osknExample

October 6, 2023

In this notebook, I'll go through a full example of using bpnet-multi to analyze some chip-nexus data. These are the same data that were used in our original paper: Avsec, Ž., Weilert, M., Shrikumar, A. et al. Base-resolution models of transcription-factor binding reveal soft motif syntax. Nat Genet 53, 354–366 (2021). https://doi.org/10.1038/s41588-021-00782-6

You can download the data from https://zenodo.org/record/3371216#.Y0muwFLMKAQ , but be aware that it's 30 GB of data. I (Charles McAnany) also have a local copy at Stowers, so if you're at Stowers, just let me know and I'll point you in the right direction. I've copied the idr-optimal-set.summit.bed files and the counts.neg.bw and counts.pos.bw files from the downloaded data into my working directory.

[2]:
```
import json
import matplotlib.pyplot as plt
plt.rcParams['figure.figsize'] = [5,4]
plt.rcParams['figure.dpi'] = 150
import numpy as np


# This is specific to running the job on teak (my workstation) since I need to
#add bedtools to my path.
import os
os.environ["PATH"] = os.environ["PATH"] + ":/n/apps/CentOS7/bin/"
import pybedtools
import pysam
import pyBigWig
import h5py
```

[3]:
```
#Here, I'll set a few constants that will be applicable throughout the project.
WORKING_DIRECTORY="/n/projects/cm2363/bpreveal/test/oskn"
SRC_DIR="/n/projects/cm2363/bpreveal/src"
GENOME_FASTA="/n/data1/genomes/indexes/mm10/mm10.fa"
TF_NAMES = ["oct4", "sox2", "klf4", "nanog"] #The names of the factors we'll␣
 ↪use.
                                             #For consistency, I'm always going
                                             #to use lowercase.
TEST_CHROMS = ["chr" + str(x) for x in [1,8,9]]
VAL_CHROMS = ["chr" + str(x) for x in [2,3,4]]
TRAIN_CHROMS = ["chr" + str(x) for x in [5,6,7,10,11,12,13,14,15,16,17,18,19]]
```

[4]:
```
HEADER_NOGPU="""#!/usr/bin/env zsh
#SBATCH --job-name {jobName:s}
#SBATCH --ntasks={ntasks:d}
#SBATCH --nodes=1
#SBATCH --mem={mem:d}gb
#SBATCH --time={time:s}
#SBATCH --output=logs/{jobName:s}_%A_%a.out
#SBATCH --partition=compute
#SBATCH --array=1-{numJobs:d}%10

source /home/cm2363/.bashrc
source /home/cm2363/.zshrc
module load bpreveal
module load bedtools
module load meme

"""
def jobsNonGpu(tasks, jobName, ntasks, mem, time):
    cmd = HEADER_NOGPU.format(jobName=jobName, ntasks=ntasks, mem=mem,
                              time=time, numJobs=len(tasks))
    for i, task in enumerate(tasks):
        cmd += "if [[ ${{SLURM_ARRAY_TASK_ID}} == {0:d} ]] ; then\n".format(i+1)
        cmd += "    {0:s}\n".format(task)
        cmd += "fi\n\n"
    with open(WORKING_DIRECTORY+"/{0:s}.slurm".format(jobName), "w") as fp:
        fp.write(cmd)
```

[5]:
```
HEADER_GPU="""#!/usr/bin/env zsh
#SBATCH --job-name {jobName:s}
#SBATCH --ntasks={ntasks:d}
#SBATCH --nodes=1
#SBATCH --mem={mem:d}gb
#SBATCH --time={time:s}
#SBATCH --output=logs/{jobName:s}_%A_%a.out
#SBATCH --partition=gpu
#SBATCH --gres gpu:1
#SBATCH --array=1-{numJobs:d}%4

source /home/cm2363/.bashrc
source /home/cm2363/.zshrc
module load bpreveal
module load bedtools
module load meme

"""
def jobsGpu(tasks, jobName, ntasks, mem, time):
    cmd = HEADER_GPU.format(jobName=jobName, ntasks=ntasks, mem=mem,
```

```
                            time=time, numJobs=len(tasks))
    for i, task in enumerate(tasks):
        cmd += "if [[ ${{SLURM_ARRAY_TASK_ID}} == {0:d} ]] ; then\n".format(i+1)
        cmd += "    {0:s}\n".format(task)
        cmd += "fi\n\n"
    with open(WORKING_DIRECTORY+"/{0:s}.slurm".format(jobName), "w") as fp:
        fp.write(cmd)
```

[6]:
```
!ls -l {WORKING_DIRECTORY}
```

```
total 484385
drwxrwxr-x  2 cm2363 domain users       385 Jun 21 09:25 bed
lrwxrwxr-x  1 cm2363 domain users        15 Jun 23  2022 bpnet-pub -> bpnet-pub-
local
drwxrwxr-x  9 cm2363 domain users       367 Feb 24  2023 bpnet-pub-local
drwxrwxr-x  2 cm2363 domain users         0 Jun  2  2022 bw
-rwxrwxr-x  1 cm2363 domain users      1280 Oct  2 18:44 cwmScan.json
-rwxrwxr-x  1 cm2363 domain users       573 Oct  2 18:45 cwm.slurm
drwxrwxr-x  2 cm2363 domain users       433 Jun 21 09:09 data
-rwxrwxr-x  1 cm2363 domain users   1065133 Jun 23 13:47 er2.log
-rwxrwxr-x  1 cm2363 domain users   1060005 Jun 22 22:49 er.log
drwxrwxr-x  2 cm2363 domain users         0 Sep 30 17:23 hits
drwxrwxr-x  2 cm2363 domain users       126 Jun 21 09:27 input
-rwxrwxr-x  1 cm2363 domain users       870 Jun 22 20:52 interpretFlat.slurm
-rwxrwxr-x  1 cm2363 domain users     11748 Feb 27  2023 jobScript.sge
-rwxrwxr-x  1 cm2363 domain users      8595 Jun 24  2022 jobScriptSplit.sge
drwxrwxr-x  2 cm2363 domain users       859 Sep 15 14:16 json
drwxrwxr-x  3 cm2363 domain users      3711 Oct  2 18:45 logs
-rwxr-xr-x  1 cm2363 domain users       197 Sep 30 17:20
melanieMetaclusters.json
drwxrwxr-x  9 cm2363 domain users       439 Jun 21 15:38 models
drwxrwxr-x 10 cm2363 domain users       275 Oct  6 13:18 modisco
-rwxrwxr-x  1 cm2363 domain users      2653 Jun 23 11:09 modiscoReport.slurm
-rwxrwxr-x  1 cm2363 domain users      3262 Jun 22 21:09 modisco.slurm
-rwxrwxr-x  1 cm2363 domain users       432 Jun  3  2022 notes.txt
drwxrwxr-x  2 cm2363 domain users      1355 Jun 21 09:05 old-json
-rw-r--r--  1 cm2363 domain users 396529920 Jun 22 22:42 pisa.fa
drwxrwxr-x  2 cm2363 domain users       963 Jun 23 13:48 pred
-rwxrwxr-x  1 cm2363 domain users       641 Jun 22 20:53 predictCombined.slurm
-rwxr-xr-x  1 cm2363 domain users       287 Jun 23 13:43 predictFasta.json
-rwxrwxr-x  1 cm2363 domain users       491 Jun 22 20:51 predictSolo.slurm
-rwxrwxr-x  1 cm2363 domain users       521 Jun 22 20:51
predictTransformation.slurm
-rwxrwxr-x  1 cm2363 domain users       848 Jun 22 20:51 predToBigwigBias.slurm
-rwxrwxr-x  1 cm2363 domain users      4434 Jun 22 20:53
predToBigwigCombined.slurm
-rwxrwxr-x  1 cm2363 domain users       862 Jun 22 20:51
predToBigwigTransform.slurm
```

```
-rwxrwxr-x  1 cm2363 domain users       487 Jun 22 20:50
prepareBedNonPeaks.slurm
-rwxrwxr-x  1 cm2363 domain users       478 Jun 22 20:50 prepareBedPeaks.slurm
-rwxrwxr-x  1 cm2363 domain users      1070 Jun 22 20:51
prepareTrainingData.slurm
-rwxrwxr-x  1 cm2363 domain users    218012 Oct  2 15:58 profile.log
drwxrwxr-x  4 cm2363 domain users       329 Oct  3 16:32 scan
drwxrwxr-x  2 cm2363 domain users      1351 Jun 22 21:07 shap
-rwxrwxr-x  1 cm2363 domain users      1986 Jun 22 21:02 shapToBigwig.slurm
-rwxrwxr-x  1 cm2363 domain users      2646 Jun 22 21:04 shapToNumpy.slurm
-rwxrwxr-x  1 cm2363 domain users    200926 Oct  2 15:59 times.dat
-rwxrwxr-x  1 cm2363 domain users       498 Jun 22 20:51 trainCombined.slurm
-rwxrwxr-x  1 cm2363 domain users       483 Jun 22 20:51 trainSolo.slurm
-rwxrwxr-x  1 cm2363 domain users       522 Jun 22 20:51
trainTransformation.slurm
-rwxrwxr-x  1 cm2363 domain users     25279 Oct  2 18:11 x100
-rwxrwxr-x  1 cm2363 domain users     25279 Oct  2 18:36 x101
drwxrwxr-x  2 cm2363 domain users       815 Feb 27  2023 zenodo
```

[7]:
```
!ls -lh {WORKING_DIRECTORY}/data
```

```
total 4.3G
-rw-r----- 1 cm2363 domain users 3.5M Jun 21 08:56 klf4.narrowPeak
-rw-r----- 1 cm2363 domain users 350M Jun 21 08:55 klf4.neg.bw
-rw-r----- 1 cm2363 domain users 350M Jun 21 08:55 klf4.pos.bw
-rw-r----- 1 cm2363 domain users 3.5M Jun 21 08:56 nanog.narrowPeak
-rw-r----- 1 cm2363 domain users 398M Jun 21 08:56 nanog.neg.bw
-rw-r----- 1 cm2363 domain users 398M Jun 21 08:56 nanog.pos.bw
-rw-r----- 1 cm2363 domain users 1.6M Jun 21 08:57 oct4.narrowPeak
-rw-r----- 1 cm2363 domain users 551M Jun 21 08:57 oct4.neg.bw
-rw-r----- 1 cm2363 domain users 551M Jun 21 08:57 oct4.pos.bw
-rw-r----- 1 cm2363 domain users 154M Jun 21 09:09 patchcap.neg.bw
-rw-r----- 1 cm2363 domain users 154M Jun 21 09:09 patchcap.pos.bw
-rw-r----- 1 cm2363 domain users 676K Jun 21 09:03 sox2.narrowPeak
-rw-r----- 1 cm2363 domain users 301M Jun 21 08:57 sox2.neg.bw
-rw-r----- 1 cm2363 domain users 300M Jun 21 08:58 sox2.pos.bw
```

[7]:
```
#The first thing I need to do is prepare input files in order to train a bias␣
 ↪model.
#But what shall I use for bias? I have two options: I can either use background
#regions from the actual chip-nexus experiments, or I can use the patchcap␣
 ↪track.
#If I were to use background regions, I'd have to have a stringent way to␣
 ↪determine
#when a region is not bound, and the data are noisy enough that this might be a
#tough call. I'll train up the bias model on patchcap data instead.

#In order to train that model, I'll need a couple things:
```

```
# 1. The bias data. I'm going to just use the patchcap bigwigs from the paper, no biggie.
# 2. A set of regions to train on. I'll make these in a minute.
# 3. A model architecture. I have to decide on this right now, because
#    it will determine the size of the regions I train on.

#I'll use a standard BPNet architecture, but with few filters since it's learning
#something so simple. With a 9-layer network, and a 25 bp input filter and 25 bp
#output filter, using 1000 bp output windows, I can calculate the input size:
```

[8]:
```
OUTPUT_LENGTH=1000
input_length_str = !{SRC_DIR}/lengthCalc.py --output-len {OUTPUT_LENGTH} \
                                            --n-dil-layers 9 \
                                            --conv1-kernel-size 25 \
                                            --profile-kernel-size 25
INPUT_LENGTH=int(input_length_str[0])
print(INPUT_LENGTH)
RECEPTIVE_FIELD=INPUT_LENGTH - OUTPUT_LENGTH+1
print(RECEPTIVE_FIELD)
MAX_JITTER = 100
```

```
3092
2093
```

[9]:
```
#Okay, great. I need to make sure that the regions I train on have valid DNA
#within 3092/2 bases of the middle of the window.
#For clarity, here are some dimensions:

#
#
#            |<---  2093 bp (Receptive field) --->|
#   |<---------------------   3092 bp (Input length) ------------------------>|
#   SEQUENCESEQUENCESEQUENCESEQUENCESEQUENCESEQUENCESEQUENCESEQUENCESEQUENCE
#   \        \                                  /                       /
#     \        \                              /                       /
#       \        \                          /                       /
#         \        \                      /                       /
#           \        \                  /                       /
#             \        \              /                       /
#               \        \          /                       /
#                 \        \      /                       /
#                   \        \  /                       /
#                     \        \/                      /
#                      PROFILEPROFILEPROFILEPROFILEPROFIL
#                      |<--- 1000 bp (Output length) --->|
#    |<--- 1046 bp --->|
```

```
#
# During training, we also shift the regions around by a little bit, a process
# called jittering. We'll use a maximum jitter of 100.
```

[10]:
```
#In order to generate bias regions, I need to get the actual training regions.
#This is not really part of bpreveal, but I do have a few utility scripts in
#the repo to help with this.
#I'm going to combine the called peaks, make sure there's valid genome under
#all of them (i.e., no "N" nucleotides within the receptive field.),
#then split them into train, validation, and test splits.
```

[9]:
```
bigwigFileNames = [WORKING_DIRECTORY + "/data/" + tfName + "." + strand + ".bw"
                   for tfName in TF_NAMES
                   for strand in ["pos", "neg"]]
print(bigwigFileNames)
summitBedFnames = [WORKING_DIRECTORY + "/data/" + tfName + ".narrowPeak"
                   for tfName in TF_NAMES]
print(summitBedFnames)
#And I need to make bigwig specs, for the upcoming json.
#The bigwig spec needs to list max and min quantiles.
bwSpec = [{"file-name" : fname, "max-quantile" : 1, "min-counts" : 1}
          for fname in bigwigFileNames]
```

```
['/n/projects/cm2363/bpreveal/test/oskn/data/oct4.pos.bw',
'/n/projects/cm2363/bpreveal/test/oskn/data/oct4.neg.bw',
'/n/projects/cm2363/bpreveal/test/oskn/data/sox2.pos.bw',
'/n/projects/cm2363/bpreveal/test/oskn/data/sox2.neg.bw',
'/n/projects/cm2363/bpreveal/test/oskn/data/klf4.pos.bw',
'/n/projects/cm2363/bpreveal/test/oskn/data/klf4.neg.bw',
'/n/projects/cm2363/bpreveal/test/oskn/data/nanog.pos.bw',
'/n/projects/cm2363/bpreveal/test/oskn/data/nanog.neg.bw']
['/n/projects/cm2363/bpreveal/test/oskn/data/oct4.narrowPeak',
'/n/projects/cm2363/bpreveal/test/oskn/data/sox2.narrowPeak',
'/n/projects/cm2363/bpreveal/test/oskn/data/klf4.narrowPeak',
'/n/projects/cm2363/bpreveal/test/oskn/data/nanog.narrowPeak']
```

[10]:
```
prepareBedPeaksConfig = {
    "bigwigs" : bwSpec,
    "splits" : {"test-chroms"  : TEST_CHROMS,
                "val-chroms"   : VAL_CHROMS,
                "train-chroms" : TRAIN_CHROMS,
                "regions" : summitBedFnames},
    "genome" : GENOME_FASTA,
    "write-counts-to" : WORKING_DIRECTORY + "/bed/peak_all.stats",
    "output-length" : OUTPUT_LENGTH,
    "input-length" : INPUT_LENGTH,
    "max-jitter" : MAX_JITTER,
    "output-prefix" : WORKING_DIRECTORY + "/bed/peak",
```

```
    "resize-mode" : "center",
    "remove-overlaps" : True,
    "overlap-max-distance" : 100,
    "verbosity" : "INFO"}

with open(WORKING_DIRECTORY + "/json/prepareBedPeaks.json", "w") as fp:
    json.dump(prepareBedPeaksConfig, fp, indent=4)
    print(json.dumps(prepareBedPeaksConfig, indent=4))
```


```
{
    "bigwigs": [
        {
            "file-name":
"/n/projects/cm2363/bpreveal/test/oskn/data/oct4.pos.bw",
            "max-quantile": 1,
            "min-counts": 1
        },
        {
            "file-name":
"/n/projects/cm2363/bpreveal/test/oskn/data/oct4.neg.bw",
            "max-quantile": 1,
            "min-counts": 1
        },
        {
            "file-name":
"/n/projects/cm2363/bpreveal/test/oskn/data/sox2.pos.bw",
            "max-quantile": 1,
            "min-counts": 1
        },
        {
            "file-name":
"/n/projects/cm2363/bpreveal/test/oskn/data/sox2.neg.bw",
            "max-quantile": 1,
            "min-counts": 1
        },
        {
            "file-name":
"/n/projects/cm2363/bpreveal/test/oskn/data/klf4.pos.bw",
            "max-quantile": 1,
            "min-counts": 1
        },
        {
            "file-name":
"/n/projects/cm2363/bpreveal/test/oskn/data/klf4.neg.bw",
            "max-quantile": 1,
            "min-counts": 1
        },
        {
```

```
            "file-name":
"/n/projects/cm2363/bpreveal/test/oskn/data/nanog.pos.bw",
            "max-quantile": 1,
            "min-counts": 1
        },
        {
            "file-name":
"/n/projects/cm2363/bpreveal/test/oskn/data/nanog.neg.bw",
            "max-quantile": 1,
            "min-counts": 1
        }
    ],
    "splits": {
        "test-chroms": [
            "chr1",
            "chr8",
            "chr9"
        ],
        "val-chroms": [
            "chr2",
            "chr3",
            "chr4"
        ],
        "train-chroms": [
            "chr5",
            "chr6",
            "chr7",
            "chr10",
            "chr11",
            "chr12",
            "chr13",
            "chr14",
            "chr15",
            "chr16",
            "chr17",
            "chr18",
            "chr19"
        ],
        "regions": [
            "/n/projects/cm2363/bpreveal/test/oskn/data/oct4.narrowPeak",
            "/n/projects/cm2363/bpreveal/test/oskn/data/sox2.narrowPeak",
            "/n/projects/cm2363/bpreveal/test/oskn/data/klf4.narrowPeak",
            "/n/projects/cm2363/bpreveal/test/oskn/data/nanog.narrowPeak"
        ]
    },
    "genome": "/n/data1/genomes/indexes/mm10/mm10.fa",
    "write-counts-to":
"/n/projects/cm2363/bpreveal/test/oskn/bed/peak_all.stats",
```

```
    "output-length": 1000,
    "input-length": 3092,
    "max-jitter": 100,
    "output-prefix": "/n/projects/cm2363/bpreveal/test/oskn/bed/peak",
    "resize-mode": "center",
    "remove-overlaps": true,
    "overlap-max-distance": 100,
    "verbosity": "INFO"
}
```

[11]:
```
#Now we can go ahead and run that script.
jobsNonGpu(["prepareBed.py {0:s}/json/prepareBedPeaks.json".
 ↪format(WORKING_DIRECTORY)],
           "prepareBedPeaks", 2, 20, "1:00:00")
#(I've run this on Cerebro.)
```

[12]:
```
def generateTilingRegions(genome, width, chromEdgeBoundary, spaceBetween,␣
 ↪allowChroms):
    chromRegions = []
    numRegions = 0
    #To use window_maker from pybedtools, I first need to create a bed
    #containing the chromosomes where I want regions made.
    for chrom in genome.references:
        if(chrom not in allowChroms):
            continue

        startPos = chromEdgeBoundary
        chromSize = genome.get_reference_length(chrom)
        stopPos = chromSize - chromEdgeBoundary
        chromRegions.append(pybedtools.Interval(chrom, startPos, stopPos))

    windows = pybedtools.BedTool(chromRegions).window_maker(w=width,
                              s=spaceBetween + width, genome='mm10')
    return windows

with pysam.FastaFile(GENOME_FASTA) as genomeFp:
    w = generateTilingRegions(genomeFp, 1000, 100000, 10000,
                              TEST_CHROMS + TRAIN_CHROMS + VAL_CHROMS)
    peaks = pybedtools.BedTool(WORKING_DIRECTORY + "/bed/peak_all.bed")
    peaksReject = pybedtools.BedTool(WORKING_DIRECTORY + "/bed/peak_reject.bed")
    allPeaks = peaks.cat(peaksReject).sort().slop(b=INPUT_LENGTH, genome='mm10')
    trimWindows = w.subtract(allPeaks, A=True)
    print("Number of peak regions: {0:d}".format(allPeaks.count()))
    trimWindows.saveas(WORKING_DIRECTORY+ "/bed/tiling_all.bed")
    print("Background window candidates: {0:d}".format(trimWindows.count()))
```

```
Number of peak regions: 85399
Background window candidates: 197805
```

[13]:
```
#Now that we have a bed file with all of our training regions in it, we can
#generate the background regions that we'll train the bias model on.
#Note that even though I'm using patchcap data for my bias track, I'm still
#going to train the bias model on unbound regions, so that any effect
#TF binding has on patchcap doesn't show up in my bias model.
#This is another script I wrote, it generates tiling regions across the
#whole genome and then removes regions that overlap your peak set,
#and also only outputs regions that fall in a set percentile of counts.

biasBigwigFnames = [WORKING_DIRECTORY + "/data/patchcap" + "." + strand + ".bw"
                    for strand in ["pos", "neg"]]
print(biasBigwigFnames)
```

```
['/n/projects/cm2363/bpreveal/test/oskn/data/patchcap.pos.bw',
'/n/projects/cm2363/bpreveal/test/oskn/data/patchcap.neg.bw']
```

[14]:
```
biasBwSpec = [{"file-name" : fname,
               "max-quantile" : 0.6,
               "min-quantile" : 0.01}
              for fname in bigwigFileNames]
biasBwSpec = biasBwSpec + [{"file-name" : fname,
                            "max-quantile" : 0.95,
                            "min-quantile" : 0.1}
                           for fname in biasBigwigFnames]
prepareBedNonPeaksConfig = {
    "bigwigs" : biasBwSpec,
    "splits" : {"test-chroms"  : TEST_CHROMS,
                "val-chroms"   : VAL_CHROMS,
                "train-chroms" : TRAIN_CHROMS,
                "regions" : [WORKING_DIRECTORY + "/bed/tiling_all.bed"]},
    "genome" : GENOME_FASTA,
    "write-counts-to" : WORKING_DIRECTORY + "/bed/nonpeak_all.stats",
    "output-length" : OUTPUT_LENGTH,
    "input-length" : INPUT_LENGTH,
    "max-jitter" : MAX_JITTER,
    "output-prefix" : WORKING_DIRECTORY + "/bed/nonpeak",
    "remove-overlaps" : False,
    "resize-mode" : "center",
    "verbosity" : "INFO"}

with open(WORKING_DIRECTORY + "/json/prepareBedNonPeaks.json", "w") as fp:
    json.dump(prepareBedNonPeaksConfig, fp)
    print(json.dumps(prepareBedNonPeaksConfig, indent=4))
```

```
{
    "bigwigs": [
        {
            "file-name":
```

```
"/n/projects/cm2363/bpreveal/test/oskn/data/oct4.pos.bw",
            "max-quantile": 0.6,
            "min-quantile": 0.01
        },
        {
            "file-name":
"/n/projects/cm2363/bpreveal/test/oskn/data/oct4.neg.bw",
            "max-quantile": 0.6,
            "min-quantile": 0.01
        },
        {
            "file-name":
"/n/projects/cm2363/bpreveal/test/oskn/data/sox2.pos.bw",
            "max-quantile": 0.6,
            "min-quantile": 0.01
        },
        {
            "file-name":
"/n/projects/cm2363/bpreveal/test/oskn/data/sox2.neg.bw",
            "max-quantile": 0.6,
            "min-quantile": 0.01
        },
        {
            "file-name":
"/n/projects/cm2363/bpreveal/test/oskn/data/klf4.pos.bw",
            "max-quantile": 0.6,
            "min-quantile": 0.01
        },
        {
            "file-name":
"/n/projects/cm2363/bpreveal/test/oskn/data/klf4.neg.bw",
            "max-quantile": 0.6,
            "min-quantile": 0.01
        },
        {
            "file-name":
"/n/projects/cm2363/bpreveal/test/oskn/data/nanog.pos.bw",
            "max-quantile": 0.6,
            "min-quantile": 0.01
        },
        {
            "file-name":
"/n/projects/cm2363/bpreveal/test/oskn/data/nanog.neg.bw",
            "max-quantile": 0.6,
            "min-quantile": 0.01
        },
        {
            "file-name":
```

```
"/n/projects/cm2363/bpreveal/test/oskn/data/patchcap.pos.bw",
            "max-quantile": 0.95,
            "min-quantile": 0.1
        },
        {
            "file-name":
"/n/projects/cm2363/bpreveal/test/oskn/data/patchcap.neg.bw",
            "max-quantile": 0.95,
            "min-quantile": 0.1
        }
    ],
    "splits": {
        "test-chroms": [
            "chr1",
            "chr8",
            "chr9"
        ],
        "val-chroms": [
            "chr2",
            "chr3",
            "chr4"
        ],
        "train-chroms": [
            "chr5",
            "chr6",
            "chr7",
            "chr10",
            "chr11",
            "chr12",
            "chr13",
            "chr14",
            "chr15",
            "chr16",
            "chr17",
            "chr18",
            "chr19"
        ],
        "regions": [
            "/n/projects/cm2363/bpreveal/test/oskn/bed/tiling_all.bed"
        ]
    },
    "genome": "/n/data1/genomes/indexes/mm10/mm10.fa",
    "write-counts-to":
"/n/projects/cm2363/bpreveal/test/oskn/bed/nonpeak_all.stats",
    "output-length": 1000,
    "input-length": 3092,
    "max-jitter": 100,
    "output-prefix": "/n/projects/cm2363/bpreveal/test/oskn/bed/nonpeak",
```

```
    "remove-overlaps": false,
    "resize-mode": "center",
    "verbosity": "INFO"
}
```

[15]:
```
#Now we can go ahead and run that script.
jobsNonGpu(["prepareBed.py {0:s}/json/prepareBedNonPeaks.json".
 ↪format(WORKING_DIRECTORY)],
           "prepareBedNonPeaks", 2, 20, "1:00:00")
```

[ ]:
```
```

1 Building the training dataset

[16]:
```
#This next step is pretty easy; we just need to pull the sequence and profile
#information into a single hdf5-format file for the training programs to use.

#We'll need to make training and validation sets for both the nonpeaks and
#peaks bed files.

configFnames = []
for split in ["train", "val"]:
    for dataset in ["peak", "nonpeak"]:
        heads = []
        for tfName in TF_NAMES:
            if(dataset == 'peak'):
                heads.append({
                    "revcomp-task-order" : "auto",
                    "bigwig-files" : [WORKING_DIRECTORY + "/data/" + tfName + ".
 ↪pos.bw",
                                      WORKING_DIRECTORY + "/data/" + tfName + ".
 ↪neg.bw"]})
            else:
                heads.append({
                    "revcomp-task-order" : "auto",
                    "bigwig-files" : [WORKING_DIRECTORY + "/data/patchcap.pos.
 ↪bw",
                                      WORKING_DIRECTORY + "/data/patchcap.
 ↪neg.bw"]})
        config = {"genome" : GENOME_FASTA,
                  "input-length" : INPUT_LENGTH,
                  "output-length" : OUTPUT_LENGTH,
                  "max-jitter" : MAX_JITTER,
                  "regions" : WORKING_DIRECTORY + "/bed/" + dataset + "_" +␣
 ↪split + ".bed",
```

```
                  "output-h5" : WORKING_DIRECTORY + "/input/" + dataset + "_" +␣
 ↪split + ".h5",
                  "reverse-complement" : True,
                  "heads" : heads,
                  "verbosity" : "DEBUG"}
        configFname =WORKING_DIRECTORY + "/json/prepareInput" + dataset + "_" +␣
 ↪split+ ".json"
        with open(configFname, "w") as fp:
            json.dump(config, fp, indent=2)
        configFnames.append(configFname)
jobsNonGpu([SRC_DIR+"/prepareTrainingData.py {0:s}".format(configFname)
                for configFname in configFnames],
            "prepareTrainingData", 2, 20, "1:00:00")
#!{SRC_DIR}/prepareTrainingData.py {configFname}
```

2 Training the bias model

[ ]:
```
#Okay, so the bed preparation step is done. I didn't spend much time
#on that since it will be specific to every system you deal with.
#But now comes the common stuff. And it's (honestly) easier.
```

[18]:
```
#To make the model config file, I'll assemble the heads first.
heads = []
for tfName in TF_NAMES:
    heads.append({"num-tasks" : 2,
                  "profile-loss-weight" : 1,
                  "head-name" : "patchcap_" + tfName,
                  "counts-loss-weight" : 10})


#And now the whole config file:
biasTrainConfig = {
    "settings" : {
        "output-prefix" : WORKING_DIRECTORY + "/models/solo",
        "epochs" : 200,
        "max-jitter" : 100,
        "early-stopping-patience" : 20,
        "batch-size" : 128,
        "learning-rate" : 0.004,
        "learning-rate-plateau-patience" : 5,
        "architecture" : {
            "architecture-name" : "bpnet",
            "input-length" : INPUT_LENGTH,
            "output-length" : OUTPUT_LENGTH,
```

```
            "model-name" : "patchcap",
            "model-args" : "",
            "filters" : 16,
            "layers" : 9,
            "input-filter-width" : 25,
            "output-filter-width" : 25
        }
    },
    "train-data" : WORKING_DIRECTORY + "/input/nonpeak_train.h5",
    "val-data" : WORKING_DIRECTORY + "/input/nonpeak_val.h5",
    "heads" : heads,
    "verbosity" : "WARNING"
}

print(json.dumps(biasTrainConfig, indent=4))

with open(WORKING_DIRECTORY + "/json/trainBias.json", "w") as fp:
    json.dump(biasTrainConfig, fp, indent=4)
```


```
{
    "settings": {
        "output-prefix": "/n/projects/cm2363/bpreveal/test/oskn/models/solo",
        "epochs": 200,
        "max-jitter": 100,
        "early-stopping-patience": 20,
        "batch-size": 128,
        "learning-rate": 0.004,
        "learning-rate-plateau-patience": 5,
        "architecture": {
            "architecture-name": "bpnet",
            "input-length": 3092,
            "output-length": 1000,
            "model-name": "patchcap",
            "model-args": "",
            "filters": 16,
            "layers": 9,
            "input-filter-width": 25,
            "output-filter-width": 25
        }
    },
    "train-data":
"/n/projects/cm2363/bpreveal/test/oskn/input/nonpeak_train.h5",
    "val-data": "/n/projects/cm2363/bpreveal/test/oskn/input/nonpeak_val.h5",
    "heads": [
        {
            "num-tasks": 2,
            "profile-loss-weight": 1,
```

```
            "head-name": "patchcap_oct4",
            "counts-loss-weight": 10
        },
        {
            "num-tasks": 2,
            "profile-loss-weight": 1,
            "head-name": "patchcap_sox2",
            "counts-loss-weight": 10
        },
        {
            "num-tasks": 2,
            "profile-loss-weight": 1,
            "head-name": "patchcap_klf4",
            "counts-loss-weight": 10
        },
        {
            "num-tasks": 2,
            "profile-loss-weight": 1,
            "head-name": "patchcap_nanog",
            "counts-loss-weight": 10
        }
    ],
    "verbosity": "WARNING"
}
```

[19]:
```
#!{SRC_DIR}/trainSoloModel.py {WORKING_DIRECTORY}/json/trainBias.json
jobsGpu(["trainSoloModel.py {0:s}".format(WORKING_DIRECTORY + "/json/trainBias.
 ↪json")],
        "trainSolo", 10, 100, "10:00:00")
```

[ ]:
```
#(I've deleted many pages of output from the training program)
#We should look at how well the model did.
```

[20]:
```
!{SRC_DIR}/makeLossPlots.py --json {WORKING_DIRECTORY}/models/solo.history.json␣
 ↪\
                            --output {WORKING_DIRECTORY}/models/solo.png
```

[ ]:
```
#It's pretty clear that the model overlearned, even with only sixteen filters.
#Interesting. It would be great if the training and validation losses were
#more similar, but it's not a lethal flaw since we don't need to interpret
#the bias model. We should, however, make predictions from it and calculate
#some metrics.
```

3 Evaluating the bias model

[21]:
```
#First, we need to make predictions with the bias model. That's another json␣
↪file...
biasPredictConfig = {
    "settings" : {
        "genome" : GENOME_FASTA,
        "output-h5" : WORKING_DIRECTORY + "/pred/patchcap.h5",
        "batch-size" : 128,
        "heads" : 4,

        "architecture" : {
            "model-file" : WORKING_DIRECTORY + "/models/solo.model",
            "input-length" : INPUT_LENGTH,
            "output-length" : OUTPUT_LENGTH
        }
    },
    "bed-file" : WORKING_DIRECTORY + "/bed/peak_all.bed",
    "verbosity" : "DEBUG"
}


with open(WORKING_DIRECTORY + "/json/predictBias.json", "w") as fp:
    json.dump(biasPredictConfig, fp)
```

[22]:
```
jobsGpu(["makePredictionsBed.py {0:s}".format(WORKING_DIRECTORY + "/json/
↪predictBias.json")],
        "predictSolo", 1, 50, "10:00:00")
```

[23]:
```
#And now I need to convert that hdf5 file into a bigwig.
predCmd = "predictToBigwig.py " +\
          "--h5 {0:s}/pred/patchcap.h5 " +\
          "--bw {0:s}/pred/patchcap_{1:s}.bw "+\
          "--head-id 0 --task-id {2:d} --mode profile --verbose"

jobsNonGpu([predCmd.format(WORKING_DIRECTORY, strand[0], strand[1]) for strand␣
↪in [("positive", 0), ("negative", 1)]],
           "predToBigwigBias", 2, 20, "1:00:00")
```

[25]:
```
#Note that I've only written bigwigs for the first head - since all heads were␣
↪trained on the same data, I'm going to assume each head performed equally␣
↪well.
```

[24]:
```
#We can now calculate some standard metrics on our predictions, though we don't␣
↪yet have anything to compare these to.
```

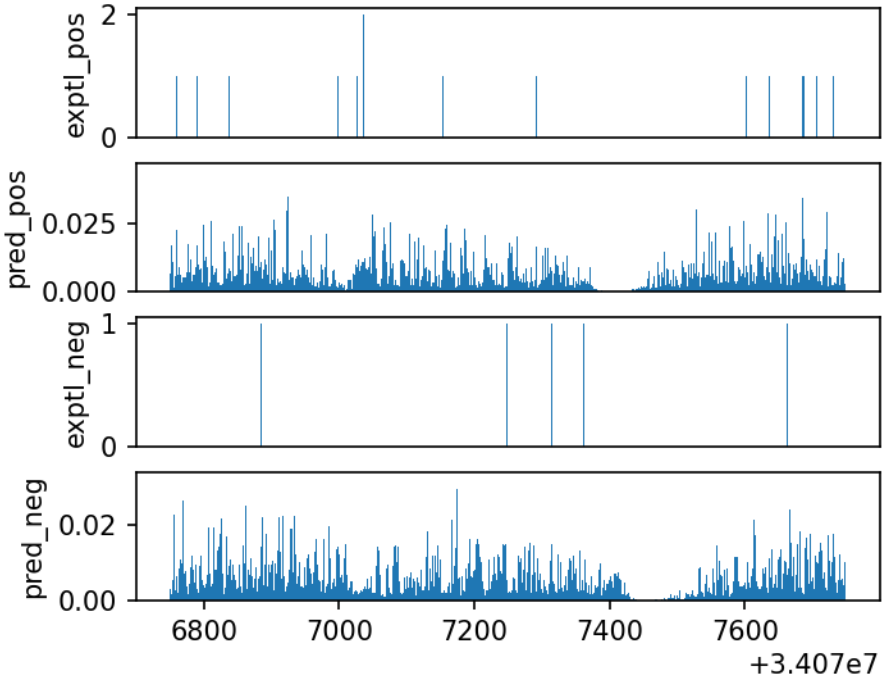
```
!{SRC_DIR}/metrics.py --reference {WORKING_DIRECTORY}/data/patchcap.pos.bw␣
↪--pred {WORKING_DIRECTORY}/pred/patchcap_positive.bw --regions␣
↪{WORKING_DIRECTORY}/bed/peak_all.bed --threads 20 --apply-abs
```

```
reference /n/projects/cm2363/bpreveal/test/oskn/data/patchcap.pos.bw predicted
/n/projects/cm2363/bpreveal/test/oskn/pred/patchcap_positive.bw regions
/n/projects/cm2363/bpreveal/test/oskn/bed/peak_all.bed
100%|                | 106640/106640 [00:10<00:00, 10276.16it/s]
metric                0.000000%      25.000000%      50.000000%      75.000000%
100.000000% regions
mnll                        nan             nan             nan             nan
nan 106640
jsd                         nan             nan             nan             nan
nan 106640
pearsonr                    nan             nan             nan             nan
nan 106640
spearmanr                   nan             nan             nan             nan
nan 106640
Process Process-22:
Traceback (most recent call last):
  File "/scratch/bpreveal-teak/lib/python3.10/multiprocessing/process.py", line
314, in _bootstrap
    self.run()
  File "/scratch/bpreveal-teak/lib/python3.10/multiprocessing/process.py", line
108, in run
    self._target(*self._args, **self._kwargs)
  File "/n/projects/cm2363/bpreveal/src/metrics.py", line 149, in receiveThread
    countsPearson = scipy.stats.pearsonr(referenceCounts, predictedCounts)
  File "/scratch/bpreveal-teak/lib/python3.10/site-
packages/scipy/stats/_stats_py.py", line 4451, in pearsonr
    normxm = linalg.norm(xm)
  File "/scratch/bpreveal-teak/lib/python3.10/site-
packages/scipy/linalg/_misc.py", line 146, in norm
    a = np.asarray_chkfinite(a)
  File "/scratch/bpreveal-teak/lib/python3.10/site-
packages/numpy/lib/function_base.py", line 627, in asarray_chkfinite
    raise ValueError(
ValueError: array must not contain infs or NaNs
```

[25]:
```
#Let's also take a quick look at the generated bigwigs.

def plotBws(bwNames, titles, chrom, start, stop):

    for i, bwName in enumerate(bwNames):
        plt.subplot(100*len(bwNames)+10+(i+1))
        bw = pyBigWig.open(bwName)
        bwVals = np.nan_to_num(bw.values(chrom, start, stop))
```

```
        #plt.xlim(0,stop-start)
        plt.bar(range(start, stop), bwVals, width=1)
        plt.ylabel(titles[i])
        if(i < len(bwNames)-1):
            plt.xticks([])
```

[26]:
```
plotBws([WORKING_DIRECTORY + "/data/patchcap.pos.bw",
         WORKING_DIRECTORY + "/pred/patchcap_positive.bw",
         WORKING_DIRECTORY + "/data/patchcap.neg.bw",
         WORKING_DIRECTORY + "/pred/patchcap_negative.bw"],
        ["exptl_pos", "pred_pos", "exptl_neg", "pred_neg"], "chr1", 34076750,␣
↪34077750)
```



[ ]:
```
#Huh. With so little patchcap data, it's really hard to tell if the model is␣
↪doing a good job.
#In any event, it's time to train the transformation model up.
```

4 Training the transformation model

[27]:
```
heads = []
for tfName in TF_NAMES:
    heads.append({"num-tasks" : 2,
                  "profile-loss-weight" : 1,
                  "head-name" : "patchcap_" + tfName,
                  "counts-loss-weight" : 10})

transformationTrainConfig = {
    "settings" : {
        "output-prefix" : WORKING_DIRECTORY + "/models/transformation",
        "epochs" : 200,
        "early-stopping-patience" : 20,
        "batch-size" : 128,
        "learning-rate" : 0.04, #Note the very aggressive LR; we can do this␣
 ↪because there are so few parameters.
        "learning-rate-plateau-patience" : 5,
        "solo-model-file" : WORKING_DIRECTORY + "/models/solo.model",
        "input-length" : INPUT_LENGTH,
        "output-length" : OUTPUT_LENGTH,
        "max-jitter" : 100,
        "profile-architecture" : {
            "name" : "simple",
            "types" : ["linear", "sigmoid"]},
        "counts-architecture" : {
            "name" : "simple",
            "types" : ["linear", "sigmoid"]}},

    "train-data" : WORKING_DIRECTORY+ "/input/peak_train.h5",
    "val-data" : WORKING_DIRECTORY + "/input/peak_val.h5",
    "heads" : heads,
    "verbosity" : "INFO"
}

print(json.dumps(transformationTrainConfig, indent=2))

with open(WORKING_DIRECTORY + "/json/trainTransformation.json", "w") as fp:
    json.dump(transformationTrainConfig, fp)
```

```
{
  "settings": {
    "output-prefix":
"/n/projects/cm2363/bpreveal/test/oskn/models/transformation",
    "epochs": 200,
    "early-stopping-patience": 20,
    "batch-size": 128,
    "learning-rate": 0.04,
```

```
    "learning-rate-plateau-patience": 5,
    "solo-model-file":
"/n/projects/cm2363/bpreveal/test/oskn/models/solo.model",
    "input-length": 3092,
    "output-length": 1000,
    "max-jitter": 100,
    "profile-architecture": {
      "name": "simple",
      "types": [
        "linear",
        "sigmoid"
      ]
    },
    "counts-architecture": {
      "name": "simple",
      "types": [
        "linear",
        "sigmoid"
      ]
    }
  },
  "train-data": "/n/projects/cm2363/bpreveal/test/oskn/input/peak_train.h5",
  "val-data": "/n/projects/cm2363/bpreveal/test/oskn/input/peak_val.h5",
  "heads": [
    {
      "num-tasks": 2,
      "profile-loss-weight": 1,
      "head-name": "patchcap_oct4",
      "counts-loss-weight": 10
    },
    {
      "num-tasks": 2,
      "profile-loss-weight": 1,
      "head-name": "patchcap_sox2",
      "counts-loss-weight": 10
    },
    {
      "num-tasks": 2,
      "profile-loss-weight": 1,
      "head-name": "patchcap_klf4",
      "counts-loss-weight": 10
    },
    {
      "num-tasks": 2,
      "profile-loss-weight": 1,
      "head-name": "patchcap_nanog",
      "counts-loss-weight": 10
    }
```

```
  ],
  "verbosity": "INFO"
}
```

[28]:
```
jobsGpu(["trainTransformationModel.py {0:s}".format(WORKING_DIRECTORY + "/json/
 ↪trainTransformation.json")],
       "trainTransformation", 10, 60, "10:00:00")
#!{SRC_DIR}/trainTransformationModel.py {WORKING_DIRECTORY}/json/
 ↪trainTransformation.json
```

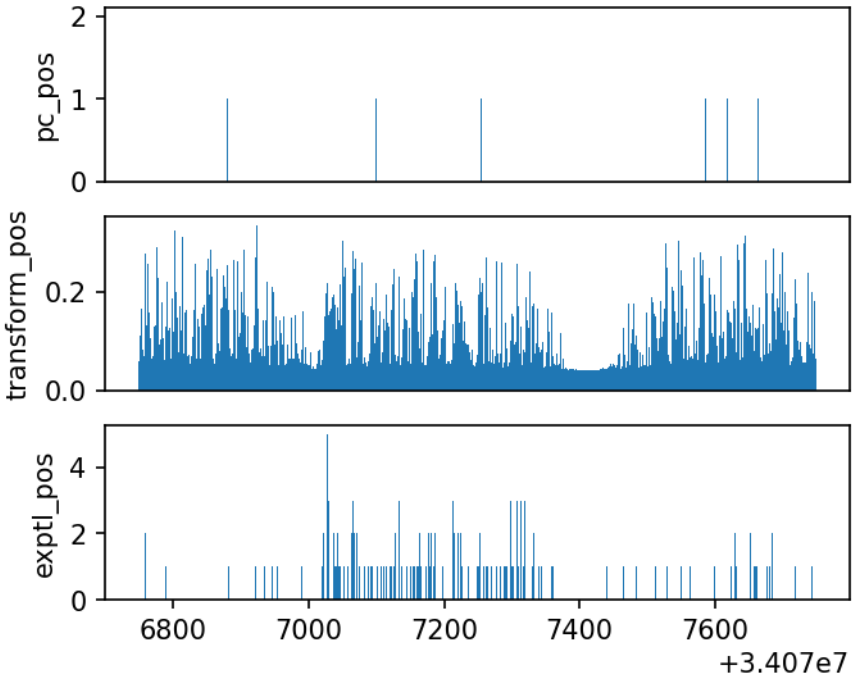
[29]:
```
#Let's go ahead and make predictions...
transformPredictConfig = {
    "settings" : {
        "genome" : GENOME_FASTA,
        "output-h5" : WORKING_DIRECTORY + "/pred/transform.h5",
        "batch-size" : 128,
        "heads" : 4,

        "architecture" : {
            "model-file" : WORKING_DIRECTORY + "/models/transformation.model",
            "input-length" : INPUT_LENGTH,
            "output-length" : OUTPUT_LENGTH
        }
    },
    "bed-file" : WORKING_DIRECTORY + "/bed/peak_all.bed",
    "verbosity" : "DEBUG"
}

print(transformPredictConfig)

with open(WORKING_DIRECTORY + "/json/predictTransformation.json", "w") as fp:
    json.dump(transformPredictConfig, fp)
```

```
{'settings': {'genome': '/n/data1/genomes/indexes/mm10/mm10.fa', 'output-h5':
'/n/projects/cm2363/bpreveal/test/oskn/pred/transform.h5', 'batch-size': 128,
'heads': 4, 'architecture': {'model-file':
'/n/projects/cm2363/bpreveal/test/oskn/models/transformation.model', 'input-
length': 3092, 'output-length': 1000}}, 'bed-file':
'/n/projects/cm2363/bpreveal/test/oskn/bed/peak_all.bed', 'verbosity': 'DEBUG'}
```

[30]:
```
jobsGpu(["makePredictionsBed.py {0:s}".format(WORKING_DIRECTORY + "/json/
 ↪predictTransformation.json")],
       "predictTransformation", 1, 50, "10:00:00")

predCmd = "predictToBigwig.py " +\
          "--h5 {0:s}/pred/transform.h5 " +\
          "--bw {0:s}/pred/transform_{1:s}.bw "+\
          "--head-id 0 --task-id {2:d} --mode profile --verbose"
```

```
jobsNonGpu([predCmd.format(WORKING_DIRECTORY, strand[0], strand[1]) for strand␣
 ↪in [("positive", 0), ("negative", 1)]],
           "predToBigwigTransform", 2, 20, "1:00:00")
#!{SRC_DIR}/makePredictionsBed.py {WORKING_DIRECTORY}/json/
 ↪predictTransformation.json
```

[31]:
```
plotBws([WORKING_DIRECTORY + "/data/patchcap.pos.bw",
         WORKING_DIRECTORY + "/pred/transform_positive.bw",
         WORKING_DIRECTORY + "/data/nanog.pos.bw"],
        ["pc_pos", "transform_pos", "exptl_pos"], "chr1", 34076750, 34077750)
```



[ ]:
```
#Of course these aren't a good match, but that's partly the point - the bald␣
 ↪spot that the patchcap model predicts seems to also be present in the Nanog␣
 ↪and Oct4 experimental data,
#and this suggests that that bald spot is an artifact.
```

5 Training the combined model

[32]:
```
heads = []
for tfName in TF_NAMES:
    heads.append({"num-tasks" : 2,
                  "profile-loss-weight" : 1,
```

```
                    "head-name" : "combined_" + tfName,
                    "counts-loss-weight" : 10,
                    "use-bias-counts" : False})


#And now the whole config file:
combinedTrainConfig = {
    "settings" : {
        "output-prefix" : WORKING_DIRECTORY + "/models/joint",
        "epochs" : 200,
        "early-stopping-patience" : 20,
        "batch-size" : 128,
        "learning-rate" : 0.004,
        "learning-rate-plateau-patience" : 5,
        "max-jitter" : 100,
        "transformation-model" : {
            "transformation-model-file" : WORKING_DIRECTORY + "/models/
 ↪transformation.model"
        },
        "architecture" : {
            "architecture-name" : "bpnet",
            "input-length" : INPUT_LENGTH,
            "output-length" : OUTPUT_LENGTH,
            "model-name" : "joint",
            "model-args" : "",
            "filters" : 64,
            "layers" : 9,
            "input-filter-width" : 25,
            "output-filter-width" : 25
        }
    },
    "train-data" : WORKING_DIRECTORY + "/input/peak_train.h5",
    "val-data" : WORKING_DIRECTORY + "/input/peak_val.h5",
    "heads" : heads,
    "verbosity" : "INFO"
}

print(json.dumps(combinedTrainConfig, indent=2))

with open(WORKING_DIRECTORY + "/json/trainCombined.json", "w") as fp:
    json.dump(combinedTrainConfig, fp)
```


```
{
  "settings": {
    "output-prefix": "/n/projects/cm2363/bpreveal/test/oskn/models/joint",
    "epochs": 200,
    "early-stopping-patience": 20,
    "batch-size": 128,
```

```
    "learning-rate": 0.004,
    "learning-rate-plateau-patience": 5,
    "max-jitter": 100,
    "transformation-model": {
      "transformation-model-file":
"/n/projects/cm2363/bpreveal/test/oskn/models/transformation.model"
    },
    "architecture": {
      "architecture-name": "bpnet",
      "input-length": 3092,
      "output-length": 1000,
      "model-name": "joint",
      "model-args": "",
      "filters": 64,
      "layers": 9,
      "input-filter-width": 25,
      "output-filter-width": 25
    }
  },
  "train-data": "/n/projects/cm2363/bpreveal/test/oskn/input/peak_train.h5",
  "val-data": "/n/projects/cm2363/bpreveal/test/oskn/input/peak_val.h5",
  "heads": [
    {
      "num-tasks": 2,
      "profile-loss-weight": 1,
      "head-name": "combined_oct4",
      "counts-loss-weight": 10,
      "use-bias-counts": false
    },
    {
      "num-tasks": 2,
      "profile-loss-weight": 1,
      "head-name": "combined_sox2",
      "counts-loss-weight": 10,
      "use-bias-counts": false
    },
    {
      "num-tasks": 2,
      "profile-loss-weight": 1,
      "head-name": "combined_klf4",
      "counts-loss-weight": 10,
      "use-bias-counts": false
    },
    {
      "num-tasks": 2,
      "profile-loss-weight": 1,
      "head-name": "combined_nanog",
      "counts-loss-weight": 10,
```

```
      "use-bias-counts": false
    }
  ],
  "verbosity": "INFO"
}
```

[33]:
```
jobsGpu(["trainCombinedModel.py {0:s}".format(WORKING_DIRECTORY + "/json/
 ↪trainCombined.json")],
        "trainCombined", 10, 60, "10:00:00")
#!{SRC_DIR}/trainCombinedModel.py {WORKING_DIRECTORY}/json/trainCombined.json
```

[34]:
```
#Let's look at the losses...
!{SRC_DIR}/makeLossPlots.py --json {WORKING_DIRECTORY}/models/joint.history.
 ↪json --output {WORKING_DIRECTORY}/models/joint.png
```

[ ]:
```
#It's overfitting a bit, maybe next time I'll try with fewer filters.
#But now's the time to make predictions.
```

[35]:
```
combinedPredictConfig = {
    "settings" : {
        "genome" : GENOME_FASTA,
        "output-h5" : WORKING_DIRECTORY + "/pred/combined.h5",
        "batch-size" : 128,
        "heads" : 4,

        "architecture" : {
            "model-file" : WORKING_DIRECTORY + "/models/joint_combined.model",
            "input-length" : INPUT_LENGTH,
            "output-length" : OUTPUT_LENGTH
        }
    },
    "bed-file" : WORKING_DIRECTORY + "/bed/peak_all.bed",
    "verbosity" : "DEBUG"
}

print(combinedPredictConfig)

with open(WORKING_DIRECTORY + "/json/predictCombined.json", "w") as fp:
    json.dump(combinedPredictConfig, fp)
#For the residual model, I just need to change a few terms:
residualPredictConfig = combinedPredictConfig
residualPredictConfig["settings"]["output-h5"] = WORKING_DIRECTORY + "/pred/
 ↪residual.h5"
residualPredictConfig["settings"]["architecture"]["model-file"] =␣
 ↪WORKING_DIRECTORY + "/models/joint_residual.model"
with open(WORKING_DIRECTORY + "/json/predictResidual.json", "w") as fp:
    json.dump(residualPredictConfig, fp)
```

```
{'settings': {'genome': '/n/data1/genomes/indexes/mm10/mm10.fa', 'output-h5':
'/n/projects/cm2363/bpreveal/test/oskn/pred/combined.h5', 'batch-size': 128,
'heads': 4, 'architecture': {'model-file':
'/n/projects/cm2363/bpreveal/test/oskn/models/joint_combined.model', 'input-
length': 3092, 'output-length': 1000}}, 'bed-file':
'/n/projects/cm2363/bpreveal/test/oskn/bed/peak_all.bed', 'verbosity': 'DEBUG'}
```

[36]:
```
jobsGpu(["makePredictionsBed.py {0:s}".format(WORKING_DIRECTORY + "/json/
 ↪predictCombined.json"),
         "makePredictionsBed.py {0:s}".format(WORKING_DIRECTORY + "/json/
 ↪predictResidual.json")],
        "predictCombined", 1, 50, "10:00:00")


bwCmdBase = "predictToBigwig.py " +\
          "--h5 {wd:s}/pred/{inf:s}.h5 " +\
          "--bw {wd:s}/pred/{outf:s}.bw "+\
          "--head-id {hid:d} --task-id {tid:d} --mode profile --verbose"
bwCmds = []
for modelType in ["residual", "combined"]:
    for headid, tfname in enumerate(TF_NAMES):
        for tid, strand in enumerate(["positive", "negative"]):
            cmd = bwCmdBase.format(wd=WORKING_DIRECTORY,
                                   inf=modelType,
                                   outf=tfname + "_" + modelType + "_" + strand,
                                   hid=headid, tid=tid)
            bwCmds.append(cmd)

jobsNonGpu(bwCmds,
           "predToBigwigCombined", 2, 20, "1:00:00")

#!{SRC_DIR}/makePredictionsBed.py {WORKING_DIRECTORY}/json/predictCombined.json
#!{SRC_DIR}/makePredictionsBed.py {WORKING_DIRECTORY}/json/predictResidual.json
```

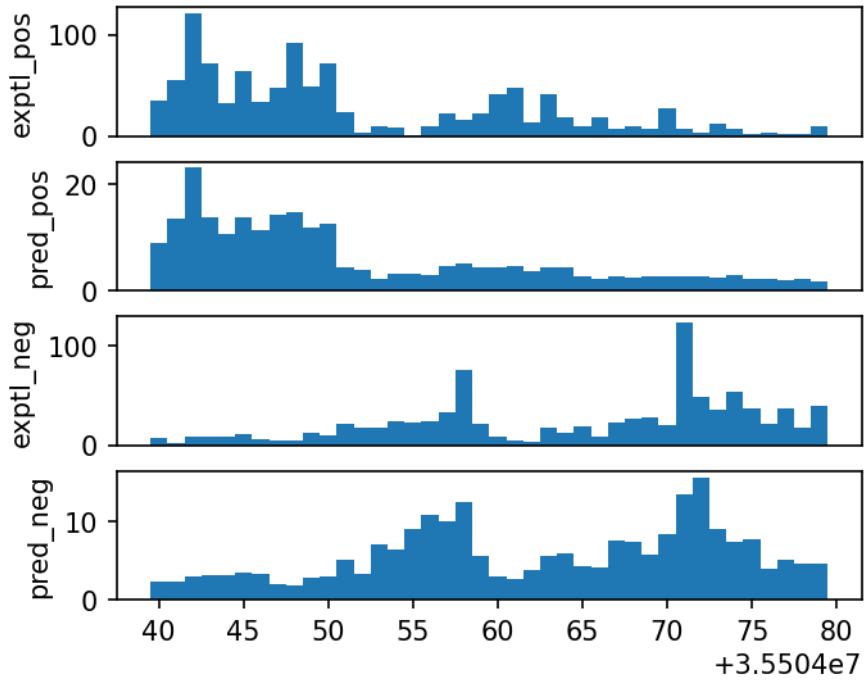
[ ]:
```

```

[ ]:
```

```

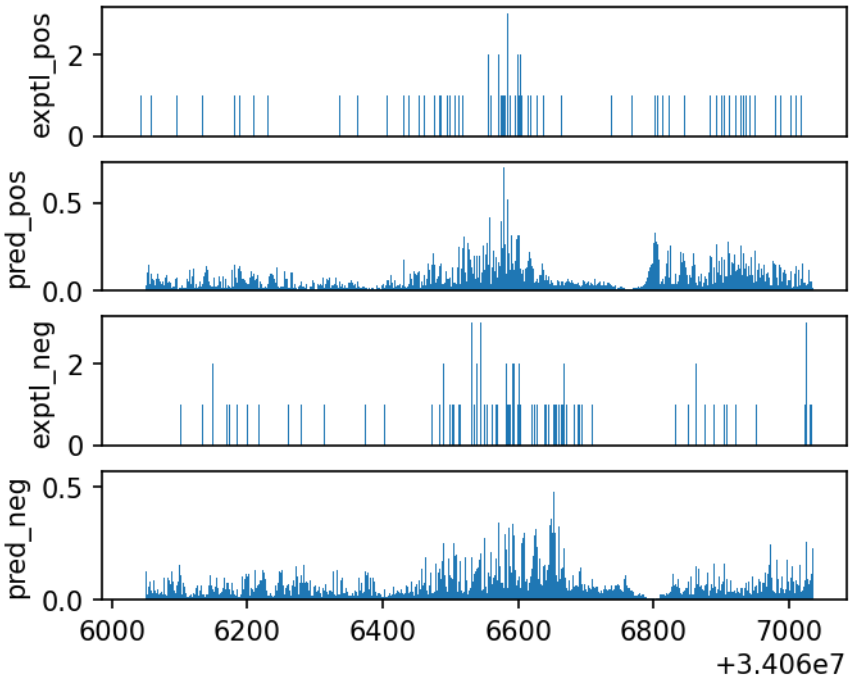
[37]:
```
def plotTfBigwigs(tfName, exptName, startPos = 34066036, span=1000,␣
 ↪chrom="chr1"):
    plotBws([WORKING_DIRECTORY + "/data/" + tfName + ".pos.bw",
             WORKING_DIRECTORY + "/pred/" + tfName + "_" + exptName +␣
 ↪"_positive.bw",
             WORKING_DIRECTORY + "/data/" + tfName + ".neg.bw",
             WORKING_DIRECTORY + "/pred/" + tfName + "_" + exptName +␣
 ↪"_negative.bw"],
```

```
            ["exptl_pos", "pred_pos", "exptl_neg", "pred_neg"], chrom,␣
↪startPos, startPos+span)
```

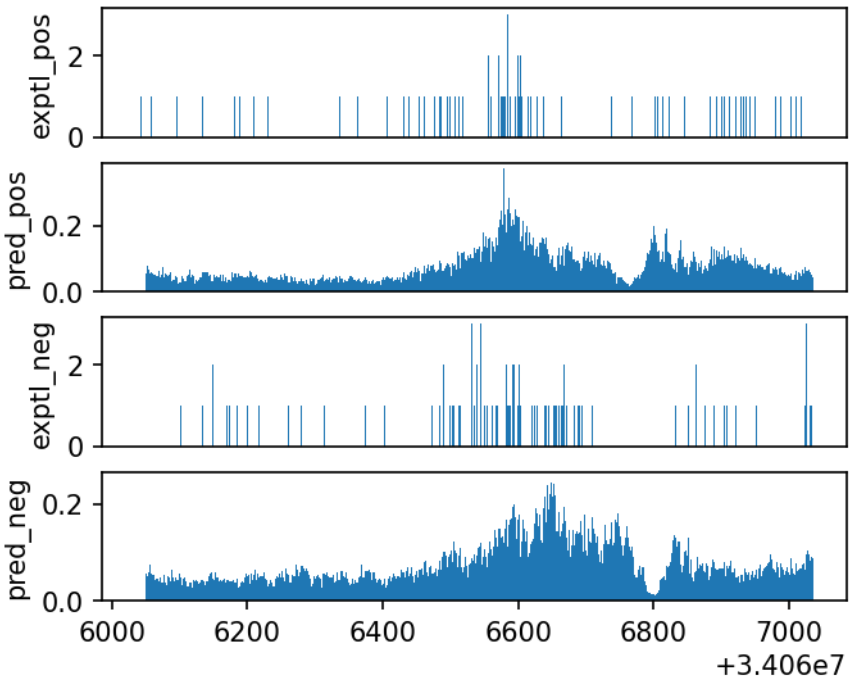
[38]:
```
plotTfBigwigs('oct4', 'residual', startPos = 35504040, span=40, chrom="chr17")
```



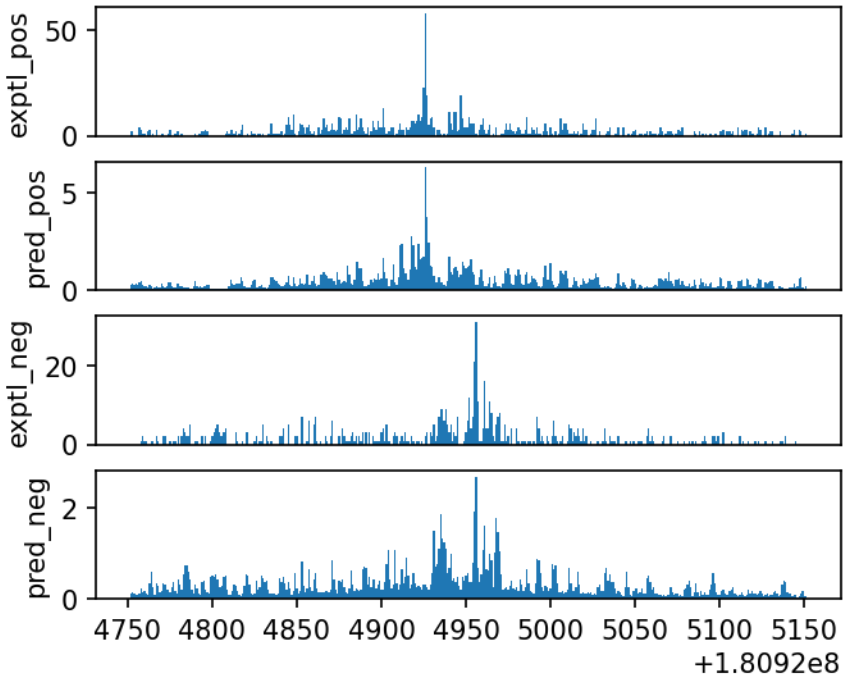
[39]:
```
plotTfBigwigs('oct4', 'combined')
```



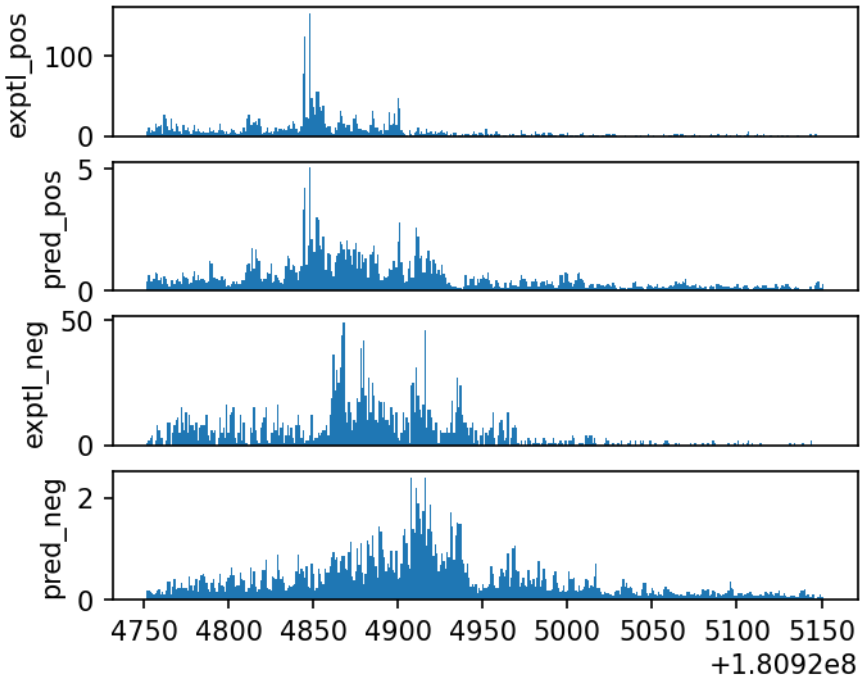
[40]:
```
plotTfBigwigs('oct4', 'residual')
```



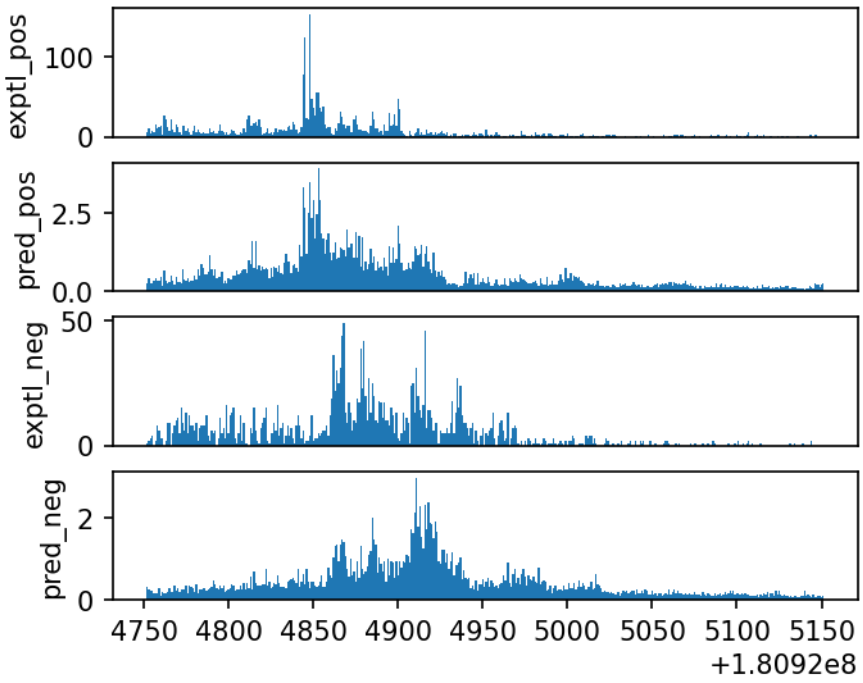
[41]:
```
#Let's look around our favorite Lefty1 enhancer...
plotTfBigwigs('oct4', 'combined', startPos = 180924752, span=400)
```



[42]:
```
plotTfBigwigs('nanog', 'combined', startPos = 180924752, span=400)
```



[43]:
```
plotTfBigwigs('nanog', 'residual', startPos = 180924752, span=400)
```



6 Deriving flat importance scores

[44]:
```
#Importance scores are needed to run motif discovery, but they're also a great␣
 ↪way to analyze what the model learned.
#Unfortunately for us, they take a while to generate.
def makeInterpretJson(tfNum):
    return {
        "genome" : GENOME_FASTA,
        "bed-file" : WORKING_DIRECTORY + "/bed/peak_all.bed",
        "model-file" : WORKING_DIRECTORY + "/models/joint_residual.model",
        "input-length" : INPUT_LENGTH,
        "output-length" : OUTPUT_LENGTH,
        "heads" : 4,
        "head-id": tfNum,
        "profile-task-ids" : [0,1],
        "profile-h5" : WORKING_DIRECTORY + "/shap/" + TF_NAMES[tfNum] +␣
 ↪"_profile.h5",
        "counts-h5" : WORKING_DIRECTORY + "/shap/" + TF_NAMES[tfNum] + "_counts.
 ↪h5",
```

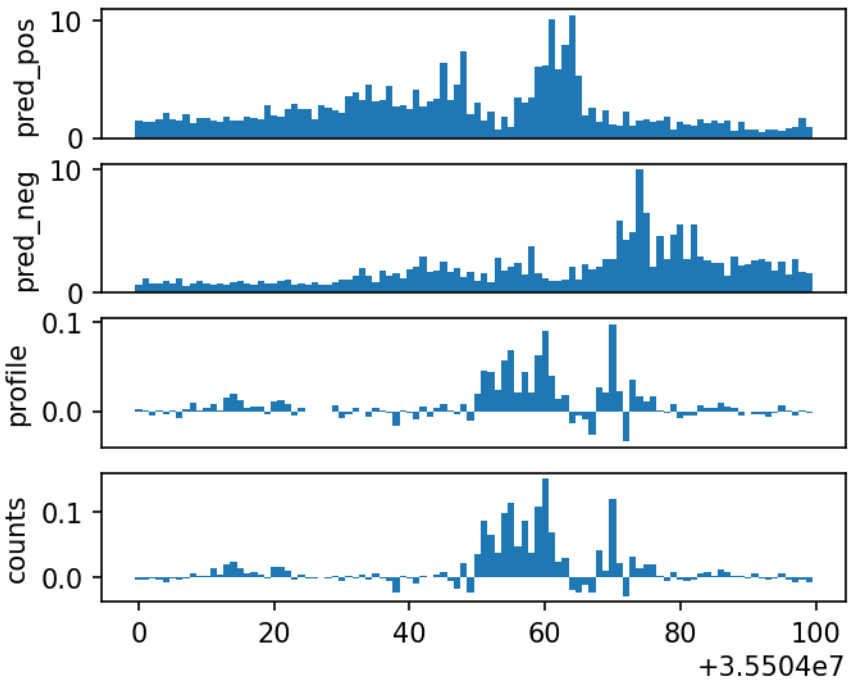
```
        "num-shuffles" : 20,
        "verbosity" : "DEBUG"}
cmds = []
for tfNum in range(len(TF_NAMES)):
    fname = WORKING_DIRECTORY + "/json/shap_" + TF_NAMES[tfNum] + ".json"
    cmds.append("interpretFlat.py {0:s}".format(fname))
    with open(fname, "w") as fp:
        json.dump(makeInterpretJson(tfNum), fp)
jobsGpu(cmds,
        "interpretFlat", 5, 50, "10:00:00")
```

[45]:
```
shapBwCmdBase = "shapToBigwig.py " +\
          "--h5 {wd:s}/shap/{tf:s}_{readout:s}.h5 " +\
          "--bw {wd:s}/shap/{tf:s}_{readout:s}.bw "+\
          "--verbose"
shapBwCmds = []
for tfname in TF_NAMES:
    for readout in ["profile", "counts"]:
        cmd = shapBwCmdBase.format(wd=WORKING_DIRECTORY,
                                   tf=tfname,
                                   readout=readout)
        shapBwCmds.append(cmd)

jobsNonGpu(shapBwCmds,
           "shapToBigwig", 2, 20, "1:00:00")
```

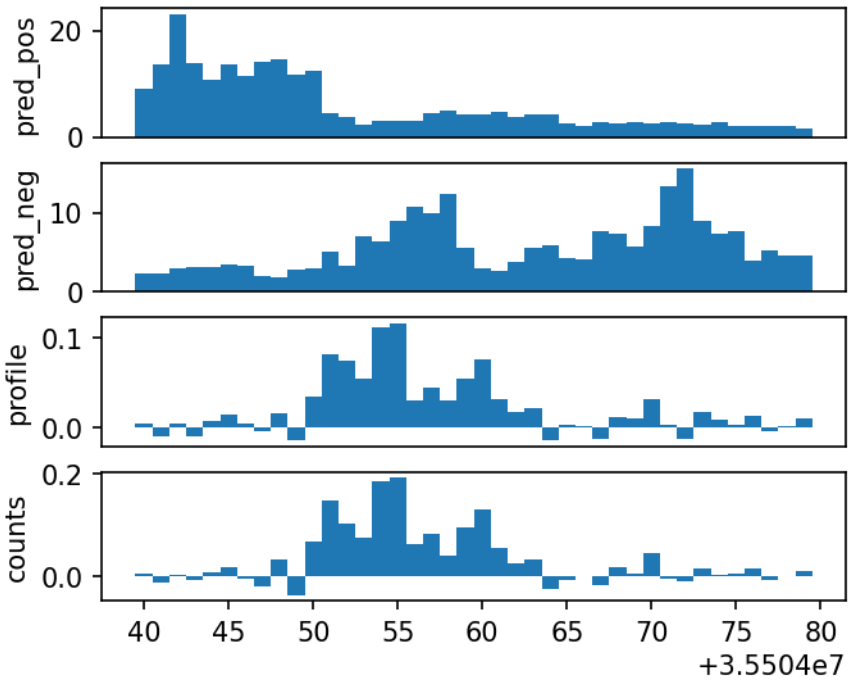
[ ]:
```

```

[46]:
```
def plotShapBigwigs(tfName, exptName, startPos = 34066036, span=1000,␣
 ↪chrom="chr1"):
    plotBws([WORKING_DIRECTORY + "/pred/" + tfName + "_" + exptName +␣
 ↪"_positive.bw",
             WORKING_DIRECTORY + "/pred/" + tfName + "_" + exptName +␣
 ↪"_negative.bw",
             WORKING_DIRECTORY + "/shap/" + tfName + "_profile.bw",
             WORKING_DIRECTORY + "/shap/" + tfName + "_counts.bw"],
            ["pred_pos", "pred_neg", "profile", "counts"], chrom, startPos,␣
 ↪startPos+span)
```

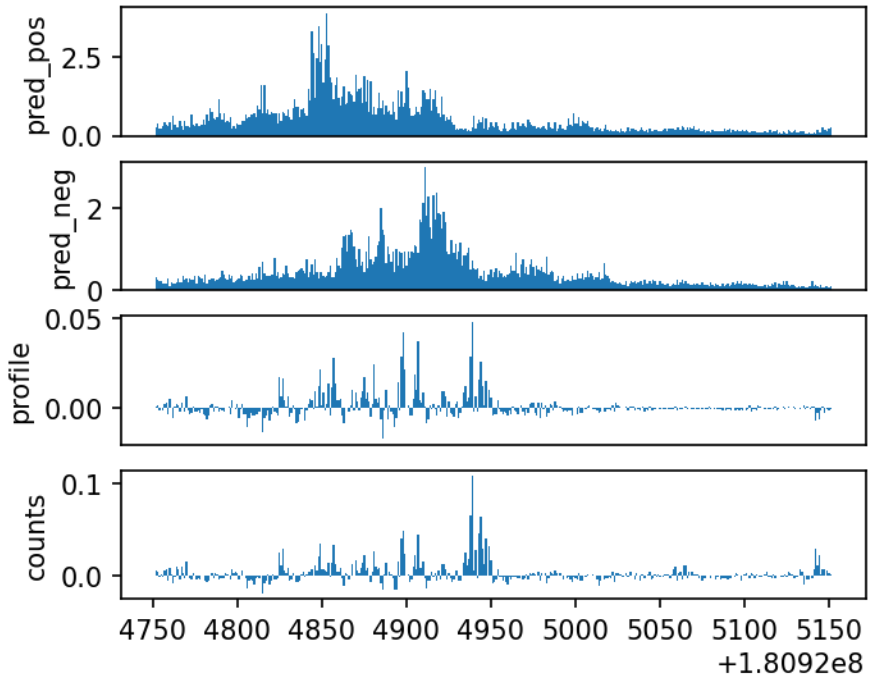
[47]:
```
plotShapBigwigs("nanog", "residual", startPos = 35504000, span=100,␣
 ↪chrom="chr17")
```



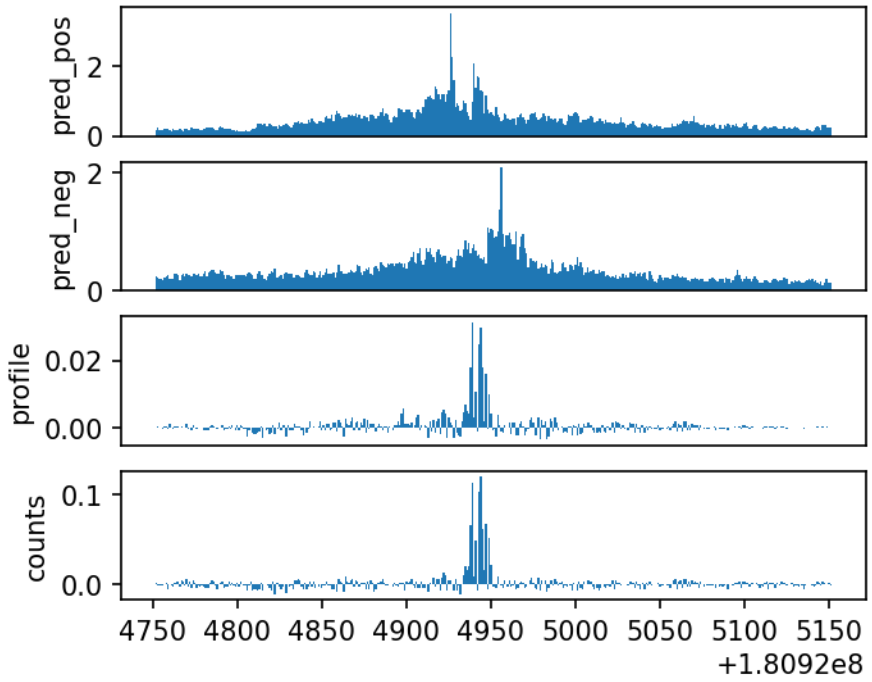
[48]:
```
plotShapBigwigs('oct4', 'residual', startPos = 35504040, span=40, chrom="chr17")
```



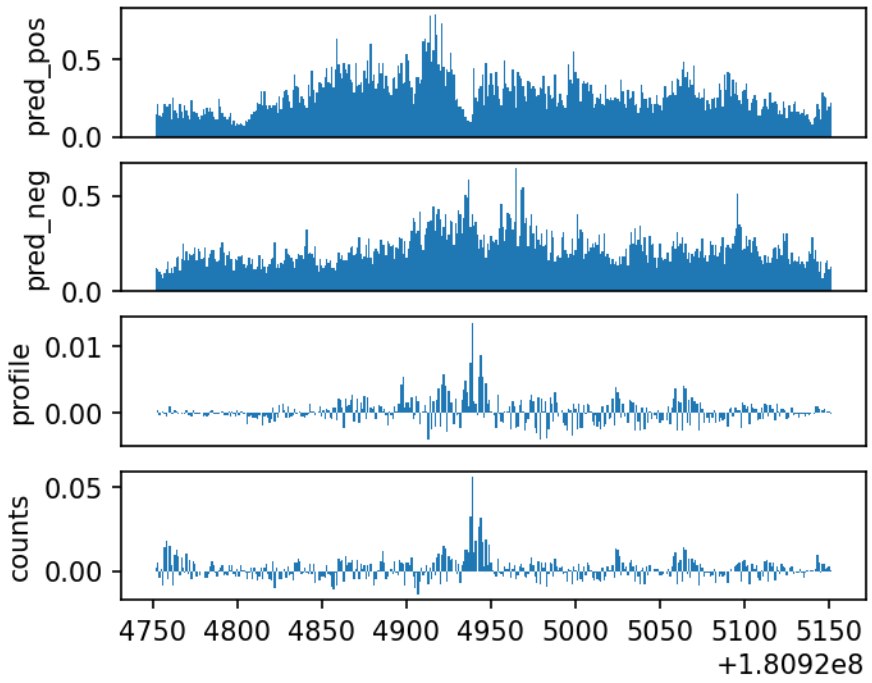
[49]:
```
plotShapBigwigs('nanog', 'residual', startPos = 180924752, span=400)
```



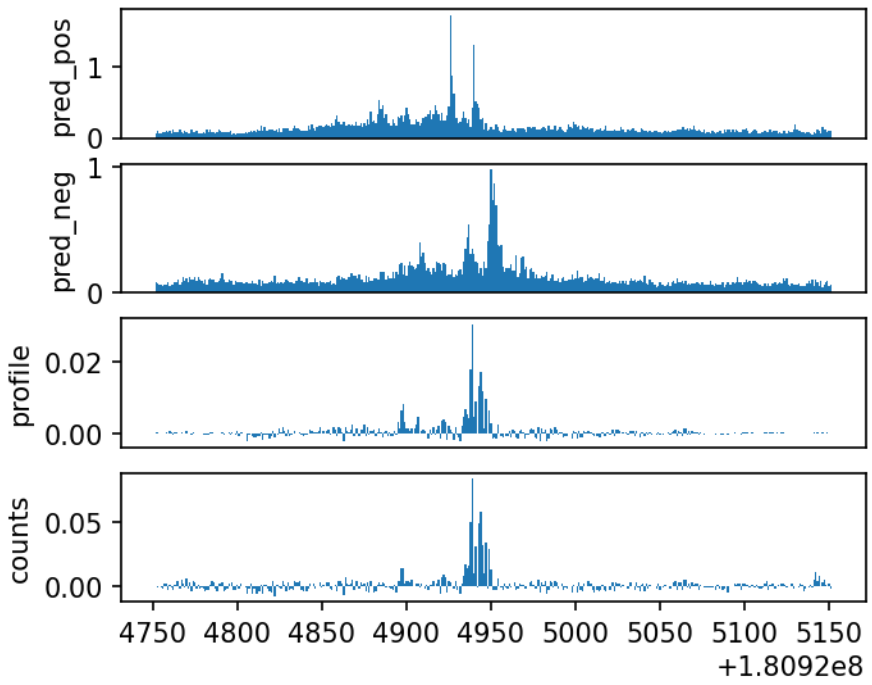
[50]:
```
plotShapBigwigs('oct4', 'residual', startPos = 180924752, span=400)
```



[51]:
```
plotShapBigwigs('klf4', 'residual', startPos = 180924752, span=400)
```

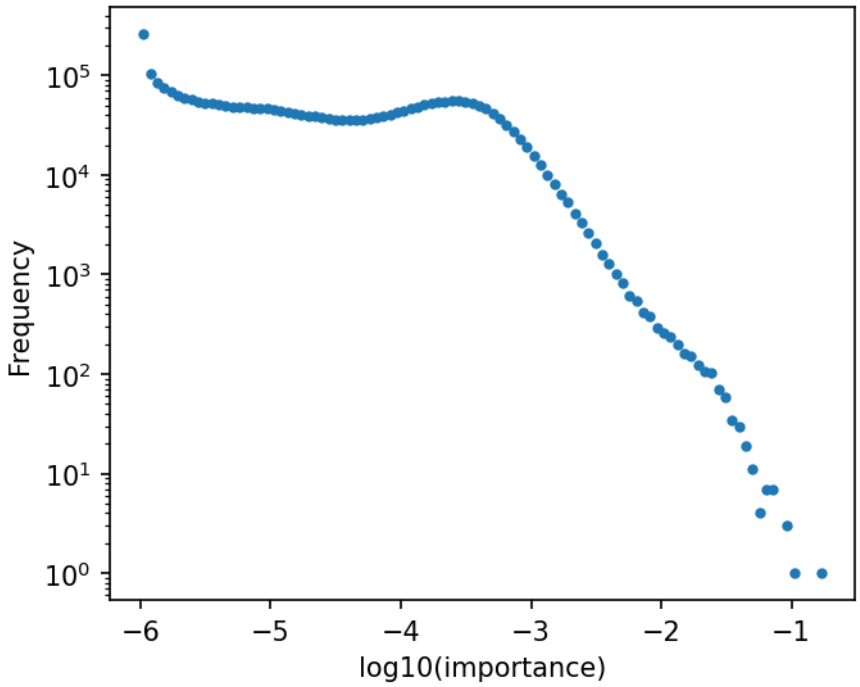


[52]:
```
plotShapBigwigs('sox2', 'residual', startPos = 180924752, span=400)
```



[53]:
```
#I'd like to know the distribution of importance scores.
def plotHistogram(bwName, chrom, start, stop):
    fp = pyBigWig.open(bwName, "r")
    dats = np.array(fp.values(chrom, start, stop))
    fp.close()
    validDats = dats[~np.isnan(dats)]
    validDats = np.abs(validDats)
    validDats = np.log10(validDats+1e-6)
    hist,edges = np.histogram(validDats, bins=100)
    plt.semilogy((edges[:-1] + edges[1:]) / 2, hist, '.')
    plt.xlabel("log10(importance)")
    plt.ylabel("Frequency")
```

[54]:
```
plotHistogram(WORKING_DIRECTORY + "/shap/oct4_profile.bw", "chr1", 10000000,␣
↪50000000)
```



[ ]:

[ ]:

[55]:
```
#Great, so we have those bigwigs and the importance hdf5. I can run Modisco now!
#The first thing to do is to export the shap scores as numpy arrays, then I run Modisco proper,
#and finally I generate reports.

shapToNumpyCmdBase = "shapToNumpy.py " +\
          "--h5 {wd:s}/shap/{tf:s}_{readout:s}.h5 " +\
          "--seqs {wd:s}/shap/seqs_{tf:s}_{readout:s}.npy "+\
          "--scores {wd:s}/shap/scores_{tf:s}_{readout:s}.npy "+\
          "--verbose"
shapToNumpyCmds = []
for tfname in TF_NAMES:
    for readout in ["profile", "counts"]:
        cmd = shapToNumpyCmdBase.format(wd=WORKING_DIRECTORY,
                                        tf=tfname,
                                        readout=readout)
        shapToNumpyCmds.append(cmd)

jobsNonGpu(shapToNumpyCmds,
```

```
            "shapToNumpy", 2, 20, "1:00:00")
```

[56]:
```
modiscoCmdBase = "mkdir -p {wd:s}/modisco/{tf:s}_{readout:s}\n" +\
          "modisco motifs " +\
              "-s {wd:s}/shap/seqs_{tf:s}_{readout:s}.npy " +\
              "-a {wd:s}/shap/scores_{tf:s}_{readout:s}.npy "+\
              "-n 50000 " +\
              "-w 1000 "+\
              "-v " +\
              "-o {wd:s}/modisco/{tf:s}_{readout:s}/modisco.h5 "
modiscoCmds = []
for tfname in TF_NAMES:
    for readout in ["profile", "counts"]:
        cmd = modiscoCmdBase.format(wd=WORKING_DIRECTORY,
                                    tf=tfname,
                                    readout=readout)
        modiscoCmds.append(cmd)

jobsNonGpu(modiscoCmds,
           "modisco", 70, 200, "5:00:00")
```

[65]:
```
reportCmdBase = "modisco report " +\
              "-i {wd:s}/modisco/{tf:s}_{readout:s}/modisco.h5 " +\
              "-o {wd:s}/modisco/{tf:s}_{readout:s}/ "+\
              "-n 2 " +\
              "-m /n/data1/JASPAR/2022/
 ↪JASPAR2022_CORE_vertebrates_non-redundant_pfms_meme.txt "

reportCmds = []
for tfname in TF_NAMES:
    for readout in ["profile", "counts"]:
        cmd = reportCmdBase.format(wd=WORKING_DIRECTORY,
                                    tf=tfname,
                                    readout=readout)
        reportCmds.append(cmd)

jobsNonGpu(reportCmds,
           "modiscoReport", 10, 20, "1:00:00")
```

[ ]:
```
```

[69]:
```
#Now let's map hits. The first thing to do is to look at the modisco results␣
 ↪and see what motifs I
#want to scan for.
# I'm going to annotate the modisco report htmls to add some meme information␣
 ↪about each matched motif.
```

```
# This tool is NOT part of bpreveal, since it's really a feature for modisco␣
 ↪and it hard-codes meme databases and stuff.
renameCmdBase = "{wd:s}/modisco/annotateModiscoHtml {wd:s}/modisco/{tf:
 ↪s}_{readout:s}/motifs.html " +\
        "> {wd:s}/modisco/{tf:s}_{readout:s}/motifs_annot.html"
for tfname in TF_NAMES:
    for readout in ["profile", "counts"]:
        cmd = renameCmdBase.format(wd=WORKING_DIRECTORY,
                                   tf=tfname,
                                   readout=readout)
        ! {cmd}
```

[71]:
```
# Now comes a rather manual step - looking over the modisco reports and finding␣
 ↪the motifs
# I want to scan for. I've selected the patterns that look interesting to me,␣
 ↪and then I'll use my
# cwm scanning tool to map those patterns back to the genome.
patternsToScan = {
    "klf4_counts" : {
        "pos" : [
            [0, "klf4"],
            [1, "octsox"],
            [2, "esrrb"],
            [3, "sox2"],
            [4, "zic3"],
            [6, "octsox"]]},
    "klf4_profile" : {
        "pos" : [
            [0, "klf4"],
            [1, "klf4klf4"],
            [2, "octsox"],
            [3, "sox2"],
            [4, "esrrb"],
            [7, "octsox"]]},
    "nanog_counts" : {
        "pos" : [
            [0, "sox2"],
            [1, "nanog"],
            [2, "octsox"],
            [4, "esrrb"],
            [7, "klf4"],
            [8, "zic3"]]},
    "nanog_profile" : {
        "pos" : [
            [0, "nanog"],
            [1, "sox2"],
            [2, "octsox"],
```

```
            [4, "klf4"]]},
    "oct4_counts" : {
        "pos" : [
            [0, "octsox"],
            [2, "sox2"],
            [3, "esrrb"],
            [4, "octsox"],
            [5, "zic3"],
            [7, "elk4"]]},
    "oct4_profile" : {
        "pos" : [
            [0, "octsox"],
            [2, "sox2"],
            [3, "klf4"],
            [4, "octsox"],
            [7, "esrrb"]]},
    "sox2_counts" : {
        "pos" : [
            [0, "sox2"],
            [1, "octsox"],
            [2, "octsox"],
            [3, "esrrb"],
            [4, "klf4"]]},
    "sox2_profile" : {
        "pos" : [
            [0, "sox2"],
            [1, "octsox"]]}}
```

[85]:
```
bgProbs = [(1-0.42) /2, 0.21, 0.21, (1-0.42) /2]
cmds = []
SCAN_BASE = SRC_DIR + "/motifScan.py {fname:s} \n    " +\
            SRC_DIR + "/motifAddQuantiles.py --seqlet-tsv {seqletTsv:s}␣
 ↪--scan-tsv {scanTsv:s}\n" +\
            "    cat {scanTsv:s} | " + \
                "cut -f 1-6 | " + \
                "tail -n +2 | " + \
                "sort -k1,1 -k2,2n -k3,3n -k4,4 -k5,5nr | "+\
                "awk '!_[$1,$2,$3,$4,$6]++' > {scanBed:s}"
#This is a three-part command. First, we scan for the motifs.
#Then, we use the motifAddQuantiles.py script to add quantile information,␣
 ↪which can be useful for
#analyzing the motifs.
#The third part, starting with `cat {scanTsv:s}` is scary, but it's just␣
 ↪extracting a bed file from
#the generated tsv files. The sort and awk lines are just there to remove␣
 ↪duplicate maps, where
```

```
#a motif is mapped to the same region several times. If you want all of the␣
 ↪called motifs,
#or don't want to deal with awk, you can remove the sort and awk parts of that␣
 ↪command.

for pat in patternsToScan.keys():
    curPats = patternsToScan[pat]
    patternSpec = []
    for mcName in curPats.keys():
        patternSpec.append({
            "metacluster-name" : mcName + "_patterns",
            "pattern-names" : ["pattern_{0:d}".format(x[0]) for x in␣
 ↪curPats[mcName]],
            "short-names" : [x[1] for x in curPats[mcName]]})
    seqletTsv =  WORKING_DIRECTORY + "/modisco/" + pat + "/seqlets_" + pat + ".
 ↪tsv"
    hitsTsv = WORKING_DIRECTORY + "/scan/" + pat + ".tsv"
    hitsBed = WORKING_DIRECTORY + "/scan/" + pat + ".bed"
    configDict = {
        "scan-settings" : {
            "scan-contrib-h5" : WORKING_DIRECTORY + "/shap/" + pat + ".h5",
            "hits-tsv" : hitsTsv,
            "num-threads" : 70},
        "seqlet-cutoff-settings" : {
            "seqlets-tsv" : seqletTsv,
            "modisco-h5" : WORKING_DIRECTORY + "/modisco/" + pat + "/modisco.
 ↪h5",
            "modisco-contrib-h5" : WORKING_DIRECTORY + "/shap/" + pat + ".h5",
            "patterns" : patternSpec,
            "seq-match-quantile" : 0.1,
            "contrib-match-quantile" : 0.2,
            "contrib-magnitude-quantile" : 0.1,
            "trim-threshold" : 0.3,
            "trim-padding" : 4,
            "background-probs" : bgProbs},
        "verbosity" : "INFO"}
    fname = WORKING_DIRECTORY + "/json/scan_" + pat + ".json"
    cmdStr = SCAN_BASE.format(fname = fname, seqletTsv = seqletTsv, scanTsv =␣
 ↪hitsTsv, scanBed = hitsBed)
    cmds.append(cmdStr)
    with open(fname, "w") as fp:
        json.dump(configDict, fp, indent=4)
jobsNonGpu(cmds, "motifScan", 70, 10, "10:00:00")
```

[ ]:
```
# We can now load up the generated bed files and see where our motifs are!
```

7 Making a PISA plot

[78]:
```
#In order to make a pisa plot, I need to get a list of regions I want to␣
 ↪analyze. The way the PISA script works is that I give it a fasta-format file
#of genomic regions, each region being INPUT_LENGTH long. The PISA tool will␣
 ↪then assign contributions to all of the bases in the input relative to the
#*leftmost* base in the output.
#This is important, so let me phrase it differently:



#|<- Receptive field ->|
#INPUTSEQUENCEINPUTSEQUENCEINPUTSEQUENCEINPUTSEQUENCE
#\                    /                          /
#  \                /                          /
#    \            /                          /
#      \         /                          /
#        \     /                          /
#          \ /                          /
#           OUTPUTPROFILEOUTPUTPROFILEOUTP
#           ^
#           | This O is the base that will be used to calculate the␣
 ↪contribution scores.
#It's important to not have any off-by-one problems here, so let's work it out␣
 ↪manually.
print(INPUT_LENGTH)
print(RECEPTIVE_FIELD)
```

```
3092
2093
```

[79]:
```
#Since I don't feel like doing ascii art that's quite so wide, I'm going to say␣
 ↪that the network is quite a bit smaller:
!{SRC_DIR}/lengthCalc.py --output-len 20 --n-dil-layers 3 --conv1-kernel-size 3␣
 ↪--profile-kernel-size 3
```

```
52
```

[ ]:
```
#So in this example the receptive field would be 52-20+1=33.
#And there are 16 bases of slop on each side that need to be seen by the model.
#-30     -20       -10         0        10        20        30        40        ␣
 ↪50
#V         V         V         V         V         V         V         V        ␣
 ↪ V
#09876543210987654321098765432101234567890123456789012345678901234567890123456789
#Output:                      01234567890123456789
#Input:        65432109876543210123456789012345678901234567890123456789012345
#Receptive:    654321098765432101234567890123456
```

```
#So in this case, if we want shap scores for a base at position zero, we need␣
 ↪sequence from -16 to +35 (inclusive)
```

[ ]:

[80]:
```
windowStart = 180924752
```

[81]:
```
#So I need to get windows that are 3092 bases wide, and the first 2093 bases␣
 ↪are the only ones that have a chance of affecting the output
#(since that's the receptive field for the first base.)
#The slop is (2093-1)/2 = 1046
#I want to shap starting at chr1:180924752 and I want to take 400 bases worth␣
 ↪of calculations.
def writeRegion(genome, outFp, regionStart):
    genomeStart = regionStart - 1046
    genomeEnd = genomeStart + INPUT_LENGTH
    seq = genome.fetch("chr1", genomeStart, genomeEnd)
    outFp.write(">{0:d}\n".format(regionStart))
    outFp.write(seq.upper())
    outFp.write("\n")

with open(WORKING_DIRECTORY + "/shap/pisa_regions.fa", "w") as fp:
    with pysam.FastaFile(GENOME_FASTA) as genome:
        for regionStart in range(windowStart, windowStart + 400):
            writeRegion(genome, fp, regionStart)
```

[84]:
```
#And now we bulid the json file for the PISA analysis.
for tfid in [0,3]:
    for strand in [0,1]:
        task_name = TF_NAMES[tfid] + "_" + ["positive", "negative"][strand]
        pisa_config = {"model-file" : WORKING_DIRECTORY + "/models/
 ↪joint_residual.model",
                       "sequence-fasta" : WORKING_DIRECTORY + "/shap/
 ↪pisa_regions.fa",
                       "num-shuffles" : 20,
                       "head-id" : tfid, #(That's the nanog head)
                       "task-id" : strand,
                       "output-h5" : WORKING_DIRECTORY + "/shap/pisa_" +␣
 ↪task_name + ".h5",
                       "input-length" : INPUT_LENGTH,
                       "output-length" : OUTPUT_LENGTH,
                       "make-predictions" : True,
                       "verbosity" : "WARNING"}
```

```
      with open(WORKING_DIRECTORY + "/json/pisa_" + task_name + ".json", "w")␣
↪as fp:
          json.dump(pisa_config, fp)
      !{SRC_DIR}/interpretPisaFasta.py {WORKING_DIRECTORY}/json/
↪pisa_{task_name}.json
```

```
  0%|                                                    | 0/400 [00:00<?,
?it/s]WARNING:tensorflow:From /scratch/bpreveal-teak/lib/python3.10/site-
packages/tensorflow/python/util/deprecation.py:561: calling function (from
tensorflow.python.eager.polymorphic_function.polymorphic_function) with
experimental_relax_shapes is deprecated and will be removed in a future version.
Instructions for updating:
experimental_relax_shapes is deprecated, use reduce_retracing instead
2023-06-21 16:41:44.844777: W tensorflow/c/c_api.cc:291] Operation
'{name:'AssignVariableOp_7' id:463 op device:{requested: '/device:CPU:0',
assigned: ''} def:{{{node AssignVariableOp_7}} =
AssignVariableOp[_has_manual_control_dependencies=true, dtype=DT_FLOAT,
validate_shape=false, _device="/device:CPU:0"](total_3, Identity_7)}}' was
changed by setting attribute after it was run by a session. This mutation will
have no effect, and will trigger an error in the future. Either don't modify
nodes after running them or create a new session.
WARNING:tensorflow:From /n/projects/cm2363/bpreveal/src/shap.py:147: The name
tf.keras.backend.get_session is deprecated. Please use
tf.compat.v1.keras.backend.get_session instead.

/scratch/bpreveal-teak/lib/python3.10/site-
packages/keras/engine/training_v1.py:2357: UserWarning: `Model.state_updates`
will be removed in a future version. This property should not be used in
TensorFlow 2.0, as `updates` are applied automatically.
  updates=self.state_updates,
2023-06-21 16:41:45.003989: W tensorflow/c/c_api.cc:291] Operation
'{name:'solo_profile_combined_oct4/BiasAdd' id:820 op device:{requested: '',
assigned: ''} def:{{{node solo_profile_combined_oct4/BiasAdd}} =
BiasAdd[T=DT_FLOAT, _has_manual_control_dependencies=true,
data_format="NHWC"](solo_profile_combined_oct4/Conv1D/Squeeze,
solo_profile_combined_oct4/BiasAdd/ReadVariableOp)}}' was changed by setting
attribute after it was run by a session. This mutation will have no effect, and
will trigger an error in the future. Either don't modify nodes after running
them or create a new session.
100%|                    | 400/400 [00:16<00:00, 23.77it/s]
  0%|                                                    | 0/400 [00:00<?,
?it/s]WARNING:tensorflow:From /scratch/bpreveal-teak/lib/python3.10/site-
packages/tensorflow/python/util/deprecation.py:561: calling function (from
tensorflow.python.eager.polymorphic_function.polymorphic_function) with
experimental_relax_shapes is deprecated and will be removed in a future version.
Instructions for updating:
experimental_relax_shapes is deprecated, use reduce_retracing instead
2023-06-21 16:42:01.974539: W tensorflow/c/c_api.cc:291] Operation
```

```
'{name:'AssignVariableOp_16' id:481 op device:{requested: '/device:CPU:0',
assigned: ''} def:{{{node AssignVariableOp_16}} =
AssignVariableOp[_has_manual_control_dependencies=true, dtype=DT_FLOAT,
validate_shape=false, _device="/device:CPU:0"](count_8, Identity_16)}}' was
changed by setting attribute after it was run by a session. This mutation will
have no effect, and will trigger an error in the future. Either don't modify
nodes after running them or create a new session.
WARNING:tensorflow:From /n/projects/cm2363/bpreveal/src/shap.py:147: The name
tf.keras.backend.get_session is deprecated. Please use
tf.compat.v1.keras.backend.get_session instead.

/scratch/bpreveal-teak/lib/python3.10/site-
packages/keras/engine/training_v1.py:2357: UserWarning: `Model.state_updates`
will be removed in a future version. This property should not be used in
TensorFlow 2.0, as `updates` are applied automatically.
  updates=self.state_updates,
2023-06-21 16:42:02.134817: W tensorflow/c/c_api.cc:291] Operation
'{name:'solo_profile_combined_oct4/BiasAdd' id:820 op device:{requested: '',
assigned: ''} def:{{{node solo_profile_combined_oct4/BiasAdd}} =
BiasAdd[T=DT_FLOAT, _has_manual_control_dependencies=true,
data_format="NHWC"](solo_profile_combined_oct4/Conv1D/Squeeze,
solo_profile_combined_oct4/BiasAdd/ReadVariableOp)}}' was changed by setting
attribute after it was run by a session. This mutation will have no effect, and
will trigger an error in the future. Either don't modify nodes after running
them or create a new session.
100%|                      | 400/400 [00:17<00:00, 23.48it/s]
  0%|                                                        | 0/400 [00:00<?,
?it/s]WARNING:tensorflow:From /scratch/bpreveal-teak/lib/python3.10/site-
packages/tensorflow/python/util/deprecation.py:561: calling function (from
tensorflow.python.eager.polymorphic_function.polymorphic_function) with
experimental_relax_shapes is deprecated and will be removed in a future version.
Instructions for updating:
experimental_relax_shapes is deprecated, use reduce_retracing instead
2023-06-21 16:42:19.328994: W tensorflow/c/c_api.cc:291] Operation
'{name:'AssignVariableOp_8' id:465 op device:{requested: '/device:CPU:0',
assigned: ''} def:{{{node AssignVariableOp_8}} =
AssignVariableOp[_has_manual_control_dependencies=true, dtype=DT_FLOAT,
validate_shape=false, _device="/device:CPU:0"](count_4, Identity_8)}}' was
changed by setting attribute after it was run by a session. This mutation will
have no effect, and will trigger an error in the future. Either don't modify
nodes after running them or create a new session.
WARNING:tensorflow:From /n/projects/cm2363/bpreveal/src/shap.py:147: The name
tf.keras.backend.get_session is deprecated. Please use
tf.compat.v1.keras.backend.get_session instead.

/scratch/bpreveal-teak/lib/python3.10/site-
packages/keras/engine/training_v1.py:2357: UserWarning: `Model.state_updates`
will be removed in a future version. This property should not be used in
```

```
TensorFlow 2.0, as `updates` are applied automatically.
  updates=self.state_updates,
2023-06-21 16:42:19.485970: W tensorflow/c/c_api.cc:291] Operation
'{name:'solo_profile_combined_oct4/BiasAdd' id:820 op device:{requested: '',
assigned: ''} def:{{{node solo_profile_combined_oct4/BiasAdd}} =
BiasAdd[T=DT_FLOAT, _has_manual_control_dependencies=true,
data_format="NHWC"](solo_profile_combined_oct4/Conv1D/Squeeze,
solo_profile_combined_oct4/BiasAdd/ReadVariableOp)}}' was changed by setting
attribute after it was run by a session. This mutation will have no effect, and
will trigger an error in the future. Either don't modify nodes after running
them or create a new session.
100%|                      | 400/400 [00:16<00:00, 23.83it/s]
  0%|                                                        | 0/400 [00:00<?,
?it/s]WARNING:tensorflow:From /scratch/bpreveal-teak/lib/python3.10/site-
packages/tensorflow/python/util/deprecation.py:561: calling function (from
tensorflow.python.eager.polymorphic_function.polymorphic_function) with
experimental_relax_shapes is deprecated and will be removed in a future version.
Instructions for updating:
experimental_relax_shapes is deprecated, use reduce_retracing instead
2023-06-21 16:42:36.500236: W tensorflow/c/c_api.cc:291] Operation
'{name:'AssignVariableOp_16' id:481 op device:{requested: '/device:CPU:0',
assigned: ''} def:{{{node AssignVariableOp_16}} =
AssignVariableOp[_has_manual_control_dependencies=true, dtype=DT_FLOAT,
validate_shape=false, _device="/device:CPU:0"](count_8, Identity_16)}}' was
changed by setting attribute after it was run by a session. This mutation will
have no effect, and will trigger an error in the future. Either don't modify
nodes after running them or create a new session.
WARNING:tensorflow:From /n/projects/cm2363/bpreveal/src/shap.py:147: The name
tf.keras.backend.get_session is deprecated. Please use
tf.compat.v1.keras.backend.get_session instead.

/scratch/bpreveal-teak/lib/python3.10/site-
packages/keras/engine/training_v1.py:2357: UserWarning: `Model.state_updates`
will be removed in a future version. This property should not be used in
TensorFlow 2.0, as `updates` are applied automatically.
  updates=self.state_updates,
2023-06-21 16:42:36.663830: W tensorflow/c/c_api.cc:291] Operation
'{name:'solo_profile_combined_oct4/BiasAdd' id:820 op device:{requested: '',
assigned: ''} def:{{{node solo_profile_combined_oct4/BiasAdd}} =
BiasAdd[T=DT_FLOAT, _has_manual_control_dependencies=true,
data_format="NHWC"](solo_profile_combined_oct4/Conv1D/Squeeze,
solo_profile_combined_oct4/BiasAdd/ReadVariableOp)}}' was changed by setting
attribute after it was run by a session. This mutation will have no effect, and
will trigger an error in the future. Either don't modify nodes after running
them or create a new session.
100%|                      | 400/400 [00:16<00:00, 23.53it/s]
```

[ ]:

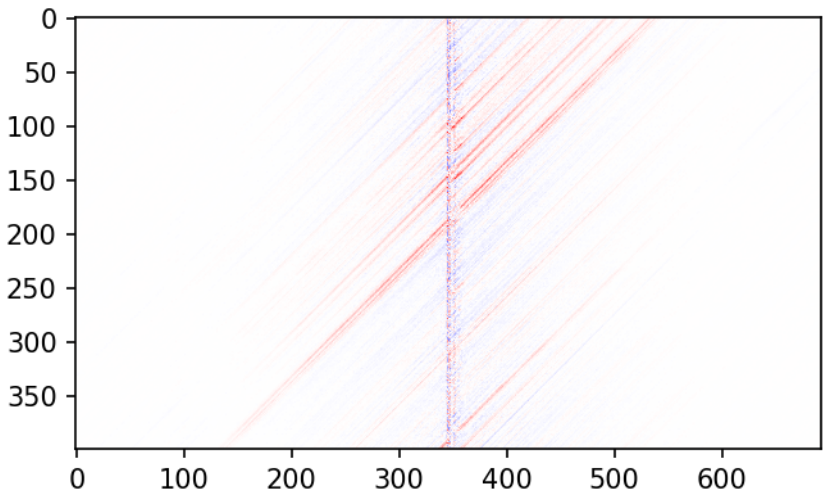
[87]:
```
#Let's take a look at the pisa results!
with h5py.File(WORKING_DIRECTORY + "/shap/pisa_nanog_positive.h5", "r") as fp:
    pisaDescriptions = list(fp["descriptions"])
    pisaSequences = np.array(fp["sequence"])
    pisaShap = np.array(fp["shap"])
    pisaInputPred = np.array(fp["input_predictions"])
    pisaInputPred = np.array(fp["shuffle_predictions"])
```

[88]:
```
pisaVals = np.sum(pisaShap,axis=2)
print(pisaVals.shape)
```

```
(400, 2092)
```

[89]:
```
pisaSpan = 0.4
plt.imshow(pisaVals[:,700:-700], vmin=-pisaSpan, vmax=pisaSpan, cmap='bwr')
```

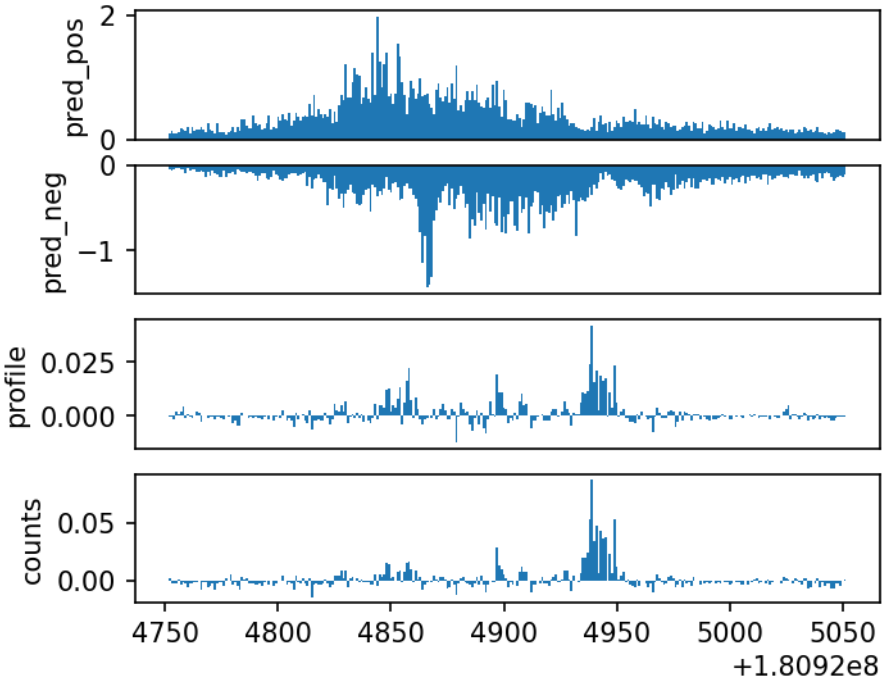
[89]: <matplotlib.image.AxesImage at 0x7f5942ccbe50>



[90]:
```
#The axes can be kind of hard to interpret on a pisa plot, so I'm going to␣
 ↪shear it real quick.
def plotPisa(taskName, cutMiddle, cutLengthX, cutLengthY, colorSpan=1.0):
    with h5py.File(WORKING_DIRECTORY + "/shap/pisa_" + taskName + ".h5", "r")␣
 ↪as fp:
        pisaShap = np.array(fp["shap"])
    pisaVals = np.sum(pisaShap,axis=2)
    numRegions = pisaVals.shape[0]
    shearMat = np.zeros((numRegions, pisaVals.shape[1] + numRegions))
    for i in range(0, numRegions):
```

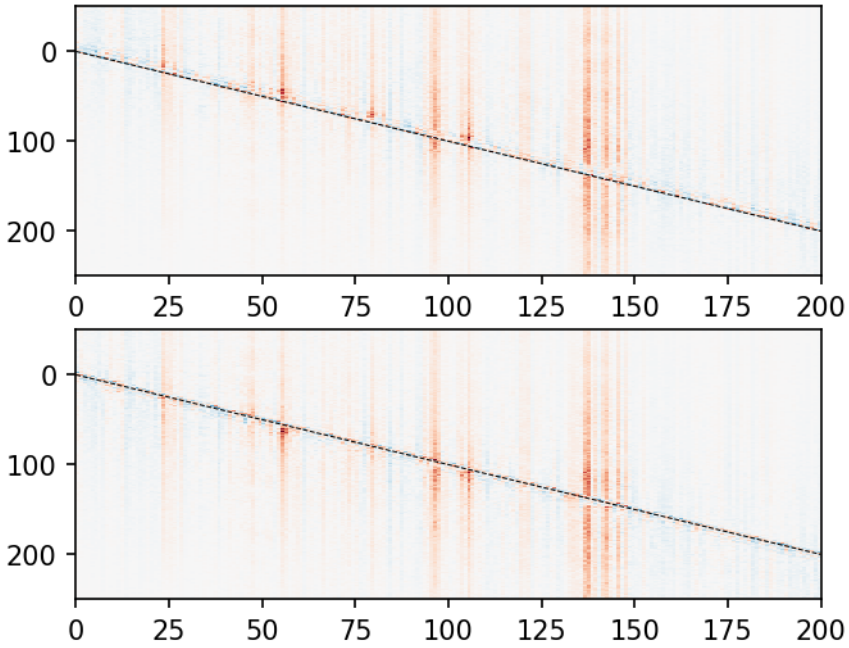
```
        offset = i
        shearMat[i, offset:offset+pisaVals.shape[1]] = pisaVals[i]
    print(shearMat.shape)
    cutStartX = cutMiddle-cutLengthX//2
    cutStartY = cutMiddle - cutLengthY//2
    plotMat = shearMat[cutStartY:cutStartY + cutLengthY,RECEPTIVE_FIELD//
↪2+cutStartX:RECEPTIVE_FIELD//2+cutStartX + cutLengthX]
    axStartY = (cutLengthX - cutLengthY)//2
    axStopY = axStartY + cutLengthY
    extent=[0, cutLengthX, axStopY, axStartY]
    plt.imshow(plotMat, vmin=-colorSpan, vmax=colorSpan, extent=extent,␣
↪cmap='RdBu_r', aspect='auto', interpolation='nearest')
    #And let's get the sequence for that:
    plt.plot([0,cutLengthX], [0,cutLengthX], 'k--', lw=0.5)
    if(cutLengthX < 40):
        with pysam.FastaFile(GENOME_FASTA) as genome:
            seq = genome.fetch("chr1", windowStart+cutStartX,␣
↪windowStart+cutStartX + cutLengthX)
            print(seq)
        plt.xticks(range(0,cutLengthX), labels=seq);
```

[138]:
```
#Let's remind ourselves of what the nanog binding looked like...
plotShapBigwigs('nanog', 'residual', startPos = windowStart, span=300)
```



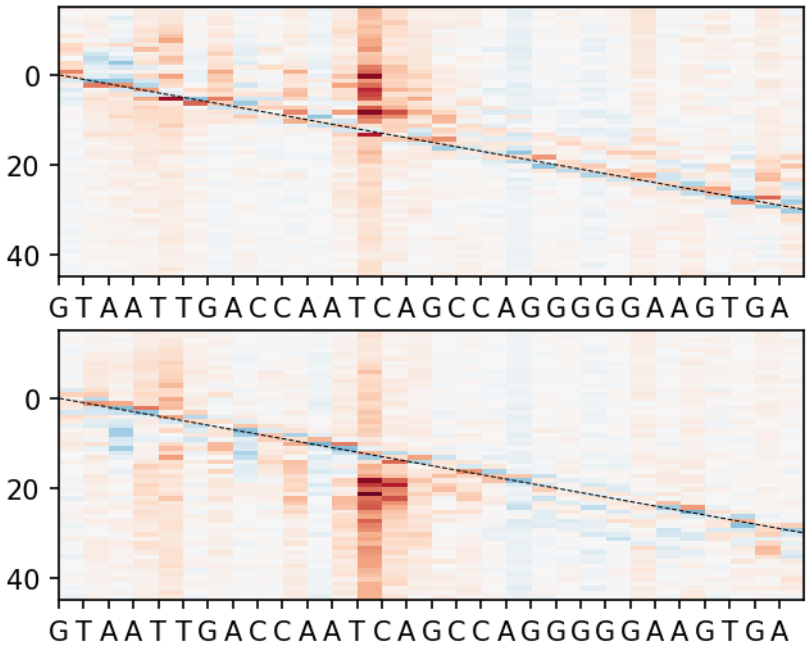
[91]:
```
plt.subplot(211)
plotPisa("nanog_positive", 150, 200, 300, colorSpan = 0.5)

plt.subplot(212)
plotPisa("nanog_negative", 150, 200, 300, colorSpan = 0.5)
```

```
(400, 2492)
(400, 2492)
```



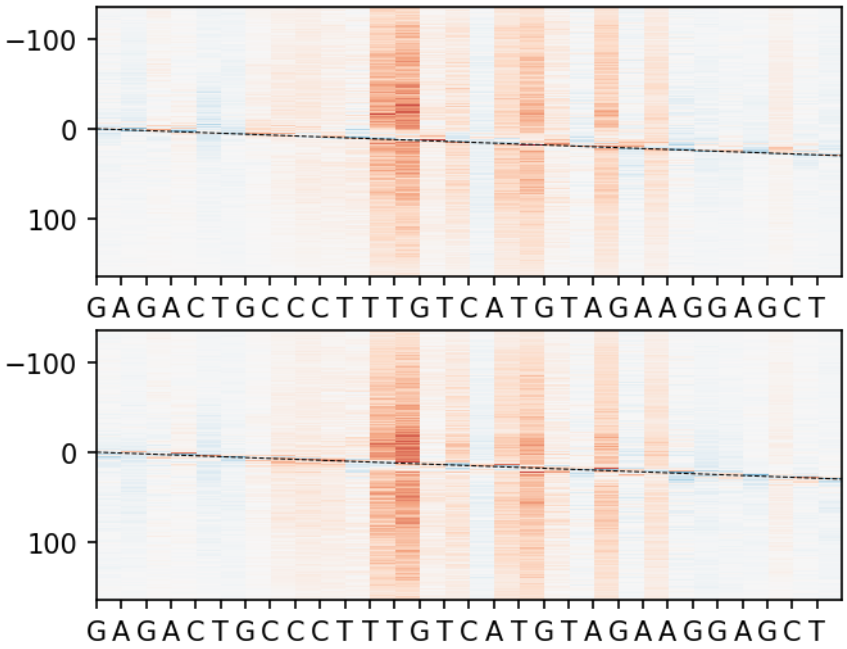
[92]:
```
#One thing is pretty striking. The motif at ~100 bp has a directional effect,␣
 ↪that is, the importance toward the positive peak is upstream of the
#motif and the importance of the negative peak is downstream. But the motif at␣
 ↪~190 doesn't seem to be directional, and it seems to have a larger
#reach. Let's zoom in!
plt.subplot(211)
plotPisa("nanog_positive", 108, 30, 60, colorSpan = 0.5)
plt.subplot(212)
plotPisa("nanog_negative", 108, 30, 60, colorSpan = 0.5)
```

```
(400, 2492)
GTAATTGACCAATCAGCCAGGGGGAAGTGA
(400, 2492)
GTAATTGACCAATCAGCCAGGGGGAAGTGA
```



[93]:
```
plt.subplot(211)
plotPisa("nanog_positive", 190, 30, 300, colorSpan = 0.5)
plt.subplot(212)
plotPisa("nanog_negative", 190, 30, 300, colorSpan = 0.5)
```

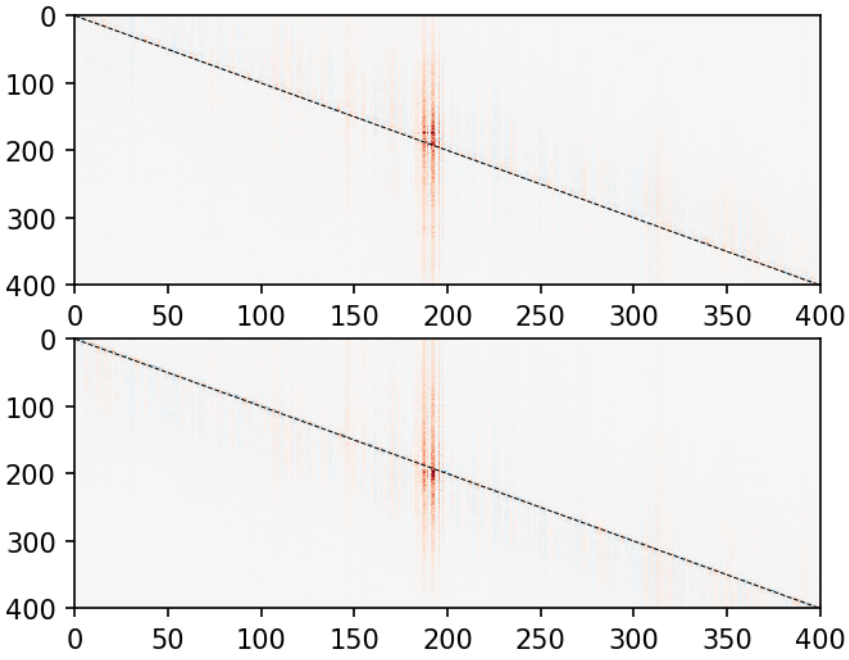
```
(400, 2492)
GAGACTGCCCTTTGTCATGTAGAAGGAGCT
(400, 2492)
GAGACTGCCCTTTGTCATGTAGAAGGAGCT
```



[ ]:
```
#Indeed, this motif looks very different!
```

[94]:
```
plt.subplot(211)
plotPisa("oct4_positive", 200, 400, 400, colorSpan = 0.5)
plt.subplot(212)
plotPisa("oct4_negative", 200, 400, 400, colorSpan = 0.5)
```

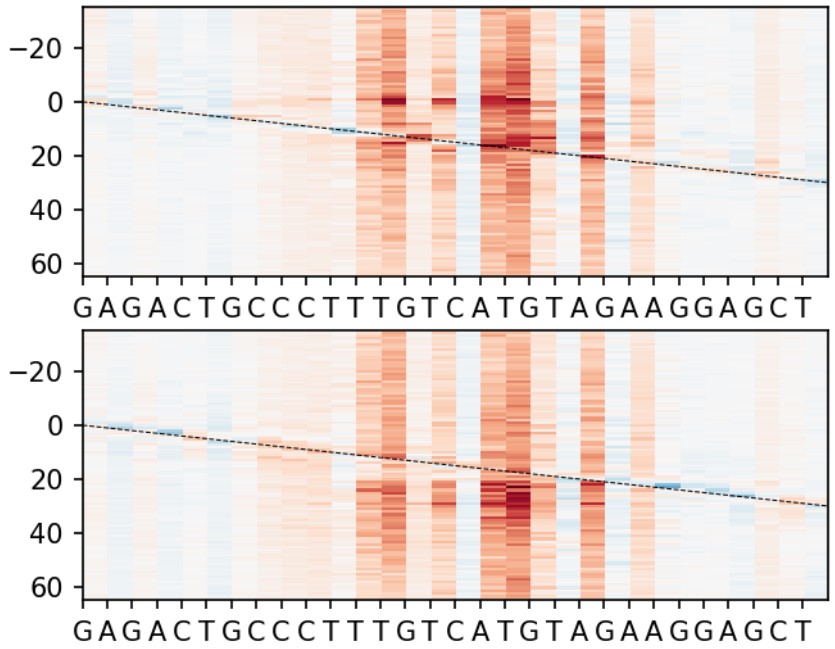
```
(400, 2492)
(400, 2492)
```



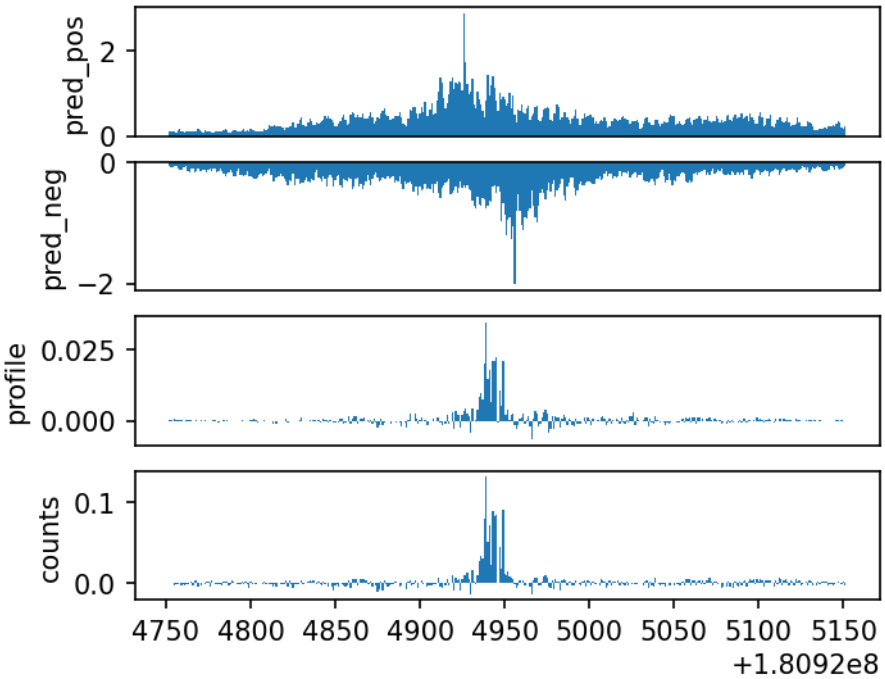
[ ]:
```
#Ah, that might explain why the motif acts differently - it's a motif for a␣
↪different protein altogether! Let's zoom in on the Oct4 motif.
```

[96]:
```
plt.subplot(211)
plotPisa("oct4_positive", 190, 30, 100, colorSpan = 0.5)
plt.subplot(212)
plotPisa("oct4_negative", 190, 30, 100, colorSpan = 0.5)
```

```
(400, 2492)
GAGACTGCCCTTTGTCATGTAGAAGGAGCT
(400, 2492)
GAGACTGCCCTTTGTCATGTAGAAGGAGCT
```



[144]:
```
#As a reminder, let me pull up the Oct4 tracks:
plotShapBigwigs('oct4', 'residual', startPos = 180924752, span=400)
```



[ ]:

[ ]:

[ ]:

[ ]:

[ ]: