

•Epigenomic analysis using massively-parallel sequencing

•**Masako Suzuki DVM PhD**

Center for Epigenomics

Department of Genetics

Albert Einstein College of Medicine, Bronx, NY



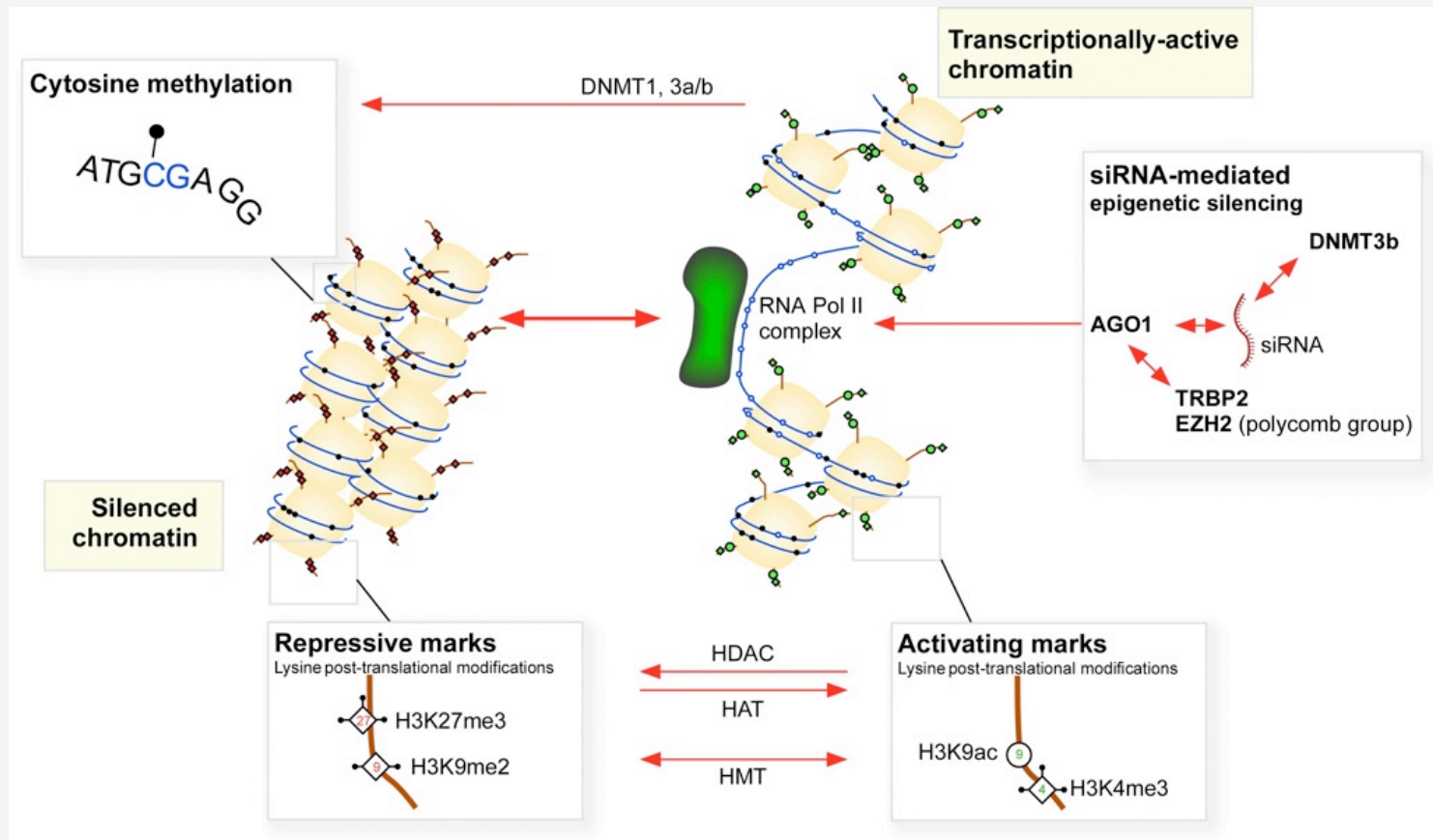
Overview of presentation

- **Introduction of Epigenomics**
- **Center for epigenomics Einstein**
 - ✓ New DNA methylation assay development
- **Data management using WikiLIMs**
 - ✓ Sample submission and retrieving results
 - ✓ Electronic lab notebook



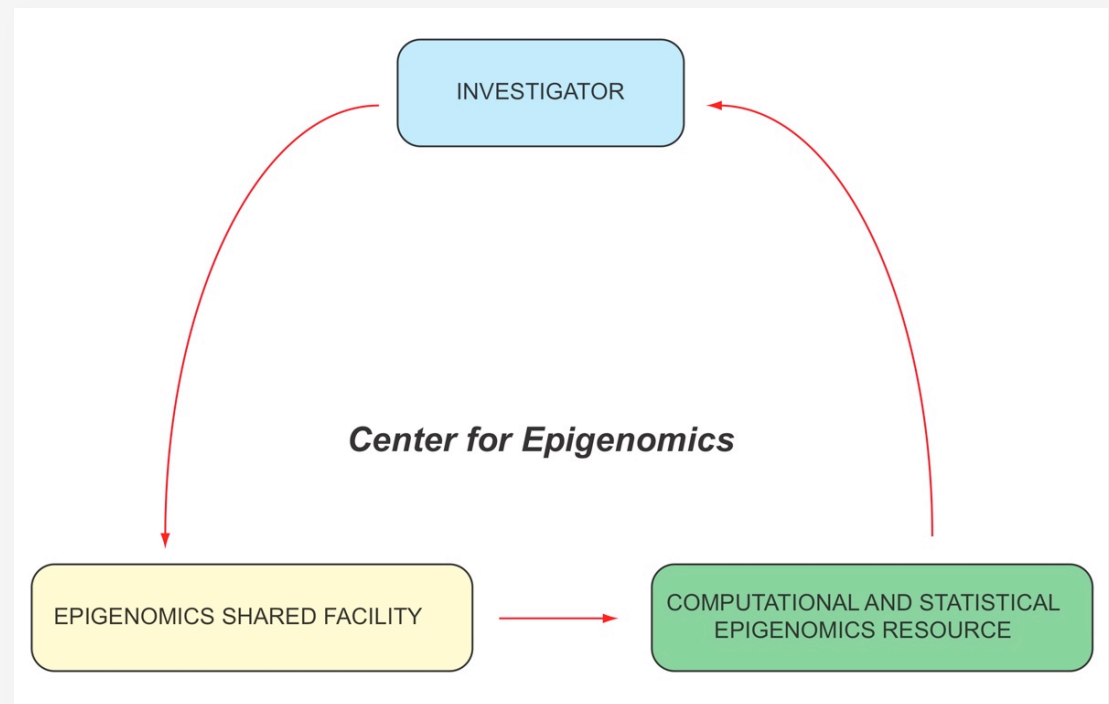
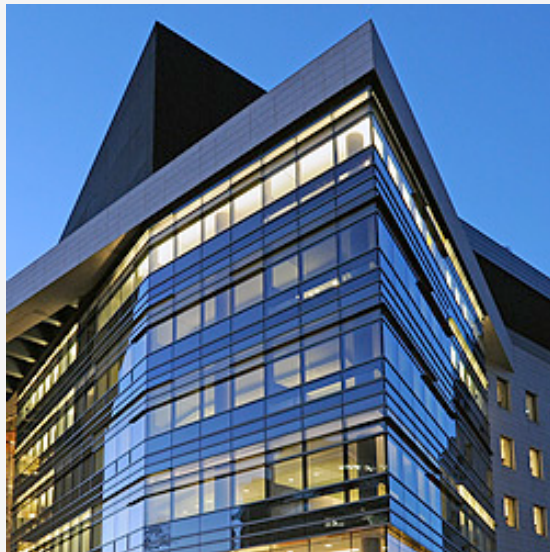
The epigenome, a complex system

•Epigenetic regulators



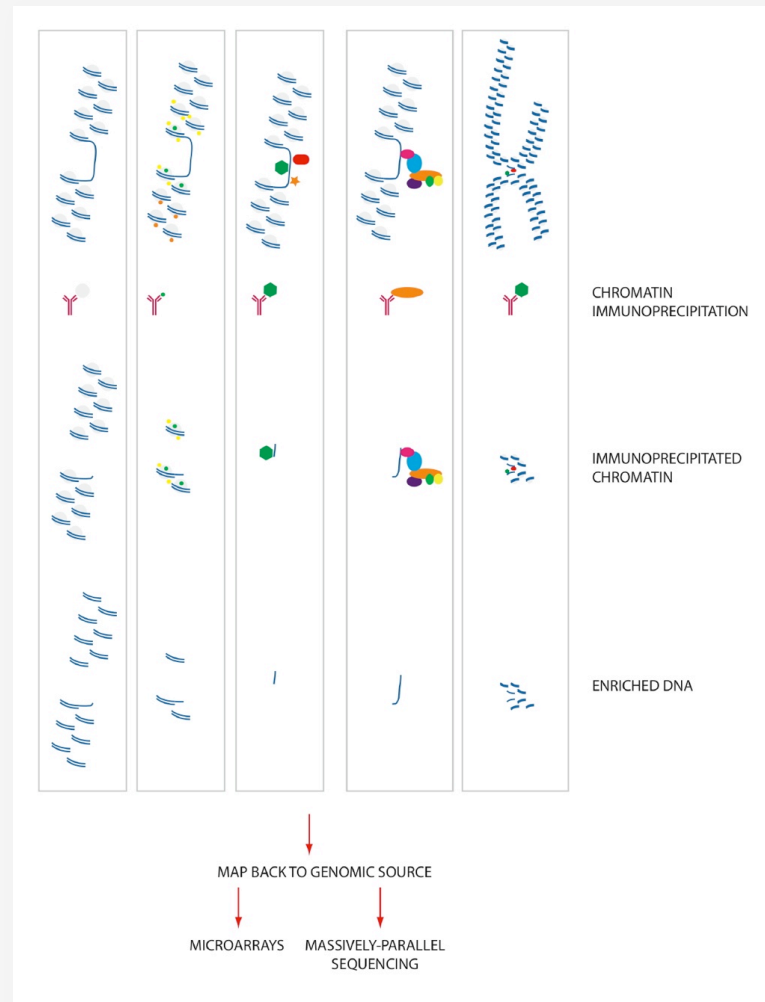
The Einstein Center for Epigenomics

- Epigenomic dysregulation in human disease
- Novel technologies
- Data management and analysis



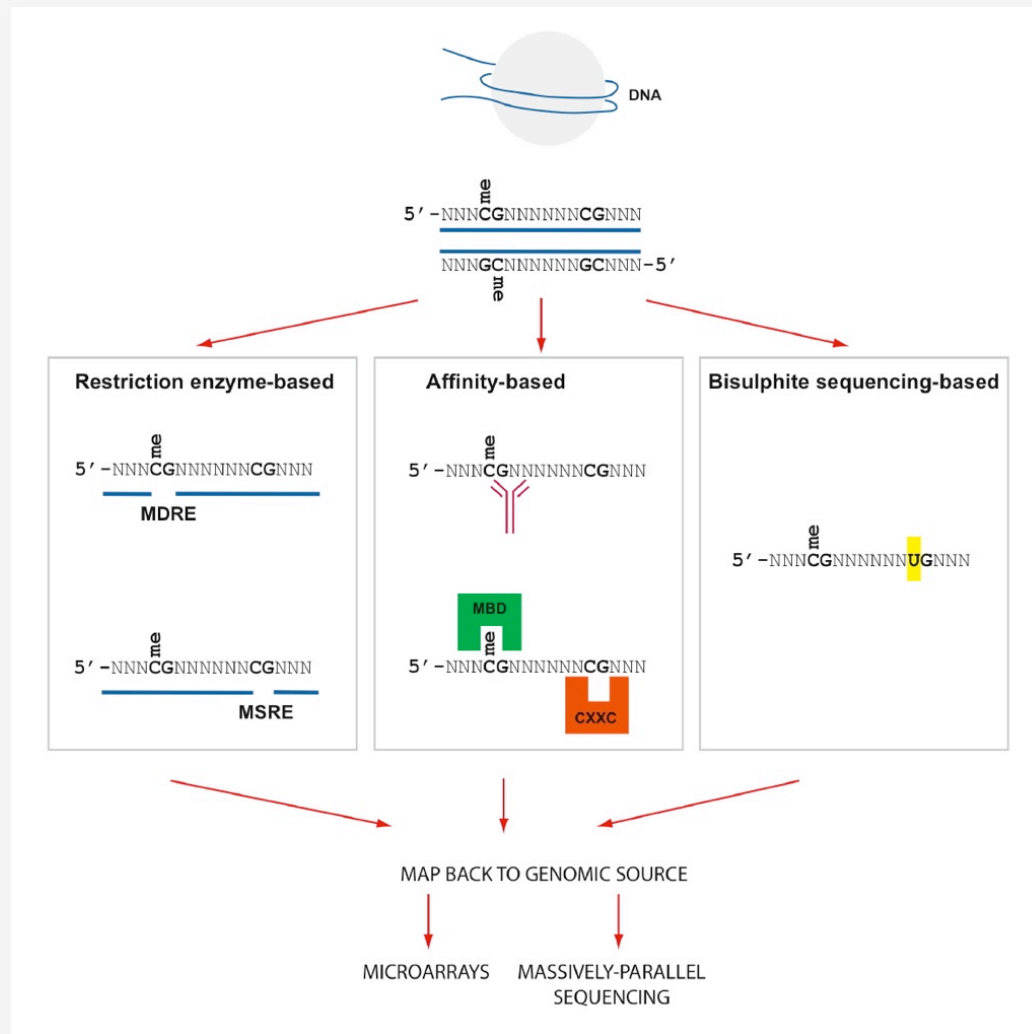
Epigenomic assays

- Chromatin immunoprecipitation (ChIP) assays



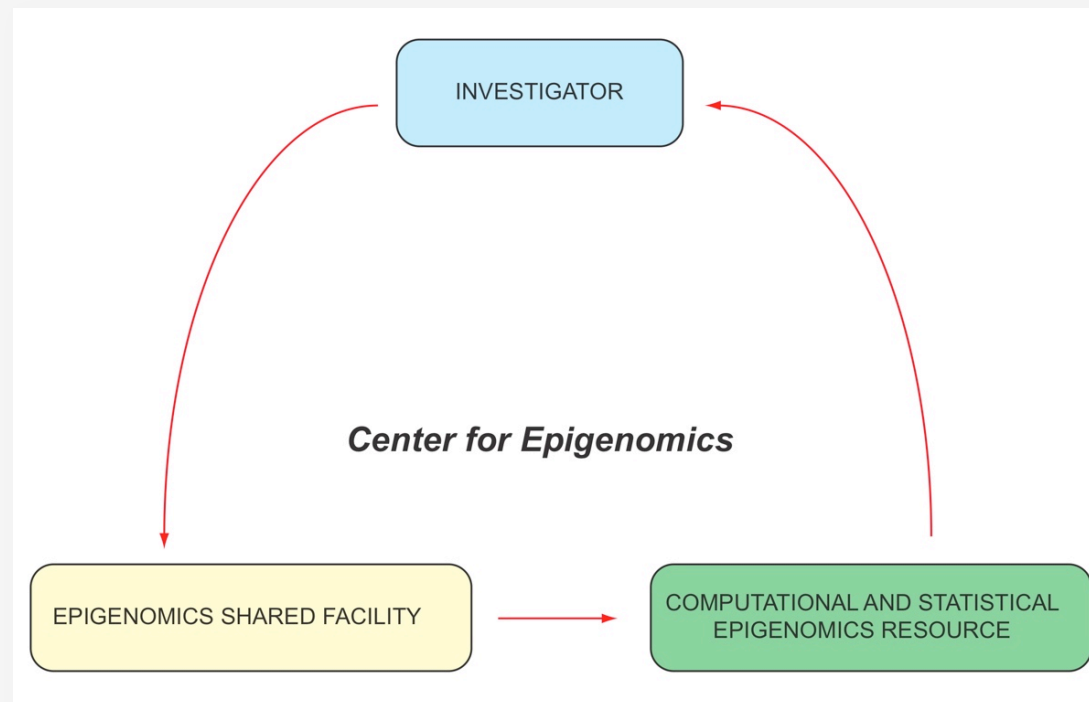
Epigenomic assays

- Cytosine methylation assays

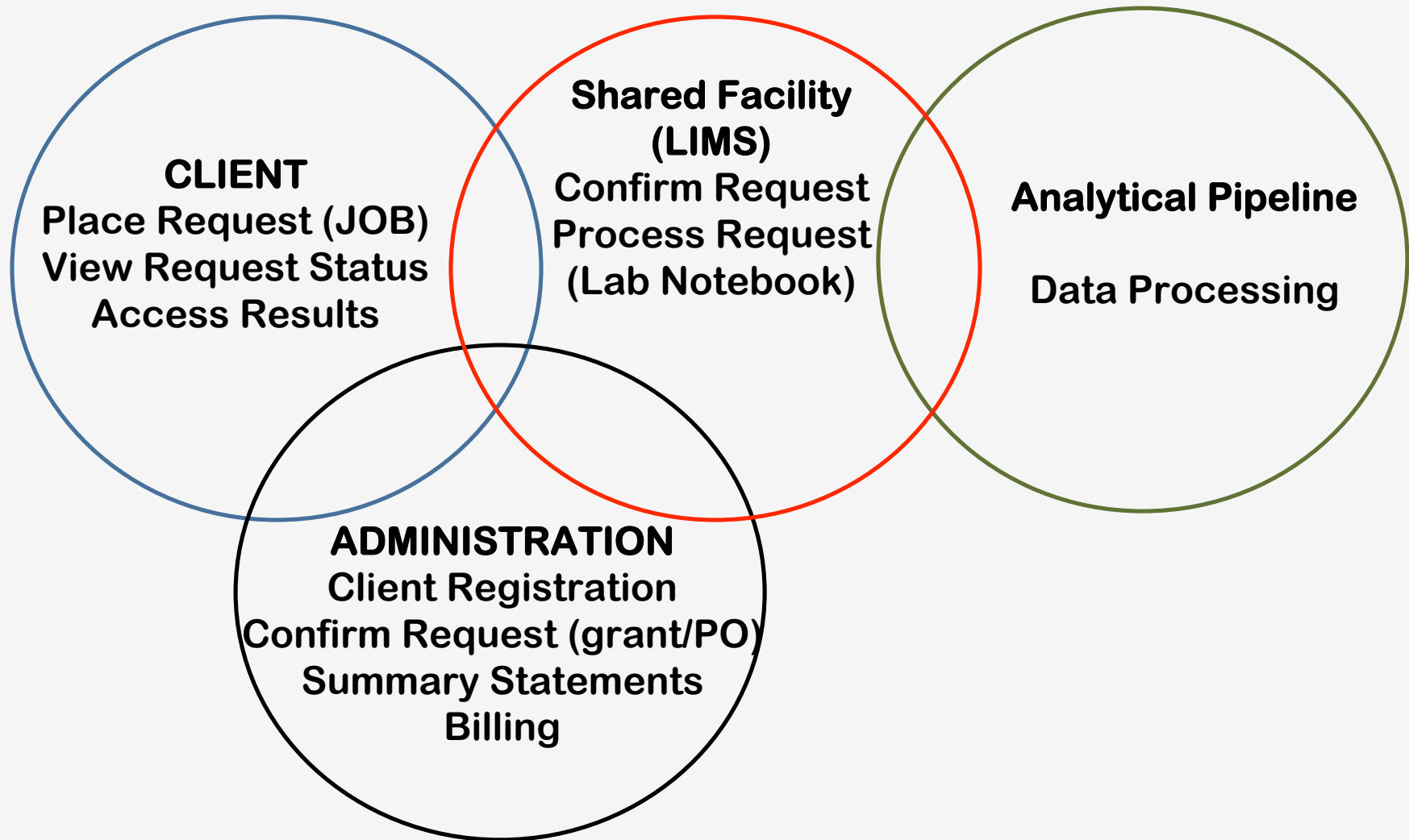


The Einstein Center for Epigenomics

- Sample submission & analysis need to be integrated
- Development of such a system at Einstein

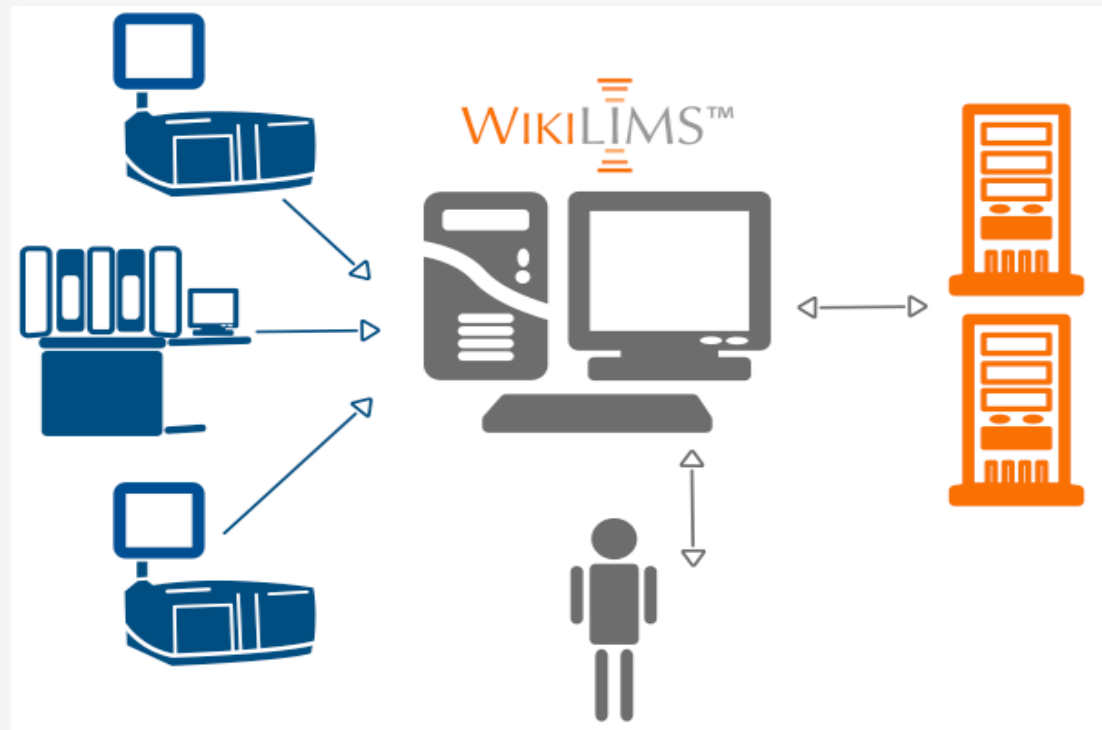


EPIGENOMICS SHARED FACILITY

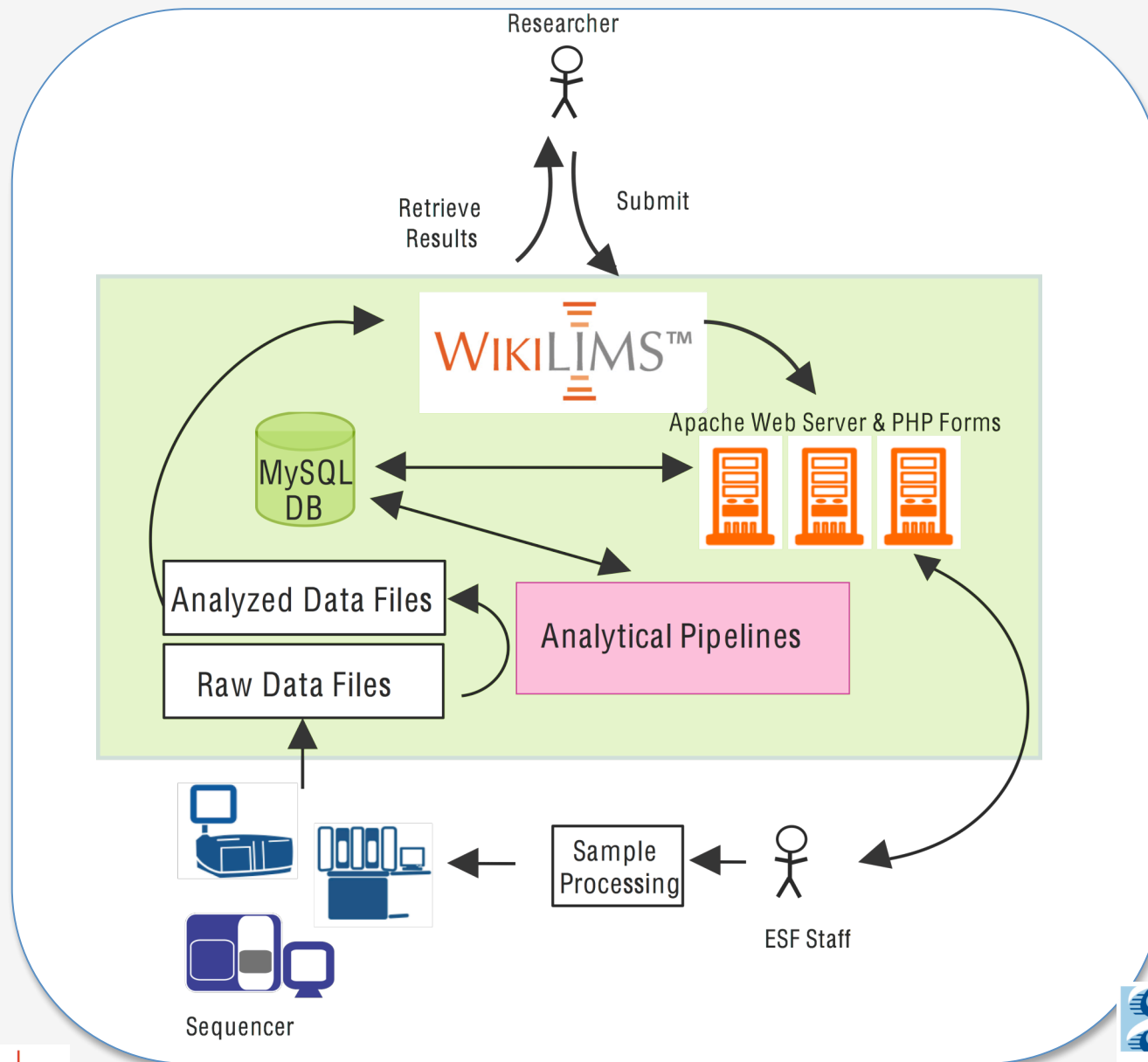


WikiLIMs:


- A system that is quickly constructed and easily revised
- A system that can handle many data types
- A modular and transparent system
- A Web-based system



WikiLIMs:



WikiLIMs: User main page



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User:Msuzuki



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Note: This is a temporary homepage, all users will have a special home page created for them in the near future with links to their projects, job submissions and results.

To submit a sample, click the button below.

Sample Submission

Projects

Completed Projects

Assay Type	Submitted by	Date Completed	Results Link
ChipSeq	msuzuki	08/31/09	s_1_eland_multi.txt.gz
ChipSeq	msuzuki	08/31/09	s_2_eland_multi.txt.gz
Nimblegen	Nathalie Lailler	06/08/09	090603_MM8_FY1-2-Chip2.1-Analysis.zip
Nimblegen	Nathalie Lailler	06/08/09	090603_MM8_FY3-4-Chip2.1-Analysis.zip
Illumina	Nathalie Lailler	2009-07-17	090717_HWI-EAS438_42DCEAAXX_8.tar.gz

WikiLIMs:Sample submission management

Contact & Billing

Contact & Billing

Sample Setup

New Sample Details

How many inputs are you submitting:

Please fill all fields:

Inputs:

	Name	Material	Library Tag	Size(bp) 200-500	Amt.(µg) 10ng	Conc.(ng/ul) 1-100	A260/280 1.8	A260/230 1.7	Vol.	Buffer	Antibody	PCR Primers (+)	PCR Primers (-)	Genome
1	<input type="text"/>	--Material--	--Tag--	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	TE	none(input)	--Primer Pair--	--Primer Pair--	--Genome--

Samples:

	Name	Material	Library Tag	Size(bp) 200-500	Amt.(µg) 10ng	Conc.(ng/ul) 1-100	A260/280 1.8	A260/230 1.7	Vol.	Buffer	Antibody	PCR Primers (+)	PCR Primers (-)	Genome	Match to Input
1	<input type="text"/>	--Material--	--Tag--	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	TE	--Antibody--	--Primer Pair--	--Primer Pair--	--Genome--	no input
2	<input type="text"/>	--Material--	--Tag--	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	TE	--Antibody--	--Primer Pair--	--Primer Pair--	--Genome--	no input


Comments:

<< SUBMIT >>

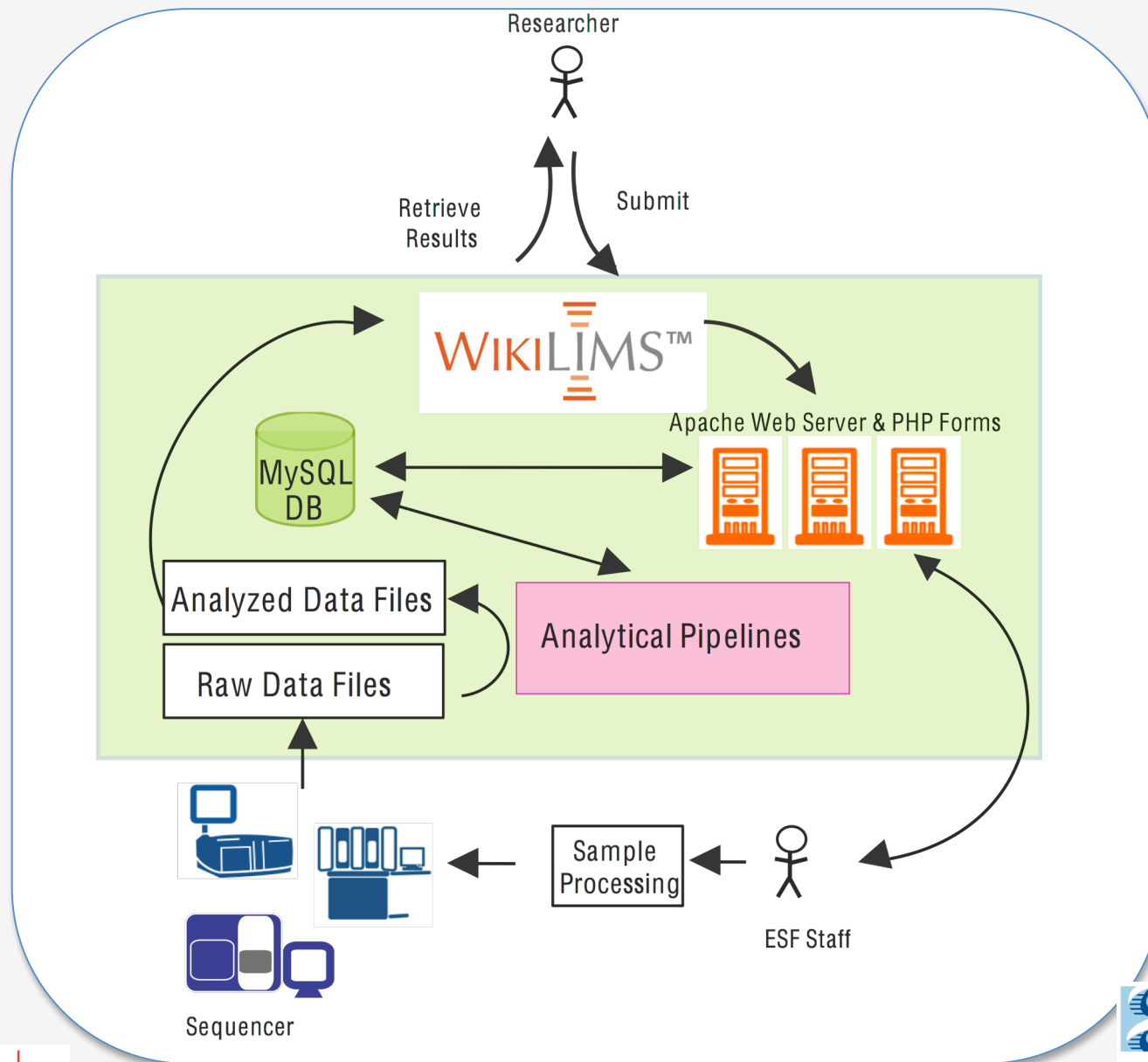
PCR primers: [Add](#)

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WikiLIMs:



WikiLIMs: Sequence data management



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 - 1.1 Sequencing and Alignment Results
 - 1.2 Peak Finding Results

Job description

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- **Job Name**
 - CHP-SEQ with anti-GATA1
- **Assay Type**
 - ChIP-Seq
- **Submitted By**
 - Masako Suzuki (Greally Lab)
- **Submitted Date**
 - 07/13/09
- **Completed Date**
 - 09/04/09
- [Click to Show Charts of Job Quality](#)

Sequencing and Alignment Results

[\[edit\]](#)

Flowcell ID	Sequencing Summary	Sample Name	Lane	Raw Data File	Alignment Result
42DCEAAXX	Click to show	ES_no_cytokines_INPUT	lane_1	Click to download	Show in Genome Browser
		ES_plus_cytokines_INPUT	lane_2	Click to download	Show in Genome Browser
FC42AHHAAXX	Click to show	ES_plus_cytokines_anti-gata1	lane_3	Click to download	Show in Genome Browser
		ES_no_cytokines_anti-gata1	lane_4	Click to download	Show in Genome Browser
		ES_plus_cytokines_anti-gata1	lane_5	Click to download	Show in Genome Browser

Peak Finding Results

[\[edit\]](#)

Sample Name	Sample Type	Flowcell ID	Lane	Result (1 sample)	Result (2 samples)
ES_no_cytokines_INPUT	Input	42DCEAAXX	lane_1	Click to download Show raw.bed in Genome Browser Show peaks.bed in Genome Browser	Click to download Show raw.bed (sample1) in Genome Browser Show raw.bed (sample2) in Genome Browser
ES_no_cytokines_anti-gata1	IP	FC42AHHAAXX	lane_4	Click to download Show raw.bed in Genome Browser	
ES_no_cytokines_INPUT	Input	42DCEAAXX	lane_1	Click to download Show raw.bed in Genome Browser Show peaks.bed in Genome Browser	Click to download Show raw.bed (sample1) in Genome Browser Show raw.bed (sample2) in Genome Browser Show peaks.bed in Genome Browser
ES_plus_cytokines_INPUT	Input	42DCEAAXX	lane_2	Click to download Show raw.bed in Genome Browser Show peaks.bed in Genome Browser	

WikiLIMs: Sequence data management

Summary Information For Experiment 090717_HWI-EAS438_42DCEAAXX

Chip Summary

Machine	HWI-EAS438
Run Folder	090717_HWI-EAS438_42DCEAAXX
Chip ID	unknown

Chip Results Summary

Clusters	Clusters (PF)	Yield (kbases)
89175116	33045159	1189626

Lane Parameter Summary

Lane	Sample ID	Sample Target	Sample Type	Length	Filter	Chast. Thresh.	Num Tiles	Tiles
1	unknown	ucsd_mm_9	ELAND_EXTENDED	35	'((FAILED_CHASTITY<=1.000000))'	0.600000	100	Lane 1
2	unknown	ucsd_mm_9	ELAND_EXTENDED	35	'((FAILED_CHASTITY<=1.000000))'	0.600000	100	Lane 2
3	unknown	ucsd_mm_9	ELAND_EXTENDED	35	'((FAILED_CHASTITY<=1.000000))'	0.600000	100	Lane 3
4	unknown	ucsd_mm_9	ELAND_EXTENDED	35	'((FAILED_CHASTITY<=1.000000))'	0.600000	100	Lane 4
5	unknown	Hs19	ELAND_EXTENDED	35	'((FAILED_CHASTITY<=1.000000))'	0.600000	100	Lane 5
6	unknown	Hs19	ELAND_EXTENDED	35	'((FAILED_CHASTITY<=1.000000))'	0.600000	100	Lane 6
7	unknown	Scer288c	ELAND_EXTENDED	35	'((FAILED_CHASTITY<=1.000000))'	0.600000	100	Lane 7
8	unknown	ucsd_mm_9	ELAND_EXTENDED	35	'((FAILED_CHASTITY<=1.000000))'	0.600000	100	Lane 8

Lane Results Summary

Lane Info		Tile Mean +/- SD for Lane							
Lane	Lane Yield (kbases)	Clusters (raw)	Clusters (PF)	1st Cycle Int (PF)	% intensity after 20 cycles (PF)	% PF Clusters	% Align (PF)	Alignment Score (PF)	% Error Rate (PF)
1	85438	75939 +/- 9990	23972 +/- 6961	12 +/- 3	72.20 +/- 12.78	31.73 +/- 8.04	0.92 +/- 0.07	0.44 +/- 0.06	7.23 +/- 0.49
2	139730	106688 +/- 9424	38813 +/- 7938	15 +/- 4	72.12 +/- 14.20	36.46 +/- 6.93	1.38 +/- 0.12	0.74 +/- 0.08	5.68 +/- 0.51

WikiLIMs: Sequence data management

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 - ChIP-Seq
- Submitted By**
 - Masako Suzuki (Greally Lab)
- Submitted Date**
 - 07/13/09
- Completed Date**
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- [Click to Show Charts of Job Quality](#)**

Sequencing and Alignment Results

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		ES_plus_cytokines_anti-gata1	lane_5	Click to download	Show in Genome Browser

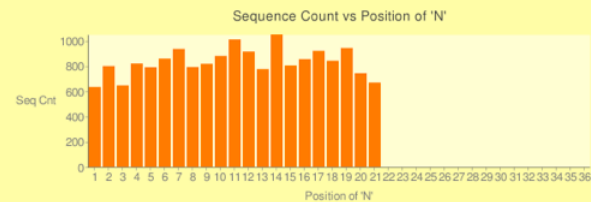
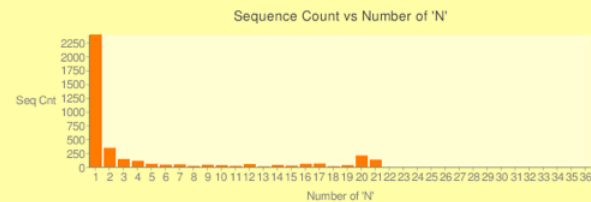
Peak Finding Results

Sample Name	Sample Type	Flowcell ID	Lane	Result (1 sample)	Result (2 samples)
ES_no_cytokines_INPUT	Input	42DCEAAXX	lane_1	Click to download <div>Show raw.bed in Genome Browser Show peaks.bed in Genome Browser</div>	Click to download <div>Show raw.bed (sample1) in Genome Browser Show raw.bed (sample2) in Genome Browser</div>
ES_no_cytokines_anti-gata1	IP	FC42AHHAAXX	lane_4	Click to download <div>Show raw.bed in Genome Browser</div>	
ES_no_cytokines_INPUT	Input	42DCEAAXX	lane_1	Click to download <div>Show raw.bed in Genome Browser Show peaks.bed in Genome Browser</div>	Click to download <div>Show raw.bed (sample1) in Genome Browser Show raw.bed (sample2) in Genome Browser Show peaks.bed in Genome Browser</div>
ES_plus_cytokines_INPUT	Input	42DCEAAXX	lane_2	Click to download <div>Show raw.bed in Genome Browser Show peaks.bed in Genome Browser</div>	

WikiLIMs: Sequence data management

Sequence Quality Statistics

ES no cytokines INPUT (flowcell: 42DCEAAXX lane 1)




Run Quality Parameters

These quality metrics are based principally on single read 36bp sequencing of human DNA. The optimal results will vary according to experiment type.

Metric	Result	Uniformity (across tiles)	Notes
Total Yield			Result is 139730 (target is >1Gbases).
Raw Cluster Count			Result is 106688 +/- 9424 (target is >20,000).
% Clusters Passing Filter (PF)			Result is 36.46 +/- 6.93 (target is > 70%). Low % may be indicative of high cluster number (clusters too close together to obtain a clean signal in early cycles)
% Clusters PF that Align Uniquely to Reference			Result is 1.38 +/- 0.12. Optimal value dependent on read-length, genome sequenced and completeness of reference. For 30mers and the human genome, < 80% may be normal.
% Error Rate of Clusters PF			Result is 5.68 +/- 0.51. Should be ~1.5% but in any case, as low as possible.
% Phasing			Result is 0.6900. Should be ~0.5% to no more than 1% but in any case, as low as possible.
% Prephasing			Result is 0.5400. Should both be ~0.5% to no more than 1% but in any case, as low as possible.
First Cycle Intensity			Result is 15 +/- 4. Should be >1000.
20th Cycle Intensity as % of First			Result is 72.12 +/- 14.20. Should be >50%. If too high, suspect relatively low first cycle intensity

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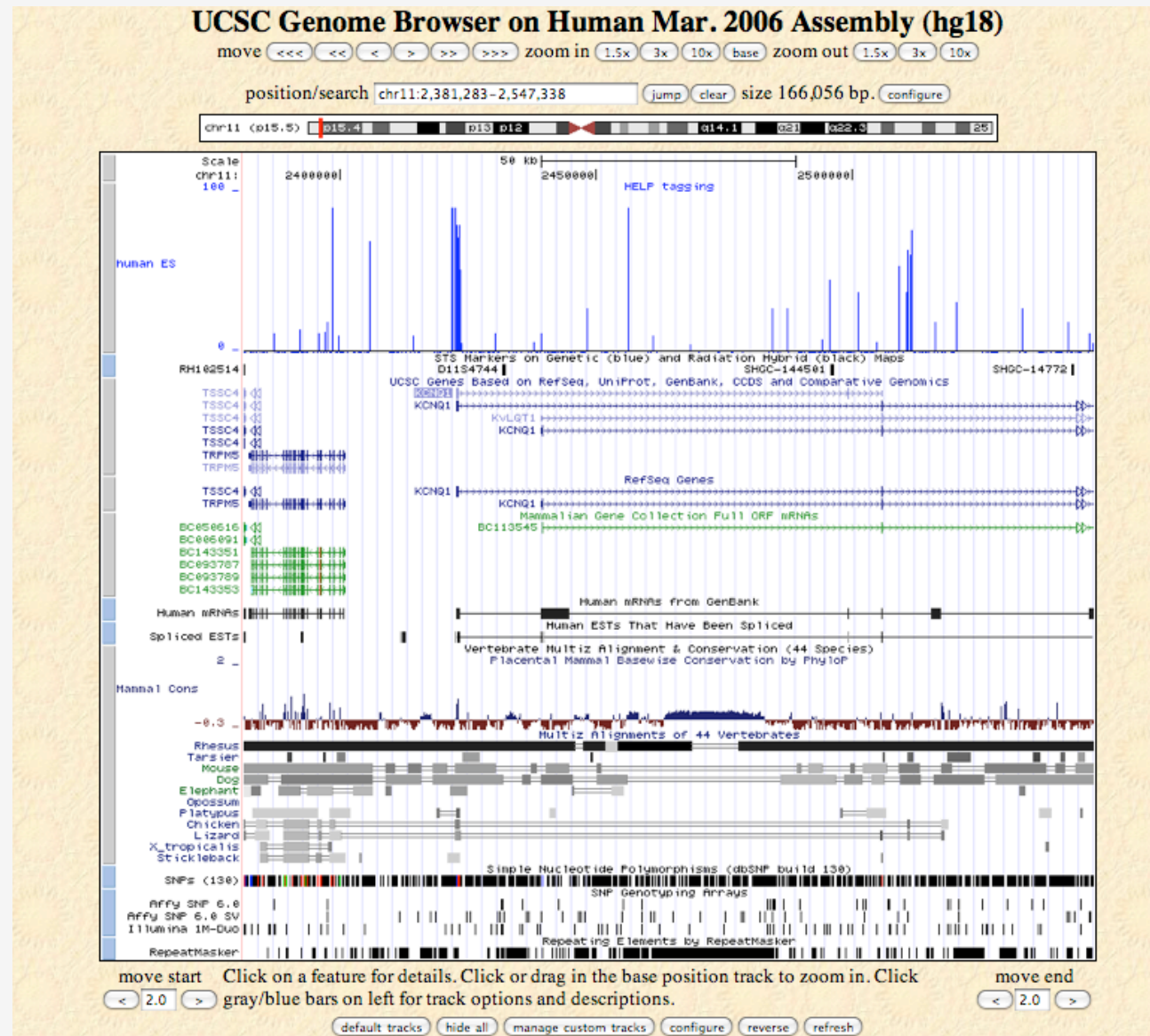
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WikiLIMs: Sequence data management



WikiLIMs: electronic lab notebook



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UserPageTest

Use the tabs below to:

- Create new projects and sample submissions,
- View results,
- Track run status,
- Write to your lab notebook

Note: Project submissions are not set up yet so "Create a Project" button and results are based on Illumina Run data

[Projects](#) [Lab Notebook](#) [Lab Homepage](#) [Links](#)

[Create a New Project](#)

Current Projects

(Click Project Title to add a new submission to this project or see status)

	Entry date	Run date	Machine	Kilobases Sequenced
090213 HWI-HWI-EAS438 30H7JAAXX	13 May 2009 13:24:17	13 February 2009	HWI-EAS438	1,803,841


Completed Projects

(Click Project Title to view results)

	Entry date	Run date	Machine	Kilobases Sequenced
090417 HWI-HWI-EAS438 30PBFAAXX	13 May 2009 13:36:50	17 April 2009	HWI-EAS438	1,339,058
090401 HWI-HWI-EAS438 30FMBAAAXX	13 May 2009 13:36:36	1 April 2009	HWI-EAS438	1,336,405
090226 HWI-HWI-EAS438 30H5GAAXX	13 May 2009 13:36:21	26 February 2009	HWI-EAS438	376,885
090213 HWI-HWI-EAS438 30H7JAAXX	13 May 2009 13:24:17	13 February 2009	HWI-EAS438	1,803,841
090203 HWI-HWI-EAS438 30FRTAAXX	13 May 2009 13:35:55	3 February 2009	HWI-EAS438	1,039,678



WikiLIMs: electronic lab notebook



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UserPageTest/New Protocols

Genomic DNA extraction protocol

Buffer and reagent:


- Genomic DNA extraction buffer (250ml):
 - 1M Tris.Cl (pH 8.0) 2.5ml
 - 0.5M EDTA (pH 8.0) 50 ml
 - Pancreatic RNase 5 mg
 - 10% SDS 12.5 ml
- Adjust pH to 8.0 and adjust volume to 250ml with ddH2O
- Saturated phenol (pH 8.0)
- 10M ammonium acetate (NH4Ac)

Protocol:

1. Weigh 0.5-1g fresh tissue and put in mortar. Add liquid nitrogen to snap freeze tissue and blend tissue to powder.
2. Add 10 ml genomic DNA extraction buffer in 50 ml tube and put tissue powder in.
3. Invert tube to submerge tissue powder and incubate at 37c for 1 hour.
4. Add 50 ul proteinase K (20mg/ml stock), mix gently.
5. Incubate in 50c water bath for 3 hours, shake gently.
6. Let stand in room temperature for 30 min to equilibrate to room temperature.
7. Add 10 ml Phenol, mix gently for 10 min.
8. Centrifuge at 3000 rpm x 15 min.
9. Transfer the viscous aqueous phase to a new tube using a wide-pore glass pipette.
10. Repeat phenol extraction for 2 times or more.
11. Add 2 ml ammonium acetate (10M), mix gently.
12. Add 2 volume of ethanol (in room temperature). Swirl gently and you will see genomic DNA start to form the white mass. Transfer genomic DNA to a new tube by using a "U" shape pipette.
13. Air dry for 5-10min to drive off ethanol and dissolve in ddH2O or TE buffer.


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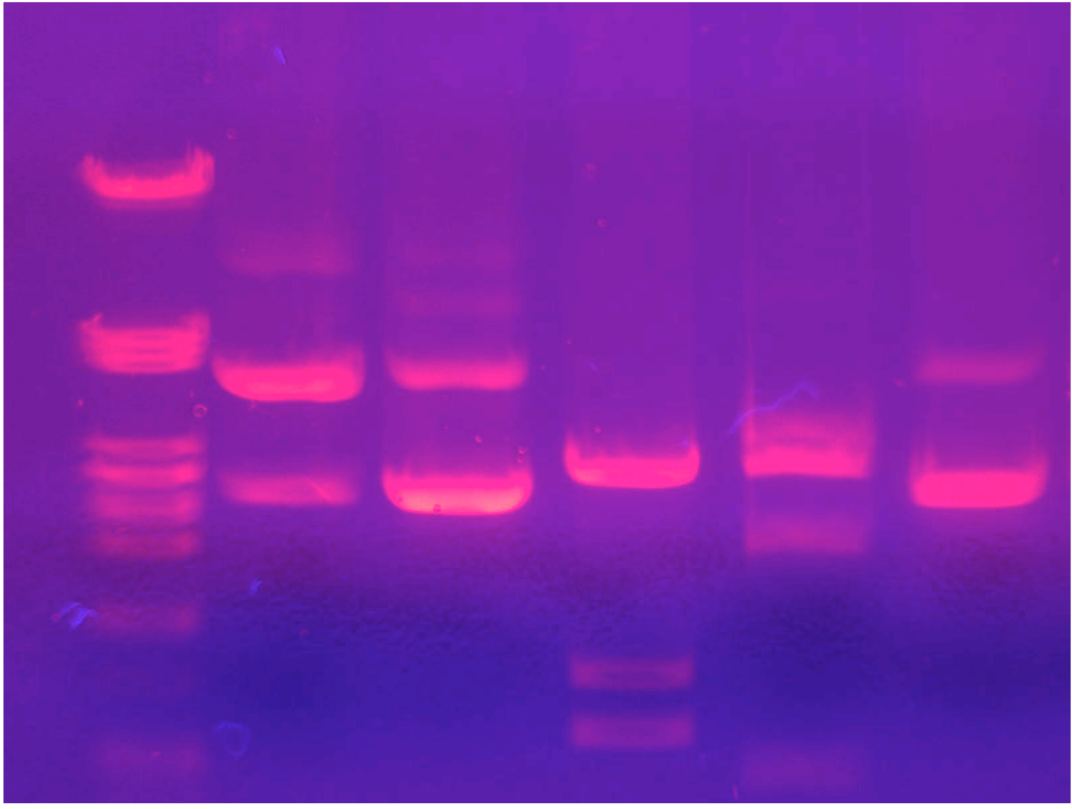
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UserPageTest/Presentation Images



Description

Gel electrophoresis: 6 "DNA-tracks". In the first row (left), DNA with known fragment sizes was used as a reference. Different bands indicate different fragment sizes (the smaller, the faster it travels, the lower it is in the image); different intensities indicate different concentrations (the brighter, the more DNA). DNA was made visible using ethidium bromide and ultraviolet light.

Summary

Development of new epigenomic assays critically dependent on analytical capacity

Provision of these assays to researchers depends further on integration of data generation and data analysis services

WikiLIMS used to provide interface to analytical services

Automated analytical pipelines increase efficiency of services

These systems are inherently expandable and adaptable



Greally Lab

Dr. John Greally
Dr. Niki Athanasiadou
Dr. Niru Narayanan
Edyta Stasiek
Marién Pascual
Maria-Paz Ramos
Esther Berko
Kevin Lau

Former lab personnel

Dr. Mayumi Oda
Dr. Khulan Batbayar
Dr. Jacob Glass
Dr. Priti Tewari
Dr. Reid Thompson

Einstein

Dr. Melissa J. Fazzari
**Computational and Statistical
Epigenomic Resources**

Dr. Andrew McLellan
Dr. Robert Dubin
Pilib Ó Broin
Qiang Jing (A.J.)

Bouhassira Lab

Dr. Eric Bouhassira
Dr. Emmanuel Olivier

Epigenomics Shared Facility

Dr. Shahina Maqbool
Raul Orea
Gael Westby

Bioteam

Dr. Stan Gloss
Dr. Brian Osborne
Dr. Adam Kraut

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