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NGenomeSyn

Manual

NGenomeSyn: an easy-to-use and flexible tool for publication-ready visualization of syntenic relationships across multiple genomes

Version 1.40 documentation

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Contents

1 Introduction

NGenomeSyn is a visualization tool for syntenic links across Multiple Genomes with highly customizable settings, including stroke, fill, position, scale, rotation, genomic coordinates for chromosome/scaffold, link colors *etc*, which enable quick exploration the data.

The advantages of this tool include

1. tens of customizable genomes (≥2)
2. flexible and customizable layout
3. high customization for order, colors or other settings for each genome
4. allowing setting for movement, rotation, scaling *etc* for each genome, which could generate particular layouts, such as triangle, quadrangle, pentagon *etc*
5. high customization for color, transparency *etc* for link information
6. Zoom in on specific region of interest

The program provides several examples for learning using seven rice genomes (genome size: ~400 Mbp) and two yeast genomes (genome size: ~12 Mbp).

2 Download and Installation

2.1 Download

The latest source code and binaries of **NGenomeSyn** are freely available at the follow website:
For Linux/Unix/macOS system:

https://github.com/hewm2008/NGenomeSyn

2.2 Prerequisites

The prerequisites for **NGenomeSyn** include:

- **Perl** and **SVG** module (Note: SVG module is optional because related modules have been included in ***bin/svg-kit*** directory).

- convert: converting ***svg*** to ***png***

2.3 Installation

Users can install it with the following commands:

```
git clone https://github.com/hewm2008/NGenomeSyn.git
cd NGenomeSyn; chmod -R 755 bin/*
./bin/NGenomeSyn  -h        ### run help
```

3 Examples

Six examples are provided and all of which use real data to generate graphs. Some important functions are illustrated below:

Examples	Description
example1	An integrating **pipeline** including data preparation and visualizing of two genomes for the simplest usage
example2	Horizontal layout of 3 or more genomes, genome layout adjustment and special region **highlight**
example3	Link settings, five link styles, genome layout adjustment for particular shape (triangle)
example4	**ZoomRegion** function of local gene **structure** (CDS, UTR) collinearity
example5	The comprehensive configuration for horizontal layout of more than three genomes (>3)
example6	quick identification of genetic deletion in some breeds (pan-genome frequently analysis) to **solve biological** problems

Notes: All test data were downloaded from the real data, and shown in the **Example/RealData** directory, and The file (**00.ReadMe**) contains the URL where the data is downloaded

3.1 Two genomes

For the simplest usage, only two genomic locus files and link file are required to be provided and defined in the configuration file. Please refer to the Example/ example1 for details.

Command:

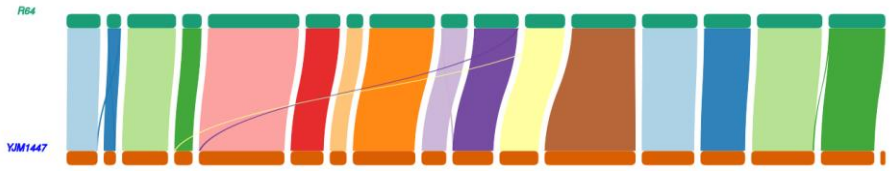
```
../../bin/NGenomeSyn -InConf  in.conf -OutPut OUT
```

Configuration file (in.conf):

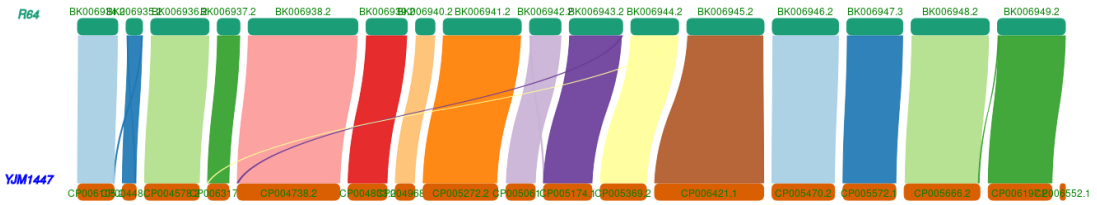
```
## in.conf in Examples/example1
```

```
SetParaFor = global
GenomeInfoFile1=Pa64.len
GenomeInfoFile2=YJM1447.len
LinkFileRef1VsRef2=R64_YJM1447.link
```

OutPut:



User could add or adjust attributes, such as opacity, ***color*** etc.
For example, we could show names of chromosome by adding a line: ***ChrNameShow=1***



Run2.sh also provides an **integrating pipeline** (GetTwoGenomeSyn.pl) for data preparation and visualization.

```
wget -c ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/146/045/GCA_000146045.2_R64/GCA_000146045.2_R64_genomic.fna.gz
wget -c ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/977/955/GCA_000977955.2_Sc_YJM1447_v1/GCA_000977955.2_Sc_YJM1447_v1_genomic.fna.gz
perl ../../bin/GetTwoGenomeSyn.pl -InGenomeA GCA_000146045.2_R64_genomic.fna.gz -InGenomeB GCA_000977955.2_Sc_YJM1447_v1_genomic.fna.gz -OutPrefix A2B -MappingBin minimap2 -BinDir /hwfssz4/BC_PUB/Software/08.Centos7/minimap2-2.23/ -MinLenA 1000 -MinLenB 1000
```

3.2 Horizontal layout of three or more genomes

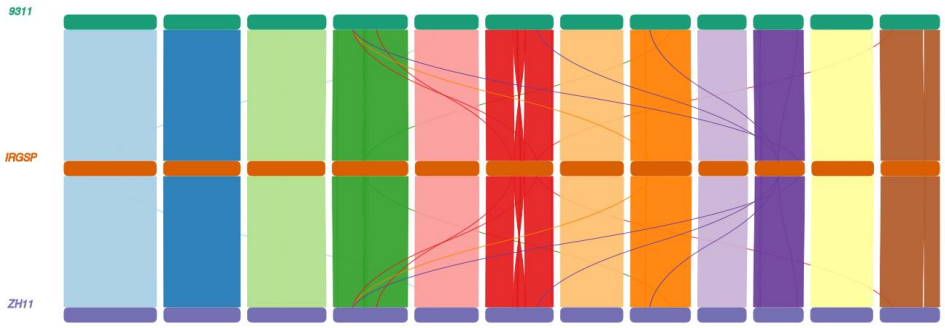
Test data used in example2 is the same in example1

Please refer to the **Examples/example2** for details.

Configuration

```
##in1.conf
SetParaFor = global
GenomeInfoFile1=9311.len                    ### the first genome (Genome1:9311)
GenomeInfoFile2=IRGSP.len                   ### the second genome (Genome2:IRGSP)
GenomeInfoFile3=ZH11.len       ### the  third genome (Genome3:ZH11)
LinkFileRef2VsRef1=IRGSP_9311.link ### link file between Ref2 and Ref1
LinkFileRef2VsRef3=IRGSP_ZH11.link ### link file between Ref2 and Ref3
```

OutPut:

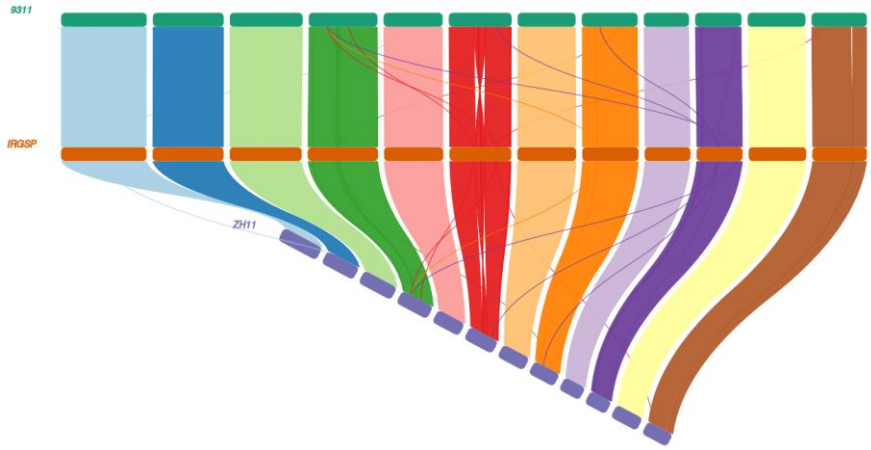


We also could adjust a genome with movement, zoom in/out, rotation to generate a particular layout.

In example2: we adjusted 3th genome by zoom in (***ZoomChr=0.5***), rotation with 28 degrees (***RotateChr=28***) and movement (***ShiftX=60,ShiftY=80***).

```
### in2.conf
CanvasHeightRitao=1.6   ## increase height of canvas
SetParaFor =Genome3      ## set for Genome3
ZoomChr=0.5              ## 50% of the default chr length
RotateChr=28             ## rotation of chr with 28 degrees clockwise
ShiftX=60
ShiftY=-80             ## move to (60,-80)
```

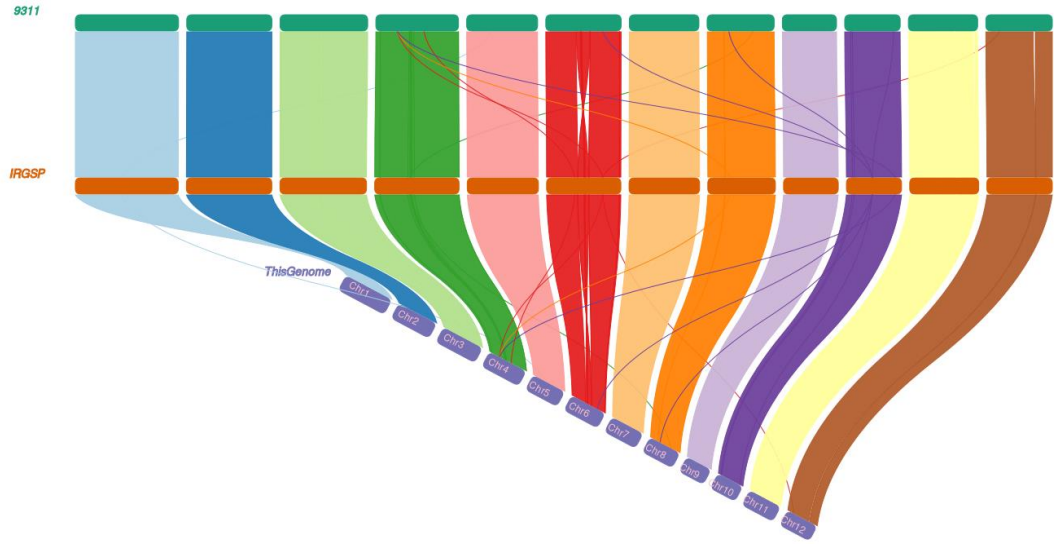
OutPut:



Other attributes (show name of chromosome, color and move) could also be added.

```
ChrNameShow=1
ChrNameColor=pink
#ChrNameShiftX=-5
ChrNameShiftY=35
GenomeName="ThisGenome"          ##rename genome name
```

OutPut:



In addition, we also could highlight a specific region by setting in the **SpeRegion.bed** file

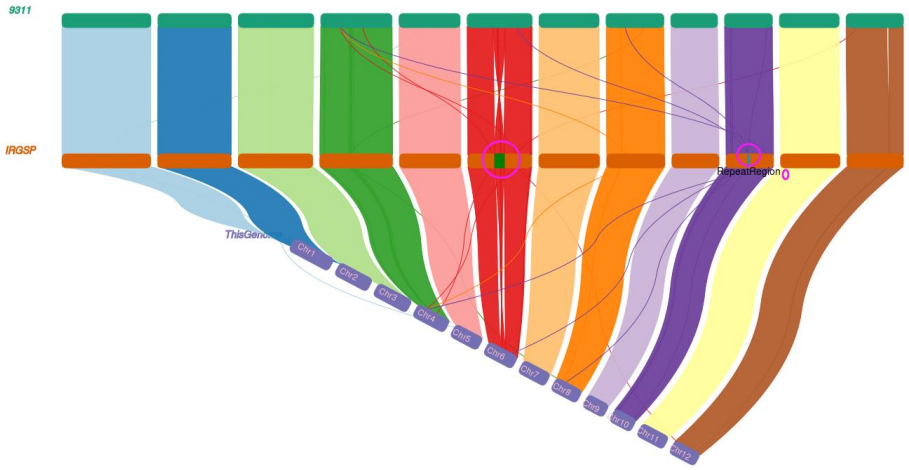
```
## Spe.region
## chr start end key=value
```

```
Chr10    10036207  11920932  fill="#3d84a8"      SN=RepeatRegion
Chr6     13071427  17863436  fill=green stroke=green
```

and set in the configure file by adding a line ***SpeRegionFile=SpeRegion.bed*** under the flag "*SetParaFor = Genome2*".

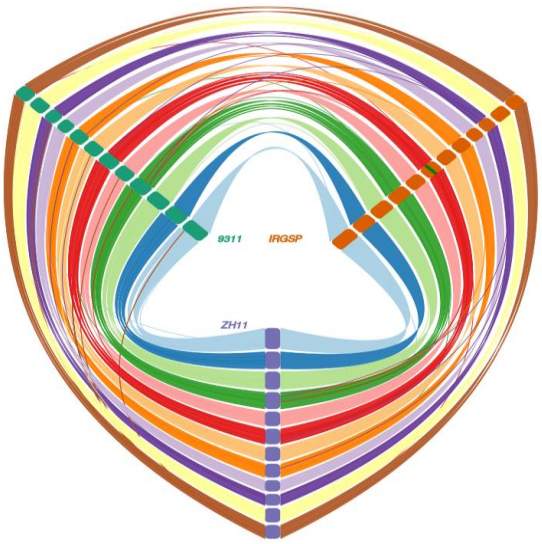
Note: if you want to display some **text** in this region, you can add an attribute of **SN**=Text.

OutPut:



At the same time, we can also move (**MoveX Y**) the three genomes to the middle of the canvas, and **rotate** the genomes at -135, -45 and 90 degrees respectively. As a result, the link style is changed to ***UpUp***. (for more information please refer to 4.2.3 section)

Please refer to the ***in3.conf*** for details



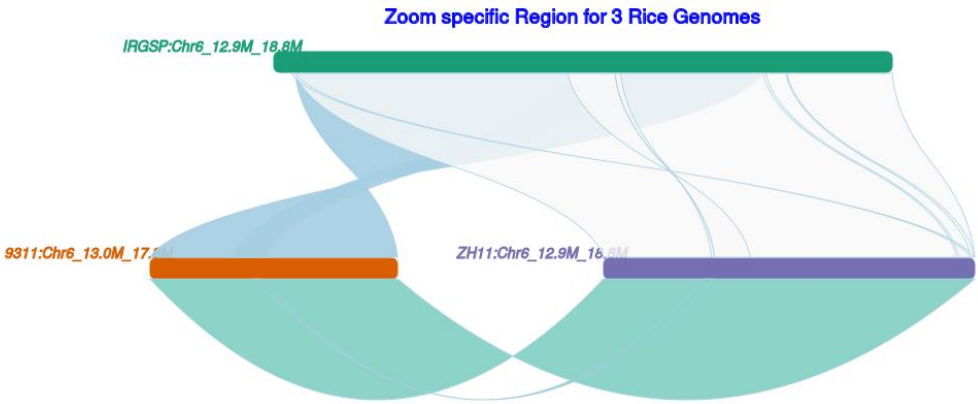
3.3 Design link styles for flexible layout

Five link styles were defined in this tool, "DownDown", "UpUp", "DownUp","UpDown" and “line”. (for more information please refer to 4.2.3 section);

Please refer to the **Examples/example3** for details.
one example is:

```
SetParaFor =Link3
HeightRatio=1.5
StyleUpDown=DownDown ## DownDown UpUp DownUp UpDown   ## line
Reverse=1
```

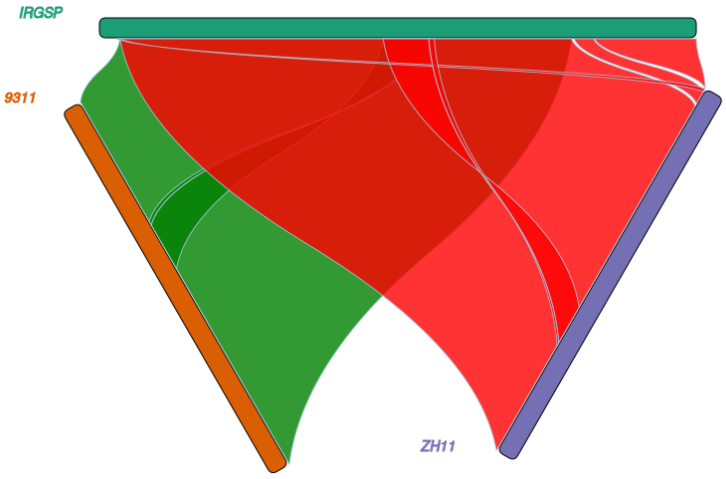
OutPut



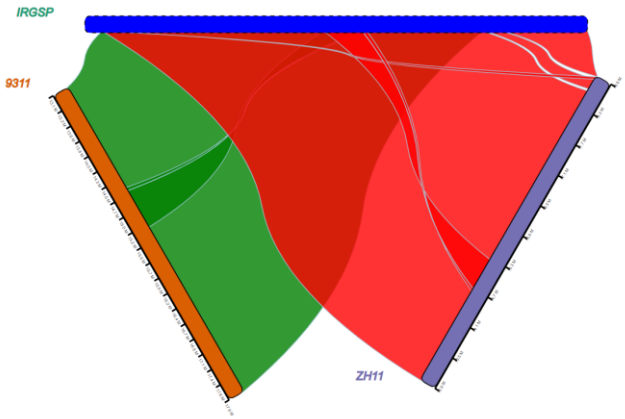
We also could set ***MoveToX/MoveToY, ZoomChr, RotateChr=60/-60*** for triangular layout

```
### in2.conf in Examples/example3
SetParaFor = Genome2
RotateChr=60
MoveToX=390
MoveToY=150
ZoomChr=0.35

SetParaFor = Genome3
MoveToX=810
MoveToY=500
ZoomChr=0.35
RotateChr=-60

SetParaFor=Link3
StyleUpDown=UpUp
HeightRatio=0.8
```

OutPut:



We also added the parameters to display a genomic coordinate (***ShowCoordinates=1),*** axes breaks (***ScaleNum=20***) and axes label precision decimal point (***LablePrecision***) etc, for each genome. Adjustment some of these parameters, NGenomeSyn generated a figure listed below:



3.4 A flexible layout of seven genomes

In general, this tool could generate a publication-quality and complex layout for tens of genomes.

We also could arrange to obtain complex layout below similar as Zheng et al, 2022. To test this, we analyzed seven rice genomes, adjusted parameters and produced a figure listed below.

Configuration:

```
### in.conf in Examples/example4  ###
############################ global variable settings ########################
 SetParaFor = global
 GenomeInfoFile1= ../RealData/Rices/IRGSP.len   ## GenomeInfoFile 1 : IRGSP japonica
 GenomeInfoFile2= ../RealData/Rices/ZH11.len    ## GenomeInfoFile 2 means the 2th genome is ZH11
 GenomeInfoFile3= ../RealData/Rices/9311.len    ## GenomeInfoFile 3 means the 3th genome is 9311
 GenomeInfoFile4= ../RealData/Rices/TM.len
 GenomeInfoFile5= ../RealData/Rices/KY131.len
 GenomeInfoFile6= ../RealData/Rices/YX1.len
 GenomeInfoFile7= ../RealData/Rices/Basmati1.len

 LinkFileRef1VsRef2= ../RealData/Rices/IRGSP_ZH11.link         ## First : Link1
 LinkFileRef1VsRef3= ../RealData/Rices/IRGSP_9311.link         ## second appearance :Link2
 LinkFileRef2VsRef3= ../RealData/Rices/ZH11_9311.link          ## Link3
 LinkFileRef4VsRef5= ../RealData/Rices/TM_KY131.link           ## Link4
 LinkFileRef6VsRef7= ../RealData/Rices/YX1_Basmati1.link       ## Link5

SetParaFor=Link3                ## setting parameters for the Link3
 StyleUpDown=DownDown
 HightRation=1.2                ## High ratio of links expand or shrink(For DownDown or UpUP)
 fill=grey                      ## fill Color set to grey
 stroke=grey
 ShortLinkLineRefA=10           ## the link line to RefA becomes a little shorter (negative lengthens)
 ShortLinkLineRefB=10           ## the link line to RefB becomes a little shorter (negative lengthens)

SetParaFor=Link4                ## setting parameters for the Link4; LinkAL, works on all links
 StyleUpDown=DownDown
 HightRation=1.2                ## High ratio of links expand or shrink(For DownDown or UpUP)
 fill=grey
 stroke=grey
 ShortLinkLineRefA=10
 ShortLinkLineRefB=10

 SetParaFor=Link5               ## setting
StyleUpDown=DownDown
HightRation=1.2                 ## High ratio of links expand or shrink(For DownDown or UpUP)
 fill=grey
 stroke=grey
 ShortLinkLineRefA=10
```

```
ShortLinkLineRefB=10

LinkFileRef2VsRef4= ../RealData/Rices/ZH11_TM.link              ## Link6
LinkFileRef4VsRef6= ../RealData/Rices/TM_YX1.link               ## Link7

SetParaFor=Link6                                    ## setting parameters for the Link6
fill="#5CA76D"                                      ## set the Links Color
stroke="#5CA76D"

SetParaFor=Link7                                    ## setting parameters for the Link7
fill="#5CA76D"                                      ## set the Links Color
stroke="#5CA76D"

LinkFileRef3VsRef5= ../RealData/Rices/9311_KY131.link           ## Link8
LinkFileRef5VsRef7= ../RealData/Rices/KY131_Basmati1.link       ## Link9

SetParaFor=Link8                                    ## setting parameters for the Link8
fill="#4B73B7"                                      ## set the Links Color
stroke="#4B73B7"

SetParaFor=Link9                                    ## setting parameters for the Link9
fill="#4B73B7"                                      ## set the Links Color
stroke="#4B73B7"

left=200                    ## The length of the space on the left side of the canvas is 200
right=200                   ## The length of the space on the right side of the canvas is 200

SetParaFor = GenomeALL              ## setting parameter for the ALL genome
ZoomChr=0.4                 ## Reduce(<1) the lenghth of chromosomes 0.4 of the original
GenomeNameSizeRatio=2.0

SetParaFor = Genome1                ## setting parameter for the 1st genome
ShiftY=100                          ## shift Y coordinates down 100

SetParaFor = Genome2                ## setting parameter for the 2st genome
RotateChr=-10       ## Move the starting point to (100,570), and rotate 10 degrees counterclockwise
MoveToX=100
MoveToY=570
```

```
SetParaFor = Genome3              ## setting parameter for the 3st genome
RotateChr=10            ## Move the starting point to (850,470), and rotate 10 degrees clockwise
MoveToX=850
MoveToY=470

SetParaFor = Genome4               ## setting parameter for the 4st genome
RotateChr=-10                      ## Move the starting point to (100,870), and rotate 10 degrees counterclockwise
MoveToX=100
MoveToY=870

SetParaFor = Genome5               ## setting parameter for the 5st genome
RotateChr=10                       ## Move the starting point to (850,770), and rotate 10 degrees
MoveToX=850
MoveToY=770

SetParaFor = Genome6               ## 6st genome
RotateChr=-10          ## Move the starting point to (100,1170), and rotate 10 degrees counterclockwise
MoveToX=100
MoveToY=1170

SetParaFor = Genome7              # 7st Genome
RotateChr=10
MoveToX=850
MoveToY=1070
```

OutPut：

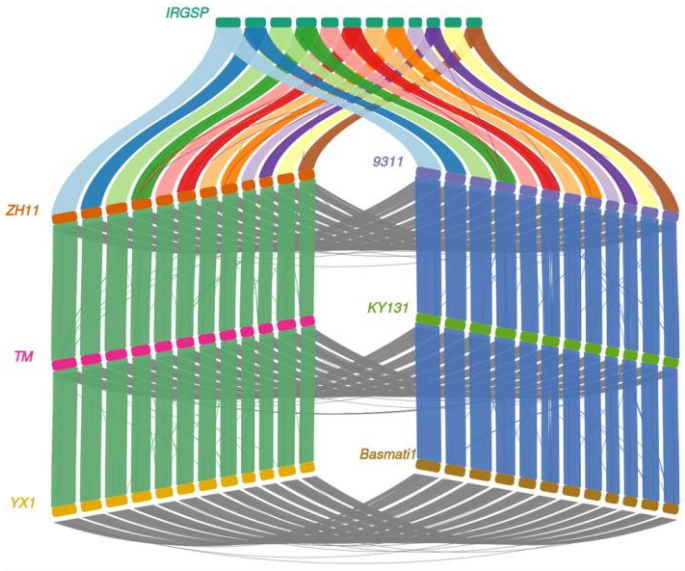


Figure in the example 4

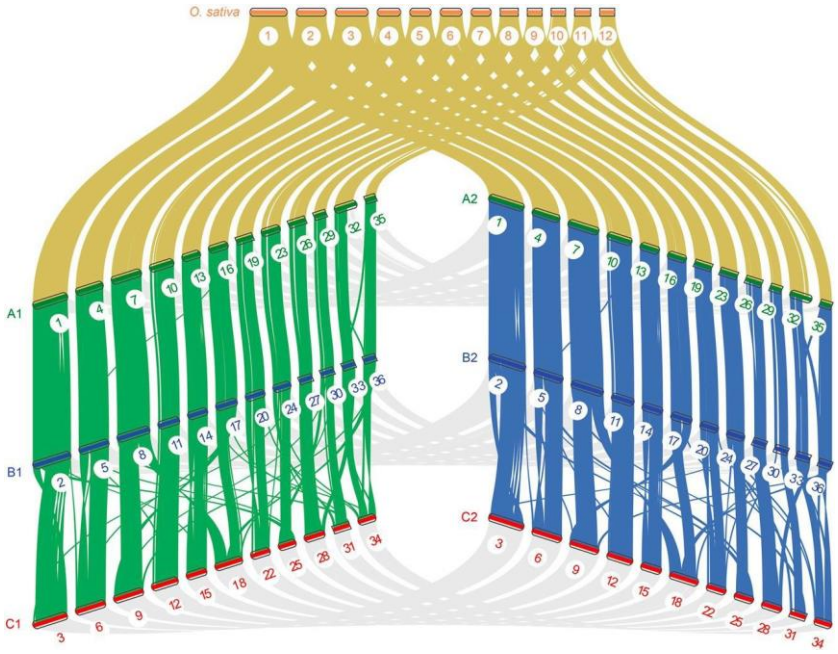


Figure was retrieved from Figure2 in Zheng et al, 2022

3.5 Highlight of gene structure in local synteny and ZoomRegion

In v0.21, we provided an attribute (***SpeRegionWidthRatio***) to control the width of the ***SpeRegion***, supporting highlights of gene structures (e.g. CDS, UTR etc). Feature types (CDS, UTR, mRNA) could be defined in the fourth column of the given SpeRegionFile. NGenomeSyn could detect and distinguish these elements using distinct colors and height relative to the rectangular chromosome.

In the latest version, the function of ***ZoomRegion*** has been added. Users could set the parameter ***ZoomRegion*** with the format "ZoomRegion=***chr:Start:End***", to display a local synteny in on specific region of interest.

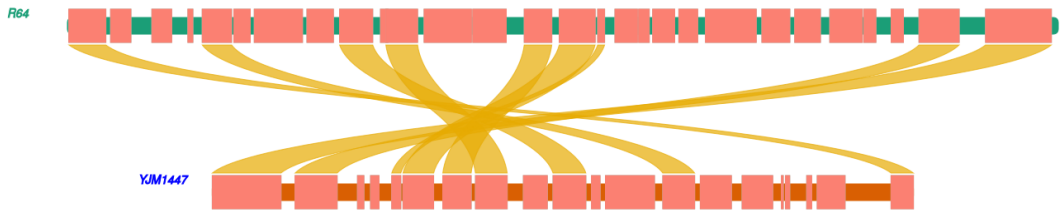
Please refer to the **Examples/example5** for details.

One example is:

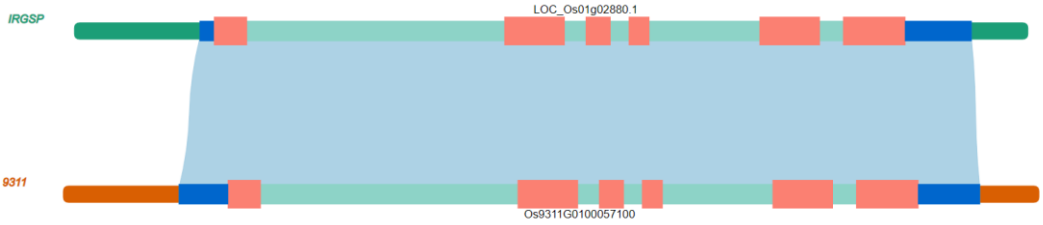
```
### in.conf in  Examples/example5 ###
SetParaFor = global
GenomeInfoFile1=R64.len                          ##  GenomeInfoFile 1 For R64
GenomeInfoFile2=YJM1447.len                      ##  GenomeInfoFile 2 For YJM1447
LinkFileRef1VsRef2=R64_YJM1447.gene.pairwise.A2B.link            ##  Link1

SetParaFor = Genome1
SpeRegionFile=R64_YJM1447.gene.pairwise.A.geneInfo.bed  ## special regions files, highlight these Regions
ChrNameColor=green           ##  set Chr Name Color to green
ChrNameShiftY=10             ##  Chr Name text move down 10
SpeRegionWidthRatio=1.5        ## Increase the height of special Region by 1.5 times of the original
NormalizedScale=1
ZoomRegion=BK006937.2:35800:82600   ##  Zoom this specific Region

SetParaFor = Genome2     ##  setting parameter  for the 2st genome
ChrNameColor=green
ChrNameShiftY=30
GenomeNameColor=blue
```

```
SpeRegionWidthRatio=1.5
SpeRegionFile = R64_YJM1447.gene.pairwise.B.geneInfo.bed ## special regions files, highlight these Regions
NormalizedScale=1
ZoomRegion=CP004738.2:1403880:1437000  ##  Zoom this specific Region

SetParaFor =Link1        ## setting parameters for the Link1
OutsideWidthDeta=13      ## ratio for increase or reduce the distance between ## link and RefA
stroke-opacity=0.7       ## Color opacity set to 0.7
fill-opacity=0.7
stroke="rgb(230,171,2)"   ## fill Color set to "rgb(230,171,2)"
fill="rgb(230,171,2)"
stroke-width=1
```

Figures generated in example 5 were listed below:



OUT1.png in the example 5



OUT2.png in the example 5

3.6 Practical application (solve biological issue)

In the pan-genome study, multiple genomes are *de novo* assembled from different individuals (accessions) independently, and it is often important to identify genetic structural variation (insertion and deletion) of different individuals (accessions) in a certain region to investigate whether link

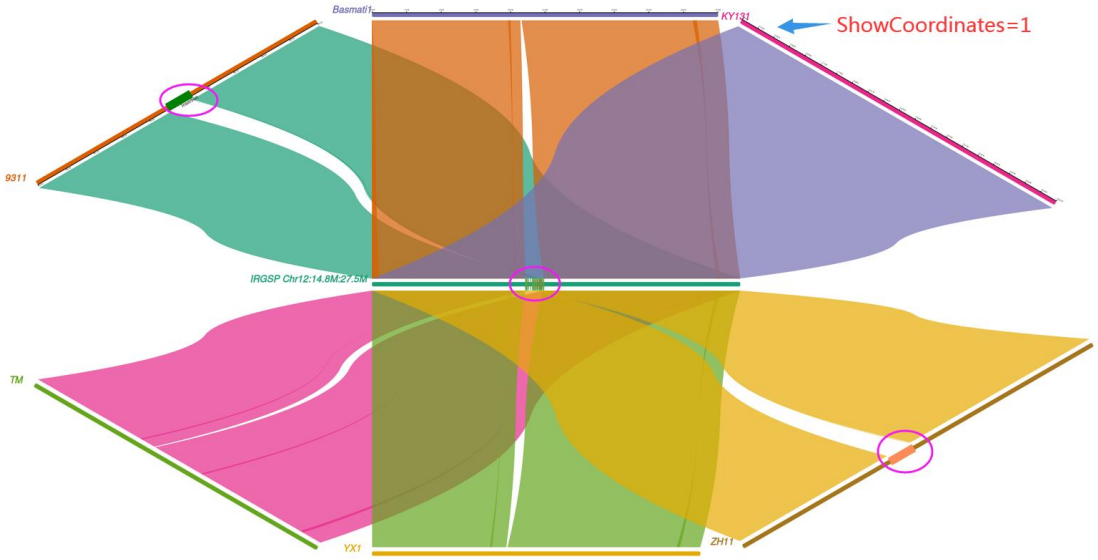
genetic variations to candidate genes that are responsible for important traits. The following example is an identification of some structural variations (insertion, deletion) in several rice accessions compared to the chromosome 12 of the reference rice genome ("IRGSP"). For example, a segment insertion occurs in the accessions of "9311" and "ZH1".

The configuration is listed as following:

```
SetParaFor = global
GenomeInfoFile1=IRGSP.len    ## GenomeInfoFile 1 :  IRGSP  japonica
GenomeInfoFile2=9311.len     ## GenomeInfoFile 2 means the 2th genome is 9311
GenomeInfoFile3=Basmati1.len
GenomeInfoFile4=KY131.len
GenomeInfoFile5=TM.len       ## GenomeInfoFile 5 means the 5th genome is TM
GenomeInfoFile6=YX1.len      ## GenomeInfoFile 6 means the 6th genome is  YX1
GenomeInfoFile7=ZH11.len     ## GenomeInfoFile 7 means the 7th genome is ZH11

LinkFileRef1VsRef2=IRGSP_9311.link       ## Link1
LinkFileRef1VsRef3=IRGSP_Basmati1.link   ## Link2
LinkFileRef1VsRef4=IRGSP_KY131.link      ## Link3
LinkFileRef1VsRef5=IRGSP_TM.link         ## Link4
LinkFileRef1VsRef6=IRGSP_YX1.link        ## Link5
LinkFileRef1VsRef7=IRGSP_ZH11.link       ## Link6

CanvasHeightRitao=1.6    # increase canvas height to 1.7 of the original
CanvasWidthRitao=2.6     # increase canvas Width to 2.6 of the original

SetParaFor =GenomeALL    ## setting parameter for the ALL genome
ChrWidth=12
NormalizedScale=1        ## use the custome scale (=1) or the same with default genomes (=0)
SpeRegionWidthRatio=2    ## Increase the height of special Region by 2 times of the original
GenomeNameSizeRatio=2    ## the font of the chr name is enlarged by 2 times of the original

SetParaFor = Genome1     ##  setting parameter  for the 1st genome
MoveToX=1400
MoveToY=1000
SpeRegionFile=Data_SpeRegion/IRGSP.bed    ##  special regions files,For highlight these Regions
ZoomRegion=Chr12:14822000:27530800        ##  Zoom this specific Region,ignore Others
GenomeName="IRGSP Chr12:14.8M:27.5M"
GenomeNameShiftX=-130

SetParaFor = Genome2
MoveToX=400
MoveToY=700
RotateChr=-30
ZoomRegion=Chr12:14878000:26029400        ##  Zoom this specific Region,ignore Others
SpeRegionFile=Data_SpeRegion/9311.bed     ##  special regions to highlight

SetParaFor = Genome3
MoveToX=1400
MoveToY=200
ZoomRegion=Chr12:15829400:27774200
ShowCoordinates=1
ScaleUpDown=Up   ## Show Coordinates . with other para [ScaleNum=10 ScaleUpDown ScaleUnit Labe   lUnit LablefontsizeRatio   ]



SetParaFor = Genome4
MoveToX=2500
MoveToY=220
RotateChr=30
ZoomRegion=Chr12:13998000:26530600      ##  Zoom this specific Region,ignore Others

SetParaFor = Genome5
MoveToX=400
MoveToY=1300
RotateChr=30
ZoomRegion=Chr12:14942000:26205200

SetParaFor = Genome6
MoveToX=1400
MoveToY=1800
ZoomRegion=Chr12:15125500:26457200

SetParaFor = Genome7
RotateChr=-30
MoveToX=2500
MoveToY=1780
```

```
SpeRegionFile=Data_SpeRegion/ZH11.bed    ##  special regions to highlight
ZoomRegion=Chr12:14217800:28156500


SetParaFor=LinkALL         ## setting parameters for the ALL Link
fill-opacity=0.7           ##  Color opacity set to 0.7
stroke-opacity=0.7
SetParaFor=Link1           ## setting parameters for the Link1
OutsideWidthDeta=-23       ## the link line to RefA and RefB becomes a little shorter
fill="rgb(27,158,119)"     ##  fill Color set to "rgb(red,green,blue)"
stroke="rgb(27,158,119)"
SetParaFor=Link2           ## setting parameters for the Link2
OutsideWidthDeta=-23
fill="rgb(217,95,2)"       ##  fill Color set to "rgb(red,green,blue)"
stroke="rgb(217,95,2)"
SetParaFor=Link3           ## setting parameters for the Link3
OutsideWidthDeta=-23
stroke-width=0
fill="rgb(117,112,179)"
stroke="rgb(117,112,179)"
SetParaFor=Link4           ### setting parameters for the Link4
OutsideWidthDeta=13
stroke="rgb(231,41,138)"
fill="rgb(231,41,138)"
stroke-width=0
SetParaFor=Link5
OutsideWidthDeta=13
fill="rgb(102,166,30)"
stroke="rgb(102,166,30)"
stroke-width=0
SetParaFor=Link6
OutsideWidthDeta=13
stroke="rgb(230,171,2)"
fill="rgb(230,171,2)"
stroke-width=0
```

OutPut:



4 Usage and parameter guides

4.1 Running NGenomeSyn

Usage

```
./bin/NGenomeSyn
        Version:1.38        hewm2008@gmail.com
                        Options
                        -InConf       <s> : InPut Configuration File
                        -OutPut       <s> : OutPut svg file result

                        -help           : Show more help with more parameter
```

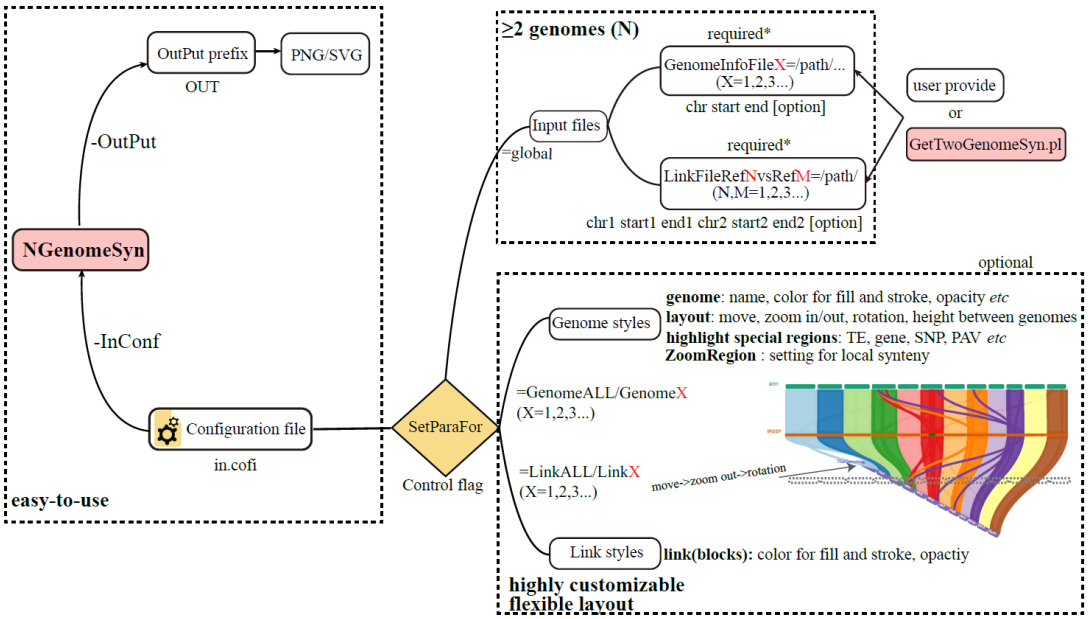
Arguments

- InConf: InPut Configuation file
- OutPut: Output name, default ***out.svg***
- help

Note: we also provide an i**ntegrating pipeline (*bin/GetTwoGenomeSyn.pl*)** for easy preparation link information for NGenomeSYn from two genomes (fasta format) using either the genomic aligner: ***MUMmer(nucmer)*** or ***minimap2*** program or directly convert output from Minimap2 (bin/GetTwoGenomeSyn.pl Paf2Link), MUMmer (bin/GetTwoGenomeSyn.pl Coords2Link) or MCScanX (MCScanX2Link.pl). Please refer to run1.sh in the example 1.

4.2 Parameter guides in the configure file

The main parameters in configuration can be overviewed in this figure:


OutPut prefix
PNG/SVG
OUT
-OutPut
NGenomeSyn
-InConf
Configuration file
in.cofi
easy-to-use
SetParaFor
Control flag
≥2 genomes (N)
required*
GenomeInfoFileX=/path/...
(X=1,2,3...)
chr start end [option]
Input files
=global
required*
LinkFileRefNvsRefM=/path/
(N,M=1,2,3...)
chr1 start1 end1 chr2 start2 end2 [option]
user provide
or
GetTwoGenomeSyn.pl
optional
Genome styles
genome: name, color for fill and stroke, opacity etc
layout: move, zoom in/out, rotation, height between genomes
highlight special regions: TE, gene, SNP, PAV etc
ZoomRegion : setting for local synteny
=GenomeALL/GenomeX
(X=1,2,3...)
=LinkALL/LinkX
(X=1,2,3...)
move->zoom out->rotation
Link styles
link(blocks): color for fill and stroke, opactiy
highly customizable
flexible layout

4.2.1 parameters

SetParaFor could be set as "global|GenomeALL|GenomeX(e.g. Genome1, Genome2, LinkALL,Link1 ...)".

No	example	help
1	GenomeInfoFileX=/path/Ref.len	(X=1,2,3...) setting for GenomeX
2	LinkFileRefNVsRefM=/path/N_M.link	link file between GenomeN and GenomeM, could be set for multiple times
3	#ZoomChr=1.0	ratio for the GenomeX, 1 for equal [default]
4	#RotateChr=0	rotation of the GenomeX
5	#MoveX MoveY ShiftX=0 ShiftY=0	GenomeX was move to (X,Y)
6	#ChrWidth=20	width of GenomeX
7	#LinkWidth=180	the link width between two genomes
8	#ChrSpacing=10	spacing between chr/scaffolds in the GenomeX
9	#ZoomRegion	Zoom the specific region, format (ZoomRegion=chr2:1000:5000)

4.2.2 Others

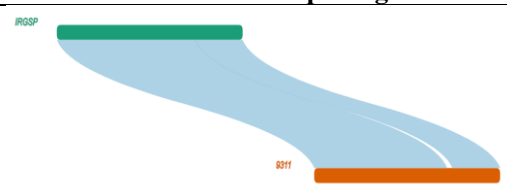
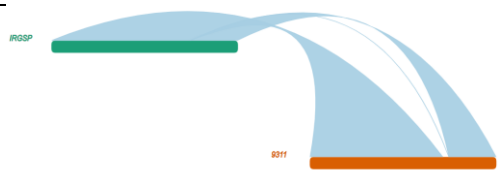
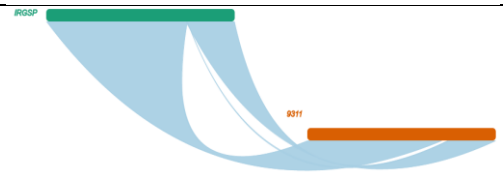
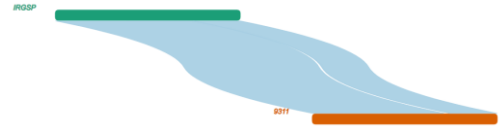
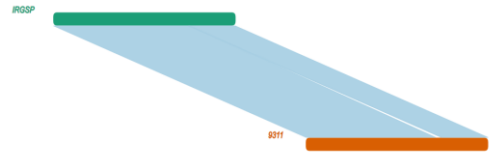
Required parameter must be given and **optional** parameters could be uncommented # to change the default settings.

```
SetParaFor = global
############## global and required parameters ###### ################
GenomeInfoFile1=./RefA.len      ### set path for Genome1
GenomeInfoFile2=./RefB.len      ### set path for Genome2

LinkFileRef1VsRef2=./RefA_RefB.link     ### link information between Genome1 and Genome2

################    canvas and figure    # ################
#body=1200    ### size of canvas with width and height. plot region:          (up/down/left/right)=(55,25,100,120)
#up=55
#down=25
#left=100
#right=120
#CanvasHeightRitao=1.0        ## adjust height of the plot
#NoPng=1       ##no output the png file
```

```
##############  adjust genome setting  ##############
SetParaFor = Genome1   ## setting parameters  for GenomeALL/GenomeX
#ZoomChr=1.0 ## adjust chr length, 1 for equal; >1 for enlarge; <1 for
#RotateChr=30 ## rotate the chr with 30 degrees
#ShiftX=0
#ShiftY=0                          ##
#MoveToX        MoveToY          ## move the start of chr to (X,Y)  similar to ShiftX and ShiftY



#ChrWidth=20  ## the height of chromosome
#LinkWidth=180                     ## link height between this genome and next     genome
#ChrSpacing=10                     ## spacing width of chr/scaffolds
#NormalizedScale=0                 ##  use the custome scale (=1) or the same with default genomes (=0)
#SpeRegionFile=/path/spe.bed ## input file for highlighted regions[chr start end key1=value1] in the                genome.
#ZoomRegion              ## Zoom the specific Region,format (ZoomRegion=chr2:1000:5000)



#GenomeNameRatio
#GenomeName
## GenomeName                  GenomeNameSizeRatio          GenomeNameColor              GenomeNameShiftX GenomeNameShiftY
## ChrNameShow                 ChrNameShiftX ChrNameShiftY ChrNameSizeRatio             ChrNameColor ChrNameRotate
##ShowCoordinates=1  ## Show Coordinates; other para [ScaleNum=10 ScaleUpDown ScaleUnit LabelUnit LablefontsizeRatio  RotateAxisText  ]


SetParaFor = Genome2          ##


##############  adjust link  setting  ##############
SetParaFor=Link1                  ### setting for the link  (Link1, ...LinkX) or LinkALL
#StyleUpDown=UpDown          ## UpDown                       DownUp          UpUp            DownDown line
#Reverse=1                         ## reverse links
#HeightRatio=1.0                   ### ratio of link height relative to the  default
## other attributes:                fill|stroke|stroke-opacity|fill-opacity|stroke-width
```

4.2.3 Link styles

Five link styles were defined in this tool, "DownDown", "UpUp", "DownUp","UpDown" and “line”.

StyleUpDown	Example Figure
StyleUpDown=UpDown HeightRatio=1.0 **(default)**	
StyleUpDown=UpUp HeightRatio=1.5	
StyleUpDown=DownDown HeightRatio=1.5	
StyleUpDown=DownUp HeightRatio=1.0	
StyleUpDown=line	

4.3 Input

Two Types of Input Files

A: genome information, we define the suffix of **GenomeInfoFile** as ***.len***.
This input file mainly stores the length of chromosome (scaffold), the number of records and the order of chromosome (scaffold).
Note: At least two genomes are required.

B : **Link File**, We define this type file with suffix name as ***.link***.
This file defines link relationships between two genomes.

4.3.1 *.len : genomic information (required)

More than one genome (≥2) are required.
GenomeInfoFile format:

```
# chr       start      end        key1=value1          key2=value2        key3=value3
chrB1       1          1000000
chrB3       1          1000000    fill="#F8F8F8"       stroke="#F8F8F8"
chrB2       1000000    1          fill=red     fill-opacity=0.5          ...
```

We define the suffix name of these files as ***xx.len*** , like *RefA.len RefB.len …*

comments:

- the first three columns are [chr start end], but also accept [chr end start]. Attributes were set as key=value e.g. ***fill=red*** **stroke-dasharray="5,5"**
- chr **orders** are determined according to your input, e.g. **B1 B3 B2**. Among them, the 1 of B2 is on the end column, that is, the **reverse complement** Syn of B2 is performed,
- fill color and opacity could be set: ***fill=red fill-opacity=1***
- stroke color, width, opacity could be set: ***stroke-width=0 stroke=black stroke-opacity=1***

Important tips A:

a. if end < start, e.g. chrB2, then the **reverse complement** Synteny of chrB2 on the Figure is performed.

4.3.2 *.link : link information (required)

LinkFileRef1VsRef2 format:

```
chrB1 1 1000000 LG01 100000 1
chrB3 1 1000000 LG02 1 1000000 fill="#F8F8F8"
chrB2 100 1 LG03 500 8000 fill=red fill-opacity=0.5
```

we define the suffix name of these files as ***xx.link*** , like *RefA_vs_RefB.link RefC_vs_RefB.link*…

comments:

- **LinkFile** is link relationship between GenomeX and GenomeY (RefXX Vs Ref YY)
- the first 6 columns are [chrA StartA EndA chrB StartB EndB

key=value] , **key=value** is link attribute, such as ***fill fill-opacity stroke …***

- Ref XX Vs Ref YY can be set for more than one times

Important tips B:

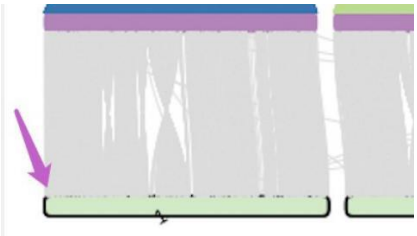
Attributes (***fill stroke stroke-opacity fill-opacityh stroke-width*)** setting for a link file could be set in the **SetParaFor=Link1** section while attributes for special links of interest should be set in the LinkFile.

• highlight specific region on the chr/scaffold (optional)
some regions of interest (such as gene loss at a genome while present in other genomes) could be highlighted.
the highlighted regions file (Spe) format is similar the setting for genome information file.

```
chrX 13022 13303 fill=green stroke=green
chrX 17203 17490 fill=green stroke=green
chrX 26639 26926 fill=green stroke=green
chrX 30599 30886 fill=green stroke=green
chrX 47918 48236 fill=blue stroke=blue
```

5 FAQs

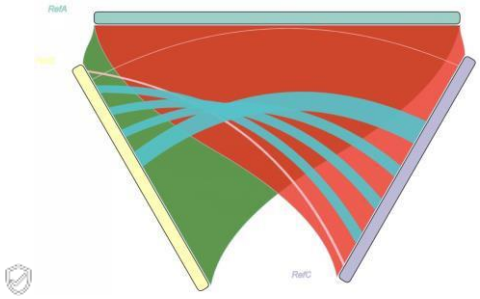
- The chr of NGenomeSyn will be overcover, how to make an adjustment? As follows



Reply

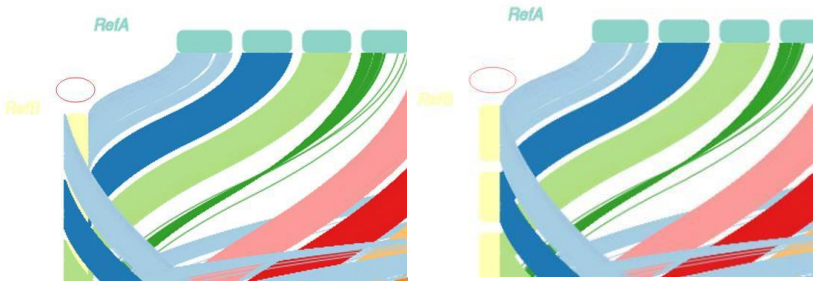
The reason why this border will be covered is that the *stroke-width=xx* of chr, the reason that the outer border of xx is too large, then the method that needs to be adjusted at this time is to reduce the distance between the top and bottom of the link layer, in the corresponding link layer settings Add the

hidden parameter **OutsideWidthDeta=xx** (xx is recommended to be the same number as stroke-width), such as adding **OutsideWidthDeta=2** to each link layer of example3 as follows:



In addition, two more hidden parameters, **ShortLinkLineRefA** and **ShortLinkLineRefB**, are provided to shorten or lengthen the two genome junctions above and below the link. Such as adding parameters to link

The changes before and after **ShortLinkLineRefB=20** are as follows:



- 5.2 Transparency does not work or there are jagged lines

Reply

See this website.

SomeOne say that NGenomeSyn transparency cannot be adjusted. I also adjusted it for a long time and found that this is the reason why the graph does not look transparent, that is, the main problem is the data, but it does not look transparent, but it is actually transparent. For example, 1-100 vs 1-100 if the data It becomes 1 vs 1, 2 vs 2, 3 vs 3 ... 100 vs 100 Due to the canvas problem, many links are stacked together, so the transparency is lost. So I suggest that everyone try to combine this situation into a large The block block (abnormally fragmented and collinear blocks are filtered out, etc.) Because of this situation in minmap2 comparison, this processing is very necessary. At the same time, this processing can make the svg file smaller, and the small segment interval **merges** the large interval link. will not affect png.

6 Contact

if any question about NGenomeSyn , please emails to:

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- join the ***QQ Group : 125293663***

QQ group：***125293663***

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