1.Circos Installation

 *Please install perl before installation of Circos as Ciros uses perl modules for its excecution.

Follow the process for setting up the circos in Linux (Globalization)

First, download Circos. The contents of the distribution are described below.

You don't need to move or edit any files in the main distribution.

2.Installing Circos on UNIX

Assuming that you want to install in `ROOT=~/software/circos`,

```
> cd ~
> mkdir software
> mkdir software/circos
> cd software/circos
# download Circos and place the archive in the directory
> ls
-rw-r--r-- 1 martink users 26725778 Jun  6 11:04 circos-0.67-pre4.tgz
# unpack
> tar xvfz circos-0.67-pre4.tgz
...
circos-0.67-pre4/data/karyotype/karyotype.arabidopsis.txt
circos-0.67-pre4/data/karyotype/karyotype.zeamays.txt
circos-0.67-pre4/data/karyotype/karyotype.oryzasativa.txt
# make a symlink to current
> ln -s circos-0.67-pre4 current
> ls
drwxr-xr-x 10 martink users     4096 Jun  6 11:06 circos-0.67-pre4/
-rw-r--r--  1 martink users 26725778 Jun  6 11:04 circos-0.67-pre4.tgz
lrwxrwxrwx  1 martink users       16 Jun  6 11:07 current -> circos-0.67-pre4/
# delete the tarball, if you want
```

To install GD and Perl modules on Ubuntu, use `apt-get`.

```
sudo apt-get -y install libgd2-xpm-dev
```

It's a good idea to add the bin/ directory in the distribution to your PATH so that you can run bin/circos from anywhere.

Assuming you have Circos in ROOT=~/software/circos/current as described above, append this to your ~/.bashrc or ~/.bash_profile.

```
export PATH=~/software/circos/current/bin:$PATH
```

You'll need to explicitly execute either `~/.bashrc` or `~/.bash_profile` for this to take effect

```
> . ~/.bashrc
# or
> . ~/.bash_profile
```

Finally, test that your `PATH` has been modified,

```
> cd ~
> echo $PATH
~/software/circos/current/bin: ...
> which circos
~/software/circos/current/bin/circos
```

For more detailed trobleshooting please follow the guide link
http://circos.ca/tutorials/lessons/configuration/distribution_and_installation/

3.Prepairing data set for plot

To plot certain parameters on Circos intial input files has to be modies as requiered. Parameters like Gene symbol, respective co-ordinates, strand information has to be arranged from provided data into excel sheet to sort data accordingly.

	A	B	C	D	E	F	G
1	CDS	dnaA	+	516	1877		
7	CDS	nnrD	-	9754	10566		
35	CDS	dus_1	-	43885	44337		
37	CDS	ricR_1	-	45307	45567		
42	CDS	dus_2	-	50015	51001		
45	CDS	ywqN_1	-	53143	53628		
62	CDS	eamA	-	75533	76405		
66	CDS	spa	-	80761	81291		
67	CDS	sarS	-	82732	83484		
68	CDS	yfhA	-	83853	84851		
69	CDS	yfiZ_1	-	84848	85843		
70	CDS	yfiY	-	85859	86851		
94	CDS	treR_1	-	111616	112356		
99	CDS	phnE_1	-	116752	117567		
100	CDS	phnE_2	-	117564	118364		
101	CDS	glnQ	-	118366	119139		
102	CDS	phnD2	-	119353	120309		
128	CDS	isdI	-	146373	146699		
129	CDS	ybaN	-	146706	147089		
143	CDS	yagU	-	167453	167947		
144	CDS	argB	-	168202	168966		
145	CDS	argJ	-	168982	170223		
146	CDS	argC	-	170235	171269		

As these are the key parametrs to sort data, we can make out input files in given format

chr1 start   stop   genesymbol / GC%

It is a good idea to plot the data based on strand information to make it less crowded, hence two files has to created for each gene_symbol and GC content.
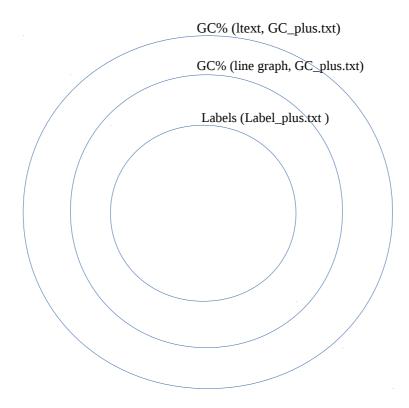
4.Generating Input files

Input file for GC content (create each for **+** and **−** strand)
(GC_plus.txt,GC_minus.txt)

chr1	516	1877	0.32966226138
chr1	2155	3288	0.327160493827
chr1	3911	5023	0.341419586703
chr1	5033	6967	0.37157622739
chr1	7004	9667	0.370495495495
chr1	10892	12406	0.369636963696
chr1	12785	14071	0.347319347319
chr1	14721	15416	0.372126436782
chr1	. . . .		

Input file for Gene symbol (create each for **+** and **−** strand)
(Label_plus.txt,Label_minus.txt)

chr1	9754	10566	nnrD
chr1	43885	44337	dus_1
chr1	45307	45567	ricR_1
chr1	50015	51001	dus_2
chr1	53143	53628	ywqN_1
chr1	75533	76405	eamA
chr1	80761	81291	spa
chr1	82732	83484	sarS
chr1	. . . .		

Karyotype file (karyo.txt)

chr	**−**	chr1	1	1	2835462	black
band	chr1	band1	band1	516	1877	black
band	chr1	band2	band2	2155	3288	vlgrey
band	chr1	band3	band3	3911	5023	black
band	chr1	band4	band4	5033	6967	vlgrey
band	chr1	band5	band5	7004	9667	black
band	chr1	band6	band6	9754	10566	vlgrey
band	. . . . .					

Karyotype file and GC content file was generated by using python script (developed for custom use where file with start,stop,strand and gene information was used as input file). GC content was calculated using start and stop position and mapping same from contig file to calculate GC percent (coustom python script written for the same)

5.Layout of CIRCOS diagram

GC% (ltext, GC_plus.txt)

GC% (line graph, GC_plus.txt)

Labels (Label_plus.txt)

Once Input files for each track are ready as per layout we need to configure the configuration file which handles all the section for input files and plots.

```
# circos.conf
karyotype = karyo.txt                              Karyotype
chromosomes_units = 1000000                        plotting
chromosomes_display_defaults = yes
<ideogram>
<spacing>
default = 0u
break = 0u
</spacing>
thickness = 80p
radius = 0.85r
show_label = yes
label_font = bold
label_with_tag = yes
label_radius = dims(ideogram,radius) + 0.05r
label_size = 48
labell_parallel = yes
label_case = upper
stroke_thickness = 3
stroke_color = black
fill = yes

show_bands = yes
fill_bands = yes
</ideogram>
<<include thicks.conf>>
<plots>
<plot>
type = text
color = black                                      Gene Lable plotting
label_font = bold                                  (plus strand)
label_size = 5p
file = Label_plus.txt
r1 = 0.900r            #label plus
```

```
r0 = 0.50r
padding = 0.5p
rpadding = 0.5p
show_links = yes
link_dims = 1p,2p,3p,2p,1p,1p
link_thickness = 0.5p
link_color = blue
label_snuggle = yes
max_snuggle_distance = 0.5r
snuggle_sampling = 1
snuggle_tolerance = 0.25r
snuggle_link_overlap_test = yes
snuggle_link_overlap_tolerance = 2p
snuggle_refine = yes
</plot>
```

```
<plot>
type      = line                          GC% plotting (plus
extend_bin = no                           strand(graph))
color     = black
fill_under = yes
thickness  = 1
file = GC_plus.txt
r0   = 0.65r              #GC PLUS
r1   = 0.75r
min  = 0
max  = 0.7
fill_color      = blue
<<include axis.conf>>
<<include background.conf>>
</plot>
```

```
<plot>
type = text                               GC% plotting (plus
color = black                             strand(text))
label_font = bold
label_size = 2p
file = GC_plus.txt
r1 = 0.75r              #label plus %%%
r0 = 0.73r
</plot>
```

```
<plot>
type = text
color = black                             Gene Lable plotting
label_font = bold                         (minus strand)
label_size = 5p
file = Label_minus.txt
r1 = 1.0r              #label minus
r0 = 0.75r
padding = 0.5p
rpadding = 0.5p
show_links = yes
link_dims = 1p,2p,3p,2p,1p,1p
link_thickness = 0.5p
link_color = green
label_snuggle = yes
max_snuggle_distance = 0.5r
snuggle_sampling = 1
snuggle_tolerance = 0.25r
snuggle_link_overlap_test = yes
snuggle_link_overlap_tolerance = 2p
snuggle_refine = yes
</plot>
```

```
<plot>
type      = line                          GC% plotting (minus
extend_bin = no                           strand(graph))
color     = black
fill_under = yes
thickness  = 1
file = GC_minus.txt
r0   = 0.90r              #GC minus
r1   = 0.995r
min  = 0
max  = 0.7
fill_color      = green
<<include axis.conf>>
<<include background.conf>>
</plot>
```

```
<plot>
type = text
color = black
```

```
label_font = bold
label_size = 2p
file = GC_minus.txt
r1 = 0.995r          #label %%
r0 = 0.97r
</plot>
</plopts>
```

GC% plotting (minus strand(text))

```
<<include etc/colors_fonts_patterns.conf>>
<image>
# Included from Circos distribution.
<<include etc/image.conf>>
</image>
<<include etc/housekeeping.conf>>
```

*config.conf

Circos program needs config files to set the directions required. The "master" config file is the circos.conf, in which we indicated the features of the figure and give the path to the files with the data.
Another suporting files has to be created which are used by config.conf

```
show_ticks = yes
show_tick_labels = yes

<ticks>
radius = dims(ideogram,radius_outer)
multiplier = 1e-6

label_offset = 5p
thickness = 3p
size = 20p

label_separation = 5p

<tick>
spacing = 0.5u
color = red
show_label = yes
label_size = 50p
label_font = bold
label_offset = 0p
format = %.1f
</tick>

<tick>
spacing = 1u
color = red
show_label = yes
label_size = 50p
label_font = bold
label_offset = 0p
format = %d
</tick>

<tick>
spacing = 0.1u
color = black
show_label = yes
label_size = 20p
label_font = bold
label_offset = 0p
format = %.1f
</tick>
</ticks>
```

*thicks.conf

Once all the requiered files are created, place them in one directory. Also 'etc' and 'font' folder in working directory as they contain other supporting conf files. Execute the following command in terminal which is open in same directory.

 $circos -conf config.conf

Two files circos.svg and circos.png will be created where svg file is high resolution file.