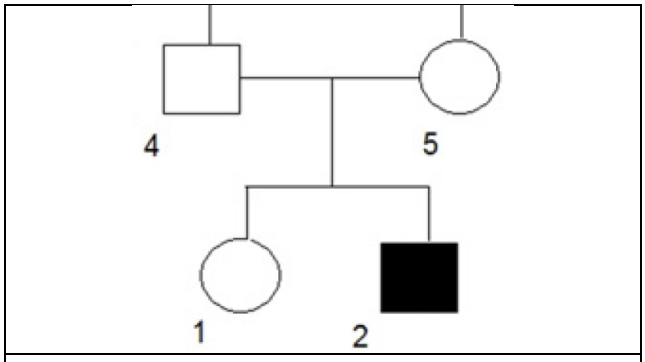
CircosVCF exercise

In this exercise, we will create and design circos plots using CircosVCF.

We will use vcf files of a published case *"X-linked elliptocytosis with impaired growth is related to mutated AMMECR1",* Basel-Vanagaite et al., Gene 2017.

This study reported a family with X-linked recessive syndrome caused by mutatation in the *AMMECR1* gene.
The samples from the family consist of: father, mother and two siblings (sister and brother). The proband is male (number 2 in the family tree), all other family members are healthy. Each family member is represented by a separate vcf file.
The syndrome is X-linked, thus the files contain only data on chromosome X.
You can find the vcf files for the exercise in the folder: *vcf exercise files.*



Pedigree of the family.
Affected individuals are represented by black filled symbols

We will create and design circos plots using CircosVCF representing the *AMMECR1* mutation in chromosome X

Do the following steps:

1. Enter to the CircosVCF website at http://legolas.ariel.ac.il/~tools/CircosVCF/
2. Press the green button “LET’S START”

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Data upload

3. In the window **Please set karyotype information** we need to choose the karyotype from UCSC or upload from file.
   In this exercise we will choose **human** karyotype, build **hg19**. Select only **chromosome X**. Press “SAVE”.
4. Press the "Annotations" button. In the window **Annotations** we can choose to add annotations from UCSC or from file (optional).
   In this exercise we will upload from file. Press "?" to see the file format.
   Now you need to create an annotation file (.txt) for ***AMMECR1*** gene located at the genomic location: X- **109507799.** Use notepad++.
5. In the window **Annotations**, choose the option "LOAD FROM FILE". Select your annotation file and Press “SAVE”.
6. Press the plus sign under the yellow arrow “Please add VCF files”.
7. Choose and add the exercise VCF files (You can add them all together).

Ring creation and design

8. Press the “Rings” button, flashing in yellow.
9. Press the plus sign under the yellow arrow “Please create new rings”

We want to create a ring for each family member (4 rings). In each ring we want to represent the SNVs (single nucleotide variants) that differ from all other family members (unique SNVs for each family member).

10. In the window **Ring Information** we will enter the name of the ring and choose it sizes (default 10%) and type (SNPs density or genotype). In this exercise we will choose **genotype.**
11. Please named the first ring “**father**”, type= **genotype**. Press “NEXT” button.
12. In the window **Ring Condition Tree** we will define the column for preview (which sample will be represented in the ring), and define the conditions that we would like to apply on all samples.
13. Please define the column for preview: **father**.
14. Press the “+Root” button.
15. In the window **Add a Condition** we can choose the condition type: AND tree, OR tree, Equal columns, Inequal columns. In this exercise the father and sister have the same genotype, thus we will define them as Equal columns. Check the box “Ignore rows with NA”. Press “SAVE”.

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16. Press the “+Root” button, now we want to add another condition thus we will choose AND tree.
17. Press the “+” button, in order to add the second condition.
18. The mother, father and brother have different genotypes, thus we will define them as Inequal columns. Check the box “Ignore rows with NA”. Press “SAVE”.
19. Press “SAVE”. The first ring will be created.
20. Next we want to create the same ring that would represent the **mother**. We can create a new ring repeating steps 9-16. A better option is to duplicate the first ring “**father**”, and change only the name and the column for preview. In In this exercise we will do the duplication.
21. Press on the first ("father") ring with the right button of the mouse. Choose “Copy”.
22. An inner ring will be created.
23. Press on the second ring with the right button of the mouse. Choose “Edit”.
24. Please change the name to “**mother**”, Press “NEXT” button.
25. In the window **Ring Condition Tree** we will change the column for preview: **mother**. Press “SAVE”.
26. In order to create rings for the **sister** and **brother** files, please repeat steps 21-25.
27. Now we have 4 rings.
28. To create the final figure press the button 
29. Please confirm your data and press "SUBMIT"
30. Please type your email, the results will be sent to this email. press "START"

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Looking at the plot

We created a plot that contains equalization to reference. In this option, a line is drawn at a corresponding SNP location. Each line represents a single SNP and is coloured based on the genotype:

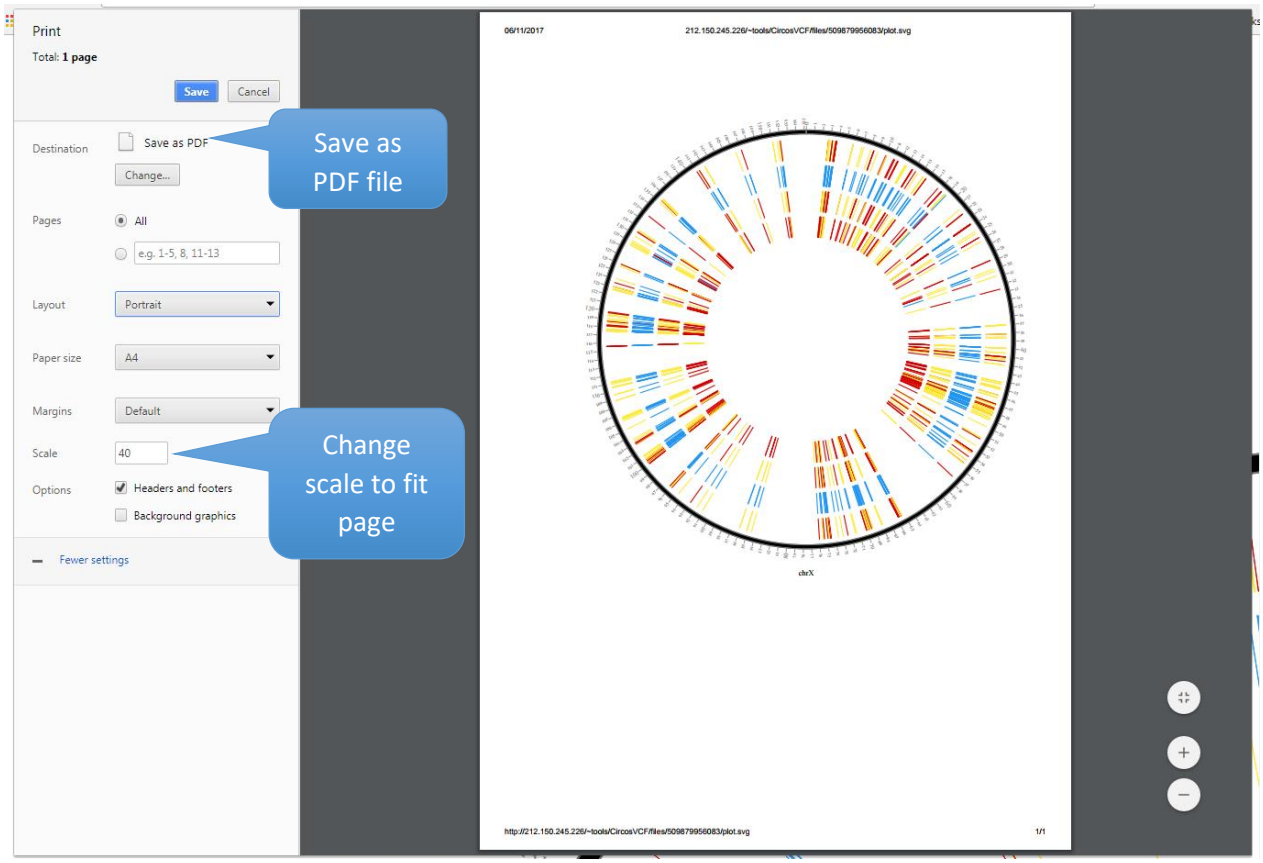
- Homozygosity to the reference allele is yellow
- Homozygosity to the alternative allele is red
- Heterozygosity is blue.

This representation option is recommended when plotting relatively small number of SNPs, or single chromosome variations.

Saving the plot

Recommended using the **Google Chrome** browser.

To save as PDF file press Ctrl P.



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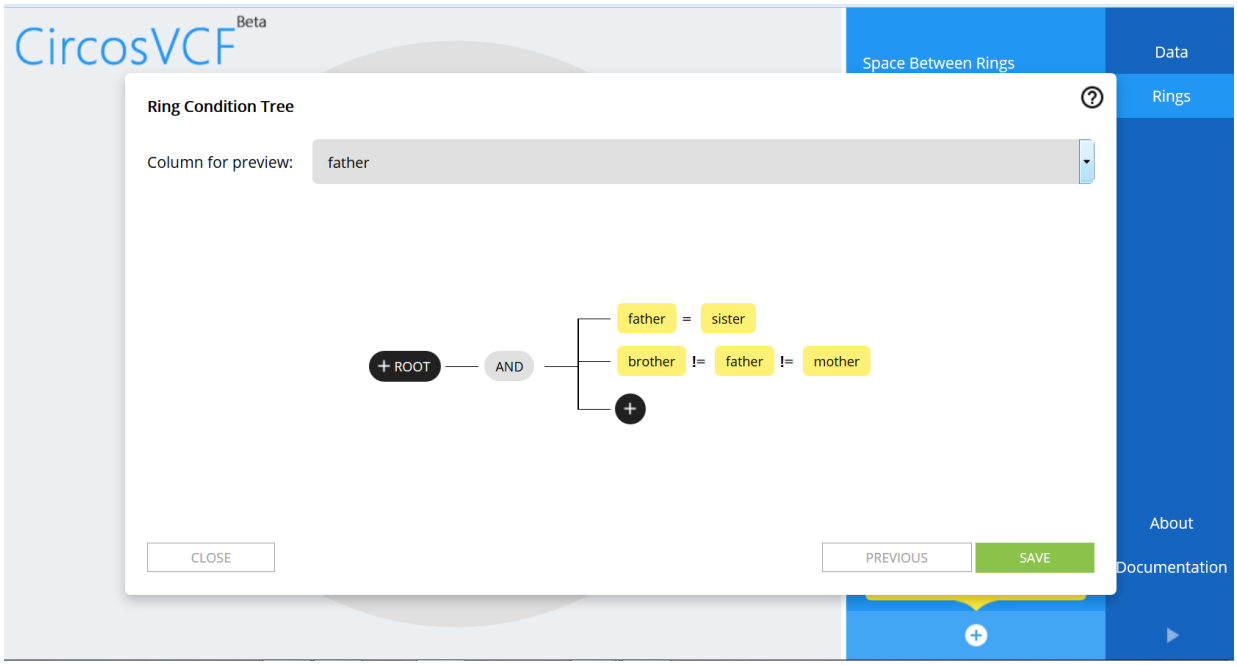
Condition tree



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