Endovir

A pipeline to identify endogenous viruses in SRA datasets

https://github.com/NCBI-Hackathons/EndoVir

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Foreword

Based on previous work

- ViruSpy [1]
- Logic mostly implemented in Bash and Perl
  $\rightarrow$ Complex to expand and adjust
- Initiated during my stay as a visiting bioinfomartician as NCBI from Oct.2017 to Dec. 2017.
- Ongoing

[1] https://github.com/NCBI-Hackathons/ViruSpy

Foreword

Standing on the shoulder of giants

- Karina Zile, karina.zile@gmail.com
- Mitchell A. Ellison II, mae92@pitt.edu
- Jacob Waldman, jaw267@pitt.edu
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- Paul G. Cantalupo, pcantalupo@gmail.com
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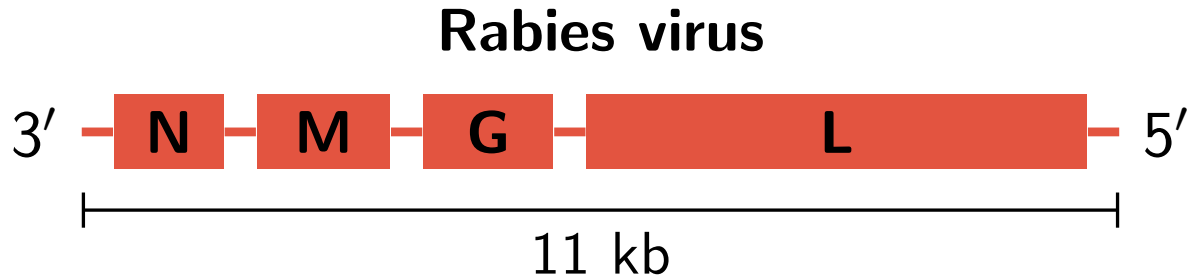
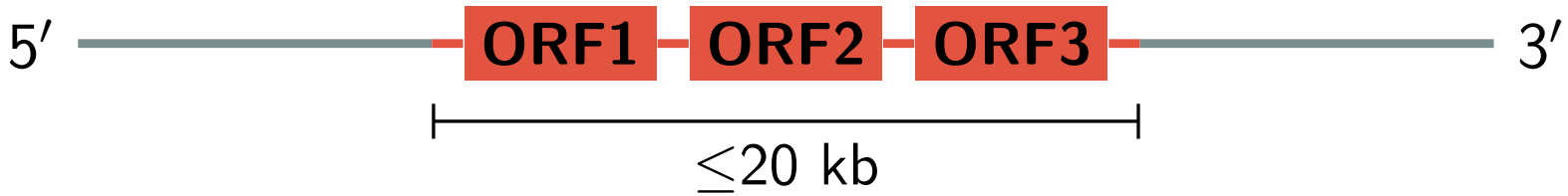
Exogenous viruses

- Integration into host genome not required for replication
- Hist genome integration occurs occasionally, e.g. Human Herpes virus 6
- Not part of the host genome
- RNA viruses: $\leq\approx$ 40 kb
- DNA viruses: $\leq\approx$ 2 Mbp



Endogenous viral element (EVEs)

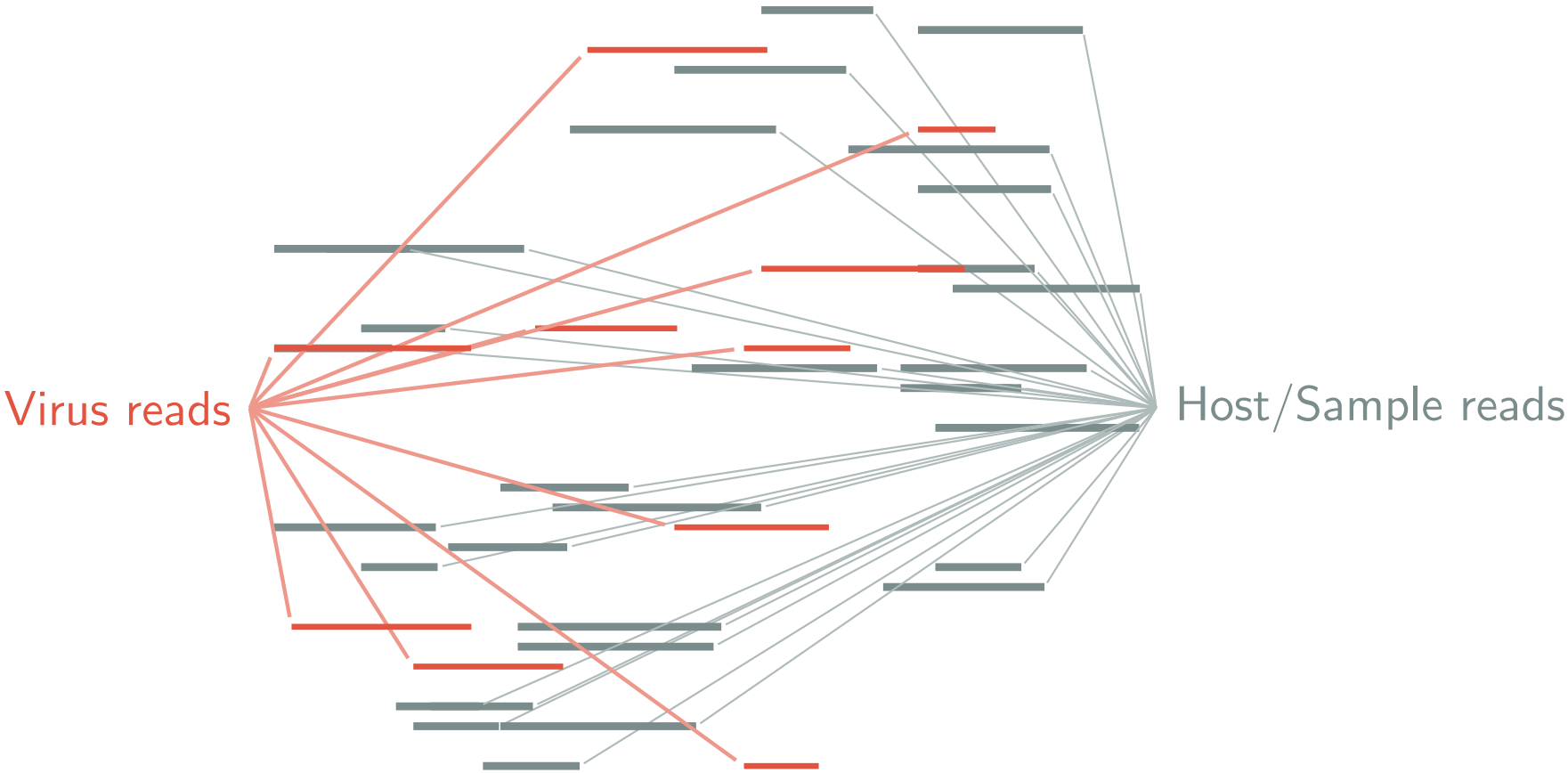


- Integration into host genome mandatory for replication
- Retroviridae, e.g. HIV
- Part of the host genome
- Not widely analyzed: complex analysis and missing data [2]
- Allowing to date virus evolution
- Characterize the mechanisms that underpin the integration of viruses into host genomes

[2] Katzourakis, A. and Gifford, R.J. (2010). PLos Genet., 6, e1001191

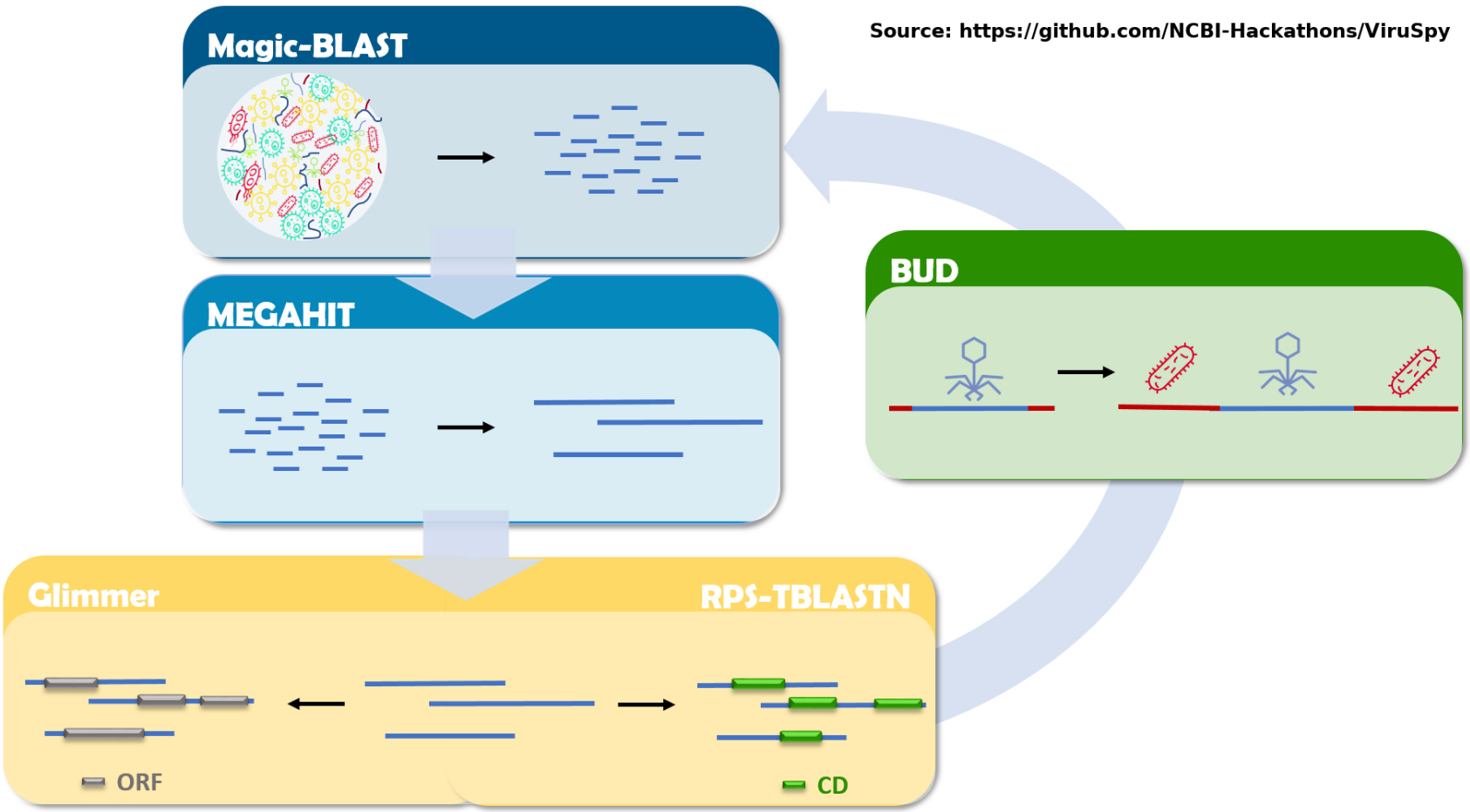
SRA data: a treasure trove for virus genomics

- SRA data contains host and virus sequences
- Exo/Endogenous and putative novel viruses



BUD algorithm

- "Building Up Domains"



Endovir

Open source	MIT licence
Modern	Implemented in Python 3 (3.6)
Independent	Using only Python standard libraries
Limited disk I/O	Using pipes where possible
Modularity	Changing external tools, e.g. assembler, without touching Endovir internals
Status	Alpha, ongoing development

Biological databases

Endovir uses two biological databases:

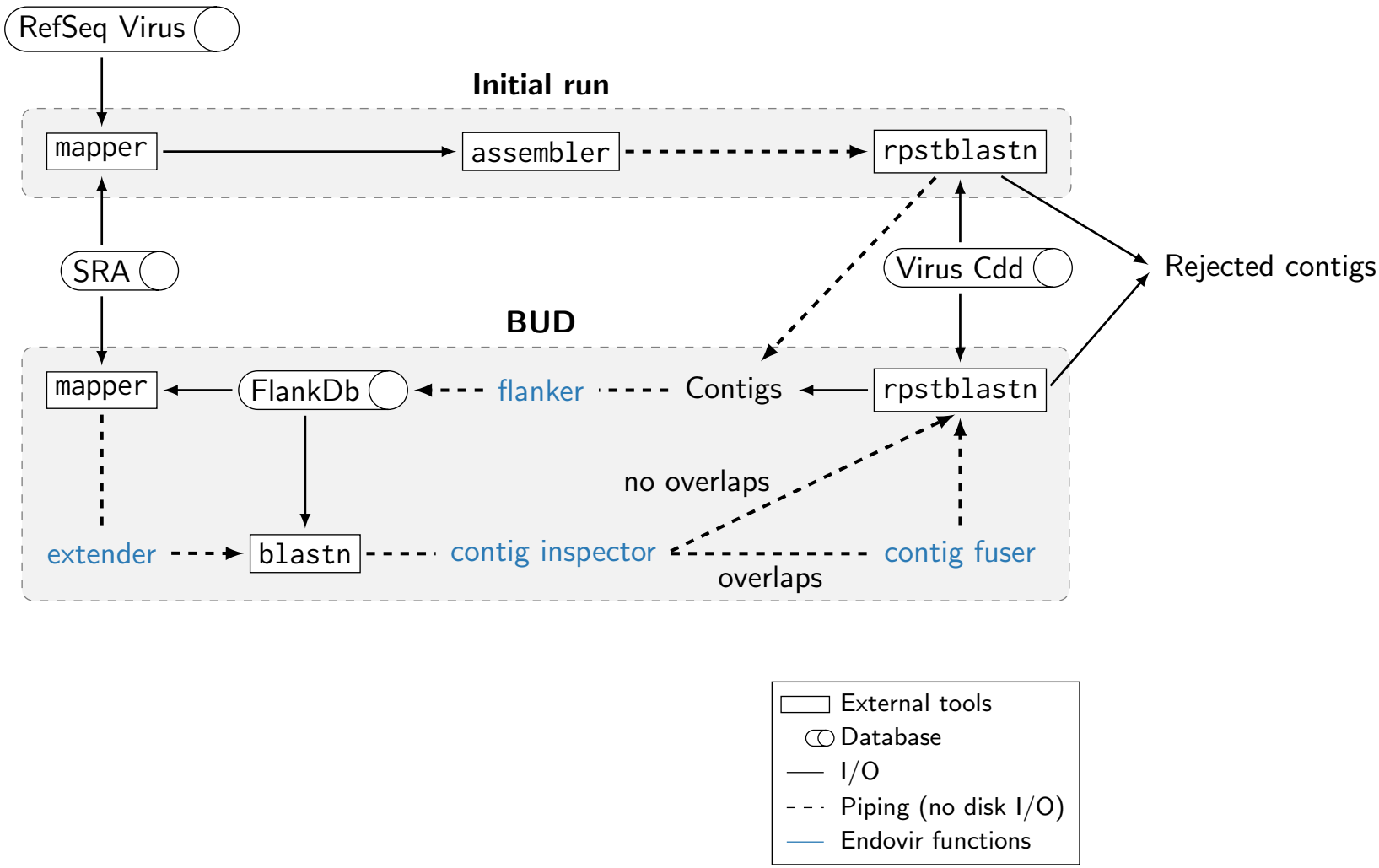
Sequences Virus genomes sequences from RefSeq

Domains virus-specific Cdd database. We provide a setup script to create the Cdd database

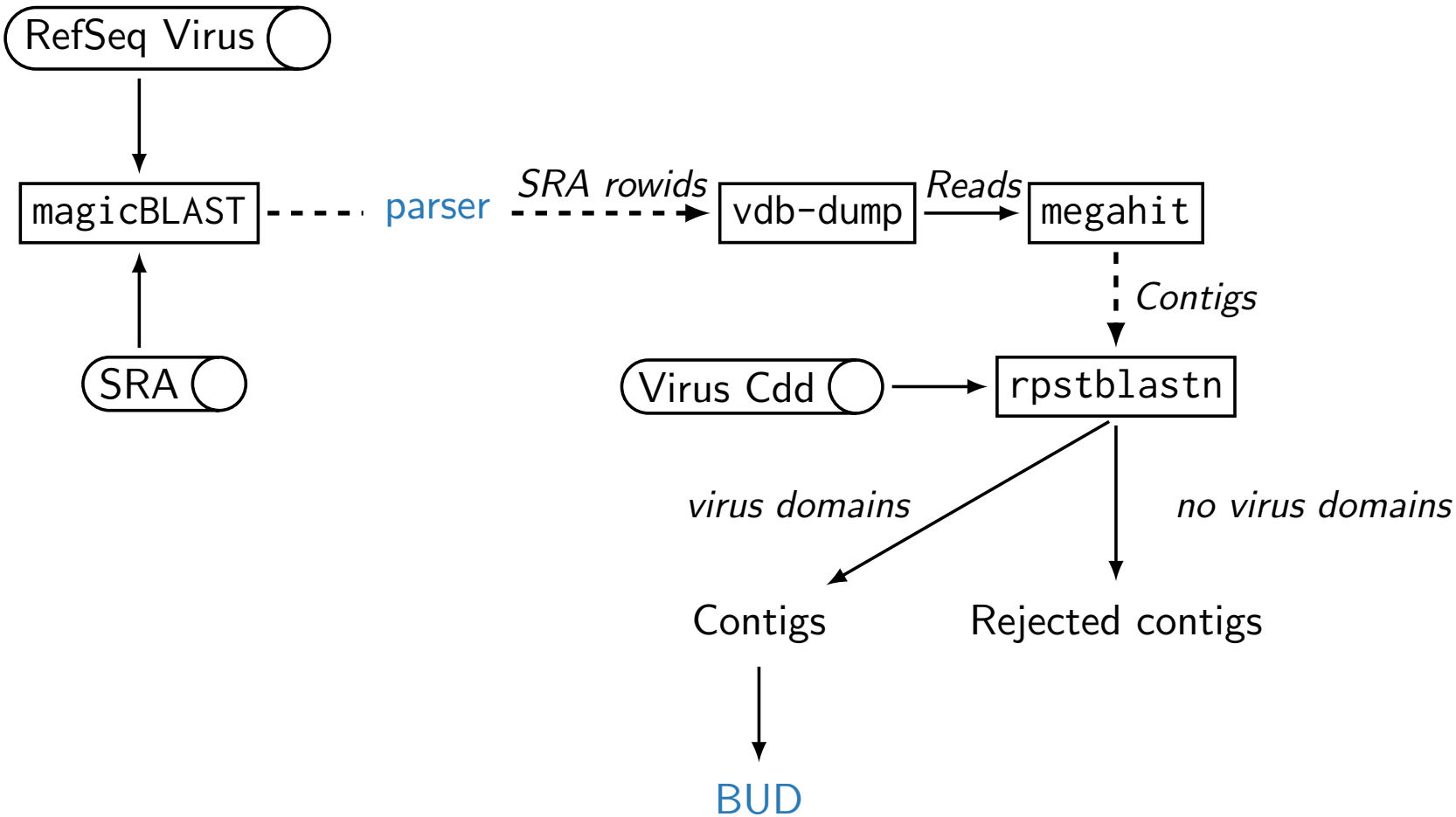
Usage

```
usage: endovir.py [-h] [-srr SRR] [--wd WD] [--max_cpu MAX_CPU]

Endovir

optional arguments:
  -h, --help            show this help message and exit
  -srr SRR              SRR number, e.g. SRR5150787
  --wd WD               Working directory for analysis
  --max_cpu MAX_CPU, -p MAX_CPU
                        Max number of cores to use. NOT YET IMPLEMENTED
```

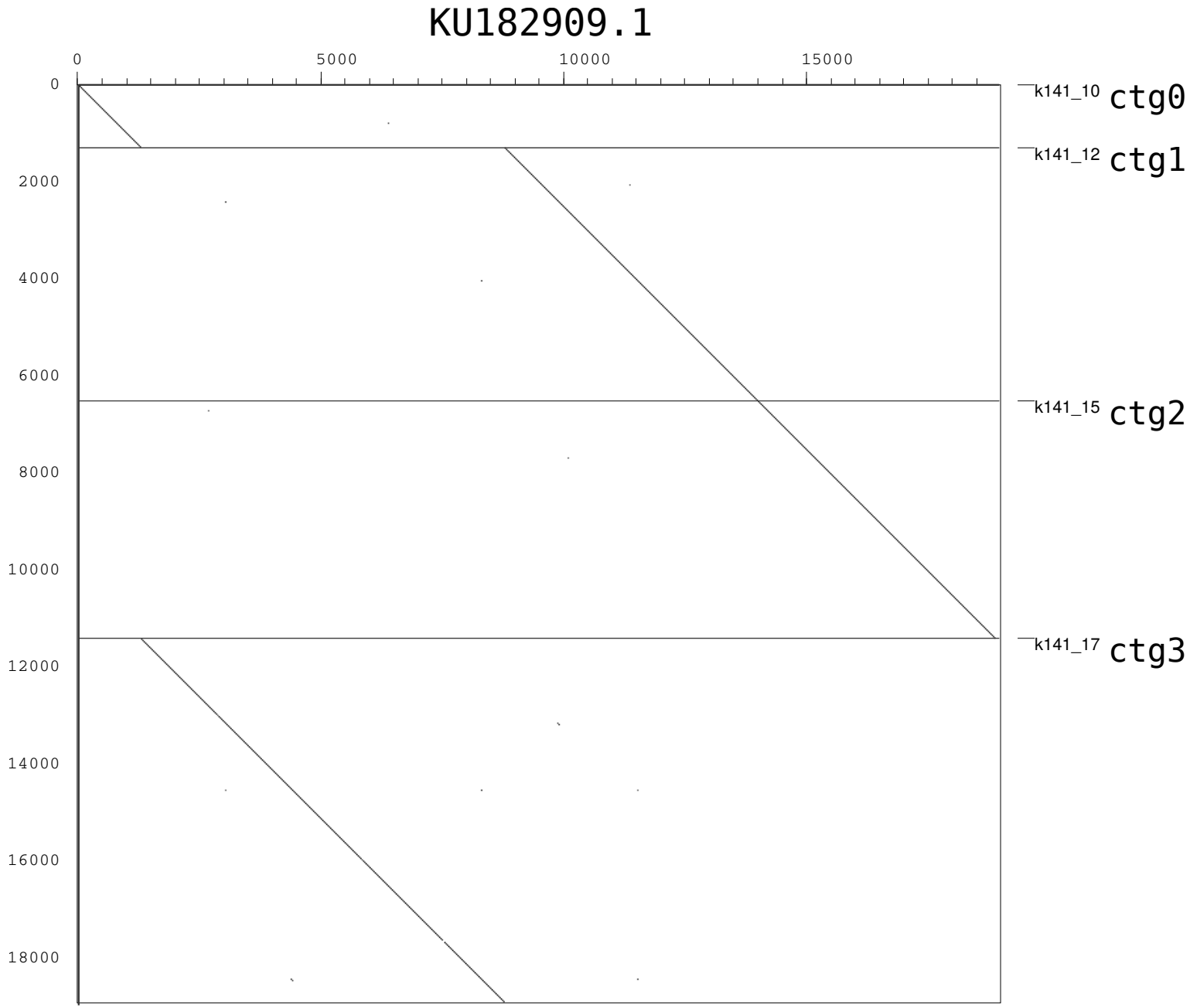
Workflow


RefSeq Virus
Initial run
mapper
assembler
rpstblastn
SRA
Virus Cdd
Rejected contigs
BUD
mapper
FlankDb
flanker
Contigs
rpstblastn
no overlaps
extender
blastn
contig inspector
contig fuser
overlaps
External tools
Database
I/O
Piping (no disk I/O)
Endovir functions

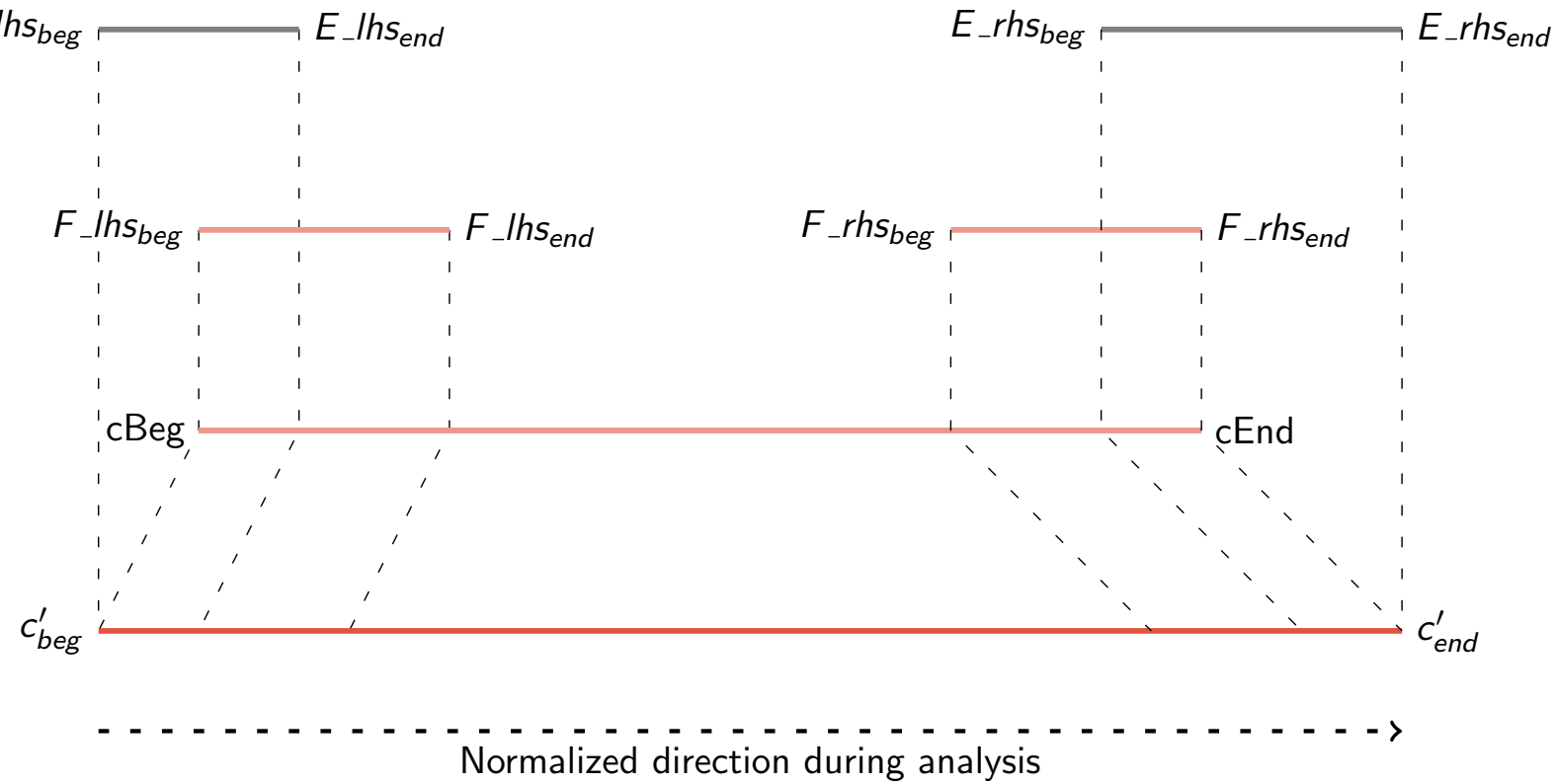
Init: current version



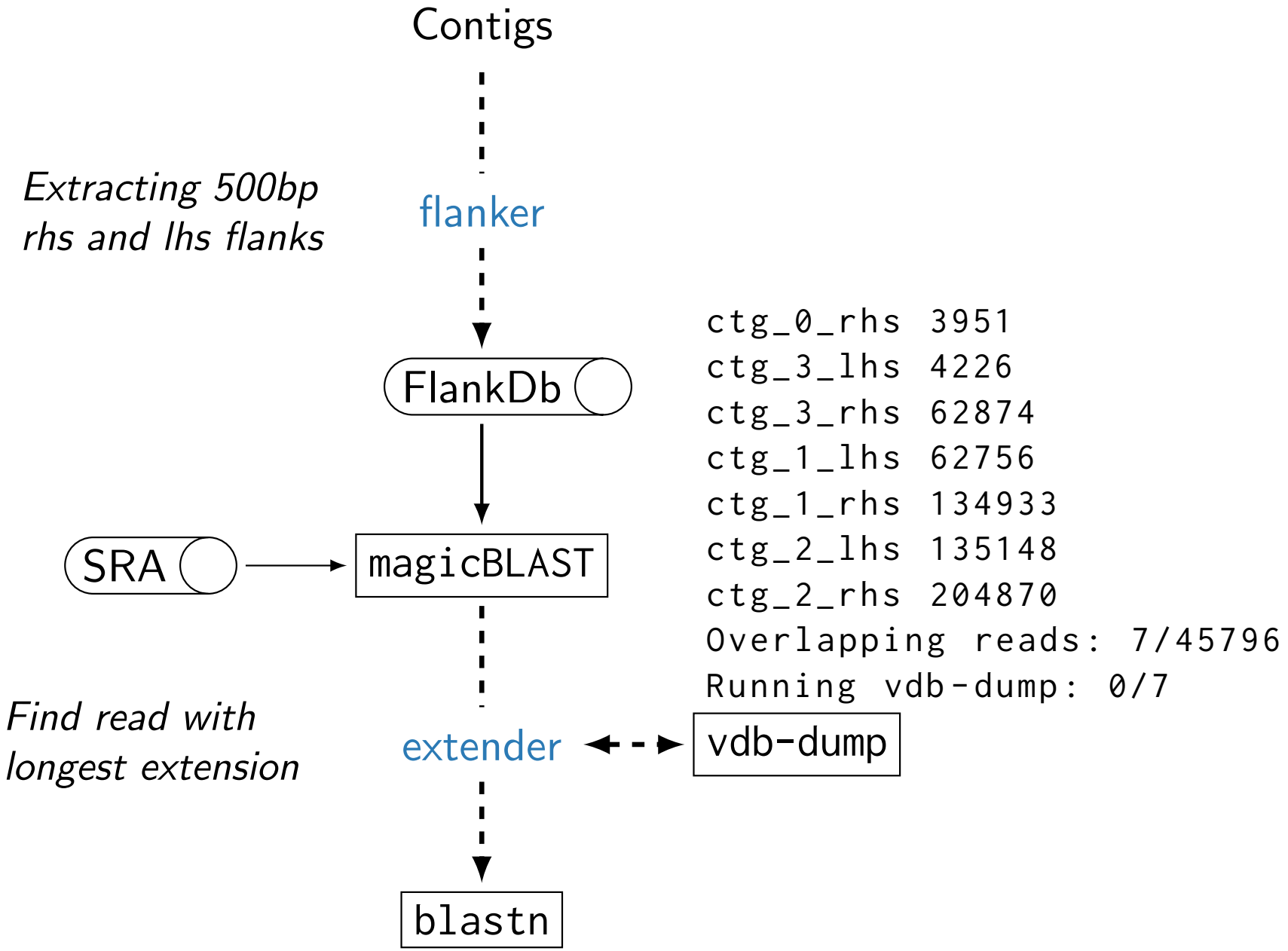
Init: SRR5150787 example (Ebola)



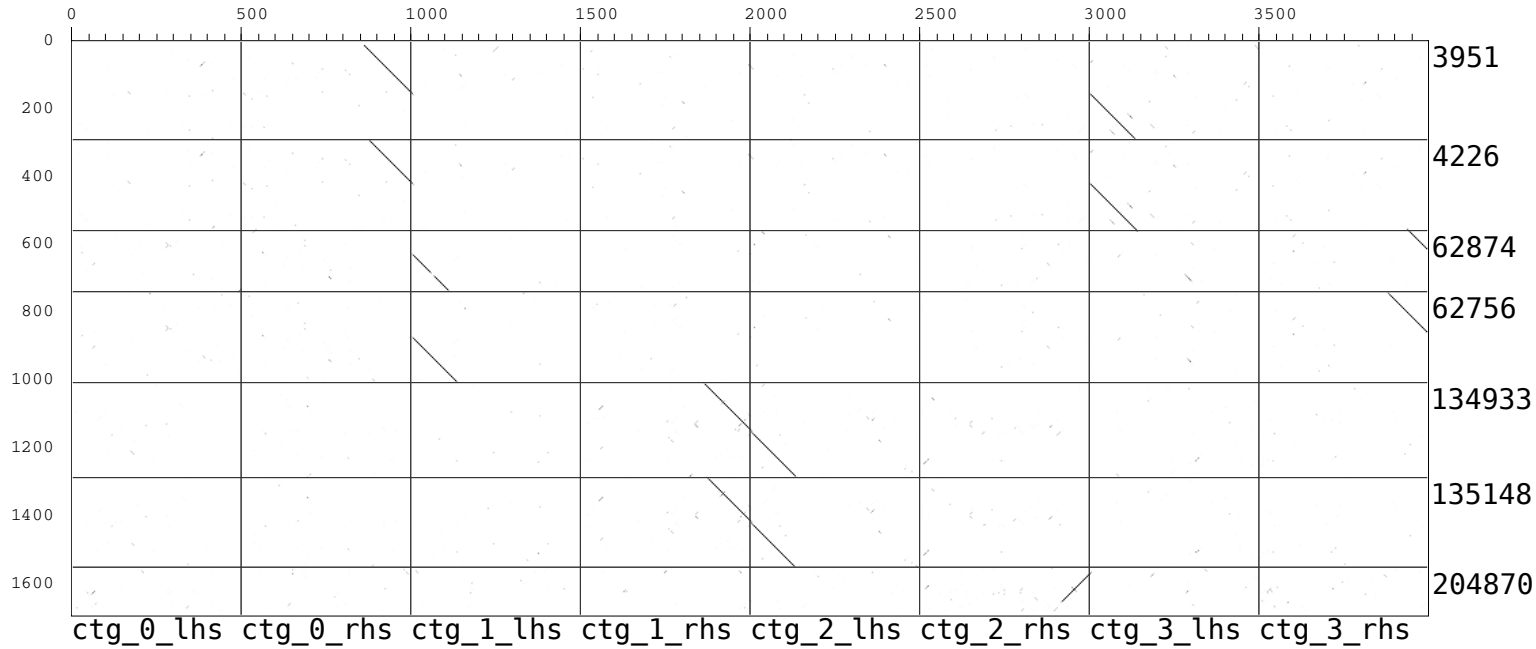
Endovir's contig model



BUD: Extending

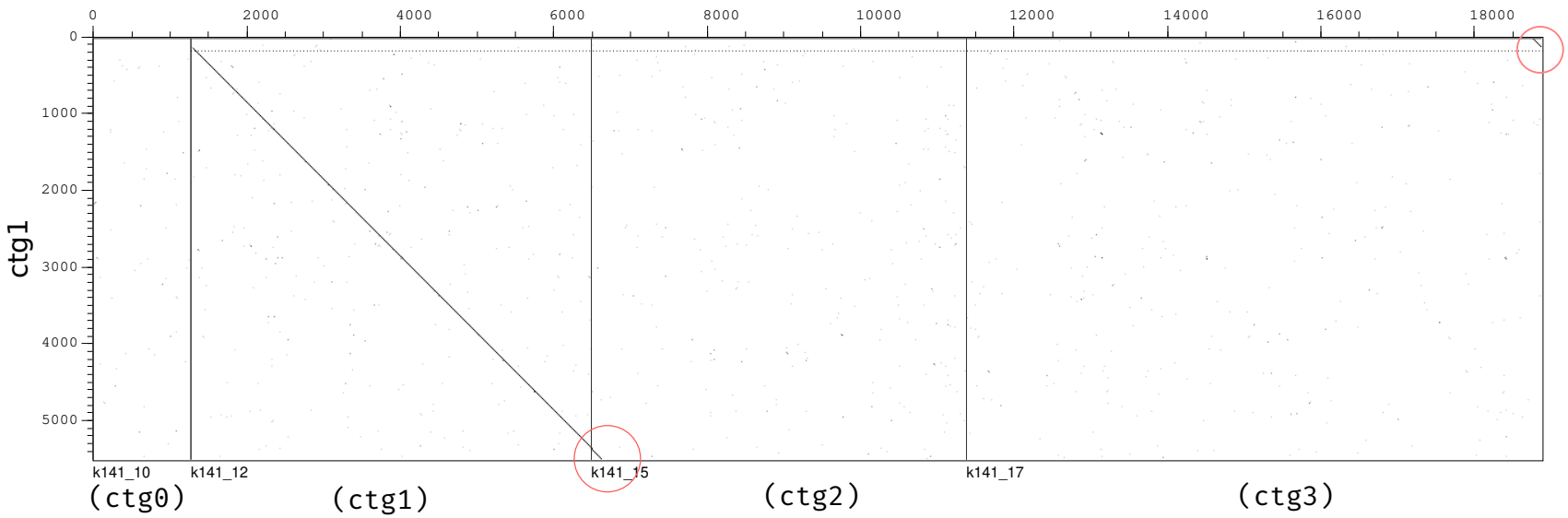


BUD: Extending

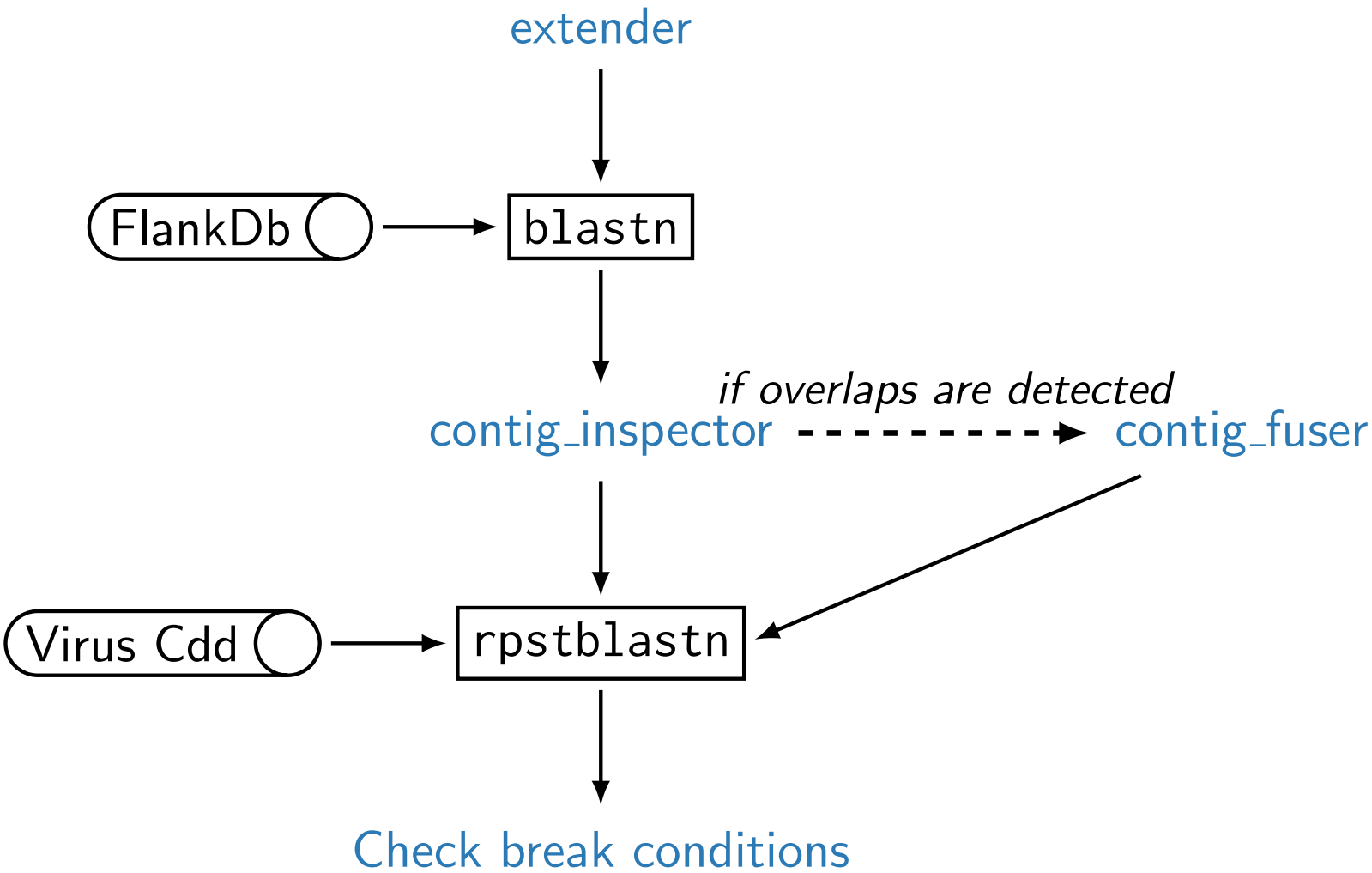



```
SRR5150787
Flank: ctg_1_lhs  0 500 500 0 0 135
Read:  62756  129 264 265 0
Ext:   0  264 267
Flank: ctg_1_rhs  4843  5343  500 0 359 499
Read:  134933 0 140 280 0
Ext:   5202 5342  282
ctg_1 5482
  lhs ctg_1_lhs : 0 500 500 129
  62756 : lhs 0 264 136 131 267
  rhs ctg_1_rhs : 4982  5482  500 139
  134933 :  rhs 5202  5342  141 141 282
```

BUD: Extending



BUD: Overlap check



BUD: Overlap check

```
Checking flanks for overlaps
Blast results: ctg_0_lhs vs. ctg_0_lhs
Not an overlap
-----------
Blast results: ctg_0_lhs vs. ctg_2_rhs
Checking ctg_0_lhs and ctg_2_rhs
ctg_2:rhs + ctg_0:lhs
LHS:  130
RHS 117
oLHS: 129
oRHS  139
Src: ctg_2  7724
 RHS_flank: 7224  7724  500
  Extension: 7544 7606  182
Dst: ctg_0  5482  0 126 0
Brk: ctg_2  7597  ctg_0 129
---------------------------------------
Blast results: ctg_0_rhs vs. ctg_1_lhs
Checking ctg_0_rhs and ctg_1_lhs
  Update: ctg_0 is now ctg_2
ctg_2 ctg_1
ctg_2:rhs + ctg_1:lhs
LHS:  130
RHS 0
oLHS: 134
oRHS  12
Src: ctg_2  7724
 RHS_flank: 7224  7724  500
  Extension: 10428  10568 0
Dst: ctg_1  5042  0 134 0
Brk: ctg_2  7589  ctg_1 134
```

BUD: Break conditions

Extending into host genome	Empty Cdd database screen
No grow	Contig was not extended
No contigs	No contigs were identified

Modularity

- Facilitating extending and adding other tools using classes which act as messenger between tool and Endovir.

```
class MappingAlignment:

  class Read:
    def __init__(self, name, start, stop, strand, qlen):

  class Flank:
    def __init__(self, name, start, stop, strand):

  def __init__(self, cols):
    self.read = self.Read(cols[0], cols[6], cols[7], cols[13], cols[15])
    self.flank = self.Flank(cols[1], cols[8], cols[9], cols[14])
    self.pident = float(cols[2])

class MagicblastParser:

  def __init__(self):
    self.alignments = []

  def parse(self, src):
    self.alignments = []
    for i in src:
      if i[0] != '#':
        self.alignments.append(MagicblastAlignment(i.strip().split('\t')))
```

Outlook

- Adjust tool parameters on the fly
- Replace SRA toolkit with NCBI's ngs-python
- Test on more diverse datasets
- Docker

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