Detecting regions with copy number variation

Starting with copy number estimates for each marker (either taken directly from the vendor's input file or calculated previously), the next step is to create a list of regions where adjacent markers share the same copy number.

Choosing a method for copy number detection

There are two algorithms available for copy number region detection: Genomic Segmentation and Hidden Markov Model (HMM). Both algorithms look for trends across multiple adjacent markers. The genomic segmentation algorithm identifies breakpoints - changes in copy number between two neighboring regions. The HMM algorithm looks for discrete changes of whole number copy number states (e.g., 0, 1, 2 … with no upper limit) and will find regions with those numbers of copies. Therefore, the HMM model performs better in cases of homogeneous samples such as clinical syndromes with underlying copy number aberrations. Genomic segmentation is preferable for heterogeneous samples such as cancer because tumor biopsies often contain "contaminating" healthy tissue and a tumor can have cells with different genomic aberrations.

Detecting amplifications and deletions with Genomic Segmentation

The number of copies of each marker created in the previous step will be used to detect the genomic regions with copy number variation, i.e., to identify amplifications and deletions across the genome.

- Select the **IC_IntensitiesSNP6pairedcopynumber** spreadsheet in the *Analysis* tab
- Select **Detect Amplifications and Deletions** from the *Copy Number Analysis* section of the workflow (Figure 1)

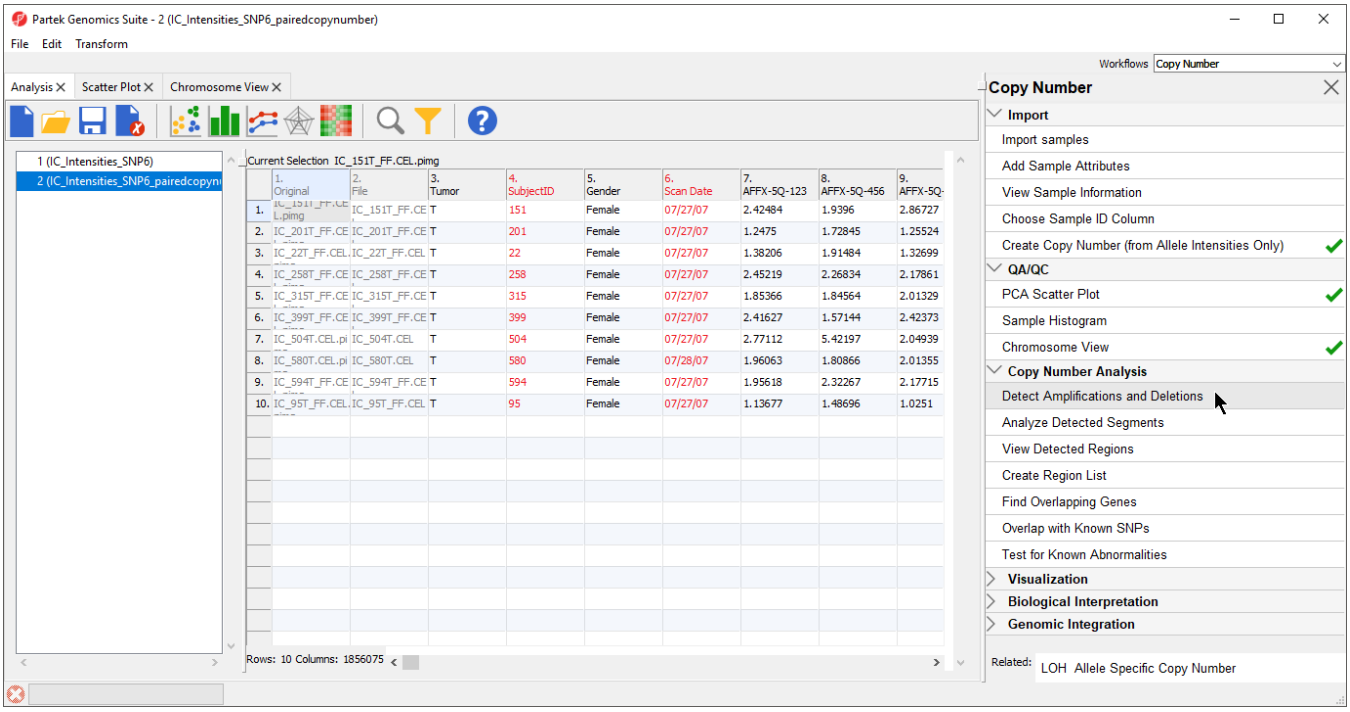


Figure 11. Invoking Detect Amplifications and Deletions

The *Detect Amplifications and Deletions* dialog will give you the option to choose *Genomic Segmentation* or *HMM Region Detection* (Figure 2).

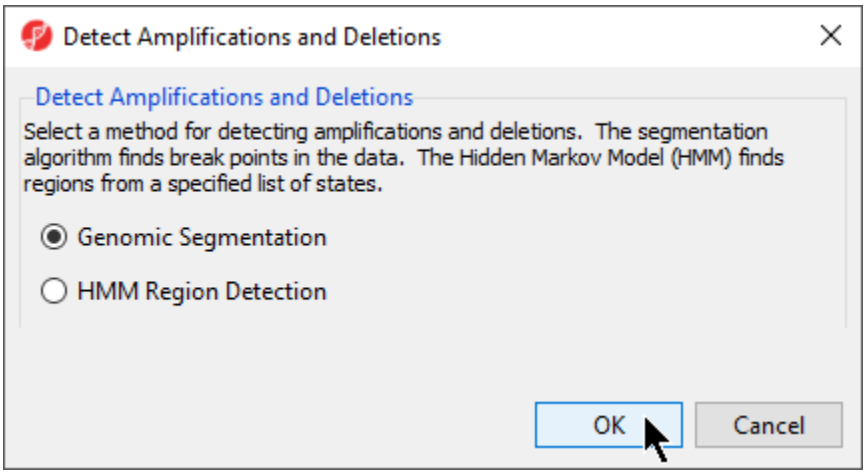


Figure 12. Select a method for detecting amplifications and deletions

- Select **Genomic Segmentation**
- Select **OK**

The *Genomic Copy Number Segmentation* dialog gives options for setting segmentation parameters and the configuring the region report (Figure 3).

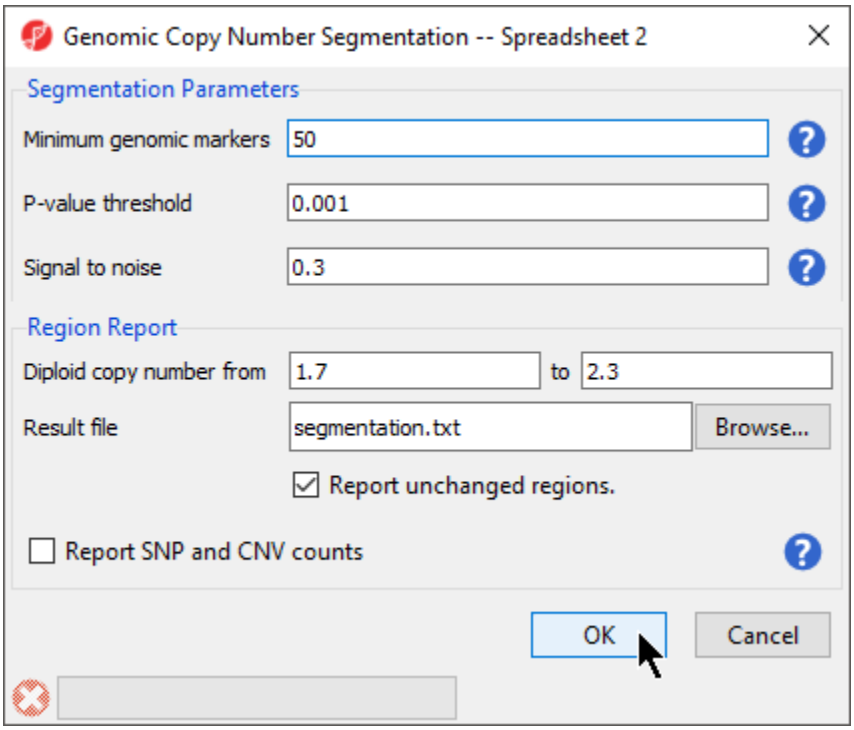


Figure 13. Configuring the Genomic Copy Number Segmentation dialog

- Set *Minimum genomic markers* to **50**
- Leave the rest of the parameters set to default values as shown (Figure 3)
- Select **OK**

The *Genomic Segmentation* task is divided into two steps. In the first step, each region is compared to an adjacent region to determine whether both have the same average copy number and whether a breakpoint can be inserted. This is determined by first using a two-sided t-test to compare the average intensities of adjacent regions and then checking whether the corresponding cut-off p-value is below the specified *P-value threshold*. The genomic size of a region is defined by the number of genomic markers in the region, *Minimum genomic markers*, while the magnitude of the significant difference between two regions is controlled by *Signal to noise,* which can be thought of as the difference in copy numbers between the regions. If the t-test is significant, the copy number of the region differs significantly from its nearest neighbors. However, a second step is needed to detemine whether the difference is due to amplificaiton or deletion. In this second step, two one-sided t-tests are used to compare the mean copy number in the region with the expected diploid copy number. For a detailed explanation of the genomic segmenetation procedure, please consult our Genomic Segmentation white paper. For more detailed information about fine-tuning the parameters of your copy number analysis, please consult our guide, Optimizing Copy Number Segmentation.

The resulting spreadsheet, *segmentation*, shows one row per genomic region per sample (Figure 4). The columns provide the following information:

1-4: Genomic location of the region

5. Sample ID

6. Description of the copy number change

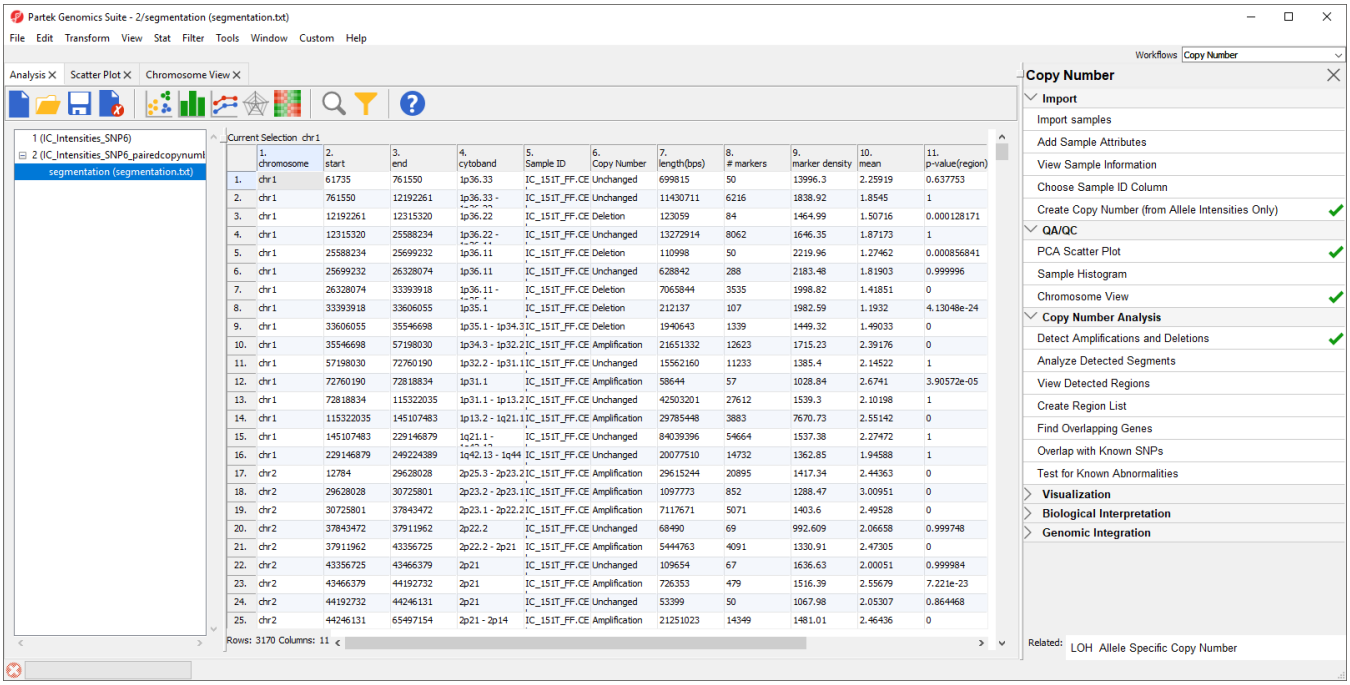
7. The length of the region (in base pairs)

8. The number of markers in the region

9. Markers density in the region (region length in base pairs divided by the number of markers)

10. Geometric mean of the copy number of all the markers in the region

11. Minimum p-value of the one-sided t-tests of the difference of the copy number in column 10 vs. the diploid range



	1. chromosome	2. start	3. end	4. cytoband	5. Sample ID	6. Copy Number	7. length(bps)	8. # markers	9. marker density	10. mean	11. p-value(region)
1.	chr1	61735	761550	1p36.33	IC_151T_FF.CE	Unchanged	699815	50	13996.3	2.25919	0.637753
2.	chr1	761550	12192261	1p36.33 -	IC_151T_FF.CE	Unchanged	11430711	6216	1838.92	1.8545	1
3.	chr1	12192261	12315320	1p36.22	IC_151T_FF.CE	Deletion	123059	84	1464.99	1.50716	0.000128171
4.	chr1	12315320	25588234	1p36.22 -	IC_151T_FF.CE	Unchanged	13272914	8062	1646.35	1.87173	1
5.	chr1	25588234	25699232	1p36.11	IC_151T_FF.CE	Deletion	110998	50	2219.96	1.27462	0.000856841
6.	chr1	25699232	26328074	1p36.11	IC_151T_FF.CE	Unchanged	628842	288	2183.48	1.81903	0.999996
7.	chr1	26328074	33393918	1p36.11 -	IC_151T_FF.CE	Deletion	7065844	3535	1998.82	1.41851	0
8.	chr1	33393918	33606055	1p35.1	IC_151T_FF.CE	Deletion	212137	107	1982.59	1.1932	4.13048e-24
9.	chr1	33606055	35546698	1p35.1 - 1p34.3	IC_151T_FF.CE	Deletion	1940643	1339	1449.32	1.49033	0
10.	chr1	35546698	57198030	1p34.3 - 1p32.2	IC_151T_FF.CE	Amplification	21651332	12623	1715.23	2.39176	0
11.	chr1	57198030	72760190	1p32.2 - 1p31.1	IC_151T_FF.CE	Unchanged	15562160	11233	1385.4	2.14522	1
12.	chr1	72760190	72818834	1p31.1	IC_151T_FF.CE	Amplification	58644	57	1028.84	2.6741	3.90572e-05
13.	chr1	72818834	115322035	1p31.1 - 1p13.2	IC_151T_FF.CE	Unchanged	42503201	27612	1539.3	2.10198	1
14.	chr1	115322035	145107483	1p13.2 - 1q21.1	IC_151T_FF.CE	Amplification	29785448	3883	7670.73	2.55142	0
15.	chr1	145107483	229146879	1q21.1 -	IC_151T_FF.CE	Unchanged	84039396	54664	1537.38	2.27472	1
16.	chr1	229146879	249224389	1q42.13 - 1q44	IC_151T_FF.CE	Unchanged	20077510	14732	1362.85	1.94588	1
17.	chr2	12784	29628028	2p25.3 - 2p23.2	IC_151T_FF.CE	Amplification	29615244	20895	1417.34	2.44363	0
18.	chr2	29628028	30725801	2p23.2 - 2p23.1	IC_151T_FF.CE	Amplification	1097773	852	1288.47	3.00951	0
19.	chr2	30725801	37843472	2p23.1 - 2p22.2	IC_151T_FF.CE	Amplification	7117671	5071	1403.6	2.49528	0
20.	chr2	37843472	37911962	2p22.2	IC_151T_FF.CE	Unchanged	68490	69	992.609	2.06658	0.999748
21.	chr2	37911962	43356725	2p22.2 - 2p21	IC_151T_FF.CE	Amplification	5444763	4091	1330.91	2.47305	0
22.	chr2	43356725	43466379	2p21	IC_151T_FF.CE	Unchanged	109654	67	1636.63	2.00051	0.999984
23.	chr2	43466379	44192732	2p21	IC_151T_FF.CE	Amplification	726353	479	1516.39	2.55679	7.221e-23
24.	chr2	44192732	44246131	2p21	IC_151T_FF.CE	Unchanged	53399	50	1067.98	2.05307	0.864468
25.	chr2	44246131	65497154	2p21 - 2p14	IC_151T_FF.CE	Amplification	21251023	14349	1481.01	2.46436	0

Figure 14. Viewing the segmentation spreadsheet

If desired, you can use *Merge Adjacent Regions* under *Tools* in the main toolbar to combine similar regions.

Visualizing regions of interest

Individual regions of interest can be visualized using *Chromosome View*.

- Right-click a row header in the *segmentation* spreadsheet
- Select **Browse to location** from the pop-up menu

Alternatively, you can visualize results at the whole chromosome level.

- Select the **segementation** spreadsheet
- Select **Chromosome View** from the *QA/QC* section of the workflow

The *Genomic Segementation* track displays the segmentation results (Figure 5). Each line in the track represents a sample. Amplified, deleted, and unchanged regions are shown in red, blue, and white, respectively. The *Profile* track now also includes information from the segmentation spreadsheet for the selected sample.

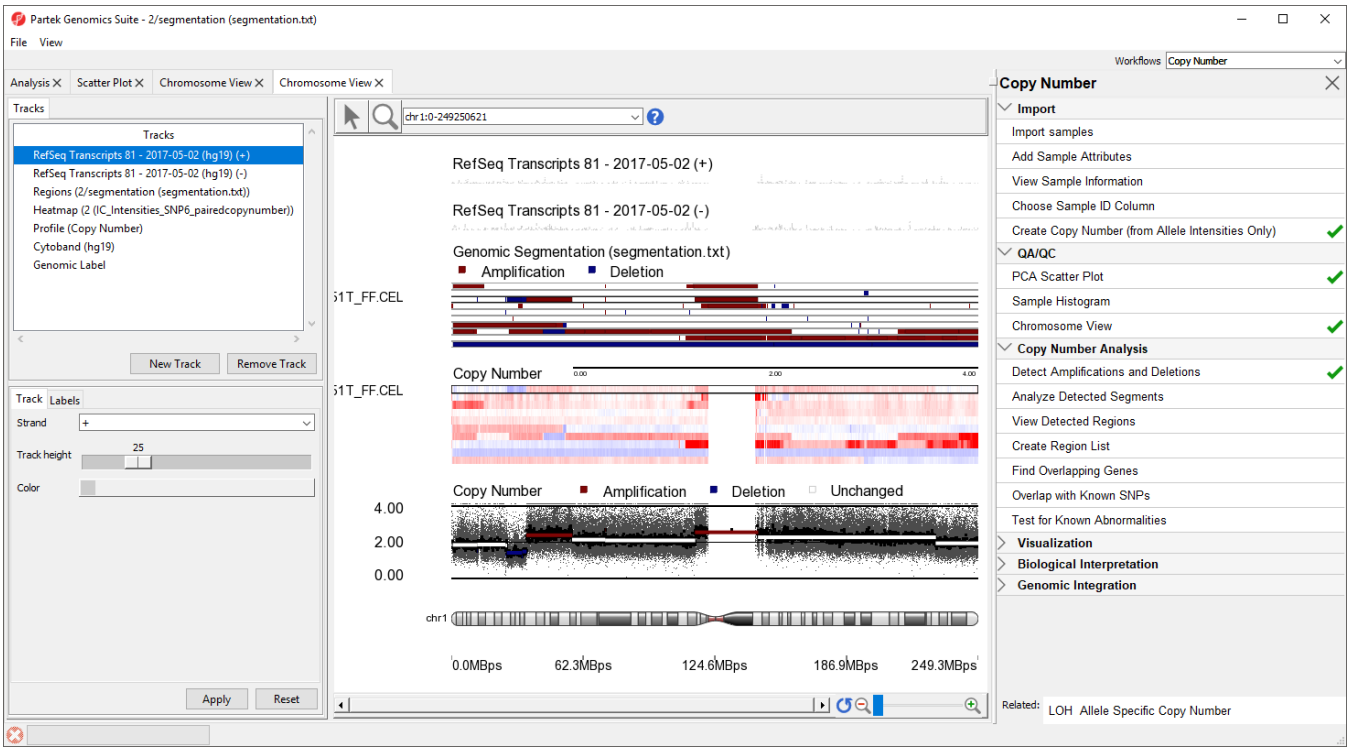


Figure 15. Segmentation results shown as regions of amplification and deletion in each sample

Analyzing shared regions of copy number variation

Amplified and deleted regions in each sample have been detected, we can compare the regions across multiple samples to detect copy number changes that are shared by multiple samples.

- Select **Analyze detected segments** from the *Copy Number Analysis* section of the workflow

The *Analyze Segments* task (Figure 6) can test for associations between copy number variations and sample categories using the ^{2} test. In this tutorial, all pairs share the sample phenotype, so we will not test for associations.

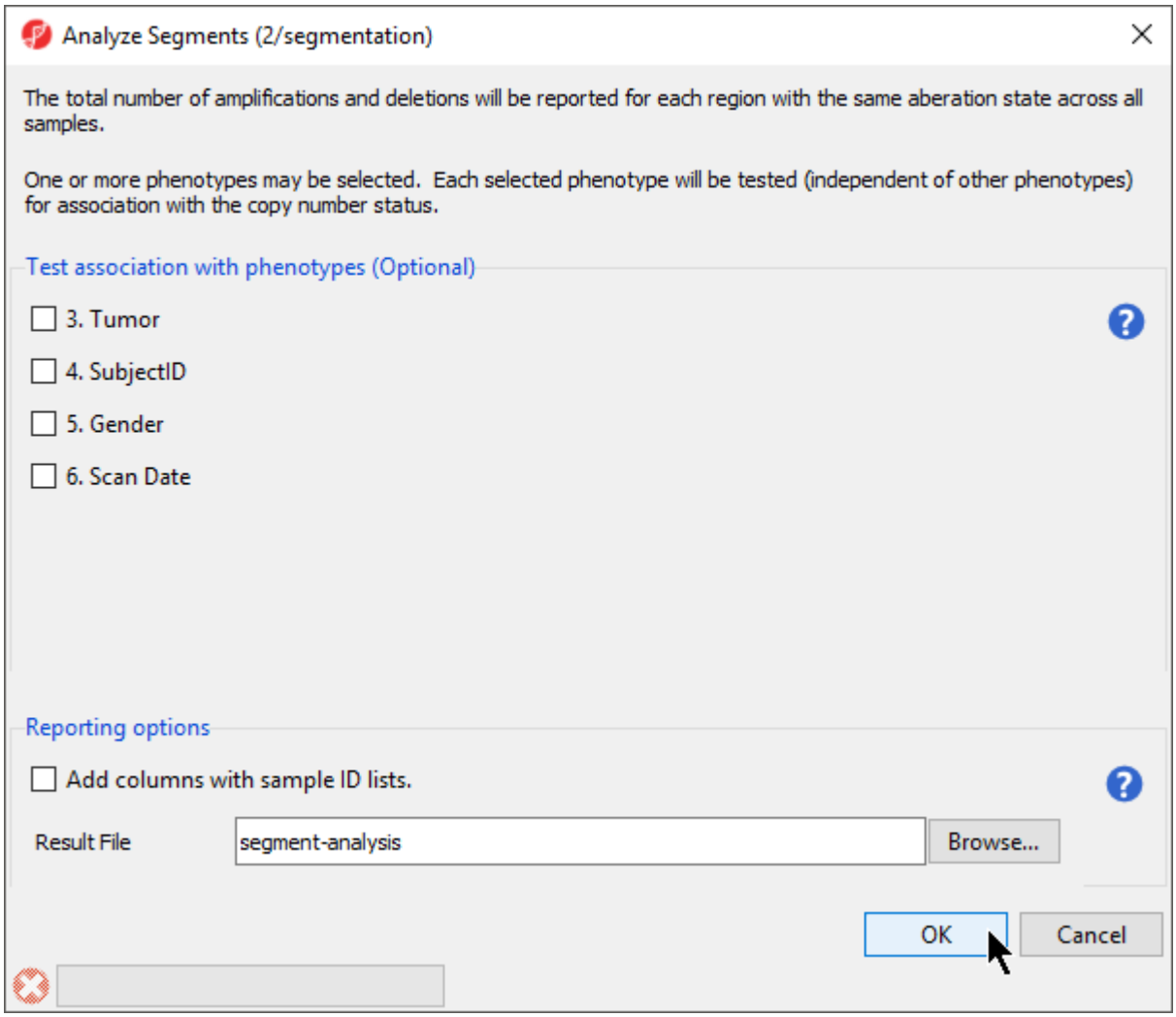


Figure 16. Viewing the Analyze segments dialog

- Leave all boxes unchecked
- Select **OK** to run the Analyze Segements task

The task generates a new spreadsheet, *summary (segment-analysis)* (Figure 7), with one region per row. The columns provide the following information:

1-4. Genomic locations of the regions

5. Total number of samples

6-7. Number of samples with amplifications and the average amplified copy number, respectively

8-9. Number of samples with deletions and the average deleted copy number, respectively

10. Total number of samples with copy number abberations

11-12. Number of samples with no change in copy number and the average copy number in those samples, respectively

13. Number of markers in the region

14. Length of the region (in base pairs)

15+. Two columns per sample - the average copy number in each sample as well as the copy number change status of the sample sample (e.g., amplified, deleted, unchanged, depending on the copy number and the threshold for unchanged defined in the *Genomic Segementation* dialog)

A "?" indicates that a region with the particular characterisitic does not exist or cannot be computed. For example, if a region is not amplified in any of the samples, the average amplified copy number will be shows as "?". This list may be filtered to contain only regions that meet user-specified criteria as discussed in the next section of the tutorial.

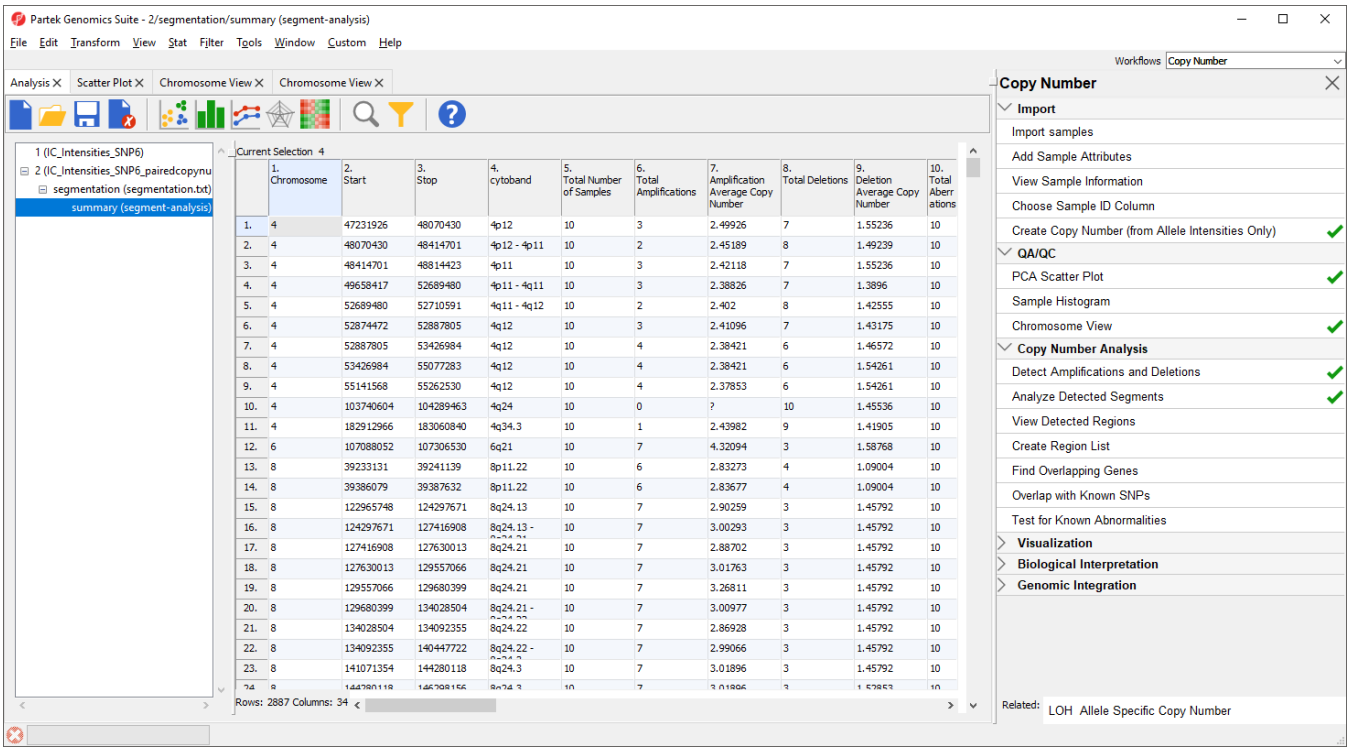


Figure 17. Viewing the results of Analyze Detected Segments

Visualizing shared regions of copy number variation

To get an overiew of the common abberations in the group of samples over the entire genome we can use *View Detected Regions.*

- Select **View Detected Regions**

The *View Detected Regions* dialog (Figure 7) allows you to select the spreadsheet with genomic regions and choose between histogram and copy number classification plots.

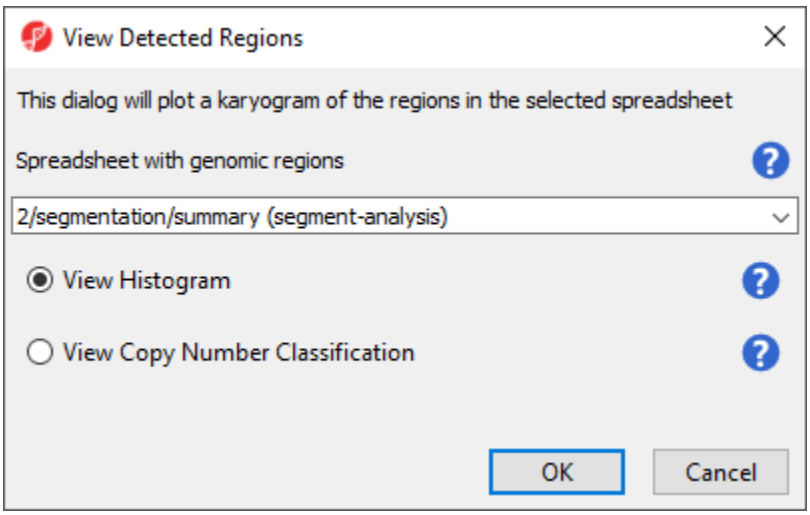


Figure 18. View Detected Regions dialog

- Select **summary (segment-analysis)** from the drop-down menu
- Select **View Histogram**
- Select **OK**

The plot will open in a new tab titled *Karyogram View* (Figure 8).

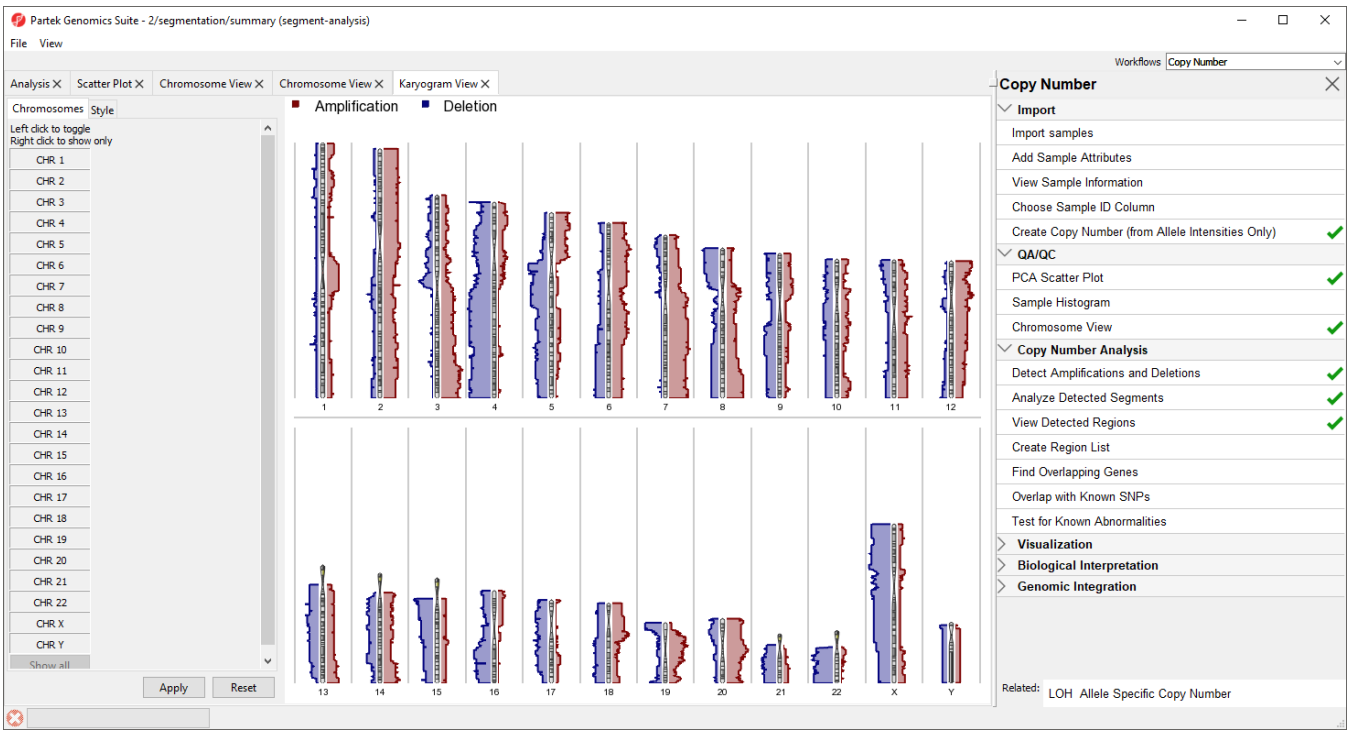


Figure 19. Viewing amplification and deletion histograms using Karyogram View

The *Karyogram View* shows each chromosome with red and blue histograms on either side corresponding to amplification and deletion, repsectively. The histogram height reflects the number of samples that share either amplification of deletion a that particular region. For example, the long arms of chromosomes 3 and 7 are amplified in the majority of samples and most samples share a deletion in the long arm of chromosome 4.

Mousing over the chromosome will give cytoband information, mousing over the histogram will give the number of shared regions at each position and the number of samples sharing the type of variation. Both the menu and display may be used to control which chromosomes are displayed; left-click in the menu to toggle a chromosome on/off and right click in the menu or graph to show only that chromosome.

Alternatively, we can use the *Copy Number Classification* plot to get a more sample-centric view.

- Select **View Detected Regions**
- Select **View Copy Number Classification**
- Select **OK**

The *Copy Number Classificaiton* also utilizes *Karyogram View* to provides an overview of all the samples and the copy number of regions on each chromosome (Figure 9).

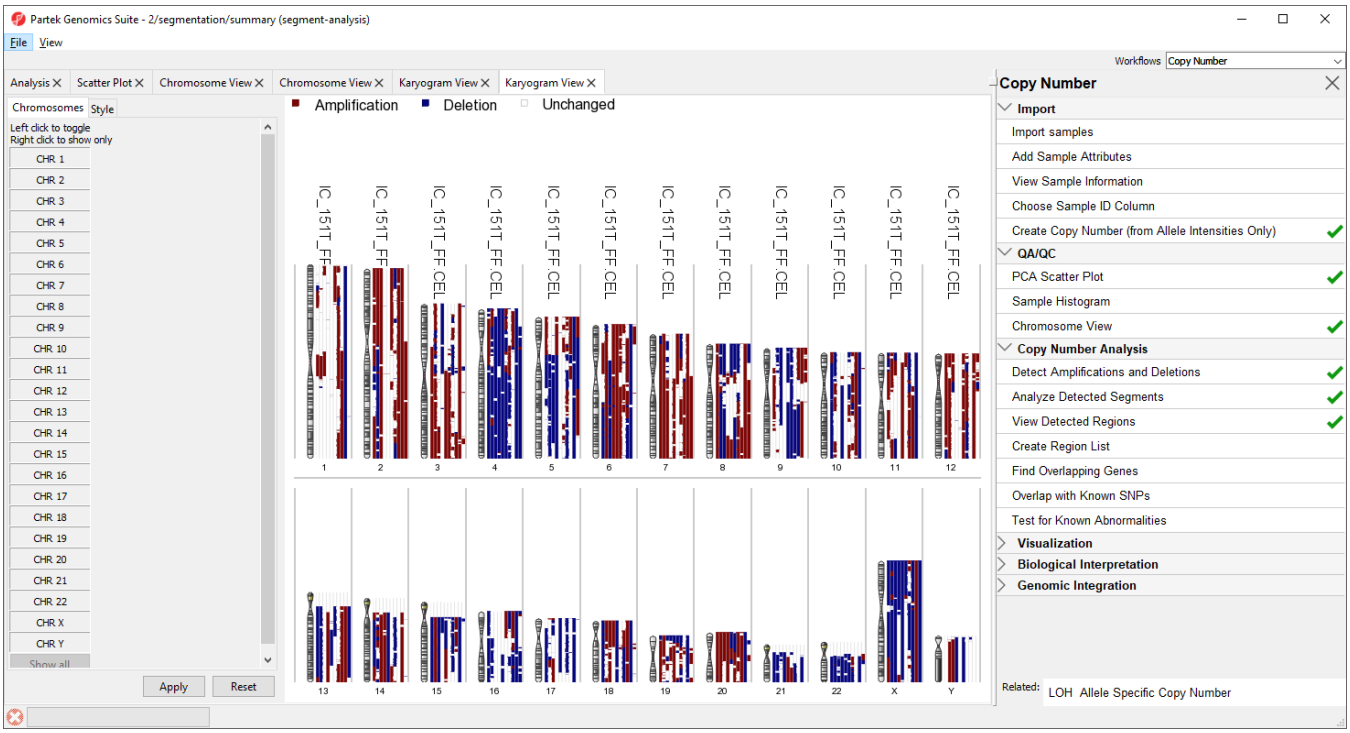


Figure 20. Viewing the Copy Number Classification plot

Each sample is drawn as a separate column next to the chromosome. Amplified regions are depicted in red, deleted regions in blue, and regions with no copy number change in white. Sample names are given accross the top of each column. For greater detail, try viewing fewer chromosomes.

« Creating Copy Number from Allele Intensities Creating a list of regions »

Additional Assistance

If you need additional assistance, please visit our support page to submit a help ticket or find phone numbers for regional support.

Your Rating: Results: 34 rates