in an interesting way, namely, to find high-priority locations at which to compute alignments. The process of identifying high-priority alignment locations can be a significant bottleneck in a read aligner. Arioc uses GPU hardware to speed up this process and thereby achieve higher throughput.

What Arioc does

Arioc accepts as input a large number — that is, tens or hundreds of millions — of short sequencing reads. For each such read, Arioc reports the location (or locations) within a given reference sequence where a highly-similar subsequence is found.

Ideally, Arioc would find a perfect and unique mapping for every read. In other words, there would be exactly one location in the reference sequence where the read sequence exactly matches the reference sequence starting at that location. In practice, of course, many reads do not map perfectly or uniquely within a given reference sequence. In this case, Arioc reports what it considers to be the "best" mapping (or mappings) for each read. Arioc does these things accurately and with high throughput.

Arioc has several "database-oriented" features in its implementation. One of Arioc's original design goals was to carry out read alignment on sequence data residing in relational database tables. For this reason, Arioc handles input and output files that are encoded so as to facilitate data transfers to and from a database management system. (Arioc's encoded input and output formats are compatible with the binary data formats used by the Microsoft SQL Server for high-speed "bulk" input and output.) This may seem like "wasted time" if the goal is simply to align sequencer reads without archiving them in a database, but in fact the additional effort involved in encoding does not appreciably affect throughput because file I/O in the read aligner runs on CPU threads concurrently with the rest of the read-processing pipeline.

Arioc was consciously implemented with the goal of handling large amounts of sequencer data and genomes the size of the human reference genome. We kept the notion of "scarcity thinking" in mind [Cooper, 2004] and made a conscious effort to avoid it! If you are processing sequencer reads on a multi-terabyte or petabyte scale, we assume that you have computers with the compute and memory capabilities required by Arioc.

What Arioc does not do

Arioc is not a tool for searching a database of short sequences and ranking those sequences by their similarity to a given sequence. (CUDASW++ [Liu et al, 2013] is the best-known GPU-accelerated tool for this.) Arioc does not operate with any sequences other than DNA; it does not understand any sequence "alphabet" other than ACGTN. It does not report SNPs or attempt to recognize variations. It does not do multiple sequence alignment or assemble sequencing reads in any way.

Finally, Arioc is not by any means a "simple", "turnkey", or "one size fits all" tool. Instead, Arioc exposes a variety of user-configurable parameters that let you experiment to optimize speed and sensitivity (or

trade one for the other). The downside to this approach is that it exposes the complexity of the read-alignment process; the benefit lies in the ability to fine-tune Arioc's performance to your own requirements.

The GPU software-development landscape is still changing. We expect Arioc to grow and adapt to newer, faster GPU hardware and software as it becomes available. We think that Arioc's approach to the read-alignment problem is sound, and that it has the potential to prove its utility as sequencers generate ever-increasing numbers of reads to align.

Installation

Arioc is compiled C++ for the NVidia CUDA environment. The same code base is used for both Windows and Linux.

Hardware requirements

Arioc requires the following minimum hardware:

- a multi-core 64-bit Intel-compatible CPU
- at least 72 gigabytes of free memory
- sufficient disk space to store reference sequences, reads, and alignment results
- at least one NVidia GPU of compute capability 3.5 or greater, with at least 5 gigabytes of GPU memory

We have tested Arioc on both GTX-series and Kepler-series GPUs.

CUDA

Arioc has been tested with the following minimum CUDA versions:

- NVidia video driver v343.98
- CUDA runtime version 6.5

Operating systems

Arioc requires a 64-bit operating system. It has been tested with the following:

- Windows 7 (64-bit)
- Windows Server 2008 R2 datacenter
- Scientific Linux v7.0

Installation

Windows

Install using the Windows installer and the Arioc setup package (AriocSetup.msi).

Linux

The Arioc executable binaries may be copied and executed without modification. If necessary, you can rebuild the Arioc binaries using the unified makefile to compile each Arioc executable separately:

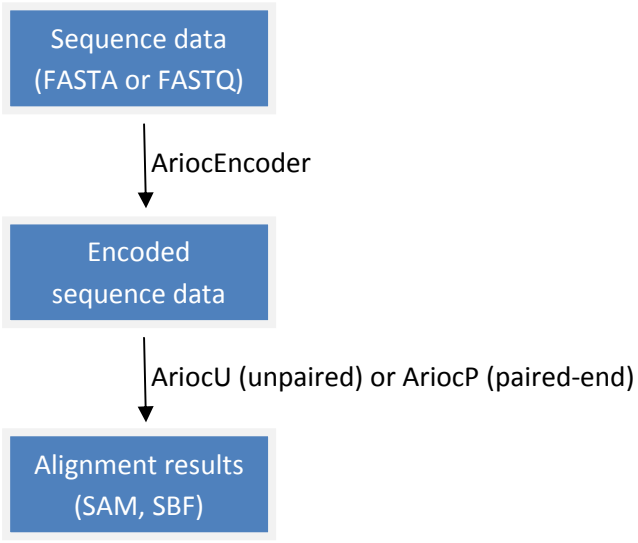
```
make AriocE
make AriocU
make AriocP
```

Arioc runtime roadmap

There are three executable binaries in the Arioc distribution:

- AriocE: encodes sequence data
- AriocU: aligns unpaired reads
- AriocP: aligns paired-end reads

Here is how they are used:



The three executables are described in detail in the sections that follow.

Arioc command-line syntax

Each of the Arioc executables obtains its runtime configuration from an XML-formatted configuration file, so the command-line syntax for running them is simple:

```
AriocE config_filename
AriocU config_filename
AriocP config_filename
```

where `config_filename` is the name of the configuration file to use. (You should, of course, use fully-qualified filenames whenever it makes sense to do so.)

Arioc error messages

In general, Arioc error messages contain some cryptic information that identifies the source-code location that detected the error, along with human-readable text that explains the nature of the error. For example, if Arioc cannot open the configuration file you specify, it emits an error message like the following:

```
44:37.416 [00001704] ApplicationException ([0x5892] AriocAppMainBase.cpp 715):
XMLDocument::Loadfile( "AriocP.cfg" ) failed with error 3: file not found
XML error details:
AriocP.cfg
```

Arioc input and output

Arioc writes status and error messages to the stderr device. In practice, this means that all output appears in the console by default. If you want to redirect console output to a file, use command-line redirection syntax. For example:

```
AriocU test23.cfg 2> test23.log
```

For sequence input and alignment results, Arioc uses files whose names you specify in the configuration file.

AriocE: encoding sequence data

The AriocE component transforms raw sequence data from FASTA or FASTQ format into the binary format read by the AriocU and AriocP executables. Encoded sequence data resides in separate files whose contents can be distinguished by filename extension:

- `$a21.sbf, $a21.rc.sbf`: encoded forward and reverse-complement bases (3 bits per base, 20 bases per 64-bit value)
- `$raw.sbf, $raw.rc.sbf`: ASCII representation of the sequence data (forward and reverse complement)
- `$sqm.sbf, $sqm.rc.sbf`: sequence metadata (FASTA or FASTQ description line)
- `$sqq.sbf, $sqq.rc.sbf`: sequence quality scores (for FASTQ-formatted input only)

For example, encoded sequence data for human chromosome 1 might reside in the following set of files:

```
hs_ref_GRCh37_chr1$a21.sbf
hs_ref_GRCh37_chr1$a21.rc.sbf
hs_ref_GRCh37_chr1$raw.sbf
hs_ref_GRCh37_chr1$raw.rc.sbf
hs_ref_GRCh37_chr1$sqm.sbf
hs_ref_GRCh37_chr1$sqm.rc.sbf
```

Before using the Arioc aligners, AriocU and AriocP, you must encode both a reference sequence and a set of reads to align. Encoding is a one-time-only procedure. You can re-use the encoded files for multiple invocations of either the AriocU or AriocP executables.

Encoding unpaired reads

Encoding reads with AriocE is straightforward. For each FASTA file, AriocE generates an $a21, $raw, and $sqm file; for FASTQ input, AriocE generates a $sqq file as well.

The configuration file you provide to AriocE, in addition to specifying input and output filenames, contains "metadata" that is passed to the AriocU or AriocP aligners:

```
<?xml version="1.0" encoding="utf-8"?>

<AriocE>

  <dataIn sequenceType="Q">
    <file referenceId="1">C:\test103\i100p_1.fastq</file>
  </dataIn>

  <dataOut>
    <path>C:\BulkData\Q\Mason\test103</path>
  </dataOut>

</AriocE>
```

Simple configuration file for AriocE (unpaired reads).

In the XML configuration file, the `sequenceType=` attribute of the `<dataIn>` element indicates that the input sequences are to be encoded as reads. The <dataIn> element also contains one or more `<file>` elements, each of which specifies the fully-qualified name of an input file. Similarly, the <dataOut> element contains a `<path>` element that contains the fully-qualified name of the output directory.

AriocE requires that you use the `referenceId=` attribute of each `<file>` element to assign a "reference ID" number to each input file. AriocE creates a unique 64-bit integer sequence identifier for every input sequence in every file you specify; the purpose of the reference ID is simply to ensure that the sequence identifier is unique across multiple input files. The reference ID can be any integer between 1 and 127.

Although it was originally intended to simplify the automation of the sequence-alignment process, the use of an XML configuration file provides flexibility in parameterization as well as an opportunity to include human-readable documentation alongside runtime parameters. Here is an example of a more complex configuration file:

```
<?xml version="1.0" encoding="utf-8"?>

<AriocE maxDOP="2">

  <!-- Note: test 103 data consists of 100nt reads simulated using Mason -->
  <dataIn sequenceType="Q" dataSourceId="1" samplingRatio="0.1">
    <rg CN="JHU PHA" DS="test 103" PL="ILLUMINA" />
    <file referenceId="1" ID="1" SM="sample 1: 11221">C:\test103\i100p_1.fastq</file>
    <file referenceId="2" ID="2" SM="sample 1: 11222">C:\test103\i100p_2.fastq</file>
  </dataIn>

  <dataOut>
    <path>C:\BulkData\Q\Mason\test103</path>
  </dataOut>

</AriocE>
```

Configuration file for AriocE (unpaired reads) with comments, optional parameters (XML attributes), and SAM-style read groups.

This configuration encodes two different files. It also specifies an optional parameter (`maxDOP=`) that controls the number of concurrent CPU threads used by AriocE; with a multi-core CPU and multiple input files, AriocE can encode multiple files concurrently and thereby run faster than it would on a single CPU thread. Finally, the `samplingRatio=` parameter causes AriocE to encode a randomly-sampled subset of the input reads:

This configuration file also illustrates how AriocE associates read group information with reads. AriocE merges the specified read group attributes (CN, DS, PL, ID, and SM) and copies them to its own "private" metadata file, which resides in the output directory. The AriocU and AriocP aligners use the read group information to generate appropriate @RG headers and RG tags in SAM-formatted output.

Here is a summary of the optional XML elements and attributes that control the runtime configuration of AriocE:

Element	Attribute	Description
<AriocE>	maxDOP	Number of concurrent CPU threads
<dataIn>	dataSourceId	Data source ID (between 0 and 4,194,303)
	samplingRatio	Sampled fraction of reads encoded (maximum 1.0)
	parseQname	Encodes only the specified whitespace-separated field in each read's description.
<rg>	ID CN DS DT FO KS LB PG PI PL PU SM	SAM read group fields (common to all input files)
<file>	ID CN DS DT FO KS LB PG PI PL PU SM	SAM read group fields (merged with fields in the <rg> element)

Optional elements and attributes in the AriocE configuration file for encoding unpaired reads.

Encoding paired-end reads

The AriocE configuration file for paired-end reads uses the same parameters as the configuration file for unpaired reads, but it uses an additional XML attribute (mate=) to indicate the files in which mate 1 and mate 2 are found.

```
<?xml version="1.0" encoding="utf-8"?>

<AriocE>

  <dataIn sequenceType="Q">
    <file referenceId="1" mate="1">C:\test103\paired\i100p_1.fastq</file>
    <file referenceId="1" mate="2">C:\test103\paired\i100p_2.fastq</file>
  </dataIn>

  <dataOut>
    <path> C:\BulkData\Q\Mason\test103</path>
  </dataOut>

</AriocE>
```

Simple configuration file for AriocE (paired-end reads).

You can encode multiple input files (or, more exactly, pairs of input files) by using the referenceId= and mate= attributes of the <file> element:

```
<?xml version="1.0" encoding="utf-8"?>

<AriocE maxDOP="6">

  <dataIn sequenceType="Q" dataSourceId="110114" samplingRatio="0.01">
    <file referenceId="5" mate="1">E:\yh110114\s_5_1_sequence.txt</file>
    <file referenceId="5" mate="2">E:\yh110114\s_5_2_sequence.txt</file>
    <file referenceId="6" mate="1">E:\yh110114\s_6_1_sequence.txt</file>
    <file referenceId="6" mate="2">E:\yh110114\s_6_2_sequence.txt</file>
    <file referenceId="7" mate="1">E:\yh110114\s_7_1_sequence.txt</file>
    <file referenceId="7" mate="2">E:\yh110114\s_7_2_sequence.txt</file>
  </dataIn>

  <dataOut>
    <path>C:\BulkData\Q\yh110114</path>
  </dataOut>

</AriocE>
```

Configuration file for AriocE (paired-end reads) with multiple input files and optional parameters.

Encoding a reference sequence

Encoding a reference sequence or genome with AriocE is similar to encoding reads. But in addition to creating encoding sequence data and metadata, AriocE builds a set of hash tables that are used in both AriocU and AriocP.

Input files

If you are encoding a genome that consists of two or more reference sequences (chromosomes or other genome subunits), place each reference sequence in a separate file prior to encoding. For example, to encode the human genome, use a separate input file for each chromosome rather than concatenating all of the chromosome sequences into a single file.

AriocE recognizes reference sequence files in FASTA format only.

Two-pass encoding

AriocE builds hash tables in a straightforward manner: At every location in each reference, the encoder extracts a seed whose length and pattern is specified in the configuration file. A numerical hash function is applied to each seed and the reference-sequence location is appended to a list of such locations for the seed's hash value. Other configuration-file parameters control the number of bits in a hash value (and thus the size of the hash table) as well as the maximum number of reference-sequence locations recorded for a given hash value.

Internally, both the AriocU (unpaired) and AriocP (paired-end) aligners implement a pipeline in which nongapped alignments for each read are computed prior to gapped alignments. The aligners use different hash tables for nongapped (spaced-seed) alignment and for gapped (seed-and-extend) alignment. This means that you must execute AriocE twice to fully encode a reference sequence. The idea is to make it possible to use AriocE multiple times to create hash tables with different characteristics and then choose among those hash tables when you execute the AriocU or AriocP aligners.

Output directory layout

The output from each invocation of AriocE should occupy a single directory that contains encoded sequence files as well as a subdirectory for each hash table. If, for example, you run AriocE twice (once for a nongapped hash table and once for a gapped hash table), and configure it to write its output to a directory named /GRCh37, the resulting directory layout would look like this:

```
/GRCh37              encoded sequence files (*.sbf)
/GRCh37/ssi84_2_30   hash table for nongapped (spaced-seed) aligner
/GRCh37/hsi20_0_30   hash table for gapped (seed-and-extend) aligner
```

Here is an example of an AriocE configuration file that builds a nongapped (spaced-seed) hash table for the human genome:

```
<?xml version="1.0" encoding="utf-8"?>

<AriocE seed="ssi84_2_30" maxDOP="32" maxJ="*">

  <dataIn sequenceType="R" dataSourceId="0" filePath="/dss004/R/GRCh37.p5"
uriPath="ftp://ftp.ncbi.nlm.nih.gov/genomes/H_sapiens/ARCHIVE/BUILD.37.3/Assembled_chromosomes
/seq/">
    <file referenceId="1">hs_ref_GRCh37.p5_chr1.fa</file>
    <file referenceId="2">hs_ref_GRCh37.p5_chr2.fa</file>
    <file referenceId="3">hs_ref_GRCh37.p5_chr3.fa</file>
    <file referenceId="4">hs_ref_GRCh37.p5_chr4.fa</file>
    <file referenceId="5">hs_ref_GRCh37.p5_chr5.fa</file>
    <file referenceId="6">hs_ref_GRCh37.p5_chr6.fa</file>
    <file referenceId="7">hs_ref_GRCh37.p5_chr7.fa</file>
    <file referenceId="8">hs_ref_GRCh37.p5_chr8.fa</file>
    <file referenceId="9">hs_ref_GRCh37.p5_chr9.fa</file>
    <file referenceId="10">hs_ref_GRCh37.p5_chr10.fa</file>
    <file referenceId="11">hs_ref_GRCh37.p5_chr11.fa</file>
    <file referenceId="12">hs_ref_GRCh37.p5_chr12.fa</file>
    <file referenceId="13">hs_ref_GRCh37.p5_chr13.fa</file>
    <file referenceId="14">hs_ref_GRCh37.p5_chr14.fa</file>
    <file referenceId="15">hs_ref_GRCh37.p5_chr15.fa</file>
    <file referenceId="16">hs_ref_GRCh37.p5_chr16.fa</file>
    <file referenceId="17">hs_ref_GRCh37.p5_chr17.fa</file>
    <file referenceId="18">hs_ref_GRCh37.p5_chr18.fa</file>
    <file referenceId="19">hs_ref_GRCh37.p5_chr19.fa</file>
    <file referenceId="20">hs_ref_GRCh37.p5_chr20.fa</file>
    <file referenceId="21">hs_ref_GRCh37.p5_chr21.fa</file>
    <file referenceId="22">hs_ref_GRCh37.p5_chr22.fa</file>
    <file referenceId="23">hs_ref_GRCh37.p5_chrX.fa</file>
    <file referenceId="24">hs_ref_GRCh37.p5_chrY.fa</file>
    <file referenceId="25">hs_ref_GRCh37.p5_chrMT.fa</file>
  </dataIn>

  <dataOut>
    <path>/dss004/BulkData/R/NCBI/GRCh37.p5</path>
  </dataOut>

</AriocE>
```

Configuration file for AriocE (reference sequences) for a nongapped (spaced-seed) hash table.

To build the corresponding gapped (spaced-seed) hash tables, change the parameters specified in the XML attributes of the `<AriocE>` element and re-execute AriocE:

```
<AriocE seed="hsi20_0_30" maxDOP="32" maxJ="200">
```

Here is a list of additional configuration-file parameters for reference-sequence encoding:

Element	Attribute	Description
<AriocE>	maxDOP	Number of concurrent CPU threads
	maxJ	Maximum hash table bucket size ("big bucket" threshold)
	seed	One of the following values: ssi84_2_29 (seed width 84, 2 mismatches, 29-bit hash) ssi84_2_30 (seed width 84, 2 mismatches, 30-bit hash) hsi20_0_29 (seed width 20, 0 mismatches, 29-bit hash) hsi20_0_30 (seed width 20, 0 mismatches, 30-bit hash)
<dataIn>	dataSourceId	Data source ID (between 0 and 4,194,303)
	filePath	Parent directory for files specified in <file> elements
	uriPath	Original URI for reference sequences (copied to the UR field in the @SQ records in SAM-formatted output)

Optional elements and attributes in the AriocE configuration file for encoding reference sequences or a genome.

Most of these parameters are self-evident, but the seed= and maxJ= values deserve careful attention.

seed=. The seed= value for the nongapped hash table should typically be ssi84_2_30 (seed width 84, up to 2 mismatches, 30-bit hash values); for the gapped hash table, the value should be hsi20_0_30 (seed width 20, no mismatches, 30-bit hash values). It is possible to trade memory space for speed: with 29-bit hash values, the aligners use only about 36 gigabytes of memory, but alignment throughput decreases because of the need to resolve more hash-table collisions.

maxJ=. The maxJ= parameter trades speed for sensitivity. For the nongapped hash table, this effect is barely noticeable. In this case, you may simply omit the maxJ= parameter so that the maximum size of a hash table bucket is unlimited.

For the gapped hash table, however, maximum throughput is obtained with a value of about 16 (but with less sensitivity), whereas maximum sensitivity can be achieved by using values of 250 and up (but with a corresponding decrease in throughput). When encoding a genome such as the human genome that contains numerous repetitive regions, you should always specify a value for maxJ= . This causes AriocE to optimize the hash table by pruning repetitive regions of the genome where adjacent seeds hash to "big buckets"; this optimization improves throughput significantly with very little decrease in sensitivity for reads that map to repetitive regions.

AriocU: aligning unpaired reads

Before running AriocU, use AriocE to encode a reference sequence and hash tables (see AriocE: encoding sequence data, page 10).

To run AriocU, use a configuration file that specifies where to find encoded reference-sequence and read files, where to write alignment-result files, and how to parameterize the aligner:

```
<?xml version="1.0" encoding="utf-8"?>

<AriocU gpuMask="0x00000001" batchSize="64k" maxDOP="24">

  <R>C:\BulkData\R\NCBI\GRCh37.p5</R>

  <nongapped seed="ssi84_2_30" />

  <gapped seed="hsi20_0_30" Wmxgs="2_6_5_3" Vt="400" />

  <Q filePath="C:\BulkData\Q\mason\test203">
    <unpaired referenceId="1">
      <file>i250p_1</file>
    </unpaired>
  </Q>

  <A overwrite="true">
    <sam report="mu">C:\BulkData\A\mason</sam>
  </A>

</AriocU>
```

Simple configuration file for AriocU.

Each of the XML elements in the configuration file controls a different aspect of the read-alignment pipeline:

- `<AriocU>`: GPU devices, GPU memory, and CPU threads
- `<R>`: encoded reference sequence files
- `<nongapped>`: seed type for nongapped alignments
- `<gapped>`: seed type and scoring parameters for gapped alignments
- `<Q>`: encoded read files
- `<A>`: output files and formats

The following table summarizes the basic configuration parameters:

Element	Attribute	Description
<AriocU>	gpuMask	Bits corresponding to GPU devices
	batchSize	Number of reads per batch per GPU
	maxDOP	Number of concurrent CPU threads
<nongapped>	seed	Seed used for AriocE nongapped reference-sequence encoding
	maxA	Maximum number of reported nongapped mappings per read
<gapped>	seed	Seed used for AriocE gapped reference-sequence encoding
	Wmxgs	Smith-Waterman scoring parameters
	Vt	Minimum reportable alignment score
	maxA	Maximum number of reported gapped mappings per read
<Q>	maxQ	Limits the number of reads aligned (see Tuning for speed and sensitivity, page 22)
<unpaired>	referenceId	Reference ID used for AriocE read encoding
<A>	overwrite	"true" to overwrite existing result file(s)
<sam>	report	Alignment result type(s) to report in SAM format in the specified output directory

XML elements and attributes in the AriocU configuration file.

Some of these configuration parameter values are self-evident, but others demand careful attention:

gpuMask=. The gpuMask= parameter is a bit mask (specified as a hexadecimal value) that indicates which GPU device(s) are to be used by AriocU. Each bit in the parameter corresponds to a GPU device identifier. If there is only one GPU in the computer, the value of gpuMask= should be 1. To run AriocU on two or more GPUs, set the corresponding bits in gpuMask=.

batchSize=. The batchSize= parameter determines how many reads AriocU can process concurrently per GPU. Batch sizes are limited by available GPU memory, but the amount of GPU memory needed to compute alignments cannot be precisely predicted because it depends on the number of Smith-Waterman computations AriocU must perform in its search for satisfactory mappings for every read — and this is something you don't know until you try it.

With some data, we have observed overall speed increases on the order of 10% with "optimally large" batch sizes, but batch size has less effect on speed than other "tuning" parameters (see Tuning for speed and sensitivity, page 2219). If you do want to try to find an optimal batch size for your data, we suggest that you start with a batch size of about 32k. If AriocU runs without memory-allocation errors, progressively increase the batch size and re-run AriocU until it runs out of GPU memory or until its throughput does not improve with increasing batch size.

The batchSize= parameter may be specified either as a positive integer (e.g., "25000") or as a multiple of 1024 (e.g., "128K").

maxDOP=. AriocU uses CPU threads for file input/output and for post-processing alignment data (computing mapping qualities, formatting alignment results, and so on). AriocU's throughput is generally limited by GPU speed rather than CPU speed, but in general, you should let AriocU use as many CPU threads as you have available.

Wmxgs=. This parameter specifies four Smith-Waterman scoring parameters as non-negative integers separated by commas or underscores. The four values indicate the score for a match, mismatch, gap open, and gap space respectively. For example, "2,6,5,3" means match=+2; mismatch=-6; gap open=-5; gap space=-3.

Vt=. This parameter indicates the minimum reportable Smith-Waterman alignment score. You can specify `Vt=` in one of two ways: as a non-negative integer threshold score (e.g., "100") or as a function of read length described in a Bowtie-style function parameter: "[LSG],b,a", where the initial letter indicates the function type (L: linear; S: square root; G: natural log), b is a constant term and a is the coefficient. For example, `Vt="S,75,2.5"` specifies a reportable alignment score threshold of `75 + 2.5*sqrt(read length)`.

report=. Use the `report=` parameter to filter reported alignment results. You can use any combination of `m` (mapped) and `u` (unmapped). For example, to report both mapped and unmapped results in the same output file, use `report="mu"`. To report the same results to two separate files, use two `<sam>` elements, one with `report="m"` and one with `report="u"`.

AriocP: aligning paired-end reads

You must use AriocE to encode a reference sequence and hash tables (see AriocE: encoding sequence data, page 10) before you run AriocP.

The AriocP configuration file is similar to the one that AriocU uses. The important differences have to do with parameters that control the alignment of paired-end reads:

```
<?xml version="1.0" encoding="utf-8"?>

<AriocP gpuMask="0x00000001" batchSize="48k" maxDOP="24">

  <R>/BulkData/R/NCBI/GRCh37.p5</R>

  <nongapped seed="ssi84_2_30" />

  <gapped seed="hsi20_0_30" Wmxgs="2,6,5,3" Vt="100" />

  <Q filePath="/BulkData/Q/mason/test103">
    <paired referenceId="1">
      <file>i100p_1</file>
      <file>i100p_2</file>
    </paired>
  </Q>

  <A overwrite="true" pairOrientation="c" pairCollision="oc">
    <sam report="cdru">/BulkData/A/mason</sam>
  </A>

</AriocP>
```

Simple configuration file for AriocP.

Each of the XML elements in the configuration file controls a different aspect of the read-alignment pipeline:

- `<AriocP>`: GPU devices, GPU memory, and CPU threads
- `<R>`: encoded reference sequence files
- `<nongapped>`: seed type for nongapped alignments
- `<gapped>`: seed type and scoring parameters for gapped alignments
- `<Q>`: encoded read files
- `<A>`: output files and formats

The following table summarizes the basic configuration parameters:

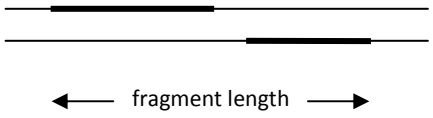
Element	Attribute	Description
<AriocP>	gpuMask	Bits corresponding to GPU devices
	batchSize	Number of reads per batch per GPU
	maxDOP	Number of concurrent CPU threads
<nongapped>	seed	Seed used for AriocE nongapped reference-sequence encoding
	maxA	Maximum number of reported nongapped mappings per read
<gapped>	seed	Seed used for AriocE gapped reference-sequence encoding
	Wmxgs	Smith-Waterman scoring parameters
	Vt	Minimum reportable alignment score
	maxA	Maximum number of reported gapped mappings per read
<Q>	maxQ	Limits the number of reads aligned (see Tuning for speed and sensitivity, page 22)
<paired>	referenceId	Reference ID used for AriocE read encoding
<A>	overwrite	"true" to overwrite existing result file(s)
	pairFragmentLength	Distance between the ends of a paired-end mapping
	pairOrientation	Specifies the relative orientation (convergent/divergent/same) of the mates in a pair.
	pairCollision	Indicates whether mates may overlap, cover, or dovetail.
<sam>	report	Alignment result type(s) to report in SAM format in the specified output directory

XML elements and attributes in the AriocP configuration file.

Most of the AriocP configuration parameters control the same behaviors as they do with AriocU. The important differences are related to the management of paired-end alignments:

Vt=. The Vt= parameter is the minimum reportable alignment score for each individual mate in a pair, not the combined alignment scores for both mates.

pairFragmentLength=. The pairFragmentLength= parameter specifies a range of acceptable fragment-length values. AriocP computes the pair fragment length as a SAM TLEN, that is, the distance between the "upstream" and "downstream" positions of a pair of mappings:



pairOrientation=. This parameter specifies the expected orientation of the mates in a pair:

- convergent: the "upstream" mate (that is, the mate at the lower-numbered location on the reference sequence) maps to the forward strand and the opposite mate maps to the reverse-complement strand
- divergent: the upstream mate maps to the reverse-complement strand and the opposite mate to the forward strand
- same: both mates map to the same strand

pairCollision=. The pairCollision= parameter indicates whether the mappings of a pair of mates may overlap, cover, or dovetail each other:

report=. The report= parameter value can be any combination of the following (similar to Bowtie's paired-end alignment result categories):

- c (concordant): both mates in a pair have reportable mappings and meet the user-specified constraints for orientation and fragment length
- d (discordant): each mate has a unique mapping, but the mappings do not meet the user-specified paired-end constraints
- r (rejected): both mates have mappings and at least one of the mates has two or more mappings, but no combination of the mappings meets the user-specified paired-end constraints
- u (unmapped): one or both of the mates has no reportable mapping

You can write alignment results to two, three, or four different files by using multiple <sam> elements, each with a different, mutually-exclusive subset of the four alignment-result categories.

Tuning for speed and sensitivity

Arioc supports a variety of parameters you can use to control its behavior and balance between speed and sensitivity.

The verboseMask= and maxQ= parameters

The verboseMask= parameter (used with the <AriocE>, <AriocU>, or <AriocP> element) controls the amount of detail in the output of each of the Arioc executables. Specify the parameter value as a 32-bit hexadecimal bitmap:

Bits	Hexadecimal	Description
31	0x80000000	Output to stderr
30	0x40000000	Output to Windows debugger (OutputDebugString)
29	0x20000000	Emit timestamps
11	0x00000800	Trace main loop iterations
9	0x00000200	Trace CUDA (GPU) memory management
8	0x00000100	Trace host (CPU) memory management
4	0x00000010	Detailed trace
3	0x00000008	Basic trace
2	0x00000004	Detailed performance metrics
1	0x00000002	Basic performance metrics
0	0x00000001	Banners and exceptions

Bit flags in the verboseMask= parameter.

For performance tuning, use verboseMask=0xE0000807 to see time-stamped intermediate results and summary timings.

The maxQ= parameter (used with the <Q> element) halts read alignment after the specified number of reads (for AriocU) or pairs (for AriocP) have been processed per GPU.

How to make Arioc more sensitive

Arioc finds more high-scoring mappings when it evaluates potential alignments at more reference-sequence locations. Here are several ways to broaden Arioc's "search space":

AriocE: Build the seed-and-extend hash table with a higher value for maxJ=. In effect, this increases the maximum size of a hash table bucket. This affects "big bucket" hash values, that is, seeds that derive from highly-repetitive regions and that are thus associated with many reference-sequence locations.

Increasing maxJ= has no effect on most hash table buckets because seeds that are not associated with highly-repetitive regions of the reference hash to very few reference-sequence locations anyway. Thus, with increased maxJ=, AriocU and AriocP are able to find additional mappings primarily for a few noisy reads. (By "noisy", we mean that a read contains enough mismatches and indels that only a few of its seeds remain intact. If those intact seeds happen to be associated with "big buckets" in the hash table, the aligner is more likely to find a mapping if you use a larger value for maxJ=.)

With larger values for `maxJ=`, AriocU and AriocP spend more time evaluating additional seed locations. With the human reference genome, speed starts to decrease sharply with `maxJ=` values above 500 or 1000, with relatively small improvements in sensitivity.

AriocU, AriocP: To obtain greater sensitivity from the AriocU and AriocP aligners, you can increase the value of any or all of the following parameters:

Element	Attribute	Description
`<gapped>`	`AtN`	Maximum number of nongapped mappings per read
	`seedDepth`	Number of seed iterations
	`AtG`	Maximum number of gapped mappings per read

Configuration parameters useful for AriocU and AriocP performance tuning.

To understand how to use these parameters, consider how the aligners process a read.

First, the aligner uses periodic spaced seeds to find nongapped mappings that contain no more than two mismatches. The value of `AtN=` is a threshold for the number of nongapped mappings; if the aligner finds at least that number of nongapped mappings for a read, it excludes the read from further processing by the gapped aligner. This parameter setting makes a difference for the occasional read whose best nongapped mapping has a lower alignment score than the read's best gapped mapping. You can find the higher-scoring gapped mappings for such reads by setting `AtN=` to a value of 2 or more.

After searching for nongapped mappings, the aligner uses seed-and-extend to find gapped mappings for the remaining reads (i.e., those that do not have the minimum number of nongapped mappings). For each such read, the aligner iteratively chooses seeds, starting with the set of seeds that are immediately adjacent to each other (that is, the seeds at positions 0, 20, 40... in the read). If none of these seeds leads to a successful mapping, the seed interval is halved and the new set of seeds is evaluated. Since Arioc uses 20nt seeds, six such "interval halving" iterations are needed to evaluate every possible seed location in the read. You can alter this behavior by setting the `seedDepth=` parameter, which limits the number of seed iterations to a number between 1 and 5. The Arioc aligners run significantly faster with smaller values of `seedDepth=`, but they also find high-scoring mappings for fewer reads.

Arioc abandons the search for seed-and-extend mappings for a read when it finds a sufficient number of high-scoring mappings. You can use the `AtG=` parameter to force Arioc to keep searching for higher-scoring mappings; the aligner will not give up on a read until it finds at least the number of gapped mappings specified in `AtG=` (or until it runs out of seeds).

Because Arioc computes potential alignments in parallel, neither `AtN=` nor `AtG=` is precisely deterministic. For example, when you set AtN= to 2, Arioc might nevertheless find 10 high-scoring nongapped mappings for a particular read, simply because all 10 mappings were discovered in parallel.

References

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