Using UCSC Tools for Browsing and Data Mining ENCODE Data

Aims

- Learn to locate and display ENCODE data in the UCSC Genome
 Browser
- Learn to retrieve ENCODE data from the UCSC Genome Browser database using the Table Browser data retrieval tool

Introduction

The University of California Santa Cruz (UCSC) Genome Browser athttp://genome.ucsc.edu is a web-based set of tools providing access to a database of genome sequence and annotations for visualization, comparison and analysis by the scientific, medical and academic communities. The primary mission of the site is to provide timely and convenient open access to high-quality human genome sequence and annotations in a framework that enables easy exploration from genome-wide down to the base level. Annotation datasets, or 'tracks', on the human genome cover conservation and evolutionary comparisons, gene models, regulation, expression, epigenetics and tissue differentiation, variation, phenotype and disease associations. A substantial contributor to our mission has been participation in the ENCODE project as the designated data repository in the ENCODE Pilot (2003-2007) and as the Data Coordination Center (DCC) in the ENCODE whole-genome data production phase (2007-2011). All production ENCODE data is routed to UCSC for validation, guality review, database storage, visualization, and dissemination to other public databases. At this time over 2700 distinct ENCODE experiments have been processed by the DCC and made publicly available.

Other organisms represented at the site include 4 non-human primates, 14 other mammals including a marsupial and a monotreme, 10 non-mammalian vertebrates and 24 non-vertebrates. The Genome Browser hosts mapping and sequence annotation tracks that describe assembly, gap and GC content for all organisms in the browser database. Additionally, for most organisms we show alignments from RefSeq genes, mRNAs and ESTs from GenBank, and other gene or gene prediction tracks such as Ensembl Genes (6). For human and mouse assemblies, we also offer a locally generated UCSC Genes track based upon RefSeq, GenBank, CCDS and UniProt data. About half of the genomes hosted at UCSC include a multiple sequence alignment track and pairwise genomic alignments between assemblies to further comparative and evolutionary investigations. Expression, regulation, variation and phenotype tracks are available for many of the assemblies. We also support user data upload and visualization, and have recently introduced a data hub mechanism allowing visualization of user data hosted remotely.

Worked Example 1: You will need to follow this through with the OpenHelix exercises at the end of this section.

Examining RNA expression in the vicinity of the TP53 gene

Browse to genome.ucsc.edu.

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	ENCODE	Welcome to the UCSC Genome Browser website. This site contains the reference sequence and working draft assemblies for a large collection of genomes. It also provides portals to the <u>ENCODE</u> and <u>Neandertal</u> projects.					
	Neandertal	We encourage you to explore these sequences with our tools. The Genome Browser zooms and scrolls over chromosomes, showing the work of annotators					
	Blat	worldwide. The <u>Gene Sorter</u> shows expression, homology and other information on groups of genes that can be related in many ways. <u>Blat</u> quickly maps yo sequence to the genome. The <u>Table Browser</u> provides convenient access to the underlying database. <u>VisiGene</u> lets you browse through a large collection of					
	Table	situ mouse and frog images to examine expression patterns. Genome Graphs allows you to upload and display genome-wide data sets.					
	Gene Sorter	The UCSC Genome Browser is developed and maintained by the Genome Bioinformatics Group, a cross-departmental team within the Center for Biomolecular Science and Engineering (CBSE) at the University of California Santa Cruz (UCSC). If you have feedback or questions concerning the tools or data on this					
	In Silico PCR	website, feel free to contact us on our <u>public mailing list</u> .					

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	NOTE: Early access to additional track data may be available on the Preview Browser.



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Worked Example 2:

Exploring TFBS and Histone Marks in the TP53 region



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Worked Example 3:

Intersect NFKB binding sites with RNA-seq using the Table Browser.

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7 Browsers Browsers br: wgEncodeReg chrom chrcc chr21 1545 chr21 1545 chr21 1545 chr21 1545 chr21 1545 chr21 1591 chr21 1591 chr21 1591 chr21 1591 chr21 1592 chr21 1592 c	Redmine TfbSCluster mStarts 8906 10,11,12,13 ,55,56,57,51 0,0,0,0,0,0 8491 10,11,12,13 ,55,56,57,55 0,0,0,0,0,0 0,0,0,0,0,0 0,0,0,0,0,0 0,0,0,0,	/cgi-bin/hg UCSC Tools ed.score chromEnd expCount 15459240 ,14,15,16 8,59,60,6 ,0,0,0,0, ,0,0,0,0, ,0,0,0,0, ,0,0,0,0	Table 5 500 and wgE exp1d NFKB ,17,18,19,20, 1,62,63,64,65 0,0,0,0,0,0,0 NFKB ,17,18,19,20, 1,62,63,64,65 0,0,0,0,0,0,0 NFKB ,17,18,19,20, 1,62,63,64,65 1,7,18,19,20, 1,62,63,64,65 1,62,64 1,64,65 1,64,65 1,64,65 1,64,65 1,64,65 1,64,65 1,64	bols ≥ encodeWiki score strand is expScores 689 . 21,22,23,24,25,26 ,66,67,68,69,70,77 30,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,	Google Docs - Home ered.name = 'nfkb thickStart 15458906 ,27,28,29,30,31,3 1,72,73,74,75,76, 0,0,0,0,0,0,0,0,0 15918491 ,27,28,29,30,31,3 1,72,73,74,75,76, 0,0,0,0,0,0,0,0,0,3 15920162 ,27,28,29,30,31,3 1,72,73,74,75,76,	Web dev thickEnd 15459240 2,33,34,35,36, 77,78,79,80,81 89,0,458,0,0,0 15918837 2,33,34,35,36, 77,78,79,80,81 15920503 2,33,34,35,36, 77,78,79,80,81	Sonar reserved 0 1 37,38,39,40, 82,83,84,85 0,0,0,0,0,0,0 0 1 37,38,39,40, 82,83,84,85 0,0,0,0,0,0,0 0 1 37,38,39,40, 82,83,84,85	» • • • • • • • • • • • • • • • • • • •	Count 0 45,46,47, ,90,91,92 ,0,0,0,0, 0 45,46,47, ,90,91,92 ,0,0,0,0, 0 45,46,47, ,90,91,92
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Table Browser

Use this program to retrieve the data ass	sociated with a track in text format, to calculate intersections between tracks, and to retrieve DNA							
sequence covered by a track. For help in using this application see <u>Using the Table Browser</u> for a description of the controls in this form, the User's Guide for general information and sample queries and the OpenHelix Table Browser tutorial for a narrated presentation of the software								
User's Guide for general information and sample queries, and the OpenHelix Table Browser tutorial for a narrated presentation of the software features and usage. For more complex queries, you may want to use Galaxy or our public MySQL server. To examine the biological function								
features and usage. For more complex queries, you may want to use <u>Galaxy</u> or our <u>public MySQL server</u> . To examine the biological function								
of your set through annotation enrichments, send the data to <u>GREAT</u> . Refer to the <u>Credits</u> page for the list of contributors and usage								
restrictions associated with these data. All tables can be downloaded in their entirety from the Sequence and Annotation Downloads page.								
clade: Mammal + genome: H	man assembly: Feb. 2009 (GRCh37/ho19) a							
group: Regulation	Intersect with Txn Factor ChIP							
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ine type retained to plain test to g	All Txn Factor ChIP records that have no overlap with CSHL Long RNA-seq							
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	Intersect bases covered by Txn Factor ChIP and/or CSHL Long RNA-seq:							
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	O Base-pair-wise intersection (AND) of Txn Factor ChIP and CSHL Long RNA-seq							
	O Base-pair-wise union (OR) of Txn Factor ChIP and CSHL Long RNA-seq							
	Check the following boxes to complement one or both tables. To complement a table means to include a base pair in the intersection/union if it is <i>not</i> included in the table.							
	Complement Txn Factor ChIP before base-pair-wise intersection/union							
	Complement CSHL Long RNA-seg before base-pair-wise intersection/union							
	(submit) cancel							
	88							

Table Browser

Use this program to retrieve the data associated with a track in text format, to calculate intersections between tracks, and to retrieve DNA sequence covered by a track. For help in using this application see <u>Using the Table Browser</u> for description of the controls in this form, the <u>User's Guide</u> for general information and sample queries, and the OpenHelix Table Browser <u>tutorial</u> for a narrated presentation of the software features and usage. For more complex queries, you may want to use <u>Galaxy</u> or our <u>public MySQL server</u>. To examine the biological function of your set through annotation enrichments, send the data to <u>GREAT</u>. Refer to the <u>Credits</u> page for the list of contributors and usage restrictions associated with these data. All tables can be downloaded in their entirety from the <u>Sequence and Annotation Downloads</u> page.

clade: Mammal genome: Human second assembly: Feb. 2009 (GRCh37/hg19)					
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NFKB at chr21:20	100082-20901104	2543	
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Exercises for the ENCODE data in the UCSC Genome Browser

1) Using RNA-seq data, examine expression of RNA in the vicinity of the TP53 gene. From the CSHL Long RNA-seq track, determine which strand is transcribed into Poly-A+ RNA and then found in the nuclear fraction of K562 and GM12878 cells.

Skills: Use RNA-seq data to evaluate RNA presence in a region; become aware of the cellular fraction data that is available.

2) In the region we are exploring, let's add transcription factor binding data and histone marks that are often found near active regulatory elements. Let's also determine if these histone marks are indicated in human embryonic stem cells.

Skills: Explore TFBS data; examine features associated with histone modifications; visualize cell type specific data.

3) Use the Table Browser to locate NKFB transcription factor binding signals that are greater than 500 on chromosome 21. Let's intersect that with RNA-seq data indicating presence of RNA in epidermal keratinocyte cells (NHEK cells).

Skills: Table Browser to query ENCODE data; use filters and intersections to generate a complex customized query of the data.

For additional guidance and ways to interact with the ENCODE data, access this open access publication in PLoS Biology: <u>http://bit.ly/plosENC</u>

Citation: The ENCODE Project Consortium (2011) A User's Guide to the Encyclopedia of DNA Elements (ENCODE). PLoS Biol 9(4): e1001046. doi:10.1371/journal.pbio.1001046

UCSC ENCODE 2 Exercises, version 1. Correspond to the data available in March 2012.

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1) Using RNA-seq data, examine expression of RNA in the vicinity of the TP53 gene. From the CSHL Long RNA-seq track, determine which strand is transcribed into Poly-A+ RNA and then found in the nuclear fraction of K562 and GM12878 cells.

Step	Action	
1	Go to the UCSC Genome Browser homepage, genome.ucsc.edu	
2	From the blue navigation links on the left side of the page, click the link for Genome Browser.	
3	From the Gateway interface, click the link that says " <u>Click here to</u> <u>reset</u> the browser user interface settings to their defaults." This will ensure that any prior activity on the Browser has been cleared out and that everyone is starting with default settings.	
4	Choose the Human February 2009 assembly. Enter the text tp53 in the gene box. Choose the TP53 item in the list. Click submit .	
5	In the TP53 region on the browser, examine the features briefly. Then click the "zoom out" 1.5x button near the top. Assess the features again.	
6	Click the "hide all" button in the middle of the resulting Genome viewer page. (We want to reduce what's in the display to reduce the burden on the servers, and to focus on our features of interest.)	
	 Add back 2 tracks to the viewer: GENCODE Genes V11 in "pack" visibility (from the Genes and Gene Predictions group) ENC RNA-seqin "show" (from the Expression group) Click a refresh button to add these tracks back to the viewer. It may take a while for this to load, as there is a lot of data here. 	
8	RNA seq data from multiple labs, cell lines, and experiment types are shown. Let's focus on the Long RNA-seq data. You can see there is signal across this region indicating RNA transcription in this region of the genome in this mode. But we'd like to distinguish which RNA-seq data corresponds to which genes in this region. Return to the RNA-seq hyperlink and click it to access	
	Individual tracks from this super-track.	
9	this time. Turn all of them to "hide" except for the CSHL Long RNA-seg menu.	
10	Click the CSHL Long RNA-seq hyperlink. Examine the options you can set to explore this track's data. (continued on next page)	



1	At the top, set the Maximum display mode to "full".			
I	Make these changes on the Long RNA-seq settings: *Set Contig view to "hide". *Poly-A+ should be checked. Uncheck "Total RNA" in the extract row. Leave the			
	other settings in that area unchanged. *Select the GM12878 cell line "nucleus" localization checkbox. *Unselect all other localization checkboxes except K562 "nucleus".			
	Click "Submit" when these changes have been made.			
1 2	Back on the viewer, examine the data. Use the select/drag feature of the left label area to move the GM12878 data sets together.			
	Image: State of the state			
1 3	Note the data which derives from the Plus strand and which from the Minus strand. It appears that the WRAP53 RNA derives from the plus strand, and the TP53 RNA from the minus strand. This will help you to orient when looking for transcription factor binding sites or other genomic features.			
Th pa	is exercise was inspired by the Figure 3 illustration in the ENCODE User Guide aper. See that figure legend and the accompanying text for more assessments of the data and the features in this region: <u>http://bit.ly/plosENC</u>	3		



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2) In the region we are exploring, let's add transcription factor binding data and histone marks that are often found near active regulatory elements. Let's also determine if these histone marks are indicated in human embryonic stem cells.

Step	Action	\checkmark	
1	On the browser view that we established in exercise 1, scroll down to the Regulation Group.		
2	Locate the ENCODE Regulation track. Choose "show" in the pulldown menu. Click a "refresh" button.		
3	 Examine the display. New data appears in the viewer beneath the RNA-seq data. *Note that the Transcription Factor ChIP-seq from ENCODE track shows data blocks, but not individual transcription factors. *Note that the H3K27Ac histone mark track appears to have multiple data sets of various colors. 		
4	Return to the ENCODE Regulation menu area. Click the hyperlink to look at the component tracks of this super-track.		
5	By default Txn Factor ChIP is visible in "dense" mode. Set that menu to "full". Click the "Submit" button.		
6	Examine the display again. Note that individual transcription factors can be identified by name using the labels on the left. Note that the letter codes near the blocks correspond to cell lines that have been used in experiments for this data. Click some of the blocks to note the cell lines and signal levels observed in them. Return to the viewer for the next steps.		
7	Click the grey control button to the left of the Layered H3K27Ac to go to the controls for that track.		
8	On this histone mark page, note that there are various cell line data sets, which have color codes. One of the lines is H1-hESC , which is a human embryonic stem cell line.		
9	Uncheck all cell line boxes except H1-hESC.		
10	Click the "Submit" button at the top to return to the genome viewer.		
11	Note that we can now see that there is signal associated with this histone mark in stem cells in this region. This was difficult to examine before because of the other color overlays.		
12	Return to the histone mark page by clicking the gray bar to the left of the browser track. Turn on or off various cell lines to view the data. Return to the viewer each time by clicking "Submit".		
13	The various data types in this region should help you to understand possible features of regulation of the genes in this area.		
This exercise was inspired by the Figure 5 illustration in the ENCODE User Guide paper. See that figure legend and the accompanying text for more assessments of the data and the features in this region: <u>http://bit.ly/plosENC</u>			



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3) Use the Table Browser to locate NKFB transcription factor binding signals that are greater than 500 on chromosome 21. Let's intersect that with RNA-seq data indicating presence of RNA in epidermal keratinocyte cells (NHEK cells).

Step	Action					\checkmark
1	From the genome browser click the navigation bar option called Tables.					
2	At the Table Browser, begin to establish the query with these choices: *Mammal, human, February 2009 *Regulation group, Txn Factor ChIP track *table wgEncodeRegTfbsClustered *region: position chr21, Click the lookup button to load the chr21 range.					
3	Next we'll set a filter. Click the "create" button.					
	We want the factor NFKB, and signals to be over 500. *in the name area chose "does" match nfkb <i>[remove the asterisk]</i> *in the score choose "is >" and type 500 in the text box					
	chromEnd is	ignorea 🖃		AND		
	name	does 💌	match nfkb	AND		
	score is	s > •	500	AND		
	massachemen	dosser	matala tenenenenen	and a start and a start and a start and a start		
	Click submit.					
4	4 Click the summary/statistics button to assess the results at this point.					
	This will provide a sense of how many results return with these settings. If					
	there were too many or too few, you might want to adjust the filters					
5	Let's take a look at the output at this point. In the "output format" area select					
Ŭ	"all fields fron	n selected	table".			
6	Click "get output" to see the results in table form. Note that the Name field					
	has our choice, and all the scores are over 500.					
	Let's intersect this data with some other data. Let's require that this NFKB data					
	also overlap with RNA-seq evidence in a particular cell type of our choice.					
/	Use the back button to go back to the Table Browser interface. It should still					
Q	nave all of your previous choices and settings.					
0	(continued on the next nade)					
	(continued on the next page)					



9	Make these choices in the "Intersect with" interface:			
	*In the group menu, select Expression.			
	*Track choice: select CSHL Long RNA-seq. (This is only because we are			
	already familiar with this track and have some of it visible in the browser. You			
	could choose any of the data sets later.)			
	*Table selection: choose nuclear NHEK polyA+ first data set. This looks			
	like: NHEK nucl pA+ + 1 (This is the CSHL Long data set Plus track)			
10	Ensure that the Intersect radio button is set to the first choice for "any			
	overlap".			
11	Click submit.			
12	This time let's choose "output format" as "hyperlinks to Genome			
	Browser ". This will allow us to quickly inspect some of the results visually.			
13	Click the "get output" button.			
14	Click on some of the links to explore the region of the browser that meets			
	these criteria. Zoom out for larger scope.			
	Do you see the NHEK data in the current view? If not, go to the ENC RNA			
	seq super-track and access the CSHL Long RNA-seq track details as			
	we did before. Select NHEK nuclear data to add it to the viewer. Submit.			
	Show or hide various expression tracks, transcription factor tracks, or any			
	other features you are interested in. You may need to turn on or off tracks in			
	the browser because they were not on when we were using it before.			



Tasks:

1.

Search for the SLC25A29 gene in UCSC and view the GENCODE geneset. How many alternative splice variants are there and what are their biotypes? What regulatory information can you find by investigating the ENCODE data tracks not only for this gene but for the adjacent locus?

2.

Search for RP4-550H1.6.1.

What biotype is this and what can you observe about this locus?

3.

Search for RPN2 and then zoom put so that you can also see C20orf132. How many alternative variants are there for each of these two loci and what are their biotypes? What can you observe about these two loci?



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Answers:

1.

There are 21 alternative splice variants for this locus. 8 protein coding, 3 NMD, 7 processes transcript and 2 retained intron.

Observations: Histone marks H3K4Me3 shows the classic dip at the tss.

There is TF binding, transcriptional evidence and exonic conservation back to zebrafish. Contrast this with the adjacent locus SLC25A47. This just has 2 coding transcripts and a pretty much identical conservation pattern, but appears to be transcriptionally silent in ENCODE cell-types. You can see the difference in histone modifications (very low levels detected), open chromatin (weak signals), TF binding (lower levels) and general transcription (background).

2.

This is a single-exon lncRNA locus that has a biotype of lincRNA (protein coding LOC in UCSC genes).

There is no alternative splicing. The conservation is quite good for a IncRNA, right back to Tenrec. Looking at the Open Chromatin marks there is a string signal from DNAseg and FAIRE, and the histone marks H3K4Me1, Me3 and K27Ac are all strong. Looking at the TF binding there is a huge pileup of factors with a very tight distribution and transcription looks high across the full-length of the transcript. If you look at polyA+ CAGE TSS there are strong signals on the minus strand, with the highest on H1-hESC and K562 and some expression (but not much) in GM12878. The polyA site and signal annotation shows very tight correspondence with the transcription level.

3.

C20orf132 has 10 alternative variants: 9 protein-coding and 1 processed transcript. RPN2 has 11 alternative variants: 9 protein-coding, 1 NMD and 1 processed transcript.

They have similar levels of conservation, with C20orf132 conserved doen to platypus and RPN2 to zebrafish. The two loci have a head to head arrangement. The histone modifications have detected high levels here and the Open Chromatin marks give a strong signal from DNAse1 and FAIRE. The TF binding has a big pileup of factors and a very tight distibution.

But, if you look at the transcription you can see that RPN2 has much higher transcription levels than C20orf132. This is shown by looking at the CAGE TSS data, as its much higher for the positive strand (RPN2) than the negative strand (C20orf132) and is true in all three cell types. There is also proteogenomic data for RPN2 e.g. peptides TGQEVVFVAEPDNK and FPEEEAPSTVLSQNLFTPK are found in both mitochondrial and nuclear fraction.