**Pipeline for calling TADs**

This a custom pipeline for identification TAD boundaries using TADbit1 tad caller. The output of different TAD callers does not always agree with each other and can vary substantially depending on the algorithm used.

1. **Generate interaction/contact matrix using HiC-Pro:**

Inputs to this custom pipeline are .matrix and \_abs.bed files generated by HiC-Pro. You should have them ready before calling TADs. The pipeline to generate interaction/contact matrices using HiC-Pro starting from FASTQ files is outlined in a separate document [here](https://pennstateoffice365-my.sharepoint.com/:w:/g/personal/spg5958_psu_edu/Eft8_zYvRE9Nqs2ADW3KdtMBbon0lrsGOVJG6IIp-ZEQ1w?e=fu9bFF).

1. **Call TADs:**

The python script to call TADs using TADbit can be downloaded from [here](https://github.com/spg5958/micro_c_analysis_Iwafuchi_lab-github_repo/blob/main/scripts/call_tads_tadbit.py). This script outputs TADs in bed files for every given chromosome at specified resolutions. Please modify input arguments at the beginning of the script (lines 13-18) as required and run the script. After a successful run you should get TAD domains bed files in output directory for each chromosome & resolution.

1. **Plot TADs:**

TADs can be visualized using ‘pyGenomeTracks’2 package. To begin, you need to create a track file that includes contact matrix and TAD domains. Below is an example of how to structure your track file, along with the command to plot the TADs using this file.

1. [x-axis]
2. where = top
4. [spacer]
6. [hic matrix]
7. file = matrix.cool
8. depth = 50000000
9. file\_type = hic\_matrix
10. colormap = coolwarm
11. transform = no
12. max\_value = 1
13. min\_value = -1
14. title = hFG-lib548+lib549\_merged-AB matrix
16. [tads]
17. file = domains.bed
18. display = triangles
19. border\_color = black
20. color = none
21. overlay\_previous = share-y
22. line\_width = 2.5

Command:

pyGenomeTracks **--**tracks tracks.ini **--**region CHR:star**-**end **--**outFileName out.png

**References:**

1. TADbit. https://github.com/3DGenomes/TADbit.

2. pyGenomeTracks. https://github.com/deeptools/pyGenomeTracks/.