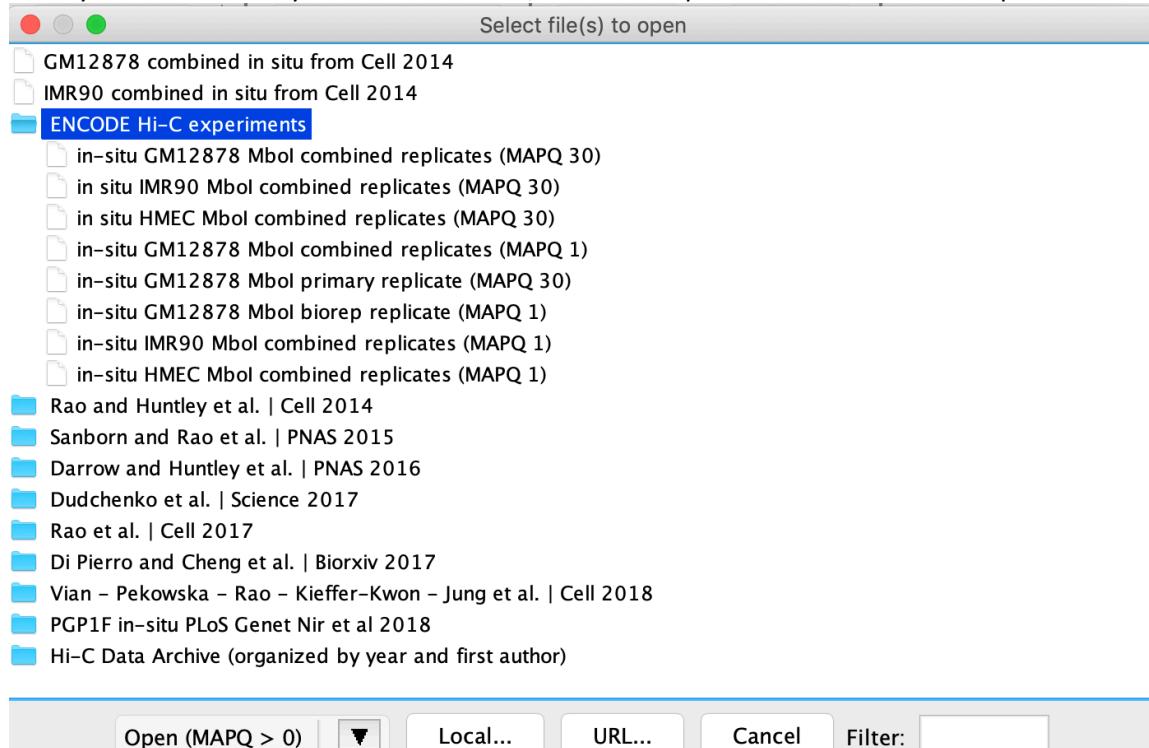


Welcome to the Juicebox tutorial! We are going to explore in depth an example of the kinds of analyses and insights you can get by visualizing 3D genomic interactions with Juicebox.

Go to <https://github.com/aidenlab/juicebox/wiki/Download> to download Juicebox.

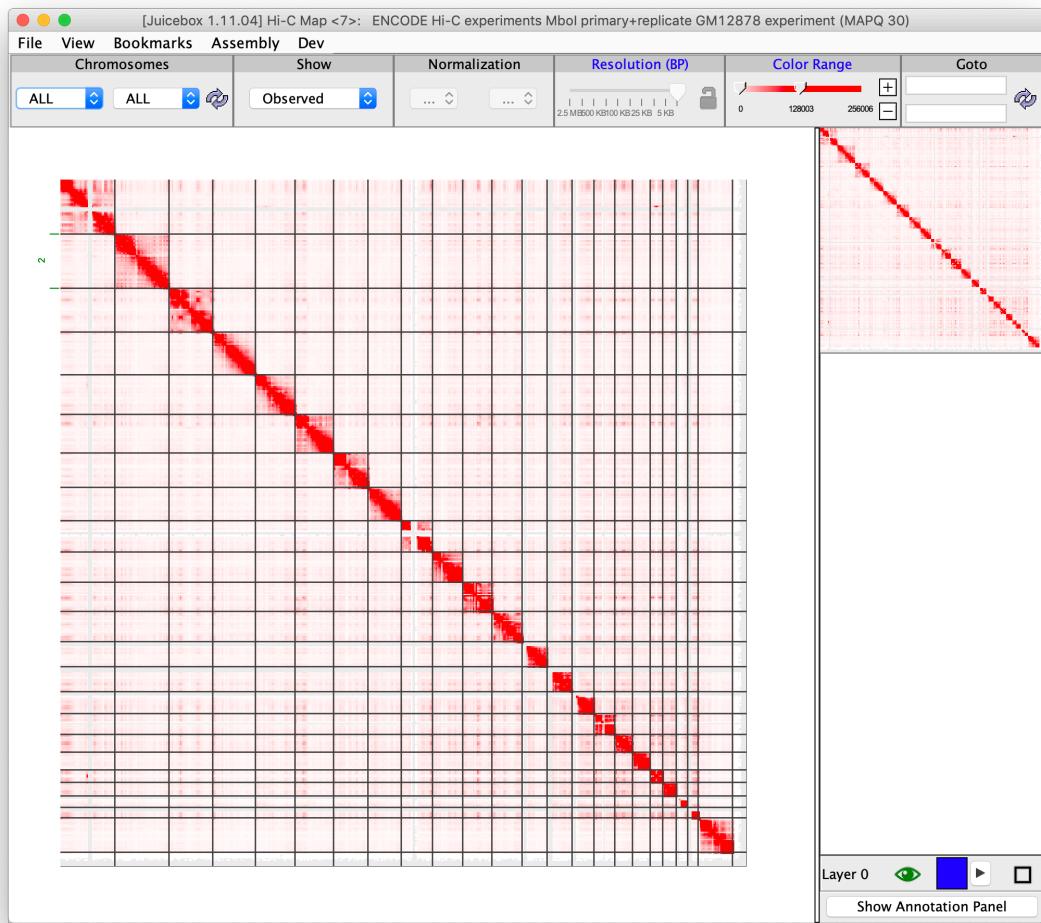
Once you've successfully launched Juicebox, click *File*→*Open* to load a new Hi-C map.



ENCODE Hi-C maps are listed under *ENCODE Hi-C experiments*. Click *in-situ GM12878 MboI combined replicates (MAPQ 30)* to open our largest map, the 6 billion read combined replicates GM12878 library.

The menu links to URLs that are automatically generated from the ENCODE API. As you can see from the dialog, you can also load a local map (useful if your internet connection is spotty) or a map via the URL.

Juicebox can load Dropbox and Google Drive URLs – so you can upload a map to Dropbox for only your collaborators to access.



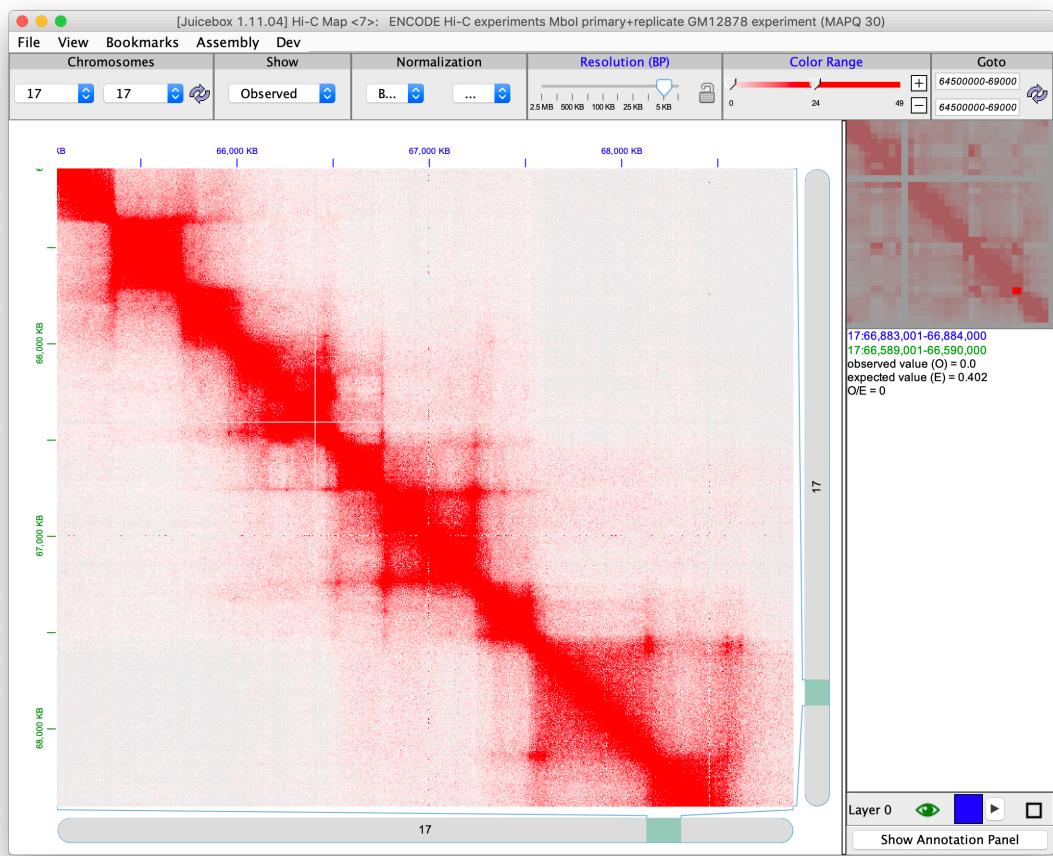
The map will load and show all chromosomes.

Click on chromosome 17. Using the selector on the left below *Normalization*, change the normalization to *Balanced*. This is a way to compensate for various biases in the data.

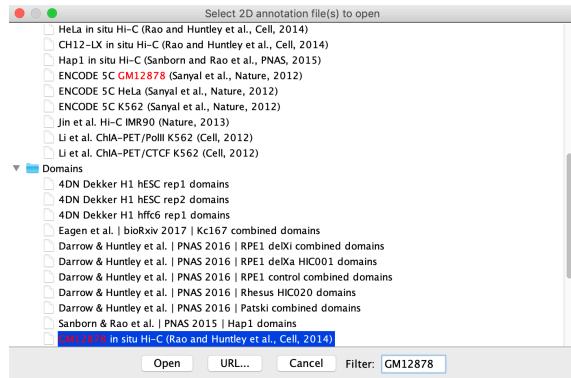
There are several ways to zoom in:

- Slide the resolution slider to 5 KB. Use your mouse to pan until your range is roughly 64,500 kb to 69,000 kb.
- Alternatively, after going to 5 KB, in the Go panel, type `17:64500000-69000000` in both boxes and hit the refresh button. Go back to 5 KB if necessary
- Or look at the text on the right as you move your mouse on the heat map. Hold down the Alt key and draw a box that encompasses 64,500 kb to 69,000 kb. You can zoom in further by clicking; the heat map will be centered at the point you click.

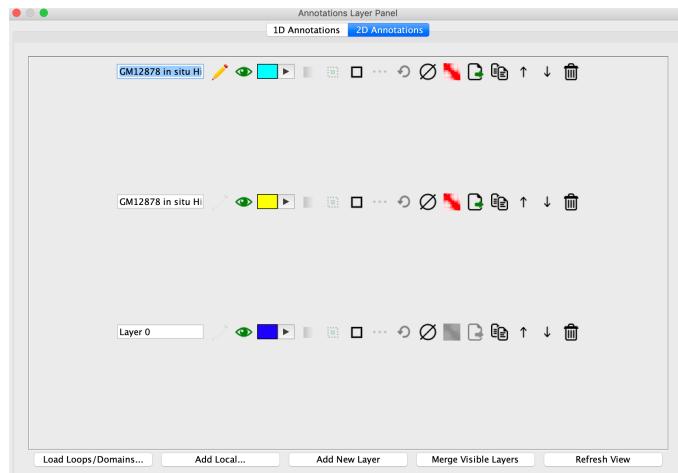
If you ever get lost, you can double click in the mini map in the upper right; you can also move the box within that mini map. You can also right click within the map and select *Jump to Diagonal*.



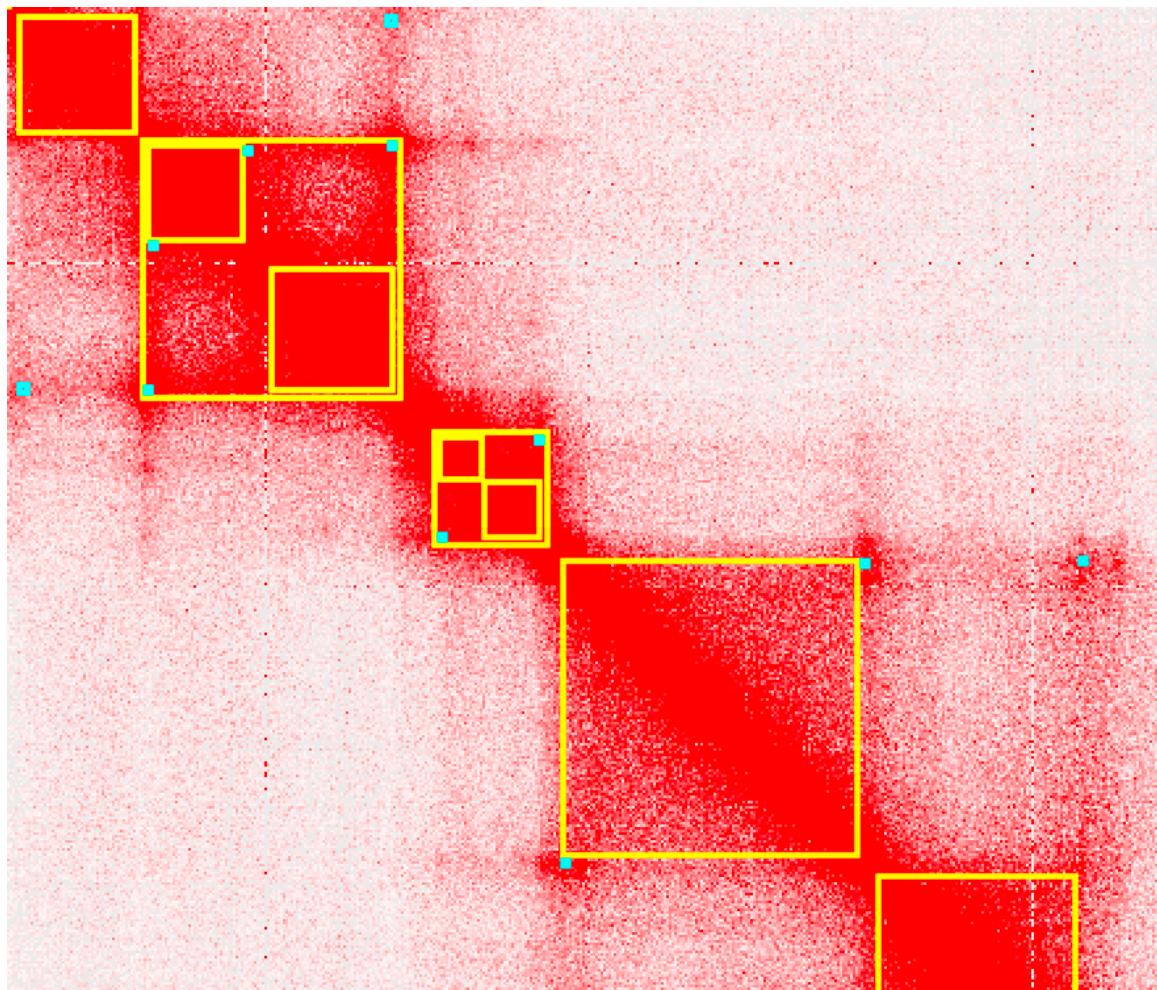
Now click *View* in the menu bar and go to *Show Annotation Panel*. Click the top tab for *2D Annotations* and then type “GM12878” into the filter box.



Select the *GM12878 in situ Hi-C* under both the *Loops* and *Domains* for this map.



Exit out of the Annotation Panel. You will see a series of yellow boxes and cyan points loaded. The cyan points denote the exact loops and so are small. These are the contact domains and loops that we found when analyzing this map. These calls were made with the automated pipeline *Juicer* and are also available via ENCODE.



Encode Production Data

Filter: GM12878 CTCF Bernstein | 14 rows

ID	Asse...	Bios...	Assay...	Target	BioRep	TechR...	OutputType	For...	Lab...	HREF	Ac...	Ex...	
<input type="checkbox"/>	/exp...	hg19	GM1...	ChIP-...	CTCF		peaks	big...	Bra...	/file...	EN...	EN...	
<input type="checkbox"/>	/exp...	hg19	GM1...	ChIP-...	CTCF		signal	big...	Bra...	/file...	EN...	EN...	
<input type="checkbox"/>	/exp...	hg19	GM1...	ChIP-...	CTCF		optimal idr thre...	big...	Bra...	/file...	EN...	EN...	
<input type="checkbox"/>	/exp...	hg19	GM1...	ChIP-...	CTCF	1	1,1	signal p-value	big...	Bra...	/file...	EN...	EN...
<input type="checkbox"/>	/exp...	hg19	GM1...	ChIP-...	CTCF	2	2,1	signal p-value	big...	Bra...	/file...	EN...	EN...
<input type="checkbox"/>	/exp...	hg19	GM1...	ChIP-...	CTCF	1	1,1	fold change ove...	big...	Bra...	/file...	EN...	EN...
<input checked="" type="checkbox"/>	/exp...	hg19	GM1...	ChIP-...	CTCF	1,2	1,1,...	signal p-value	big...	Bra...	/file...	EN...	EN...
<input type="checkbox"/>	/exp...	hg19	GM1...	ChIP-...	CTCF	2	2,1	fold change ove...	big...	Bra...	/file...	EN...	EN...
<input type="checkbox"/>	/exp...	hg19	GM1...	ChIP-...	CTCF	1,2	1,1,...	fold change ove...	big...	Bra...	/file...	EN...	EN...
<input type="checkbox"/>	/exp...	hg19	GM1...	ChIP-...	CTCF	1,2	1,1,...	peaks and back...	big...	Bra...	/file...	EN...	EN...
<input type="checkbox"/>	/exp...	hg19	GM1...	ChIP-...	CTCF	1,2	1,1,...	conservative idr...	big...	Bra...	/file...	EN...	EN...
<input type="checkbox"/>	/exp...	hg19	GM1...	ChIP-...	CTCF	2	2,1	peaks and back...	big...	Bra...	/file...	EN...	EN...
<input type="checkbox"/>	/exp...	hg19	GM1...	ChIP-...	CTCF	1,2	1,1,...	optimal idr thre...	big...	Bra...	/file...	EN...	EN...
<input type="checkbox"/>	/exp...	hg19	GM1...	ChIP-...	CTCF	1	1,1	peaks and back...	big...	Bra...	/file...	EN...	EN...

Load Cancel

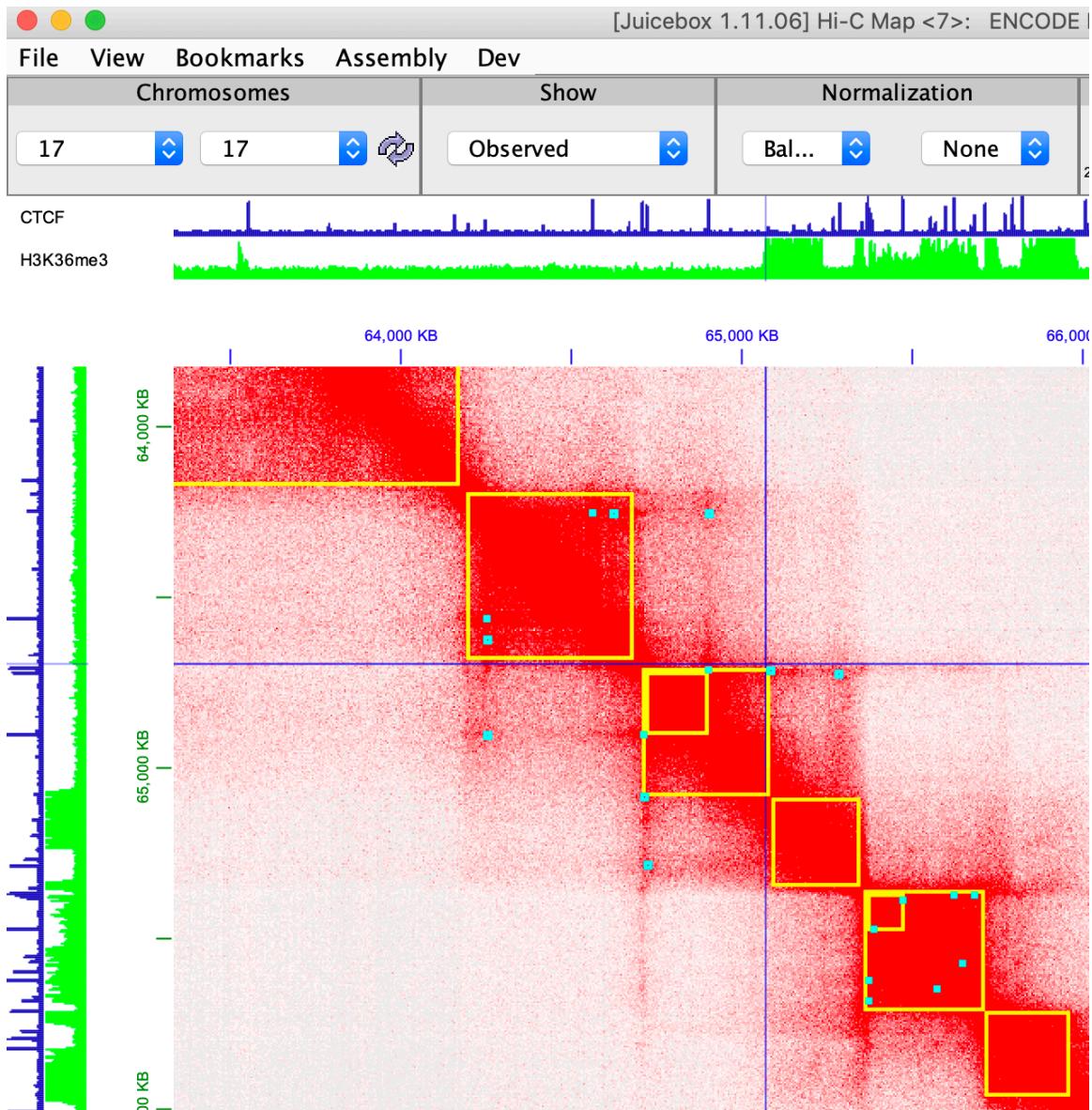
Now let's see what other annotations look like here. Click the *Show Annotation Panel* button on the lower right side of your screen and click the 1D Annotation tab at the top. Click *Load ENCODE Tracks*.

All the ENCODE tracks available for this assembly appear. We are going to filter in order to find the tracks we want to load. Type "GM12878 CTCF Bernstein" and mark the check box next to the signal p-value track. Click Load. Then *Load ENCODE Tracks* again and clear the filter and type "GM12878 H3K36me3 Bernstein" and mark the check box next the signal track. Click

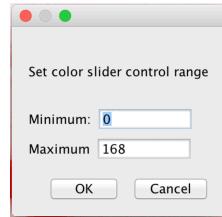
Load. Change the color for the H3K36me3 track to green by clicking the colored box next to the track. Relabel ENCFF906RJB.bigWig to H3K36me3 and ENCFF797LSC.bigWig to CTCF.

You can line up what you see in the heat map with the tracks. Right click on the heat map and select *Enable straight edge*. Then move around on the map to see the features lined up with the tracks. Press F2 to turn on and off the 2D annotations.

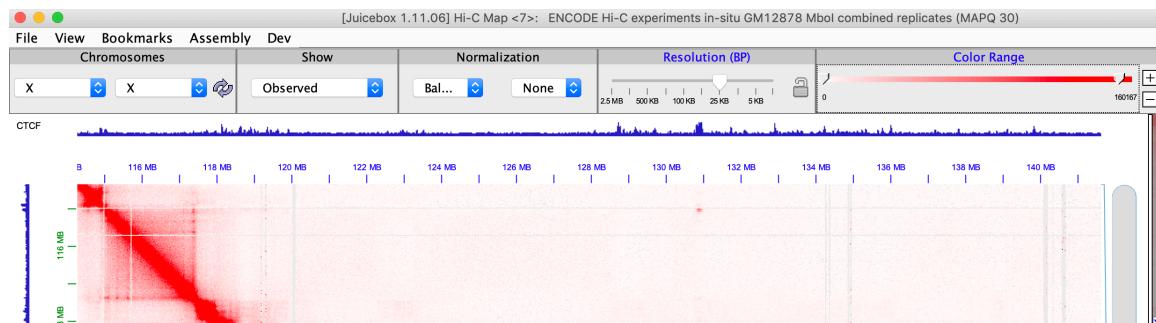
You'll notice that CTCF peaks seem to line up with focal loops in the map, and that the H3K36me3 track seems to line up with domain boundaries. These are two key findings from the *Rao and Huntley, et al. Cell 2014* paper; this discovery was made using Juicebox by qualitatively visualizing the data in just this way. Further technical analyses then showed that CTCF does seem to mediate loop formation and domains are decorated by differing epigenetic markers.



Now let's look at a different region and see what else we can discover. Turn off the straight edge. Remove the H3K36me3 track by right clicking it and clicking *Remove*. Turn off the 2D annotations by clicking F2.

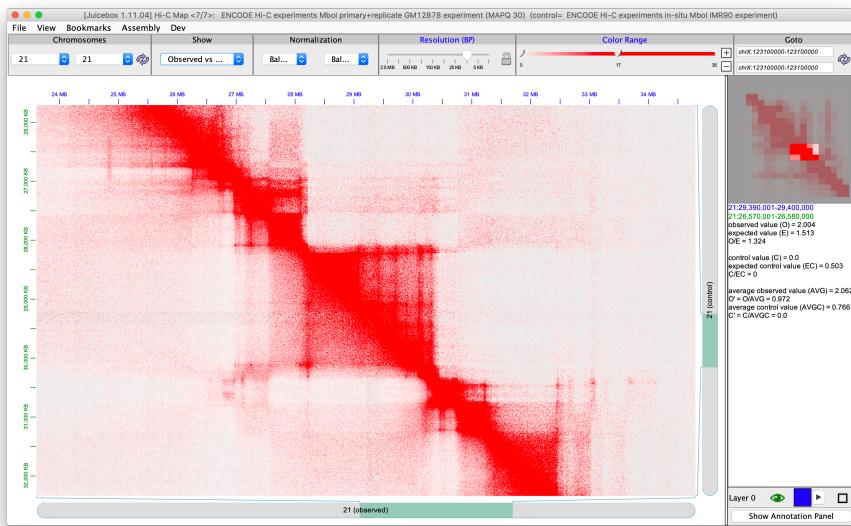


Then, in the Chromosomes menu bar, choose X for both left and right chromosomes. Click on the refresh button. Both left and top chromosomes displayed are now X. Use the Resolution menu slider and choose 25 kb. Navigate to chrX:114000000-134000000. Click on *Color range* (the label above the slider) and type 168 for the maximum value. (You can also adjust the maximum of the color range slider by clicking the + or – button.) Slide the right slider to around 160. You will see a superloop on chromosome X, around the intersection between 115Mb and 131Mb. Note also that there appears to be a series of CTCF peaks. This superloop was another discovery and led to further research into this phenomenon on the X inactive chromosome.

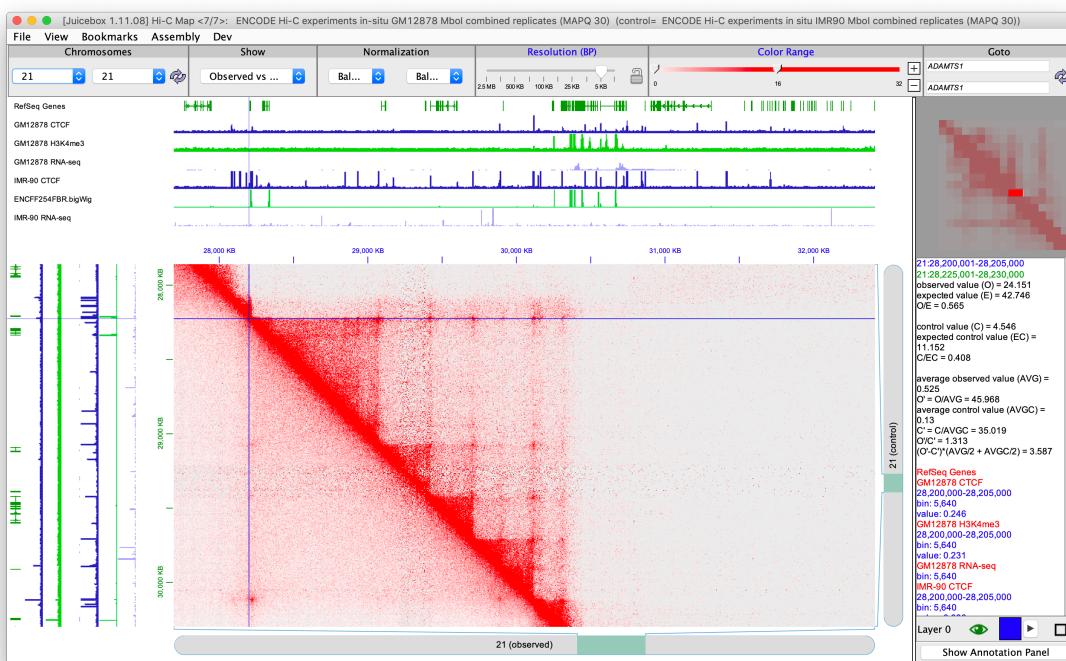


Let's load another Hi-C map and see if we can discover differences between cell lines. First go to chromosome 21. Click twice to zoom in, then draw a box while holding down the Alt key so that you're looking at 28,000 kb to 30,000 kb. Make sure the resolution is 5Kb.

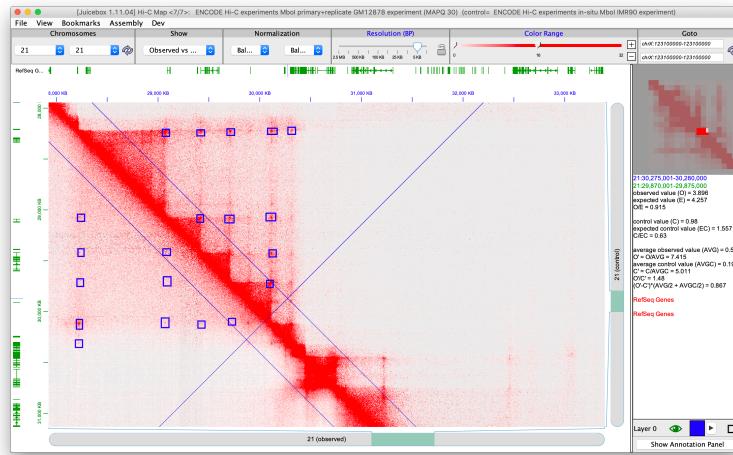
Then click *File*→*Open Control* and load the ENCODE IMR90 Mb1 MAPQ 30 map. Now go to *Show* on the toolbar and select *Control*. Change the normalization on the right to be *Balanced*. You can press F1 to toggle between Observed and Control. As you can see, the regions look quite different. Another way to examine the data is the VS view. Go to *Show* on the toolbar and select *Observed vs Control*. Observed is on the X axis (below the diagonal), control is on the Y axis (above the diagonal). You can see in the *Show* list that there are several different ways to compare two maps visually.



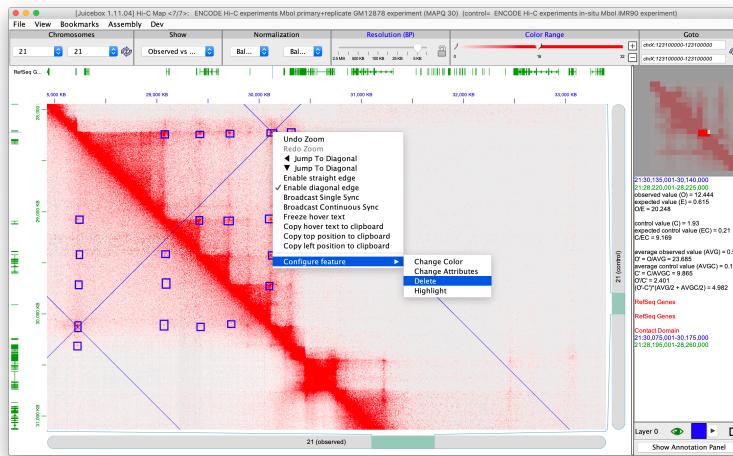
Go to *Show Annotation Panel* and in the 1D tab, click *Load Basic Annotations...* Load the Genes. Then click *Load ENCODE* and load a GM12878 RNA-seq, H3K4me3, and CTCF tracks, and the IMR90 CTCF, RNA-seq, and H3K4me3 signal tracks. You'll notice that there is a peak in the RNA-seq track for IMR-90 on the gene ADAMTS1 and that there's a peak in the IMR-90 H3K4me3 track; neither of these are present on the respective GM12878 tracks. H3K4me3 is an activation mark. ADAMTS1 encodes a protein involved in fibroblast migration and is inactive in GM12878 but active in IMR90.



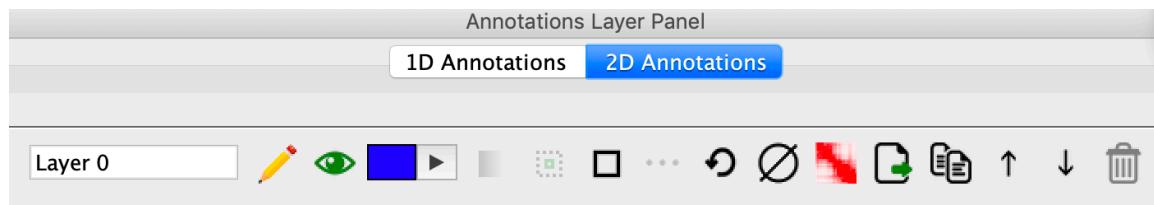
We can record the differences we see in the maps. Right click the heat map and select *Enable diagonal edge*. Then you can hand annotate the differential loops you see by holding down shift and drawing a box.



Here we've made a mistake. To remove an annotation, right click when within the box and select *Configure feature* and then *Delete*.



You can also configure by clicking *Show Annotation Panel* and then the 2D Annotations tab. The current layer is Layer 0.



You can rename the annotation by typing the box. Clicking the pencil will make the layer editable (this is useful when you import annotations). The eye icon is for turning visibility on and off. The color box allows you to change the color. The shaded icon makes the annotation more

transparent. The dotted box artificially enlarges the annotation. The solid box allows you to toggle through only showing the annotation above or below the diagonal. The ellipsis enables a mode whereby not all annotations are painted at the same time, useful when there are a large number of annotations. The backwards circle undoes the last annotation and the circle with a slash clears all annotations in this layer. The mini heat map creates a submap (more on this later). The page with a green arrow allows you to export the annotations to a file and the two pages duplicates this layer. Finally, the arrows allow you to move the annotation up and down in the list and the trash can deletes it.

There are many other features to explore in Juicebox Desktop. Right click in the heat map to see some of them. Within the File menu, you can look at Dataset Metrics or export the current screen as an image. The View menu gives some options for looking at the data, including the Submapping function (detailed here: <https://github.com/aidenlab/Juicebox/wiki/Submapping>). Within the Annotations dialog, you can load your own 1D and 2D annotations. The Bookmarks menu allows you to save a location to quickly go back to it between sessions. The Assembly menu is for using Juicebox for Assembly Tools to correct genome assemblies: <https://youtu.be/Nj7RhQZHM18>

Juicebox on the Web

We also have a lightweight version of Juicebox available on the web. If you're running out of time, just click the long link at the end of the document, which will load this example.

Otherwise, open a browser and type <https://aidenlab.org/juicebox>

Juicebox on the Web is run via client side Javascript. Any hic file can be loaded from a URL – and in particular, hic files uploaded to Dropbox, Google Drive, Amazon S3, or any web server can be streamed. You can even browse 3D maps on your mobile phone!

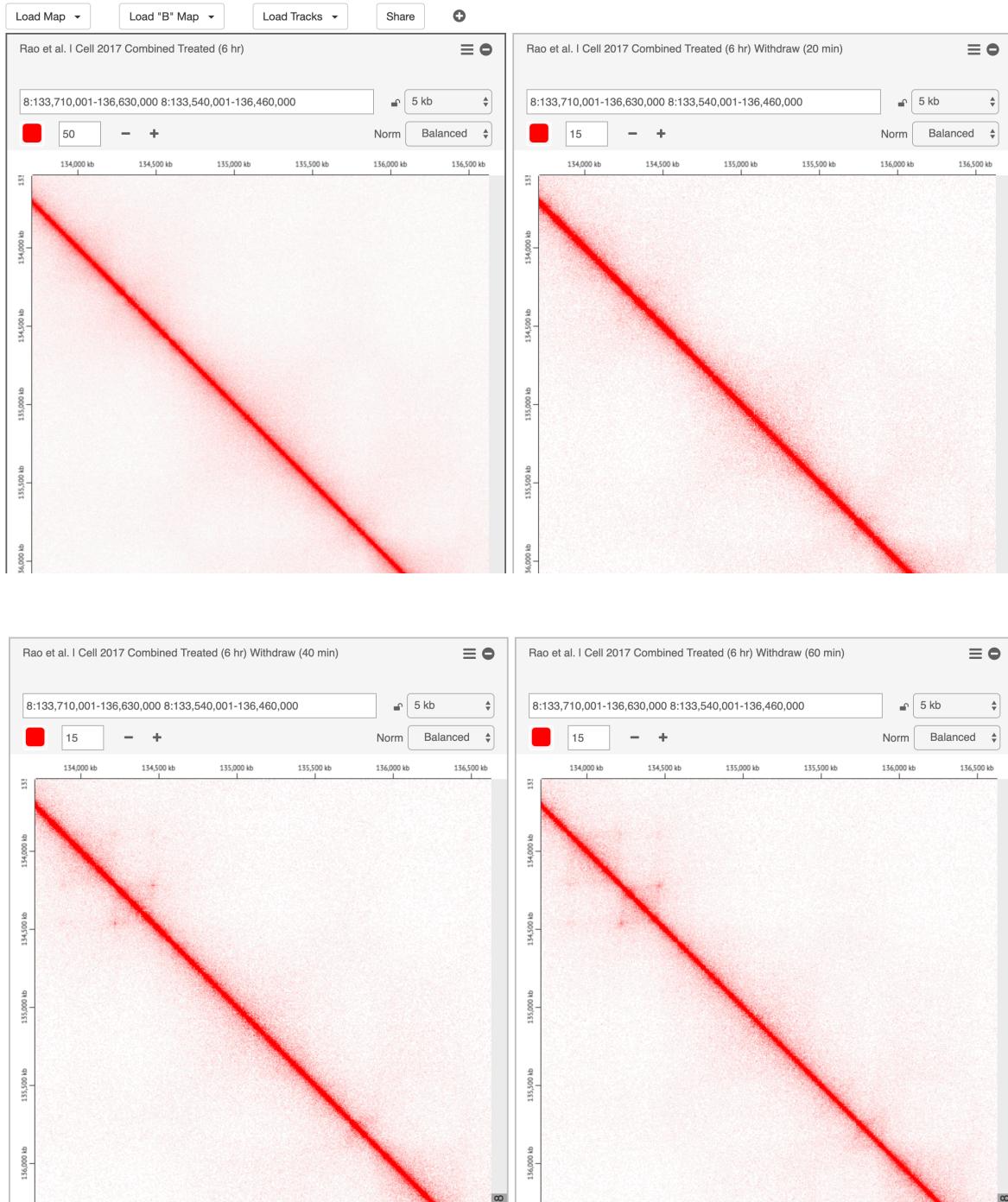
Juicebox on the Web makes it easy to browse maps side-by-side and to share exactly what you're looking at with collaborators. The core functionality of browsing contact maps together with epigenetic marks is the same.

Here we're going to recreate a figure from *Rao et al., Cell 2017*. It will be an interactive figure, enabling you to easily verify the findings. This paper looked at what happens to genome architecture when you remove cohesin and then introduce it again.

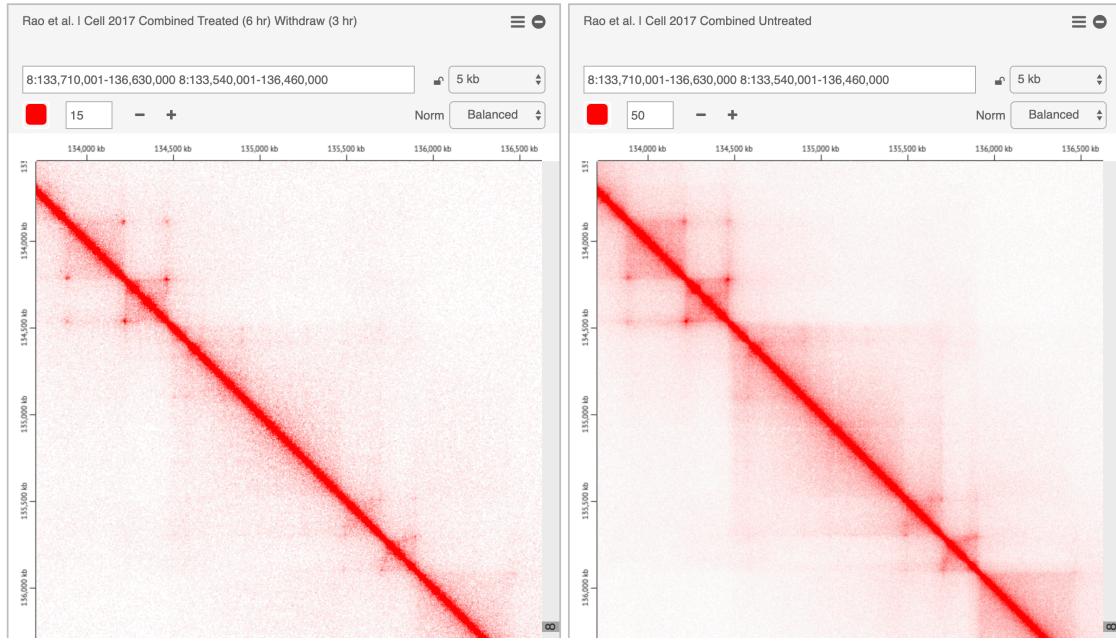
Click *Load Map* → *Select Map File*, type “Rao” and select the map *Combined Treated (6hr)*. Double click chromosome 8. Zoom in to between 133,800kb and 134,600kb. You zoom in as you would in Juicebox Desktop (drawing boxes, double clicking, manually setting resolution) – with the additional capability of pinch zoom on a mobile device.

Set the normalization to *Balanced* and the color scale value to 50.

Now load a map next to this one by clicking the + button. A new window appears to the right. You know which window you're operating on because there's a dark line around it. Click *Load Map* → *Select Map File*, type “Rao” and select *Combined Treated (6hr) Withdraw (20min)*. Set normalization to *Balanced* and the color scale value to 15.



Repeat these steps again, selecting in order: *Combined Treated (6hr) Withdraw (40min)*, *Combined Treated (6hr) Withdraw (60min)*, *Combined Treated (6hr) Withdraw (3hr)*, and *Combined Untreated*. The following maps will load below if there's no room next to the previous map. The final map is deep like the first and should have a color scale value of 50.



As you can see, withdrawing the auxin treatment allows loops to form again, as they exist in untreated form. You can play around with color scale and location to verify this wasn't cherry picked.

On the next page is the sharable link; usually these are short, but this link is too long for bit.ly.

Click *Load Tracks* at the top to load ENCODE tracks alongside these maps. You can also click the icon at the top left to add 2D Annotations to the map. Explore the icons to see what other capabilities exist in Juicebox on the Web.

Further resources

Juicebox Desktop code and documentation: <https://github.com/aidenlab/juicebox/wiki>

Juicebox on the Web documentation: <https://igvteam.github.io/juicebox.js/>

Embedding your own Juicebox: <https://igvteam.github.io/juicebox.js/docs/embedding>

Juicebox for Assembly Tools demo: <https://youtu.be/IMmVp8FodmY>

Juicebox for Assembly Tools tutorial: <https://youtu.be/Nj7RhQZHM18>

And please post on our forum with any questions: aidenlab.org/forum.html

<a href="https://aidenlab.org/juicebox/?juicebox=%7BhicUrl%253Dhttps%253A%252F%252Fs3.amazonaws.com%252Fhicfiles%252Fhiseq%252Fdegron%252Ftreated_6hr%252Funsynchronized%252Fcmbined.hic%2526name%253DRao%252Bet%252Bal.%252B%257C%252BCell%252B2017%252BCombined%252BTreated%252B(6%252Bhr)%2526state%253D8%252C8%252C8%252C26741.9999%252C26707.9999%252C1%252CKR%2526colorScale%253D50%252C255%252C0%252C0%2526nvi%253D33255540403%252C36479%2526caption%253DType%252Ba%252Bcaption%252Bhere.%7D%2C%7BhicUrl%253Dhttps%253A%252F%252Fs3.amazonaws.com%252Fhicfiles%252Fhiseq%252Fdegron%252Ftime_course%252Fdeep%252F20min_withdraw_combined.hic%2526name%253DRao%252Bet%252Bal.%252B%257C%252BCell%252B2017%252BCombined%252BTreated%252B(6%252Bhr)%252BWithdraw%252B(20%252Bmin)%2526state%253D8%252C8%252C8%252C26741.9999%252C26707.9999%252C1%252CKR%2526colorScale%253D15%252C255%252C0%2526nvi%253D11078436933%252C36479%2526caption%253DType%252Ba%252Bcaption%252Bhere.%7D%2C%7BhicUrl%253Dhttps%253A%252F%252Fs3.amazonaws.com%252Fhicfiles%252Fhiseq%252Fdegron%252Ftime_course%252Fdeep%252F40min_withdraw_combined.hic%2526name%253DRao%252Bet%252Bal.%252B%257C%252BCell%252B2017%252BCombined%252BTreated%252B(6%252Bhr)%252BWithdraw%252B(40%252Bmin)%2526state%253D8%252C8%252C8%252C26741.9999%252C26707.9999%252C1%252CKR%2526colorScale%253D15%252C255%252C0%252C0%2526nvi%253D11031830038%252C36479%2526caption%253DType%252Ba%252Bcaption%252Bhere.%7D%2C%7BhicUrl%253Dhttps%253A%252F%252Fs3.amazonaws.com%252Fhicfiles%252Fhiseq%252Fdegron%252Ftime_course%252Fdeep%252F60min_withdraw_combined.hic%2526name%253DRao%252Bet%252Bal.%252B%257C%252BCell%252B2017%252BCombined%252BTreated%252B(6%252Bhr)%252BWithdraw%252B(60%252Bmin)%2526state%253D8%252C8%252C8%252C26741.9999%252C26707.9999%252C1%252CKR%2526colorScale%253D15%252C255%252C0%252C0%2526nvi%253D10312725340%252C36479%2526caption%253DType%252Ba%252Bcaption%252Bhere.%7D%2C%7BhicUrl%253Dhttps%253A%252F%252Fs3.amazonaws.com%252Fhicfiles%252Fhiseq%252Fdegron%252Ftime_course%252Fdeep%252F180min_withdraw_combined.hic%2526name%253DRao%252Bet%252Bal.%252B%257C%252BCell%252B2017%252BCombined%252BTreated%252B(6%252Bhr)%252BWithdraw%252B(3%252Bhr)%2526state%253D8%252C8%252C8%252C26741.9999%252C26707.9999%252C1%252CKR%2526colorScale%253D15%252C255%252C0%252C0%2526nvi%253D10038610214%252C36479%2526caption%253DType%252Ba%252Bcaption%252Bhere.%7D%2C%7BhicUrl%253Dhttps%253A%252F%252Fs3.amazonaws.com%252Fhicfiles%252Fhiseq%252Fdegron%252Funtreated%252Funsynchronized%252Fcombined.hic%2526name%253DRao%252Bet%252Bal.%252B%257C%252BCell%252B2017%252BCombined%252BTreated%252BUntreated%2526state%253D8%252C8%252C8%252C26741.9999%252C26707.9999%252C1%252CKR%2526colorScale%253D50%252C255%252C0%2526nvi%253D30439217680%252C36479%2526caption%253DType%252Ba%252Bcaption%252Bhere.%7D