

Data Analysis & Management of High-throughput Sequencing Data

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Current Issues

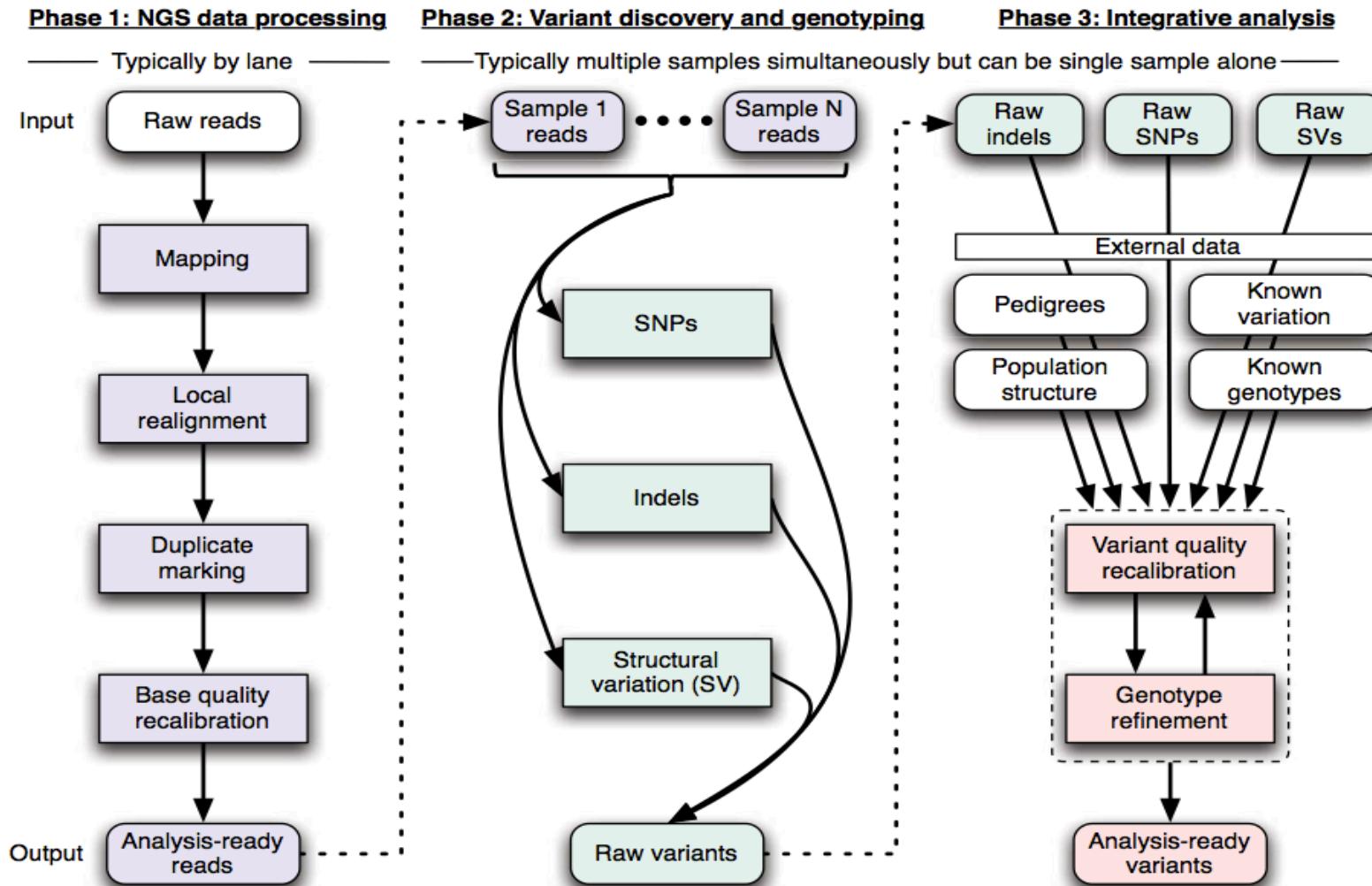


Will Computers Crash Genomics?

New technologies are making sequencing DNA easier and cheaper than ever, but the ability to analyze and store all that data is lagging

you-go service, accessible from one's own desktop, that provides rented time on a large cluster of machines that work together in parallel as fast as, or faster than, a single powerful computer. "Surviving the data deluge means computing in parallel," says Michael

Current Issues



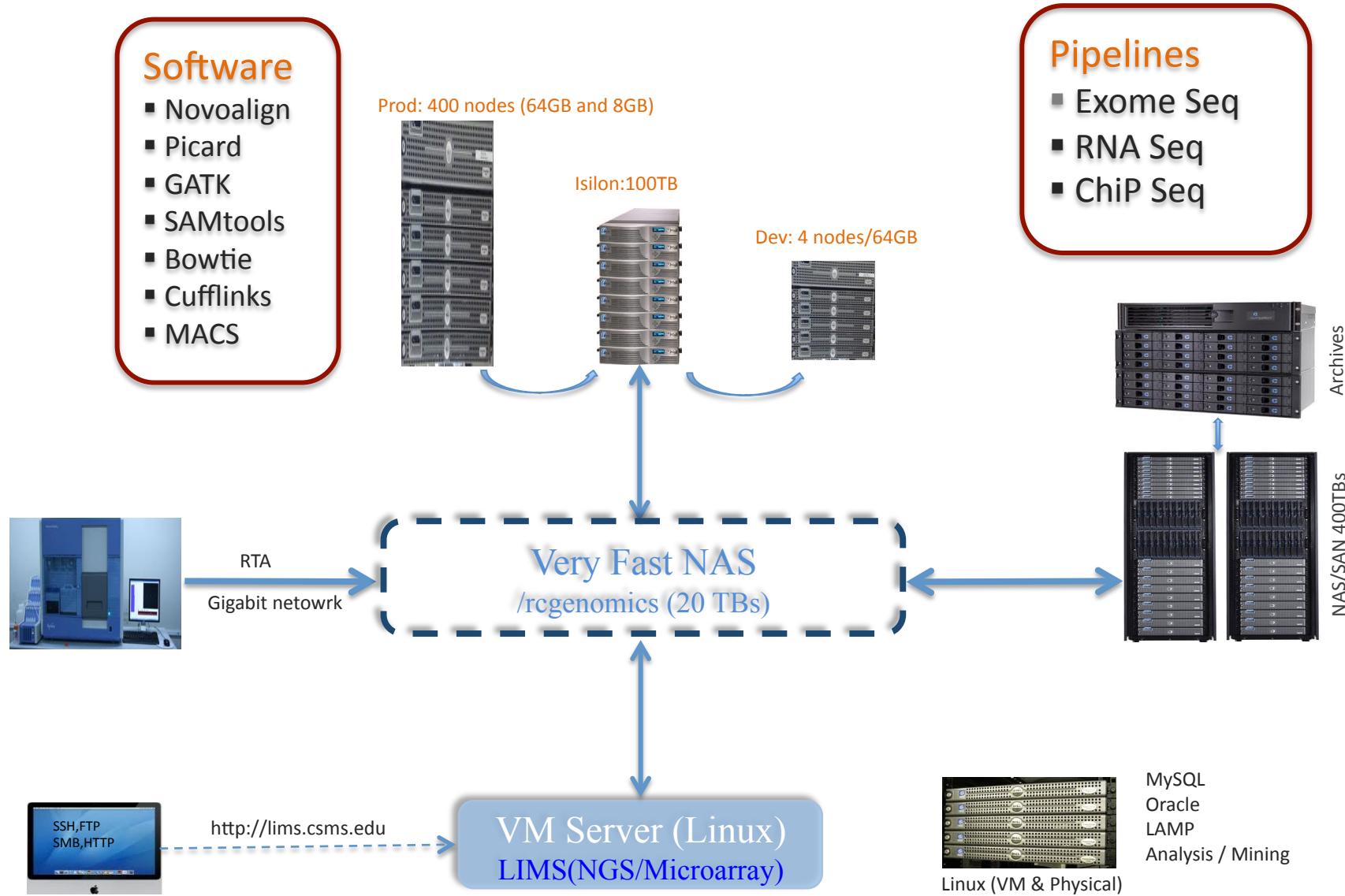
The “QSEQ” file

- Number files per run: $120 \times 8 \times (1|2) = 960 | 1920$ files
 - Number of reads per run: $\sim 40M \times 8 \times (1|2) = \sim 320M | 640M$
 - Total nucleotides (BPs): $320M \times 150 \times (1|2) = \sim 48 | 96$ billions



What am I going to do with my sequencing data?

Overview Infrastructure



Overview Infrastructure

Software

- Novoalign
- Picard
- GATK
- SAMtools
- Bowtie
- Cufflinks
- MACS

Prod: 400 nodes (64GB and 8GB)



Isilon:100TB



Dev: 4 nodes/64GB



Pipelines

- Exome Seq
- RNA Seq
- ChiP Seq

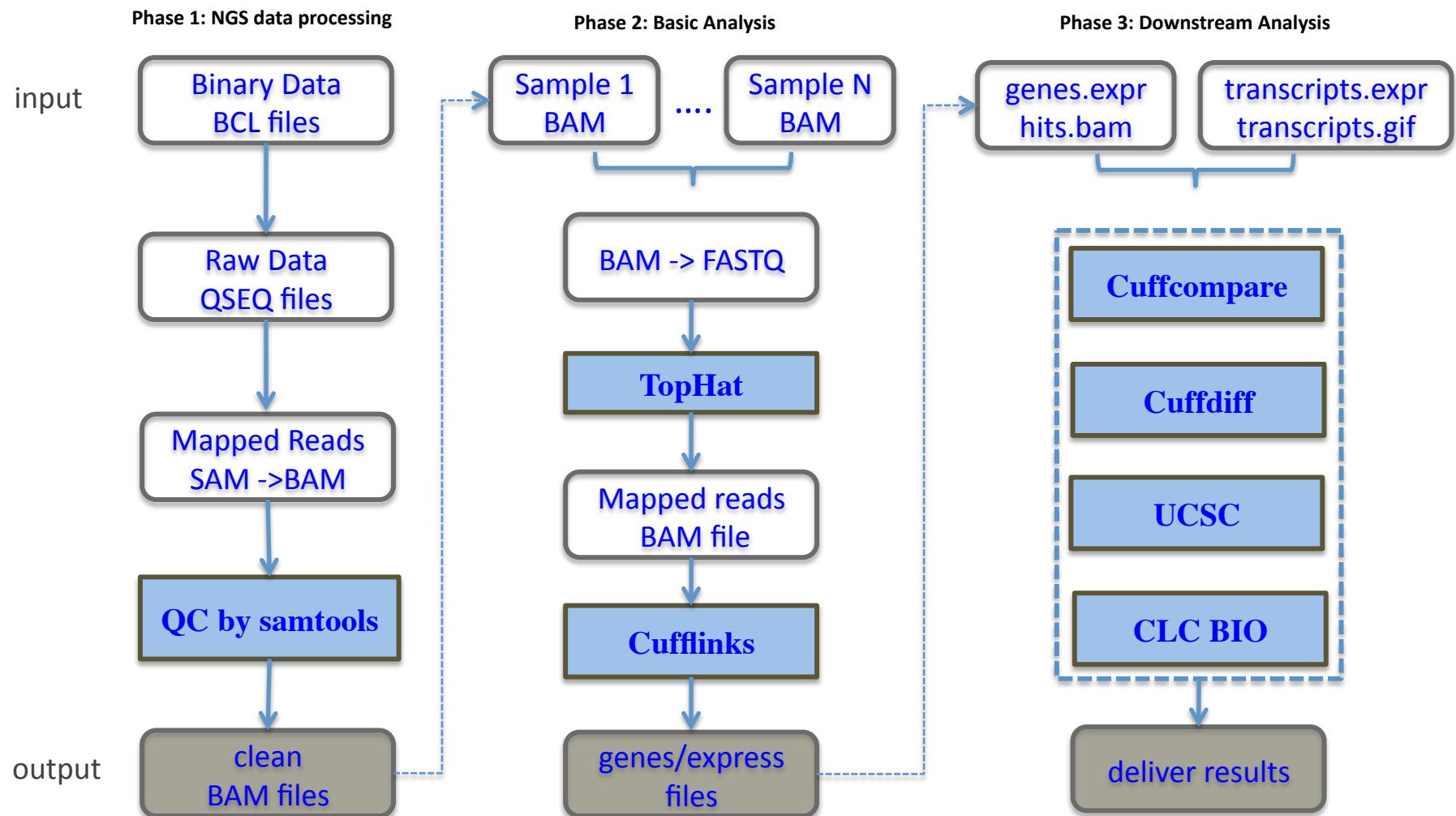
Archives



NAS/SAN 400TBs

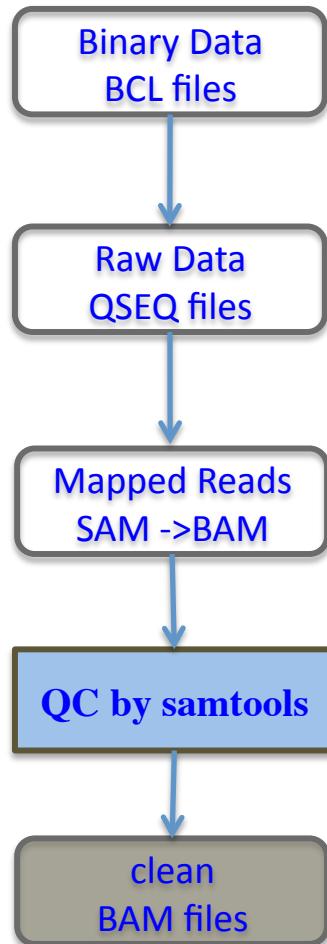
- $(3 \text{ nodes} \times 75\text{G}) + (4 \text{ nodes} \times 65\text{G}) = 485\text{Gs}$
- $(3 \text{ nodes} \times 24\text{CPU}) + (4 \text{ nodes} \times 16) = 120\text{CPUs}$
- $>300 \text{ nodes} \times 2 \text{ CPU} = >600\text{CPUs}$
- $>300 \text{ nodes} \times 4\text{G} = > 1200\text{Gs}$
- Fast NAS storage capacity $\sim 100\text{TBs}$
- Other storage devices $\sim 400\text{TBs}$

RNA-Seq Analysis Pipeline



Data Processing

Phase 1: NGS data processing

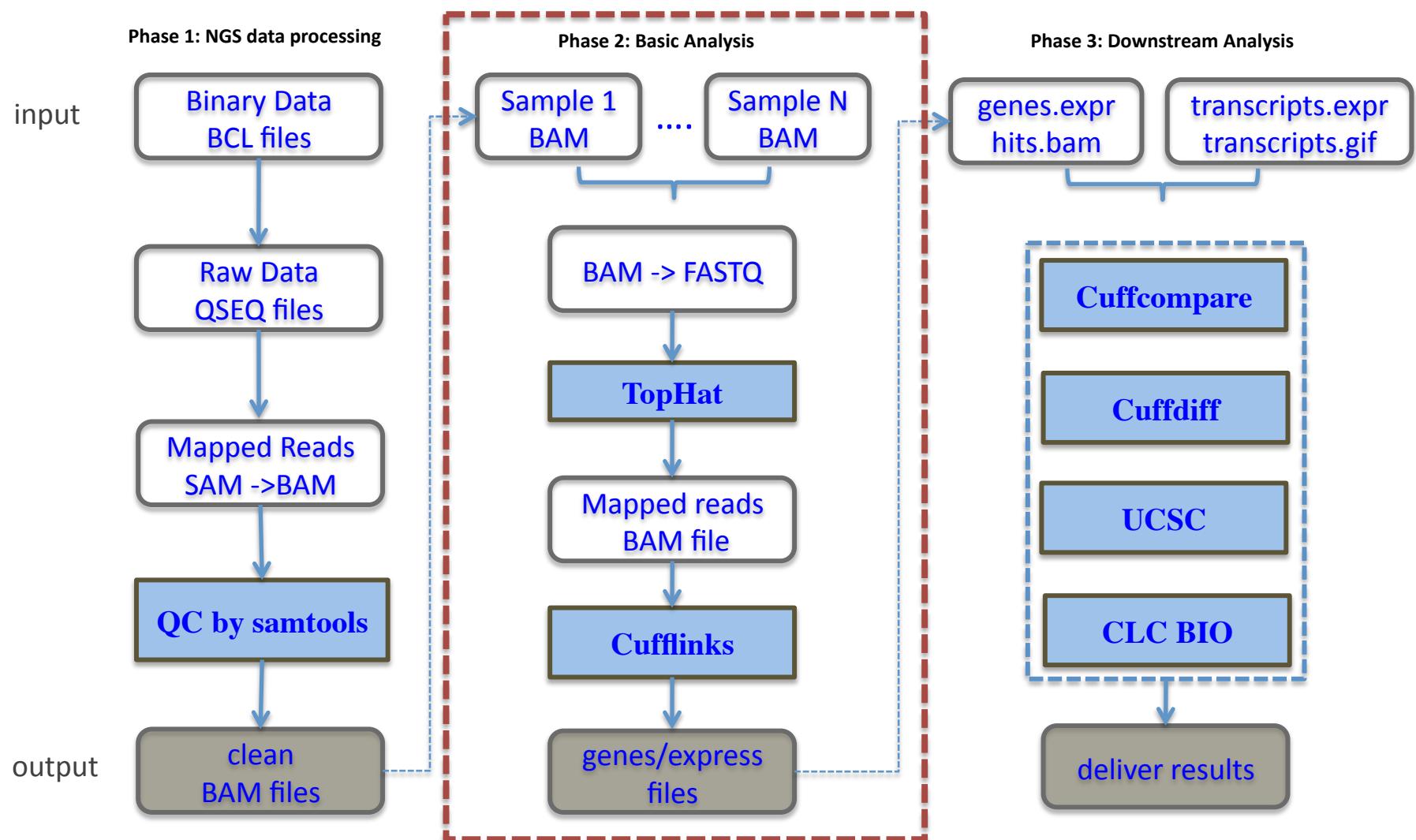


- QC / Data filtering
 - Bin and remove indexes
 - Remove adapters (if any)
 - Remove duplicate
 - Removal of non-mapped sequences
 - Filter out read mapping to ribosomal RNA
 - Percentage of ribosomal?
- QSEQ files -> Aligned files (BAM)
 - Parallel processing (MPI)
 - What is in the alignment files?

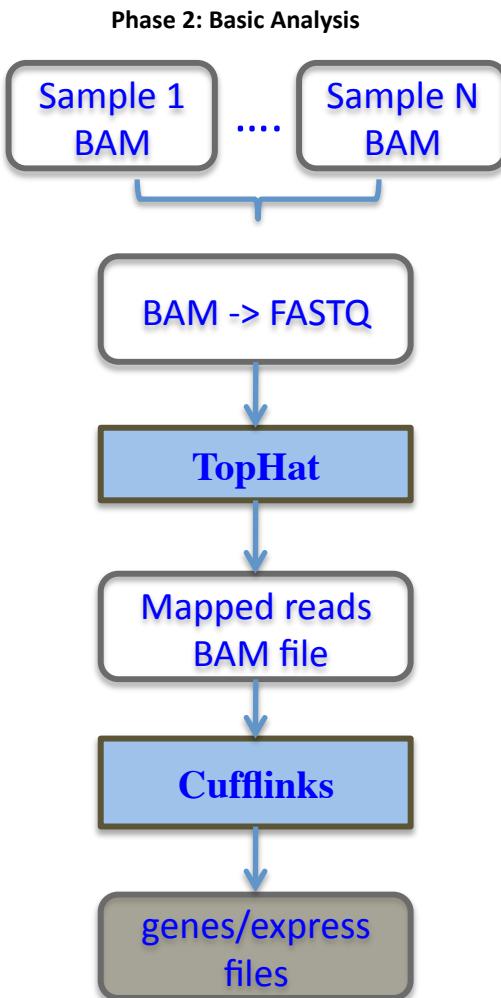
The “BAM/SAM” file

- BAM (Binary Alignment Mapping)
 - SAM (Sequence Alignment Mapping)
 - Standardized output for alignment
 - Contains all required information for downstream analysis

RNA-Seq Analysis Pipeline



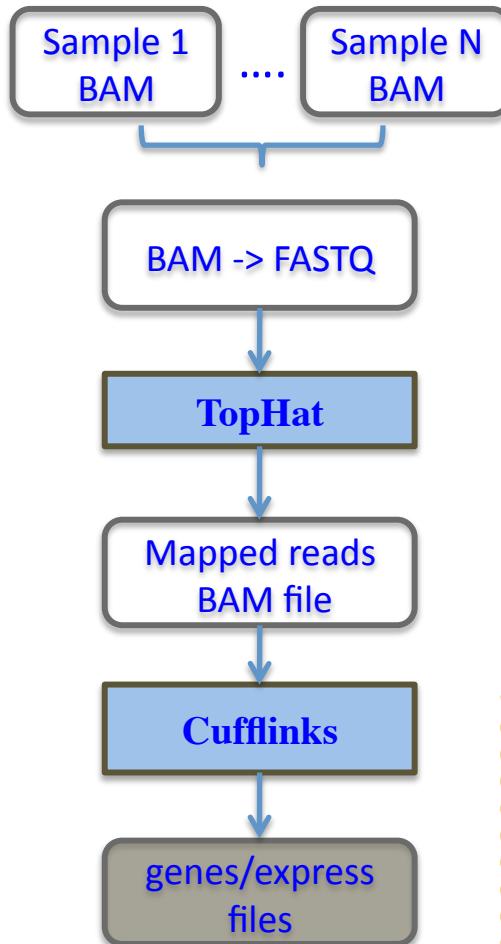
RNA-Seq Data Analysis



- One BAM file per sample
- Tophat
 - bowtie : align short reads
 - splice junction identifier
- Cufflinks
 - Uses annotation file to count transcripts and isoforms
- Output files
 - Gene expression : gene.expr
 - Transcript expression : transcripts.expr
 - Transcript in GTF format : transcripts.gft

RNA-Seq Analysis

Phase 2: Basic Analysis



Genes expression – genes.expr

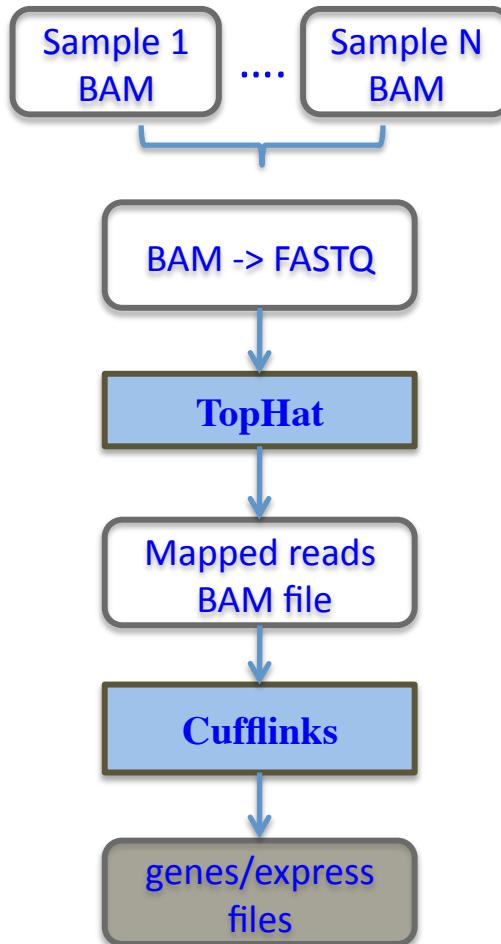
gene_id	bundle_id	chr	left	right	FPKM	FPKM_conf_lo	FPKM_conf_hi	status
CUFF.553	359292	chr1	1337362	1337500	55.407	40.5198	70.2942	OK
CUFF.633	359323	chr1	1589678	1590169	5.69524	0.9223	10.4682	OK
CUFF.717	359361	chr1	2333696	2334528	80.592	62.6374	98.5466	OK
CUFF.753	359363	chr1	2335535	2336291	6.23938	1.24363	11.2351	OK
CUFF.1445	359706	chr1	8045039	8045178	136.386	113.029	159.743	OK
CUFF.1483	359722	chr1	8086269	8086361	433.406	391.769	475.042	OK
CUFF.2055	359968	chr1	11085748	11086628	5.71943	0.936364	10.5025	OK
CUFF.2233	360066	chr1	11969707	11969966	23.9575	14.1682	33.7468	OK
CUFF.2243	360068	chr1	12018603	12020748	48.576	34.6367	62.5153	OK
CUFF.2405	360065	chr1	11968214	11969176	97.8562	78.5901	117.122	OK
CUFF.2407	360065	chr1	11968226	11968603	24.5166	21.6205	27.4127	OK
CUFF.3339	360561	chr1	16133828	16133942	292.549	258.341	326.757	OK
CUFF.3341	360561	chr1	16134070	16134194	347.513	310.229	384.796	OK
CUFF.4009	360861	chr1	19983356	19983683	15.4176	7.56455	23.2706	OK

transcriptions expression – transcripts.expr

trans_id	bundle_id	chr	left	right	FPKM	FMI	frac	FPKM_conf_lo	FPKM_conf_hi	coverage	length	effective_length	status
CUFF.553.1	359292	chr1	1337362	1337500	55.407	1	1	40.5198	70.2942	14.0625	138	64	OK
CUFF.633.1	359323	chr1	1589678	1590169	5.69524	1	1	0.9223	10.4682	2.19011	491	417	OK
CUFF.717.1	359361	chr1	2333696	2334528	80.592	1	1	62.6374	98.5466	17.0455	140	66	OK
CUFF.753.1	359363	chr1	2335535	2336291	6.23938	1	1	1.24363	11.2351	2.41935	756	682	OK
CUFF.1445.1	359706	chr1	8045039	8045178	136.386	1	1	113.029	159.743	30.9829	139	65	OK
CUFF.1483.1	359722	chr1	8086269	8086361	433.406	1	1	391.769	475.042	91.6667	92	18	OK
CUFF.2055.1	359968	chr1	11085748	11086628	5.71943	1	1	0.936364	10.5025	2.23325	880	806	OK
CUFF.2233.1	360066	chr1	11969707	11969966	23.9575	1	1	14.1682	33.7468	9.35462	259	185	OK
CUFF.2243.1	360068	chr1	12018603	12020748	48.576	1	1	34.6367	62.5153	13.3562	147	73	OK
CUFF.2405.1	360065	chr1	11968214	11969176	97.8562	1	0.921245	78.5901	117.122	35.7916	962	888	OK
CUFF.2407.1	360065	chr1	11968226	11968603	24.5166	1	0.0787548	21.6205	27.4127	8.96713	377	303	OK

RNA-Seq Analysis

Phase 2: Basic Analysis



- Genes expression – genes.expr

gene_id	bundle_id	chr	left	right	FPKM	FPKM_conf_lo	FPKM_conf_hi	status
CUFF.553	359292	chr1	1337362	1337500	55.407	40.5198	70.2942	OK
CUFF.633	359323	chr1	1589678	1590169	5.69524	0.9223	10.4682	OK
CUFF.717	359361	chr1	2333696	2334528	80.592	62.6374	98.5466	OK
CUFF.753	359363	chr1	2335535	2336291	6.23938	1.24363	11.2351	OK
CUFF.1445	359706	chr1	8045039	8045178	136.386	113.029	159.743	OK
CUFF.1483	359722	chr1	8086269	8086361	433.406	391.769	475.042	OK
CUFF.2055	359968	chr1	11085748	11086628	5.71943	0.936364	10.5025	OK
CUFF.2233	360066	chr1	11969707	11969966	23.9575	14.1682	33.7468	OK
CUFF.2243	360068	chr1	12018603	12020748	48.576	34.6367	62.5153	OK
CUFF.2405	360065	chr1	11968214	11969176	97.8562	78.5901	117.122	OK
CUFF.2407	360065	chr1	11968226	11968603	24.5166	21.6205	27.4127	OK
CUFF.3339	360561	chr1	16133828	16133942	292.549	258.341	326.757	OK
CUFF.3341	360561	chr1	16134070	16134194	347.513	310.229	384.796	OK
CUFF.4009	360861	chr1	19983356	19983683	15.4176	7.56455	23.2706	OK

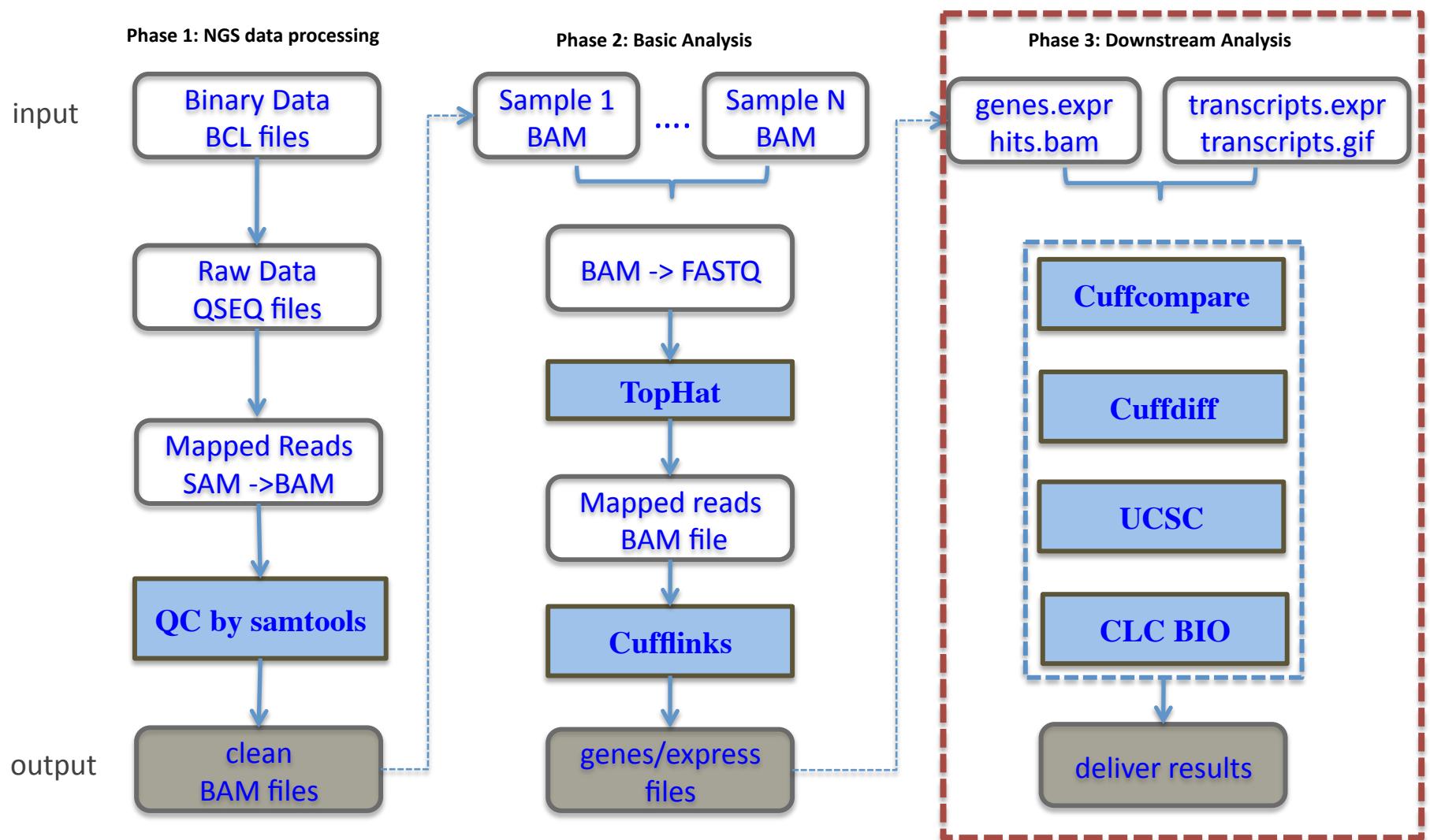
- Unit of measurement (FPKM/RPKM)

FPKM: Fragments per kilobase per million mapped reads

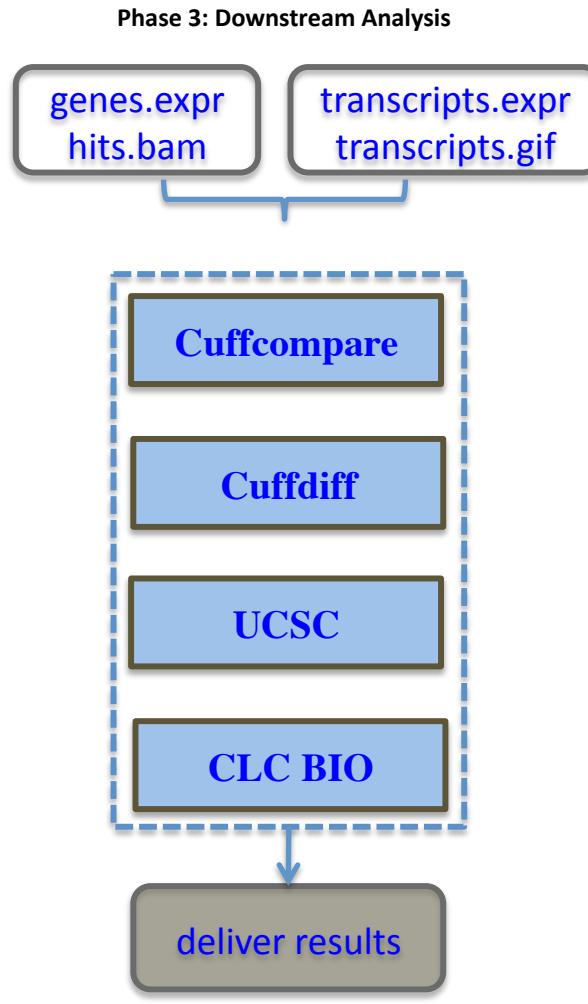
1kb transcript with 1000 alignments in a sample of 10M reads (out of which 8 million reads can be mapped) will have

$$\text{FPKM} = 1000/(1*8) = 125$$

RNA-Seq Analysis Pipeline



RNA-Seq Analysis



■ Cuffcompare

- Compare your assembled transcript to a reference annotation
- Track cufflinks transcripts across multiple experiments (e.g across time course)
- `cuffcompare -<options>`

■ Cuffdiff

- Part of cufflinks package
- Find significant changes in transcriptions, splicing, and promoter use.

■ Viewer & Annotation



UCSC Genome Bioinformatics

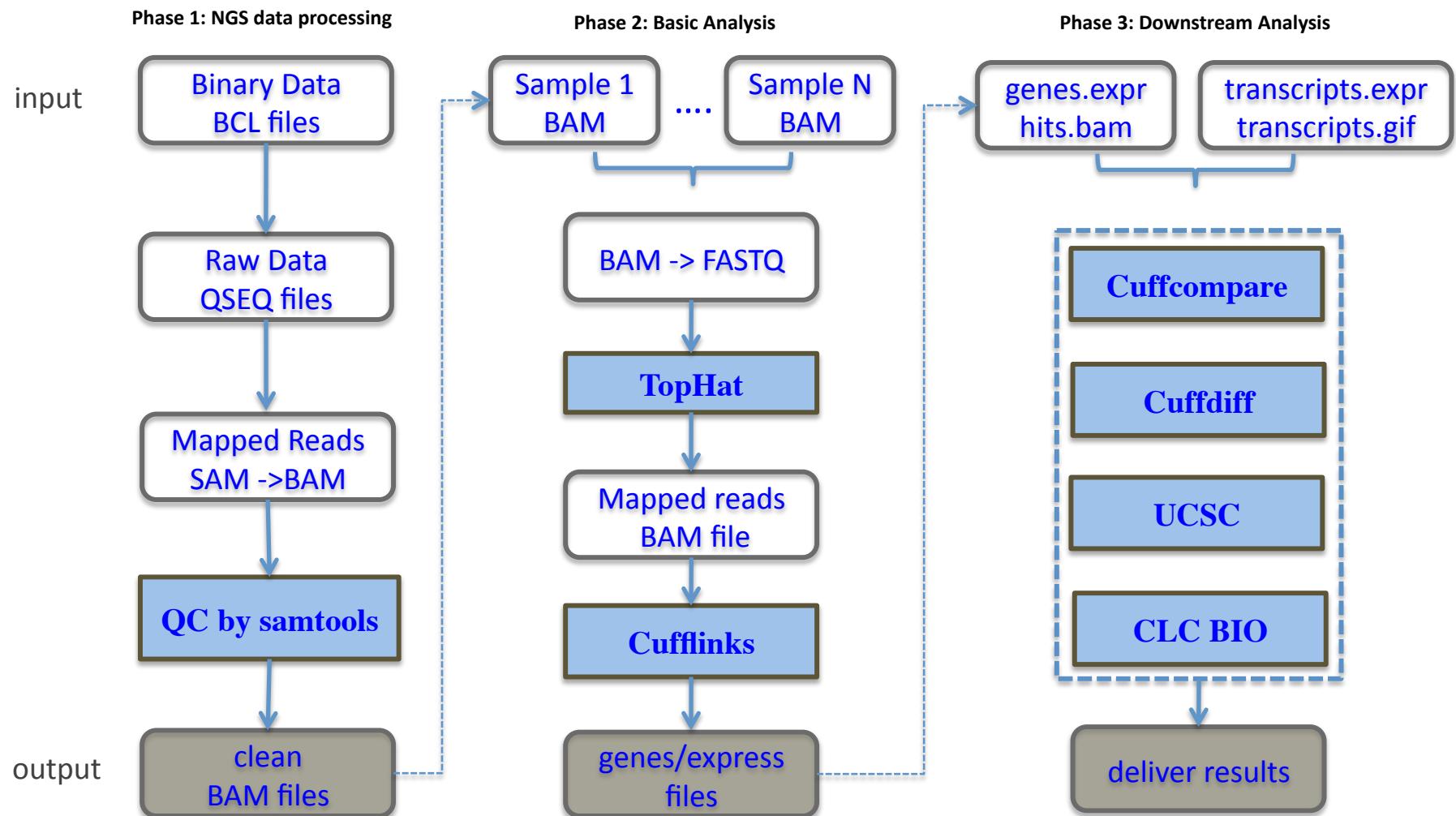


Cuffdiff Sample

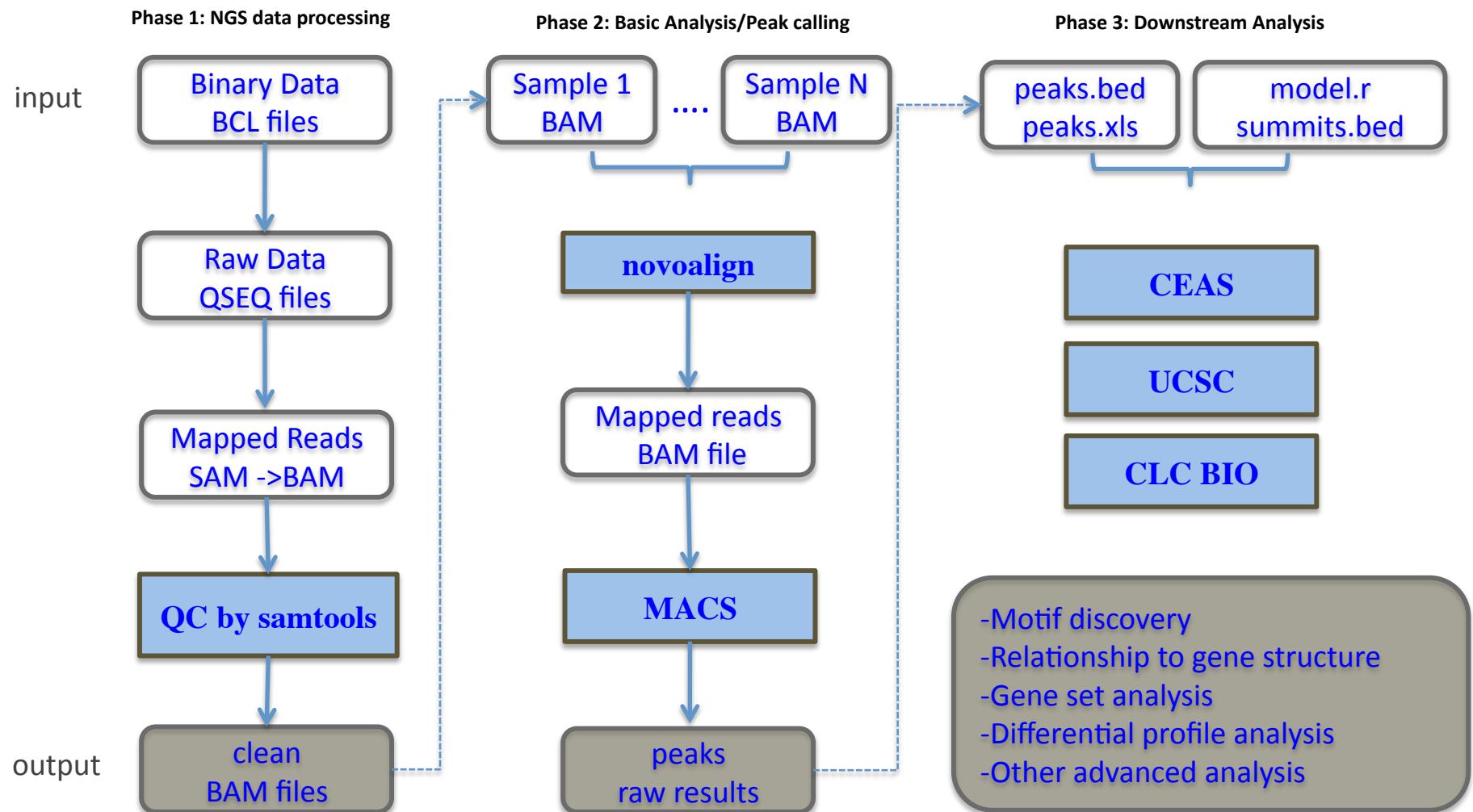
- Differential expression at the transcript 'isoform' level and at the gene level

test_id	gene	locus	sample_1	sample_2	status	value_1	value_2	ln(fold_change)	test_stat	p_value	significant
A1BG	-	chr19:58858171-58874120	N1	N2	NOTEST	10.2529	0.234601	-3.77743	2.77988	0.00543788	no
A1BG-AS1	-	chr19:58858171-58874120	N1	N2	NOTEST	0.409548	0.143733	-1.0471	0.700639	0.483528	no
A1CF	-	chr10:52559168-52645435	N1	N2	NOTEST	1.62597	0.0812403	-2.99645	0.833498	0.404564	no
A2LD1	-	chr13:100741268-101241046	N1	N2	OK	3.59671	0.379567	-2.24874	2.77077	0.00559235	yes
A2M	-	chr12:9217772-9268558	N1	N2	OK	119.206	8.94433	-2.58983	7.52449	5.28466e-14	yes
A2ML1	-	chr12:8975149-9029377	N1	N2	NOTEST	5.31409	0.1104	-3.87401	1.27403	0.202653	no
A4GALT	-	chr22:43088126-43116876	N1	N2	NOTEST	2.04244	3.95383	0.660538	-0.766551	0.443349	no
A4GNT	-	chr3:137842559-137851229	N1	N2	NOTEST	0	0	0	1	no	
AA06	-	chr17:31340105-32483825	N1	N2	OK	0	0	0	1	no	
AAA1	-	chr7:34386123-34917944	N1	N2	NOTEST	0	0	0	1	no	
AAAS	-	chr12:53701239-53715412	N1	N2	NOTEST	2.05004	1.43175	-0.358957	0.329559	0.741733	no
AACS	-	chr12:125549924-125627871	N1	N2	NOTEST	1.94065	1.9749	0.0174931	-0.0173068	0.986192	no
AACSP1	-	chr5:178191863-178203277	N1	N2	NOTEST	0	0	0	1	no	
AADAC	-	chr3:151347319-151546276	N1	N2	NOTEST	6.49447	0.217239	-3.39771	2.05452	0.0399257	no
AADACL2	-	chr3:151347319-151546276	N1	N2	NOTEST	0	0.184128	1.79769e+308	1.79769e+308	0	no
AADACL3	-	chr1:12776117-12788726	N1	N2	NOTEST	0	0.0983329	1.79769e+308	1.79769e+308	0	no
AADACL4	-	chr1:12704565-12727097	N1	N2	NOTEST	0	0.164182	1.79769e+308	1.79769e+308	0	no
AADAT	-	chr4:170981372-171011372	N1	N2	NOTEST	0.534774	0.965577	0.590883	-0.34657	0.728914	no
AAGAB	-	chr15:67493366-67547074	N1	N2	OK	3.89801	3.85409	-0.0113311	0.0157741	0.987415	no
AAK1	-	chr2:69685126-69870977	N1	N2	OK	1.2863	1.61644	0.228455	-0.193547	0.846531	no
AAMP	-	chr2:219128851-219134893	N1	N2	OK	6.0599	12.165	0.696869	-1.40155	0.161051	no
AANAT	-	chr17:74449432-74466199	N1	N2	NOTEST	0	0	0	1	no	
AARS	-	chr16:70286296-70323412	N1	N2	OK	9.25038	13.0024	0.34047	-0.79155	0.428623	no
AARS2	-	chr6:44266462-44281063	N1	N2	NOTEST	0.837631	0.785632	-0.0640899	0.0408067	0.96745	no
AARSD1	-	chr17:41102542-41132545	N1	N2	NOTEST	2.05187	2.159	0.0508964	-0.0522021	0.958368	no
AASDH	-	chr4:57204456-57253638	N1	N2	NOTEST	1.53196	2.03171	0.282332	-0.263855	0.791892	no
AASDHPPPT	-	chr11:105921824-105969419	N1	N2	OK	2.66529	4.5202	0.528243	-0.774947	0.438371	no
AASS	-	chr7:121713597-121784344	N1	N2	OK	2.95671	3.41433	0.143903	-0.181143	0.856255	no
AATF	-	chr17:35306174-35414171	N1	N2	OK	2.20866	3.13149	0.349121	-0.397319	0.691132	no
AATK	-	chr17:79008946-79156964	N1	N2	NOTEST	0.439836	0.0532832	-2.11078	1.46564	0.142746	no
ABAT	-	chr16:8768443-8878432	N1	N2	NOTEST	4.5175	0.252426	-2.8846	1.41036	0.158434	no

RNA-Seq Analysis Pipeline



ChiP-Seq Analysis Pipeline

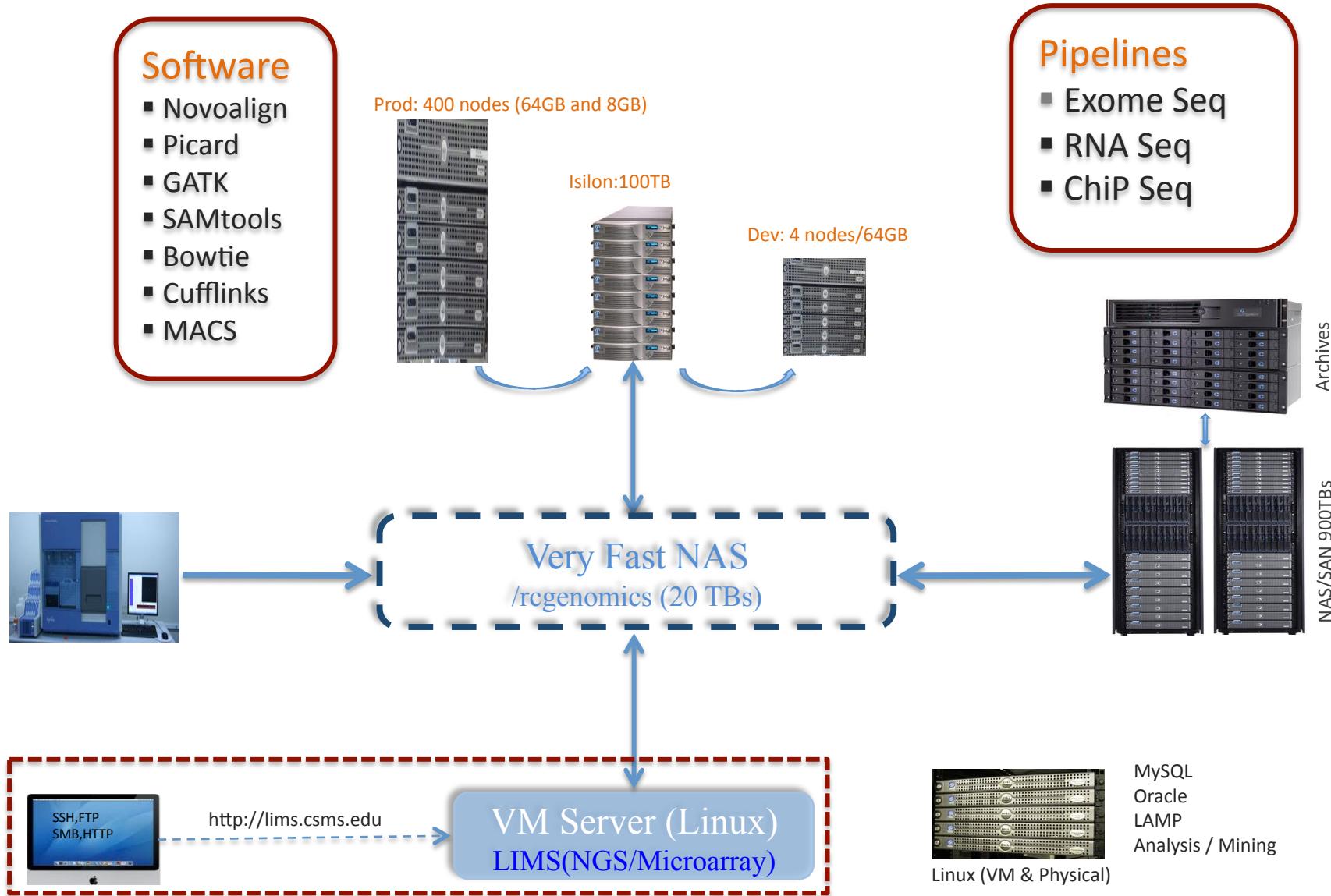


MACS: Model-base Analysis ChiP-seq / **CEAS:** Cis-regulatory Element Annotation System



I can start my research now.

Overview Infrastructure



Data Management

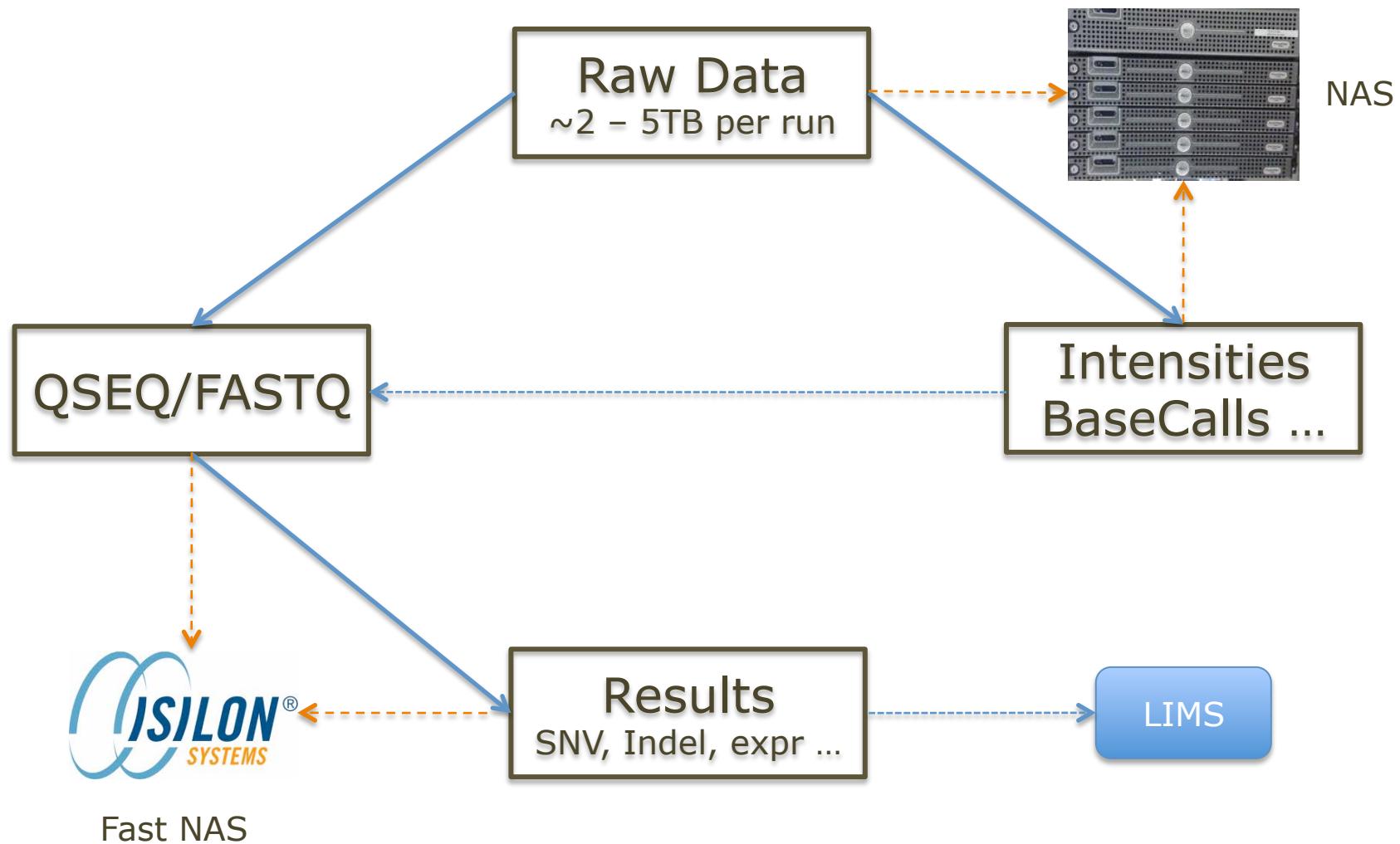
Data storage

- Understand NGS data
- Not all data equally important
- How data gets storage?

User access

- LIMS (Laboratory Informatics Management System)
- Service Request
- Access data

Data Storage



We are located @ <http://genomics>

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COMPUTATIONAL

[IT Infrastructure](#)[LIMS](#)[Applications](#)

Welcome to the Genomics Core Facility

Next Generation Sequencing Library prep and Analysis

WHEN: Friday, October 7th, 2011:1 pm to 3:30 pm

WHERE: Davis Room 1004

Next-gen sequencing services available using the Genome Analyzer IIx

The Genomics core focus is on gleaning gene expression information, regulatory information, sequence information on a genome wide scale for investigators. Our mission goal is to make these expensive and complex technologies and data accessible and biologically interpretable for clinical and basic scientists enabling interrogation of the genome with little experience in sample preparation, quality control, and analysis. To do this, we develop general but complete biological and analysis pipelines to fit the general needs and enable most genome-wide investigations. For more sophisticated or custom designs we work actively in collaboration with other CSMC cores and resources to facilitate the investigation.

The Genomics core has seen rapid investment in a number of new Microarray, Sequencing, qPCR technologies as well as computational and software resources to enable this highly desired applications most of these are listed below. In 2012, with the increased demand and continual development and launch of new technologies and applications, we are expanding our staff to 6 people which include 6 bench scientists and 2 Informatics scientists will manage the workflows

Contact Us

**8723 Alden Dr
Steven Spielberg Building Room 141
Los Angeles, California 90048**

Lab Phone: (310) 423-4066
Fax: (310) 248-8141
Email: genomics@csmc.edu (all inquiries)

[Direction:](#) ([Gmap](#))

Park or walk to the parking lot in front of the Steven Spielberg Building.

Access to the Genomics Core is by secure access only. Please call to set up an appointment for services or consultations. For access to the building please use the campus phone in the lobby and dial the lab number **(310)423-4066**.

FIND A DOCTOR

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1-800-233-2771

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Access the NGS LIMS

Home → Research & Education → Research Cores → Genomics Core →

Laboratory Information Management System

What is LIMS?

A Laboratory Information Management System (LIMS) is a software system that is for management of samples, laboratory users, data, instruments, data workflow.

USER LOGIN

MICROARRAY LIMS

- Use the Microarray LIMS for Sample Submission and Data Retrieval.
- Use your Email User ID and password to login.

Email User ID (e.g funariv)

Password

Login

NEXT GENERATION SEQUENCING LIMS

- Use your username and password has assigned to you. Please [contact us](#) for new account.

Username

Password

Login

NGS LIMS

FOR PATIENTS FOR MEDICAL PROFESSIONALS RESEARCH & EDUCATION

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GO

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Search

Add Page

Configure Page

Data Import

Admin

History

Help

October 5, 2011 Admin

Logout

[Illumina Run Summary] -> [Manage Users] -> [Editing Sample] ->

Home

Add Page

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- Add Project

Flowcells

- Create Flowcell
- Flowcell Summary

Group Data

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- PI
- Project
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Results

- Sequence Files
- Analysis Results

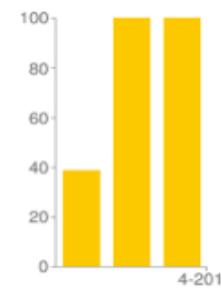
Run Data

- Illumina Runs
- Illumina Lanes
- Illumina Flowcells
- Gerald Runs
- Gerald Lanes

My Illumina Run(s) :

Summary page showing all Illumina runs in the database, their status and their sequence yield (if any).

Total kbases sequenced 63,531



KBases sequenced per month.

Page size: 20

Illumina Run	Run Date	Kb Sequenced	Status	Illumina Flowcell
110819 HWUSI-EAS1764 FC 69J4BAAXX	2011-08-19	26,620	HAS BUSTARD	FC 69J4BAAXX
110321 HWUSI-EAS1764 00007 FC 629KCAAXX	2011-03-21	26,620	HAS SEQUENCE	
101230 HWUSI-EAS1764 00004 FC	2010-12-30	10,291	HAS SEQUENCE	Flowcell EAS1764 2010-12-30

NGS LIMS – Add Sample

Home > Research & Education > Research Cores > Genomics Core >

Search Add Page Configure Page Data Import Admin History Help

October 5, 2011 Admin Logout [Pisarska_110321_lane8] -> [Editing Sample] ->

Adding Sample

* Name Pisarska N2 lane8 [Get New Name](#)

Analysis RNA [\[Add Analysis\]](#)

Barcode n/a

Comments

Concentration 10 mM

Description

Gerald Analysis

* Group Margaret Pisarska [\[Add Group\]](#)

* Group Member

Admin pisarskam nguyenqxx johnp spurkal	< Add Remove > << Add All Remove All >>	cohnd csmcrownjor dcohn funariv hondas kayej kirinoy qlng2000 qnguyen reinsteine
---	--	---

[\[Add Group_Member\]](#)

* Project pisarska project [\[Add Project\]](#)

Ref Genome hg18 [\[Add Species\]](#)

* Species Human

* Status COMPLETED

Type

* = Required field

Left Sidebar:

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 - Illumina Flowcells
 - Gerald Runs
 - Gerald Lanes

NGS LIMS – Add Sample

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Search Add Page Configure Page Data Import Admin History Help October 5, 2011 Admin Logout [Pisarska_110321_lane8] ➔ [Editing Sample] ➔

Sample:Pisarska 110321 lane8

Date Created	2011-08-09
Group	Margareta Pisarska
Group Member	brownjor, funariv, johnp, nguyenqxx, pisarskam, qnguyen, spurkal
Project	pisarska project
Species	Human
Status	COMPLETED
Type	mRNA

Existing libraries : Genomics_110321_lane8 Genomics_lib_110321_lane8 Genomics_lib_110321_lane8_dan-cohn Pisarska_lib_110321_lane8 [Add Library]

List of entries that reference this page

Library Genomics_lib_110321_lane8, Genomics_110321_lane8, Genomics_lib_110321_lane8_dan-cohn, Pisarska_lib_110321_lane8,

[Edit Sample](#) [Add Sample](#) [Clone Sample](#) [Show page history](#)

Home

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PI

Project

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Machine

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Run Data

- Illumina Runs
- Illumina Lanes
- Illumina Flowcells
- Gerald Runs
- Gerald Lanes

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- Add Project

Flowcells

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- Flowcell Summary

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- PI
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Results

- Sequence Files
- Analysis Results

Run Data

- Illumina Runs
- Illumina Lanes
- Illumina Flowcells
- Gerald Runs
- Gerald Lanes

Adding Library

Name [Get New Name](#)

Bioanalyser File [Browse...](#)

Control

Group [\[Add Group\]](#)

Group Member [\[Add Group_Member\]](#)

Index

Project [\[Add Project\]](#)

Recipe

Sample [\[Add Sample\]](#)

* Species [\[Add Species\]](#)

Status

Type [\[Add Type\]](#)

* = Required field

[Cancel](#) [Add new Library](#)

NGS LIMS – Create a FlowCell

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[Editing Illumina_Flowcell] -> [FC_69J4BAAXX] ->

Home

Add Page

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Flowcells

- Create Flowcell 
- Flowcell Summary

Group Data

- Group
- Group Member
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- Library
- Machine
- Species

Results

- Sequence Files
- Analysis Results

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Editing Illumina Flowcell

[Dev form]

* Name: FC 69J4BAAXX

Illumina Run: 110819 HWUSI-EAS1764 FC 69J4BAAXX

* Read Length: 157

Sample Sheet:

* Status: COMPLETED

* = Required field

Lane	Library no	Library	Index	Species	Type	Fragment Size	
1	1	reinsteine R11-203A		Human	Single End		<input type="button" value="add library to lane"/>
2	1	reinsteine R11-203D		Human	Paired End		<input type="button" value="add library to lane"/>
3	1	reinsteine R11-203I		Human	Paired End		<input type="button" value="add library to lane"/>
4	1	reinsteine R11-203G		Human	Paired End		<input type="button" value="add library to lane"/>
5	1	genomics R09 Exome		Human	Paired End		<input type="button" value="add library to lane"/>
6	1	reinsteine R11-076A	1	Human	Paired End		<input type="button" value="add library to lane"/>
6	2	reinsteine R11-076N	2	Human	Paired End		<input type="button" value="add library to lane"/>
6	3	reinsteine R11-076Q	3	Human	Paired End		<input type="button" value="add library to lane"/>
7	1	kayej sample lane 7		Rat	Paired End		<input type="button" value="add library to lane"/>
8	1	genomics N2 minus DSN		Human	Control		<input type="button" value="add library to lane"/>

NGS LIMS – Get Results

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My Analysis Results(s) :

Page size: 20

Analysis Results	Library	Group
Dan Cohn 06-2011 exome lane 7		Dan Cohn
FC 629KCAAXX lane 1	Genomics lib 110321 lane1	Genomics Core
FC 629KCAAXX lane 2	Pisarska lib 110321 lane2	Margareta Pisarska
FC 629KCAAXX lane 3	Pisarska lib 110321 lane3	Margareta Pisarska
FC 629KCAAXX lane 4	Pisarska lib 110321 lane4	Margareta Pisarska
Kayej FC 69J4BAAXX Lane 7	kayej sample lane 7	kayej lab

Analysis Results ←

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NGS LIMS – Get Results

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Analysis Results:FC 629KCAAXX lane 1

BAM	EXOMEL1CLC.RG.bam
BAM index	EXOMEL1CLC.RG.bam.bai
Date Created	2011-08-10
Date Modified	2011-08-10
Group	Genomics Core
Indel	EXOMEL1CLC.indel.vcf
Library	Genomics lib 110321 lane1
SNV	EXOMEL1CLC.snv.vcf

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None

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Questions?