

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\\narthrititis",  
+ "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)))

```

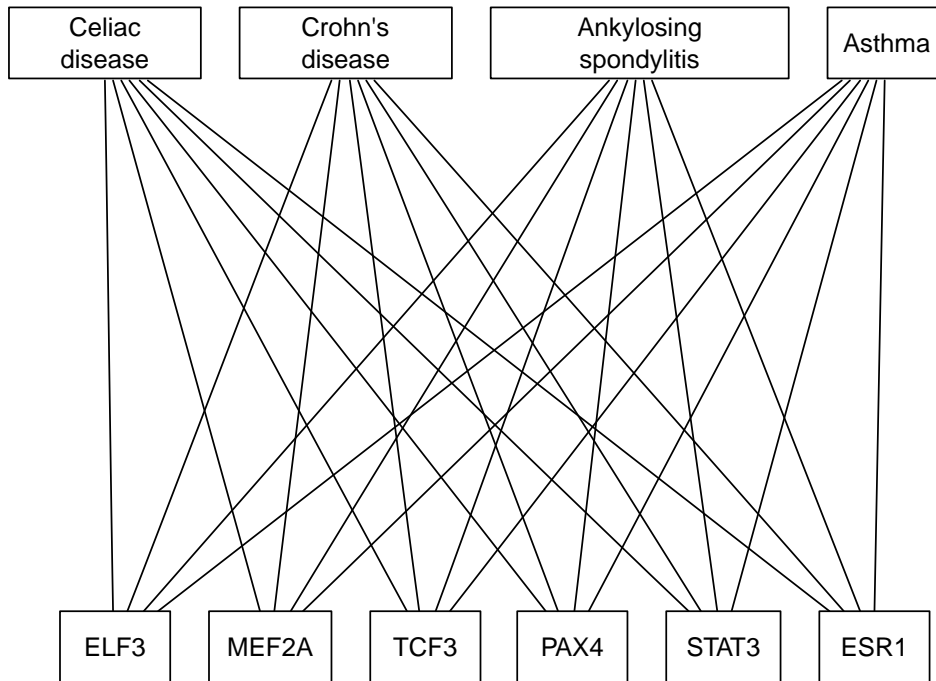


Figure 1: A complete bipartite graph for arbitrarily selected subsets of the autoimmune disorders and TFs found in Figure 4A of Maurano et al.

```
[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]	TAACCCTAACCCCTAACCCCAACCCCAACCC								
[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	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 <character>	p.Value <numeric>	Pvalue_mlog <numeric>	p.Value..text. <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 <numeric>	X95..CI..text. <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. <character>	CNV <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 <numeric>	dclass <character>	score <numeric>	tfstart <integer>	tfend <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(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(htag)
+   writeFasta(DNAStringSet(altg), file=faTargFun(htag))
+ }

```

7 Session information

```
> sessionInfo()
```

```

R version 3.6.0 (2019-04-26)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: Ubuntu 18.04.2 LTS

```

```
Matrix products: default
```

```

BLAS: /home/biocbuild/bbs-3.9-bioc/R/lib/libRblas.so
LAPACK: /home/biocbuild/bbs-3.9-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_0.24.0
- [2] doParallel_1.0.14
- [3] iterators_1.0.10
- [4] foreach_1.4.4
- [5] gwascat_2.16.0
- [6] Homo.sapiens_1.3.1
- [7] TxDb.Hsapiens.UCSC.hg19.knownGene_3.2.2
- [8] org.Hs.eg.db_3.8.2
- [9] GO.db_3.8.2
- [10] OrganismDbi_1.26.0
- [11] GenomicFeatures_1.36.0
- [12] AnnotationDbi_1.46.0
- [13] Biobase_2.44.0
- [14] GenomicRanges_1.36.0
- [15] GenomeInfoDb_1.20.0
- [16] IRanges_2.18.0
- [17] S4Vectors_0.22.0
- [18] Rgraphviz_2.28.0
- [19] graph_1.62.0
- [20] BiocGenerics_0.30.0

loaded via a namespace (and not attached):

- [1] SummarizedExperiment_1.14.0 progress_1.2.0
- [3] lattice_0.20-38 rtracklayer_1.44.0
- [5] blob_1.1.1 XML_3.98-1.19
- [7] RBGL_1.60.0 rlang_0.3.4
- [9] DBI_1.0.0 BiocParallel_1.18.0
- [11] bit64_0.9-7 matrixStats_0.54.0
- [13] GenomeInfoDbData_1.2.1 stringr_1.4.0
- [15] zlibbioc_1.30.0 Biostrings_2.52.0
- [17] codetools_0.2-16 memoise_1.1.0
- [19] biomaRt_2.40.0 Rcpp_1.0.1
- [21] BiocManager_1.30.4 DelayedArray_0.10.0
- [23] XVector_0.24.0 bit_1.1-14
- [25] Rsamtools_2.0.0 hms_0.4.2
- [27] digest_0.6.18 stringi_1.4.3
- [29] tools_3.6.0 bitops_1.0-6
- [31] magrittr_1.5 RCurl_1.95-4.12
- [33] RSQLite_2.1.1 crayon_1.3.4
- [35] pkgconfig_2.0.2 Matrix_1.2-17
- [37] prettyunits_1.0.2 assertthat_0.2.1
- [39] httr_1.4.0 R6_2.4.0

[41] GenomicAlignments_1.20.0 compiler_3.6.0

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 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.