Wardrobe

Experiment Management System

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What do we need

- store experiment description, all the data and analysis steps in a formalized way
- automated data downloading
- automated quality control and preliminary analysis
- ability to use published data with our pipelines
- share our own data with co-workers
- sophisticated analysis
- high quality plots and UCSC genome browser visualization
- easy way to add novel types of analysis
- professor friendly interface
- all of the above have to be integrated

Off the shelf products are not comprehensive enough

- LabGuru, iLabber, PerkinElmer (electronic lab notebooks) - do not integrated with analyses
- Illumina basespace, Galaxy (online bioinformatics analysis) - you have to upload data, have basic knowledge about tools, problems with personal genomic data sharing, difficult to restore analysis steps
- GeneSpring, GoldenHelix commercial desktop/server tools - do not have NGS epigenetic analysis
- Ensemble and UCSC Genome Browsers - there are no integrated analyses, not enough description

EMS life cycle

Preliminary analysis
fully automated

Advanced analysis
project dependent

comprehensive description

RNA-Seq/ChIP-Seq data

from core

external source

corresponding analysis (pipeline)

genome browser

preliminary results

quality control

project designer

Types of analysis

DESeq / DESeq2

MANorm/Diffbind

Average Tag Density

...

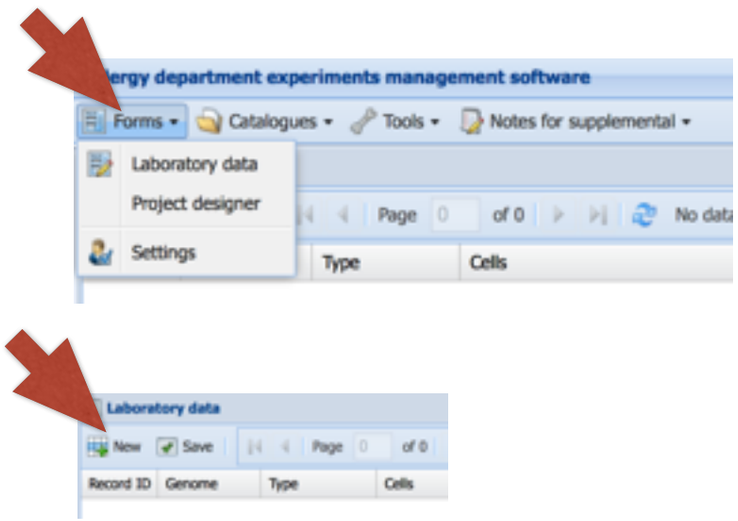
sophisticated results

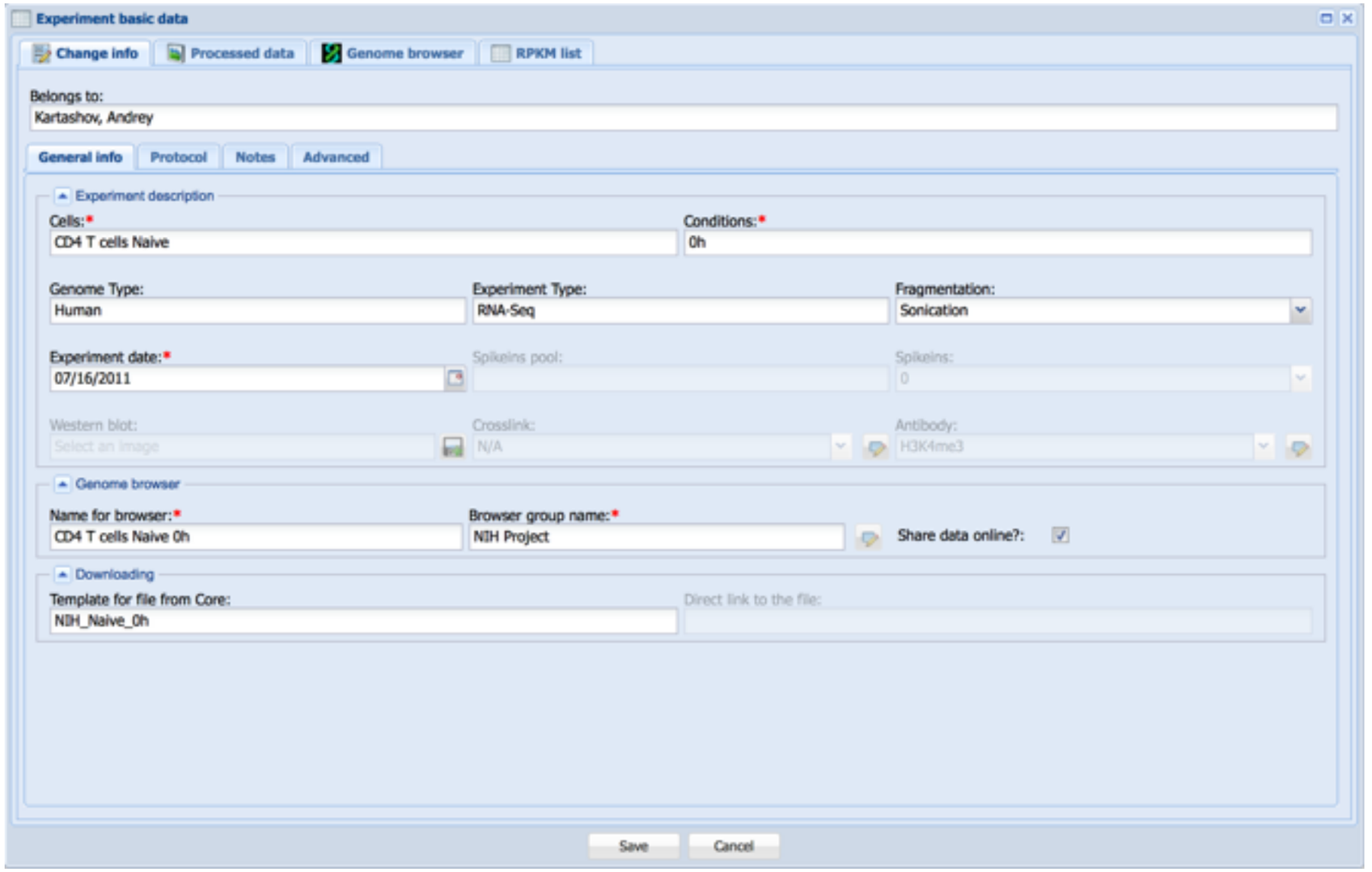
Consider an example

- Data
  - CD4 Naive Cells 0m RNA-Seq
  - CD4 Naive Cells 150m RNA-Seq
  - CD4 Naive Cells H3K4Me3 ChIP-Seq
  - CD4 Effector Cells H3K4Me3 ChIP-Seq
- Task
  - Compare RNA-Seq (find differentially expressed genes)
  - Compare epigenetic modifications (differentially enriched regions)
  - Compare epigenetic modifications for specifically expressed genes

Experiment Description

Flexible and convenient way
to describe an experiment





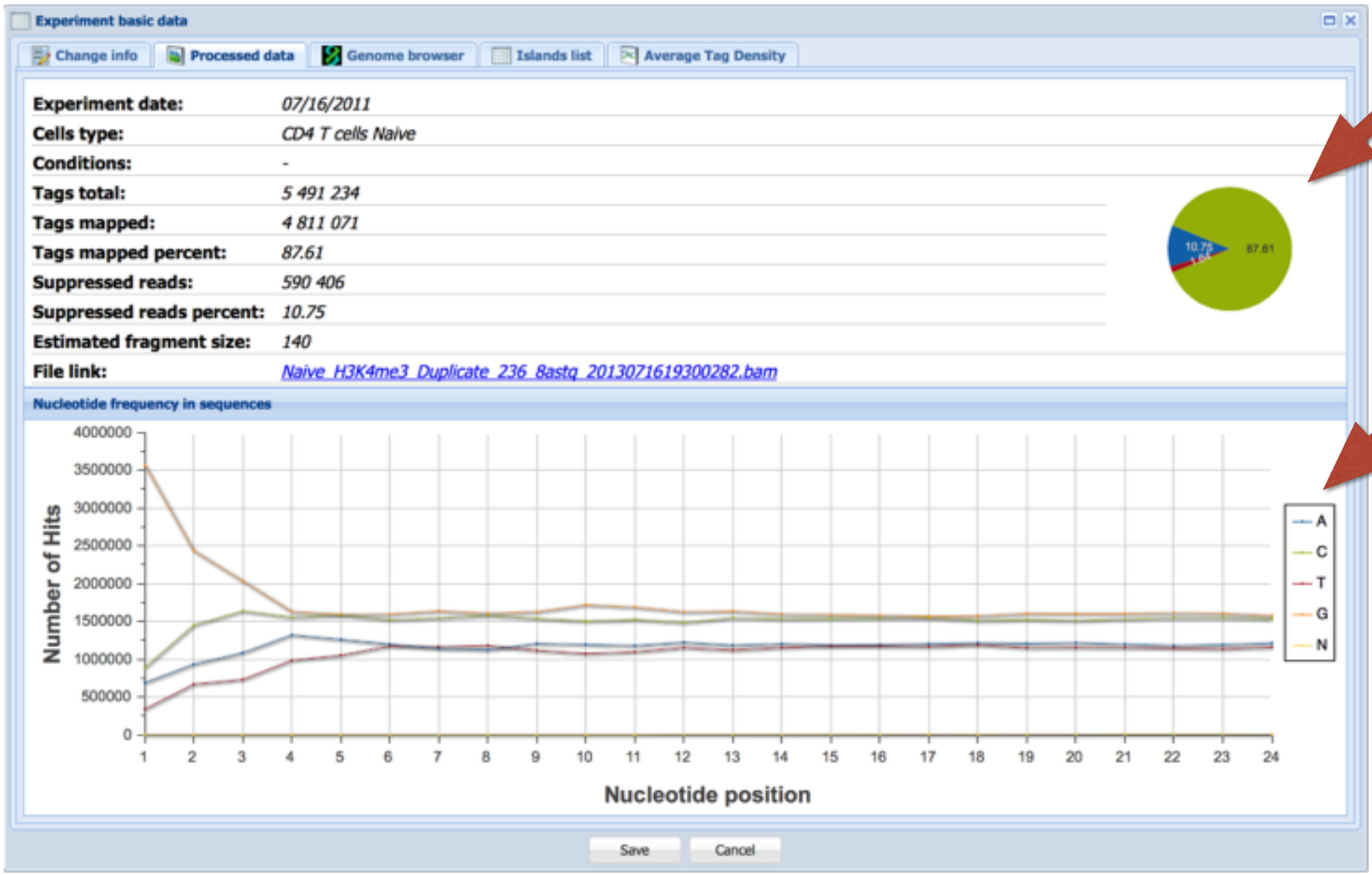
Preliminary Automated Analysis

- RNA and ChIP
  - download data
  - quality control
  - upload to local UCSC genome browser

- RNA
  - mapping data by tophat
  - calculating RPKMs
- RNA With Spike In
  - Linear regression, shows scatter plot

- ChIP
  - mapping data by bowtie
  - identification of islands by MACS (1/2)
  - Average Tag Density
  - Islands distribution

Quality Control (DNA)



Mapping statistics

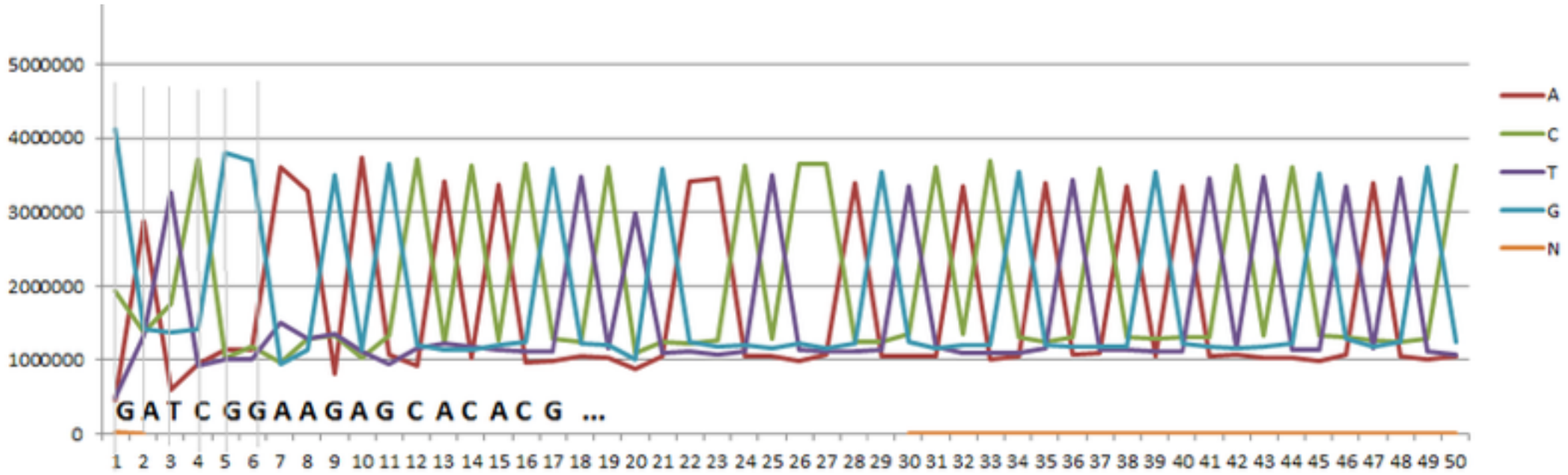
- Total tags mapped
- Suppressed reads
- Unmapped reads

Base call frequency

Nucleotide domination at position

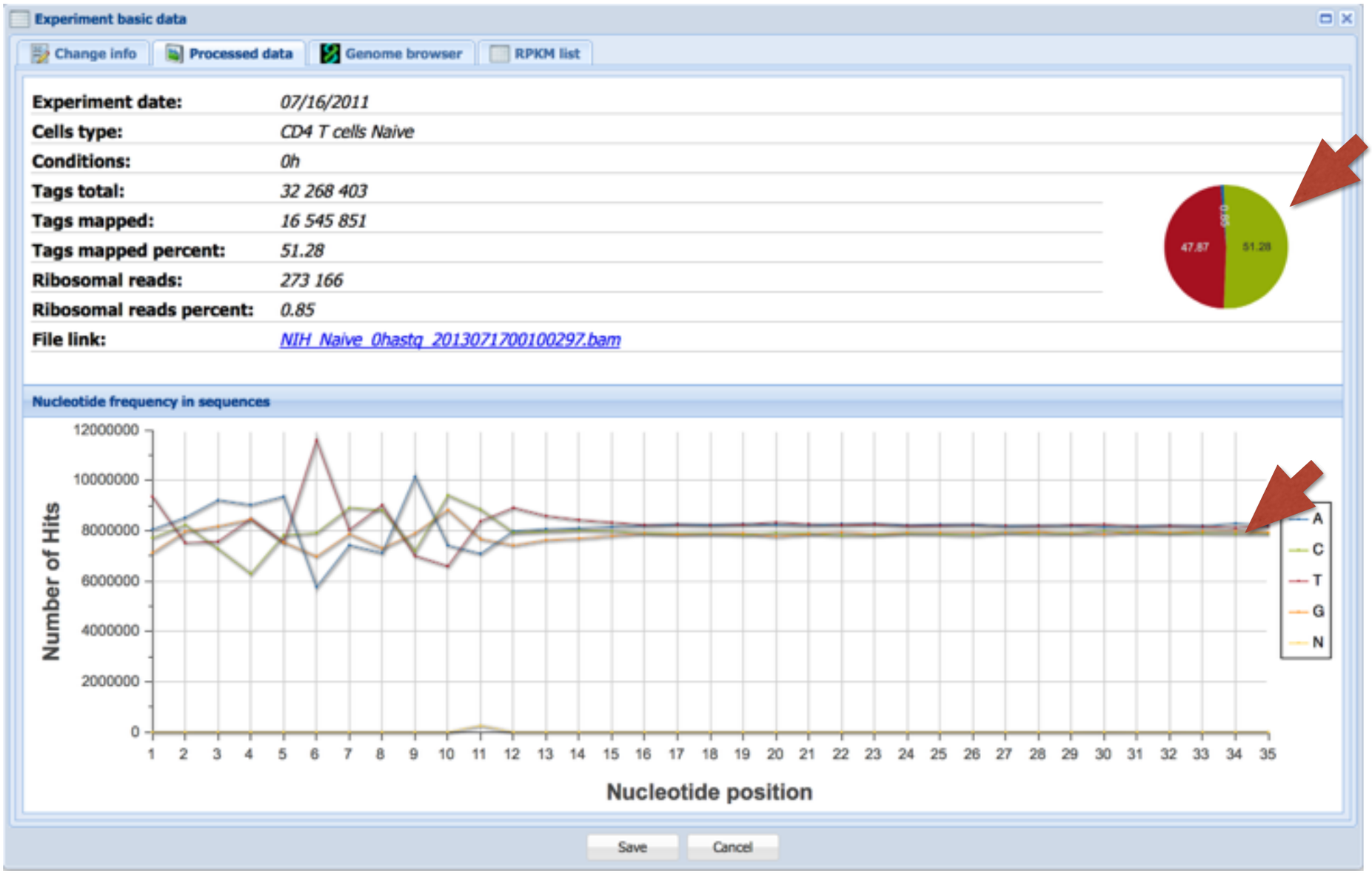
Base Call Frequency RNA/DNA

Perfect example of an adaptor contamination



In cases of adapter contamination, peaks reflect the sequence of adapters.

Quality Control (RNA)



Mapping statistics

- Total tags mapped
- Ribosomal contamination
- Non-uniquely mapped and unmapped reads

Base call frequency

Nucleotide domination at position

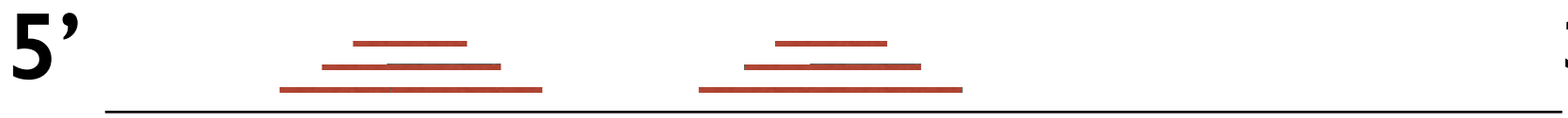
UCSC genome browser

- Data is uploaded directly to a local mirror of UCSC genome browser and available as a track
- Data is normalized to a total number of mapped reads
- Data is represented as a coverage
  - DNA:
    - for a pair-end libraries original fragment size is used
    - for a single-read estimated fragment size is used
  - RNA: read size is used

UCSC genome browser
Coverage

DNA: Estimated fragment size for single reads, original fragment size for pair-end reads

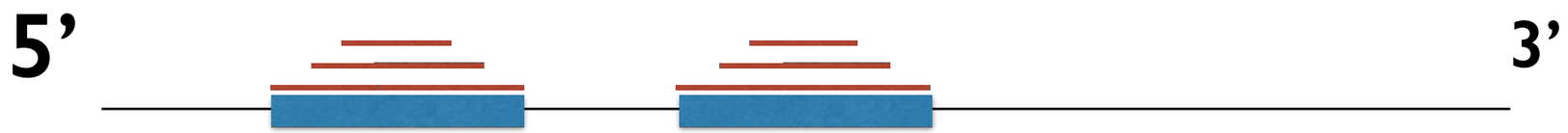
RNA: read length



UCSC genome browser
Coverage

DNA: Estimated fragment size for single reads, original fragment size for pair-end reads

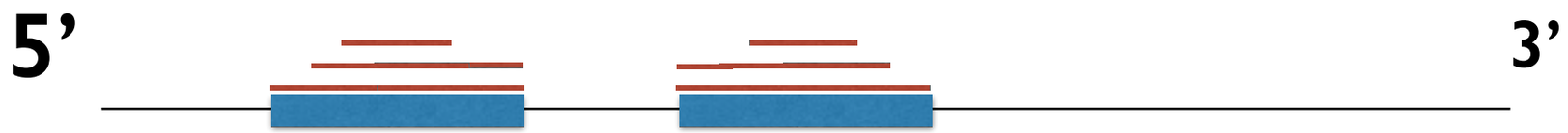
RNA: read length



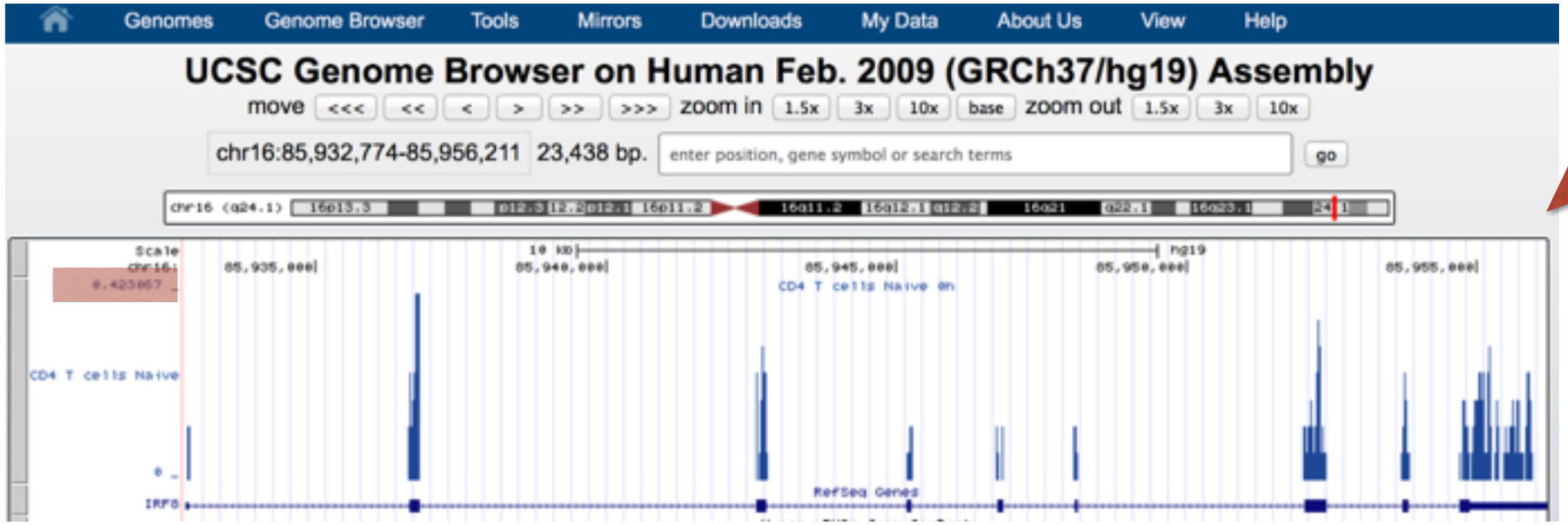
UCSC genome browser
Coverage

DNA: Estimated fragment size for single reads, original fragment size for pair-end reads

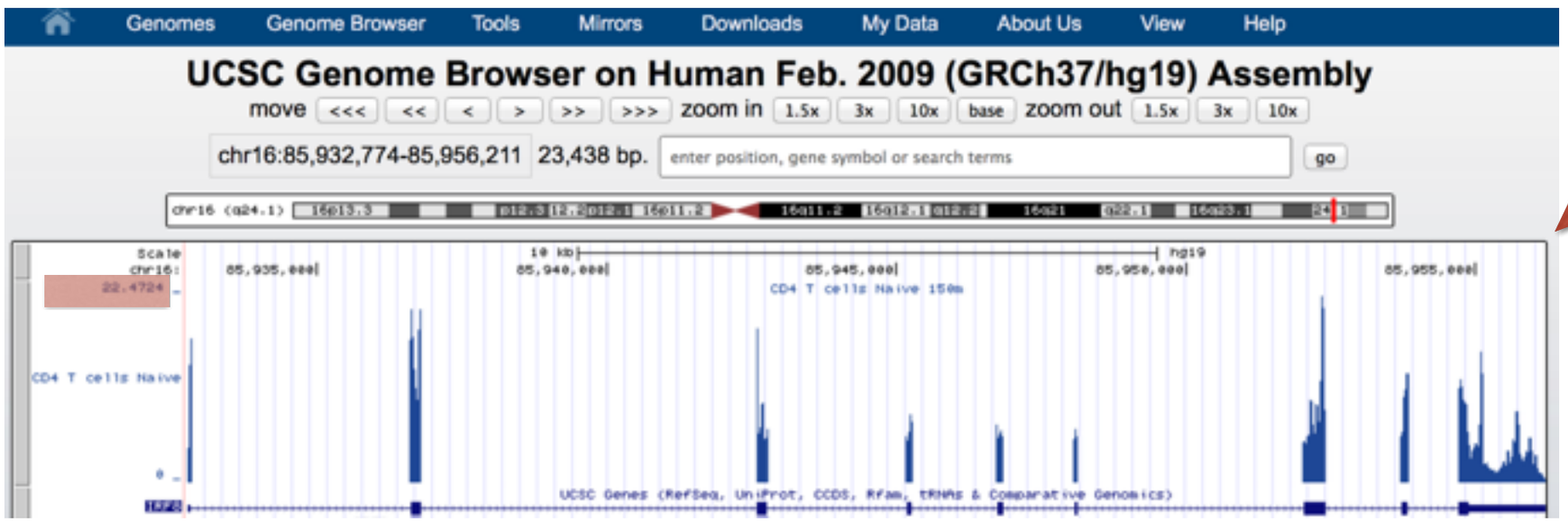
RNA: read length



UCSC genome browser RNA

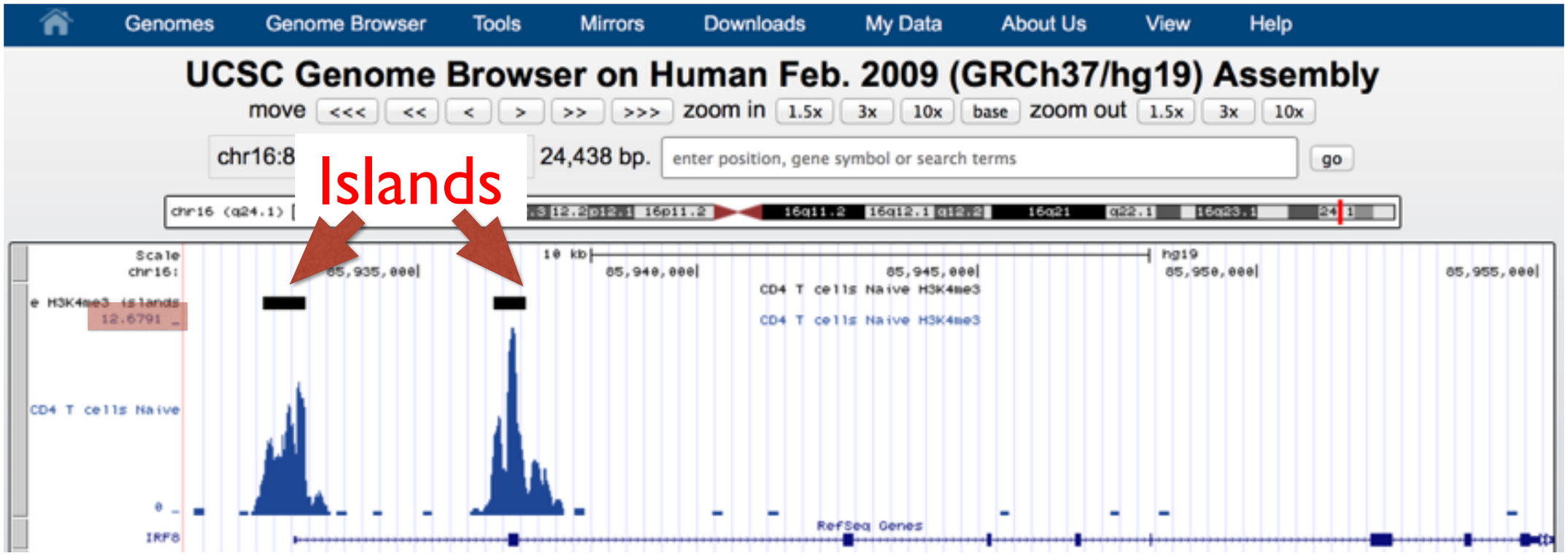


IRF8
0 time point
0.42 Normalized lvl
2.271 RPKM



IRF8
150m time point
22.47 Normalized lvl
149.363 RPKM

UCSC genome browser DNA



IRF8
0 time point
12.6791 Normalized lvl
2 Islands were found

Calculating RPKMs

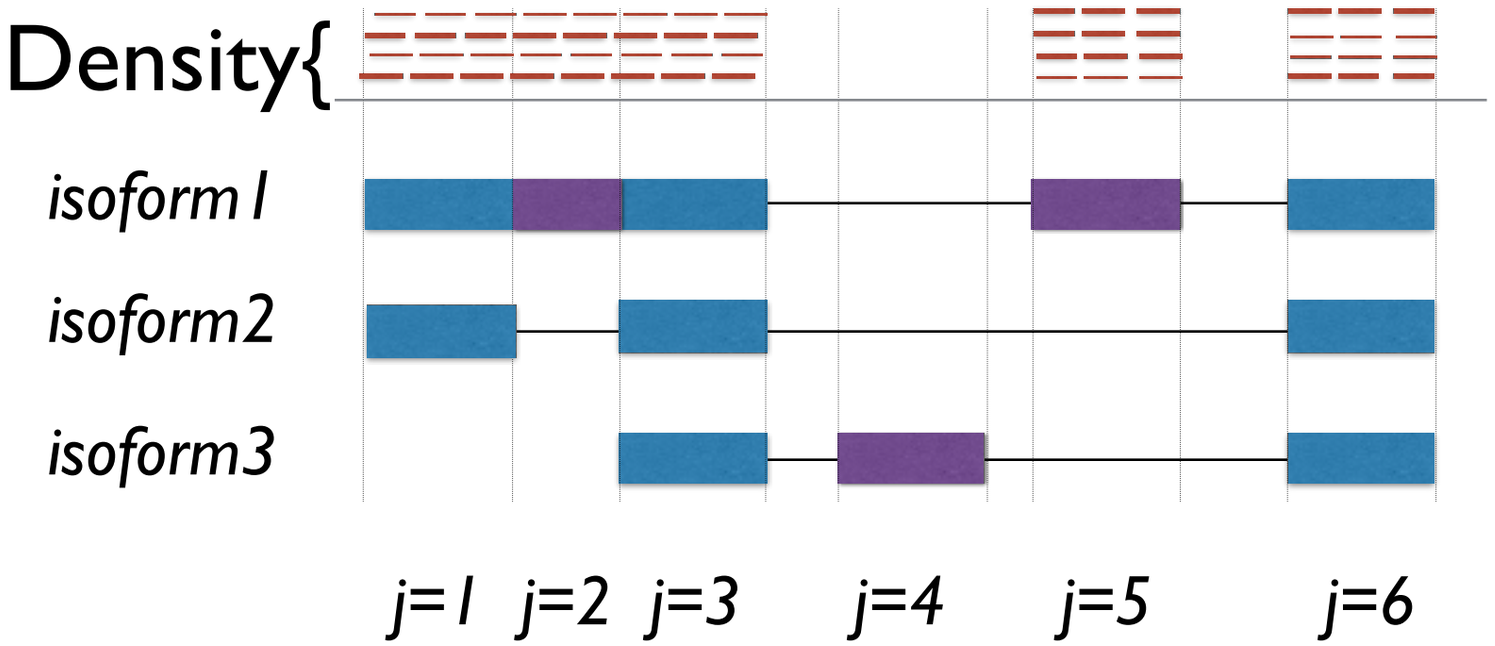
RPKM = Reads Per Kilobase of transcript length per Millionth of mapped reads

Existing tools:

- HTSeq-count
- Cufflinks
- Genespring
- IQSeq
- …

Our objective is to estimate RPKMs with respect to the current annotation and to quantify expression precisely as possible.

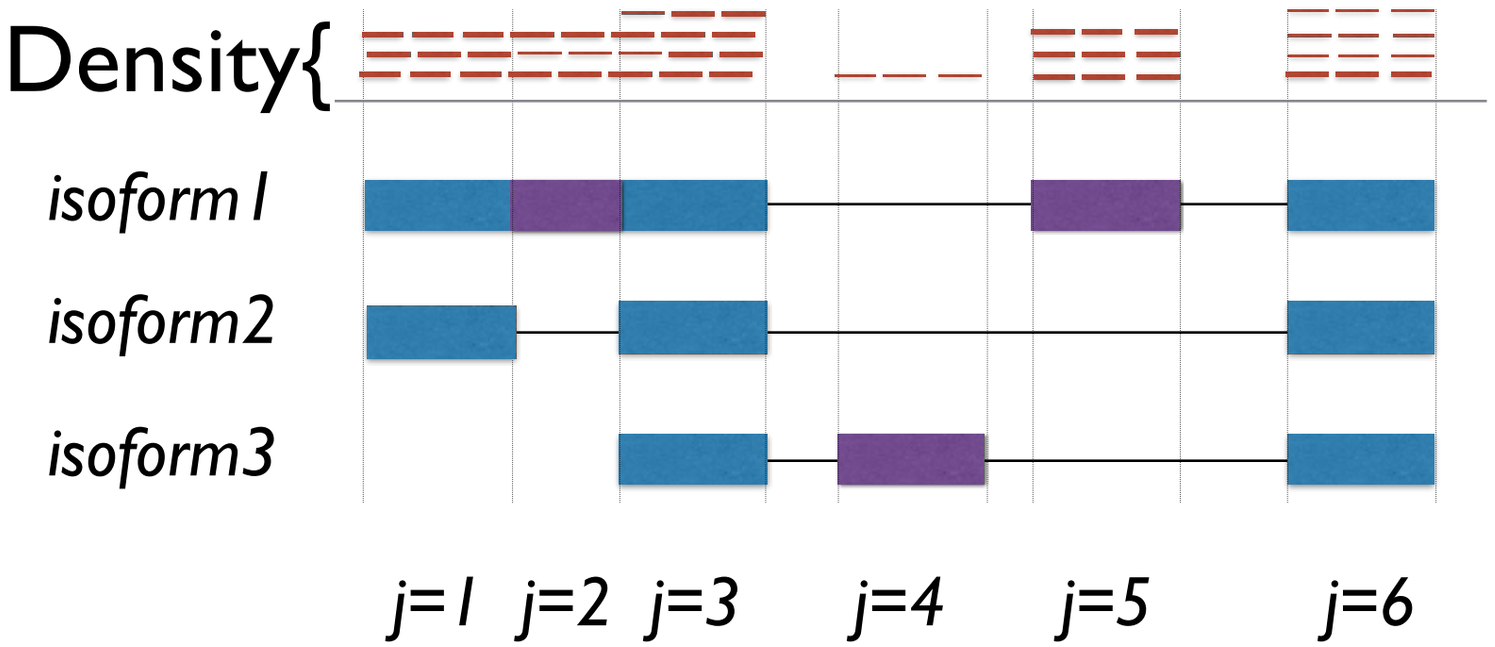
Calculating RPKMs



1. Densities across exons within the same isoform have to be the same
2. Sum of exon densities for the same region should be equal to the total density for that region

density $d_{i_j} = \frac{N_{i_j}}{L_j}$, where N_{i_j} is a number of reads in a region J of isoform I and L is the length of the region J

Calculating RPKMs



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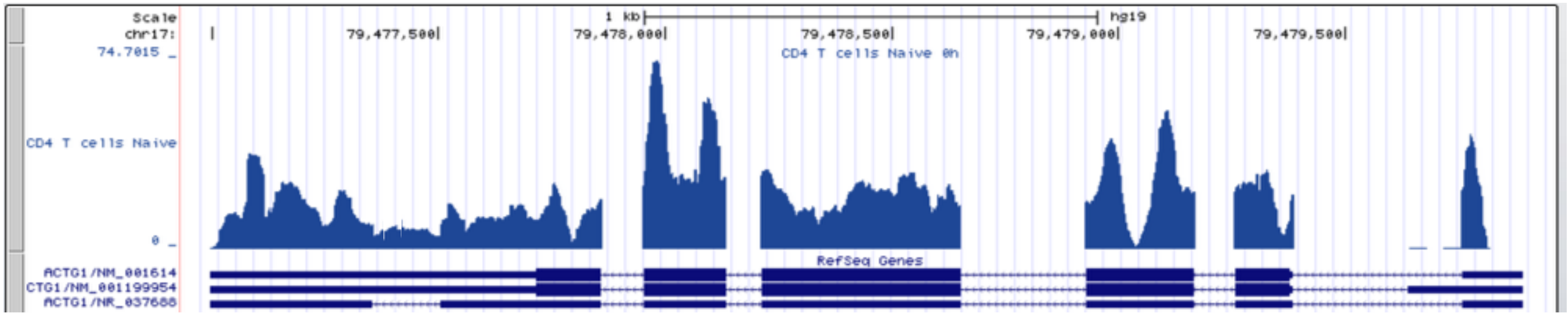
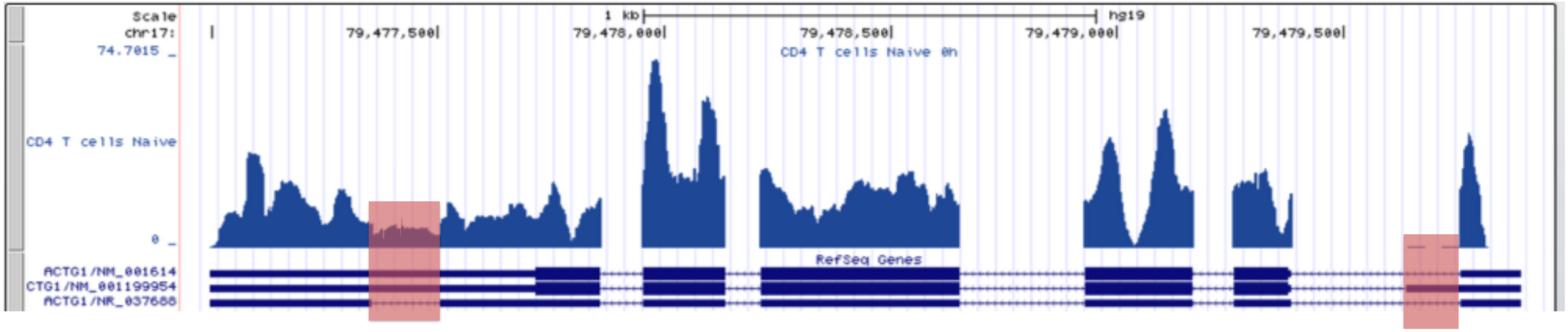
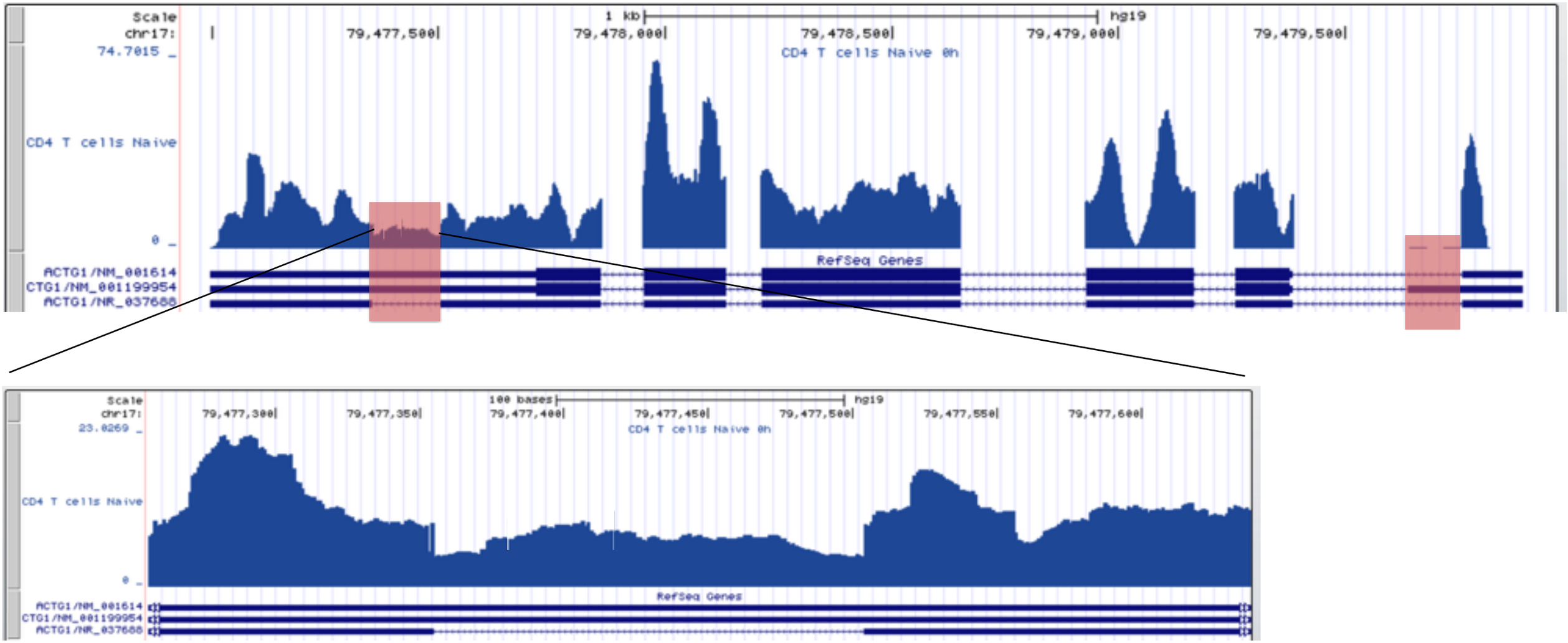
Calculating RPKMs



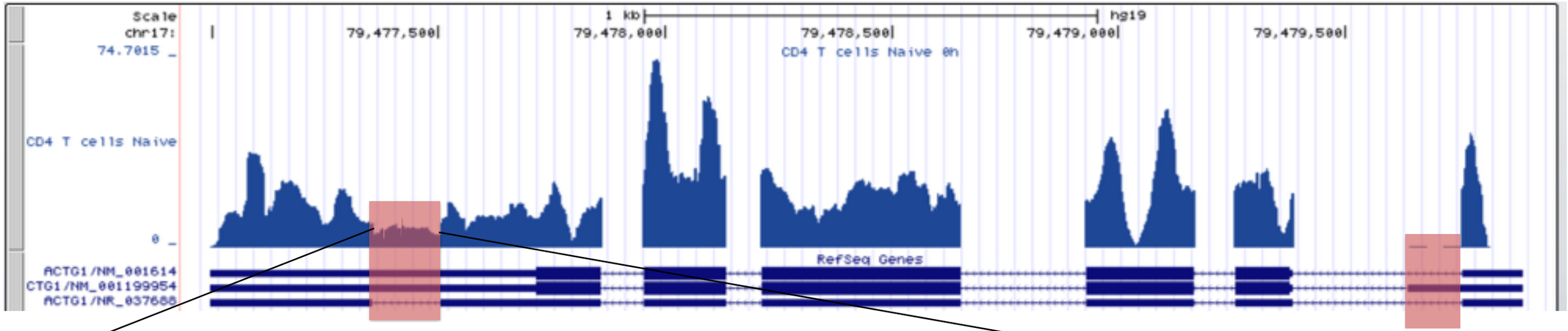
Calculating RPKMs

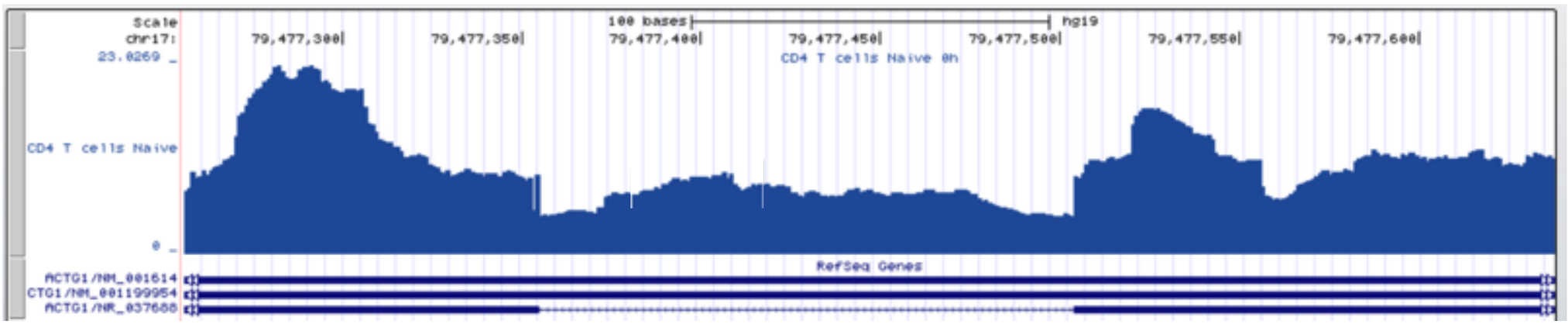


Calculating RPKMs



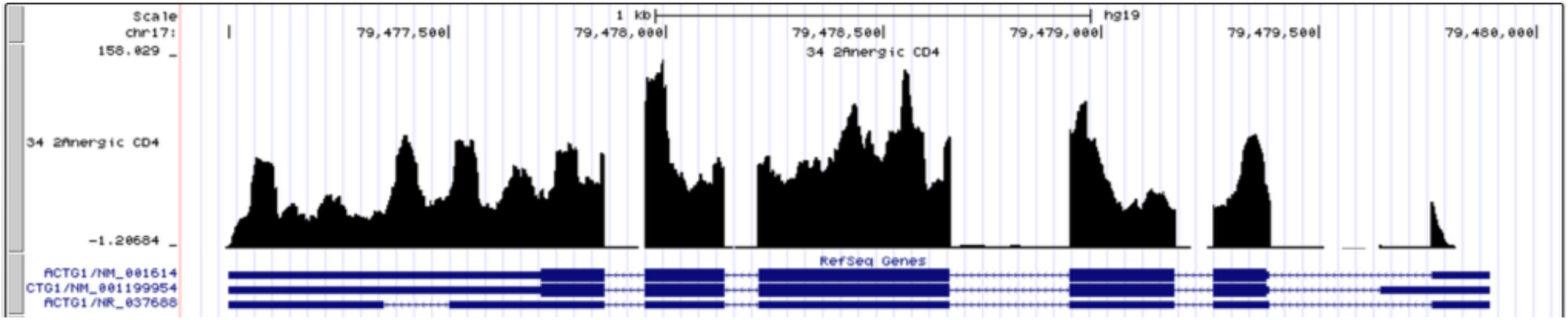
Calculating RPKMs





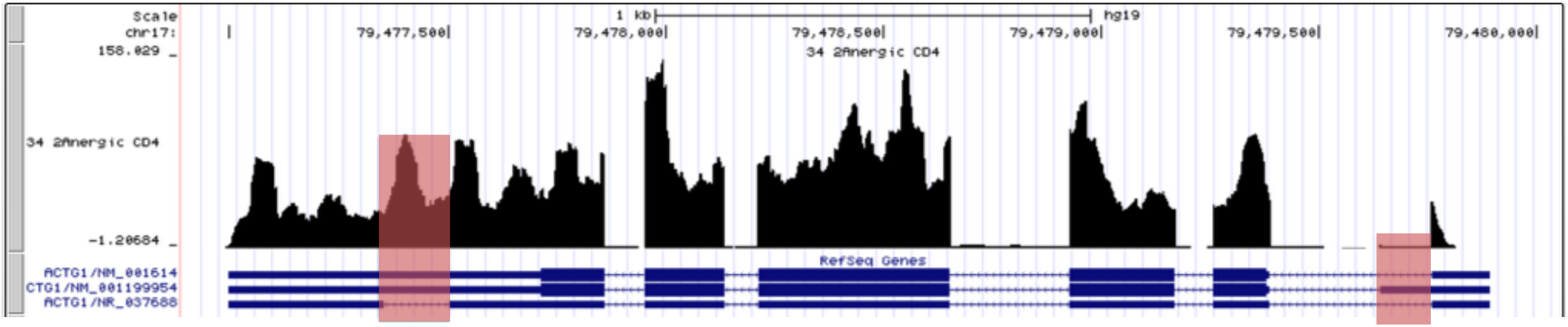
RefseqId	*GeneId*	RPKM
NR_037688	ACTG1	375.31
NM_001614	ACTG1	187.015
NM_001199954	ACTG1	4.151

Calculating RPKMs



RefseqId	*GeneId*	RPKM
NM_001614	ACTG1	994.948
NR_037688	ACTG1	118.744
NM_001199954	ACTG1	14.3

Calculating RPKMs

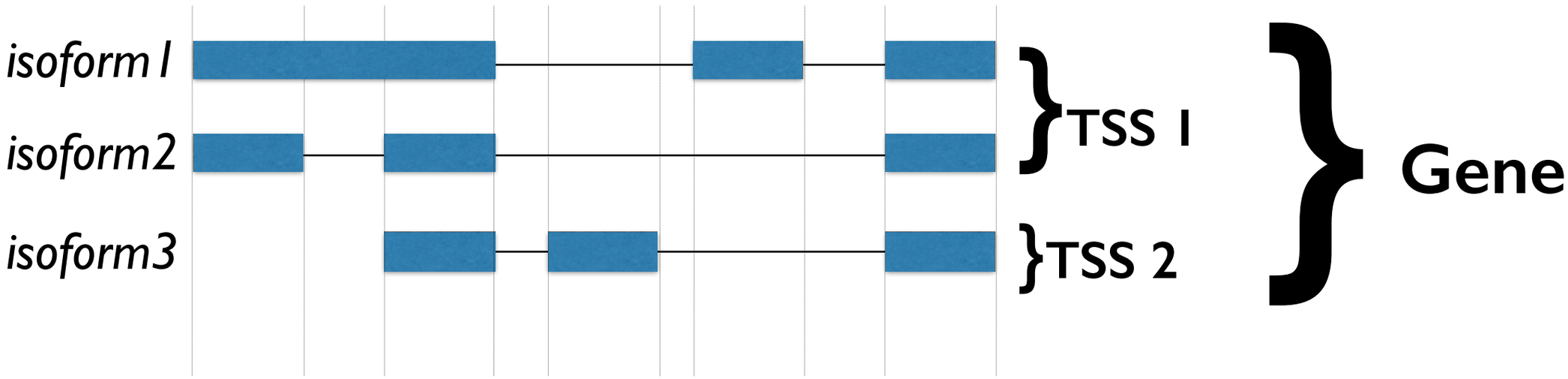


RefseqId	*GeneId*	RPKM
NM_001614	ACTG1	994.948
NR_037688	ACTG1	118.744
NM_001199954	ACTG1	14.3

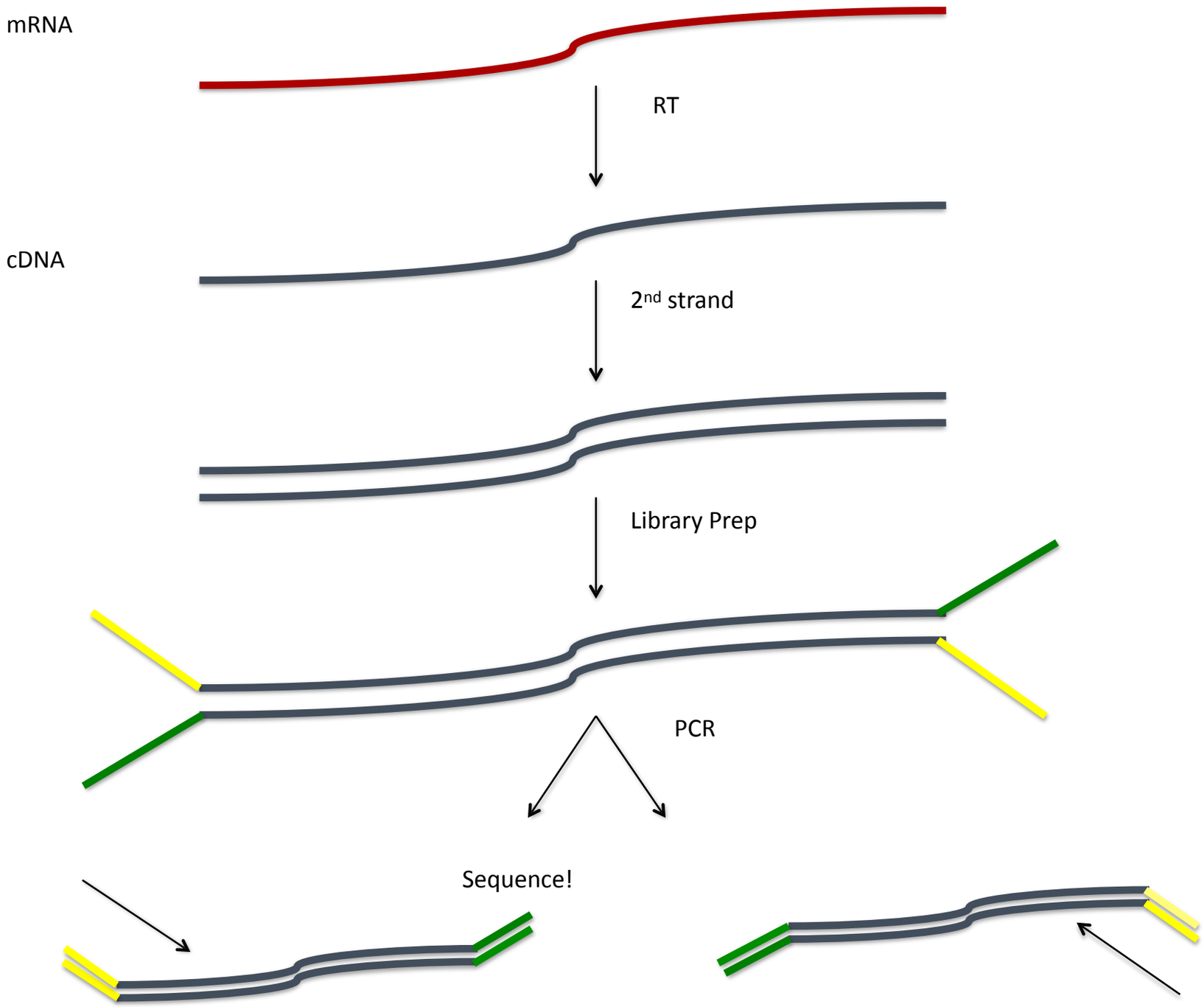
Different RPKM grouping

With respect to annotation, RPKMs can be grouped by

- Isoforms
- Gene name, sum up all isoform's RPKMs for the same gene name
- Transcription Start Site, sum up all isoform's RPKMs for common TSS

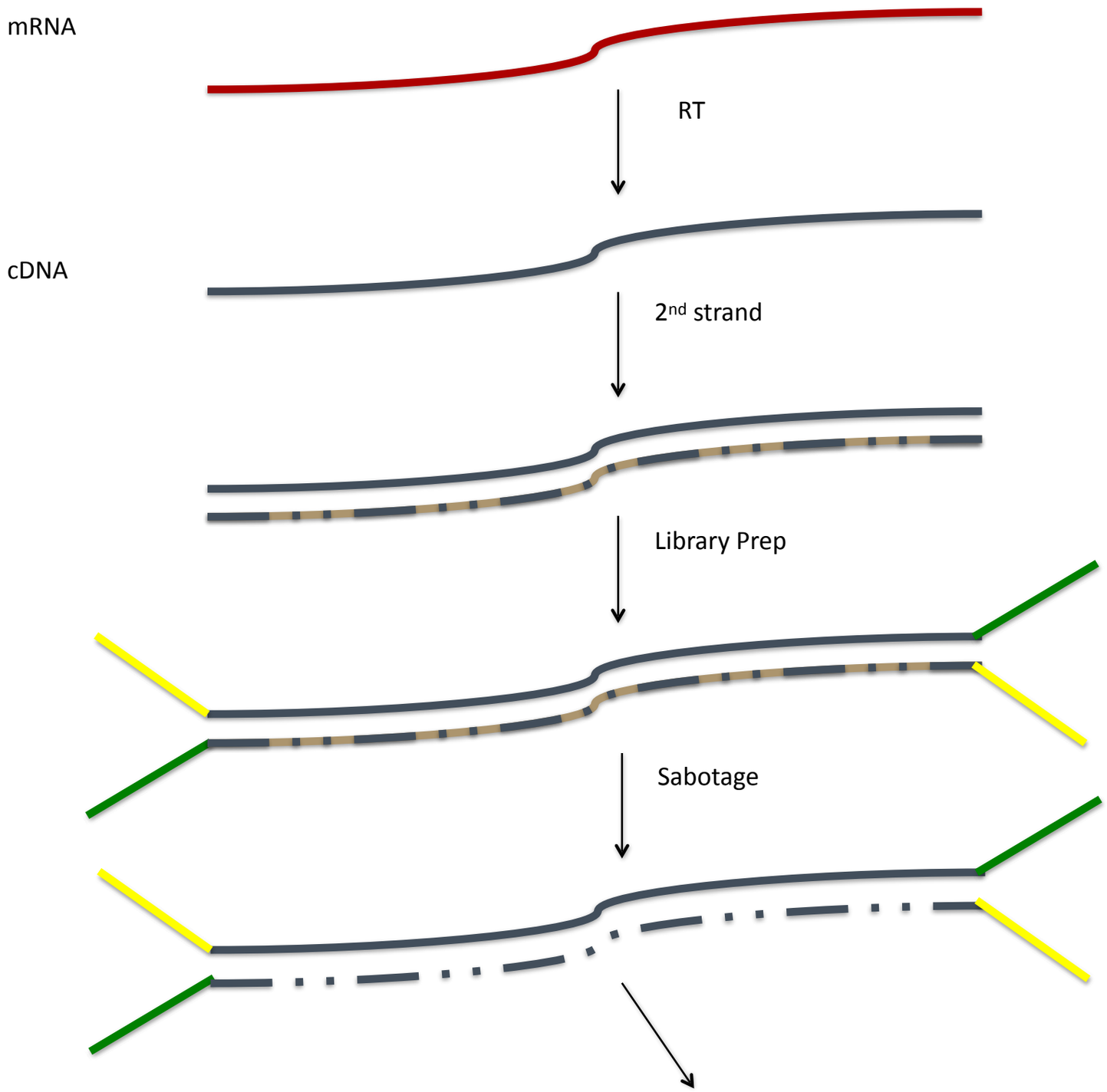


Mapping dUTP RNA-Seq libraries



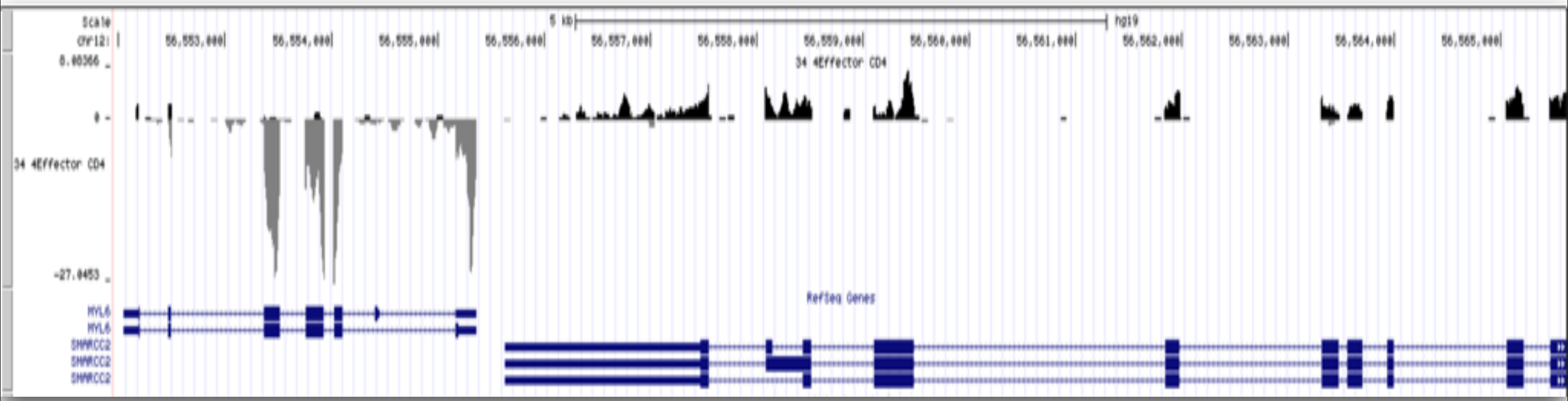
Phillip Dexheimer

Mapping dUTP RNA-Seq libraries



Phillip Dexheimer

Mapping dUTP RNA-Seq libraries



Distinguishing reads between positive and negative strand helps to increase accuracy.

RPKMs List

Experiment basic data

Change info | Processed data | Genome browser | RPKM list

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RefseqId	GeneId	chrom	txStart	txEnd	strand	RPKM
NM_001030	RPS27	chr1	153963239	153964631	+	10983.7
NM_001035267,NM_021104	RPL41	chr12	56510374	56511616	+	6310.114
NM_001402	EEF1A1	chr6	74225473	74230755	-	6214.92
NM_003295	TPT1	chr13	45911304	45915297	-	5689.13
NR_026712	RPL13AP5	chr19	49990865	49995096	+	5254.85
NM_001015	RPS11	chr19	49999622	50002969	+	5191.6
NR_039666	MIR4461	chr5	134263729	134263802	+	5002.48
NM_000980	RPL18A	chr19	17970687	17974133	+	4583.93
NM_001004	RPLP2	chr11	809936	812876	+	4492.28
NM_001020	RPS16	chr19	39923847	39926618	-	4132.23
NM_000989	RPL30	chr8	99053938	99057818	-	4040.19
NM_001003,NM_213725	RPLP1	chr15	69745159	69747884	+	3894.541
NM_001028	RPS25	chr11	118886422	118889057	-	3763.92
NM_000991,NM_001136134,NM_0...	RPL28	chr19	55897300	55903451	+	3709.856
NM_000967,NM_001033853	RPL3	chr22	39708887	39715670	-	3661.28
NM_000998	RPL37A	chr2	217363520	217366188	+	3632.94
NM_022551	RPS18	chr6	33239852	33244281	+	3587.15
NM_000975,NM_001199802	RPL11	chr1	24018269	24022915	+	3561.91
NM_002952	RPS2	chr16	2012062	2014827	-	3529.34
NM_000981	RPL19	chr17	37356536	37360980	+	3391.06
NM_000999,NM_001035258	RPL38	chr17	72199795	72206019	+	3320.06
NM_001016	RPS12	chr6	133135708	133138703	+	3230.98
NM_004048	B2M	chr15	45003685	45010357	+	3149.52
NM_001010	RPS6	chr9	19376254	19380235	-	3080.08
NM_001031	RPS28	chr19	8386384	8387280	+	2808.47

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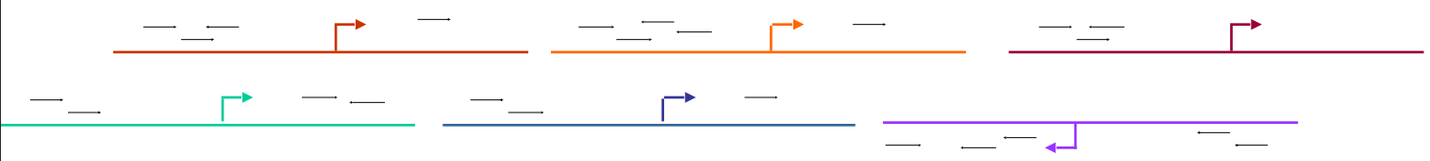
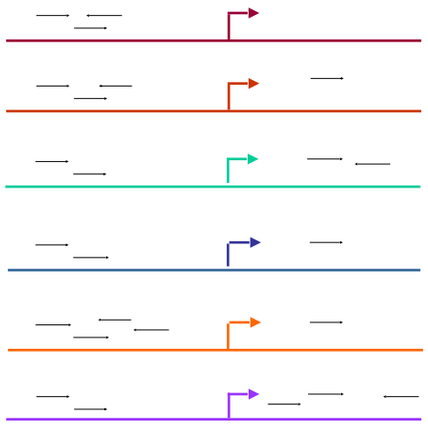
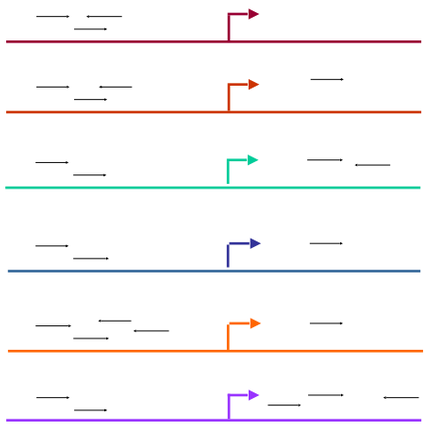
Average tag density profiles as part of the quality control



Average tag density profiles as part of the quality control

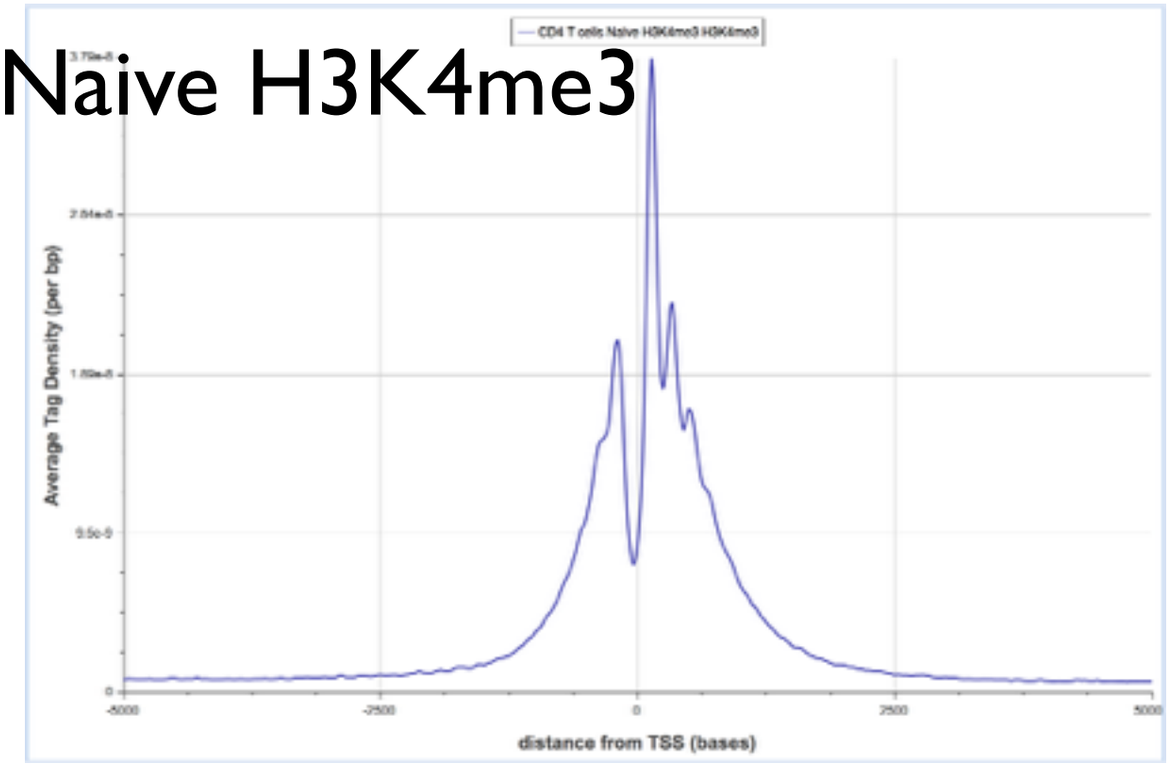


Average tag density profiles as part of the quality control

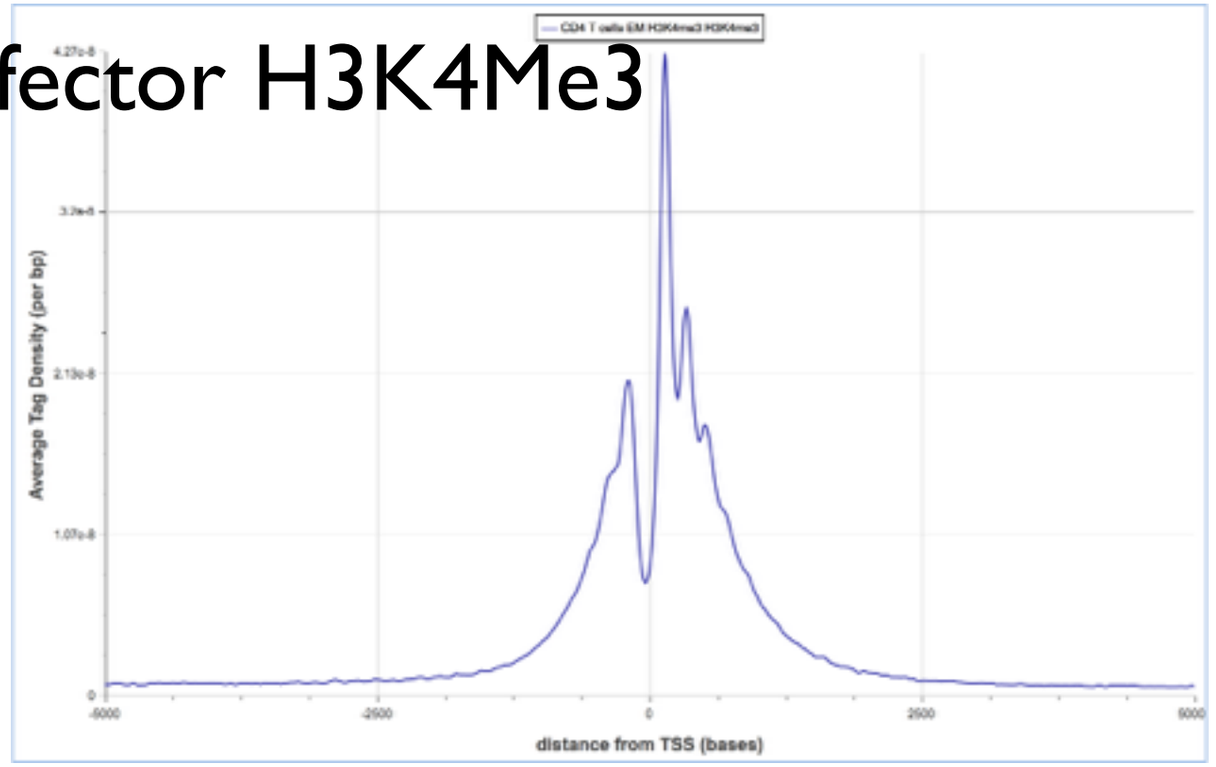


Same lvl of enrichment,
allows direct comparison

Naive H3K4me3



Effector H3K4Me3



Islands: MACS output

Experiment basic data

Change info | Processed data | Genome browser | Islands list | Average Tag Density | Islands Distribution

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chrom	start	end	length	pileup	abssummit	log10p	foldenrich	log10q
chr8	103540671	103541261	591	90	103540903	110.86	28.918	101.671
chr6	27356237	27357176	940	88	27356587	90.386	20.231	82.753
chr1	100231906	100232260	355	67	100232075	87.554	28.618	80.037
chr7	66385785	66386133	349	91	66385955	87.471	18.036	80.014
chr12	4714005	4714507	503	71	4714129	81.013	22.981	73.867
chr17	19266076	19266320	245	61	19266195	79.12	27.032	72.1
chr3	113775628	113776193	566	82	113775736	78.121	17.298	71.145
chr11	31531338	31532072	735	82	31531464	75.95	16.449	69.113
chr3	143566514	143567324	811	80	143566755	75.937	17.077	69.104
chr1	39491775	39492761	987	77	39492150	75.863	18.07	69.058
chr2	122732810	122733078	269	53	122732936	75.826	29.357	69.03
chr17	48449923	48450555	633	64	48450425	75.474	23.421	68.697
chr3	9290167	9291349	1183	77	9291044	75.196	17.787	68.448
chr6	144415939	144416715	777	77	144416572	74.804	17.621	68.085
chr1	85086328	85086758	431	58	85086623	74.367	25.879	67.683
chr2	16789987	16790447	461	61	16790332	74.274	24.266	67.593
chr13	96130960	96131384	425	57	96131085	74.066	26.232	67.411
chr18	53144545	53145368	824	71	53144818	73.916	19.474	67.271
chr11	62521392	62521691	300	60	62521489	73.605	24.4	66.984
chr7	100888117	100888388	272	70	100888251	72.641	19.275	66.083
chr19	3434996	3435253	258	62	3435125	72.56	22.814	66.008
chr10	69609279	69609962	684	63	69609419	70.941	21.465	64.492
chr19	41869785	41870112	328	62	41869925	70.561	21.731	64.151
chr19	11639475	11639946	472	54	11639813	70.135	25.511	63.744
chr12	121453800	121454272	473	57	121454131	69.484	23.59	63.107

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Islands distribution

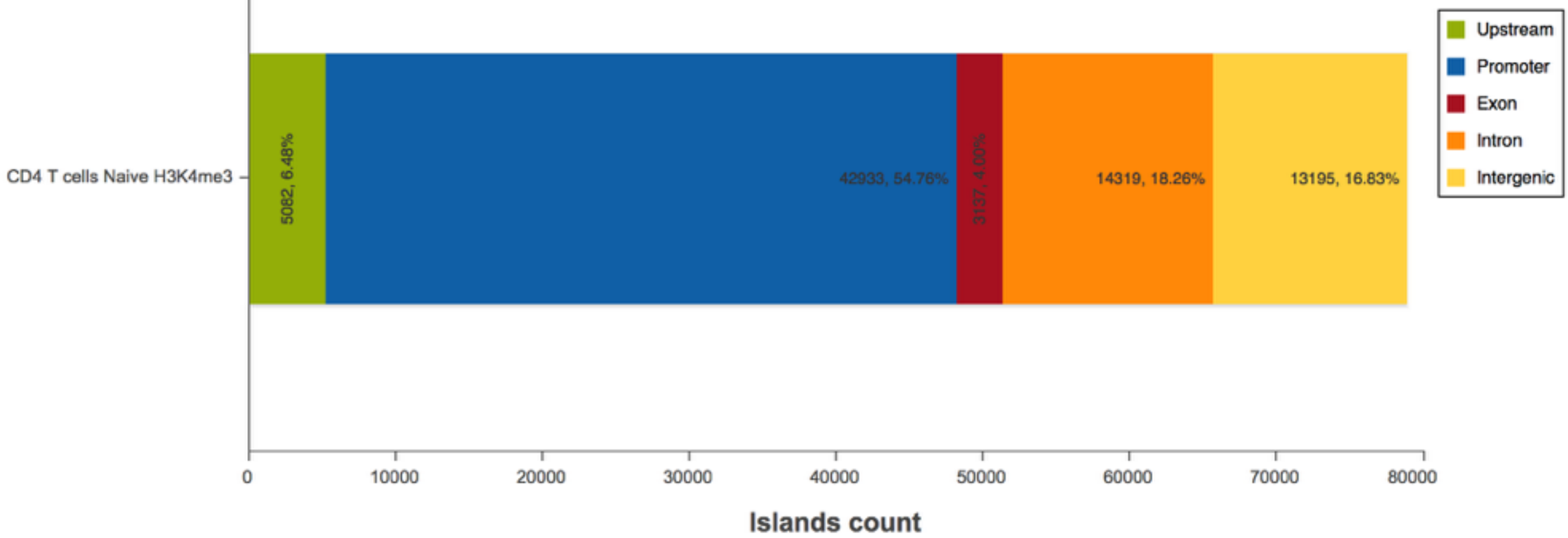
Experiment basic data

Change info | Processed data | Genome browser | Islands list | Average Tag Density | Islands Distribution

Save Chart

Islands Distribution

Upstream is [TSS-20k,TSS-2k]
Promoter is [TSS-2k,TSS+2k]



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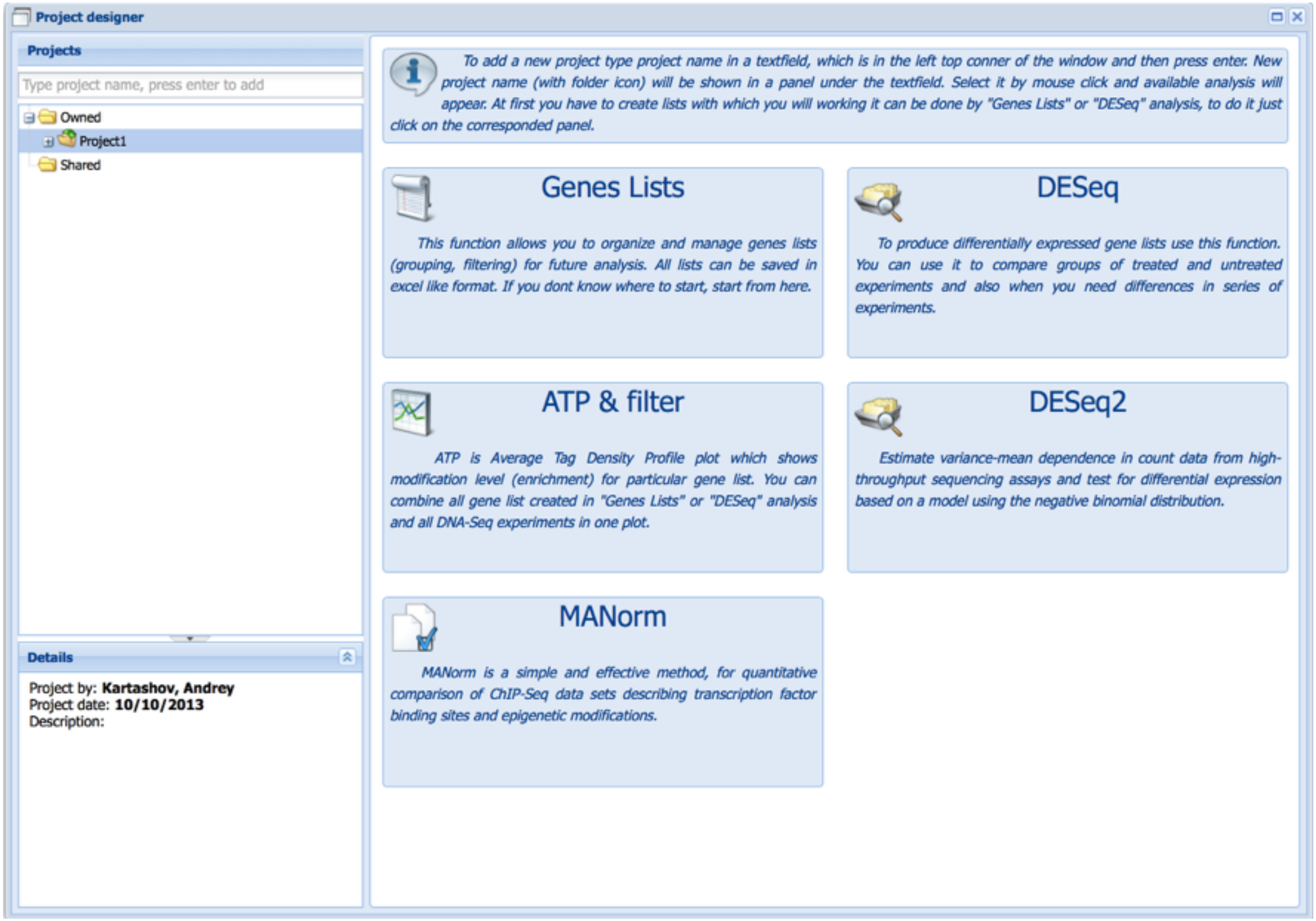
Advanced Analysis

- RNA
  - DESeq / DESeq2
- ChIP
  - MANorm / DiffBind

- RNA and ChIP
  - Average Tag Density

Project Designer

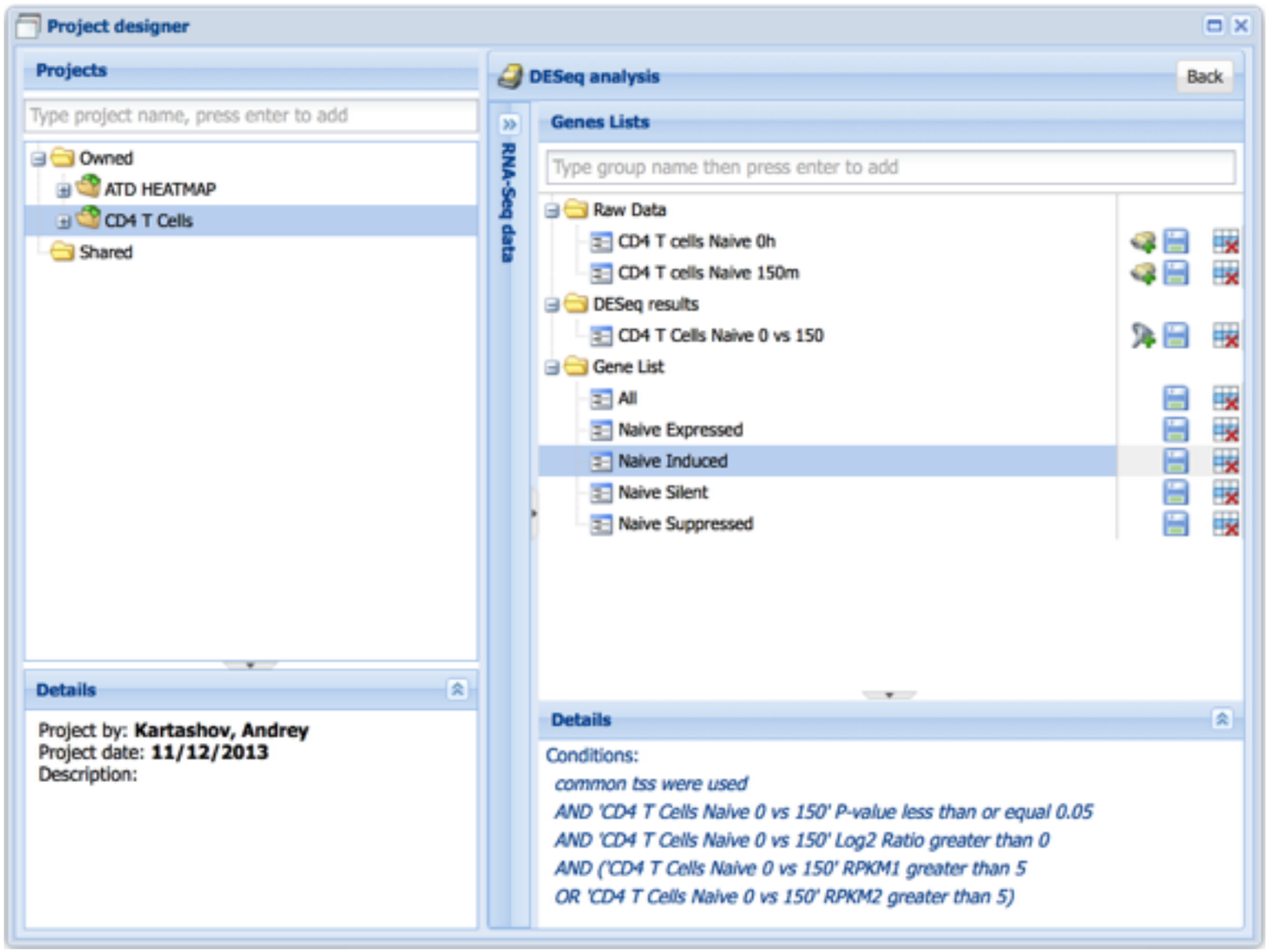


Defined lists of genes

- All - all genes grouped by TSS
- Silent are genes which expression level less then 2 RPKM at point 0
- Expressed are genes which expression level greater then 8 RPKM at point 0
- Induced are genes which expression level has been significantly increased during 150m treatment, but were silent at 0 time point
- Suppressed are genes which expression level has been significantly decreased during 150m treatment and were expressed at 0 time point

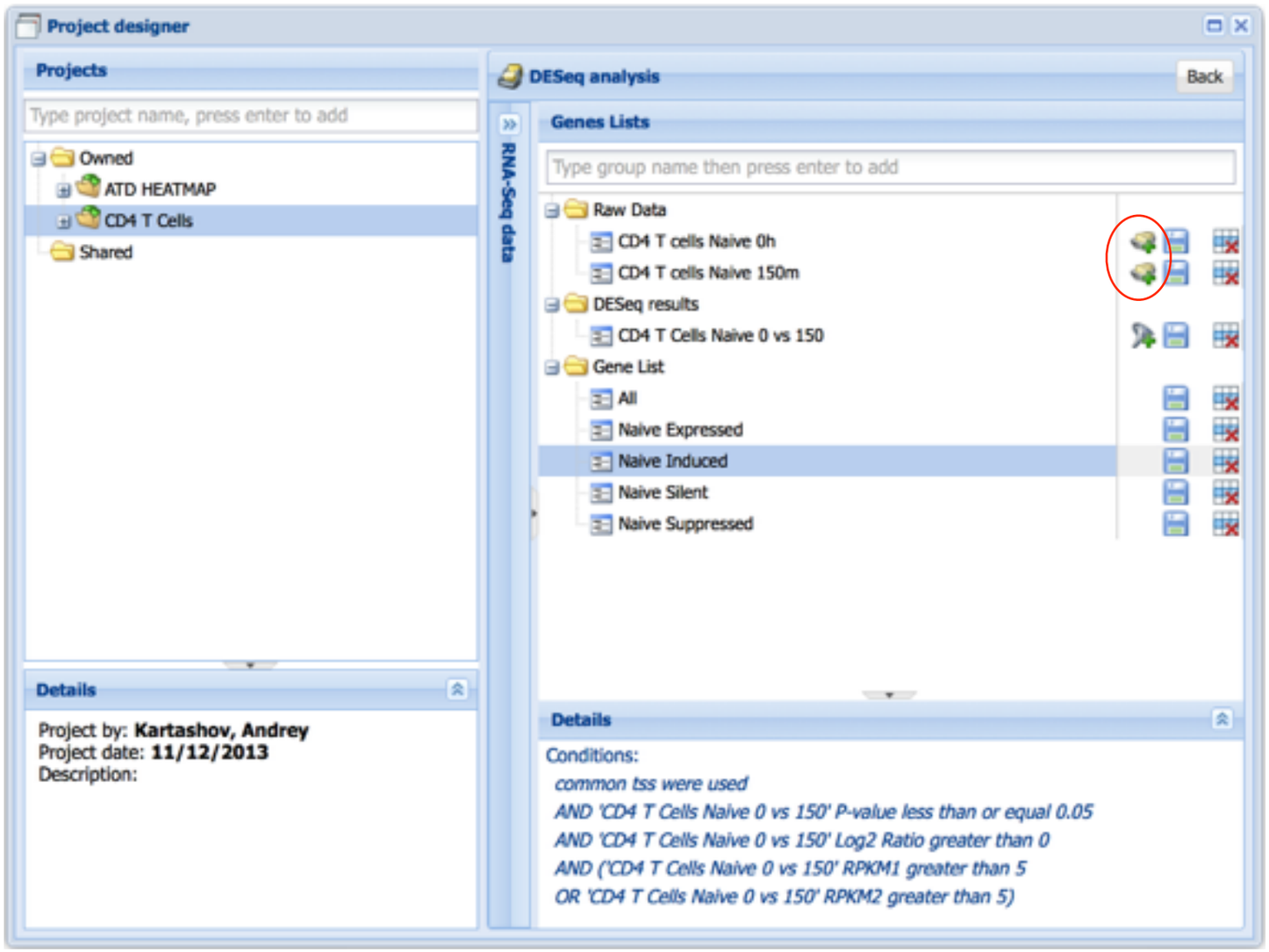
DESeq/DESeq2

(Differential Gene Expression Analysis)



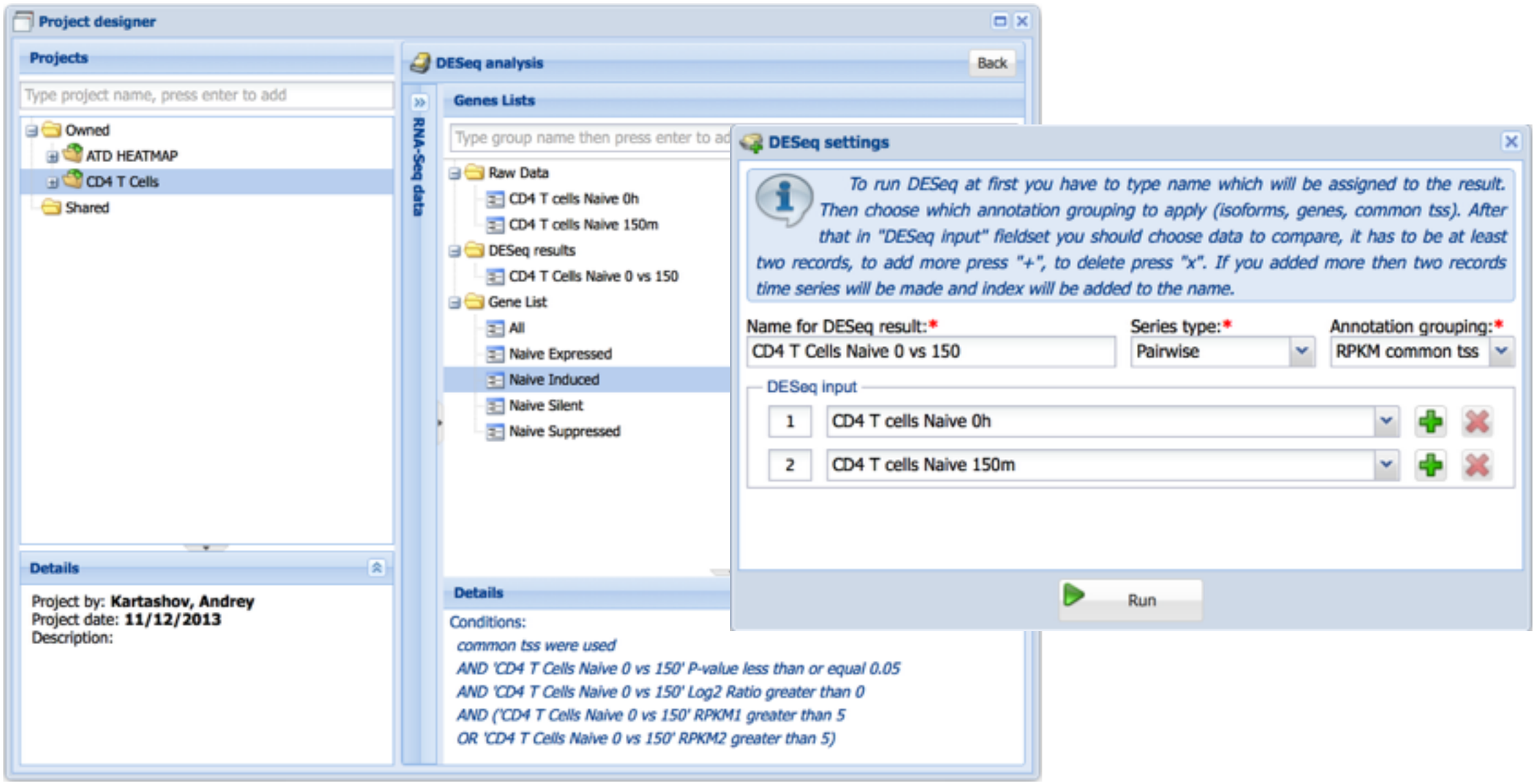
DESeq/DESeq2

(Differential Gene Expression Analysis)



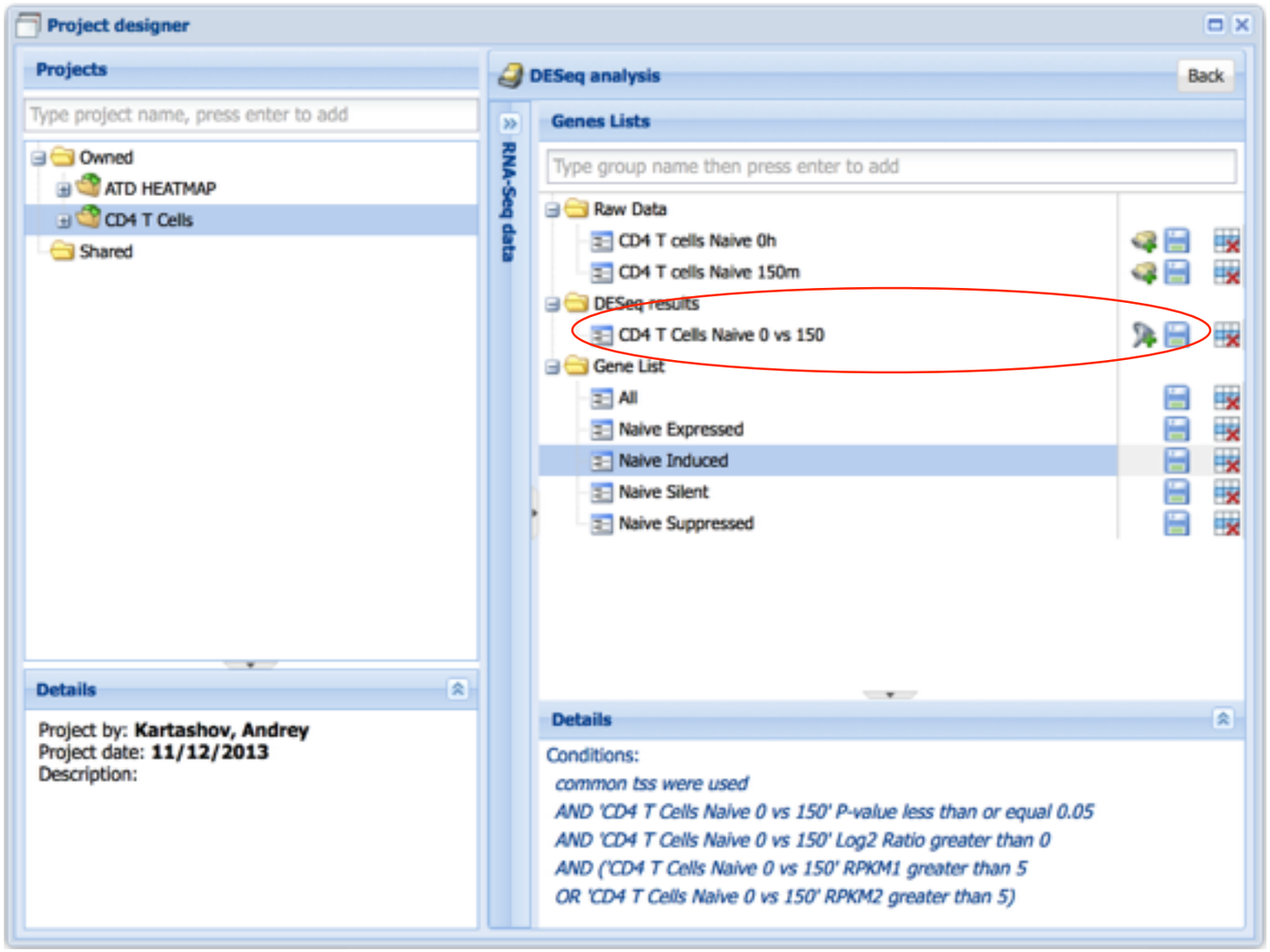
DESeq/DESeq2

(Differential Gene Expression Analysis)



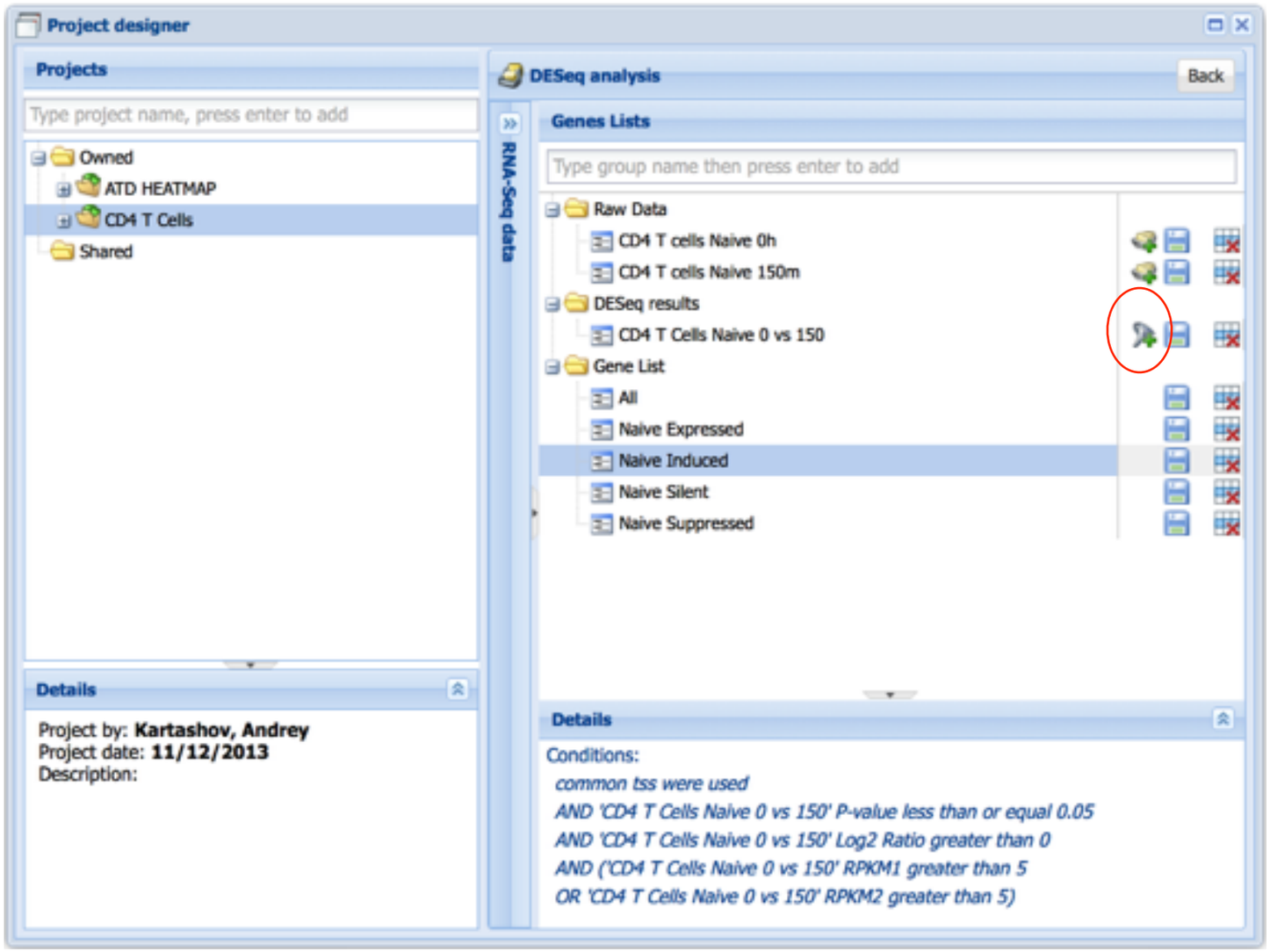
DESeq/DESeq2

(Differential Gene Expression Analysis)



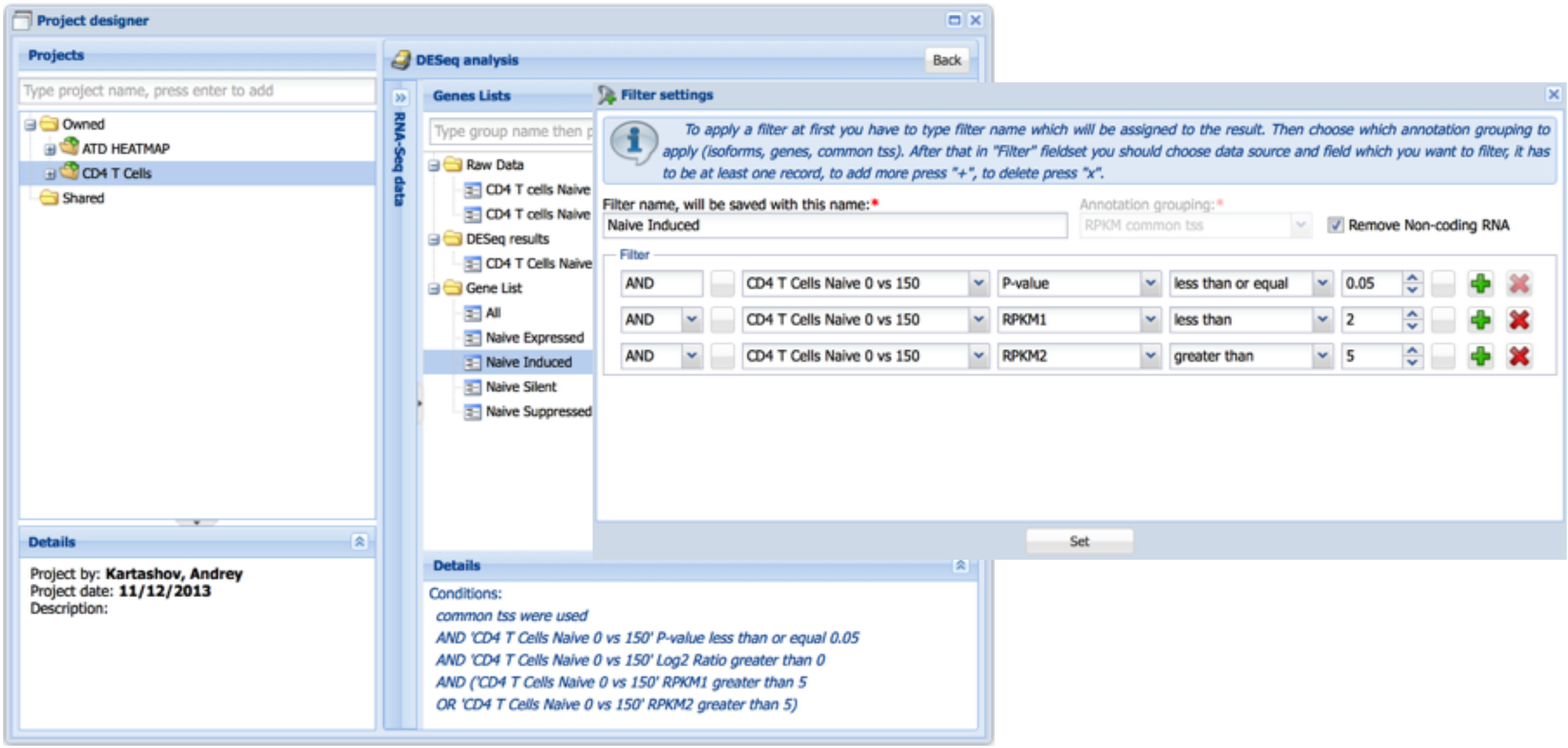
DESeq/DESeq2

(Differential Gene Expression Analysis)



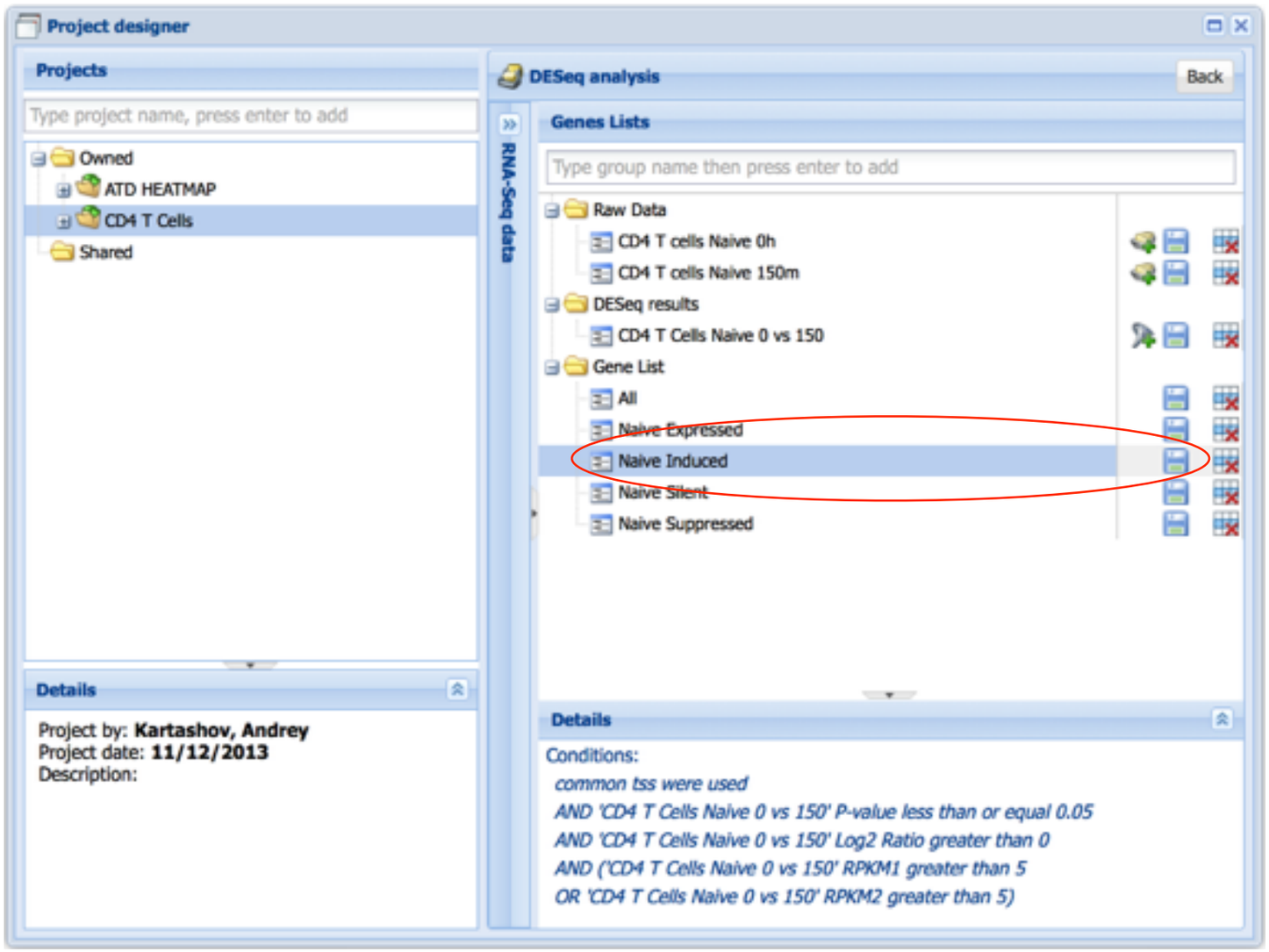
DESeq/DESeq2

(Differential Gene Expression Analysis)

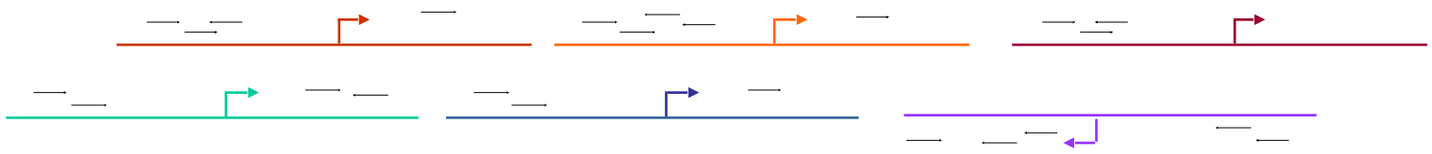


DESeq/DESeq2

(Differential Gene Expression Analysis)



Average tag density profiles are used to study groups of genes



Average tag density profiles are used to study groups of genes

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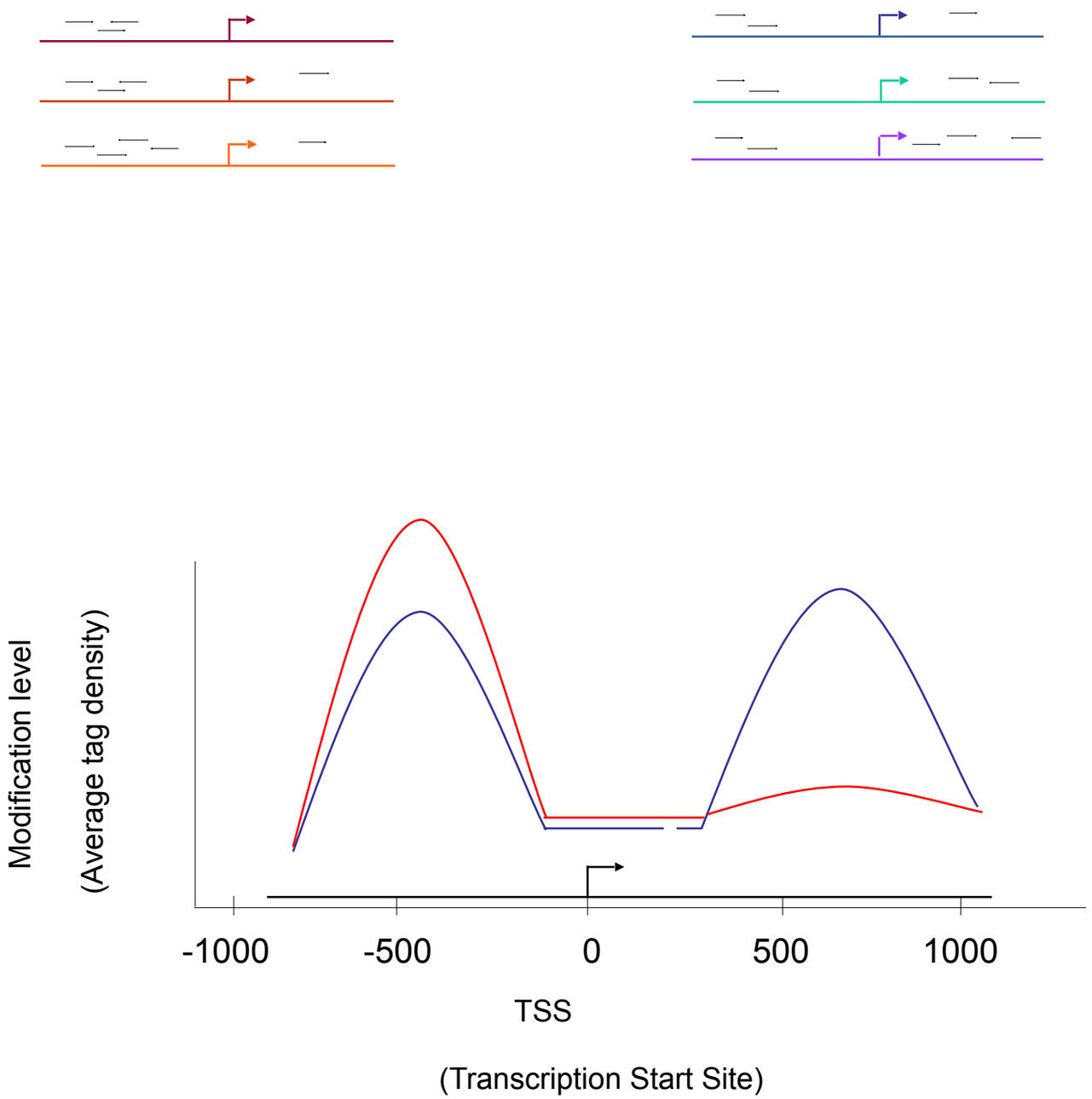
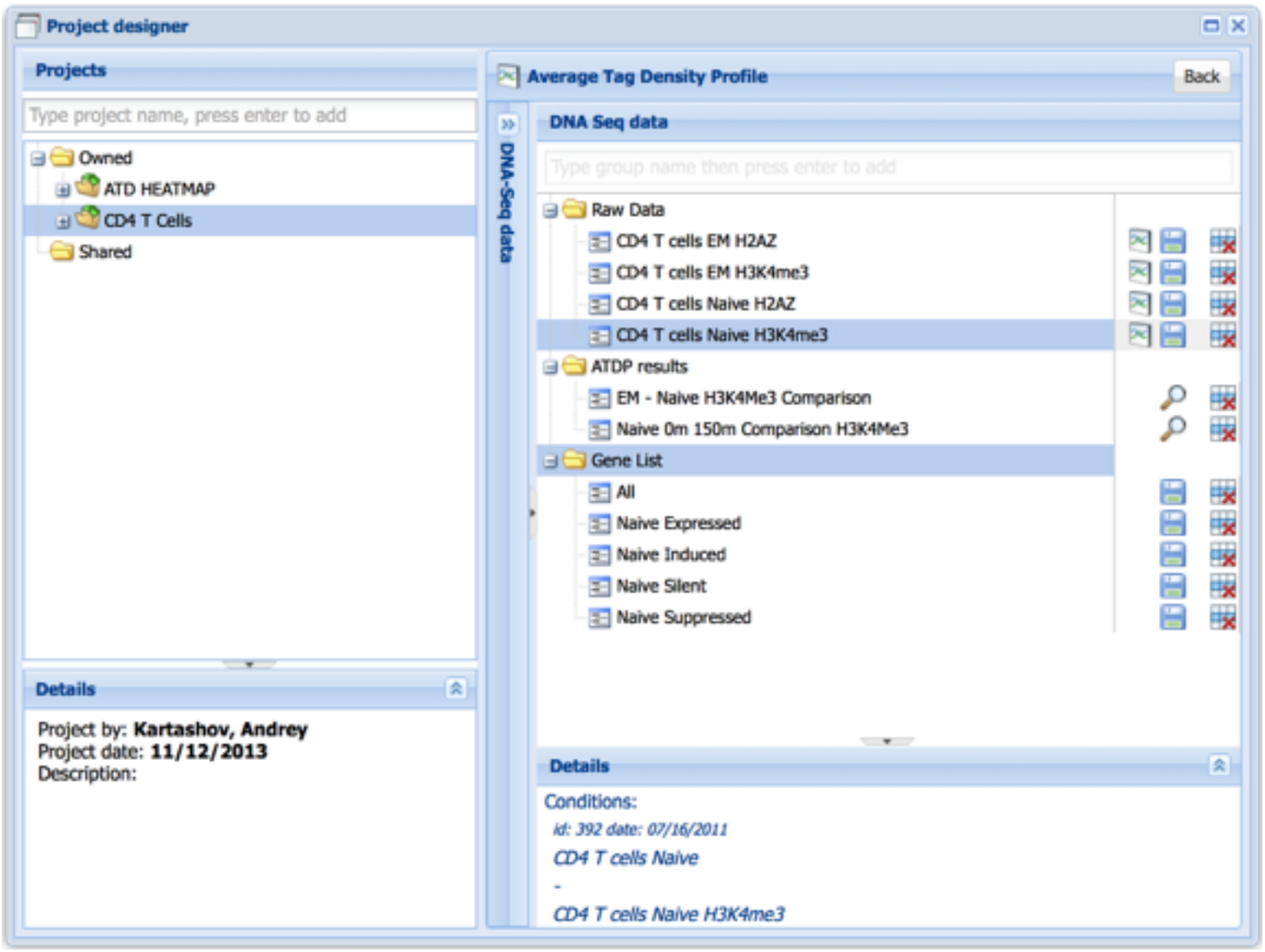


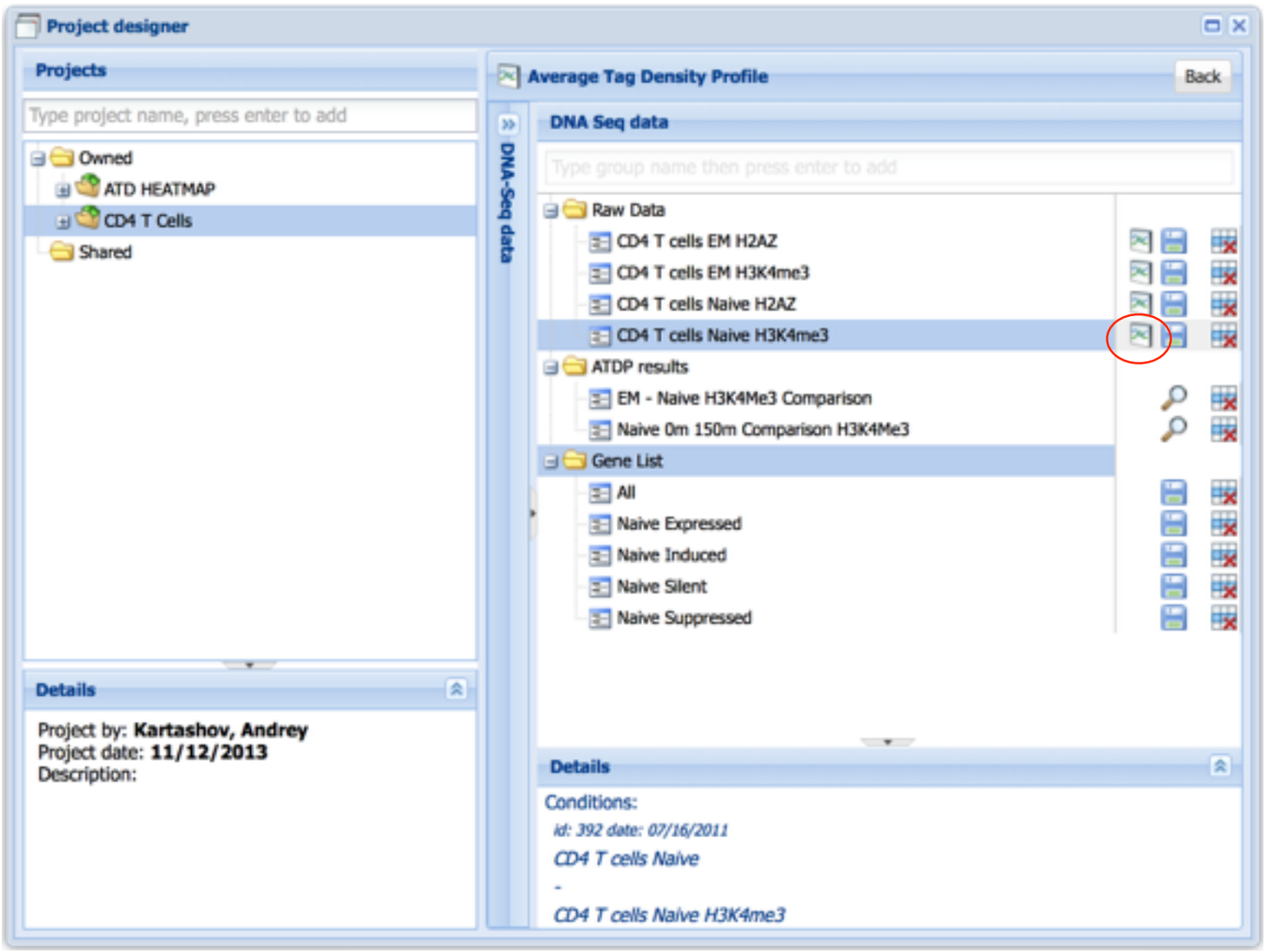
Average Tag Density Profile

(Average Tag Density Profiles are used to study groups of genes)



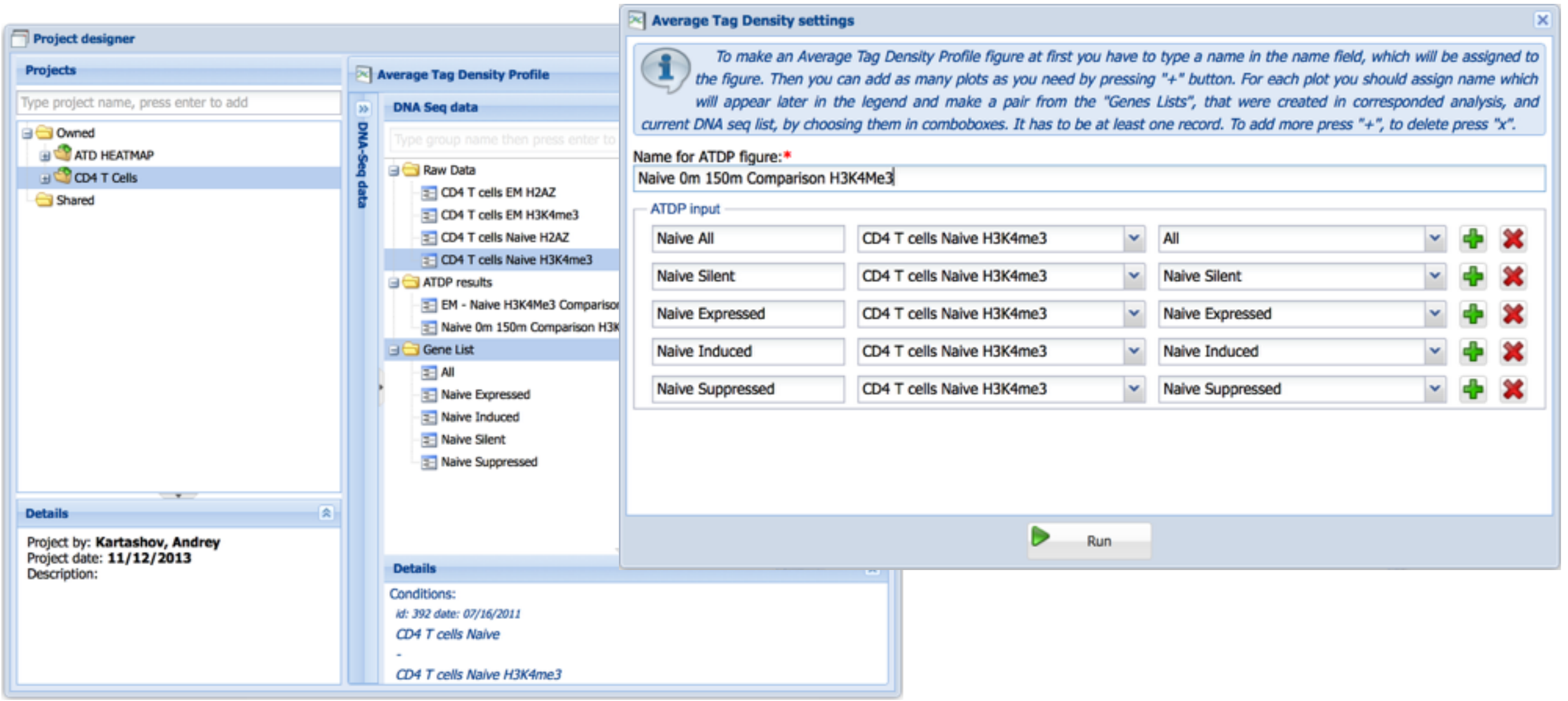
Average Tag Density Profile

(Average Tag Density Profiles are used to study groups of genes)



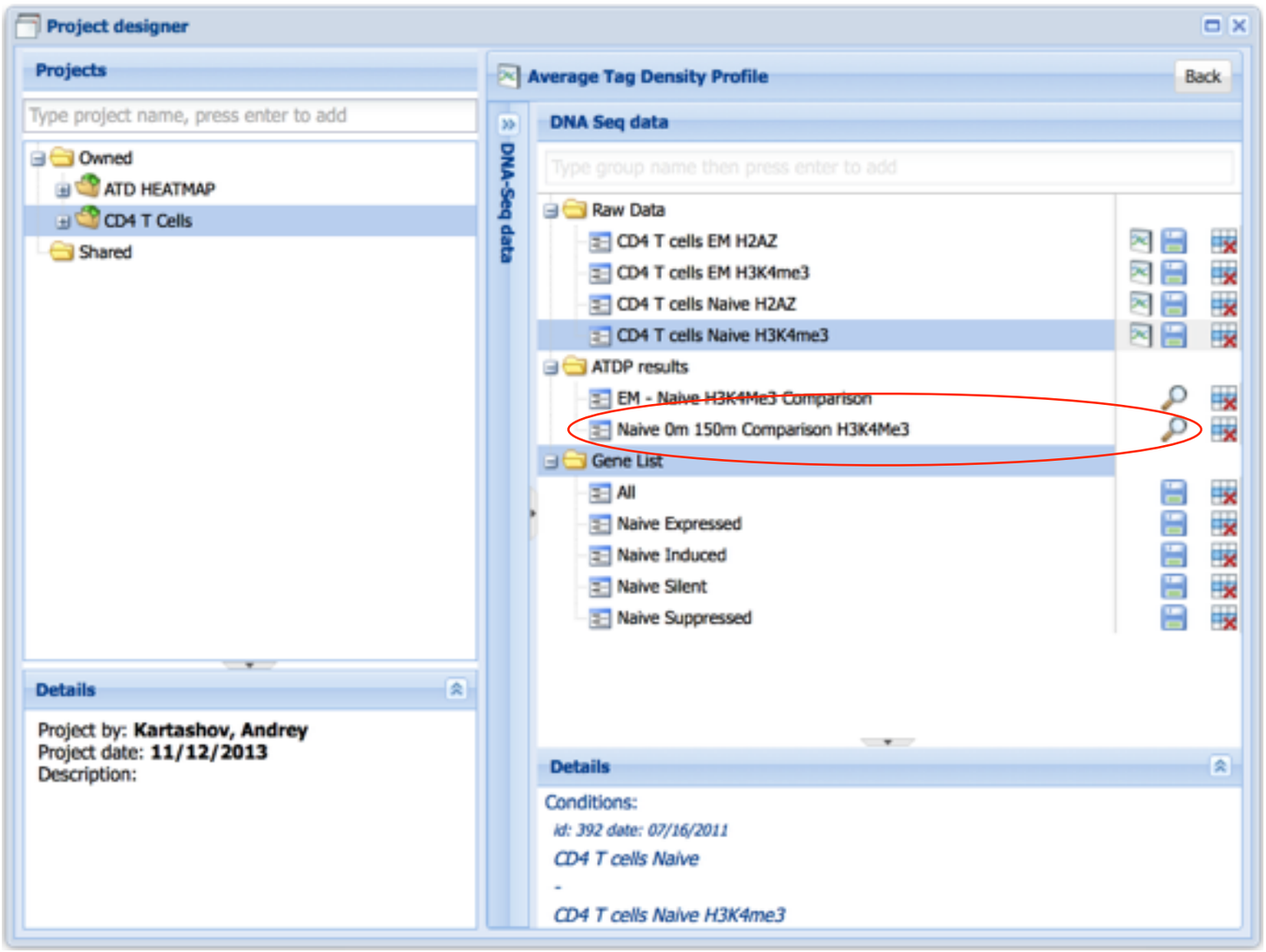
Average Tag Density Profile

(Average Tag Density Profiles are used to study groups of genes)



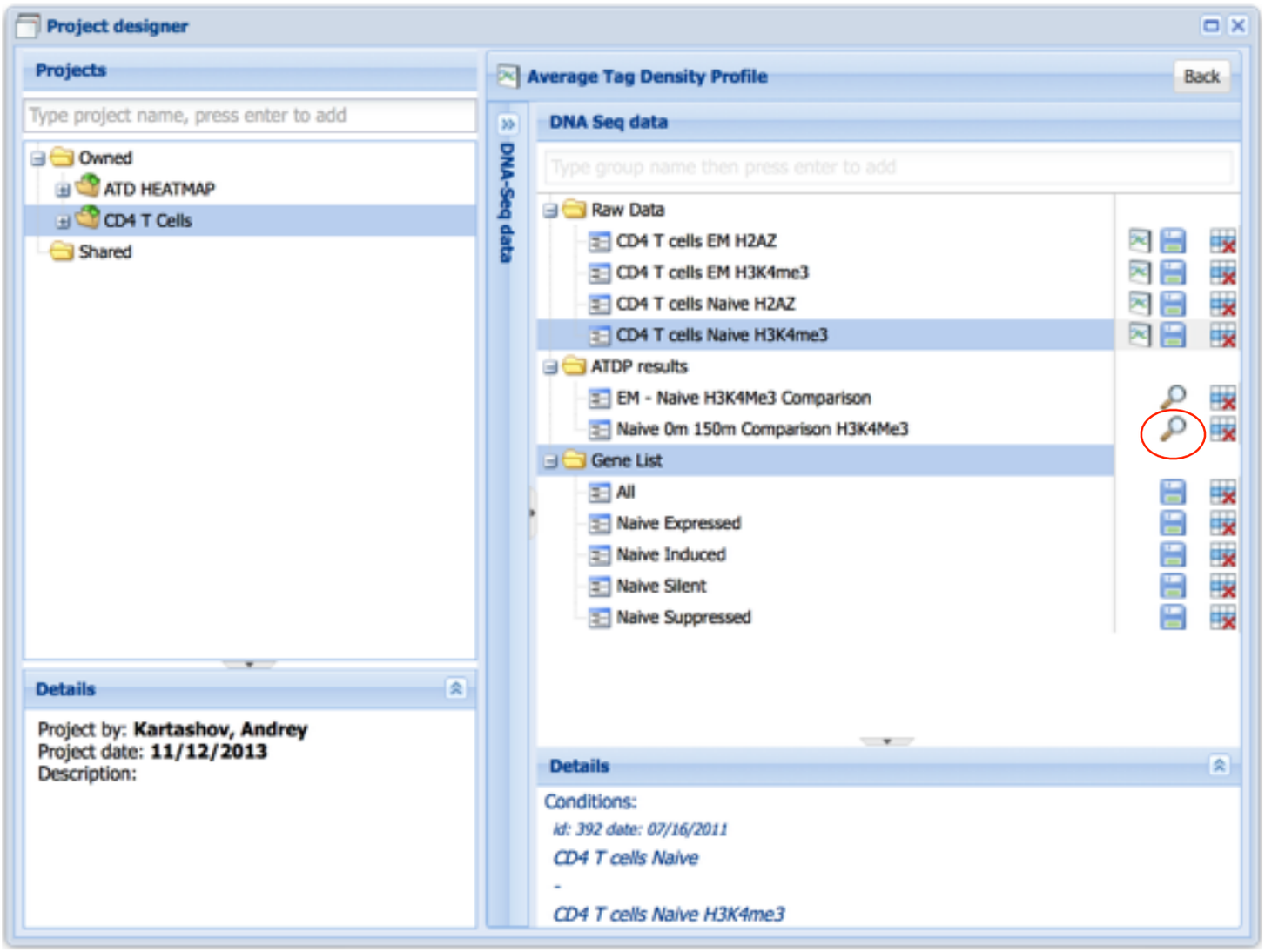
Average Tag Density Profile

(Average Tag Density Profiles are used to study groups of genes)



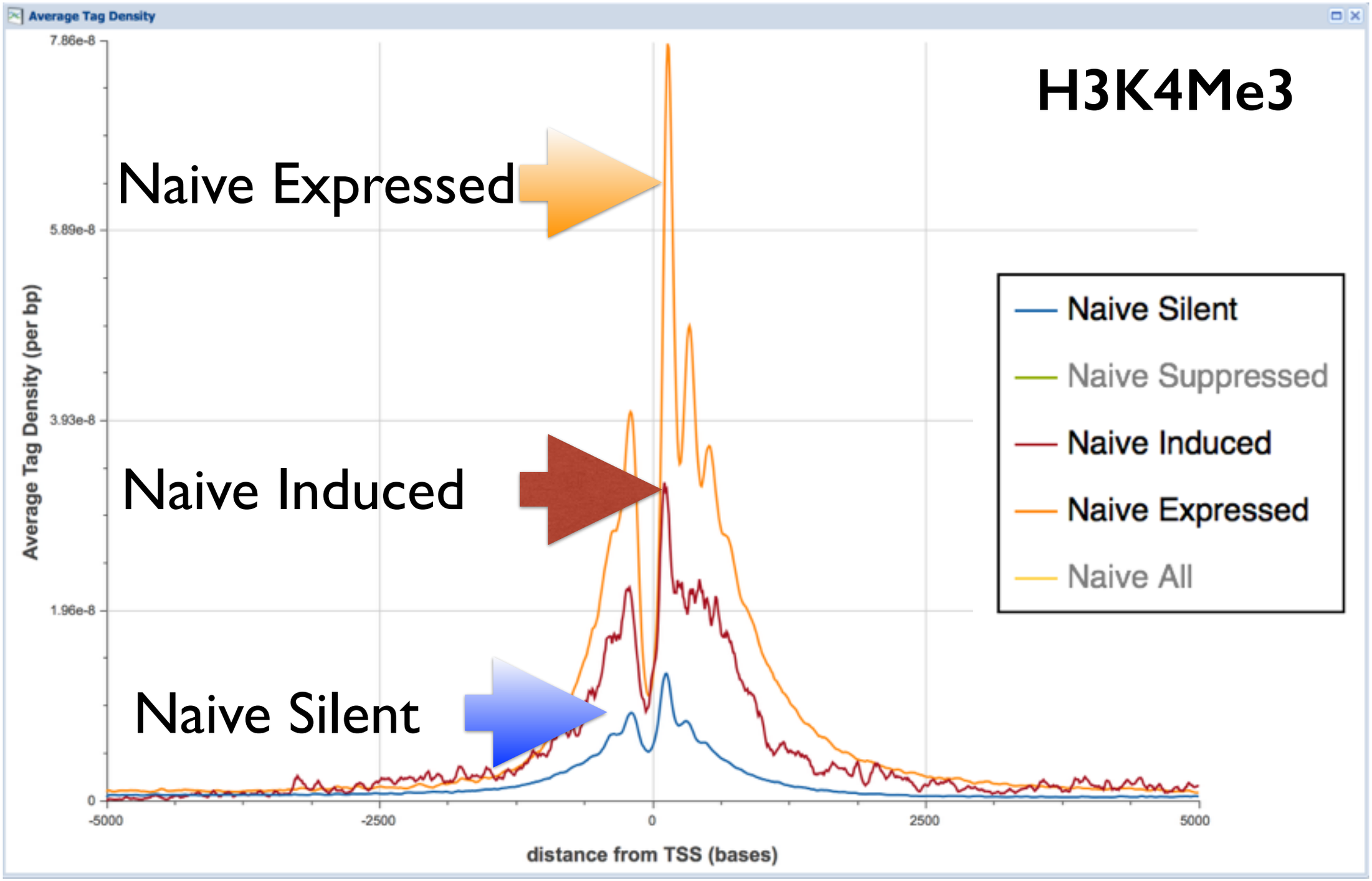
Average Tag Density Profile

(Average Tag Density Profiles are used to study groups of genes)



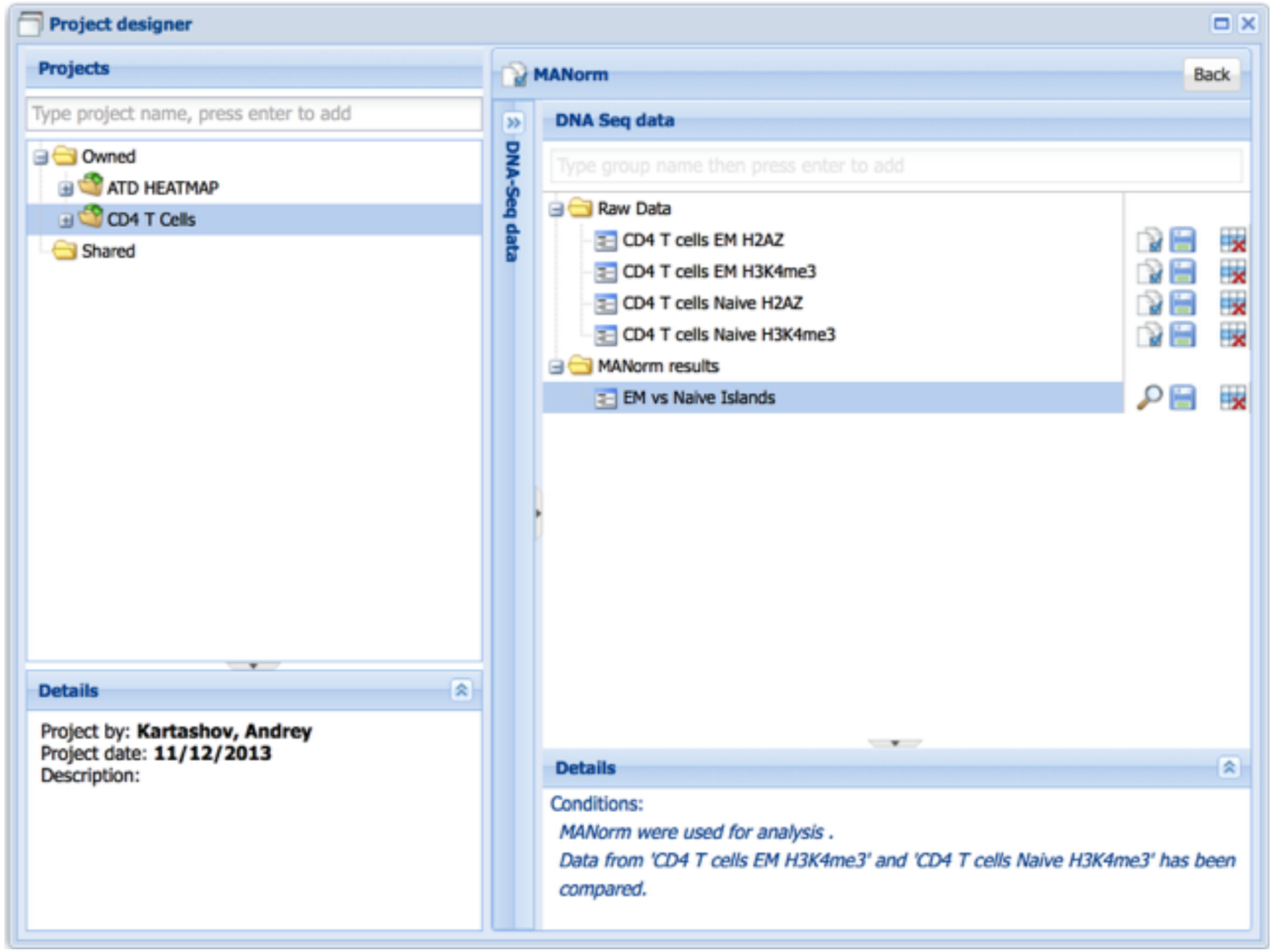
Average Tag Density Profile

(Average Tag Density Profiles are used to study groups of genes)



To compare ChIP-Seq experiments with the same modification we use MANorm

MANorm


Project designer
Projects
Type project name, press enter to add
Owned
ATD HEATMAP
CD4 T Cells
Shared
Details
Project by: Kartashov, Andrey
Project date: 11/12/2013
Description:
MANorm
Back
DNA-Seq data
DNA Seq data
Type group name then press enter to add
Raw Data
CD4 T cells EM H2AZ
CD4 T cells EM H3K4me3
CD4 T cells Naive H2AZ
CD4 T cells Naive H3K4me3
MANorm results
EM vs Naive Islands
Details
Conditions:
MANorm were used for analysis .
Data from 'CD4 T cells EM H3K4me3' and 'CD4 T cells Naive H3K4me3' has been compared.

MANorm

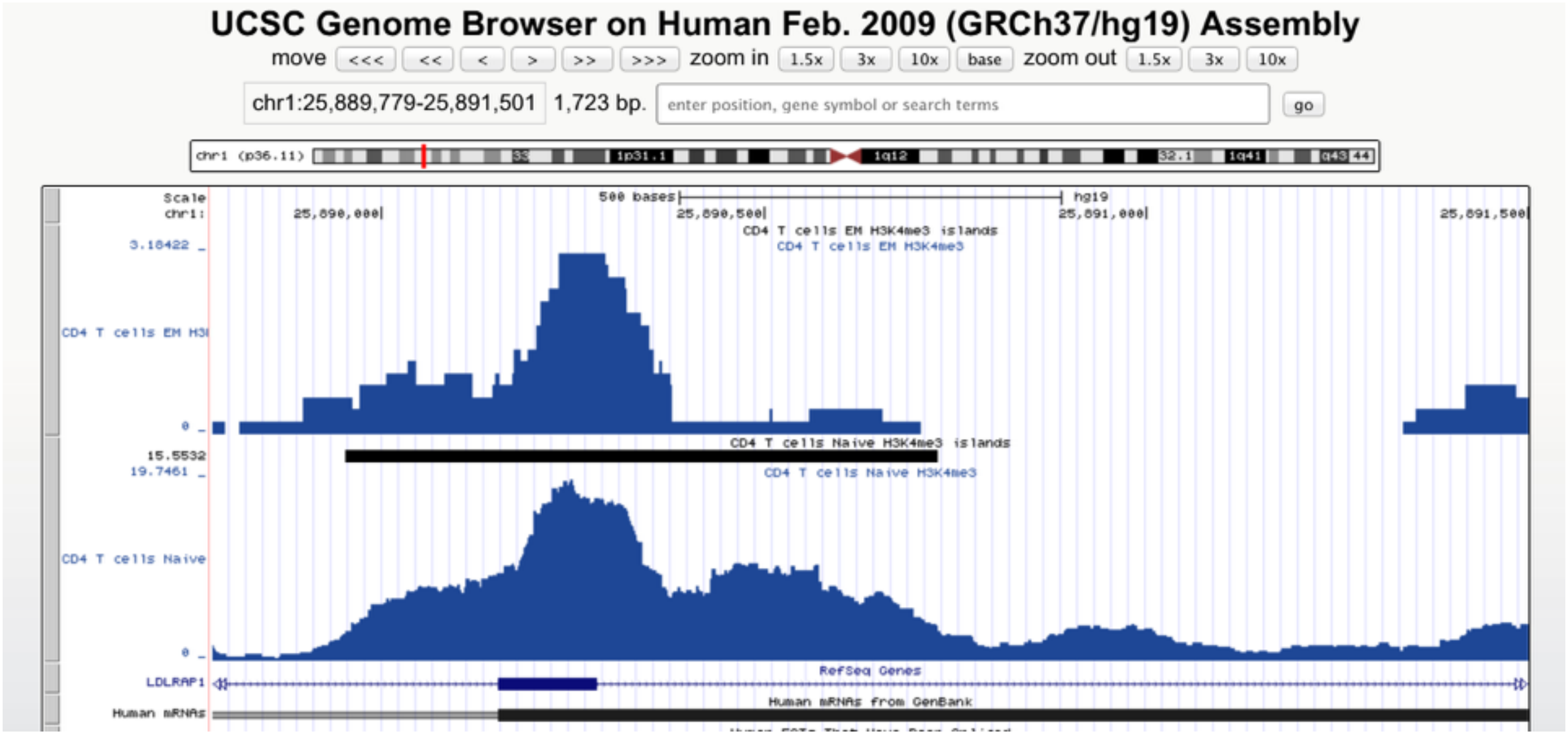
View table data

Page 1 of 33 | Displaying 1 - 100 of 3265 | jump

chrom	start	end	description	raw_read1	raw_read2	M_value_rescaled	A_value_rescaled	log10_p_value
chr15	70850864	70851764	merged_commo...	18	608	-5.0341625213...	6.7332172393799	152.06977844238
chr19	8641148	8642301	merged_commo...	592	71	2.9776721000671	7.6587610244751	95.603507995605
chr14	52734423	52735852	merged_commo...	433	37	3.45228099823	6.9740681648254	81.021423339844
chr10	51501790	51502742	unique_peak2	16	314	-4.242486000061	6.1779651641846	70.83243560791
chr12	6554077	6555005	merged_commo...	63	442	-2.8344254493...	7.3739500045776	70.400085449219
chr20	1665867	1666685	unique_peak2	5	258	-5.4527497291...	5.2904334068298	67.336303710938
chr2	100758267	100759097	merged_commo...	25	319	-3.656242609024	6.4938068389893	64.936973571777
chr20	24929770	24931051	merged_commo...	258	7	4.9603414535522	5.4801707267761	63.424602508545
chr13	100067035	100067608	unique_peak2	0	189	-7.57383537292...	3.7829377651215	54.784824371338
chr19	6668725	6669258	unique_peak1	217	5	5.1283826828003	5.1491537094116	54.70911026001
chr19	6668725	6669258	unique_peak1	217	5	5.1283826828003	5.1491537094116	54.70911026001
chr4	6691267	6692235	unique_peak1	187	1	6.501148223877	4.2505741119385	52.395317077637
chr2	75643547	75644467	unique_peak2	0	180	-7.50382566452...	3.7479329109192	52.096687316895
chr2	75643547	75644467	unique_peak2	0	180	-7.50382566452...	3.7479329109192	52.096687316895
chr2	75643547	75644467	unique_peak2	0	180	-7.50382566452...	3.7479329109192	52.096687316895
chr19	51875246	51876115	unique_peak1	191	2	5.9463605880737	4.5581426620483	51.788089752197
chr8	79716585	79717910	merged_commo...	408	75	2.3672471046448	7.4315509796143	51.5087890625
chr1	25889952	25890728	unique_peak2	25	258	-3.3511228561...	6.3412470817566	48.783340454102
chr1	25889952	25890728	unique_peak2	25	258	-3.3511228561...	6.3412470817566	48.783340454102
chr9	139869720	139870746	unique_peak1	178	2	5.8458762168884	4.5079007148743	48.233657836914
chr9	139869720	139870746	unique_peak1	178	2	5.8458762168884	4.5079007148743	48.233657836914
chr19	4301693	4302614	unique_peak2	6	192	-4.8074622154...	5.1887259483337	47.043758392334
chr19	4301693	4302614	unique_peak2	6	192	-4.8074622154...	5.1887259483337	47.043758392334
chr17	2698896	2700668	merged_commo...	849	327	1.3060750961304	9.0105895996094	46.456298828125
chr4	22516606	22517279	unique_peak2	3	170	-5.4349265098...	4.7003893852234	44.409236907959
chr4	22516606	22517279	unique_peak2	3	170	-5.4349265098...	4.7003893852234	44.409236907959
chr4	22516606	22517279	unique_peak2	3	170	-5.4349265098...	4.7003893852234	44.409236907959

Close

MANorm


UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly
move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x
chr1:25,889,779-25,891,501 1,723 bp.
enter position, gene symbol or search terms
go
chr1 (p36.11)
Scale
chr1:
500 bases
hg19
25,890,000
25,890,500
25,891,000
25,891,500
CD4 T cells EM H3K4me3 islands
CD4 T cells EM H3K4me3
3.18422
0
CD4 T cells Naive H3K4me3 islands
15.5532
19.7461
CD4 T cells Naive H3K4me3
CD4 T cells Naive
0
RefSeq Genes
LDLRAP1
Human mRNAs from GenBank
Human mRNAs