

# *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)))

```

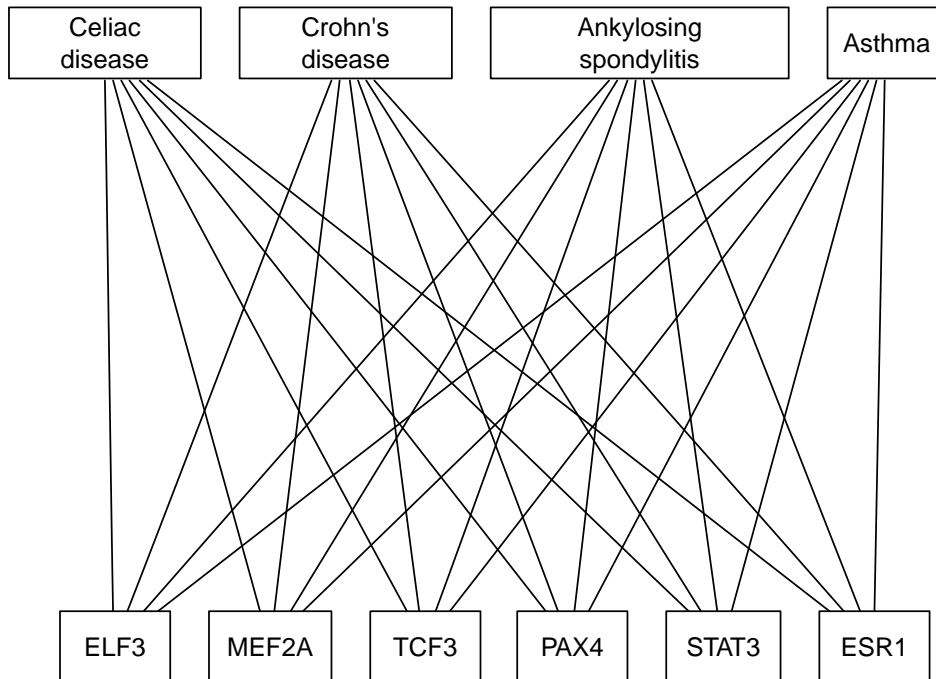


Figure 1: A complete bipartite graph for arbitrarily selected subsets of the autoimmune disorders and TFs found in Figure 4A of Maurano et al.

```
[1] 1862156
```

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

```
GRanges object with 4 ranges and 8 metadata columns:
```

	seqnames <Rle>	ranges <IRanges>	strand <Rle>	source <factor>	type <factor>	score <numeric>
[1]	chr1	10273-10302	+	fimo	nucleotide_motif	999.917
[2]	chr1	10279-10308	+	fimo	nucleotide_motif	999.962
[3]	chr1	11703-11732	-	fimo	nucleotide_motif	999.999
[4]	chr1	11704-11733	-	fimo	nucleotide_motif	999.955
	phase <integer>	Name <character>	pvalue <character>	qvalue <character>		
[1]	<NA>	+Mmusculus-JASPAR_CO..	8.35e-05	0.396		
[2]	<NA>	+Mmusculus-JASPAR_CO..	3.79e-05	0.361		
[3]	<NA>	-Mmusculus-JASPAR_CO..	8.04e-07	0.194		
[4]	<NA>	-Mmusculus-JASPAR_CO..	4.46e-05	0.368		
	sequence <character>					
[1]	TAACCCTAACCCCTAACCCCA..					
[2]	TAACCCTAACCCCAACCCCA..					
[3]	AAAAAAAATACACATGGCCAG..					
[4]	TAAAAAAAATACACATGGCCA..					

```
-----  
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)
> requireNamespace("gwascat")
> load(system.file("legacy/gwrngs19.rda", package="gwascat"))
> 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 B..		
13650	Turnbull C	06/13/2010	Nat Genet		
15145	Papaemmanuil E	08/16/2009	Nat Genet		
	Link	Study	Disease.Trait		
	<character>	<character>	<character>		
3475	<a href="http://www.ncbi.nlm...">http://www.ncbi.nlm...</a>	Large-scale genotypi..	Breast cancer		
3480	<a href="http://www.ncbi.nlm...">http://www.ncbi.nlm...</a>	Large-scale genotypi..	Breast cancer		
6963	<a href="http://www.ncbi.nlm...">http://www.ncbi.nlm...</a>	Prognostic implicati..	Non-small cell lung ..		
7155	<a href="http://www.ncbi.nlm...">http://www.ncbi.nlm...</a>	Genome-wide associat..	Lung Cancer (DNA rep..		
7480	<a href="http://www.ncbi.nlm...">http://www.ncbi.nlm...</a>	A meta-analysis of g..	Breast cancer		
12585	<a href="http://www.ncbi.nlm...">http://www.ncbi.nlm...</a>	Genome-wide associat..	Erectile dysfunction..		
13650	<a href="http://www.ncbi.nlm...">http://www.ncbi.nlm...</a>	Variants near DMRT1,..	Testicular germ cell..		

15145 <a href="http://www.ncbi.nlm...">http://www.ncbi.nlm...</a> Loci on 7p12.2, 10q2.. Acute lymphoblastic ..		Initial.Sample.Size	Replication.Sample.Size	Region	Chr_id	
		<character>	<character>	<character>	<character>	
3475	10,052	European ance..	45,290	European ance..	8q24.21	8
3480	10,052	European ance..	45,290	European ance..	11q13.1	11
6963	348	Korean ancestry ..		NR	2p23.3	2
7155	914	European ancestr..	679	European ancestr..	6q24.2	6
7480	3,666	European ances..	562	European ancestr..	20q11.22	20
12585	27	African American ..		<NA>	Xp11.4	23
13650	979	European ancestr..	664	European ancestr..	12p13.1	12
15145	907	European ancestr..		<NA>	10q21.2	10
Chr_pos.hg38	Reported.Gene.s.		Mapped_gene			
<numeric>	<character>		<character>			
3475	128182395	MIR1208, MYC	MIR1208	- LINC01263		
3480	65815595	DKFZp761E198, OVOL1,..	OVOL1-AS1	- SNX32		
6963	26303551	GPR113	HADHB	- GPR113		
7155	143622177	PHACTR2	PHACTR2			
7480	34000289	RALY, EIF2S2, ASIP	RALY			
12585	37995474	SYTL5	CXorf27	- SYTL5		
13650	14500933	ATF7IP	ATF7IP			
15145	61992400	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 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. OR.or.beta
	<character>	<numeric>	<numeric>	<character> <numeric>
3475	0.16	3e-11	10.52288	1.07
3480	0.53	9e-12	11.04576	1.05
6963	0.052	4e-06	5.39794	(Additive model) NA
7155	0.3957	7e-06	5.15490	NA
7480	0.31	1e-08	8.00000	1.16
12585	0.03	9e-06	5.04576	11.78
13650	0.62	6e-10	9.22185	1.27
15145	0.34	7e-19	18.15490	1.65
	X95..CI..text.	Platform..SNPs.passing.QC.	CNV	
	<character>	<character>	<character>	
3475	[1.04-1.10]	Illumina & Affymetri..	N	
3480	[1.04-1.08]	Illumina & Affymetri..	N	
6963	NR	Affymetrix [271,817]	N	
7155	NR	Illumina [303,669]	N	
7480	[1.10-1.22]	Illumina [2,608,509]..	N	
12585	[NR]	Affymetrix [512,497]	N	
13650	[1.12-1.44]	Illumina [298,782]	N	
15145	[1.54-1.76]	Illumina [291,473]	N	
	num.Risk.Allele.Frequency	dclass	score	tfstart tfend
	<numeric>	<character>	<numeric>	<integer> <integer>
3475	0.1600	Breast	999.985	129194621 129194650
3480	0.5300	Breast	999.952	65583065 65583094
6963	0.0520	Lung	999.987	26526415 26526444
7155	0.3957	Lung	999.939	143943292 143943321
7480	0.3100	Breast	999.928	32588075 32588104
12585	0.0300	Prostate	999.903	37854721 37854750
13650	0.6200	Testicular	999.990	14653848 14653877
15145	0.3400	ALL (ped)	999.962	63752142 63752171
	pvalue	qvalue		
	<numeric>	<numeric>		
3475	1.49e-05	0.318		



3480	4.83e-05	0.373
6963	1.25e-05	0.310
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 4.2.1 (2022-06-23)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: Ubuntu 20.04.5 LTS

```

```

Matrix products: default
BLAS:   /home/biocbuild/bbs-3.16-bioc/R/lib/libRblas.so
LAPACK: /home/biocbuild/bbs-3.16-bioc/R/lib/libRlapack.so

```

```

locale:
 [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
 [3] LC_TIME=en_GB            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] parallel stats4 grid stats graphics grDevices utils
 [8] datasets methods base

```

```

other attached packages:
 [1] vtpnet_0.38.0 doParallel_1.0.17 iterators_1.0.14
 [4] foreach_1.5.2 gwascat_2.30.0 GenomicRanges_1.50.0
 [7] GenomeInfoDb_1.34.0 IRanges_2.32.0 S4Vectors_0.36.0
[10] Rgraphviz_2.42.0 graph_1.76.0 BiocGenerics_0.44.0

```

```

loaded via a namespace (and not attached):
 [1] MatrixGenerics_1.10.0 Biobase_2.58.0
 [3] httr_1.4.4 splines_4.2.1

```

[5]	bit64_4.0.5	assertthat_0.2.1
[7]	BiocFileCache_2.6.0	blob_1.2.3
[9]	BSSgenome_1.66.0	GenomeInfoDbData_1.2.9
[11]	Rsamtools_2.14.0	yaml_2.3.6
[13]	progress_1.2.2	pillar_1.8.1
[15]	RSQLite_2.2.18	lattice_0.20-45
[17]	glue_1.6.2	digest_0.6.30
[19]	XVector_0.38.0	Matrix_1.5-1
[21]	XML_3.99-0.12	pkgconfig_2.0.3
[23]	biomaRt_2.54.0	zlibbioc_1.44.0
[25]	tzdb_0.3.0	BiocParallel_1.32.0
[27]	tibble_3.1.8	KEGGREST_1.38.0
[29]	generics_0.1.3	ellipsis_0.3.2
[31]	cachem_1.0.6	SummarizedExperiment_1.28.0
[33]	GenomicFeatures_1.50.0	cli_3.4.1
[35]	survival_3.4-0	magrittr_2.0.3
[37]	crayon_1.5.2	memoise_2.0.1
[39]	fansi_1.0.3	xml2_1.3.3
[41]	tools_4.2.1	prettyunits_1.1.1
[43]	hms_1.1.2	BiocIO_1.8.0
[45]	lifecycle_1.0.3	matrixStats_0.62.0
[47]	stringr_1.4.1	DelayedArray_0.24.0
[49]	snpStats_1.48.0	AnnotationDbi_1.60.0
[51]	Biostrings_2.66.0	compiler_4.2.1
[53]	rlang_1.0.6	RCurl_1.98-1.9
[55]	VariantAnnotation_1.44.0	rjson_0.2.21
[57]	rappdirs_0.3.3	bitops_1.0-7
[59]	restfulr_0.0.15	codetools_0.2-18
[61]	DBI_1.1.3	curl_4.3.3
[63]	R6_2.5.1	GenomicAlignments_1.34.0
[65]	dplyr_1.0.10	rtracklayer_1.58.0
[67]	fastmap_1.1.0	bit_4.0.4
[69]	utf8_1.2.2	filelock_1.0.2
[71]	readr_2.1.3	stringi_1.7.8
[73]	Rcpp_1.0.9	vctrs_0.5.0
[75]	png_0.1-7	dbplyr_2.2.1
[77]	tidyselect_1.2.0	

## 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.