kmerExtractor

Release 1.0

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CONTENTS:

A genome sequence is composed of four basic types of nucleotides: adenine (A), cytosine (C), guanine (G), and thymine (T). All possible nucleotide substrings of length k are called **k-mers**. For example, for $k = 2$ there are $4^k = 4^2 = 16$ different 2-mers corresponding to: AA, AC, AG, AT, CA, CC, CG, CT, GA, GC, GG, GT, TA, TC, TG, TT.

The kmerExtractor software calculates the k-mer frequencies for windows within the chromosomes of an organism's sequence. It takes as input a FASTA file with the nucleotide sequence of an organism separated by chromosomes. The software splits the chromosomes into windows of the size specified by the user. For a given k, the kmerExtractor computes the frequency of the k-mers in the different windows within the chromosomes. It returns a csv file indicating the chromosome, the window index, the start and end position of the window, and the frequencies of the corresponding k-mers.

CHAPTER

ONE

DOCUMENTATION

1.1 kmerExtractor module

kmerExtractor.**CGR**(*sequence*, *d=1*)

Generate the X and Y coordinates of the Chaos Game Representation (CGR) for the input sequence. Purine nucleotides (A and G) are placed on the minor diagonal, and the pyrimidine nucleotides (C and T) on the main diagonal. The CGR is built in a square of dimensions 2d x 2d with center at the coordinates (0,0). Only the four basic uppercase nucleotides (A, C, G, T) are accepted. Any other character is ignored.

Parameters

- **sequence** (*str*) – Nucleotide sequence.
- **d** (*int*) – Half of the side of the square. Default to 1.

Returns The x and y coordinates of the CGR.

Return type tuple[list[float],list[float]]

kmerExtractor.**FCGR**(*kmers*)

Organize the k-mers and their associated values in the common structure of Frequency Chaos Game Representation (FCGR), i.e., a matrix of dimensions N x N, where N = 4^(k/2); with the upper left corner equal to the k-mer of C, the upper right corner the k-mer of G, the lower left corner the k-mer of A, and the lower right corner the k-mer of T.

Parameters **kmers** (*dict*) – dictionary mapping the k-mers to a real value. All k-mers must be of the same length.

Returns FCGR matrix and the names of the k-mers in the matrix.

Return type tuple[numpy.ndarray, numpy.ndarray]

kmerExtractor.**kmers_by_window**(*filepath*, *k*, *window_size=100000*, *output_path=None*)

Calculate the frequency of k-mers by windows within each of the chromosome sequences. First, the entire sequence contained within the FASTA file is read. The sequences of each chromosome are then identified and separated. Subsequently, each chromosome is separated into windows of the specified size. Finally, the frequencies of the k-mers are calculated for each window.

Parameters

- **filepath** (*str*) – Location of the FASTA file containing the nucleotide sequence of an organism separated by chromosomes.
- **k** (*int*) – Length of k-mers.
- **window_size** (*int*) – Window size, in number of base pairs, to be used to count the frequency of k-mers within the chromosome. Must be less than or equal to the length of the smallest chromosome. Default to 100000.

- **output_path** (*str*) – Path to the directory where the output csv file will be saved. If not specified, the output file will be saved in the same directory as the input file. Default to None

Returns Dataframe indicating the chromosome, the window index, the start and end positions of the window, and the frequencies of the corresponding k-mers.

Return type pandas.DataFrame

kmerExtractor.**kmers_by_window_opt**(*filepath*, *k*, *window_size=100000*, *output_path=None*)

Calculate the frequency of k-mers by windows within each of the chromosome sequences. It calculates the k-mers, as it reads the FASTA file, optimizing space by avoiding storing the sequence.

Parameters

- **filepath** (*str*) – Location of the FASTA file containing the nucleotide sequence of an organism separated by chromosomes.
- **k** (*int*) – Length of k-mers.
- **window_size** (*int*) – Window size, in number of base pairs, to be used to count the frequency of k-mers within the chromosome. Must be greater than the number of nucleotides per line in the input FASTA file. Default to 100000
- **output_path** (*str*) – Path to the directory where the output csv file will be saved. If not specified, the output file will be saved in the same directory as the input file. Default to None

Returns Dataframe containing the k-mer frequencies for all windows of each chromosome.

Return type pandas.DataFrame

kmerExtractor.**kmers_in_sequence**(*sequence*, *k*)

Calculate the frequency of k-mers in a given sequence. Only k-mers containing the four basic uppercase nucleotides (A, C, G, T) are accepted. Any substrings of length k that have any other character in their composition will be disregarded and thus not considered as k-mers.

Parameters

- **sequence** (*str*) – Nucleotide sequence.
- **k** (*int*) – Length of k-mers.

Returns Dictionary containing the k-mer frequencies. Keys are the k-mers and values are the corresponding frequencies.

Return type dict

kmerExtractor.**plot_CGR**(*sequence*, *d=1*, *markersize=1*, *ax=None*, *figsize=(6, 6)*)

Plot the Chaos Game Representation (CGR) of the input sequence. Purine nucleotides (A and G) are placed on the minor diagonal, and the pyrimidine nucleotides (C and T) on the main diagonal. The CGR is built in a square of dimensions 2d x 2d with center at the coordinates (0,0). Only the four basic uppercase nucleotides (A, C, G, T) are accepted. Any other character is ignored.

Parameters

- **sequence** (*str*) – Nucleotide sequence.
- **d** (*int*) – Half of the side of the square in which the CGR is built. Default to 1.

Returns The Axes object containing the plot.

Return type matplotlib.axes._axes.Axes

kmerExtractor.**plot_FCGR**(*df*, *chromosome=None*, *window=None*, *figsize=(7, 6)*, *colormap='bwr'*, *ax=None*)

Plot the Frequency Chaos Game Representation (FCGR) of the k-mers in the input dataframe.

Parameters

- **df** (*pandas.DataFrame*) – Dataframe with the k-mer frequencies by windows across chromosomes.
- **chromosome** (*str*) – Name of the chromosome to plot. If None, parameter window must be None, plotting the sum of k-mers across all chromosomes. Default to None.
- **window** (*int*) – Number of the window to plot. If None, plot the sum of all windows for each chromosome. Default to None.
- **figsize** (*tuple[int,int]*) – The size in inches of the figure to create. Default to (7,6).
- **colormap** (*str*) – Matplotlib colormap name. The mapping from data values to color space. Default to 'bwr'.
- **ax** (*matplotlib.axes.Axes*) – Axes in which to draw the plot, otherwise use the currently-active Axes. Default to None.

Returns The Axes object containing the plot.

Return type matplotlib.axes._axes.Axes

kmerExtractor.**plot_kmers_across_windows**(*df*, *kmer_names*, *chromosome*, *figsize=(12, 4)*, *ax=None*)

Plot the frequencies of the given k-mers across the windows of a given chromosome.

Parameters

- **df** (*pandas.DataFrame*) – dataframe with the k-mer frequencies by window.
- **kmer_names** (*list*) – list of k-mer names to plot.
- **chromosome** (*str*) – name of the chromosome to plot.
- **figsize** (*tuple[int,int]*) – The size in inches of the figure to create. default to (12,4).
- **ax** (*matplotlib.axes._axes.Axes*) – The Axes object containing the plot. If None, a new figure and axes is created. Default to None.

Returns The Axes object containing the plot.

Return type matplotlib.axes._axes.Axes

kmerExtractor.**plot_kmers_freq_within_chromosomes**(*df*, *figsize=(15, 6)*, *ax=None*)

Plot the sum of the k-mer frequencies within each chromosome.

Parameters

- **df** (*pandas.DataFrame*) – dataframe with the k-mer frequencies by window.
- **figsize** (*tuple[int,int]*) – The size in inches of the figure to create. default to (15,6).
- **ax** (*matplotlib.axes.Axes*) – Axes in which to draw the plot, otherwise use the currently-active Axes. Default to None.

Returns The Axes object containing the plot.

Return type matplotlib.axes._axes.Axes

CHAPTER

TWO

KMEREXTRACTOR USER MANUAL

Below we present examples of how to use the main functions of the software.

2.1 Import libraries

[1]:
```
import pandas as pd
import matplotlib.pyplot as plt

# software to extract the k-mers
import kmerExtractor as kex
```

2.2 Compute k-mers

Specify the corresponding directory of the FASTA files from which you want to extract the k-mers, and the directory where you want to save the output files. Also define the window size (in base pairs) for which you want the frequency of k-mers to be counted.

In this case we will use the genome of arabidospsis, tomato and maize, with a window size of 100000 bp for all genomes.

[2]:
```
filepath_ara = '../input_data/arabidopsis_genome.fasta'
filepath_tom = '../input_data/tomato_genome.fasta'
filepath_mz = '../input_data/maize_genome.fasta'
output_path = '../output_data'
window_size = 100_000
```

Now use the `kmers_by_window_opt` function to calculate the k-mers of each genome for the k of your choice.

In the output file, the first column indicates the chromosome, the second column the window index, the third and fourth columns indicate the start and end position of the window respectively, and the rest of the 4^k columns correspond to the k-mer frequencies. This data is saved in a csv file and stored in a dataframe for downstream analysis within the tutorial.

The resulting dataframe for arabidopsis 2-mers looks like shown below.

[3]:
```
df_2mers_ara = kex.kmers_by_window_opt(filepath_ara, k=2, window_size=window_size,␣
↪output_path=output_path)
df_2mers_tom = kex.kmers_by_window_opt(filepath_tom, k=2, window_size=window_size,␣
↪output_path=output_path)
```

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```
df_2mers_mz  = kex.kmers_by_window_opt(filepath_mz,  k=2, window_size=window_size,␣
↪output_path=output_path)
df_2mers_ara
```

```
100%|| 1537509/1537509 [00:45<00:00, 33733.02it/s]
100%|| 10090326/10090326 [04:45<00:00, 35388.84it/s]
100%|| 25502007/25502007 [12:31<00:00, 33940.82it/s]
```

[3]:

	chromosome	window	start	end	AA	AC	AG	AT	CA	\
0	chr1	0	1	100000	11617	5201	5956	9480	6269	
1	chr1	1	100001	200000	10857	5241	5871	8727	6080	
2	chr1	2	200001	300000	10623	5144	6129	8658	6350	
3	chr1	3	300001	400000	9548	5117	6328	7979	6483	
4	chr1	4	400001	500000	11515	5294	5692	9068	6225	
...	...	...	...	...	...	...	...	...	...	
1190	chr5	267	26700001	26800000	11432	5154	5970	9120	6440	
1191	chr5	268	26800001	26900000	11549	5299	5782	9116	6113	
1192	chr5	269	26900001	26975502	8826	4001	4609	6658	4719	
1193	chr5	269	26900001	26975502	8826	4001	4609	6658	4719	
1194	chr5	269	26900001	26975502	8826	4001	4609	6658	4719	

	CC	CG	CT	GA	GC	GG	GT	TA	TC	TG	TT
0	3277	2412	5588	6375	2998	3460	5248	7993	6069	6253	11803
1	3516	2983	5895	6670	3174	3758	5578	7089	6544	6567	11449
2	3575	2655	6386	6617	3378	3616	5325	6964	6869	6536	11174
3	4297	2771	6834	6625	3923	4047	5310	6315	7049	6759	10614
4	3578	2638	5896	6455	3027	3592	5271	7375	6438	6422	11513
...	...	...	...	...	...	...	...	...	...	...	...
1190	3528	2620	5942	6521	3051	3289	5280	7283	6797	6262	11310
1191	3457	2948	5881	6405	3068	3254	5222	7680	6575	5965	11685
1192	2639	2158	4387	5163	2352	2573	3979	5386	4912	4726	8413
1193	2639	2158	4387	5163	2352	2573	3979	5386	4913	4726	8413
1194	2639	2158	4387	5163	2352	2573	3979	5386	4914	4726	8413

[1195 rows x 20 columns]

However, a most natural approach is to use $k = 3$, since the 3-mers represent codons, which encode for amino acids or signal the termination of protein synthesis (stop signals).

The resulting dataframe for arabidopsis 3-mers looks like below.

[4]:
```
df_3mers_ara = kex.kmers_by_window_opt(filepath_ara,k=3,window_size=window_size,output_
↪path=output_path)
df_3mers_tom = kex.kmers_by_window_opt(filepath_tom,k=3,window_size=window_size,output_
↪path=output_path)
df_3mers_mz = kex.kmers_by_window_opt(filepath_mz,k=3,window_size=window_size,output_
↪path=output_path)
df_3mers_ara
```

```
100%|| 1537509/1537509 [00:54<00:00, 28160.74it/s]
100%|| 10090326/10090326 [05:59<00:00, 28039.88it/s]
100%|| 25502007/25502007 [15:53<00:00, 26741.24it/s]
```

[4]:

	chromosome	window	start	end	AAA	AAC	AAG	AAT	ACA	\
0	chr1	0	1	100000	4415	1966	2228	3008	1926	

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```
1          chr1       1    100001    200000  4026  1914  2114  2803  1849
2          chr1       2    200001    300000  3899  1875  2138  2711  1863
3          chr1       3    300001    400000  3335  1760  2117  2336  1729
4          chr1       4    400001    500000  4629  1874  2085  2926  1929
...         ...     ...       ...       ...   ...   ...   ...   ...   ...
1190       chr5     267  26700001  26800000  4301  2000  2180  2951  1818
1191       chr5     268  26800001  26900000  4423  1999  2061  3066  1835
1192       chr5     269  26900001  26975502  3366  1522  1803  2135  1467
1193       chr5     269  26900001  26975502  3366  1522  1803  2135  1467
1194       chr5     269  26900001  26975502  3366  1522  1803  2135  1467

       ACC  ...  TCG   TCT   TGA   TGC   TGG   TGT   TTA   TTC   TTG   TTT
0     1096  ...  776  2200  2016  1007  1349  1881  2682  2169  2422  4530
1     1063  ...  959  2339  2102   957  1432  2076  2324  2283  2489  4353
2     1066  ...  932  2572  2098  1085  1390  1963  2255  2477  2322  4120
3     1110  ...  833  2586  2041  1313  1456  1949  1998  2398  2334  3884
4     1075  ...  885  2301  2109   988  1406  1919  2499  2241  2414  4359
...    ...  ...  ...   ...   ...   ...   ...   ...   ...   ...   ...   ...
1190  1114  ...  891  2453  2014  1028  1297  1922  2394  2385  2340  4191
1191  1072  ...  973  2304  1962   937  1221  1845  2695  2302  2196  4492
1192   797  ...  704  1734  1585   738   960  1443  1861  1717  1716  3119
1193   797  ...  704  1734  1585   738   960  1443  1861  1717  1716  3119
1194   797  ...  704  1734  1585   738   960  1443  1861  1717  1716  3119

[1195 rows x 68 columns]
```

2.3 Read k-mer files if already computed

Calculating k-mers can take longer for larger genomes. That's why you also have the option to load previously saved files, so you don't have to recalculate the k-mers every time you want to plot some figures or do further analysis.

[5]:
```
df_2mers_ara = pd.read_csv(output_path +'/CGRW_k2_arabidopsis_genome.csv')
df_2mers_tom = pd.read_csv(output_path +'/CGRW_k2_tomato_genome.csv')
df_2mers_mz = pd.read_csv(output_path +'/CGRW_k2_maize_genome.csv')

df_3mers_ara = pd.read_csv(output_path +'/CGRW_k3_arabidopsis_genome.csv')
df_3mers_tom = pd.read_csv(output_path +'/CGRW_k3_tomato_genome.csv')
df_3mers_mz = pd.read_csv(output_path +'/CGRW_k3_maize_genome.csv')
```

2.4 Group all windows within each chromosome

If you don't want the k-mer frequencies by windows within the chromosomes, you can get the cumulative frequencies for each chromosome as shown in the cell below. For example, the resulting dataframe for arabidopsis would have only 5 rows corresponding to its 5 chromosomes.

[6]:
```
df_2mers_ara_bychr = df_2mers_ara.groupby('chromosome').agg({k:'sum' for k in df_2mers_
↪ara.columns[4:]})
df_2mers_tom_bychr = df_2mers_tom.groupby('chromosome').agg({k:'sum' for k in df_2mers_
↪tom.columns[4:]})
```

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```
df_2mers_mz_bychr = df_2mers_mz.groupby('chromosome').agg({k:'sum' for k in df_2mers_mz.
↪columns[4:]})
df_2mers_ara_bychr
```

[6]:

```
                AA       AC       AG       AT       CA       CC      CG  \
chromosome
chr1        3519572  1588202  1794811  2806911  1923811  1016038  697362
chr2        2291405  1029105  1160640  1834416  1246754   672290  457569
chr3        2697289  1228414  1405644  2153303  1498561   804334  559027
chr4        2143926   980389  1109010  1707158  1184064   637226  439583
chr5        3140913  1410681  1608073  2510382  1712226   907201  634608

                CT       GA      GC       GG       GT       TA       TC  \
chromosome
chr1        1798090  1930160  902322  1008839  1579754  2335983  1928721
chr2        1166315  1249456  584680   660355  1026228  1527955  1256858
chr3        1396347  1516501  714668   805341  1226134  1772296  1510857
chr4        1110444  1195069  560350   630128   970513  1417419  1193357
chr5        1605965  1741333  808346   914520  1422652  2075592  1733770

                TG       TT
chromosome
chr1        1920061  3512205
chr2        1242154  2289303
chr3        1492635  2672182
chr4        1177332  2125863
chr5        1729636  3160014
```

2.5 Group all chromosomes

Furthermore, you can pool the k-mer frequencies of all the chromosomes and have the cumulative counts of the entire sequence.

[7]:

```
df_2mers_all = pd.DataFrame()
df_2mers_all['arabidopsis'] = df_2mers_ara_bychr.sum()
df_2mers_all['tomato'] = df_2mers_tom_bychr.sum()
df_2mers_all['maize'] = df_2mers_mz_bychr.sum()
df_2mers_all.T
```

[7]:

```
                  AA         AC         AG         AT         CA         CC  \
arabidopsis   13793105    6236791    7078178   11012170    7565416    4037089
tomato        85677817   37700236   41002572   75901364   45946447   25138712
maize        161563751  108257047  128688710  136989307  133796230  120957040

                  CG         CT         GA         GC         GG         GT  \
arabidopsis    2788149    7077161    7632519    3570366    4019183    6225281
tomato        11804902   40969026   42920385   18121904   25183170   37739813
maize         87977339  128781627  129723428  112442579  121098322  108465666

                  TA         TC         TG         TT
arabidopsis    9129245    7623563    7561818   13759567
```

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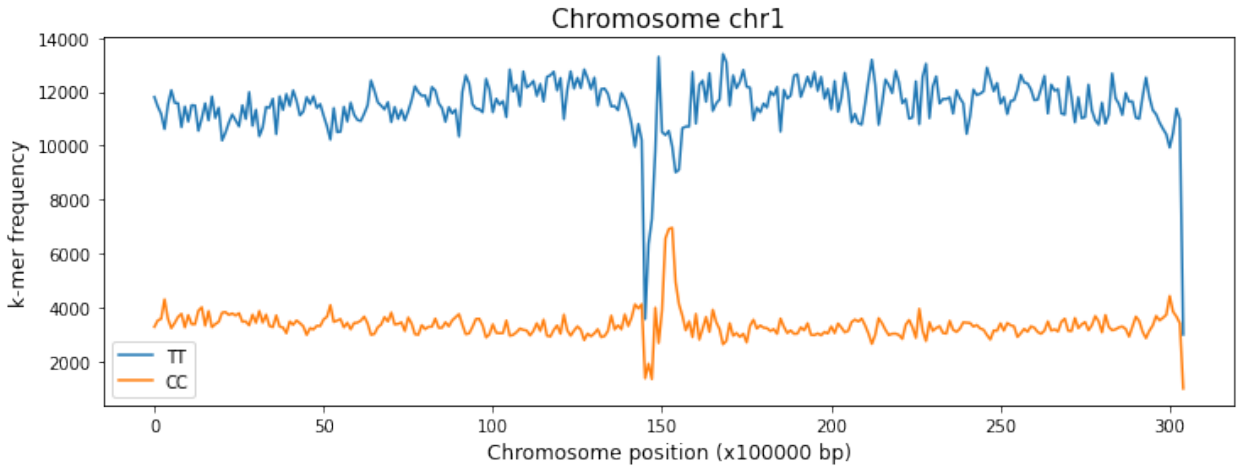
```
tomato         65737185   42898271   45974544   85737480
maize         110415392  129855155  133966157  161938131
```

2.6 k-mer figures

2.6.1 Plot k-mers across windows

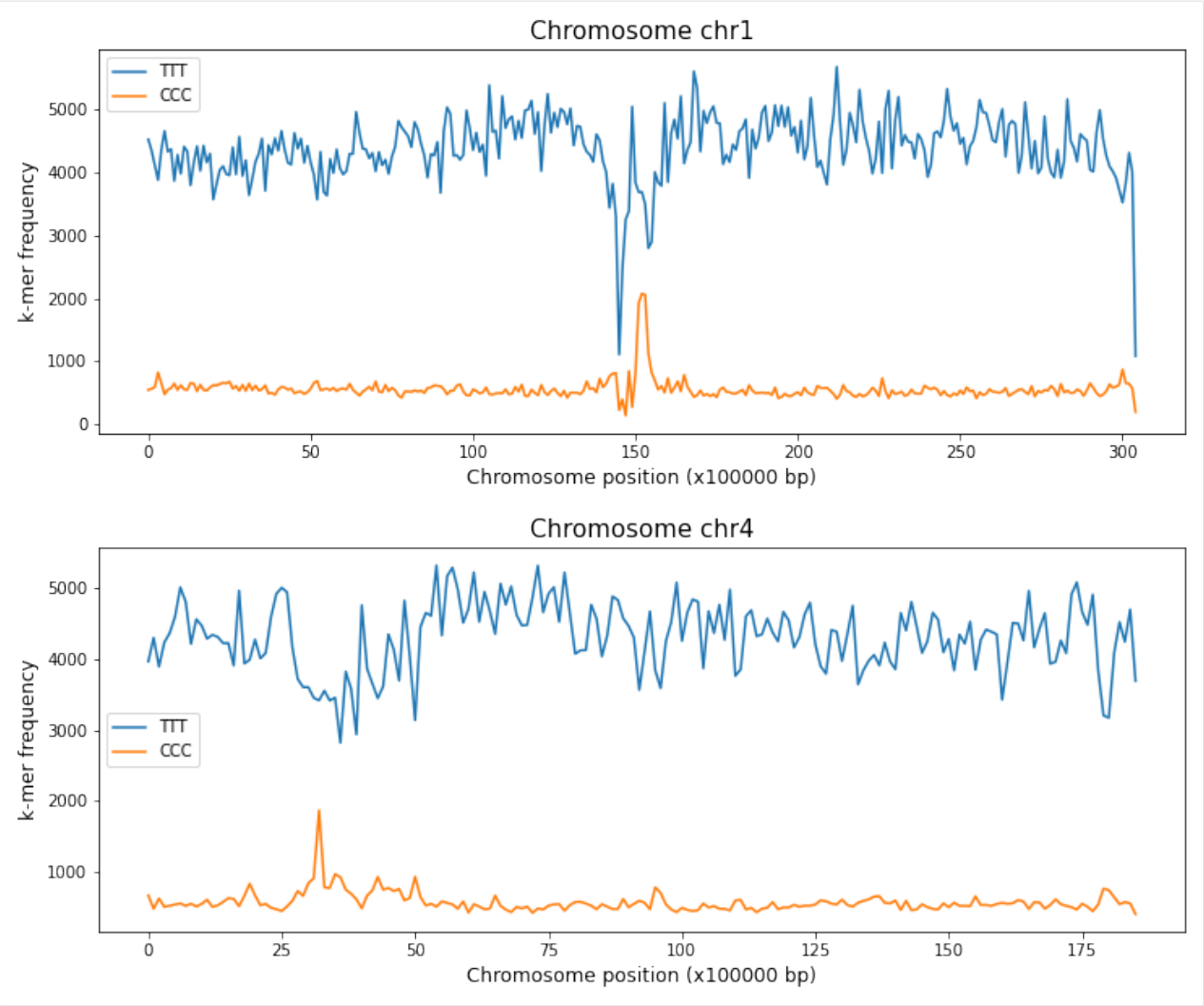
With the `plot_kmers_across_windows` function you can plot the frequencies of the k-mers of your choice across the windows of a specified chromosome.

[8]:
```
a = kex.plot_kmers_across_windows(df_2mers_ara,['TT','CC'],'chr1')
```



You can also use this function to compare, placing the plot of different chromosomes on different axes within the same figure.

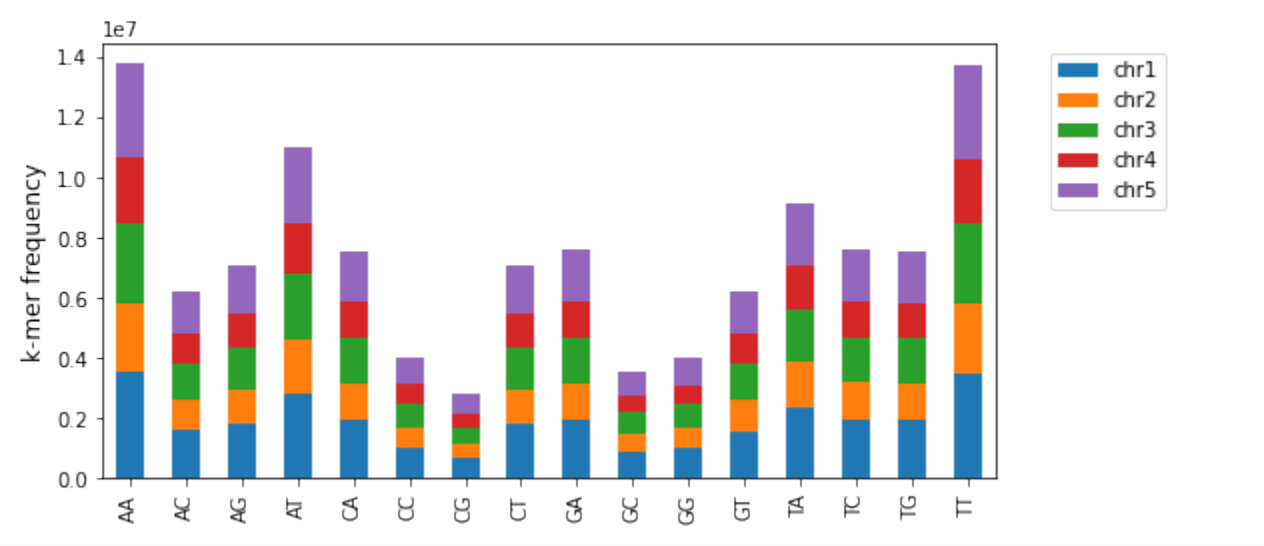
[9]:
```
fig,ax = plt.subplots(2,1,figsize=(12,10),gridspec_kw={'hspace': 0.3})

kex.plot_kmers_across_windows(df_3mers_ara,['TTT','CCC'],'chr1',ax=ax[0])
kex.plot_kmers_across_windows(df_3mers_ara,['TTT','CCC'],'chr4',ax=ax[1])

plt.show()
```



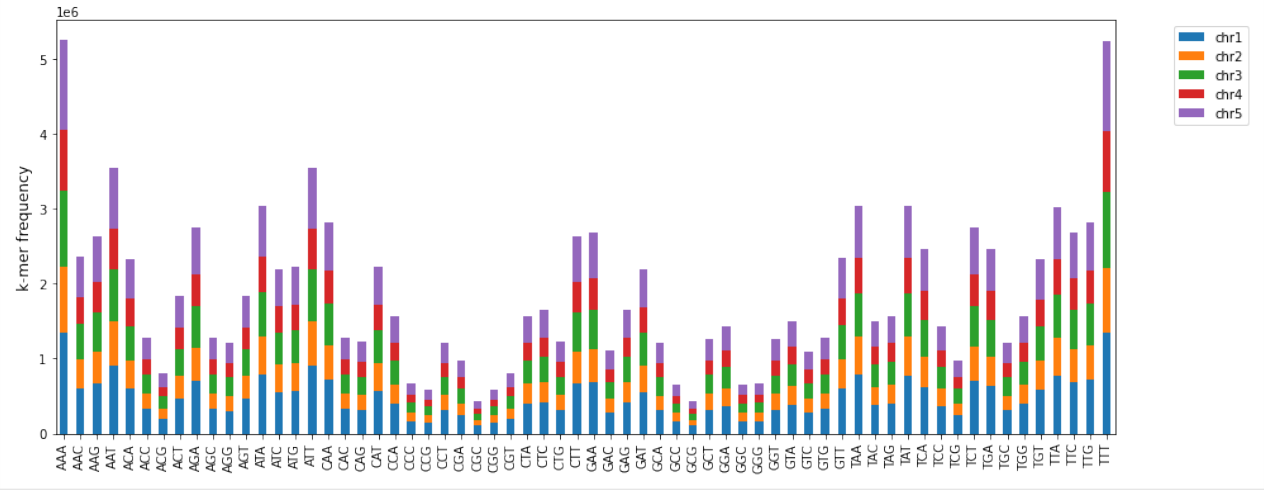
2.6.2 Plot k-mer frequencies within each chromosome

The `plot_kmers_freq_within_chromosomes` function makes a stacked bar graph of the frequency of different k-mers per chromosome. For the same k-mer, each stacked bar represents a different chromosome of the corresponding color.

[10]:
```
b = kex.plot_kmers_freq_within_chromosomes(df_2mers_ara,figsize=(8,4))
```



[11]:
```
c = kex.plot_kmers_freq_within_chromosomes(df_3mers_ara)
```



2.6.3 Plot FCGR structure

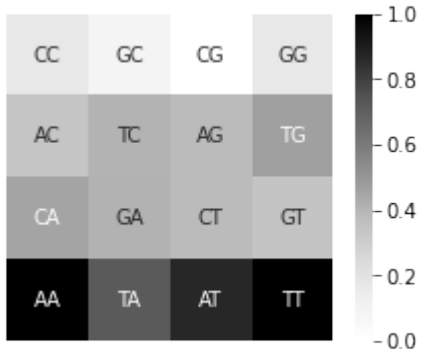
The technique to show the k-mer frequencies as images is known as Frequency Chaos Game Representation (FCGR). It corresponds to a matrix of dimensions $2^k \times 2^k$; with the upper left corner equal to the k-mer of C, the upper right corner the k-mer of G, the lower left corner the k-mer of A, and the lower right corner the k-mer of T.

The `plot_FCGR` function plots the FCGR of a sequence as a heatmap that allows to appreciate the relative frequencies of each k-mer. You can plot the FCGR for a specific chromosome and window, the cumulative frequency for an entire chromosome (if no window is specified), or the cumulative frequency for the entire genome (if neither chromosome nor window is specified).

[12]:
```
d = kex.plot_FCGR(df_3mers_ara,'chr1',window=145)
```

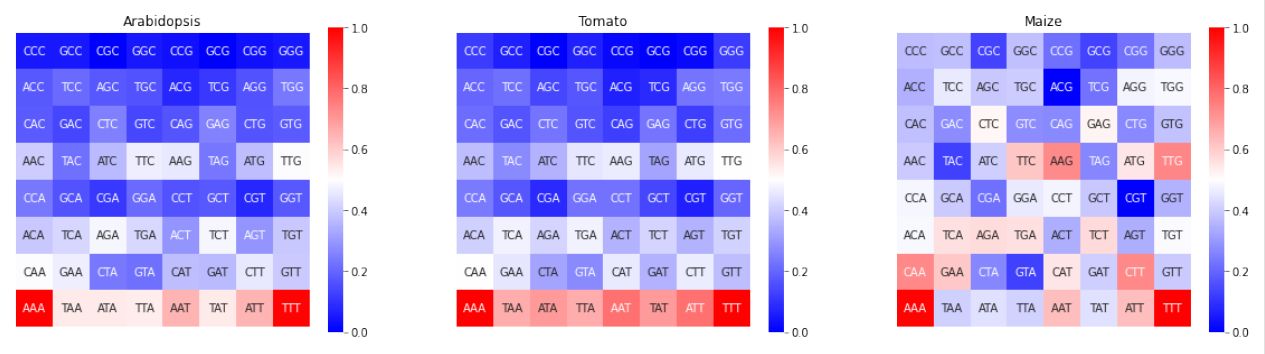
You can also adjust the size of the figure and the colormap to your preference as shown below

[13]:
```
e = kex.plot_FCGR(df_2mers_tom,'chr6',figsize=(4,3),colormap='Greys')
```



Aditionally, is possible compare the FCGR of multiple genomes as shown in the following cell

[14]:
```
fig,ax = plt.subplots(1,3,figsize=(20,5))

kex.plot_FCGR(df_3mers_ara,ax=ax[0])
kex.plot_FCGR(df_3mers_tom,ax=ax[1])
kex.plot_FCGR(df_3mers_mz,ax=ax[2])

ax[0].set_title('Arabidopsis')
ax[1].set_title('Tomato')
ax[2].set_title('Maize')

plt.show()
```

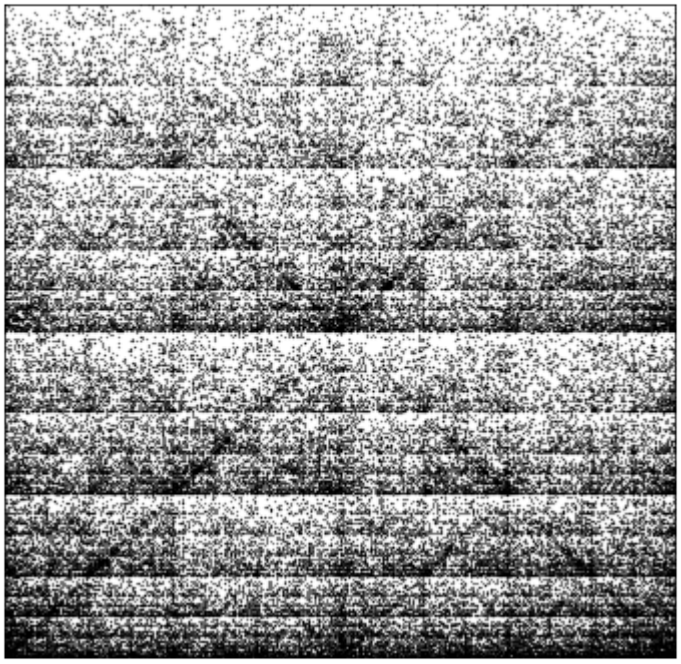


2.6.4 Plot CGR

Finally, although it is not the main objective of the software, a function to generate the CGR image of a sequence is included. In the following example, the FASTA file of the Arabidopsis genome is read and the CGR of the first 100 kb is generated.

[15]:
```
import re

# Read FASTA file with complete genome separated by chromosomes
f_in = open(filepath_ara, 'r')
all_file = f_in.read()
f_in.close()

# join lines
seq_all = "".join(all_file.split("\n"))
del all_file

# Split sequence by chromosomes
seq_bychr = re.split('>chr[1-9]*', seq_all)[1:]
del seq_all

# plot CGR of the first chromosome, first window (first 100 Kb)
f = kex.plot_CGR(seq_bychr[0][:100_000],markersize=0.2)
```

```
100%|| 100000/100000 [00:00<00:00, 1886046.78it/s]
```



CHAPTER

THREE

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