Real world analyses with bedtools.

Applied Computational Genomics, Lecture 18

https://github.com/quinlan-lab/applied-computational-genomics

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Let’s use bedtools to answer some real(ish) research questions.

Q1. Which enhancers in human embryonic stem cells are under the most genetic "constraint" (that is, have the least genetic variation)?

Q1. Which enhancers in human embryonic stem cells are under the most genetic "constraint" (that is, have the least genetic variation)?

Experimental design: what materials do we need?

1. We need a set of predicted enhancers in hESCs. ENCODE?
2. We need a deep catalog of genetic variation in the human genome. 1000 genomes?
3. We then need to count the number of genetic variants observed in each enhancer.

Q1. Which enhancers in human embryonic stem cells are under the most genetic "constraint" (that is, have the least genetic variation)?

1. We need a set of predicted enhancers in hESCs. Let's focus on chr22 for simplicity.

```
$ wget https://s3.amazonaws.com/bedtools-tutorials/web/hesc.chromHmm.bed
$ grep "^chr22" hesc.chromHmm.bed > hesc.chromHmm.chr22.bed
$ head -n 5 hesc.chromHmm.chr22.bed
chr22   16050000  16075600  13_Heterochrom/lo
chr22   16075600  16076000  8_Insulator
chr22   16076000  16084200  13_Heterochrom/lo
chr22   16084200  16084600  8_Insulator
chr22   16084600  16156800  13_Heterochrom/lo
```

Not at all enhancers.

Q1. Which enhancers in human embryonic stem cells are under the most genetic "constraint" (that is, have the least genetic variation)?

1. We need a set of predicted enhancers in hESCs. How are the enhancers labeled?

```
$ cut -f 4 hesc.chromHmm.chr22.bed | sort | uniq -c
    444 10_Txn_Elongation
   2875  11_Weak_Txn
    514  12_Repressed
   1213  13_Heterochrom/lo
     58  14_Repetitive/CNV
     29  15_Repetitive/CNV
    251  1_Active_Promoter
    671  2_Weak_Promoter
    297  3_Poised_Promoter
    149  4_Strong_Enhancer
    335  5_Strong_Enhancer
   1656  6_Weak_Enhancer
   2840  7_Weak_Enhancer
   1218  8_Insulator
    493  9_Txn_Transition
```

Let's focus on the strong enhancers

Q1. Which enhancers in human embryonic stem cells are under the most genetic "constraint" (that is, have the least genetic variation)?

1. We need a set of predicted enhancers in hESCs.

```
$ grep "Strong_Enhancer" hesc.chromHmm.chr22.bed > hesc.chromHmm.chr22.enh.bed

# sanity check
$ wc -l hesc.chromHmm.chr22.enh.bed
484
```

Q1. Which enhancers in human embryonic stem cells are under the most genetic "constraint" (that is, have the least genetic variation)?

2. We need a deep catalog of genetic variation in the human genome. 1000 genomes

```
# warning. 1.8 gigabytes. Will take a few minutes to download from 1000G FTP site
$ wget
ftp://ftp-trace.ncbi.nlm.nih.gov/1000genomes/ftp/release/20130502/ALL.wgs.phase3_shapeit2_mvncall_integrated_v5b.20130502.sites.vcf.gz

# download the tabix index of the VCF file.
$ wget
ftp://ftp-trace.ncbi.nlm.nih.gov/1000genomes/ftp/release/20130502/ALL.wgs.phase3_shapeit2_mvncall_integrated_v5b.20130502.sites.vcf.gz.tbi

# extract just the genetic variants for chromosome 22 with tabix
$ tabix -h ALL.wgs.phase3_shapeit2_mvncall_integrated_v5b.20130502.sites.vcf.gz 22 \
  > 1000g.chr22.vcf
```

Extract just chromosome 22 variants

Retain the VCF header in the output with -h

Q1. Which enhancers in human embryonic stem cells are under the most genetic "constraint" (that is, have the least genetic variation)?

3. We then need to count the number of genetic variants observed in each enhancer.

Well, now we have our enhancer file (`hesc.chromHmm.chr22.enh.bed`) and our variants file (`1000g.chr22.vcf`). How do we count the number of variants in each enhancer?

```
$ bedtools intersect -a hesc.chromHmm.chr22.enh.bed -b 1000g.chr22.vcf -c | head
***** WARNING: File hesc.chromHmm.chr22.enh.bed has inconsistent naming convention
for record:
chr22    17675000 17675600 5_Strong_Enhancer
```

Q1. Which enhancers in human embryonic stem cells are under the most genetic "constraint" (that is, have the least genetic variation)?

3. We then need to count the number of genetic variants observed in each enhancer.

What happened?

```
$ head -n 5 hesc.chromHmm.chr22.enh.bed
chr22    17675000 17675600 5_Strong_Enhancer
chr22    17679200 17679600 4_Strong_Enhancer
chr22    17680000 17681000 4_Strong_Enhancer
chr22    17681000 17681400 5_Strong_Enhancer
chr22    17714800 17717000 5_Strong_Enhancer

$ grep -v "^#" 1000g.chr22.vcf | head -n 5 | cut -f 1-6
22   16050075 rs587697622   A    G    100
22   16050115 rs587755077   G    A    100
22   16050213 rs587654921   C    T    100
22   16050319 rs587712275   C    T    100
22   16050527 rs587769434   C    A    100
```

There is greatness in the world, but there is also this.

Q1. Which enhancers in human embryonic stem cells are under the most genetic "constraint" (that is, have the least genetic variation)?

3. We then need to count the number of genetic variants observed in each enhancer.

Now we need to make the chromosome labels the same. Let's remove "chr" from the BED file with `sed`

```
$ sed -e 's/^chr//' hesc.chromHmm.chr22.enh.bed > hesc.chromHmm.chr22.enh.nochr.bed
```

1. Give sed (stream editor) an expression with -e
2. The "s" operator is to switch one pattern with another. In this case, we switch "chr" at the beginning of each line (^) with nothing "//". That is, remove it.

Q1. Which enhancers in human embryonic stem cells are under the most genetic "constraint" (that is, have the least genetic variation)?

3. We then need to count the number of genetic variants observed in each enhancer.

Now we are ready for prime time!

```
$ bedtools intersect -a hesc.chromHmm.chr22.enh.nochr.bed -b 1000g.chr22.vcf -c | head
22   17675000 17675600 5_Strong_Enhancer  25
22   17679200 17679600 4_Strong_Enhancer  16
22   17680000 17681000 4_Strong_Enhancer  41
22   17681000 17681400 5_Strong_Enhancer  11
22   17714800 17717000 5_Strong_Enhancer  60
22   17737800 17738000 5_Strong_Enhancer  8
22   17738000 17739200 4_Strong_Enhancer  50
22   17739200 17740000 5_Strong_Enhancer  40
22   17741600 17742400 4_Strong_Enhancer  29
22   17744800 17745000 5_Strong_Enhancer  2
```

200bp versus 2200bp

Ooooooh, look. Constraint!!!!

Wait a second...

Q1. Which enhancers in human embryonic stem cells are under the most genetic "constraint" (that is, have the least genetic variation)?

3. We then need to count the number of genetic variants observed in each enhancer.

We need to compute the <u>density</u> of genetic variation, not the <u>count</u>.

```
$ bedtools intersect -a hesc.chromHmm.chr22.enh.nochr.bed -b 1000g.chr22.vcf -c \
    | awk '{OFS="\t"; print $0, $5 / ($3-$2)}' \
  > hesc.chromHmm.chr22.enh.nochr.vardensity.bedgraph
```

Density is variant count (column 5) divided by the length of the region (end - start)

Q1. Which enhancers in human embryonic stem cells are under the most genetic "constraint" (that is, have the least genetic variation)?

3. We then need to count the number of genetic variants observed in each enhancer.

We need to compute the <u>density</u> of genetic variation, not the <u>count</u>.

```
$ head hesc.chromHmm.chr22.enh.nochr.vardensity.bedgraph
22    17675000    17675600    5_Strong_Enhancer     25    0.0416667
22    17679200    17679600    4_Strong_Enhancer     16    0.04
22    17680000    17681000    4_Strong_Enhancer     41    0.041
22    17681000    17681400    5_Strong_Enhancer     11    0.0275
22    17714800    17717000    5_Strong_Enhancer     60    0.0272727
22    17737800    17738000    5_Strong_Enhancer     8     0.04
22    17738000    17739200    4_Strong_Enhancer     50    0.0416667
22    17739200    17740000    5_Strong_Enhancer     40    0.05
22    17741600    17742400    4_Strong_Enhancer     29    0.03625
22    17744800    17745000    5_Strong_Enhancer     2     0.01
```

Q1. Which enhancers in human embryonic stem cells are under the most genetic "constraint" (that is, have the least genetic variation)?

3. We then need to count the number of genetic variants observed in each enhancer.

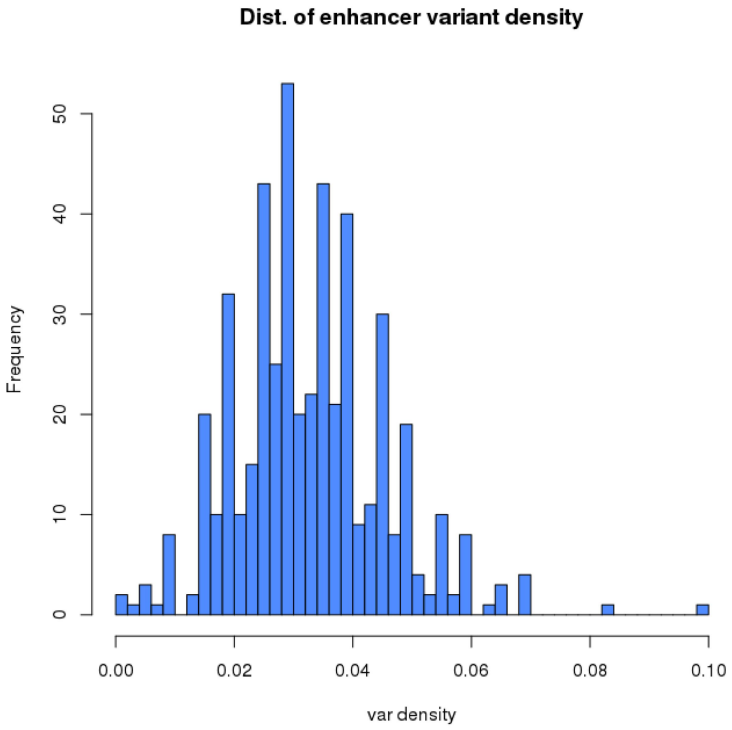
We need to compute the <u>density</u> of genetic variation, not the <u>count</u>.

Sort the enhancers by the column starting with density and ending with that column. Treat the values as numbers.

```
$ sort -k6,6n hesc.chromHmm.chr22.enh.nochr.vardensity.bedgraph | head
22    28245600    28247400    5_Strong_Enhancer     0     0
22    21846400    21847600    5_Strong_Enhancer     1     0.000833333
22    20402800    20403600    5_Strong_Enhancer     2     0.0025
22    29331400    29331600    5_Strong_Enhancer     1     0.005
22    36310454    36310654    4_Strong_Enhancer     1     0.005
22    48205736    48205936    5_Strong_Enhancer     1     0.005
22    20725800    20726200    4_Strong_Enhancer     3     0.0075
22    21795400    21797400    5_Strong_Enhancer     17    0.0085
22    17744800    17745000    5_Strong_Enhancer     2     0.01
22    29225800    29226000    5_Strong_Enhancer     2     0.01
```

Whoa! No variants from 2,504 genomes in a span of 1,800 bp!

Is approx. 0.0 variant density in an embryonic stem cell enhancer atypical?




```
$ R

> enh_constraint <- read.table('hesc.chromHmm.chr22.enh.nochr.vardensity.bedgraph', header=FALSE)
> hist(enh_constraint[,6], breaks=50, col='dodgerblue', xlab='var density', main="Dist. of enhancer variant density")
```

What sanity checks should we conduct to identify potential artifacts?

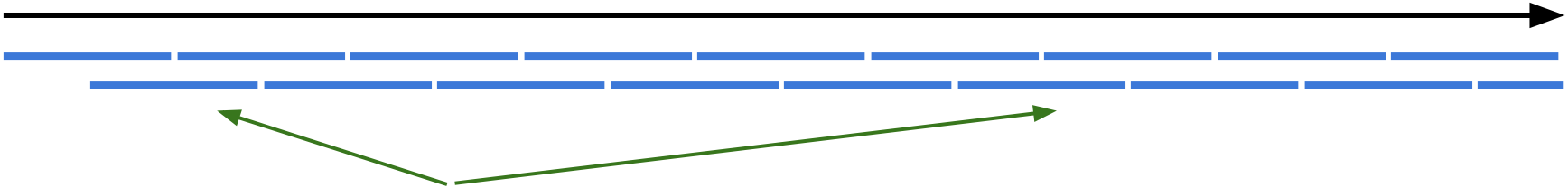
Q2. How could we compute genetic constraint for overlapping 10000bp windows along all of chromosome 22, and not just enhancers?

Q2. How could we compute genetic constraint for overlapping 10000bp windows along *all* of chromosome 22, and not just enhancers?

Experimental design: what materials do we need?

1. Now we need to create a BED file of overlapping, 10000bp windows tiled across chromosome 22.
2. We need a deep catalog of genetic variation in the human genome. 1000 genomes?
3. We then need to count the number of genetic variants observed in window.

Q2. How could we compute genetic constraint for overlapping 10000bp windows along *all* of chromosome 22, and not just enhancers?



Make equally-sized, yet overlapping windows (intervals in BED format) along each chromosome.

To do this, one MUST know the length of each chromosome. This is the purpose of a so-called "genome" file in BEDTOOLS

Q2. How could we compute genetic constraint for overlapping 1000bp windows along *all* of chromosome 22, and not just enhancers?

```
$ curl https://s3.amazonaws.com/bedtools-tutorials/web/genome.txt > human.grch37.txt

$ head human.grch37.txt | column -t
chr1                    249250621
chr10                   135534747
chr11                   135006516
chr11_gl000202_random   40103
chr12                   133851895
chr13                   115169878
chr14                   107349540
chr15                   102531392
chr16                   90354753
chr17                   81195210
```

Q2. How could we compute genetic constraint for overlapping 1000bp windows along *all* of chromosome 22, and not just enhancers?

```
$ bedtools makewindows -w 10000 -s 5000 -g human.grch37.txt | head
chr1  0     10000
chr1  5000  15000
chr1  10000 20000
chr1  15000 25000
chr1  20000 30000
chr1  25000 35000
chr1  30000 40000
chr1  35000 45000
chr1  40000 50000
chr1  45000 55000
```

10000bp windows.
5000bp "step"

Q2. How could we compute genetic constraint for overlapping 1000bp windows along *all* of chromosome 22, and not just enhancers?

Let's retain windows for just chromosome 22. And we now know we need to remove the "chr" from the chromosome label...

```
$ bedtools makewindows -w 10000 -s 5000 -g human.grch37.txt \
    | grep -w "^chr22" \
    | sed -e 's/chr//' \
  > human.grch37.22.10000w.5000s.bed

$ head human.grch37.22.10000w.5000s.bed
22    0     10000
22    5000  15000
22    10000 20000
22    15000 25000
22    20000 30000
22    25000 35000
22    30000 40000
22    35000 45000
22    40000 50000
22    45000 55000
```

Q2. How could we compute genetic constraint for overlapping 1000bp windows along *all* of chromosome 22, and not just enhancers?

Let's retain windows for just chromosome 22. And we now know we need to remove the "chr" from the chromosome label...

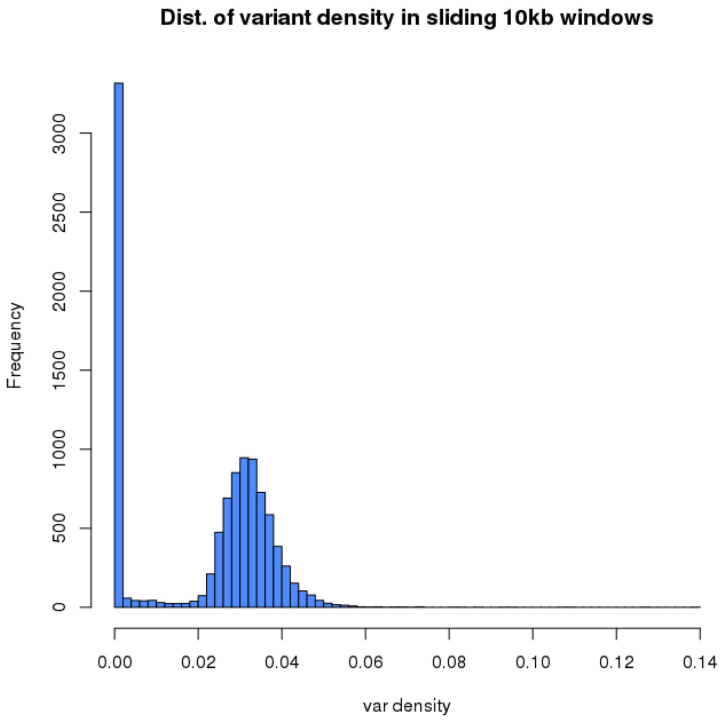
```
$ bedtools intersect -a human.grch37.22.10000w.5000s.bed -b 1000g.chr22.vcf -c \
    | awk '{OFS="\t"; print $0, $4 / ($3-$2)}' \
  > human.grch37.22.10000w.5000s.vardensity.bedgraph

$ head human.grch37.22.10000w.5000s.vardensity.bedgraph
22    0     10000 0     0
22    5000  15000 0     0
22    10000 20000 0     0
22    15000 25000 0     0
22    20000 30000 0     0
22    25000 35000 0     0
22    30000 40000 0     0
22    35000 45000 0     0
22    40000 50000 0     0
22    45000 55000 0     0
```

Number of variants in 10000bp window, then density of variants in 10000bp window.

Are these windows really constrained?

Hmmmm. Why are there so many windows with 0.0 variant density? Ideas?




```
$ R

> win_constraint <- read.table('human.grch37.22.10000w.5000s.vardensity.bedgraph', header=FALSE)
> hist(win_constraint[,5], breaks=50, col='dodgerblue', xlab='var density', main="Dist. of variant density in sliding 10kb
windows")
```

Idea. Maybe there is something about the repeat content of these windows that prevents variant detection...

To test this idea, let's download the FASTA file for chromosome 22 (GRCh37).

```
$ curl http://hgdownload.soe.ucsc.edu/goldenPath/hg38/chromosomes/chr22.fa.gz > chr22.fa.gz

$ gzip -d chr22.fa.gz

$ head -n 1 chr22.fa
>chr22

# we need to remove the "chr" from the header of the FASTA file as well.
$ sed -e 's/chr//' chr22.fa > chr22.numchrom.fa

$ head -n 1 chr22.numchrom.fa
>22
```

Idea. Maybe there is something about the repeat content of these windows that prevents variant detection...

Now that we have the chr22 FASTA, let's investigate the nucelotide content of each 10kb window with the "`getfasta`" tool.

Idea. Maybe there is something about the repeat content of these windows that prevents variant detection…

Now that we have the chr22 FASTA, let's investigate the nucelotide content of each 10kb window with the "`getfasta`*" tool.*

```
$ bedtools getfasta -bed human.grch37.22.10000w.5000s.vardensity.bedgraph -fi chr22.numchrom.fa -tab | less
22      0       10000   0       0
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN...
```

Idea. Maybe there is something about the repeat content of these windows that prevents variant detection...

Aha. Looks like masked sequence is our culprit. Let's use the "nuc" tool to just _count_ the number of Ns in the FASTA file for each 10kb window.

```
$ bedtools nuc -bed human.grch37.22.10000w.5000s.vardensity.bedgraph -fi chr22.numchrom.fa | head | column -t
#1_usercol  2_usercol  3_usercol  4_usercol  5_usercol  6_pct_at  7_pct_gc  8_num_A  9_num_C  10_num_G  11_num_T  12_num_N  13_num_oth  14_seq_len
22          0          10000      0          0          0.000000  0.000000  0        0        0         0         10000     0           10000
22          5000       15000      0          0          0.000000  0.000000  0        0        0         0         10000     0           10000
22          10000      20000      0          0          0.000000  0.000000  0        0        0         0         10000     0           10000
22          15000      25000      0          0          0.000000  0.000000  0        0        0         0         10000     0           10000
22          20000      30000      0          0          0.000000  0.000000  0        0        0         0         10000     0           10000
22          25000      35000      0          0          0.000000  0.000000  0        0        0         0         10000     0           10000
22          30000      40000      0          0          0.000000  0.000000  0        0        0         0         10000     0           10000
22          35000      45000      0          0          0.000000  0.000000  0        0        0         0         10000     0           10000
22          40000      50000      0          0          0.000000  0.000000  0        0        0         0         10000     0           10000
```

Zero variant density because there are 10000 Ns in these regions. Therefore, one cannot align reads to these regions and thus one has no power to <u>detect</u> variation in these regions.

Idea. Maybe there is something about the repeat content of these windows that prevents variant detection…

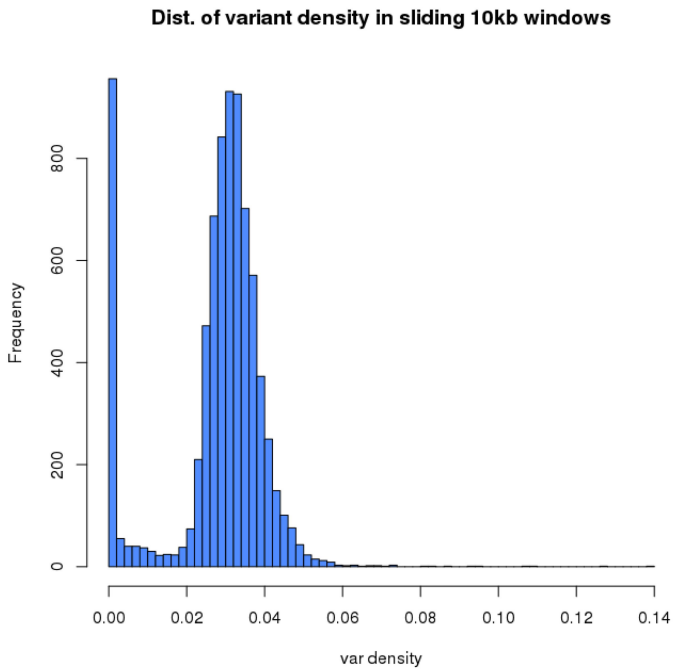
Let's use bioawk to filter out regions that have 1 or more "N" in the FASTA sequence. Recall that biowawk allows us to refer to columns in a file by <u>name</u> rather than solely by <u>number</u> in regular awk. Thanks Heng Li!

```
$ bedtools nuc -bed human.grch37.22.10000w.5000s.vardensity.bedgraph -fi chr22.numchrom.fa \
    | bioawk -H -t -c bed '$12_num_N ==0' \
  > human.grch37.22.10000w.5000s.vardensity.bedgraph.noN.bed
$ head human.grch37.22.10000w.5000s.vardensity.bedgraph.noN.bed | column -t
```

#1_usercol	2_usercol	3_usercol	4_usercol	5_usercol	6_pct_at	7_pct_gc	8_num_A	9_num_C	10_num_G	11_num_T	12_num_N	13_num_oth	14_seq_len
22	10510000	10520000	0	0	0.653900	0.346100	3800	1627	1834	2739	0	0	10000
22	10515000	10525000	0	0	0.625600	0.374400	3240	1789	1955	3016	0	0	10000
22	10520000	10530000	0	0	0.532900	0.467100	2350	2478	2193	2979	0	0	10000
22	10525000	10535000	0	0	0.568100	0.431900	2691	2093	2226	2990	0	0	10000
22	10530000	10540000	0	0	0.673900	0.326100	3280	1475	1786	3459	0	0	10000
22	10535000	10545000	0	0	0.675800	0.324200	3245	1607	1635	3513	0	0	10000
22	10540000	10550000	0	0	0.670500	0.329500	3659	1549	1746	3046	0	0	10000
22	10545000	10555000	0	0	0.656700	0.343300	3665	1598	1835	2902	0	0	10000
22	10550000	10560000	0	0	0.640300	0.359700	3369	1771	1826	3034	0	0	10000

[

Much better. Now bimodal. Any ideas?




```
$ R

> win_constraint <- read.table('human.grch37.22.10000w.5000s.vardensity.bedgraph.noN.bed', header=FALSE)
> hist(win_constraint[,5], breaks=50, col='dodgerblue', xlab='var density', main="Dist. of variant density in sliding 10kb
windows")
```

Q3. How do we know if an observed number of intersections between two datasets is statistically significant? That is, is the observation more extreme than what we would expect by chance?

Q3.1. Do GWAS SNPs overlap enhancers in hESCs more often than expected by chance?

Do GWAS SNPs overlap enhancers in hESCs more often than expected by chance?

Experimental design: what materials do we need?

1. We need hESC enhancers.
2. We need a catalog of significant SNPs from Genome Wide Association Studies (GWAS).
3. We need to measure the number of observed overlaps between the two.
4. Lastly, we need to compare the observed to what we expect to assess the significance of the relationship between these two genomic "features".

Q3. Do GWAS SNPs overlap enhancers in hESCs more often than expected by chance?

1. We need hESC enhancers. This time, let's include strong and weak enhancers

```
$ grep "Enhancer" hesc.chromHmm.bed | sed -e 's/chr//' > hesc.chromHmm.allenh.nochr.bed

$ head hesc.chromHmm.allenh.nochr.bed
1     27537 27737 6_Weak_Enhancer
1     30337 30537 6_Weak_Enhancer
1     34737 34937 7_Weak_Enhancer
1     35737 35937 7_Weak_Enhancer
1     35937 36137 6_Weak_Enhancer
1     36137 36337 7_Weak_Enhancer
1     36337 36537 5_Strong_Enhancer
1     36537 37537 6_Weak_Enhancer
1     37537 37737 7_Weak_Enhancer
1     56337 56537 6_Weak_Enhancer
```

Q3. Do GWAS SNPs overlap enhancers in hESCs more often than expected by chance?

2. We need a catalog of significant SNPs from Genome Wide Association Studies (GWAS).

```
# obtained from the UCSC Genome Browser's Table Browser
$ wget https://s3.amazonaws.com/bedtools-tutorials/web/gwas.phenotype.bed

$ sed -e 's/chr//' gwas.phenotype.bed > gwas.phenotype.nochr.bed

$ head gwas.phenotype.nochr.bed | column -t
#chrom  chromStart  chromEnd  name         pubMedID  trait
1       780396      780397     rs141175086  26955885  Morning vs. evening  chronotype
1       1005805     1005806    rs3934834    19851299  Body mass index
1       1079197     1079198    rs11260603   23382691  IgG glycosylation
1       1247493     1247494    rs12103      26192919  Ulcerative colitis
1       1247493     1247494    rs12103      26192919  Inflammatory bowel disease
1       1247493     1247494    rs12103      26192919  Crohn's disease
1       1247493     1247494    rs12103      23128233  Inflammatory bowel disease
1       1287054     1287055    rs186507655  27197191  Cancer (pleiotropy)
1       1723030     1723031    rs9660180    25673413  Body mass  index
```

Q3. Do GWAS SNPs overlap enhancers in hESCs more often than expected by chance?

3. We need to measure the number of observed overlaps between the GWAS SNPs and hESC enhancers.

```
$ wc -l gwas.phenotype.nochr.bed
39335

$ wc -l hesc.chromHmm.allenh.nochr.bed
240136

$ bedtools intersect -a gwas.phenotype.nochr.bed -b hesc.chromHmm.allenh.nochr.bed -u | wc -l
2040
```

So 5.2% (2,040 of 39335) GWAS SNPs overlap at least 1 predicted enhancer in human embryonic stem cells.
Is that interesting? How would we know?

Q3. Do GWAS SNPs overlap enhancers in hESCs more often than expected by chance?

4. Lastly, we need to compare the <u>observed</u> to what we <u>expect</u> to assess the significance of the relationship between these two genomic "features".

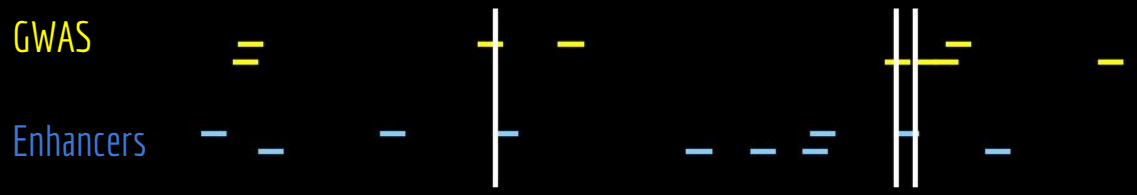
```
# observed = 2,040
$ bedtools intersect -a gwas.phenotype.nochr.bed -b hesc.chromHmm.allenh.nochr.bed -u | wc -l
2040
```

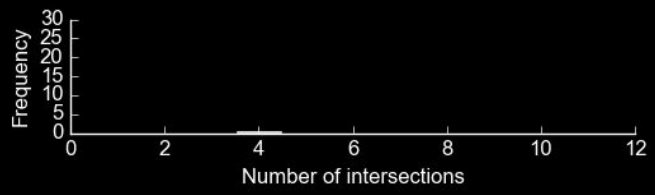
How do we derive an expectation (that is, a null hypothesis)? One way is to do a Monte Carlo simulation.

Experimental design:

1. Repeatedly (1000s of times) shuffle intervals randomly throughout the genome.
2. For each "shuffling", measure how many intersections there are.
3. Build up a distribution of the number of intersections observed for each shuffling.

Monte Carlo simulation by shuffling intervals and measuring intersections that occur "by chance"





Simulation animation from Ryan Layer (@ryanlayer)

Q3. Do GWAS SNPs overlap enhancers in hESCs more often than expected by chance?

4. Lastly, we need to compare the observed to what we expect to assess the significance of the relationship between these two genomic "features".

```
# this will take a few minutes
for i in `seq 1 100`;
do
    bedtools shuffle -i gwas.phenotype.nochr.bed -g human.grch37.nochr.txt \
      | bedtools intersect -a - -b hesc.chromHmm.allenh.nochr.bed -u \
      | wc -l \
    >> random.intersections.txt
done

sort random.intersections.txt | uniq -c
    100 0
```

">>" Append the results of each of the 100 experiments to a file.

All 100 experiments yielded 0 intersections!!!

One must be careful about defining the "domain" of an experiment

The dilemma of choosing the ideal permutation strategy while estimating statistical significance of genome-wide enrichment

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Abstract

Integrative analyses of genomic, epigenomic and transcriptomic features for human and various model organisms have revealed that many such features are nonrandomly distributed in the genome. Significant enrichment (or depletion) of genomic features is anticipated to be biologically important. Detection of genomic regions having enrichment of certain features and estimation of corresponding statistical significance rely on the expected null distribution generated by a permutation model. We discuss different genome-wide permutation approaches, present examples where the permutation strategy affects the null model and show that the confidence in estimating statistical significance of genome-wide enrichment might depend on the choice of the permutation approach. In those cases, where biologically relevant constraints are unclear, it is preferable to examine whether key conclusions are consistent, irrespective of the choice of the randomization strategy.

Keywords: *genome-wide enrichment; statistical significance; permutation strategy; null distribution*

One must be careful about defining the "domain" of an experiment

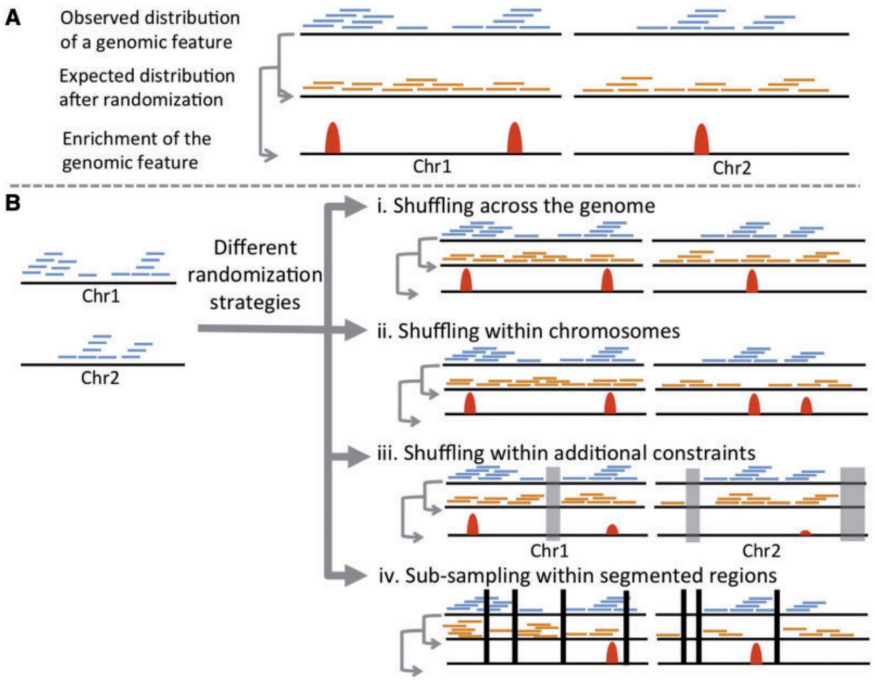

Figure 1: (**A**) The basic principle behind permutation analysis to determine genome-wide enrichment of a genomic or epigenomic feature. (**B**) A different randomization strategy can produce a different expected distribution, and hence affect statistical significance of enrichment of the feature. In (Biii), disallowed regions are masked (gray) while shuffling with additional constraints. The displayed list does not represent the exhaustive list of possible randomization strategies. A colour version of this figure is available at BIB online: http://bib.oxfordjournals.org.

Use the bedtools -incl (include regions) and -excl (exclude regions)