vtpnet: variant-transcription factor-phenotype networks

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1 Introduction

In a wide-ranging paper (PMID 22955828 Maurano et al. (2012)), Maurano and colleagues illustrate the concept of "common networks for common diseases" with a bipartite graph. One class of nodes is a set of autoimmune disorders, the other class is a set of transcription factors (TFs). In this graph, an edge exists between a disorder node and a TF node if a SNP that is significantly associated with the risk of the disorder lies in a genomic region possessing a strong match to the binding motif of the TF. This package defines tools to investigate the construction and statistical interpretation of such bipartite graphs, which we will denote VTP (variant-transcription factor-phenotype) networks.

2 Illustrative example of an unpruned VTP

The following code uses the `graphNEL` class to construct an approximation to the complete bipartite graph underlying Figure 4A of the Maurano paper; Figure 1 illustrates an arbitrary complete subgraph. The elements of `diseaseTags` are formatted to allow multiline rendering of the strings in node displays. It will be useful to distinguish a display token type and an analysis token type to simplify programming.

```
> #
> # tags formatted for display
> #
> diseaseTags = c("Ankylosing\\\nspondylitis", "Asthma",
+       "Celiac\\\ndisease", "Crohn's\\\ndisease",
+       "Multiple\\\nsclerosis", "Primary\\\nbiliary\\\ncirrhosis",
+       "Psoriasis", "Rheumatoid\\\narthritis",
+       "Systemic\\\nlupus\\\nerythematosus",
+       "Systemic\\\nsclerosis", "Type 1\\\ndiabetes",
```

```
+       "Ulcerative\\\ncolitis"
+ )
> TFtags = c("ELF3", "MEF2A", "TCF3", "PAX4", "STAT3",
+    "ESR1", "POU2F1", "STAT1", "YY1", "SP1", "CDC5L",
+    "NR3C1", "EGR1", "PPARG", "HNF4A", "REST", "PPARA",
+    "AR", "NFKB1", "HNF1A", "TFAP2A")
> # define adjacency matrix
> adjm = matrix(1, nr=length(diseaseTags), nc=length(TFtags))
> dimnames(adjm) = list(diseaseTags, TFtags)
> library(graph)
> cvtp = ugraph(aM2bpG(adjm)) # complete (V)TP network; variants not involved yet
```

3 Data on GWAS variants: their associated phenotype, locations, and other characteristics

We will use the GWAS data provided at `https://www.sciencemag.org/content/suppl/2012/09/04/science.1222794.DC1/1222794-Maurano-tableS2.txt`, which was manually imported to a GRanges instance in hg19 origin-1 coordinates.

```
> library(vtpnet)
> data(maurGWAS)
> length(maurGWAS)

[1] 5654

> names(values(maurGWAS))

[1] "name"                     "disease_trait"
[3] "disease_class"            "internally_replicated"
[5] "independently_replicated" "In_DHS"
[7] "fetal_origin"             "X.LOG.P."
[9] "sample_size"
```

4 Data on transcription factor binding sites

We have included the result of using FIMO Grant et al. (2011) to scan for motif matches for TF PAX4 as modeled in the Bioconductor *MotifDb* collection. The `-max-stored-scores` parameter was set to 10000000 so that p of up to 10^{-4} are retained.

```
> data(pax4)
> length(pax4)
```

```
> library(Rgraphviz)
> #flat = function(x, g) c(x, edges(g)[[x]])
> #sub = subGraph(unique(c(flat("Crohn's\\\ndisease", cvtp),
> #    flat("Ulcerative\\\ncolitis", cvtp))), cvtp)
> sub = subGraph(unique(c(diseaseTags[1:4], TFtags[1:6])), cvtp)
> plot(sub, attrs=list(node=list(shape="box", fixedsize=FALSE)))
> #plot(cvtp, attrs=list(graph=list(margin=c(.5,.5), size=c(4.1,4.1)),
> #   node=list(shape="box", fixedsize=FALSE, height=1)))
```

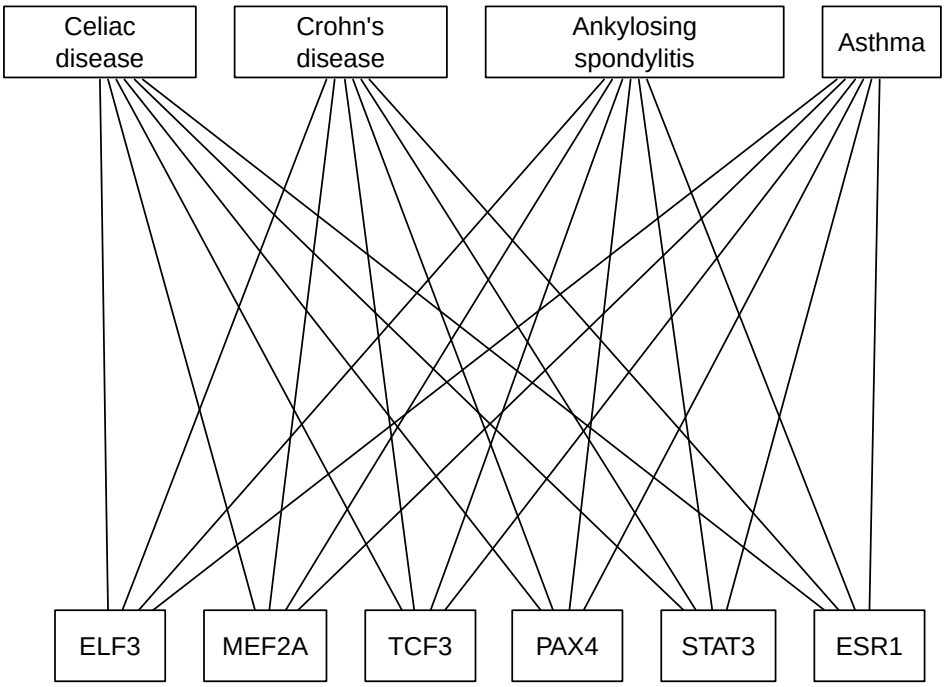

Figure 1: A complete bipartite graph for arbitrarily selected subsets of the autoimmune disorders and TFs found in Figure 4A of Maurano et al.

```
[1] 1862156
```

```
> pax4[1:4]
```

```
GRanges object with 4 ranges and 8 metadata columns:
      seqnames      ranges strand |   source            type      score
         <Rle>   <IRanges>  <Rle> | <factor>         <factor>  <numeric>
  [1]     chr1 10273-10302      + |     fimo nucleotide_motif   999.9165
  [2]     chr1 10279-10308      + |     fimo nucleotide_motif   999.9621
  [3]     chr1 11703-11732      - |     fimo nucleotide_motif 999.999196
  [4]     chr1 11704-11733      - |     fimo nucleotide_motif   999.9554
         phase                                  Name      pvalue      qvalue
     <integer>                           <character> <character> <character>
  [1]      <NA> +Mmusculus-JASPAR_CORE-Pax4-MA0068.1    8.35e-05       0.396
  [2]      <NA> +Mmusculus-JASPAR_CORE-Pax4-MA0068.1    3.79e-05       0.361
  [3]      <NA> -Mmusculus-JASPAR_CORE-Pax4-MA0068.1    8.04e-07       0.194
  [4]      <NA> -Mmusculus-JASPAR_CORE-Pax4-MA0068.1    4.46e-05       0.368
                          sequence
                       <character>
  [1] TAACCCTAACCCTAACCCCAACCCCAACCC
  [2] TAACCCTAACCCCAACCCCAACCCCAACCC
  [3] AAAAAAATACACATGGCCAGGCCCCAGCCC
  [4] TAAAAAAATACACATGGCCAGGCCCCAGCC
  -------
  seqinfo: 92 sequences from an unspecified genome; no seqlengths
```

We can also generate our own motif-match ranges. Here is an example of a parallelized search against hg19 using `matchPWM`.

```
> library(foreach)
> library(doParallel)
> registerDoParallel(cores=12)
> library(BSgenome.Hsapiens.UCSC.hg19)
> library(MotifDb)
> sn = seqnames(Hsapiens)[1:24]
> pax4 = query(MotifDb, "pax4")[[1]]
> ans = foreach(i=1:24) %dopar% {
+  cat(i)
+  subj = Hsapiens[[ sn[i] ]]
+  matchPWM( pax4, subj, "75%" )
+ }
> pax4_75 =
+  do.call(c, lapply(1:length(ans), function(x)
```

```
+   {GRanges(sn[x], as(ans[[x]], "IRanges"))}))
> save(pax4_75, file="pax4_75.rda")
```

Results of such searches retaining matches at scores of 85% and 75% of the maximum achievable score have been stored with this package.

5 Building a VTP network: one edge per phenotype

5.1 Raw matches

We can survey the entire GWAS catalog for intersection with putative PAX4 binding sites. First the two Bioconductor internal binding site sets.

```
> data(pax4_85)
> vp_pax4_85 = maurGWAS[ overlapsAny(maurGWAS, pax4_85) ]
> length(vp_pax4_85)
```

```
[1] 0
```

```
> data(pax4_75)
> vp_pax4_75 = maurGWAS[ overlapsAny(maurGWAS, pax4_75) ]
> length(vp_pax4_75)
```

```
[1] 54
```

Then the FIMO-based set.

```
> vp_pax4_fimo = maurGWAS[ overlapsAny(maurGWAS, pax4) ]
> length(vp_pax4_fimo)
```

```
[1] 67
```

The lengths reported here are the numbers of phenotypes linked to PAX4 in a VTP according to various motif matching schemes. For the two non-null results, we have

```
> u75 = unique(vp_pax4_75$disease_trait)
> ufimo = unique(vp_pax4_fimo$disease_trait)
> length(setdiff(u75, ufimo))
```

```
[1] 23
```

```
> length(setdiff(ufimo, u75))
```

```
[1] 28
```

Clearly the identification of TP links is sensitive to the approach used to locate binding sites. However, as noted in the Maurano paper, the use of matching to the reference genome without SNP injection is potentially problematic.

5.2 Filtering

It is useful to restrict the phenotypes of interest, and to map them to broader classes, and to include TFBS matching scores for the purpose of filtering edges. Here we will use the NHGRI GWAS catalog against FIMO-based (reference genome matching only) PAX4 calls.

```
> data(cancerMap)
> library(gwascat)
> data(gwrngs19)
> cangw = filterGWASbyMap( gwrngs19, cancerMap )
> getOneHits( pax4, cangw, "fimo" )

GRanges object with 8 ranges and 41 metadata columns:
        seqnames    ranges strand | Date.Added.to.Catalog  PUBMEDID
           <Rle> <IRanges>  <Rle> |            <character> <integer>
   3475     chr8 129194641      * |             09/12/2013  23535729
   3480    chr11  65583066      * |             09/12/2013  23535729
   6963     chr2  26526419      * |             01/25/2013  23144319
   7155     chr6 143943314      * |             01/15/2013  23108145
   7480    chr20  32588095      * |             11/30/2012  22976474
  12585     chrX  37854727      * |             11/15/2010  20932654
  13650    chr12  14653867      * |             07/12/2010  20543847
  15145    chr10  63752159      * |             09/04/2009  19684604
         First.Author        Date                       Journal
          <character> <character>                   <character>
   3475  Michailidou K  04/01/2013                     Nat Genet
   3480  Michailidou K  04/01/2013                     Nat Genet
   6963          Lee Y  11/08/2012                Carcinogenesis
   7155       Wang LE  10/29/2012                    Cancer Res
   7480       Siddiq A  09/13/2012                  Hum Mol Genet
  12585       Kerns SL  10/05/2010 Int J Radiat Oncol Biol Phys
  13650     Turnbull C  06/13/2010                     Nat Genet
  15145 Papaemmanuil E  08/16/2009                     Nat Genet
                                              Link
                                       <character>
   3475 http://www.ncbi.nlm.nih.gov/pubmed/23535729
   3480 http://www.ncbi.nlm.nih.gov/pubmed/23535729
   6963 http://www.ncbi.nlm.nih.gov/pubmed/23144319
   7155 http://www.ncbi.nlm.nih.gov/pubmed/23108145
   7480 http://www.ncbi.nlm.nih.gov/pubmed/22976474
  12585 http://www.ncbi.nlm.nih.gov/pubmed/20932654
  13650 http://www.ncbi.nlm.nih.gov/pubmed/20543847
```

```
15145 http://www.ncbi.nlm.nih.gov/pubmed/19684604


 3475
 3480
 6963
 7155
 7480                                                                    A meta
12585 Genome-wide association study to identify single nucleotide polymorphisms (SNPs
13650
15145
                                        Disease.Trait
                                          <character>
 3475                                   Breast cancer
 3480                                   Breast cancer
 6963                       Non-small cell lung cancer
 7155             Lung Cancer (DNA repair capacity)
 7480                                   Breast cancer
12585 Erectile dysfunction and prostate cancer treatment
13650                      Testicular germ cell cancer
15145          Acute lymphoblastic leukemia (childhood)


 3475                                                          10,052 European a
 3480                                                          10,052 European a
 6963
 7155                                          914 European ancestry non-small cell l
 7480 3,666 European ancestry cases, 28,864 European ancestry controls, 1,004 African
12585                                                                27 Afri
13650                                                             979 European
15145                                                             907 European


 3475
 3480
 6963
 7155                                                              679 European
 7480 562 European ancestry cases, 6,410 European ancestry controls, 84 Japanese ance
12585
13650
15145
          Region      Chr_id Chr_pos.hg38
```

```
      <character> <character>    <numeric>
 3475     8q24.21           8    128182395
 3480     11q13.1          11     65815595
 6963      2p23.3           2     26303551
 7155      6q24.2           6    143622177
 7480    20q11.22          20     34000289
12585      Xp11.4          23     37995474
13650     12p13.1          12     14500933
15145     10q21.2          10     61992400
                          Reported.Gene.s.         Mapped_gene
                                <character>          <character>
 3475                          MIR1208, MYC MIR1208 - LINC01263
 3480 DKFZp761E198, OVOL1, SNX32, CFL1, MUS81   OVOL1-AS1 - SNX32
 6963                                GPR113      HADHB - GPR113
 7155                             PHACTR2              PHACTR2
 7480                   RALY, EIF2S2, ASIP                 RALY
12585                                SYTL5     CXorf27 - SYTL5
13650                               ATF7IP               ATF7IP
15145                               ARID5B               ARID5B
      Upstream_gene_id Downstream_gene_id Snp_gene_ids Upstream_gene_distance
           <character>        <character>  <character>            <character>
 3475        100302281          101927774                              32.21
 3480        101927828             254122                              24.73
 6963             3032             165082                              13.09
 7155             <NA>               <NA>         9749                   <NA>
 7480             <NA>               <NA>        22913                   <NA>
12585            25763              94122                               4.16
13650             <NA>               <NA>        55729                   <NA>
15145             <NA>               <NA>        84159                   <NA>
      Downstream_gene_distance Strongest.SNP.Risk.Allele        SNPs
                   <character>                <character> <character>
 3475                   222.87              rs11780156-T  rs11780156
 3480                    18.24               rs3903072-G   rs3903072
 6963                     4.62               rs6753473-G   rs6753473
 7155                     <NA>               rs9390123-A   rs9390123
 7480                     <NA>               rs2284378-T   rs2284378
12585                    11.11                rs872690-?    rs872690
13650                     <NA>               rs2900333-C   rs2900333
15145                     <NA>               rs7089424-C   rs7089424
      Merged Snp_id_current     Context  Intergenic
      <character>    <character> <character> <character>
 3475           0       11780156  Intergenic           1
```

```
 3480           0        3903072  Intergenic           1
 6963           0        6753473  Intergenic           1
 7155           0        9390123      intron           0
 7480           0        2284378      intron           0
12585           0         872690  Intergenic           1
13650           0        2900333       UTR-3           0
15145           0        7089424      intron           0
      Risk.Allele.Frequency   p.Value      Pvalue_mlog   p.Value..text.
                <character> <numeric>        <numeric>      <character>
 3475                  0.16     3e-11 10.5228787452803
 3480                  0.53     9e-12 11.0457574905607
 6963                 0.052     4e-06 5.39794000867204 (Additive model)
 7155                0.3957     7e-06 5.15490195998574
 7480                  0.31     1e-08                8
12585                  0.03     9e-06 5.04575749056067
13650                  0.62     6e-10 9.22184874961636
15145                  0.34     7e-19 18.1549019599857
      OR.or.beta X95..CI..text.
       <numeric>    <character>
 3475       1.07   [1.04-1.10]
 3480       1.05   [1.04-1.08]
 6963       <NA>            NR
 7155       <NA>            NR
 7480       1.16   [1.10-1.22]
12585      11.78          [NR]
13650       1.27   [1.12-1.44]
15145       1.65   [1.54-1.76]
                      Platform..SNPs.passing.QC.         CNV
                                     <character> <character>
 3475 Illumina & Affymetrix [~2.6 million] (Imputed)           N
 3480 Illumina & Affymetrix [~2.6 million] (Imputed)           N
 6963                          Affymetrix [271,817]           N
 7155                            Illumina [303,669]           N
 7480                Illumina [2,608,509] (imputed)           N
12585                          Affymetrix [512,497]           N
13650                            Illumina [298,782]           N
15145                            Illumina [291,473]           N
      num.Risk.Allele.Frequency      dclass     score   tfstart     tfend
                      <numeric> <character> <numeric> <integer> <integer>
 3475                      0.16      Breast  999.9851 129194621 129194650
 3480                      0.53      Breast  999.9517  65583065  65583094
 6963                     0.052        Lung  999.9875  26526415  26526444
```

```
  7155                    0.3957        Lung  999.9387 143943292 143943321
  7480                      0.31      Breast  999.9284  32588075  32588104
 12585                      0.03    Prostate  999.9028  37854721  37854750
 13650                      0.62  Testicular  999.9895  14653848  14653877
 15145                      0.34   ALL (ped)  999.9621  63752142  63752171
         pvalue    qvalue
      <numeric> <numeric>
  3475  1.49e-05     0.318
  3480  4.83e-05     0.373
  6963  1.25e-05      0.31
  7155  6.13e-05     0.383
  7480  7.16e-05     0.388
 12585  9.72e-05     0.403
 13650  1.05e-05     0.301
 15145  3.79e-05     0.361
 -------
 seqinfo: 23 sequences from hg19 genome
```

6 Appendix: generating the ALT-injected genome image

```
> altize = function(chtag = "21",
+ #
+ # from sketch by Herve Pages, May 2013
+ #
+   slpack="SNPlocs.Hsapiens.dbSNP.20120608",
+   hgpack ="BSgenome.Hsapiens.UCSC.hg19",
+   faElFun = function(x) sub("%%TAG%%", x, "alt%%TAG%%chr"),
+   faTargFun = function(x)
+      sub("%%TAG%%", x, "alt%%TAG%%_hg19.fa")) {
+     require(slpack, character.only=TRUE)
+     require(hgpack, character.only=TRUE)
+     require("ShortRead", character.only=TRUE)
+     chk = grep("ch|chr", chtag)
+     if (length(chk)>0) {
+       warning("clearing prefix ch or chr from chtag")
+       chtag = gsub("ch|chr", "", chtag)
+       }
+     snpgettag = paste0("ch", chtag)
+     ggettag = paste0("chr", chtag)
+     cursnps = getSNPlocs(snpgettag, as.GRanges=TRUE)
```

```
+     curgenome = unmasked(Hsapiens[[ggettag]])
+     ref_allele =
+      strsplit(as.character(curgenome[start(cursnps)]),
+         NULL, fixed=TRUE)[[1L]]
+     all_alleles = IUPAC_CODE_MAP[cursnps$alleles_as_ambig]
+     alt_alleles = mapply( function(ref,all)
+       sub(ref, "", all, fixed=TRUE),
+         ref_allele, all_alleles, USE.NAMES=FALSE)
+     cursnps$ref_allele = ref_allele
+     cursnps$alt_alleles = alt_alleles
+     cursnps$one_alt = substr(cursnps$alt_alleles, 1, 1)
+     altg = list(replaceLetterAt(curgenome, start(cursnps),
+       cursnps$one_alt))
+     names(altg) = faElFun(chtag)
+     writeFasta(DNAStringSet(altg), file=faTargFun(chtag))
+ }
```

7 Session information

```
> sessionInfo()

R version 3.6.1 (2019-07-05)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: Ubuntu 18.04.3 LTS

Matrix products: default
BLAS:   /home/biocbuild/bbs-3.10-bioc/R/lib/libRblas.so
LAPACK: /home/biocbuild/bbs-3.10-bioc/R/lib/libRlapack.so

locale:
 [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
 [3] LC_TIME=en_US.UTF-8        LC_COLLATE=C
 [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
 [7] LC_PAPER=en_US.UTF-8       LC_NAME=C
 [9] LC_ADDRESS=C               LC_TELEPHONE=C
[11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C

attached base packages:
 [1] stats4    grid      parallel  stats     graphics  grDevices utils
 [8] datasets  methods   base

other attached packages:
```

```
 [1] vtpnet_0.26.0
 [2] doParallel_1.0.15
 [3] iterators_1.0.12
 [4] foreach_1.4.7
 [5] gwascat_2.18.0
 [6] Homo.sapiens_1.3.1
 [7] TxDb.Hsapiens.UCSC.hg19.knownGene_3.2.2
 [8] org.Hs.eg.db_3.10.0
 [9] GO.db_3.10.0
[10] OrganismDbi_1.28.0
[11] GenomicFeatures_1.38.0
[12] AnnotationDbi_1.48.0
[13] Biobase_2.46.0
[14] GenomicRanges_1.38.0
[15] GenomeInfoDb_1.22.0
[16] IRanges_2.20.0
[17] S4Vectors_0.24.0
[18] Rgraphviz_2.30.0
[19] graph_1.64.0
[20] BiocGenerics_0.32.0

loaded via a namespace (and not attached):
 [1] Rcpp_1.0.2                   lattice_0.20-38
 [3] prettyunits_1.0.2            Rsamtools_2.2.0
 [5] Biostrings_2.54.0            assertthat_0.2.1
 [7] zeallot_0.1.0                digest_0.6.22
 [9] BiocFileCache_1.10.0         R6_2.4.0
[11] backports_1.1.5              RSQLite_2.1.2
[13] httr_1.4.1                   pillar_1.4.2
[15] zlibbioc_1.32.0              rlang_0.4.1
[17] progress_1.2.2               curl_4.2
[19] blob_1.2.0                   Matrix_1.2-17
[21] BiocParallel_1.20.0          stringr_1.4.0
[23] RCurl_1.95-4.12              bit_1.1-14
[25] biomaRt_2.42.0               DelayedArray_0.12.0
[27] compiler_3.6.1               rtracklayer_1.46.0
[29] pkgconfig_2.0.3              askpass_1.1
[31] openssl_1.4.1                tidyselect_0.2.5
[33] SummarizedExperiment_1.16.0 tibble_2.1.3
[35] GenomeInfoDbData_1.2.2       codetools_0.2-16
[37] matrixStats_0.55.0           XML_3.98-1.20
[39] crayon_1.3.4                 dplyr_0.8.3
```

```
[41] dbplyr_1.4.2                GenomicAlignments_1.22.0
[43] bitops_1.0-6                rappdirs_0.3.1
[45] RBGL_1.62.0                 DBI_1.0.0
[47] magrittr_1.5                stringi_1.4.3
[49] XVector_0.26.0              vctrs_0.2.0
[51] tools_3.6.1                 bit64_0.9-7
[53] glue_1.3.1                  purrr_0.3.3
[55] hms_0.5.1                   BiocManager_1.30.9
[57] memoise_1.1.0
```

8 Bibliography

References

Charles E Grant, Timothy L Bailey, and William Stafford Noble. Fimo: scanning for occurrences of a given motif. *Bioinformatics (Oxford, England)*, 27(7):1017–8, Apr 2011. doi: 10.1093/bioinformatics/btr064.

Matthew T Maurano, Richard Humbert, Eric Rynes, Robert E Thurman, Eric Haugen, Hao Wang, Alex P Reynolds, Richard Sandstrom, Hongzhu Qu, Jennifer Brody, Anthony Shafer, Fidencio Neri, Kristen Lee, Tanya Kutyavin, Sandra Stehling-Sun, Audra K Johnson, Theresa K Canfield, Erika Giste, Morgan Diegel, Daniel Bates, R Scott Hansen, Shane Neph, Peter J Sabo, Shelly Heimfeld, Antony Raubitschek, Steven Ziegler, Chris Cotsapas, Nona Sotoodehnia, Ian Glass, Shamil R Sunyaev, Rajinder Kaul, and John A Stamatoyannopoulos. Systematic localization of common disease-associated variation in regulatory dna. *Science*, 337(6099):1190–5, Sep 2012. doi: 10.1126/science.1222794.