# A LAYERED GENOTYPING-BY-SEQUENCING PIPELINE USING GALAXY

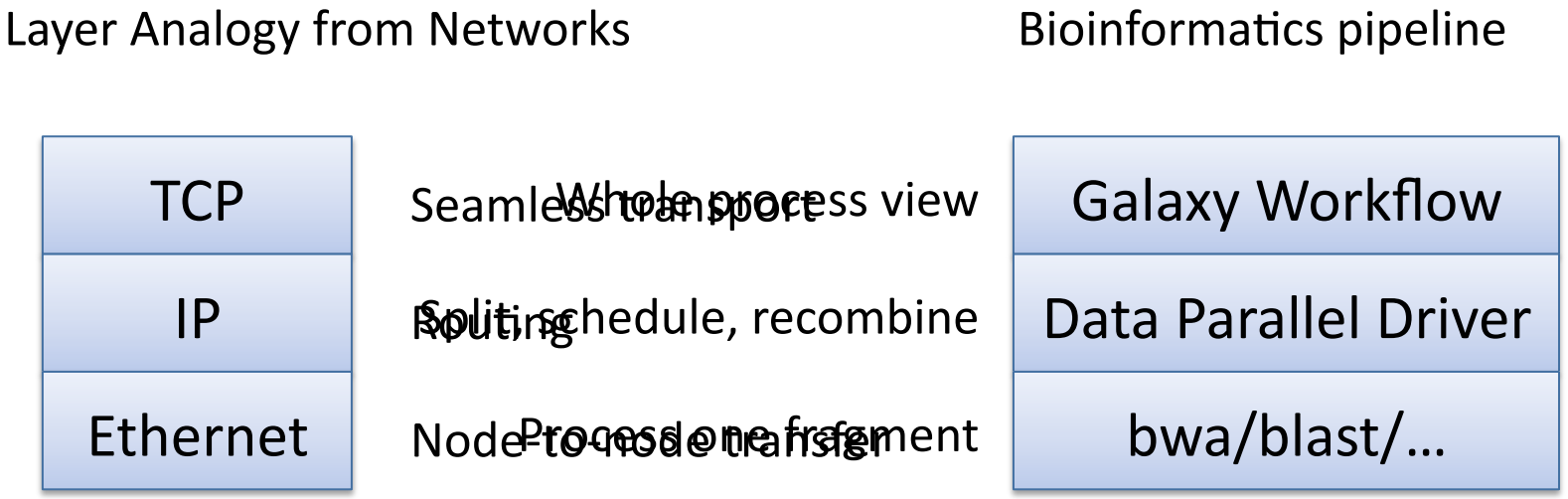
Rudiger Brauning, *Simon Guest*, Alan McCulloch, Russell Smithies
AgResearch, New Zealand

Galaxy Community Conference 2013, Oslo

agresearch

# WHAT'S THIS ALL ABOUT?

- A *layered* approach to *data parallelism*
  - Execute the *same* command in parallel on *different* pieces of the data
  - Layered => hide the details of doing this from the user
- Greatly accelerates processing on compute farm

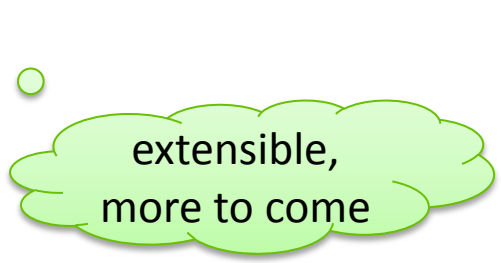



agresearch

# OUR EXAMPLE IMPLEMENTATION ON GALAXY

- We wrote a Python script called *tardis*
  - splits input files, schedules parallel jobs, recombines output
  - plumbed into Galaxy via Cheetah templates in tool XML files
  - user-switchable via checkbox on tool webpage

- Do *something* to a {fasta, fastq, sam, bam} file in parallel
  - For your choice of *something*




agresearch

## Map with BWA for Illumina (version 1.2.3)

**Will you select a reference genome from your history or use a built-in index?:**

Use a built-in index

**Select a reference genome:**

Sheep OAR31:sheep.v3.0.14th.lowercase.final.fa

**Is this library mate-paired?:**

Single-end

**FASTQ file:**

FASTQ with either Sanger-scaled quality values (fastqsanger) or Illumina-scaled quality values (fastqillumina)

**BWA settings to use:**

Commonly Used

For most mapping needs use Commonly Used settings. If you want full control use Full Parameter List

**Suppress the header in the output SAM file:**

BWA produces SAM with several lines of header information

**Use HPC cluster:**

the input file will be split, and chunks will be processed on the compute farm. The chunk outputs will be pasted together before entry into your history

**Chunk size:**

1500000

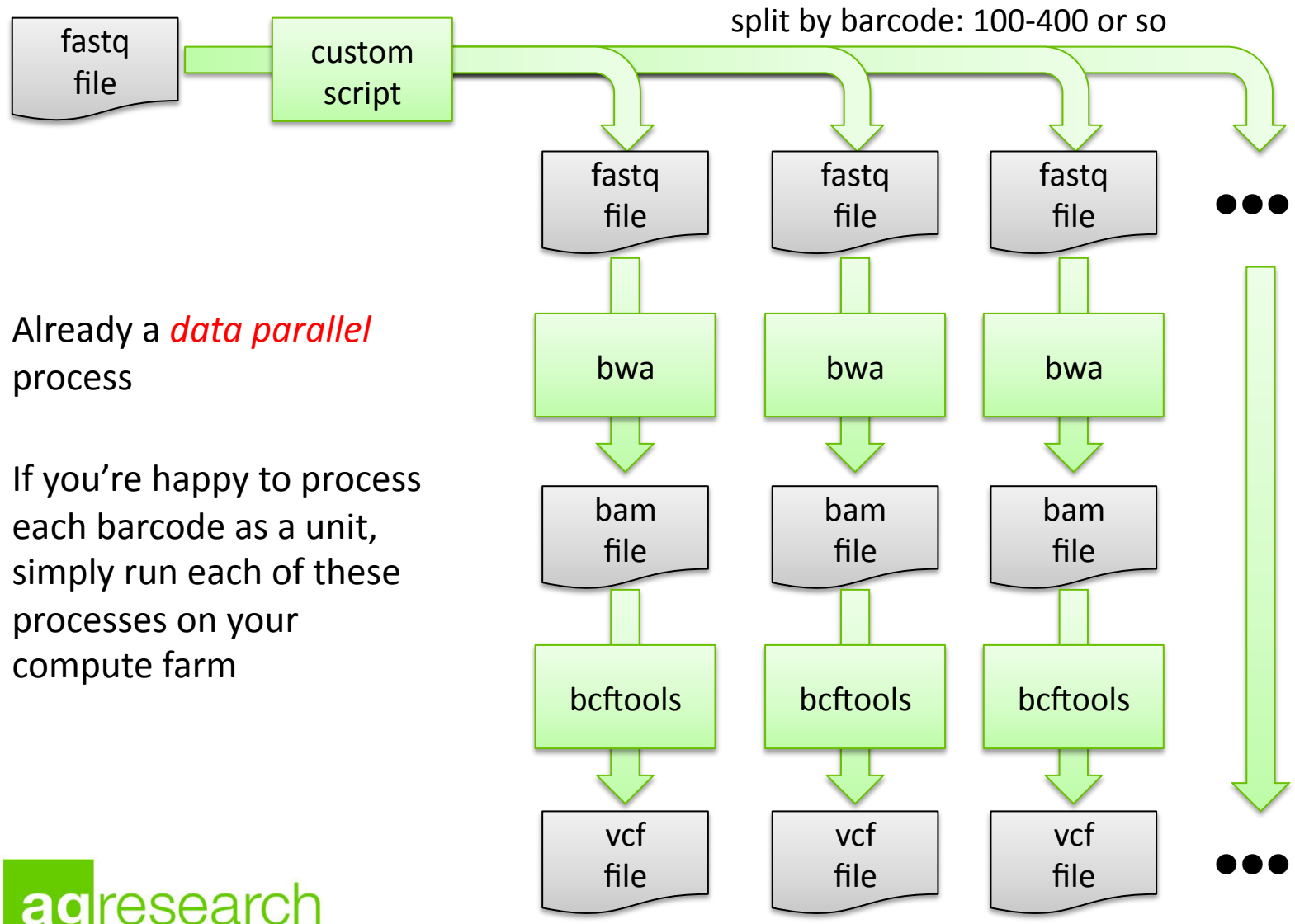
the number of sequences each split should contain

**sample rate (leave empty to process the complete file):**

for example entering .001 will randomly sample and process roughly 1 in every 1000 records

agresearch

# GENOTYPING BY SEQUENCING (GBS)

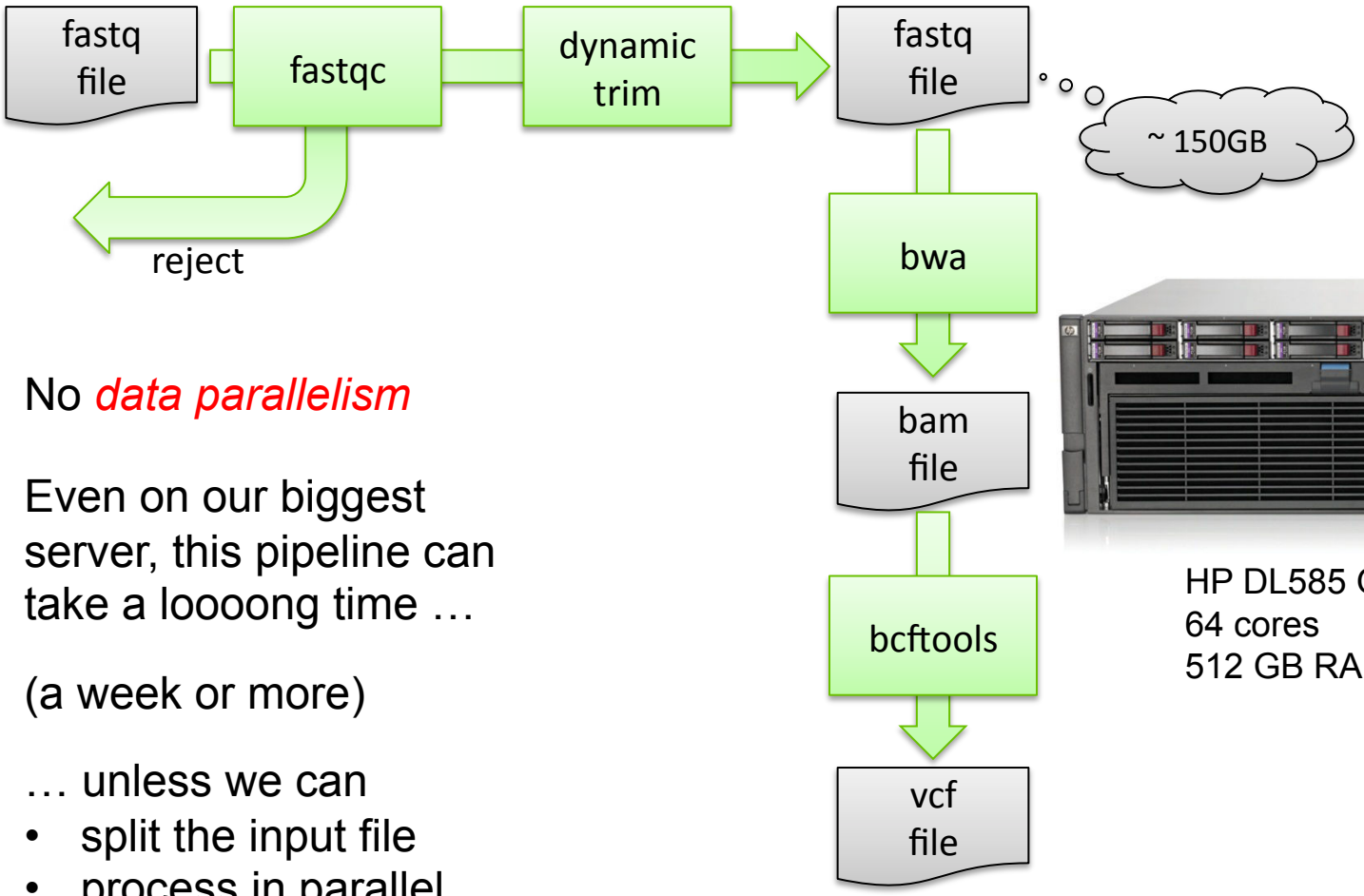



Already a *data parallel* process

If you're happy to process each barcode as a unit, simply run each of these processes on your compute farm

agresearch

# WHOLE GENOME SHOTGUN SEQUENCING (WGS)






HP DL585 G7
64 cores
512 GB RAM

No *data parallelism*

Even on our biggest server, this pipeline can take a loooong time …

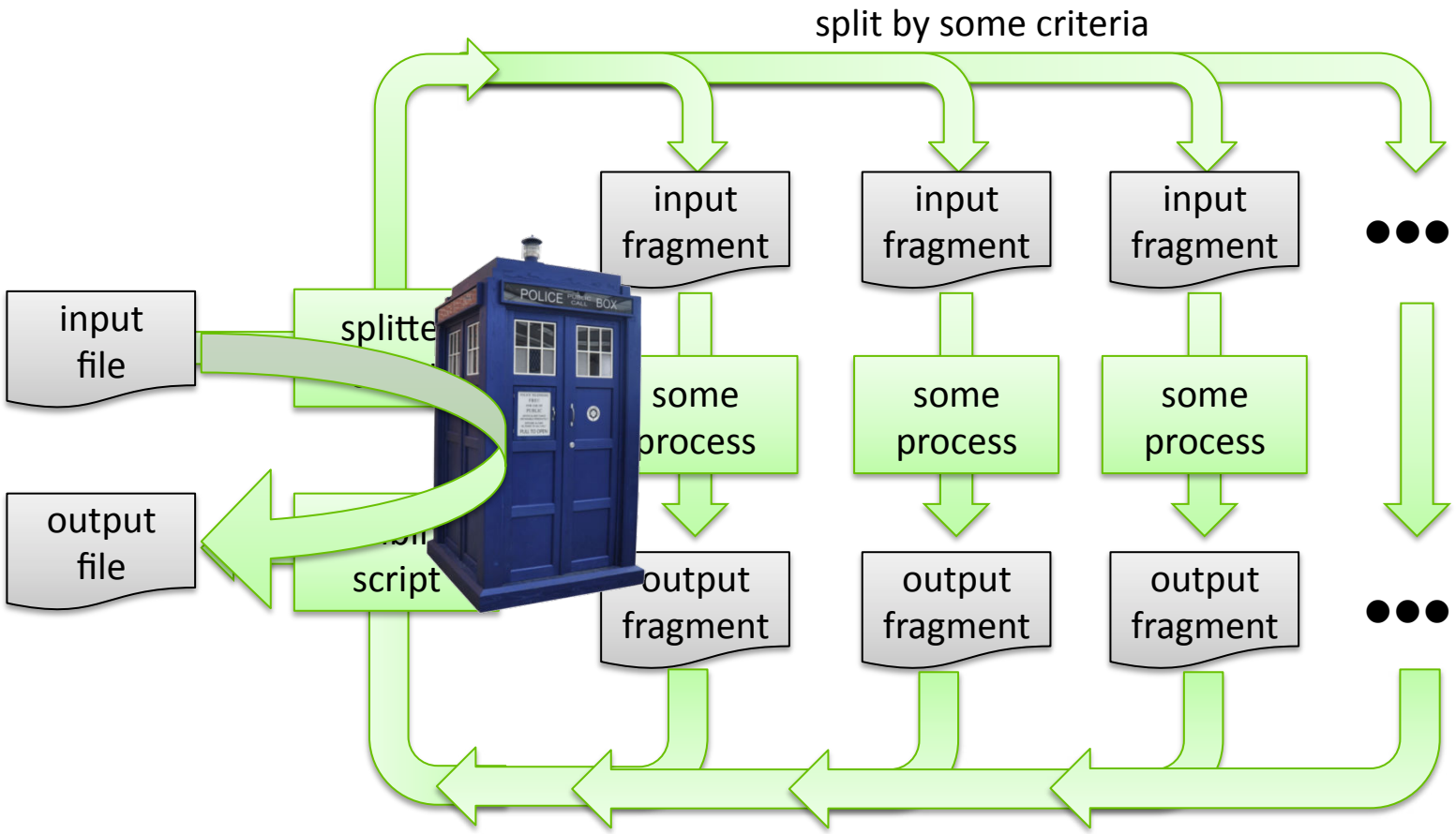
(a week or more)

… unless we can

- split the input file
- process in parallel

agresearch

# GENERAL CASE




*tardis* splits, manages cluster jobs, and recombines …

… transparently

agresearch

# AGRESEARCH COMPUTE FARM

48 x HP BL 485c,  4 core, 16 GB RAM

4 x HP DL585 G7,  48/64 core, 512 GB RAM

2 x HP DL385 G7 Fileservers, 16 core, 64 GB
200 TB storage (ZFS backed NFS over 10Gb Eth)

Key enabler:  *uniform environment*

i.e. all compute servers

- run identical software
- see same network filesystem

agresearch

# KEY TECHNOLOGIES

CentOS





AgResearch Bioinformatics RPM Repository

http://rpm.agresearch.co.nz


# END USER VIEW

## Map with BWA for Illumina (version 1.2.3)

**Will you select a reference genome from your history or use a built-in index?:**

Use a built-in index

**Select a reference genome:**

Sheep OAR31:sheep.v3.0.14th.lowercase.final.fa

**Is this library mate-paired?:**

Single-end

**FASTQ file:**

FASTQ with either Sanger-scaled quality values (fastqsanger) or Illumina-scaled quality values (fastqillumina)

**BWA settings to use:**

Commonly Used

For most mapping needs use Commonly Used settings. If you want full control use Full Parameter List

**Suppress the header in the output SAM file:**

BWA produces SAM with several lines of header information

**Use HPC cluster:**

the input file will be split, and chunks will be processed on the compute farm. The chunk outputs will be pasted together before entry into your history

**Chunk size:**

1500000

the number of sequences each split should contain

**sample rate (leave empty to process the complete file):**

for example entering .001 will randomly sample and process roughly 1 in every 1000 records

agresearch

# KNOWLEDGE FOR TOOL-WRAPPERS

```
$ tardis.py -h

  tardis is a script which "conditions" a command for execution on a cluster,
   launches the conditioned command as a series of jobs, and collects and collates the output.

  Usage :

  tardis.py [-w] [-c Chunksize] [-s SampleRate] [-d workingRootPath] [-k] [-v]
    any comand (with optional conditioning directives)

  -w : Run as part of a workflow. After launching all of the jobs, tardis waits for all outputs, which is
       then collated and merged into a single output file, as specified by the output file path.
       Without this option, the program exits immediately after launching all of the jobs, and output is left
       un-collated in the scratch folder created by this script.

  -c ChunkSize : when conditioning the input file(s), split into files each containing Chunksize
       logical records. (A logical record for a sequence file is a complete sequence. For a text file it
       is a line of text). (If the -s option is used to sample the inputs, the chunksize relates to the
       full unsampled file. For example if a chunsize of 1,000,000 is specified in combination with a
       sampling rate of .0001, then each chunks would contain 100 sequences)

  -s SampleRate : rather than process the entire input file(s), a random sample of the records is processed.
       SampleRate is the probability that a record will be sampled. For example -s .001 will result in
       roughly 1 in every 1000 logical records being sampled.  When the -s option is specified, tardis does not
       clean up the conditioned input and output - i.e. all of the fragments. These are retained to assist
       with the Q/C work that is normally associated with a sampled run.

  -d workingRootPath : create the tardis working folder under workingRootPath. If no working root is specified,
       a default location is used.

  -v : validate the run by doing a dry run. This means that the chunks , scripts and job files etc are all generated but
       the jobs are not launched. The user can inspect the script and job files to check that their
       command has been conditioned as envisaged.

  -k : keep the conditioned input and output - i.e. the input and output fragments. Noromally these are
        deleted after the output is succesfully "unconditioned" - i.e. joined back together

  -h : print usage and exit.

  see the tardis documentation for more information on the conditioning directives that are supported, and
  examples of conditioned commands.
```

agresearch

# EXAMPLE INVOCATION

```
tardis.py -c 1500000  –s .0001 -w blastn -query
   _condition_fastq2fasta_input_/dataset/
   JHI_High_Low_Sequencing_Data/L01_filtered.fastq.gz -query_gencode
   11 -db nt -outfmt 5 -evalue .00001 -out
   _condition_blastxml_output_/dataset/JHI_High_Low_Sequencing_Data/
   scratch/L01_sample.xml
```

merge XML output files

agresearch

# INPUT CONDITIONING

- Input conditioning directives are prefixed to filename arguments
- Input files that are prefixed with conditioning directives will
  - If necessary be uncompressed (e.g. fastq.gz to fastq)
  - If necessary be sampled randomly
  - If necessary be format-converted (e.g. fastq to fasta) as implied by the directive
  - Split into chunks

| | |
|---|---|
| _condition_fastq_input_ | optionally uncompress and then split |
| _condition_fasta_input_ | optionally uncompress and then split |
| _condition_fastq2fastq_input_ | optionally uncompress and then split into fasta fragments |
| _condition_pairedfastq_input_ | optionally uncompress and then split into pair-end matched fastq fragments, enforcing integrity of pair-end matching by name |
| _condition_text_input_ | optionally uncompress and then split a text file |

agresearch

# OUTPUT CONDITIONING

- Output conditioning directives are prefixed to filenames
- Output files that are prefixed with conditioning directives will be
  - Recombined
  - If necessary be compressed

| | |
|---|---|
| _condition_fastq_output_ | |
| _condition_fasta_output_ | |
| _condition_fastq2fasta_output | |
| _condition_text_output_ | concatenate the output fragments into the prefixed filename and compress. |
| _condition_sam_output_ | compress each SAM file to single merged BAM |
| _condition_pdf_output_ | combine the pdf fragments |
| _condition_blastxml_output_ | combine the blast XML fragments into a single valid blast XML output |

agresearch

# GALAXY PLUMBING IN TOOL XML FILE

```
#if str($use_hpc) == "yes":
       #if str($use_hpc_samplerate) != "":
          #set $prog = [“tardis.py -w -c ", str($use_hpc_chunksize) ,
   "-s", str($use_hpc_samplerate) , " -d /dataset/galaxy_scratch2011/
   scratch/tardis python /home/galaxy/galaxy/tools/sr_mapping/
   bwa_wrapper.py "]
       #else:
          #set $prog = ["tardis.py -w -c ", str($use_hpc_chunksize) ,
   " -d /dataset/galaxy_scratch2011/scratch/tardis python /home/
   galaxy/galaxy/tools/sr_mapping/bwa_wrapper.py "]
       #end if
       #set $prog = string.join($prog, " ")
#else
       #set $prog = "bwa_wrapper.py "
#end if

$prog …
```

agresearch

# GALAXY PLUMBING IN TOOL XML FILE (2)

```
#if str($use_hpc) == "yes":
    ## input file(s)
    #if $paired.sPaired == "paired":
       --input1=_condition_paired_fastq_input_$paired.input1
       --input2=_condition_paired_fastq_input_$paired.input2
    #else:
       --input1=_condition_fastq_input_$paired.input1
    #end if

    ## output file
    --output=_condition_uncompressedsam_output_$output
 #else:
    ## input file(s)
    --input1=$paired.input1
    #if $paired.sPaired == "paired":
       --input2=$paired.input2
    #end if

    ## output file
    --output=$output
 #end if
```

agresearch

# WHY AM I TELLING YOU THIS?

- A layered approach to data parallelism may be useful in general
- Our implementation in tardis may be of interest
- We are happy to support adoption of this work by the Galaxy community

Thanks for listening!

simon.guest@agresearch.co.nz
http://bitbucket.org/agr-bifo/tardis

agresearch