

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)))

```

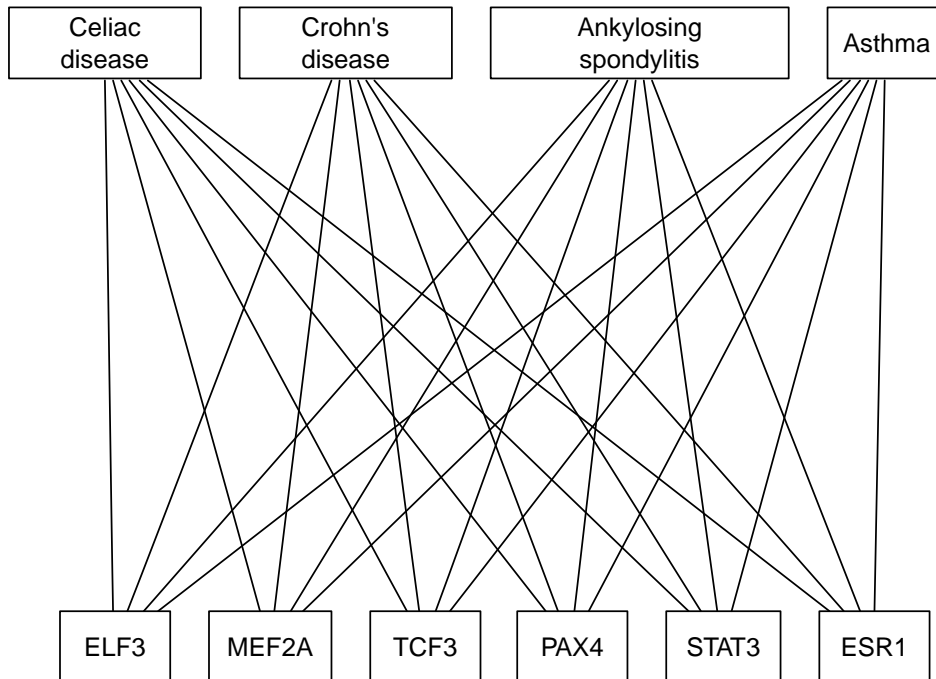


Figure 1: A complete bipartite graph for arbitrarily selected subsets of the autoimmune disorders and TFs found in Figure 4A of Maurano et al.

```
[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	nucleotide_motif	999.9165
[2]	chr1	[10279, 10308]	+	fimo nucleotide_motif	nucleotide_motif	999.9621
[3]	chr1	[11703, 11732]	-	fimo nucleotide_motif	nucleotide_motif	999.9992
[4]	chr1	[11704, 11733]	-	fimo nucleotide_motif	nucleotide_motif	999.9554
	phase	Name	pvalue	qvalue		
	<integer>	<character>	<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]	TAACCCTAACCCCTAACCCCAACCCCAACCC					
[2]	TAACCCTAACCCCAACCCCAACCCCAACCC					
[3]	AAAAAAAATACACATGGCCAGGCCCCAGCCC					
[4]	TAAAAAAAATACACATGGCCAGGCCCCAGCCC					

```
-----
```

```
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
<Rle>	<IRanges>	<Rle>	<character>
3475 chr8 [129194641, 129194641]		*	09/12/2013
3480 chr11 [65583066, 65583066]		*	09/12/2013
6963 chr2 [26526419, 26526419]		*	01/25/2013
7155 chr6 [143943314, 143943314]		*	01/15/2013
7480 chr20 [32588095, 32588095]		*	11/30/2012
12585 chrX [37854727, 37854727]		*	11/15/2010
13650 chr12 [14653867, 14653867]		*	07/12/2010
15145 chr10 [63752159, 63752159]		*	09/04/2009
PUBMEDID	First.Author	Date	Journal
<integer>	<character>	<character>	<character>
3475 23535729	Michailidou K	04/01/2013	Nat Genet
3480 23535729	Michailidou K	04/01/2013	Nat Genet
6963 23144319	Lee Y	11/08/2012	Carcinogenesis
7155 23108145	Wang LE	10/29/2012	Cancer Res
7480 22976474	Siddiq A	09/13/2012	Hum Mol Genet
12585 20932654	Kerns SL	10/05/2010	Int J Radiat Oncol Biol Phys
13650 20543847	Turnbull C	06/13/2010	Nat Genet
15145 19684604	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	Snps_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>	<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	Snps_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.	OR.or.beta
	<character>	<numeric>	<numeric>	<character>	<numeric>
3475	0.16	3e-11	10.522879		1.07
3480	0.53	9e-12	11.045757		1.05
6963	0.052	4e-06	5.397940	(Additive model)	<NA>
7155	0.3957	7e-06	5.154902		<NA>
7480	0.31	1e-08	8.000000		1.16
12585	0.03	9e-06	5.045757		11.78
13650	0.62	6e-10	9.221849		1.27
15145	0.34	7e-19	18.154902		1.65

	X95..CI..text.	Platform..SNPs.passing.QC.
	<character>	<character>
3475	[1.04-1.10]	Illumina & Affymetrix [~2.6 million] (Imputed)
3480	[1.04-1.08]	Illumina & Affymetrix [~2.6 million] (Imputed)
6963	NR	Affymetrix [271,817]
7155	NR	Illumina [303,669]
7480	[1.10-1.22]	Illumina [2,608,509] (imputed)
12585	[NR]	Affymetrix [512,497]
13650	[1.12-1.44]	Illumina [298,782]
15145	[1.54-1.76]	Illumina [291,473]

	CNV	num.Risk Allele Frequency	dclass	score	tfstart
	<character>	<numeric>	<character>	<numeric>	<integer>
3475	N	0.1600	Breast	999.9851	129194621
3480	N	0.5300	Breast	999.9517	65583065
6963	N	0.0520	Lung	999.9875	26526415
7155	N	0.3957	Lung	999.9387	143943292
7480	N	0.3100	Breast	999.9284	32588075
12585	N	0.0300	Prostate	999.9028	37854721
13650	N	0.6200	Testicular	999.9895	14653848
15145	N	0.3400	ALL (ped)	999.9621	63752142

	tfend	pvalue	qvalue
	<integer>	<numeric>	<numeric>
3475	129194650	1.49e-05	0.318
3480	65583094	4.83e-05	0.373
6963	26526444	1.25e-05	0.310

7155	143943321	6.13e-05	0.383
7480	32588104	7.16e-05	0.388
12585	37854750	9.72e-05	0.403
13650	14653877	1.05e-05	0.301
15145	63752171	3.79e-05	0.361

seqinfo: 23 sequences from hg19 genome

6 Appendix: generating the ALT-injected genome image

```
> altize = function(htag = "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", htag)
+   if (length(chk)>0) {
+     warning("clearing prefix ch or chr from htag")
+     htag = gsub("ch|chr", "", htag)
+   }
+   snpgettag = paste0("ch", htag)
+   ggettag = paste0("chr", htag)
+   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 3.3.1 (2016-06-21)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: Ubuntu 16.04.1 LTS

```

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_0.14.0
[2] doParallel_1.0.10
[3] iterators_1.0.8
[4] foreach_1.4.3
[5] gwascats_2.6.0
[6] Homo.sapiens_1.3.1
[7] TxDb.Hsapiens.UCSC.hg19.knownGene_3.2.2
[8] org.Hs.eg.db_3.4.0
[9] GO.db_3.4.0
[10] OrganismDbi_1.16.0
[11] GenomicFeatures_1.26.0
[12] AnnotationDbi_1.36.0
[13] Biobase_2.34.0
[14] GenomicRanges_1.26.0

```

[15] GenomeInfoDb_1.10.0
[16] IRanges_2.8.0
[17] S4Vectors_0.12.0
[18] Rgraphviz_2.18.0
[19] graph_1.52.0
[20] BiocGenerics_0.20.0

loaded via a namespace (and not attached):

[1] nlme_3.1-128	matrixStats_0.51.0
[3] bitops_1.0-6	httr_1.2.1
[5] RColorBrewer_1.1-2	GenomicFiles_1.10.0
[7] tools_3.3.1	backports_1.0.3
[9] R6_2.2.0	rpart_4.1-10
[11] Gviz_1.18.0	Hmisc_3.17-4
[13] DBI_0.5-1	mgcv_1.8-15
[15] colorspace_1.2-7	nnet_7.3-12
[17] gridExtra_2.2.1	GGally_1.2.0
[19] gQTLBase_1.6.0	chron_2.3-47
[21] bit_1.1-12	sendmailR_1.2-1
[23] rtracklayer_1.34.0	ggbio_1.22.0
[25] scales_0.4.0	checkmate_1.8.1
[27] BatchJobs_1.6	RBGL_1.50.0
[29] stringr_1.1.0	digest_0.6.10
[31] Rsamtools_1.26.0	foreign_0.8-67
[33] XVector_0.14.0	htmltools_0.3.5
[35] dichromat_2.0-0	base64enc_0.1-3
[37] ensemblDb_1.6.0	limma_3.30.0
[39] BSgenome_1.42.0	RSQLite_1.0.0
[41] shiny_0.14.1	BBmisc_1.10
[43] BiocInstaller_1.24.0	BiocParallel_1.8.0
[45] acepack_1.3-3.3	dplyr_0.5.0
[47] VariantAnnotation_1.20.0	RCurl_1.95-4.8
[49] magrittr_1.5	Formula_1.2-1
[51] Matrix_1.2-7.1	Rcpp_0.12.7
[53] munsell_0.4.3	stringi_1.1.2
[55] SummarizedExperiment_1.4.0	zlibbioc_1.20.0
[57] AnnotationHub_2.6.0	fail_1.3
[59] plyr_1.8.4	snpStats_1.24.0
[61] lattice_0.20-34	Biostrings_2.42.0
[63] splines_3.3.1	ffbase_0.12.3
[65] reshape2_1.4.1	codetools_0.2-15
[67] biomaRt_2.30.0	fastmatch_1.0-4

[69]	XML_3.98-1.4	biovizBase_1.22.0
[71]	latticeExtra_0.6-28	data.table_1.9.6
[73]	httpuv_1.3.3	gtable_0.2.0
[75]	reshape_0.8.5	assertthat_0.1
[77]	ggplot2_2.1.0	gQTLstats_1.6.0
[79]	mime_0.5	xtable_1.8-2
[81]	ff_2.2-13	survival_2.39-5
[83]	tibble_1.2	GenomicAlignments_1.10.0
[85]	beeswarm_0.2.3	cluster_2.0.5
[87]	interactiveDisplayBase_1.12.0	brew_1.0-6

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 Kutuyavin, 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.