Pan-cancer network analysis identifies combinations of rare somatic mutations across pathways and protein complexes

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RECOMB 2015
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Raphael Lab
Department of Computer Science &
Center for Computational Molecular Biology

Identifying cancer driver genes

Science
AAAS

Cancer Genome Landscapes
Vogelstein *et al*. (2013)

“>99.9% of mutations are passengers”

“3-8 drivers per tumor"

Identifying cancer driver genes

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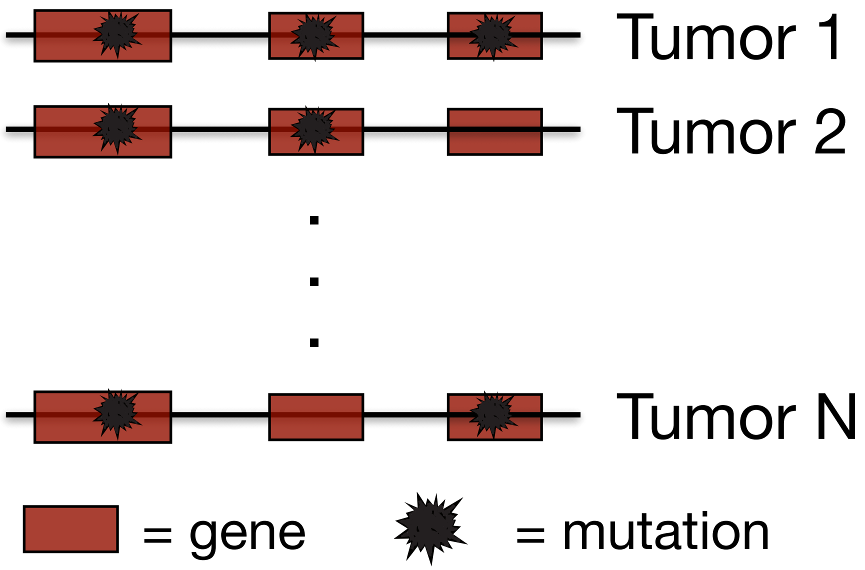
Compare variation across tumors



- Single nucleotide variants
- Copy number aberrations
- Gene expression
- ….

Identifying cancer driver genes

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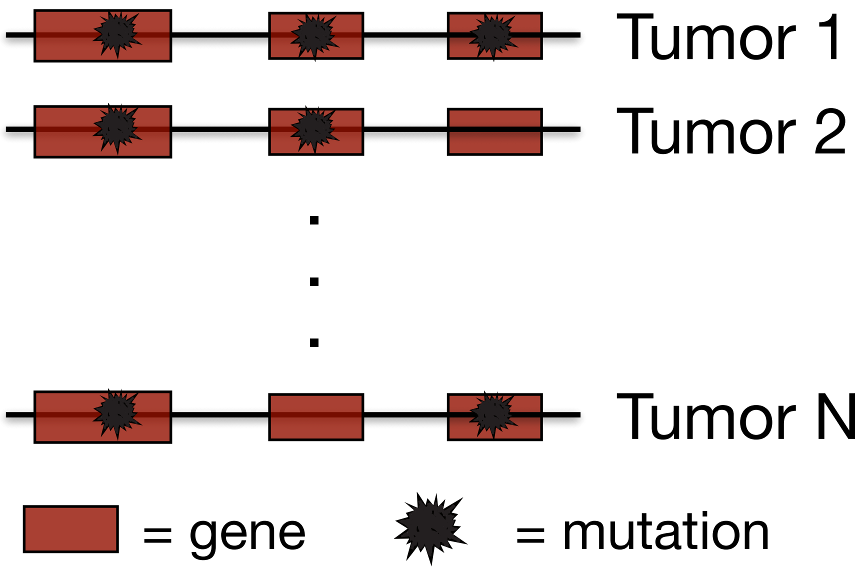

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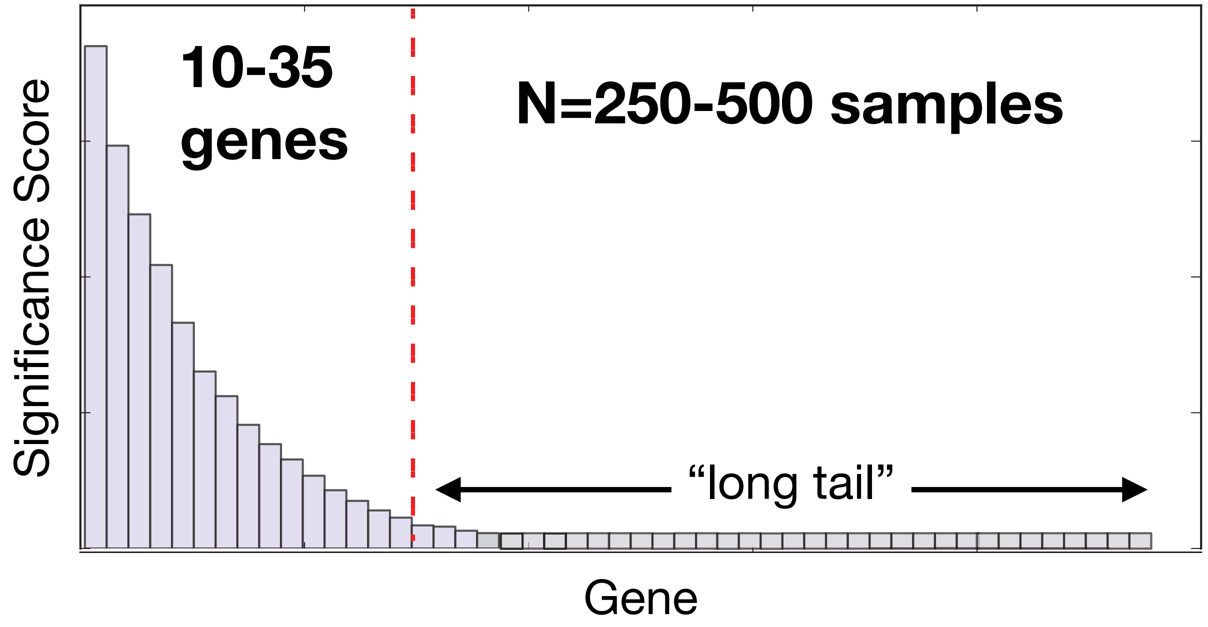

Mutations weighted by:

- Recurrence
- Gene length
- Mutation context
- Expression level
- Replication timing
- …

Cancer driver mutations target *pathways*

Driver mutations confer a growth advantage to the tumor → driver genes are members of cancer signaling pathways

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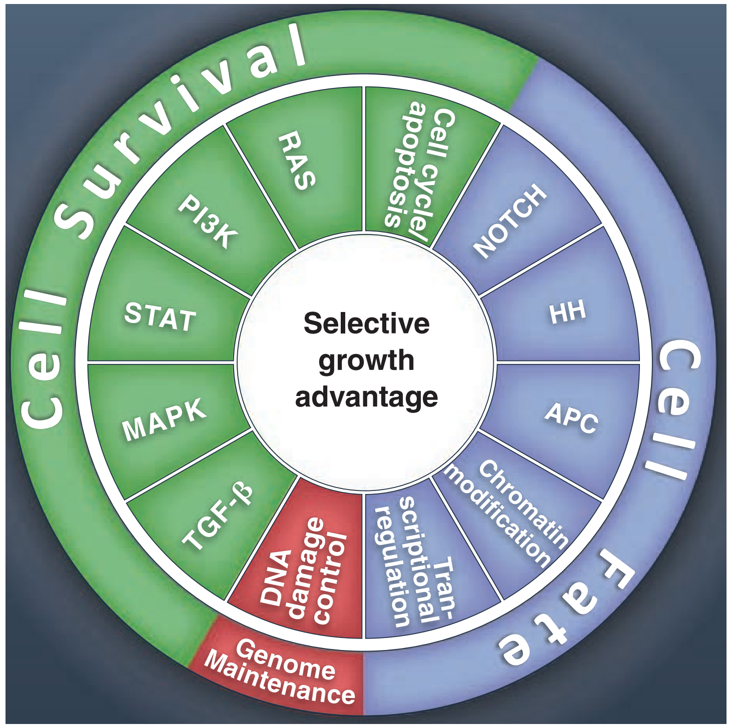

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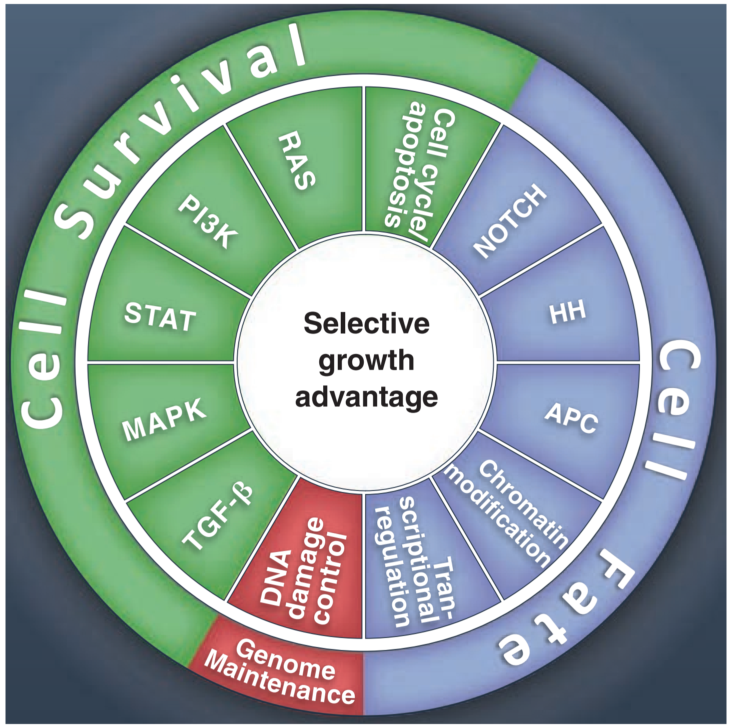

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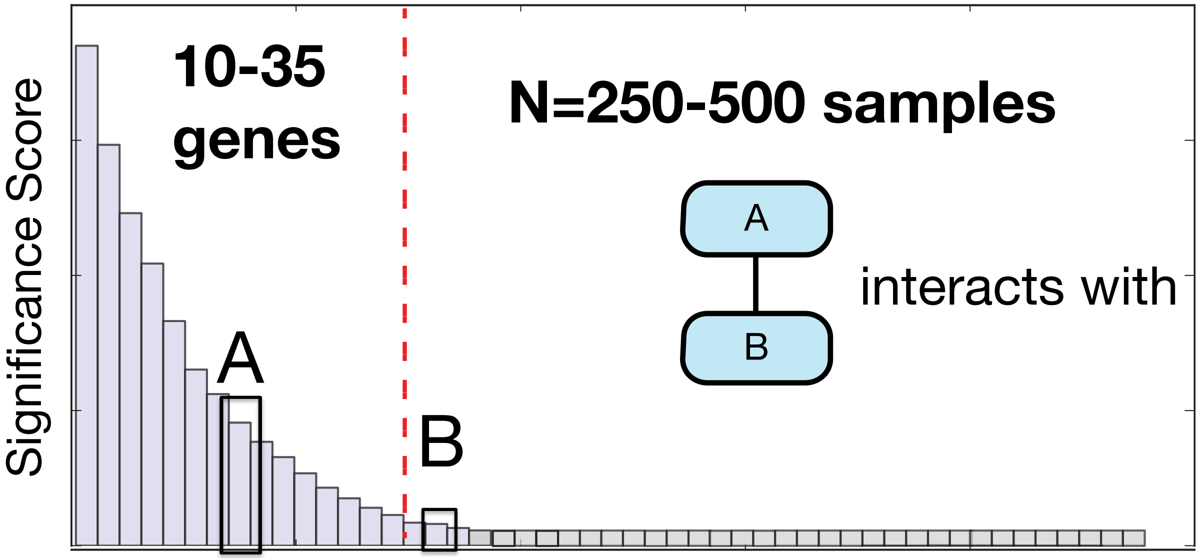

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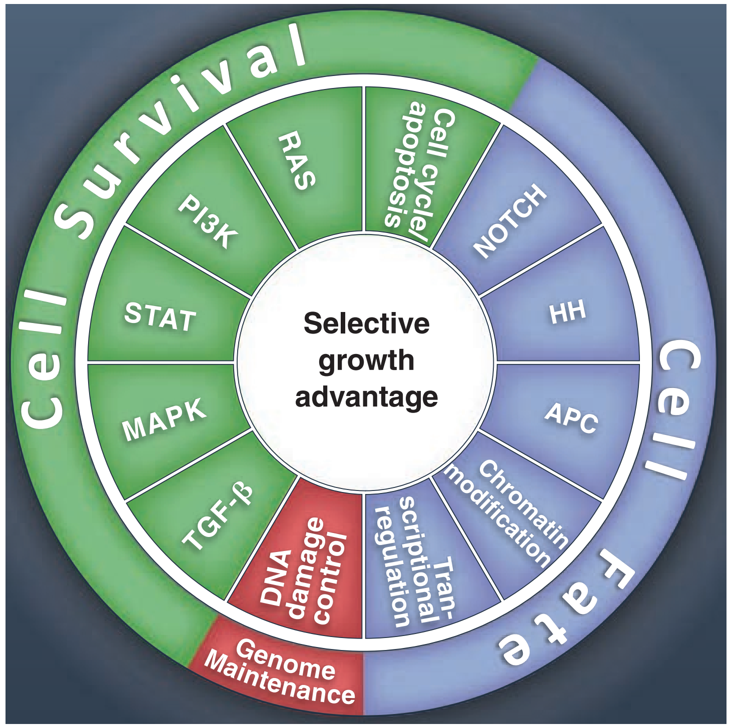

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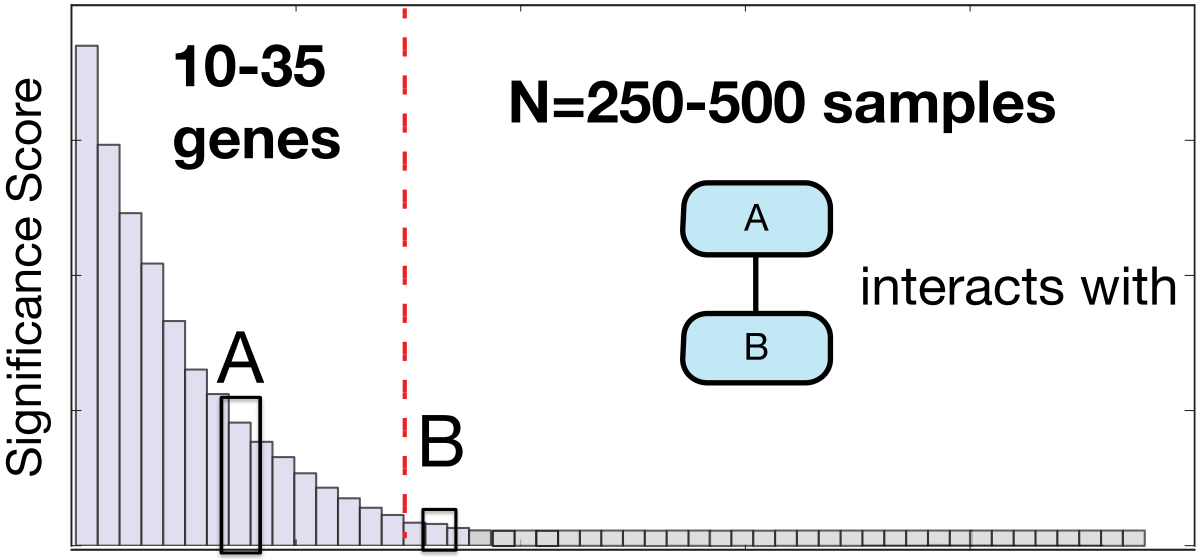
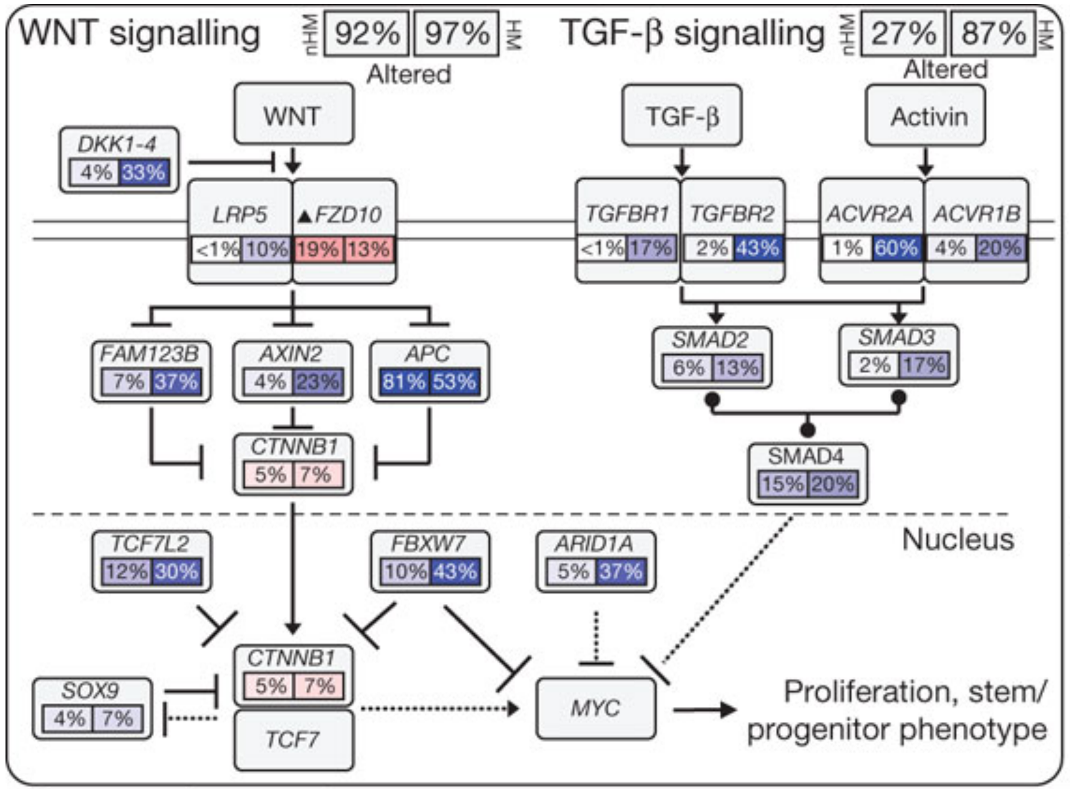


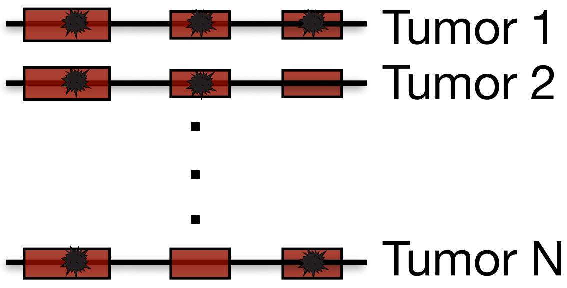


TCGA Ovarian (*Nature* 2011)

Testing known gene sets and pathways

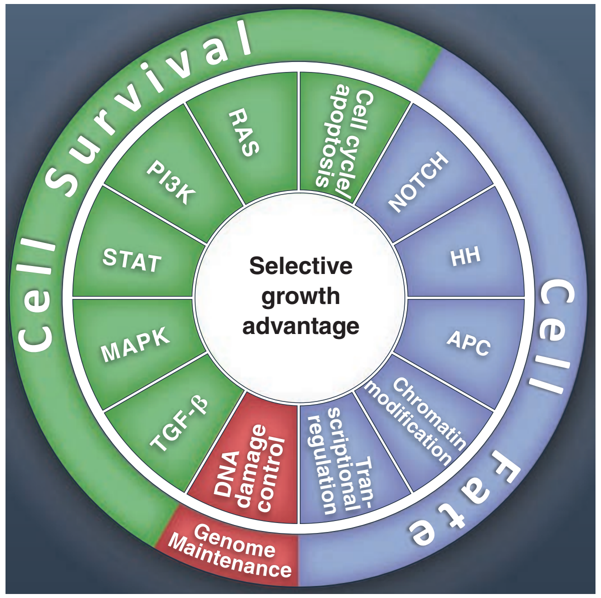
Input data

Mutation data



(e.g. most mutated genes: EGFR, KRAS, BRAF)

Gene set database



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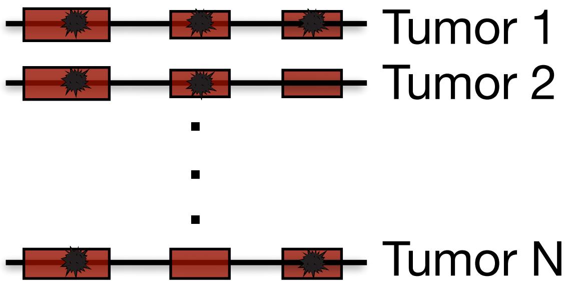

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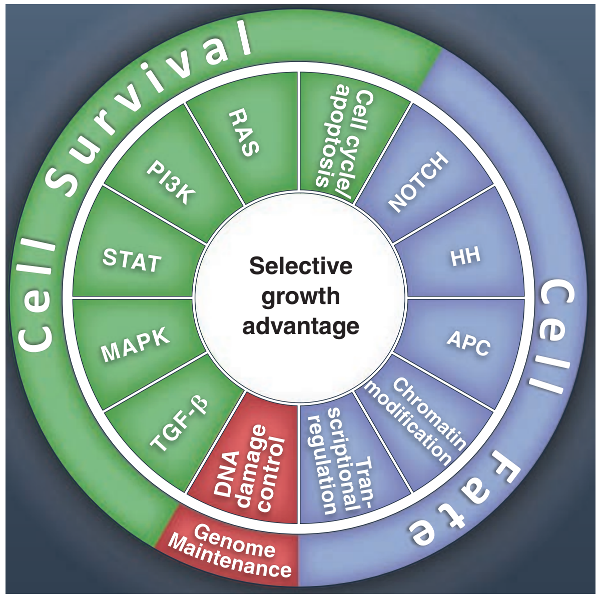

Enrichment tests

GSEA [1,2]

DAVID [3,4]

[1] Mootha *et al. Nat. Genet.* (2003).
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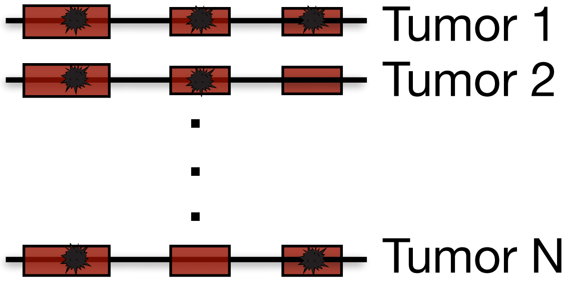

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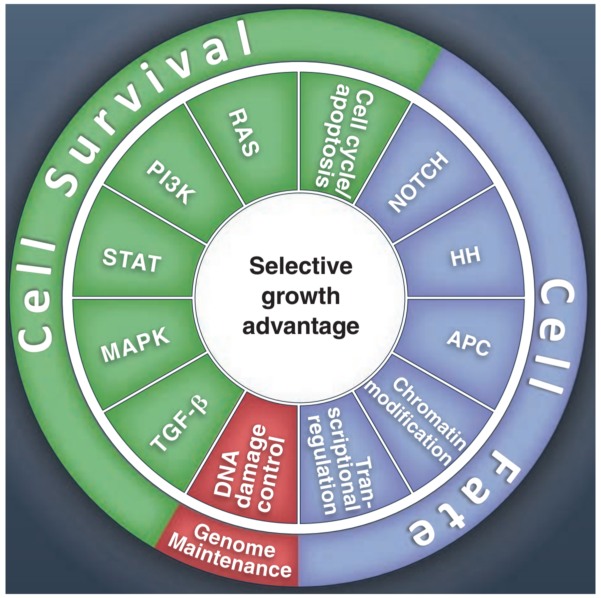

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GSEA [1,2]

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Enriched gene sets

MYC ARID1A
FBXW7 APC
SOX9 CTNNB1

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Testing known gene sets and pathways

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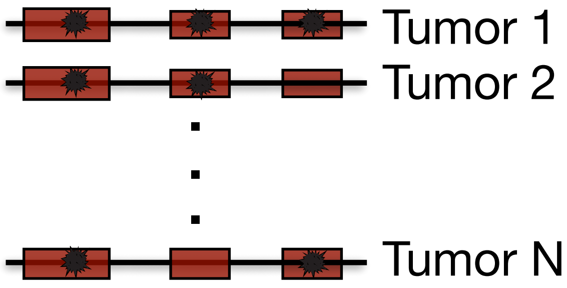

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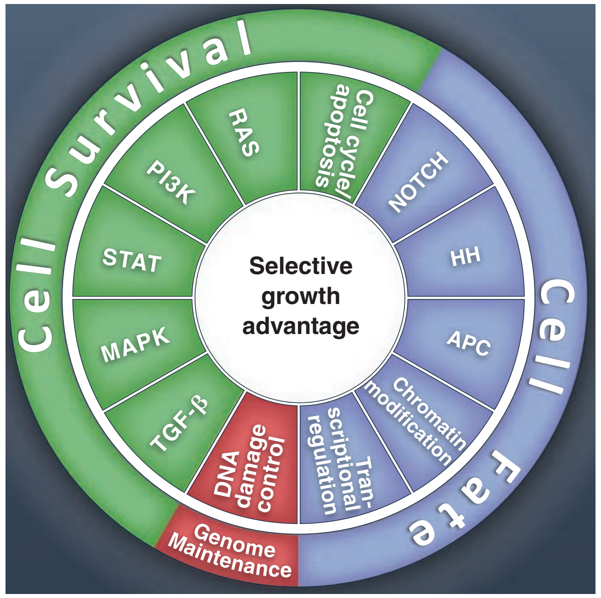

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Key drawbacks

- Novel pathways and *crosstalk*?
- Topology of interactions?
- Handling large and/or overlapping pathways?

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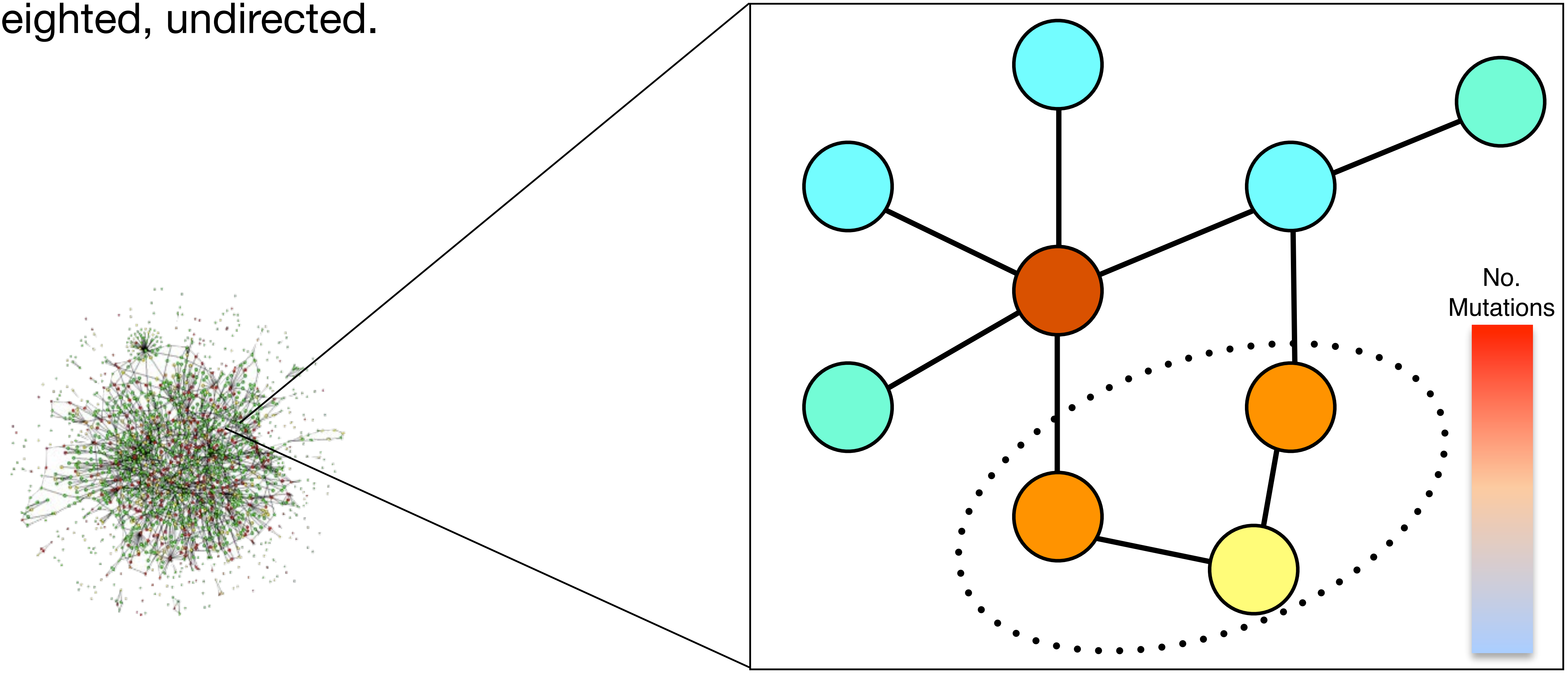
Significantly mutated subnetworks of a protein-protein interaction network

Protein-protein interaction networks

- Nodes: genes/protein
- Edges: connect genes if the proteins they encode physically interact.
- Unweighted, undirected.

Goal: identify connected subnetworks with more mutations than expected by chance.



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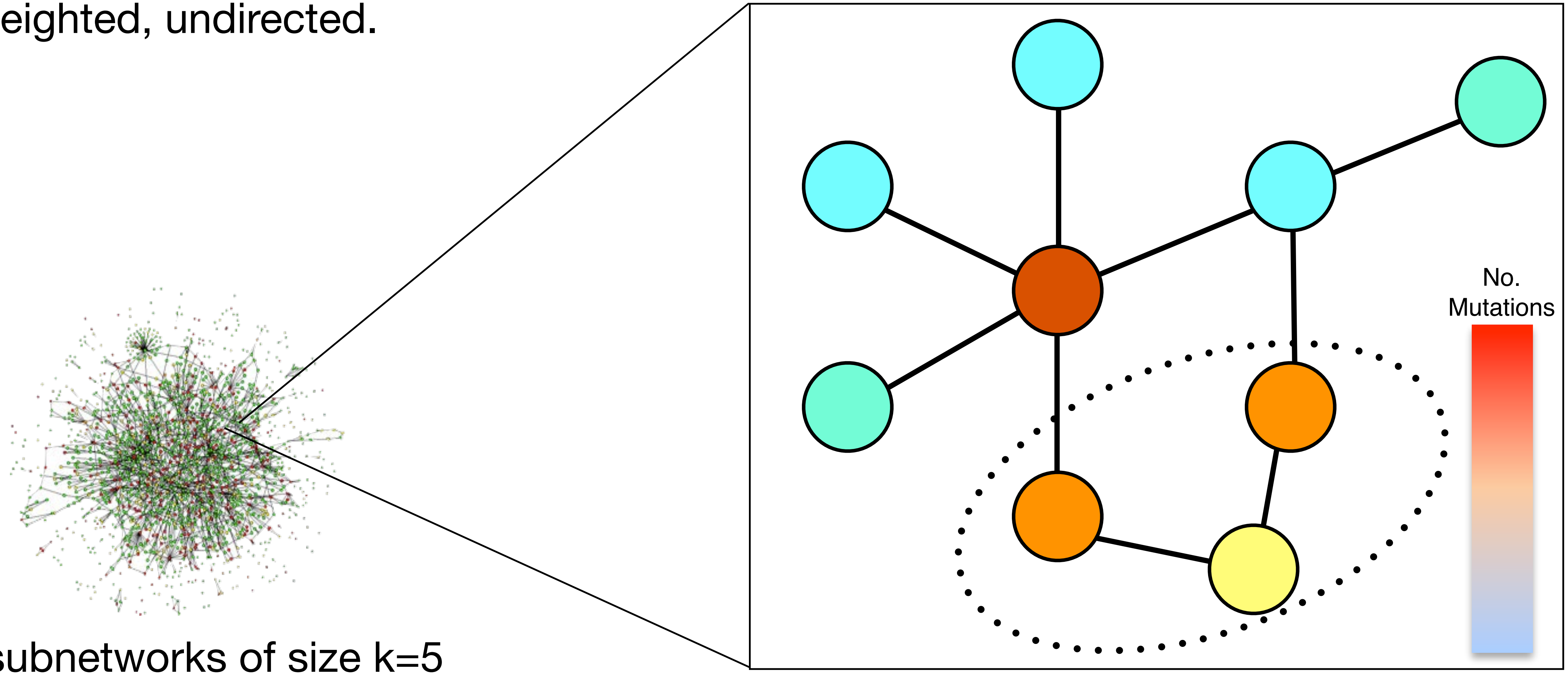


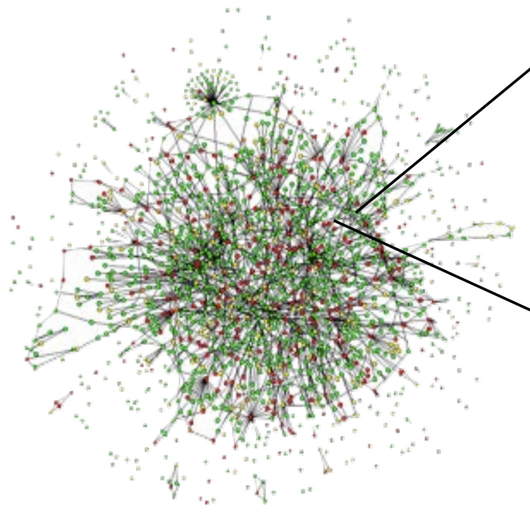
~10^{18} subnetworks of size k=5

Significantly mutated subnetworks of a protein-protein interaction network

Protein-protein interaction networks

Network	Nodes	Edges	Diameter	ASP
HPRD	9,205	36,720	**14**	**4.22**
HINT+HI2012	9,859	40,705	**14**	**4.08**
iRefIndex	12,129	91,809	**12**	**3.64**
MultiNet	14,399	109,570	**9**	**3.39**

Low diameter → Most genes have a high-scoring neighbor



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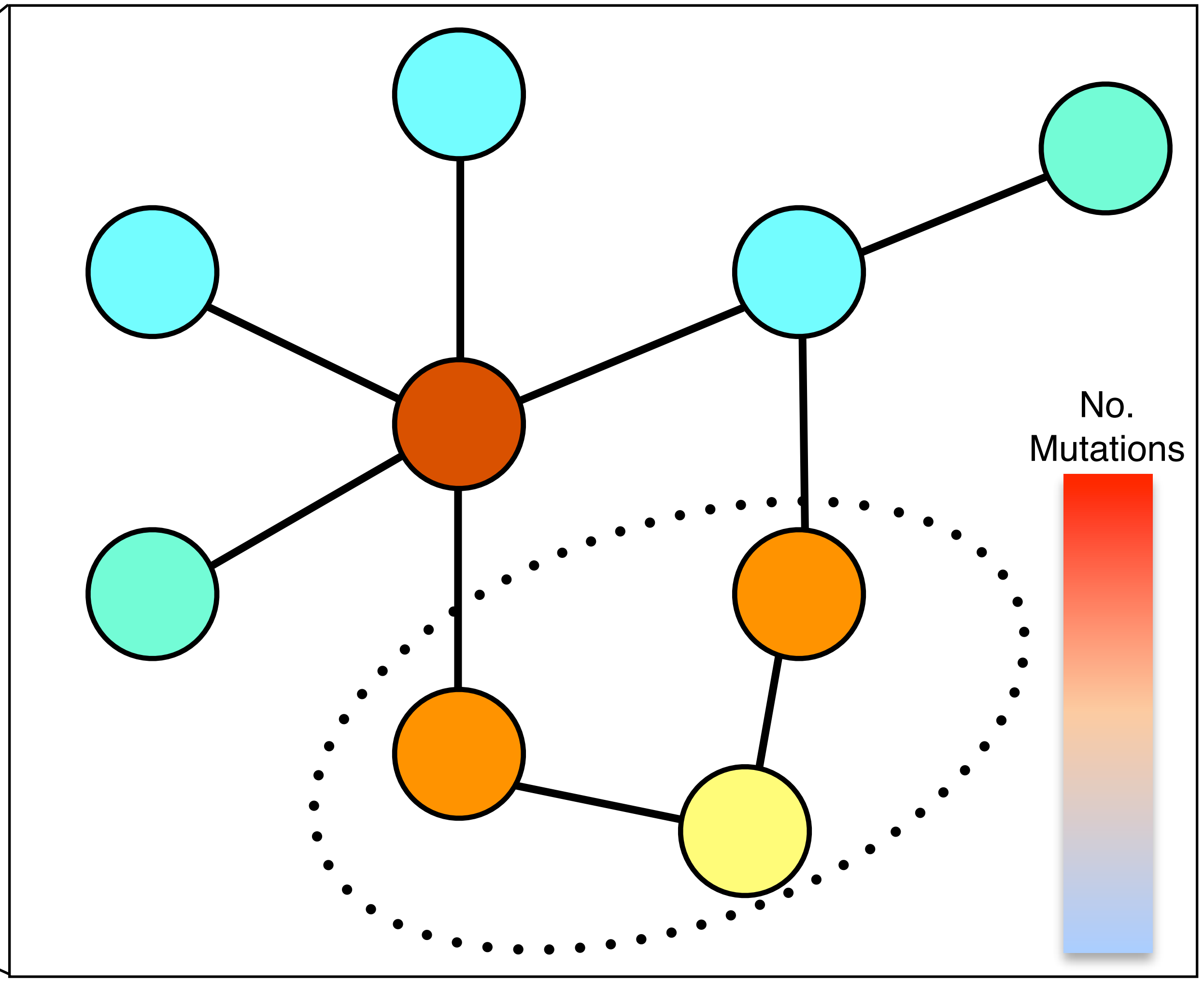

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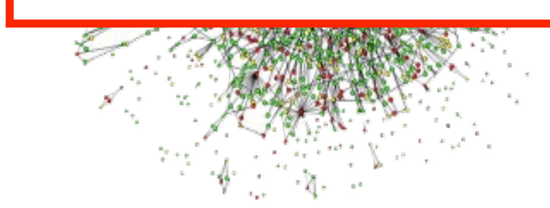

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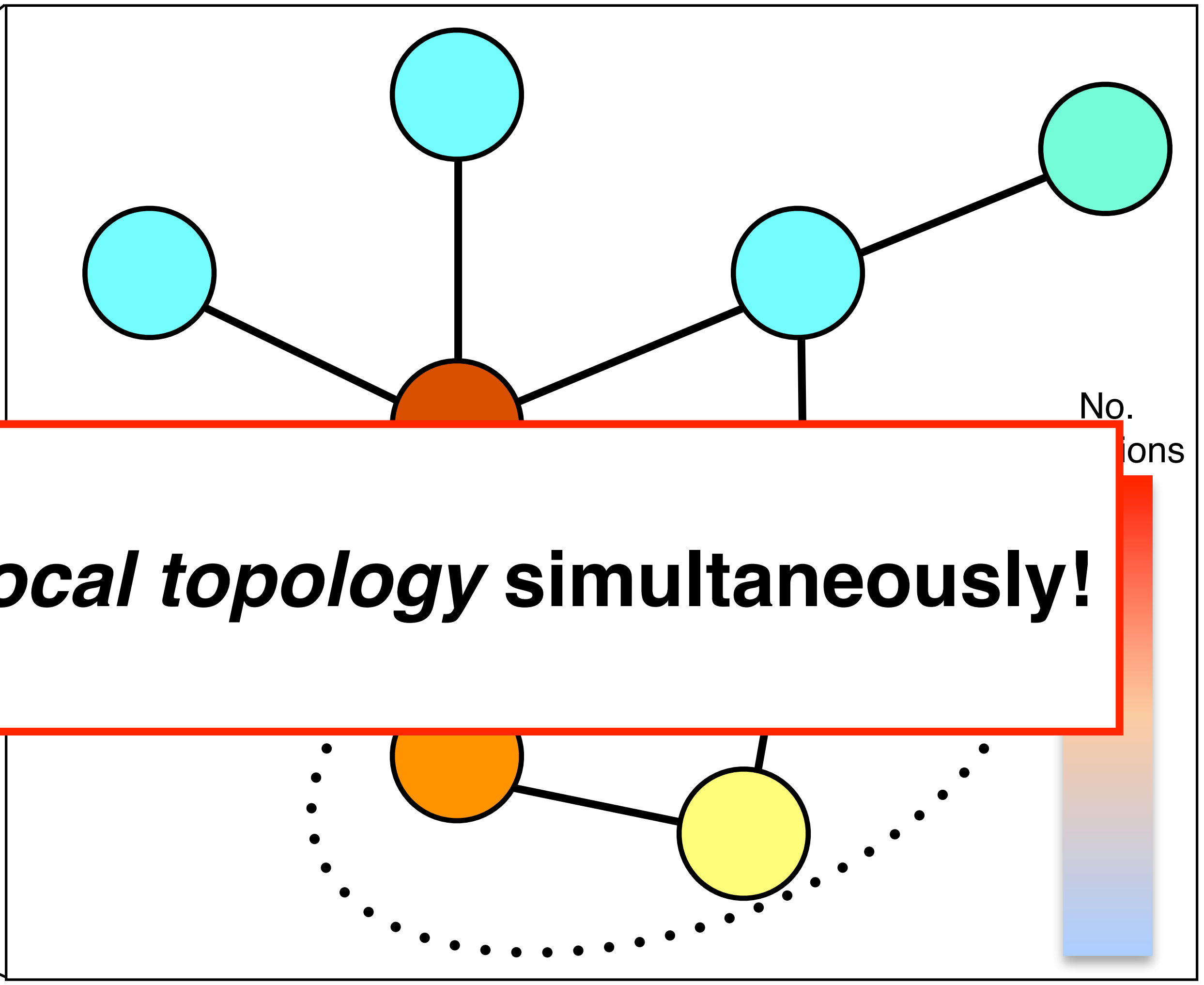
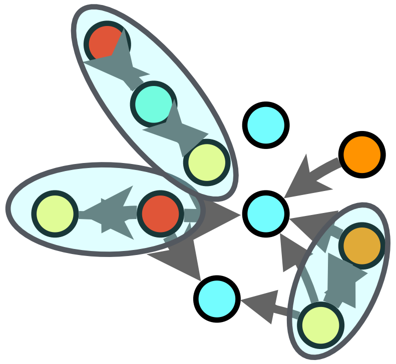


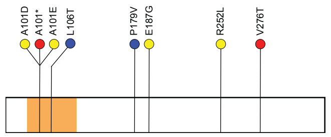
Must analyze mutations and *local topology* simultaneously!

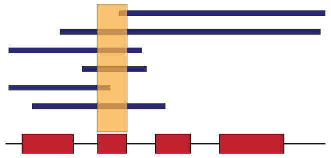
Outline

1. **A new algorithm, HotNet2.**



2. Application to TCGA Pan-Cancer data.

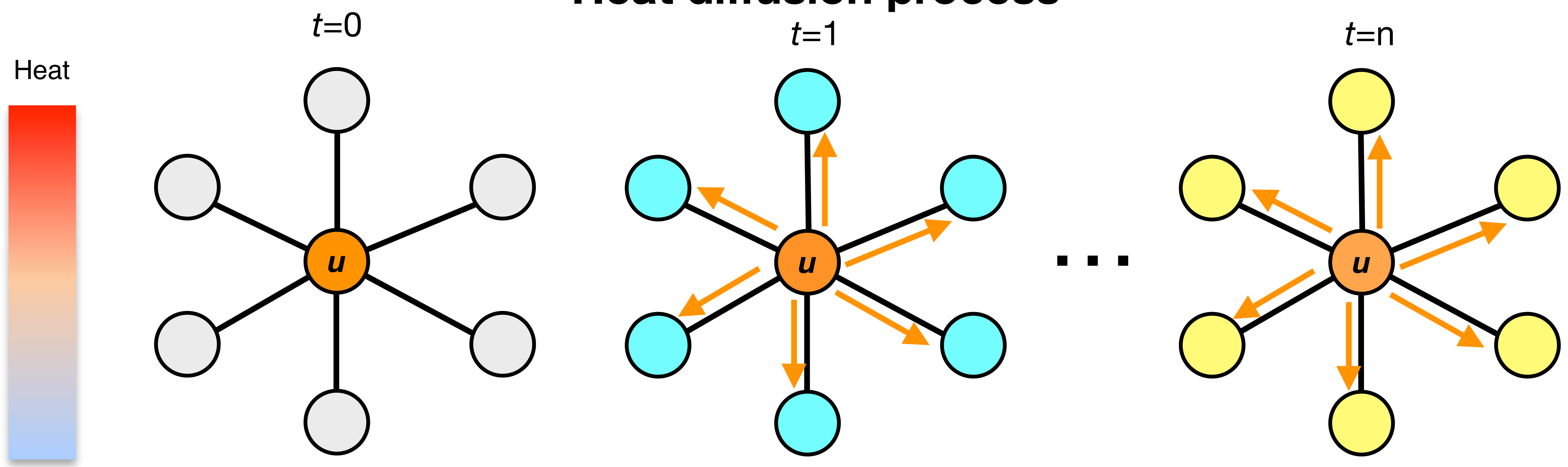




3. Comparison of HotNet2 to other methods.

Encoding mutations and graph topology with heat diffusion

Heat diffusion process

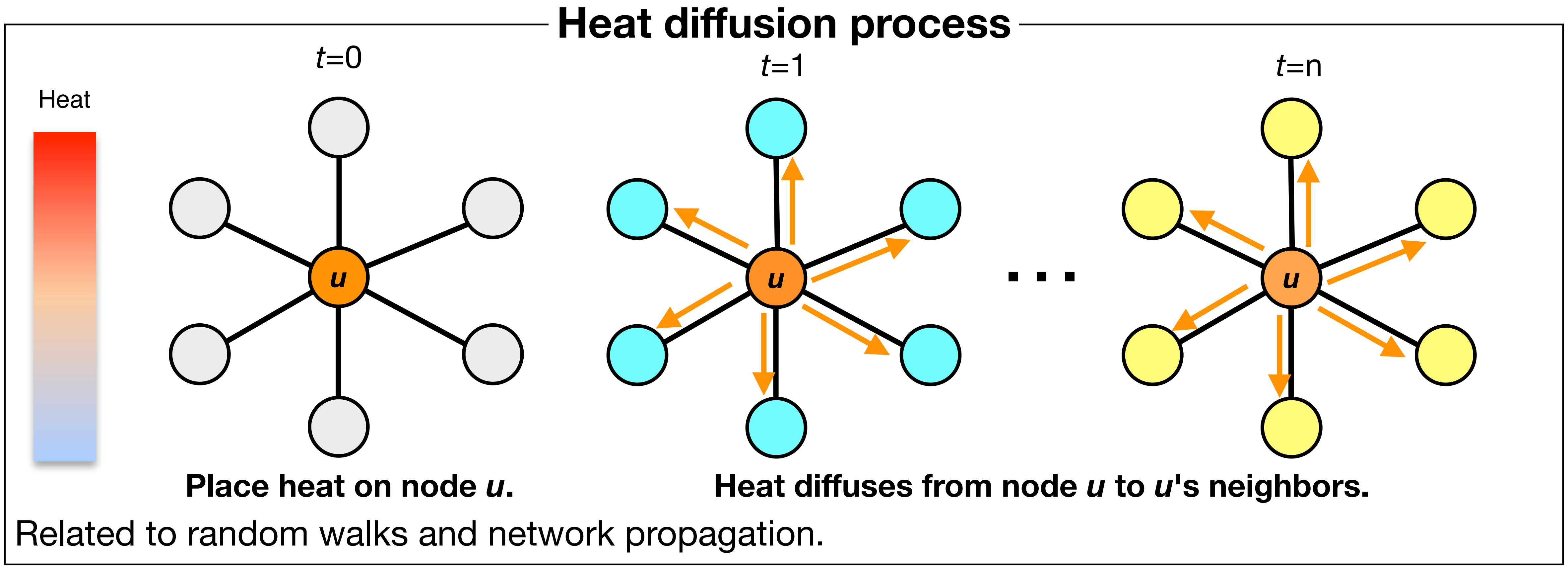


Place heat on node *u*.

Heat diffuses from node *u* to *u*'s neighbors.

Related to random walks and network propagation.

Encoding mutations and graph topology with heat diffusion



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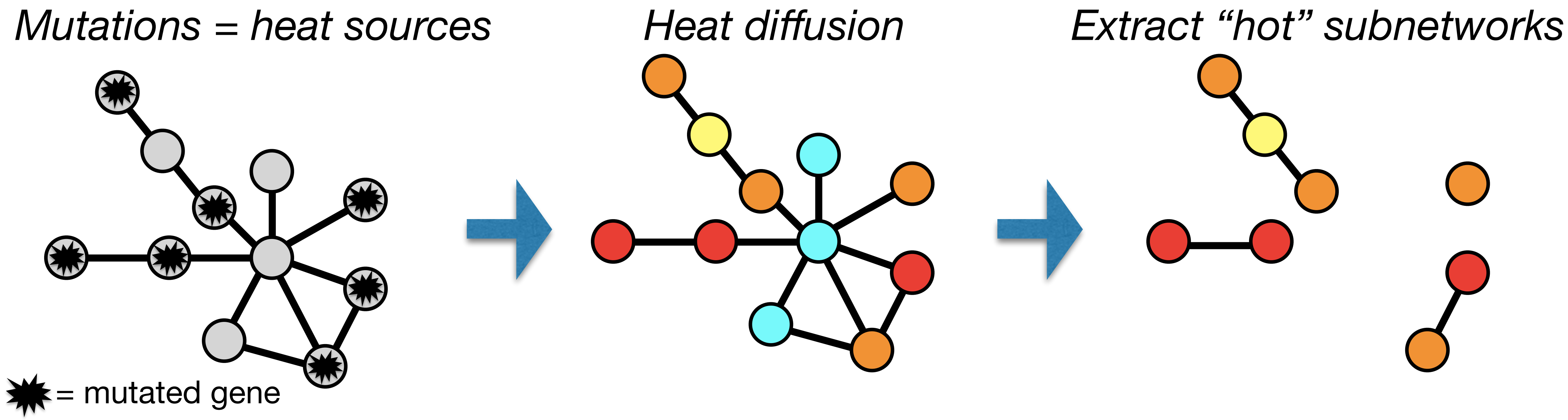
HotNet (Vandin *et al*. JCB & *RECOMB* 2010)



Mutations = heat sources

Heat diffusion

Extract “hot” subnetworks

HotNet applied to TCGA data

TCGA Papers
(~300 samples)

- Leukemia (*NEJM* 2013)
- Kidney (*Nature* 2011)
- Ovarian (*Nature* 2011)

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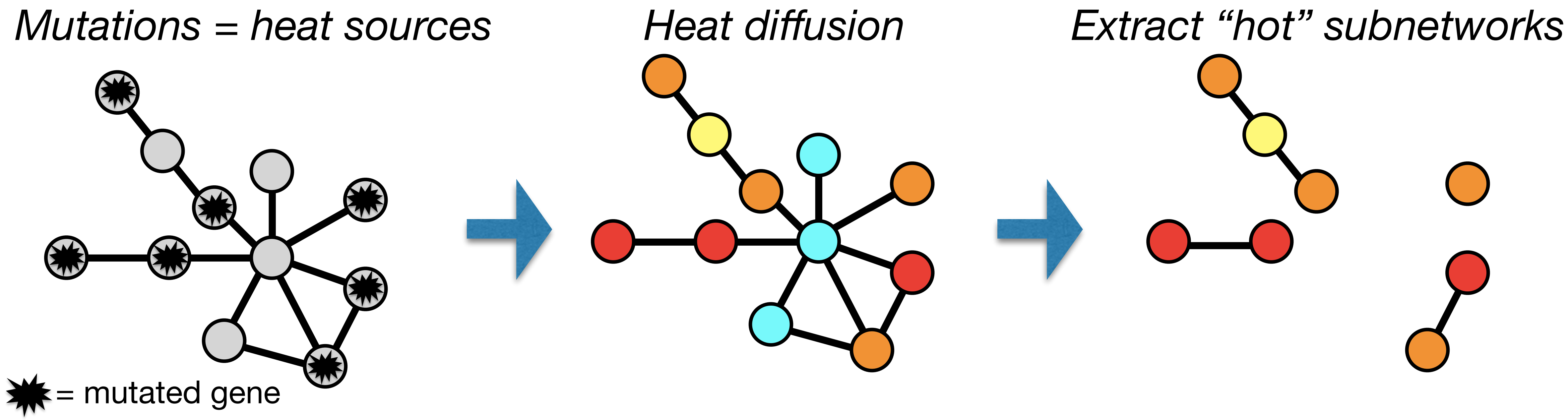

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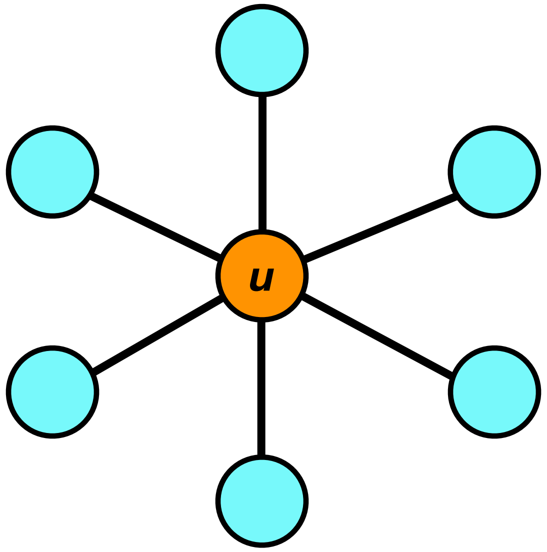
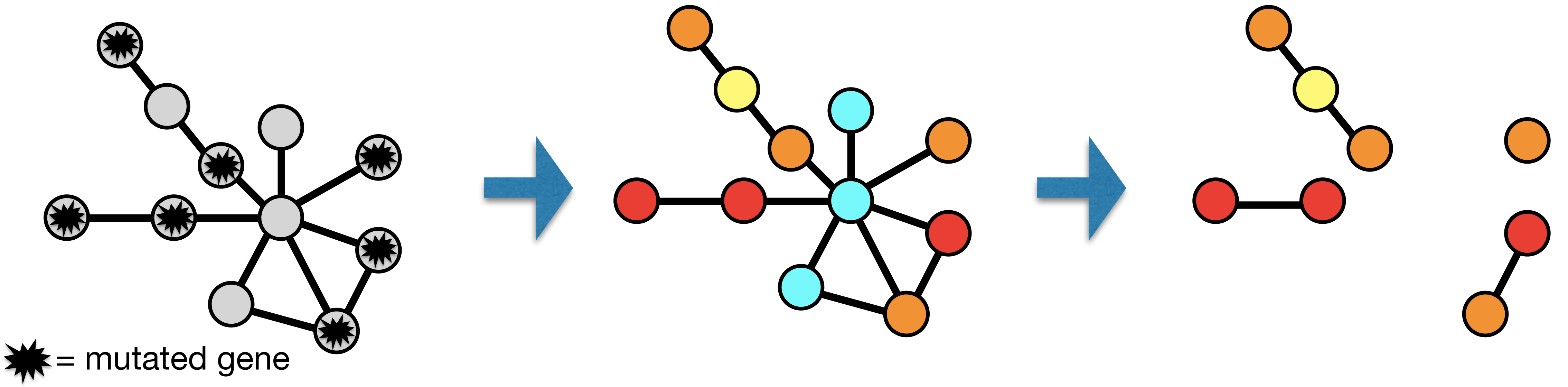
TCGA Pan-Cancer (>3000 samples)



HotNet finds many "star" subnetworks with one central, hot node

HotNet (Vandin *et al*. JCB & *RECOMB* 2010)

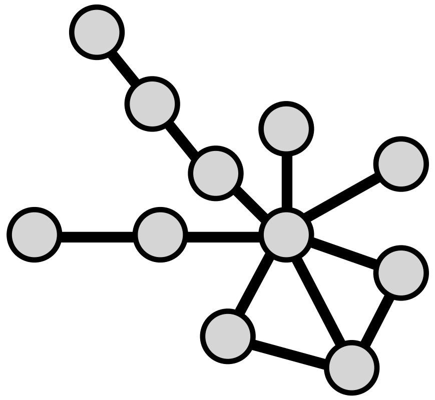
Mutations = heat sources *Heat diffusion* *Extract "hot" subnetworks*



= mutated gene

HotNet Algorithm

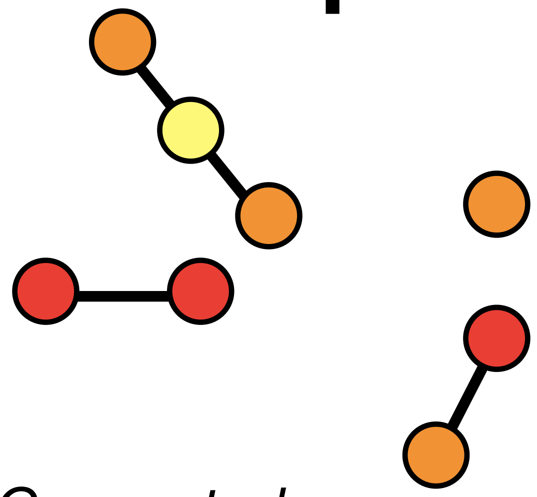
Input



$$\begin{pmatrix} h_1 \\ \vdots \\ h_n \end{pmatrix}$$

A = adjacency matrix **h** = gene scores

Output



Connected components

Threshold at δ

$$\begin{pmatrix} \text{Heat kernel} \\ f(A, t) \end{pmatrix} \begin{pmatrix} h_1 & & 0 \\ & \ddots & \\ 0 & & h_n \end{pmatrix} = \begin{pmatrix} s_{11} & \cdots & s_{1n} \\ \vdots & \ddots & \vdots \\ s_{n1} & \cdots & s_{nn} \end{pmatrix} \xrightarrow{\text{symmetrize}} \begin{pmatrix} r_{11} & \cdots & r_{1n} \\ \vdots & \ddots & \vdots \\ r_{n1} & \cdots & r_{nn} \end{pmatrix}$$

Diffusion matrix
*(**symmetric**)*

Similarity matrix
*(**asymmetric**)*

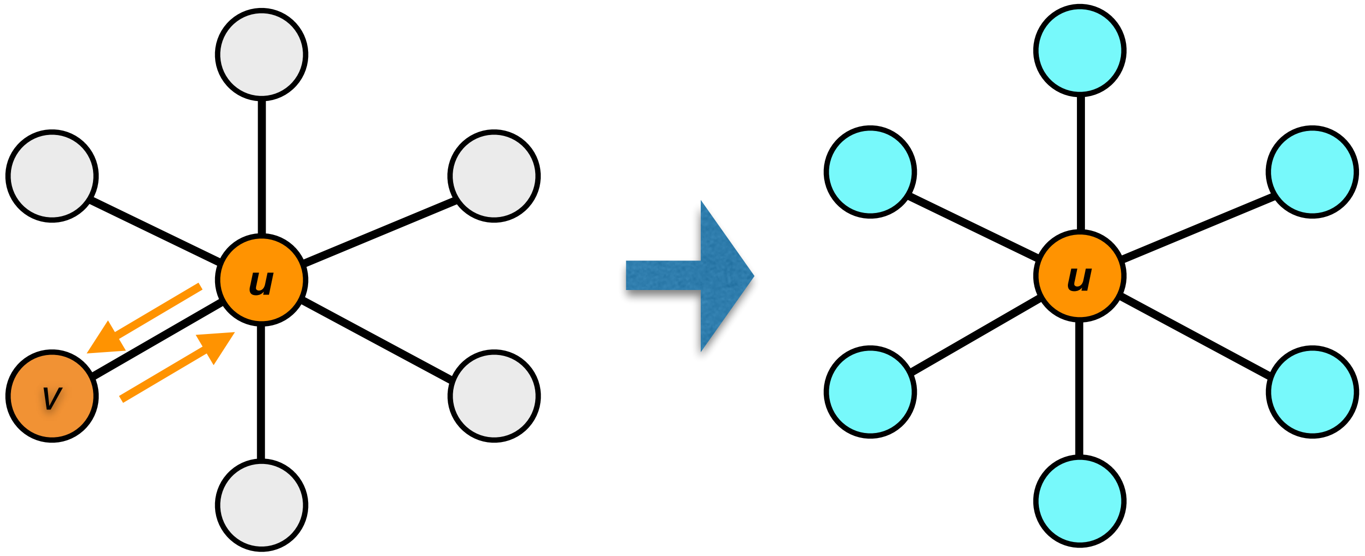
Time parameter t

s_{ij} = heat on vertex *i* at time *t* given initial heat h_j on vertex *j* at time 0.

Direction of heat is important →HotNet can fail

HotNet's heat is symmetric

u sends the same heat to *v* even though *u* has much higher degree



Star graph

Potential artifacts

Hot nodes with high degree often form large "star" subnetworks with many cold nodes

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Diffusion matrix
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Similarity matrix S
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s_{ij} = heat on vertex *i* at time *t* given initial heat h_j on vertex *j* at time 0.

HotNet2 algorithm

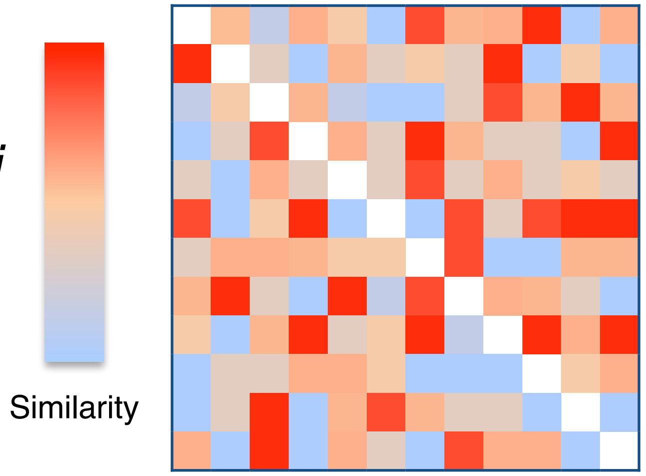
(HotNet diffusion oriented subnetworks)

Need to consider the source of heat

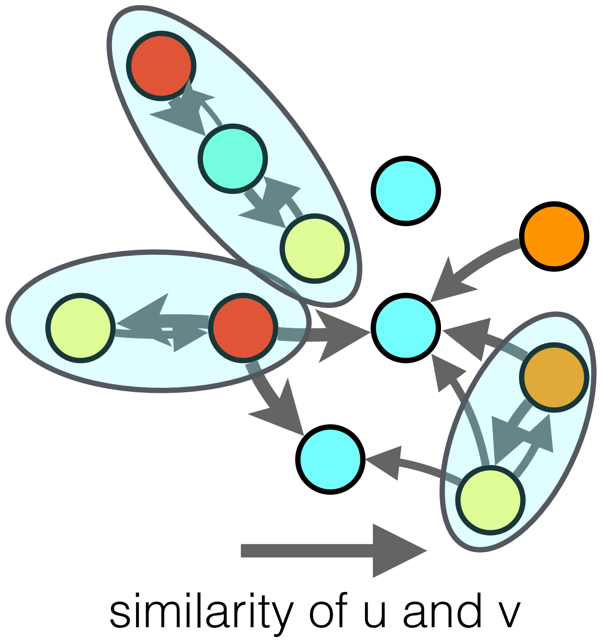
Encode directionality with asymmetric heat diffusion.

- Hot genes do not necessarily implicate their neighbors.
- Hot subnetworks have a directed path between each pair of nodes.

s_{ij} = heat on vertex *i* at *equilibrium* given initial heat h_j on vertex *j* at time 0.



Similarity matrix *S*
Asymmetric!



Identify *strongly* connected components

Leiserson, Vandin *et al. Nat. Genet.* (2015).
http://compbio.cs.brown.edu/projects/hotnet2

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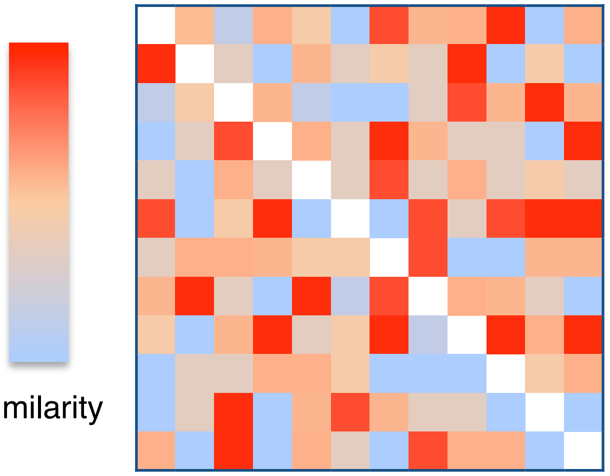

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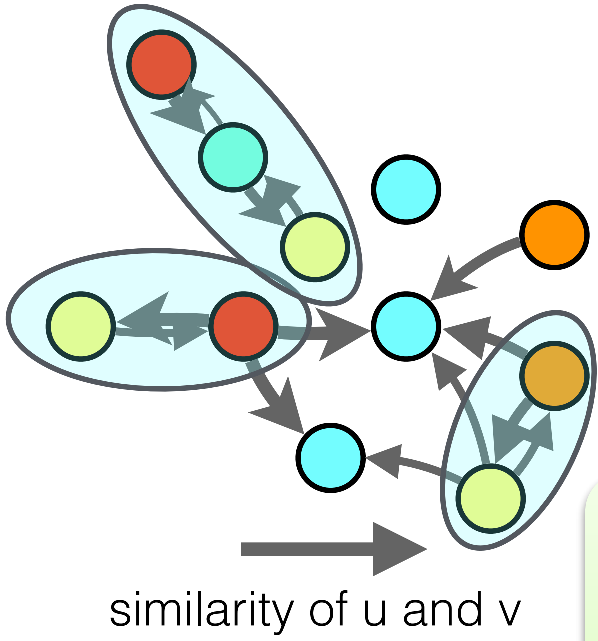

Identify *strongly* connected components

TCGA Papers

Gastric, *Nature* (2014)
Thyroid, *Cell* (2014)
…

Leiserson, Vandin *et al. Nat. Genet.* (2015).
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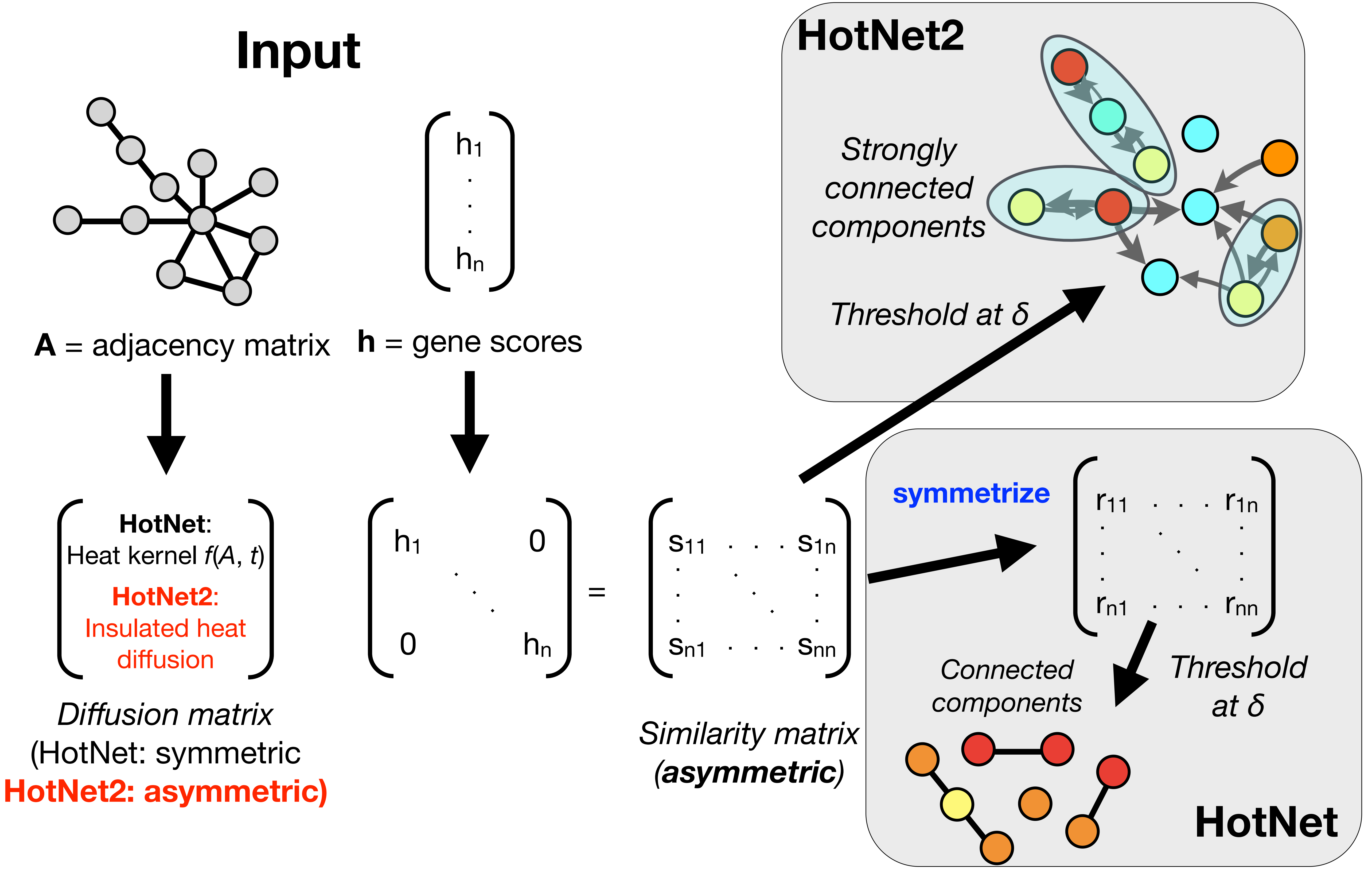
HotNet2 vs. HotNet



Input

A = adjacency matrix

h = gene scores

$$\mathbf{h} = \begin{pmatrix} h_1 \\ \vdots \\ h_n \end{pmatrix}$$

HotNet: Heat kernel *f*(*A*, *t*)

HotNet2: Insulated heat diffusion

$$\begin{pmatrix} h_1 & & 0 \\ & \ddots & \\ 0 & & h_n \end{pmatrix} = \begin{pmatrix} s_{11} & \cdots & s_{1n} \\ \vdots & \ddots & \vdots \\ s_{n1} & \cdots & s_{nn} \end{pmatrix}$$

Diffusion matrix
(HotNet: symmetric
HotNet2: asymmetric)

Similarity matrix
(***asymmetric***)

HotNet2

Strongly connected components

Threshold at δ

symmetrize

$$\begin{pmatrix} r_{11} & \cdots & r_{1n} \\ \vdots & \ddots & \vdots \\ r_{n1} & \cdots & r_{nn} \end{pmatrix}$$

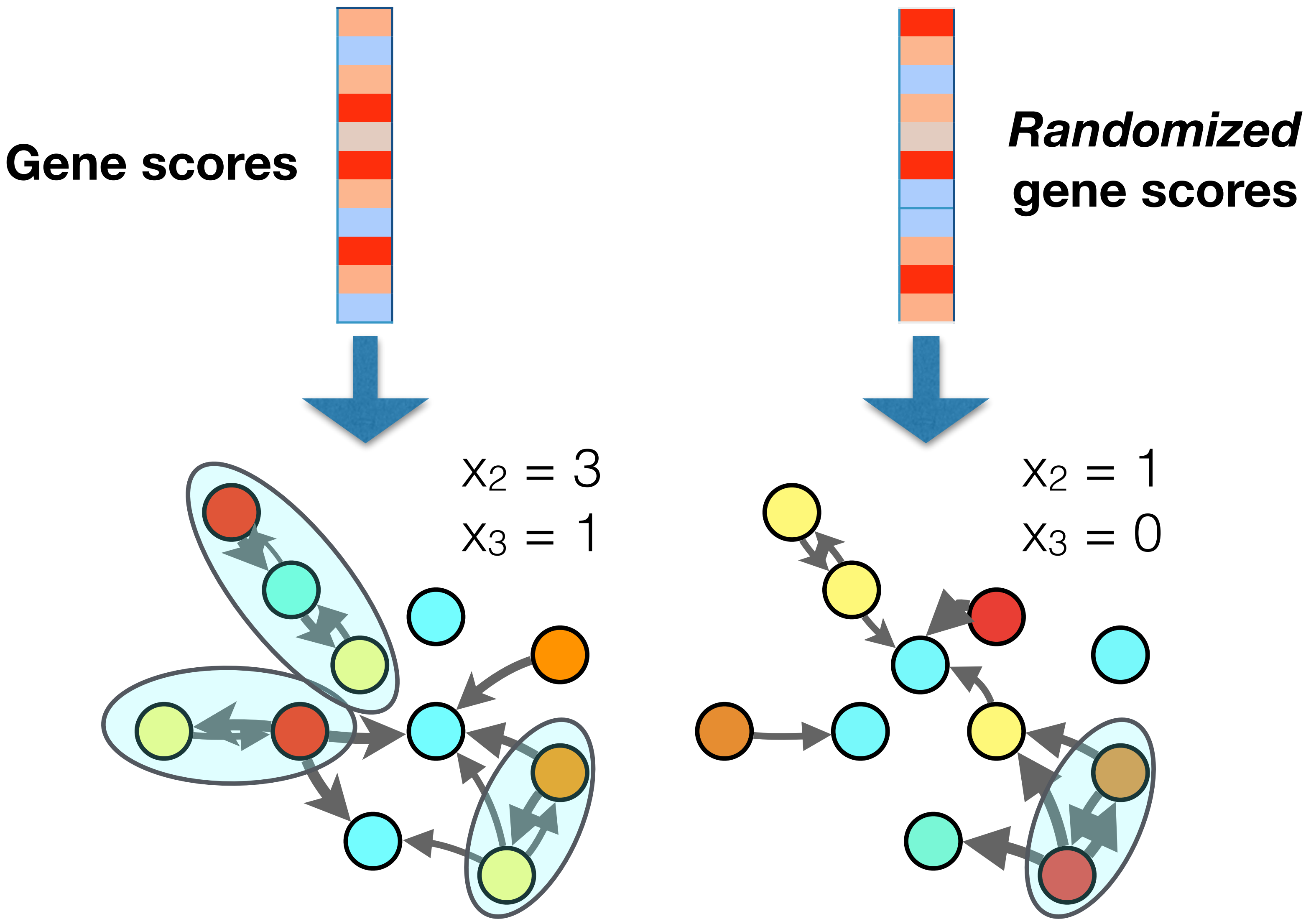
Threshold at δ

Connected components

HotNet

Statistical test

Evaluate graph partition with rigorously bounded False Discovery Rate (FDR)



Gene scores

Randomized **gene scores**

$x_2 = 3$
$x_3 = 1$

$x_2 = 1$
$x_3 = 0$

$\mathbf{X_k}$: number of subnetworks of size ≥ **k**

$\mathbf{Pr(X_k \geq x_k \mid h, \delta)}$

Leiserson, Vandin *et al. Nat. Genet.* (2015).

Outline

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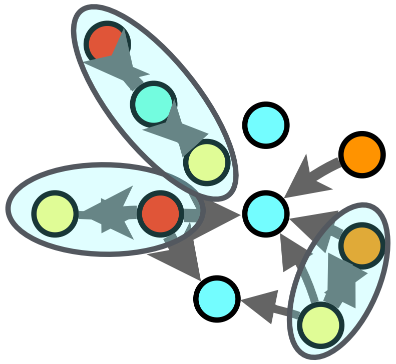

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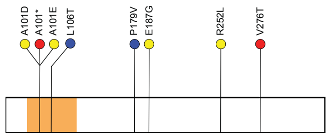
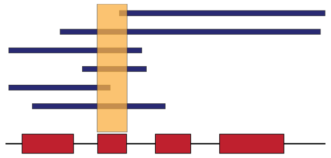




3. Comparison of HotNet2 to other methods.

TCGA Pan-Cancer

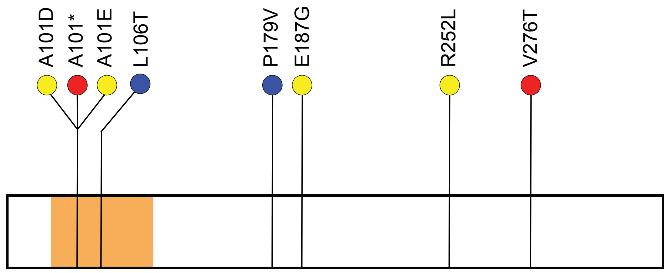
Tumor samples

Cancer	*Samples*	*Color*
BLCA	***99***	🟦
BRCA	***772***	🟦
COAD/READ	***224***	🟩
GBM	***291***	🟩
HNSC	***306***	🟥
KIRC	***417***	🟥
LAML	***196***	🟧
LUAD	***230***	🟧
LUSC	***178***	🟪
OV	***316***	🟪
UCEC	***248***	🟫

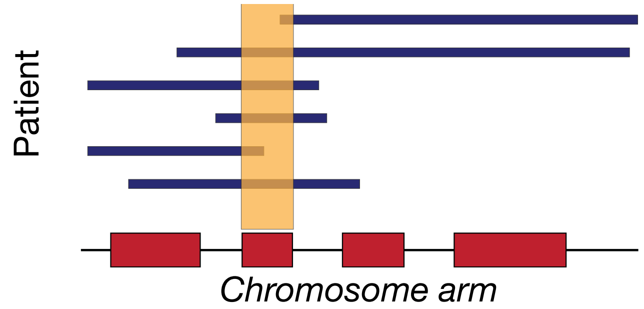
3,110 tumors of 12 cancer types

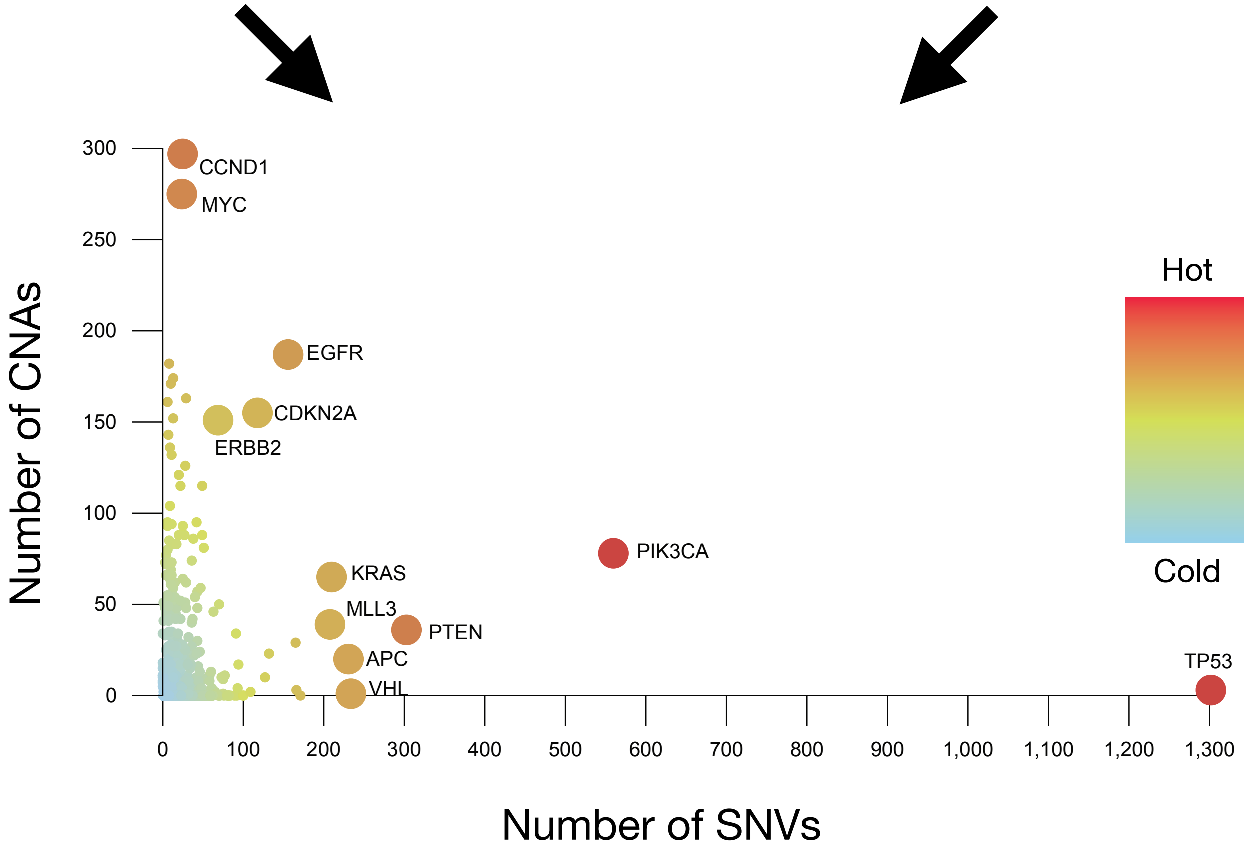
Mutations

Single nucleotide variants



Copy number aberrations

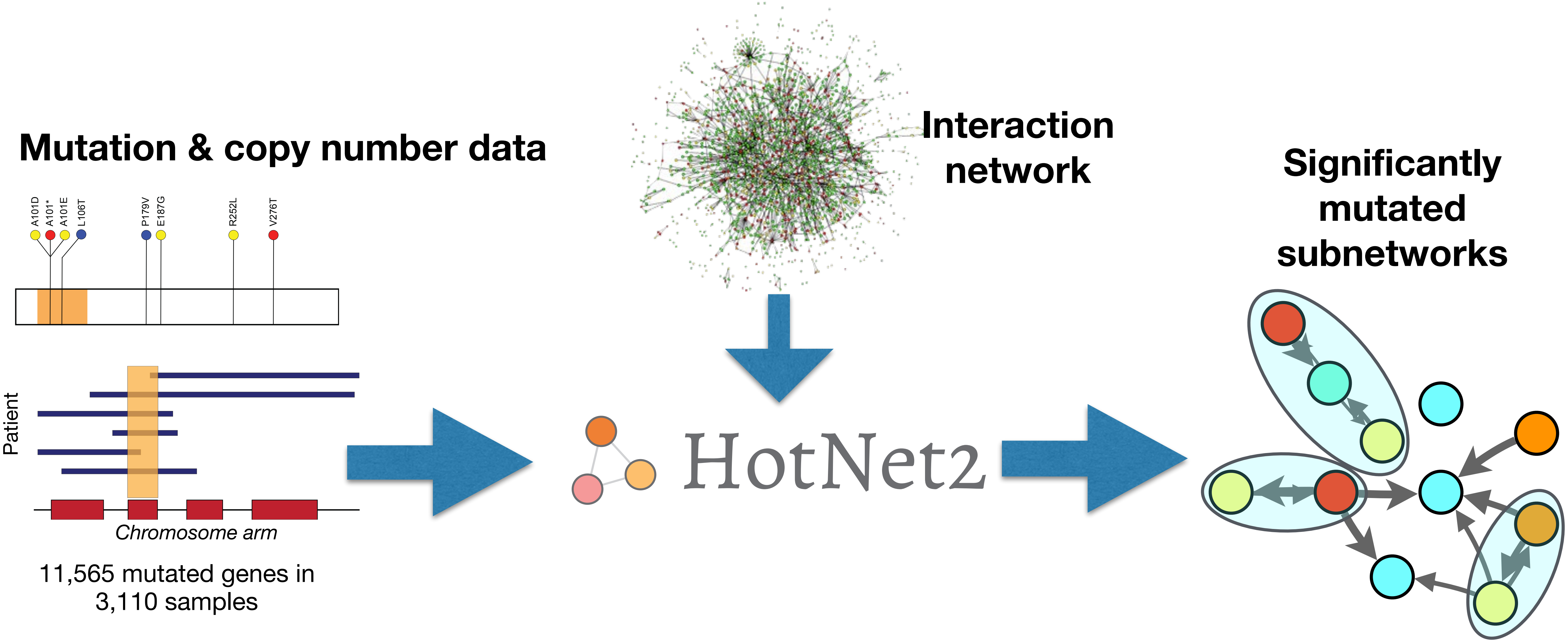




SNVs and CNAs in 3,110 samples among 11,565 *expressed* genes

Weinstein et al. *Nature Genetics* (2013)

HotNet2 runs on TCGA Pan-Cancer dataset



Mutation & copy number data

11,565 mutated genes in 3,110 samples

Interaction network

Significantly mutated subnetworks

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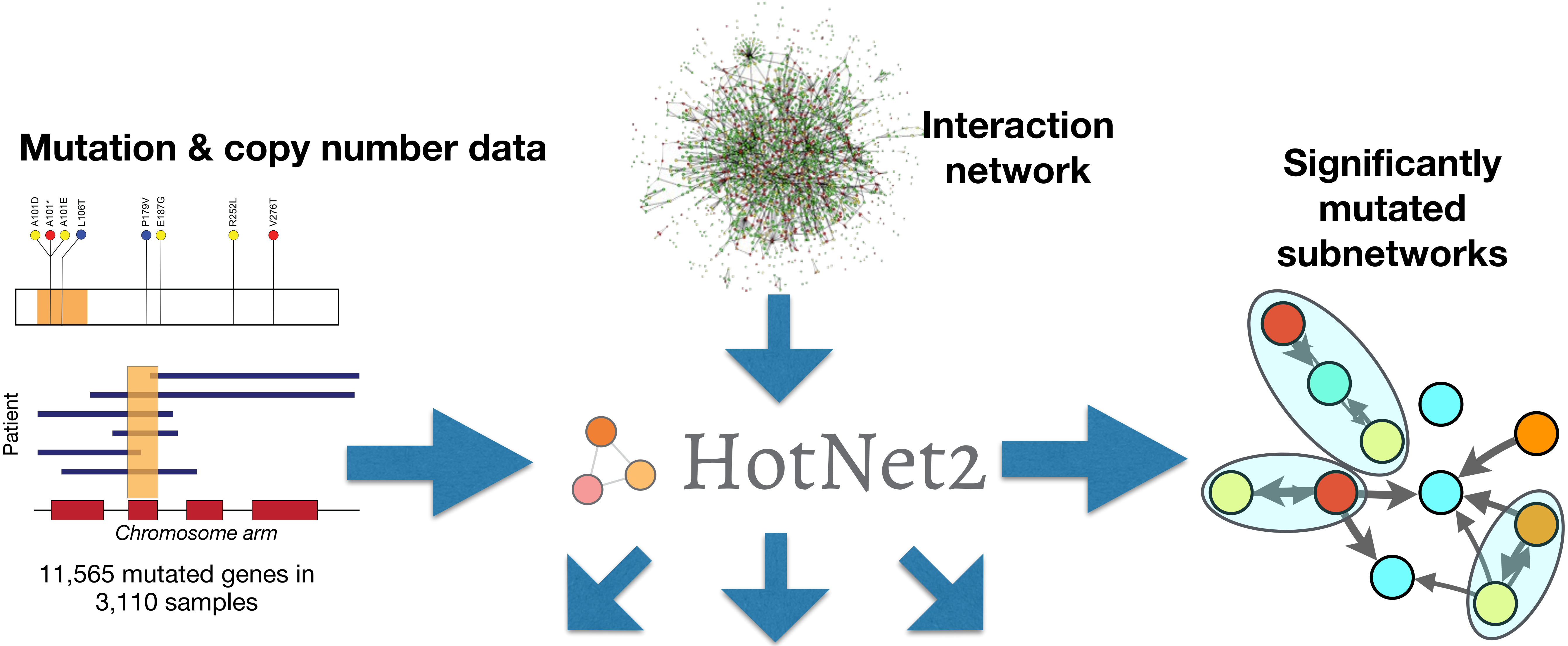
Mutation & copy number data

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HINT+HI2012 (P < 0.01)

40,704 interactions
9,858 proteins

iRefIndex 9.0 (P < 0.01)

91,808 interactions
12,128 proteins

Multinet (P < 0.01)

109,569 interactions
14,398 proteins

HotNet2 runs on TCGA Pan-Cancer dataset

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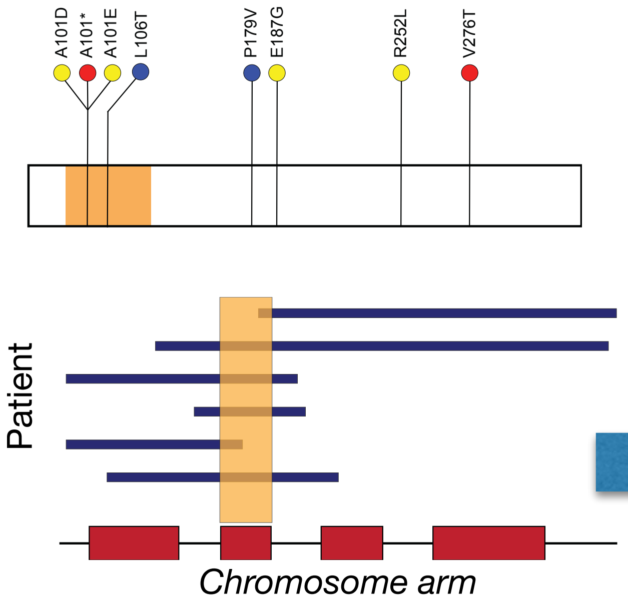

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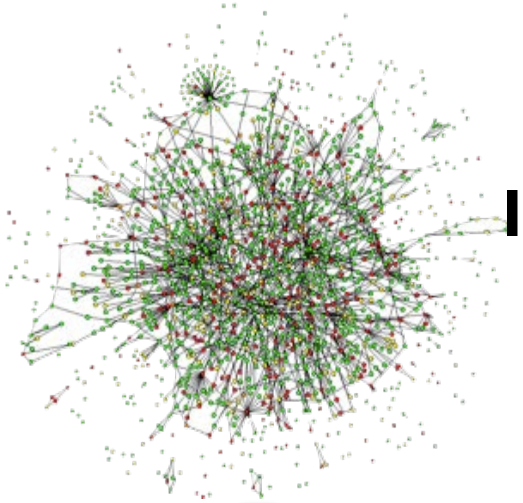
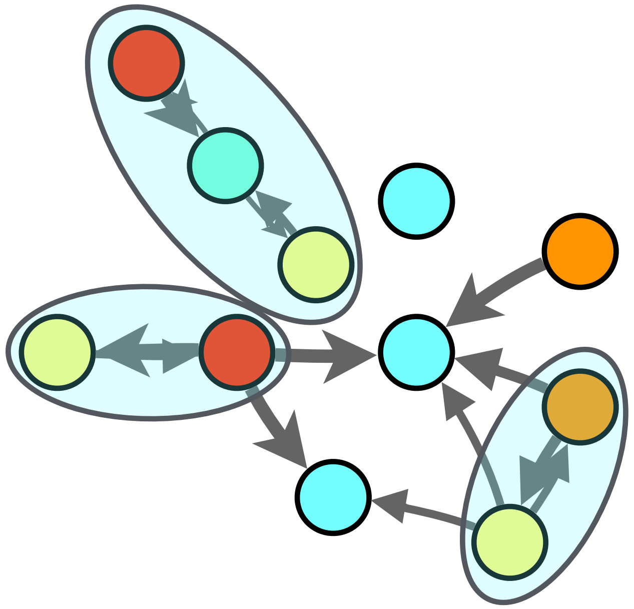


Interaction network

HotNet2

Significantly mutated subnetworks



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Consensus subnetworks

16 consensus subnetworks with ≥ 4 genes (*P*=0.004)
13 “linkers” between consensus subnetworks

HotNet2 Consensus

HotNet2 Runs
HINT+HI2012 ($P < 0.01$)
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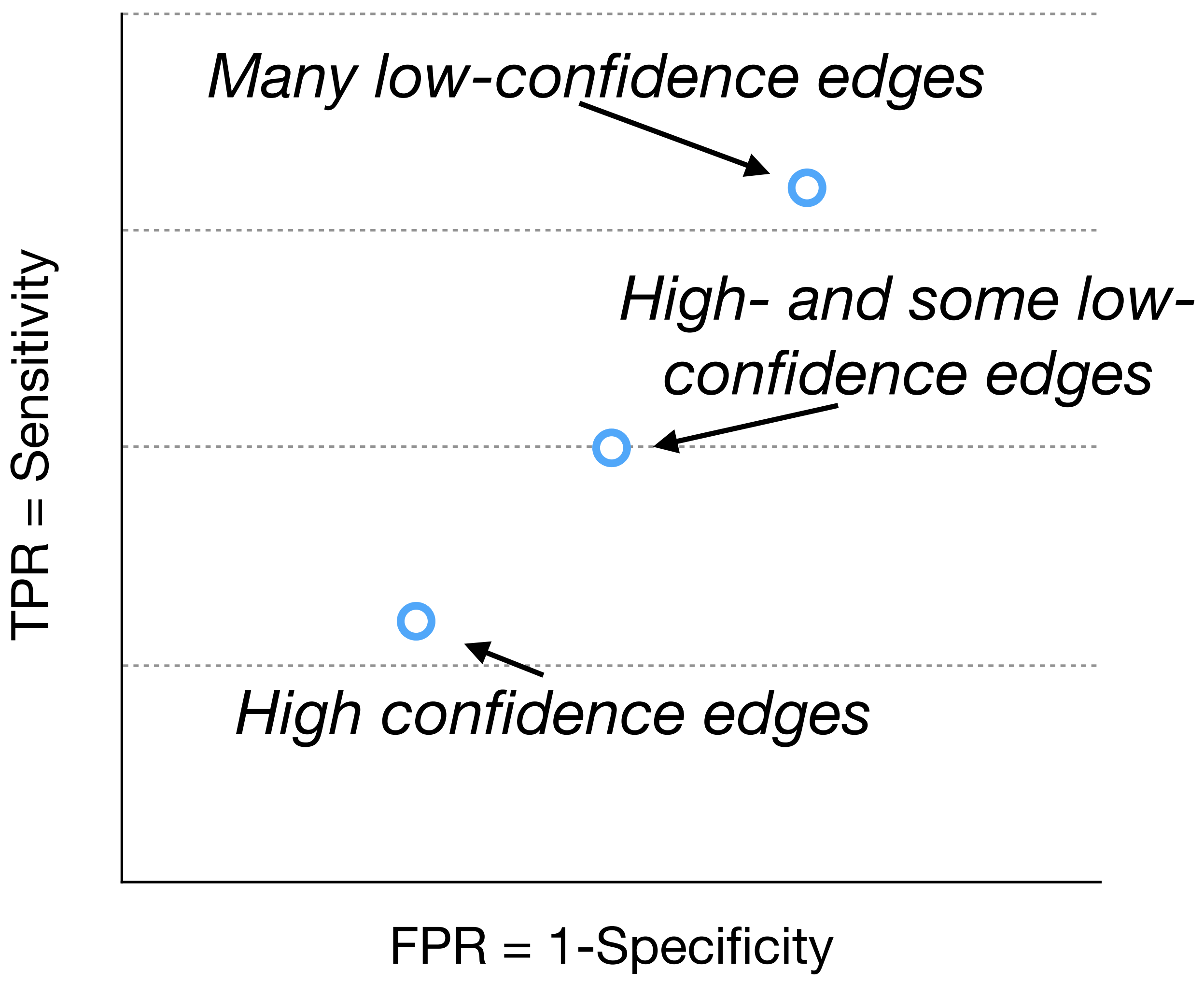

HotNet2 Consensus

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Multinet (*P* < 0.01)

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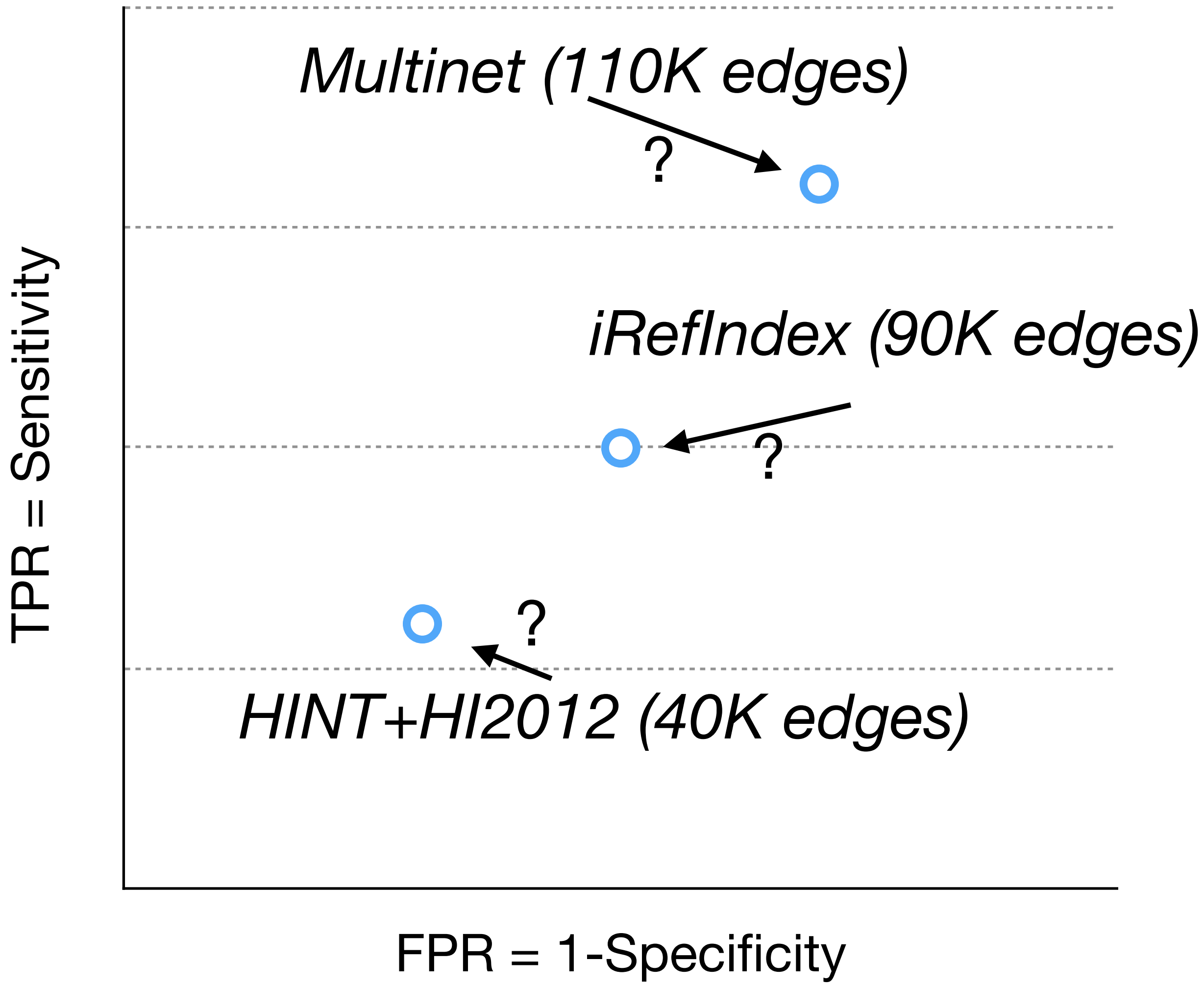

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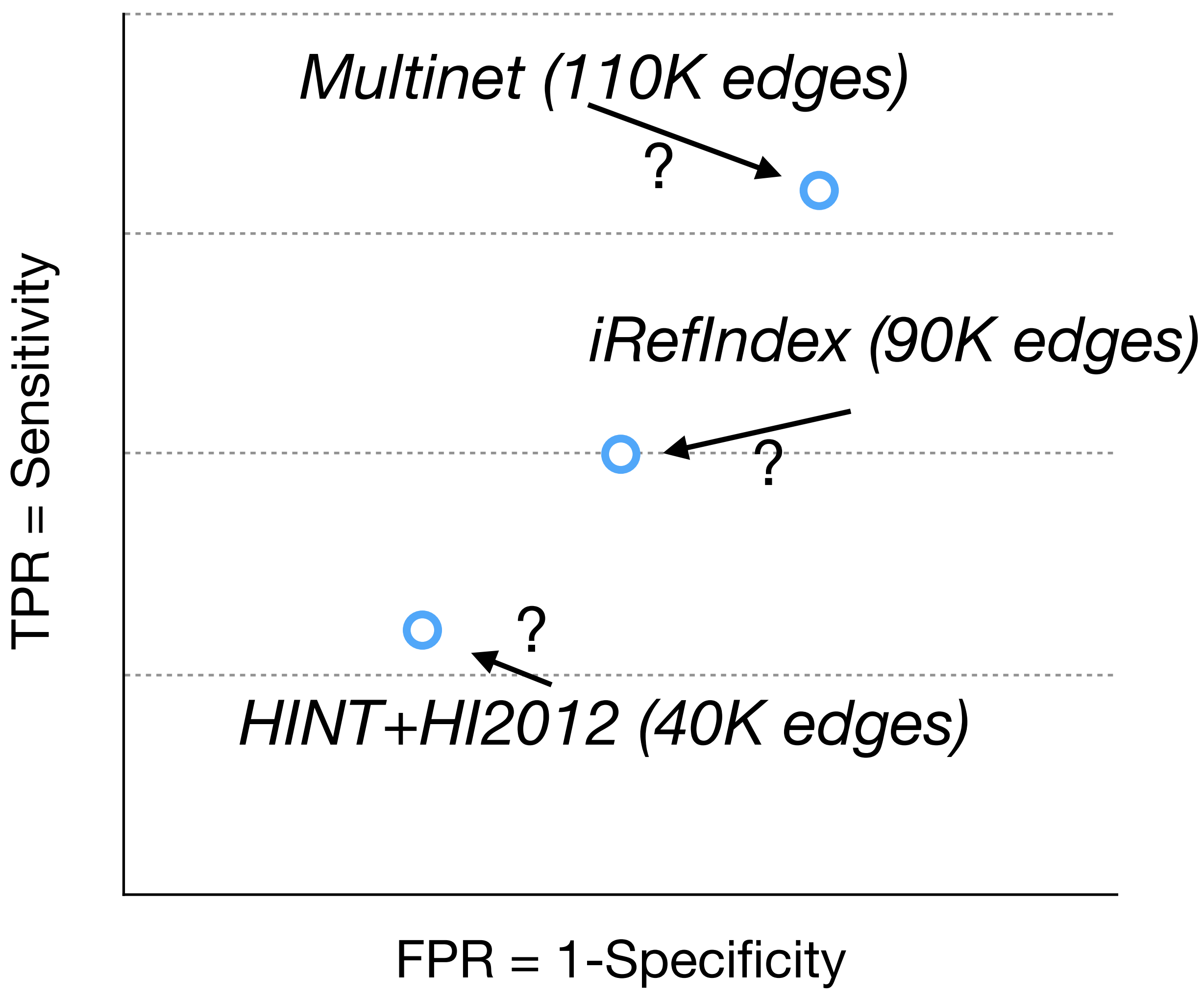
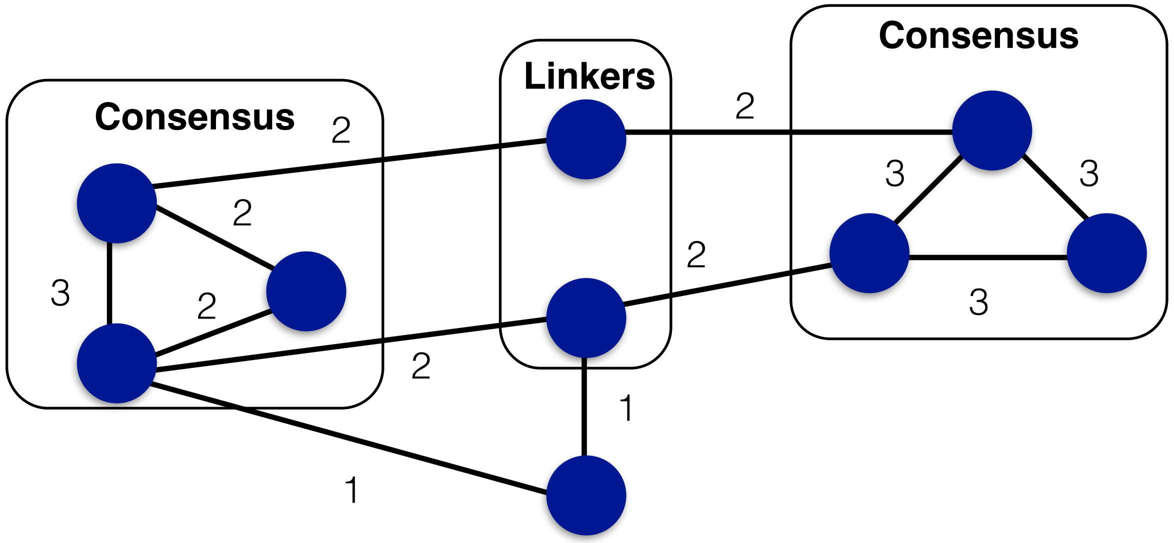
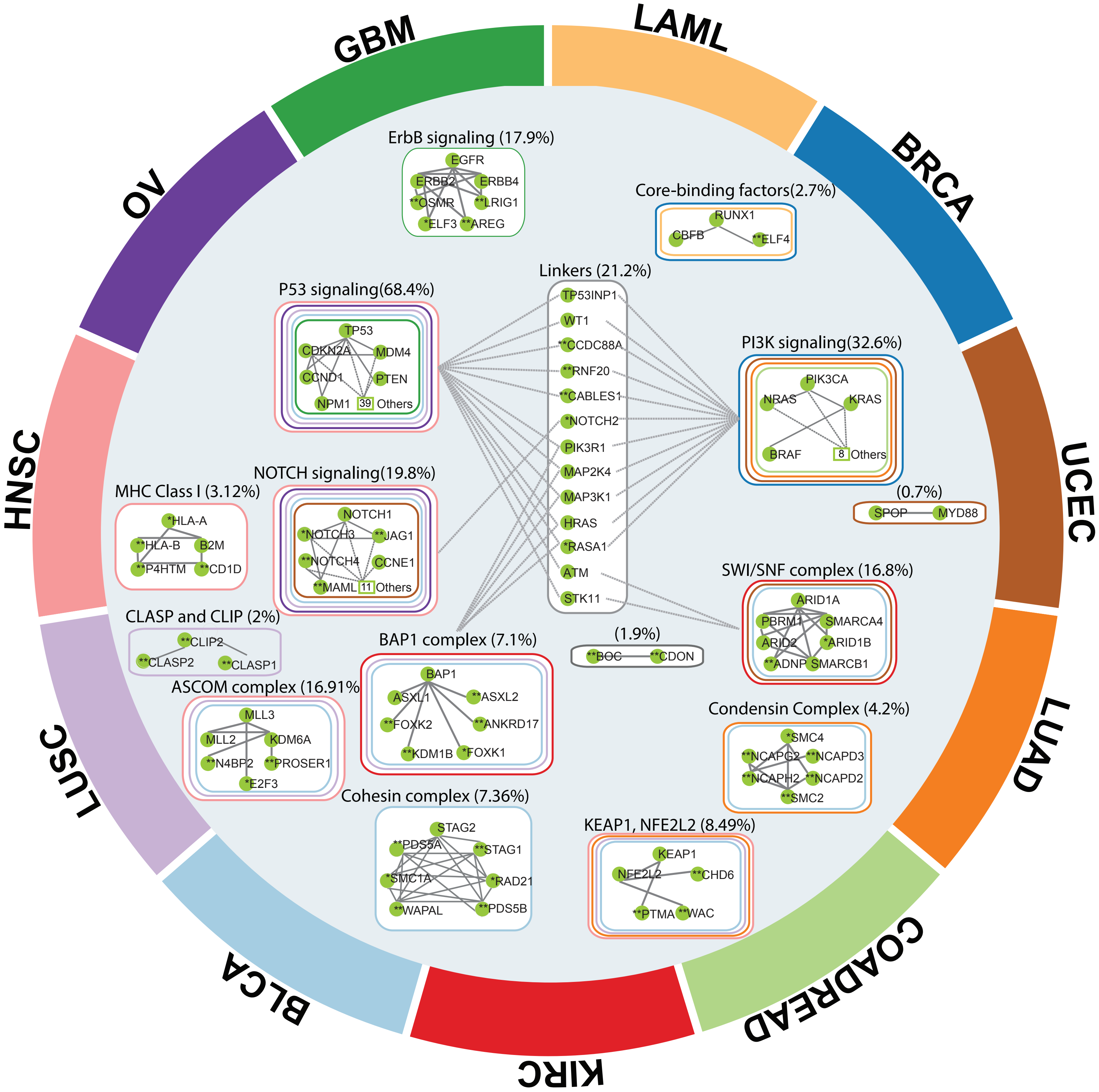


Main Idea: Incorporate low-confidence edges but give high-confidence edges more weight.



Consensus Graph
Edges connect genes identified by HotNet2 in the same subnetwork.

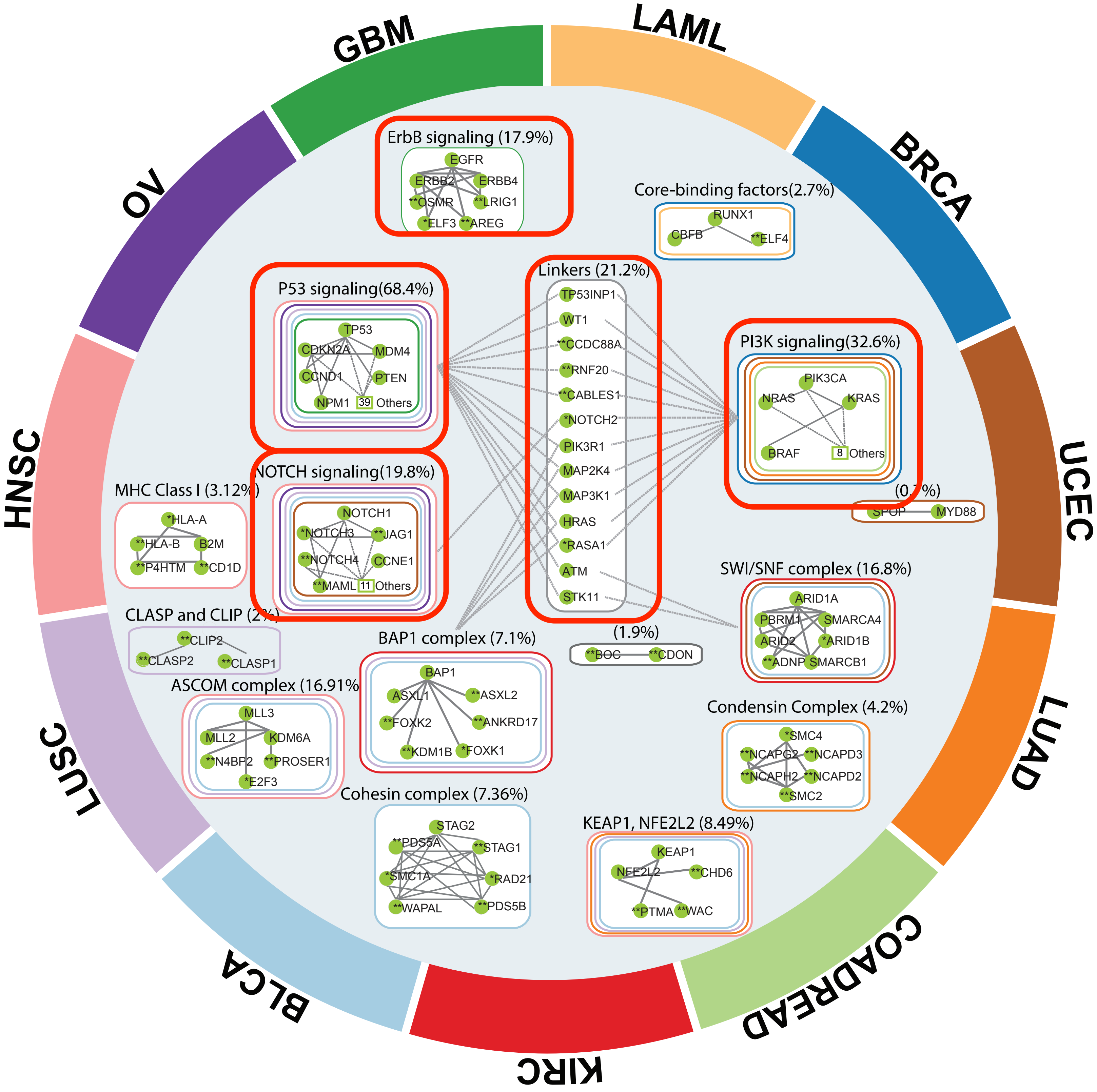
HotNet2 Consensus Subnetworks



Frequently and rarely mutated cancer genes

http://compbio.cs.brown.edu/pancancer/hotnet2

HotNet2 Consensus Subnetworks



Frequently and rarely mutated cancer genes

Well-known cancer pathways

- PI(3)K signaling
- p53 signaling
- NOTCH signaling
- ErbB signaling
- Linkers: *HRAS*, *STK11*, *ATM*

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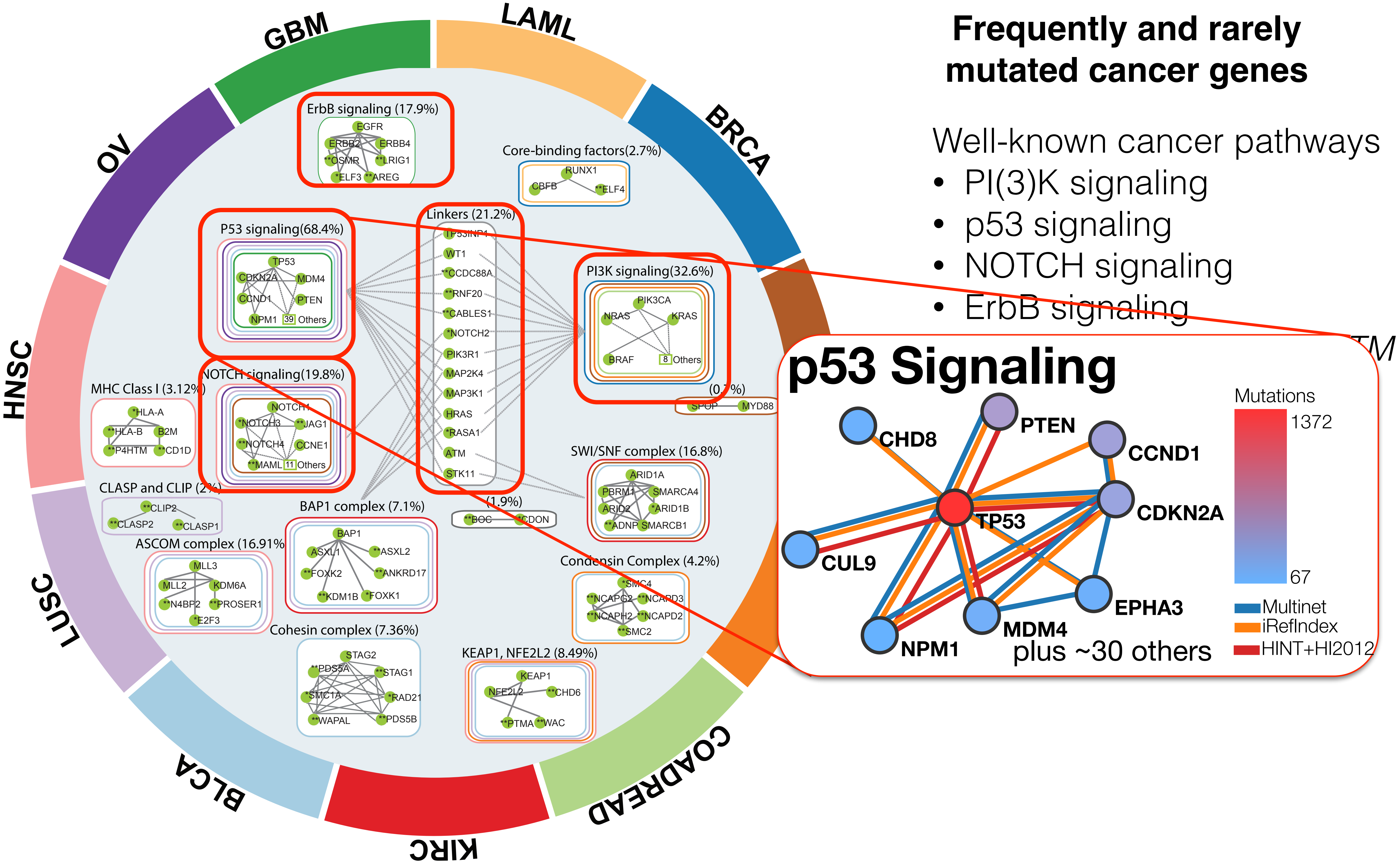

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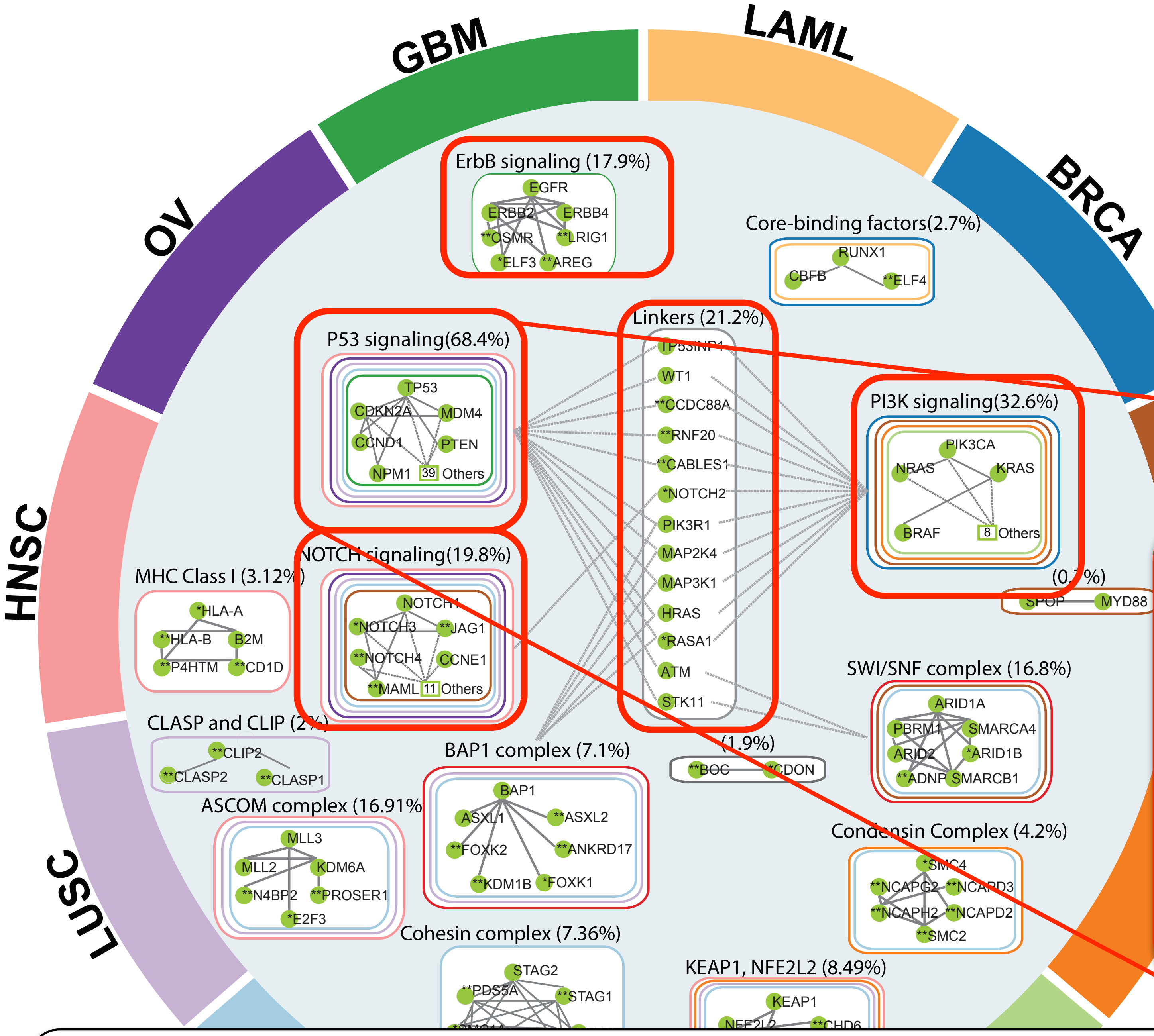

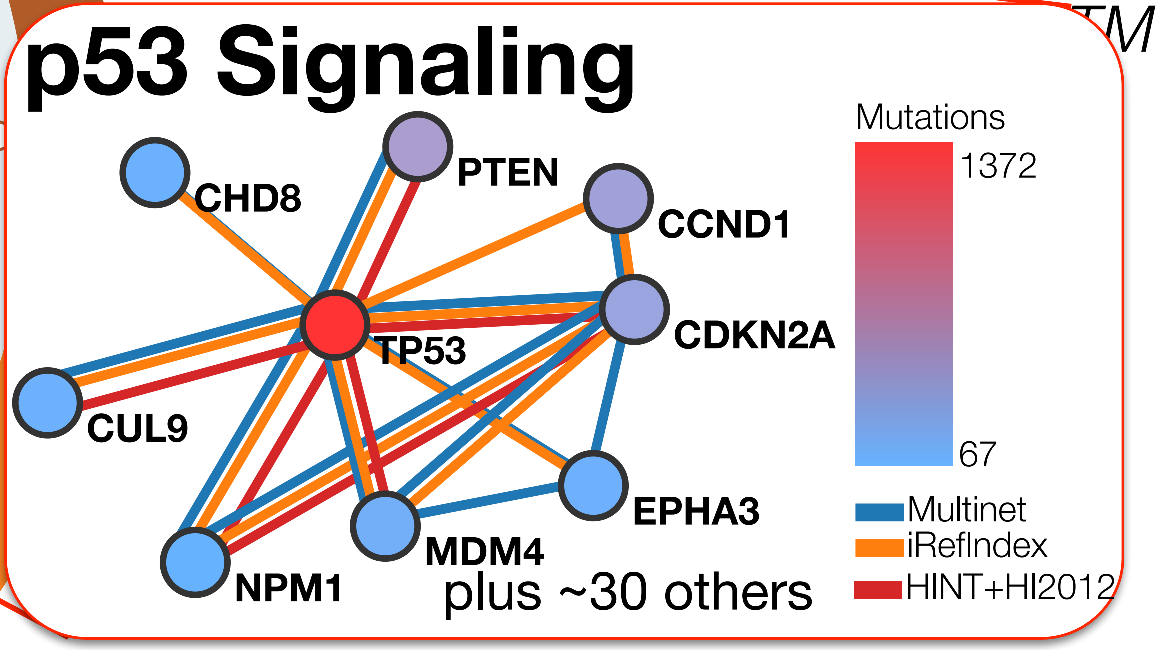

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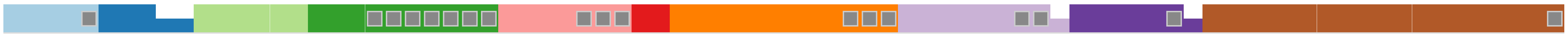
TM

CHD8 (mutated in 54 of 3110 samples)



Legend

Cancer types

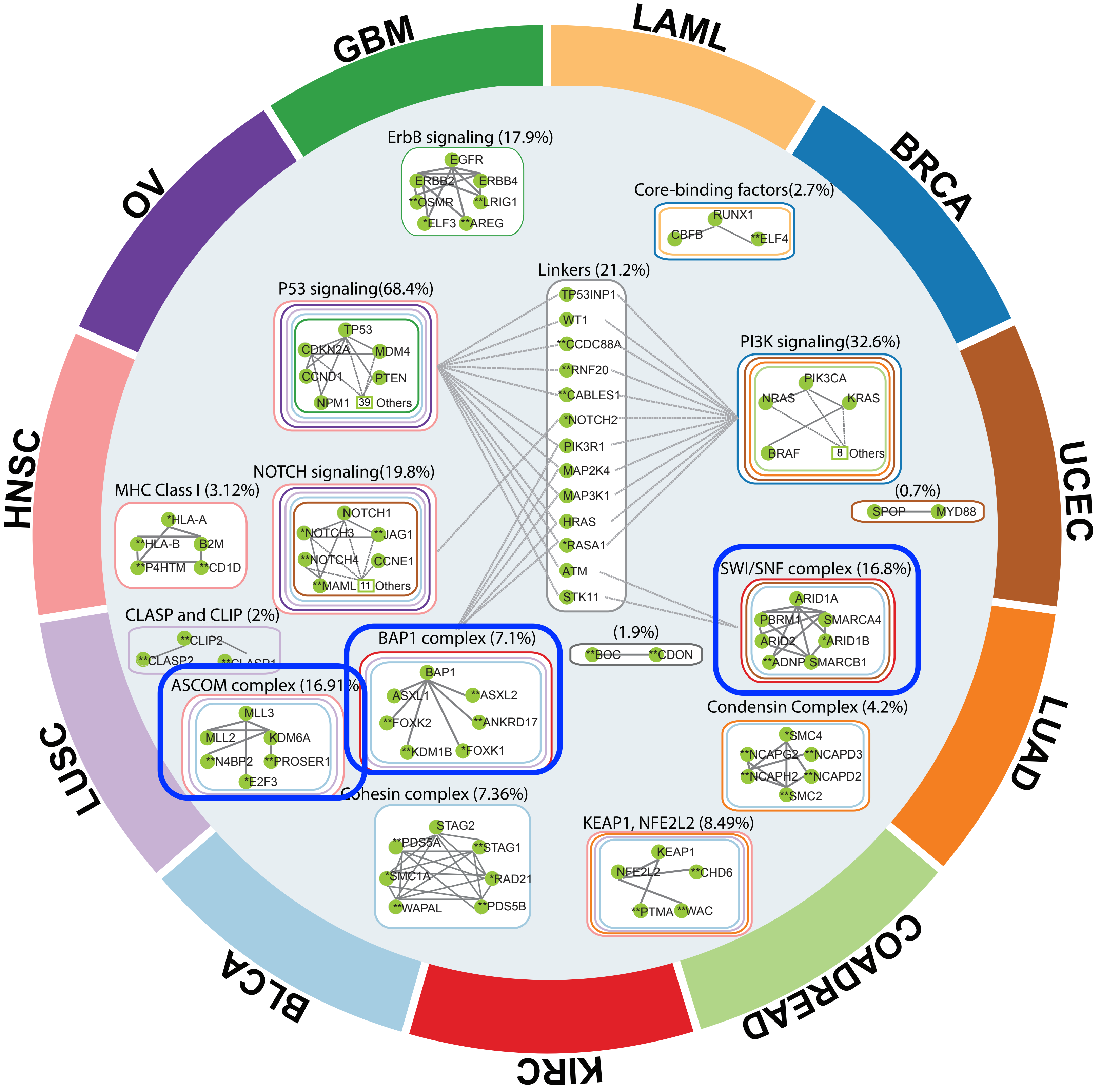
▮	BLCA	▮	BRCA	▮	COADREAD	▮	GBM	▮	HNSC
▮	KIRC	▮	LAML	▮	LUAD	▮	LUSC	▮	OV	▮	UCEC

Mutation types

▮	Inactivating SNV	▮	SNV
▮	Amplification	▮	Deletion

http://compbio.cs.brown.edu/pancancer/hotnet2

HotNet2 Consensus Subnetworks



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- SWI/SNF complex
- ASCOM complex
- BAP1 complex

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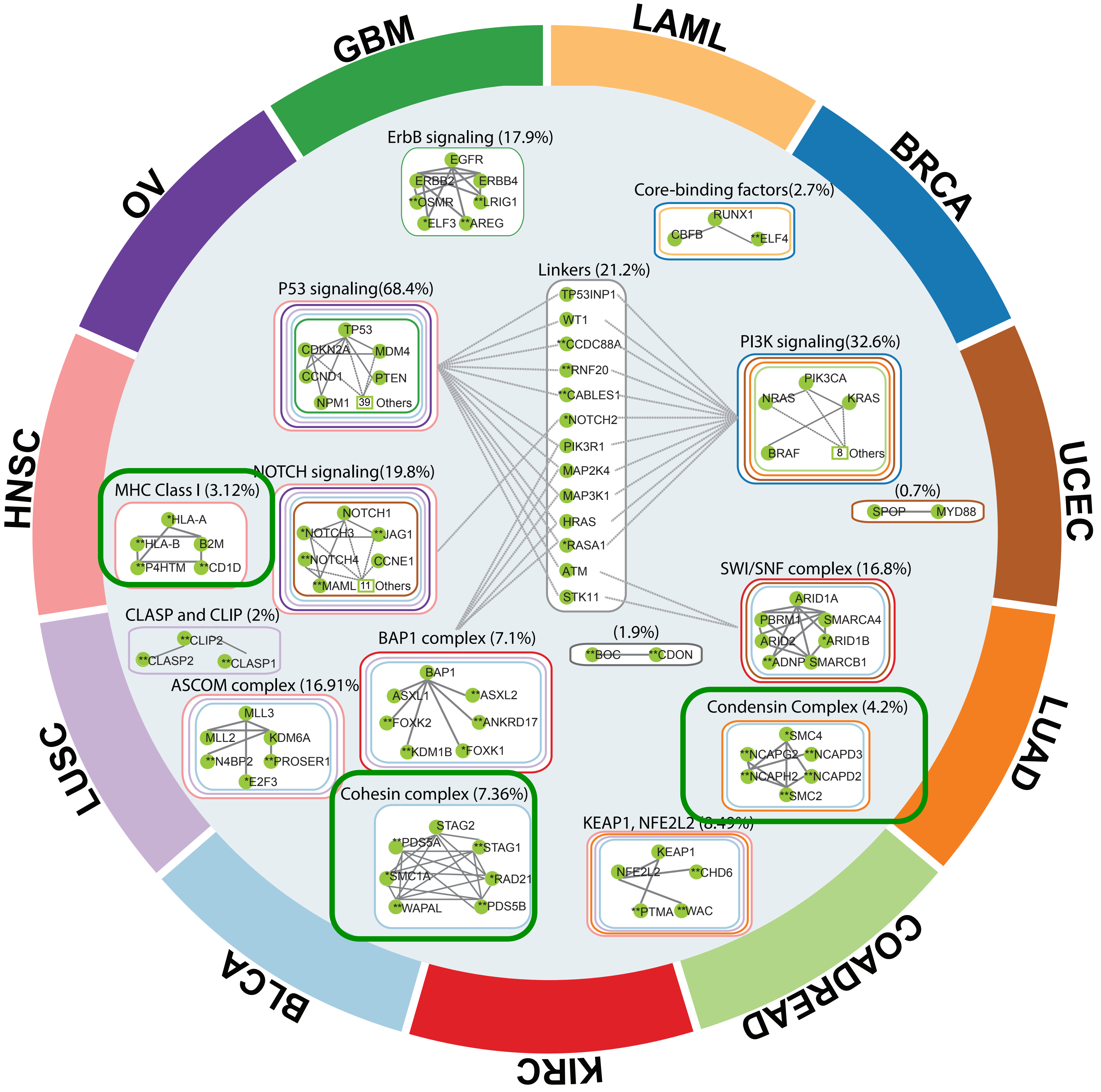

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- Cohesin complex
- Condensin complex
- MHC Class I proteins

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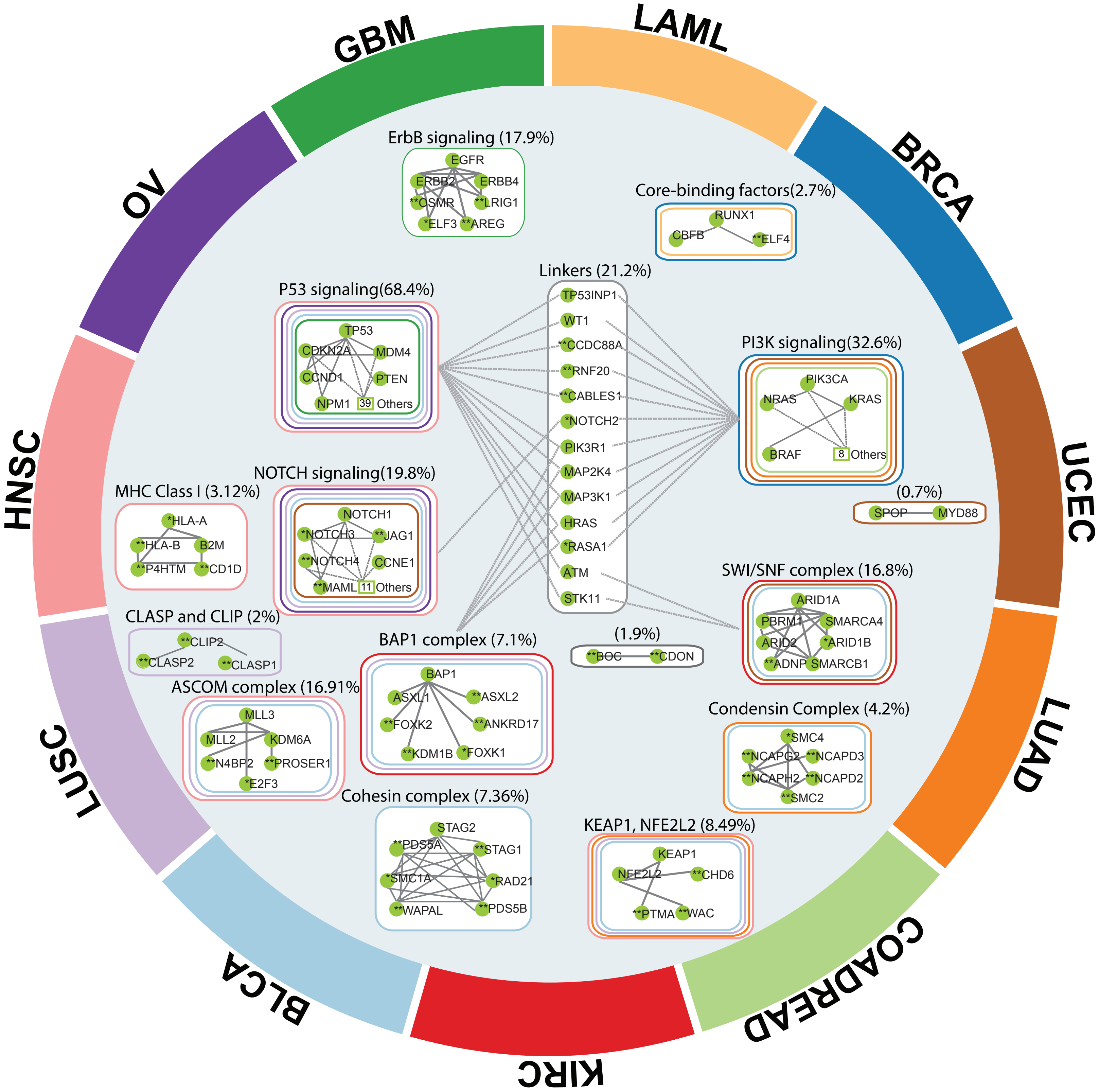
HotNet2 Consensus Subnetworks



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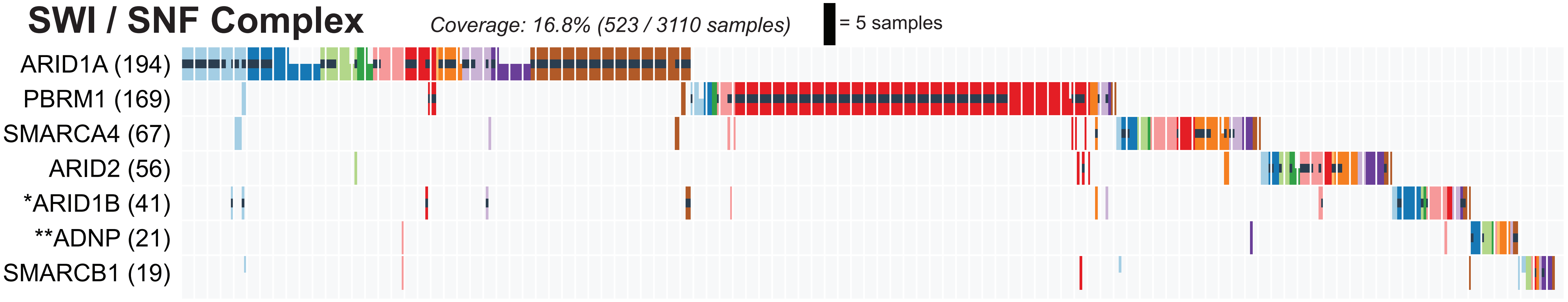
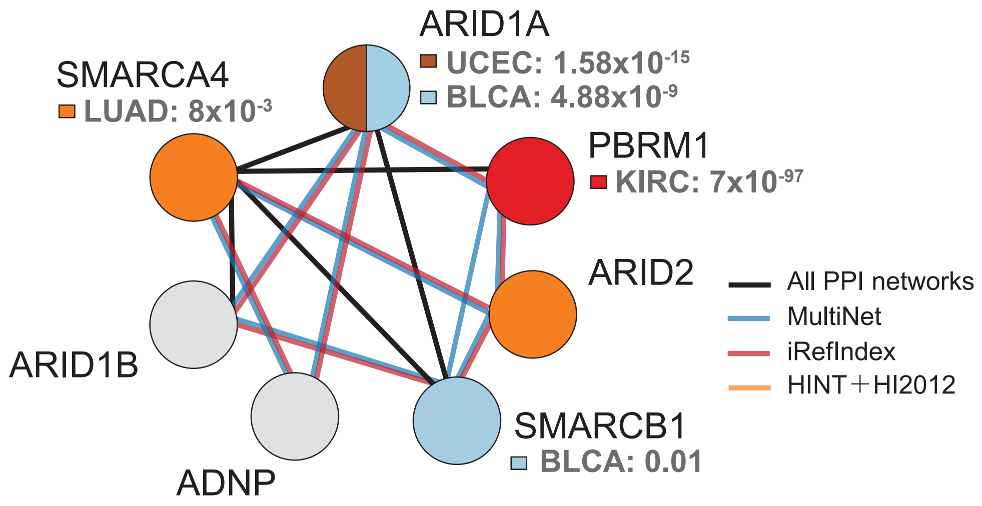
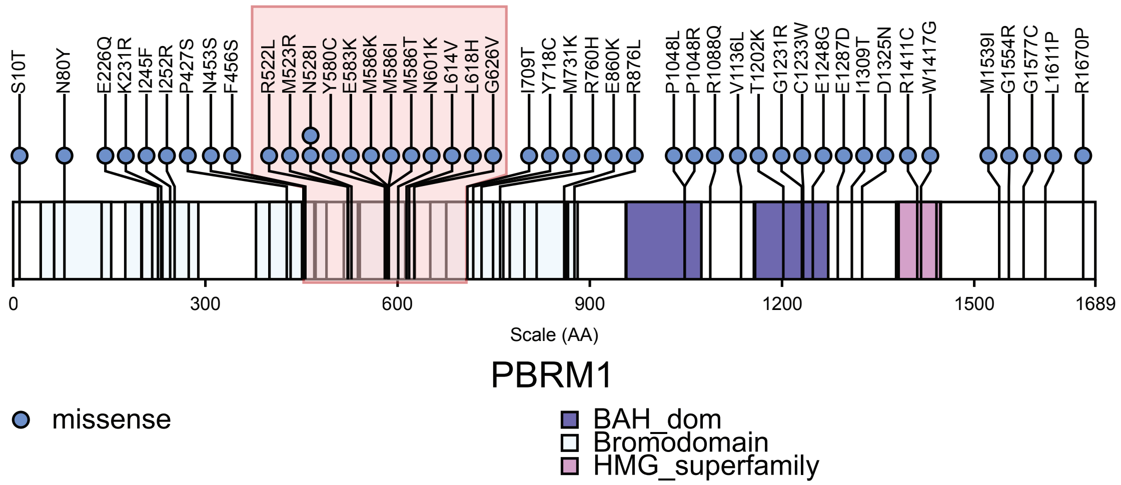
SWI/SNF complex





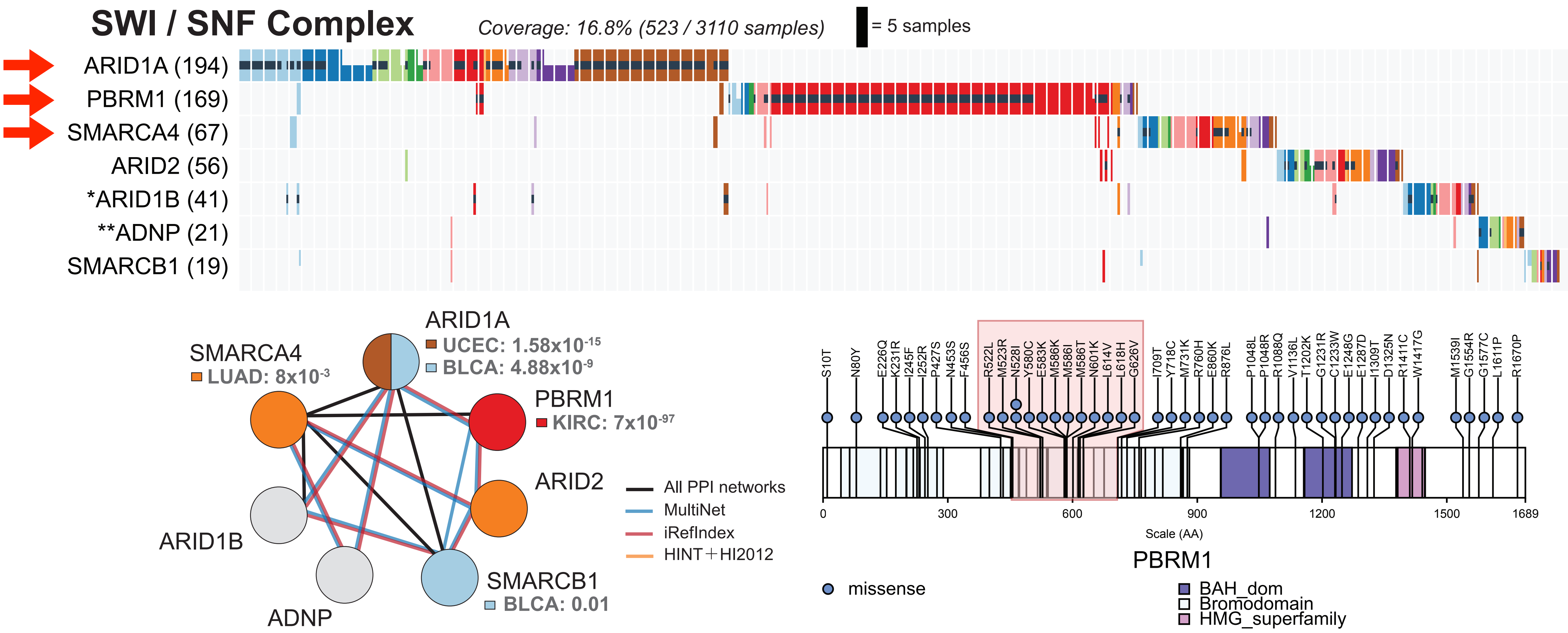


Legend

Cancer types: BLCA, BRCA, COADREAD, GBM, HNSC, KIRC, LAML, LUAD, LUSC, OV, UCEC

Mutation types: Inactivating SNV, SNV, Amplification, Deletion

SWI/SNF complex



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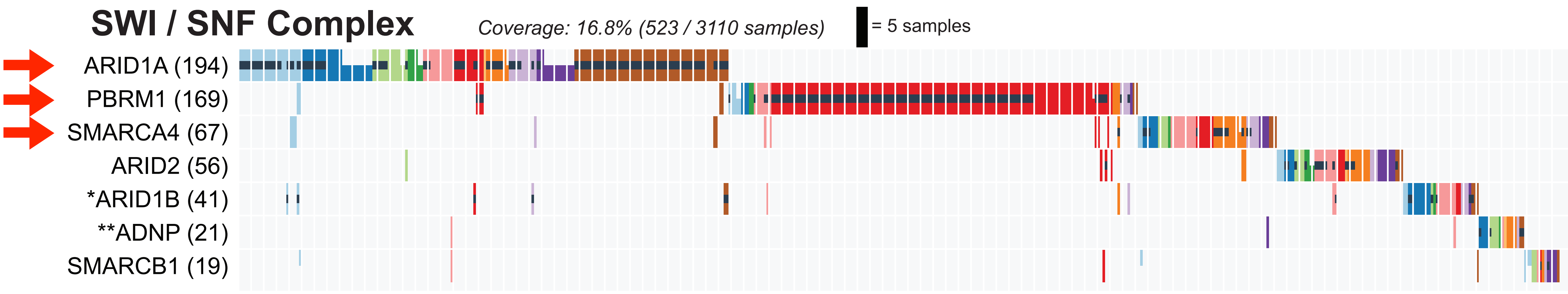
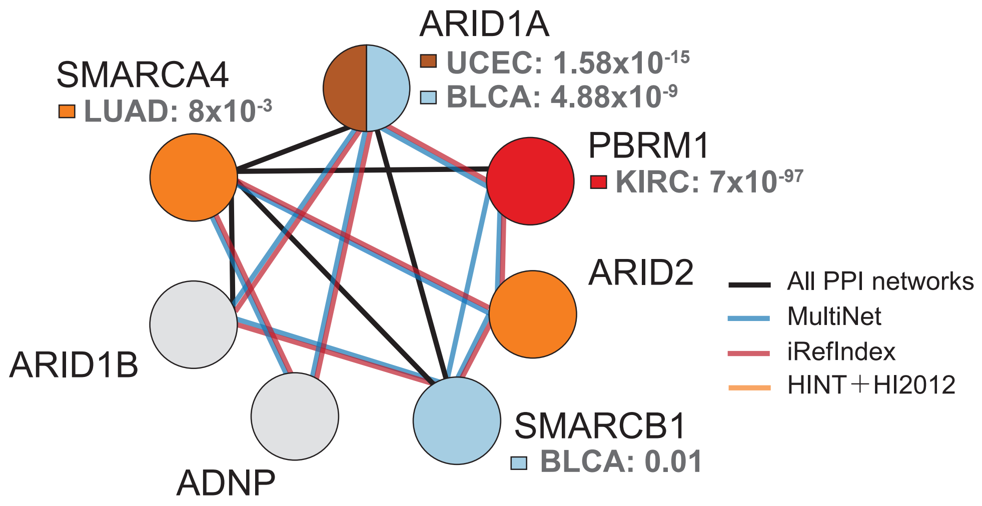
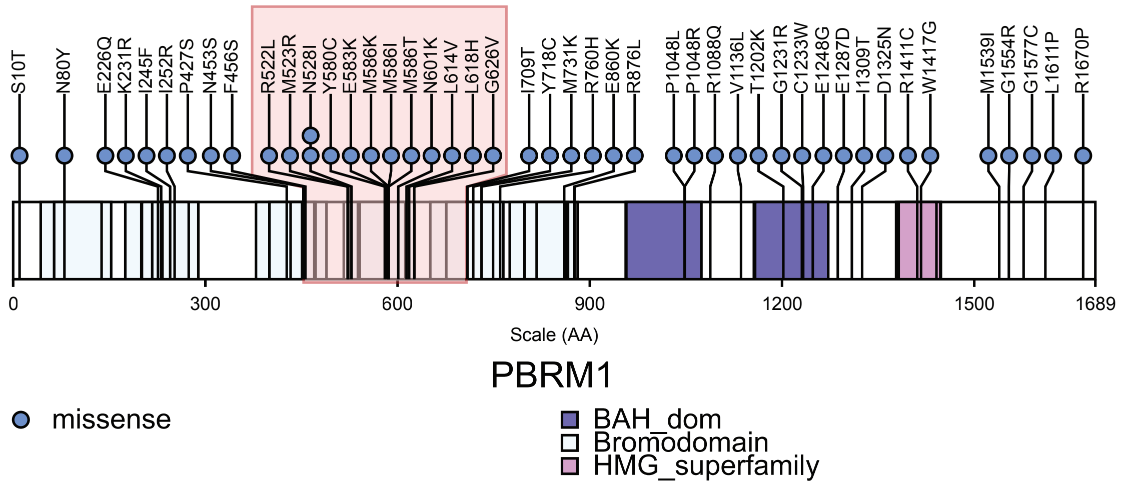
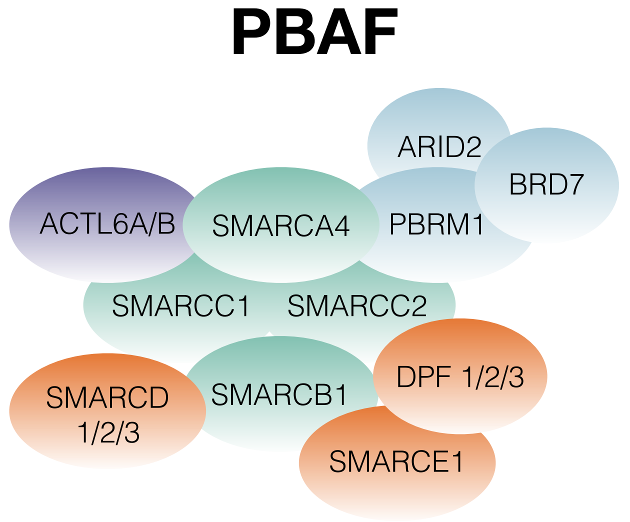
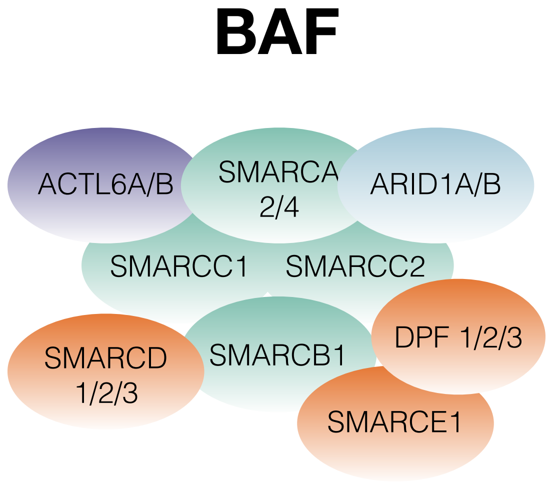










Wilson and Roberts.
Nature Reviews Cancer (2011)

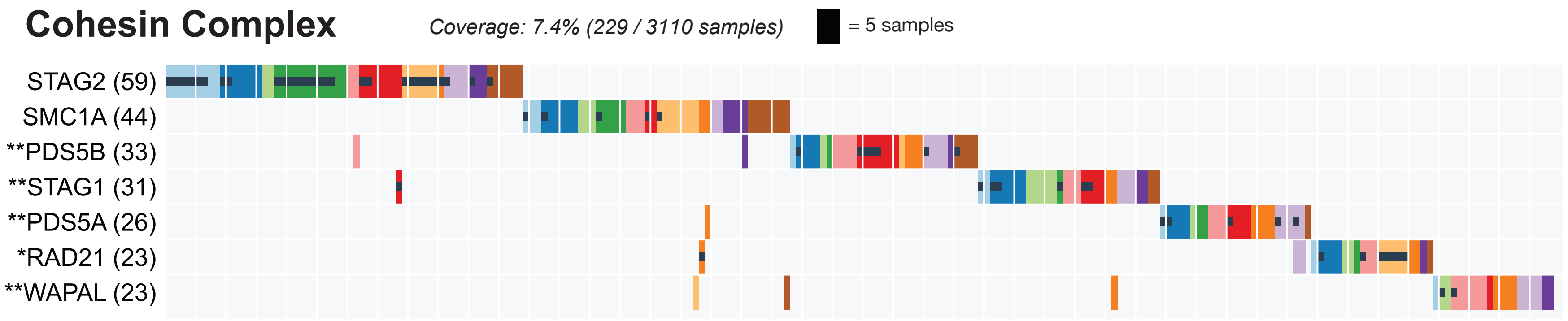
Involved in
nucleosome remodeling

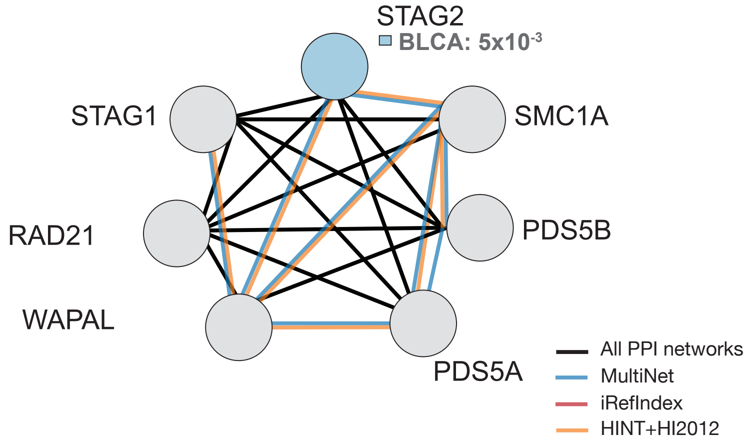
Legend

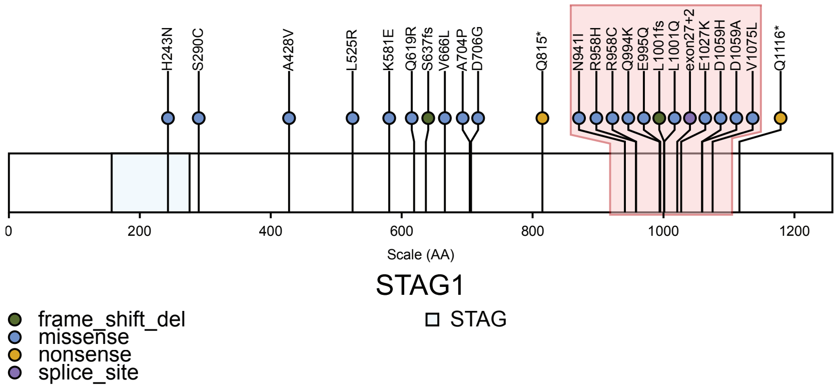
Cancer types: ■ BLCA ■ BRCA ■ COADREAD ■ GBM ■ HNSC ■ KIRC ■ LAML ■ LUAD ■ LUSC ■ OV ■ UCEC

Mutation types: ▌Inactivating SNV ▌SNV ▌Amplification ▌Deletion

Cohesin and condensin complexes

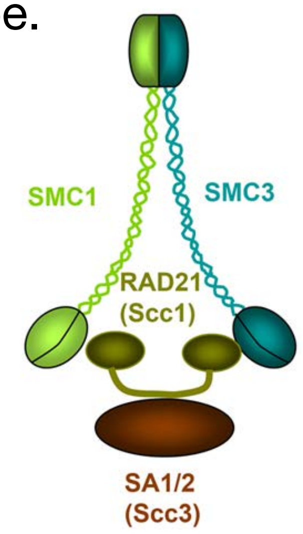






Cohesin complex

- 4/5 members of complex
- Involved in sister chromatid cohesion and gene regulation.
- Mutated in >4% of samples in each cancer type.



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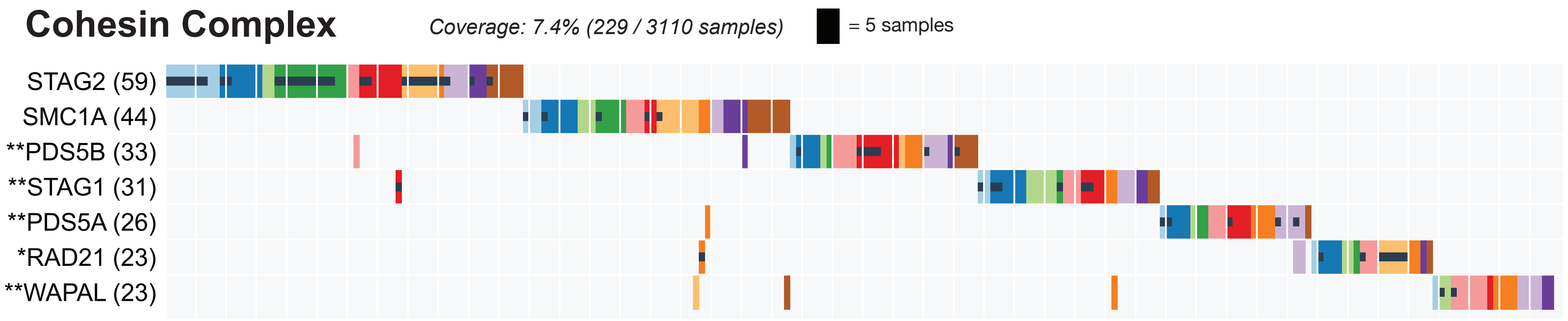

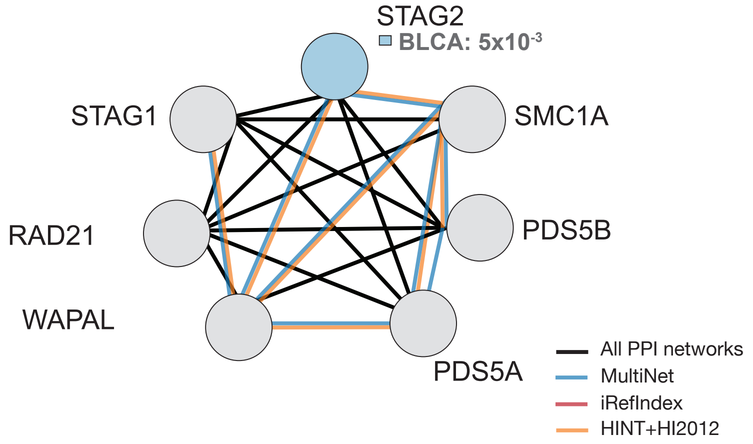

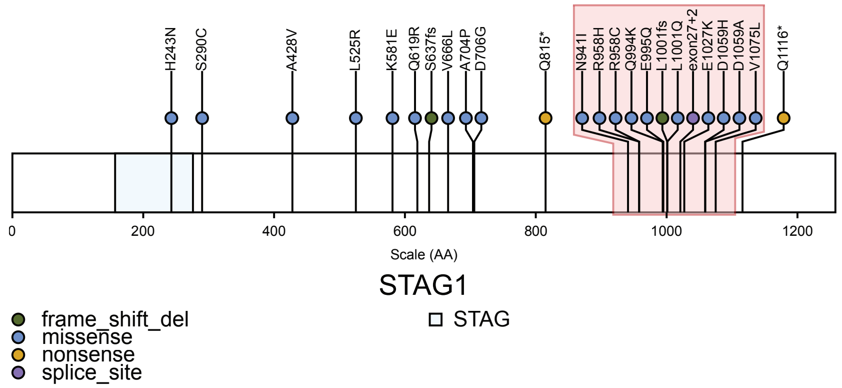

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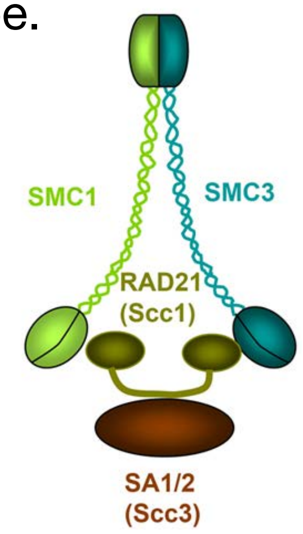
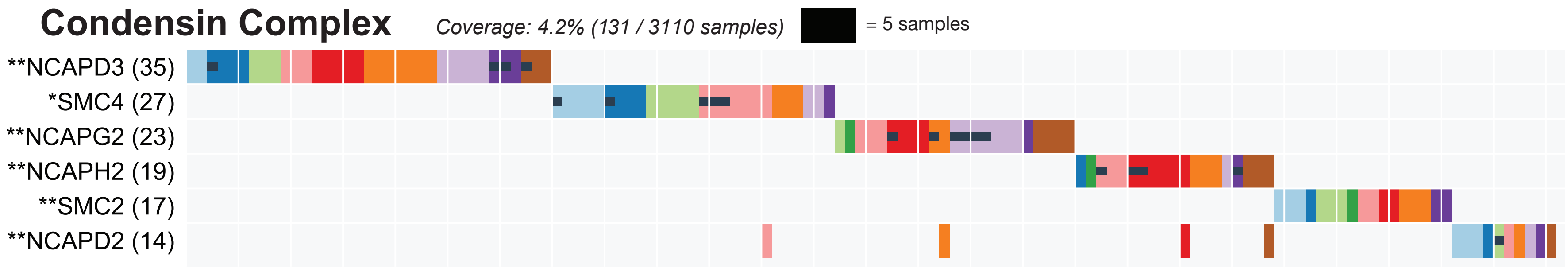
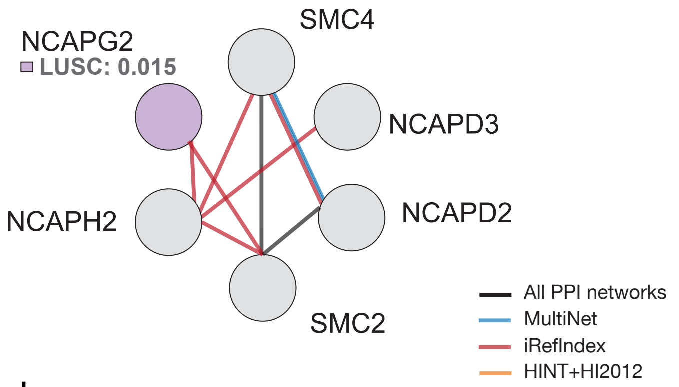
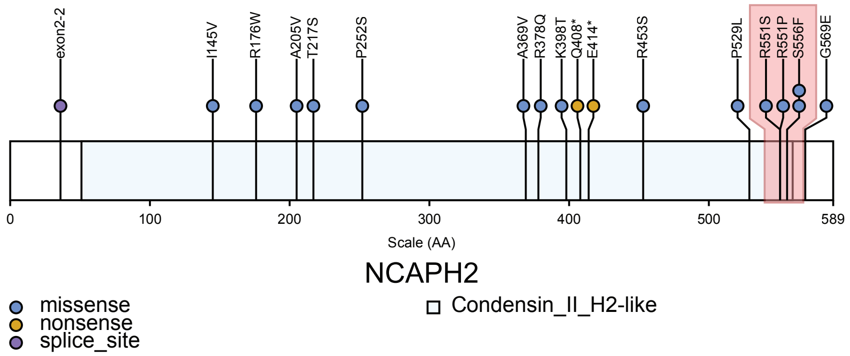








Condensin complex

- 6/8 members of complex
- Involved in sister chromatid condensation and gene regulation.
- Somatic mutations and expression validated using whole-genome sequencing and RNA-Seq

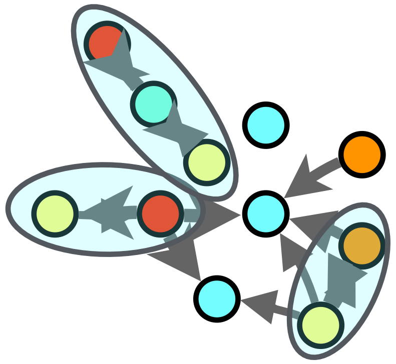
Legend

Cancer types: ▮ BLCA ▮ BRCA ▮ COADREAD ▮ GBM ▮ HNSC ▮ KIRC ▮ LAML ▮ LUAD ▮ LUSC ▮ OV ▮ UCEC

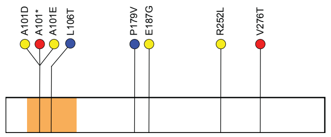
Mutation types: ▮ Inactivating SNV ▮ SNV ▮ Amplification ▮ Deletion

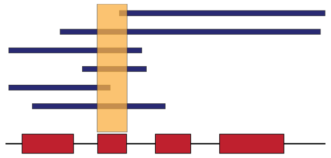
Outline

1. A new algorithm, HotNet2.



2. Application to TCGA Pan-Cancer data.





3. **Comparison of HotNet2 to similar methods.**

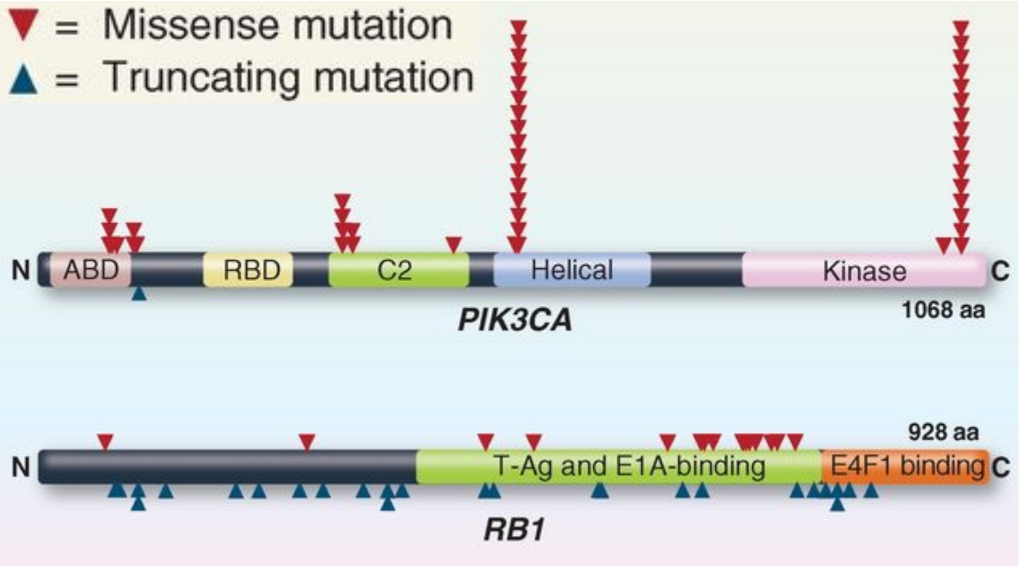
HotNet2 outperforms other methods on real data

No gold standard dataset → compare methods at identifying putative cancer genes

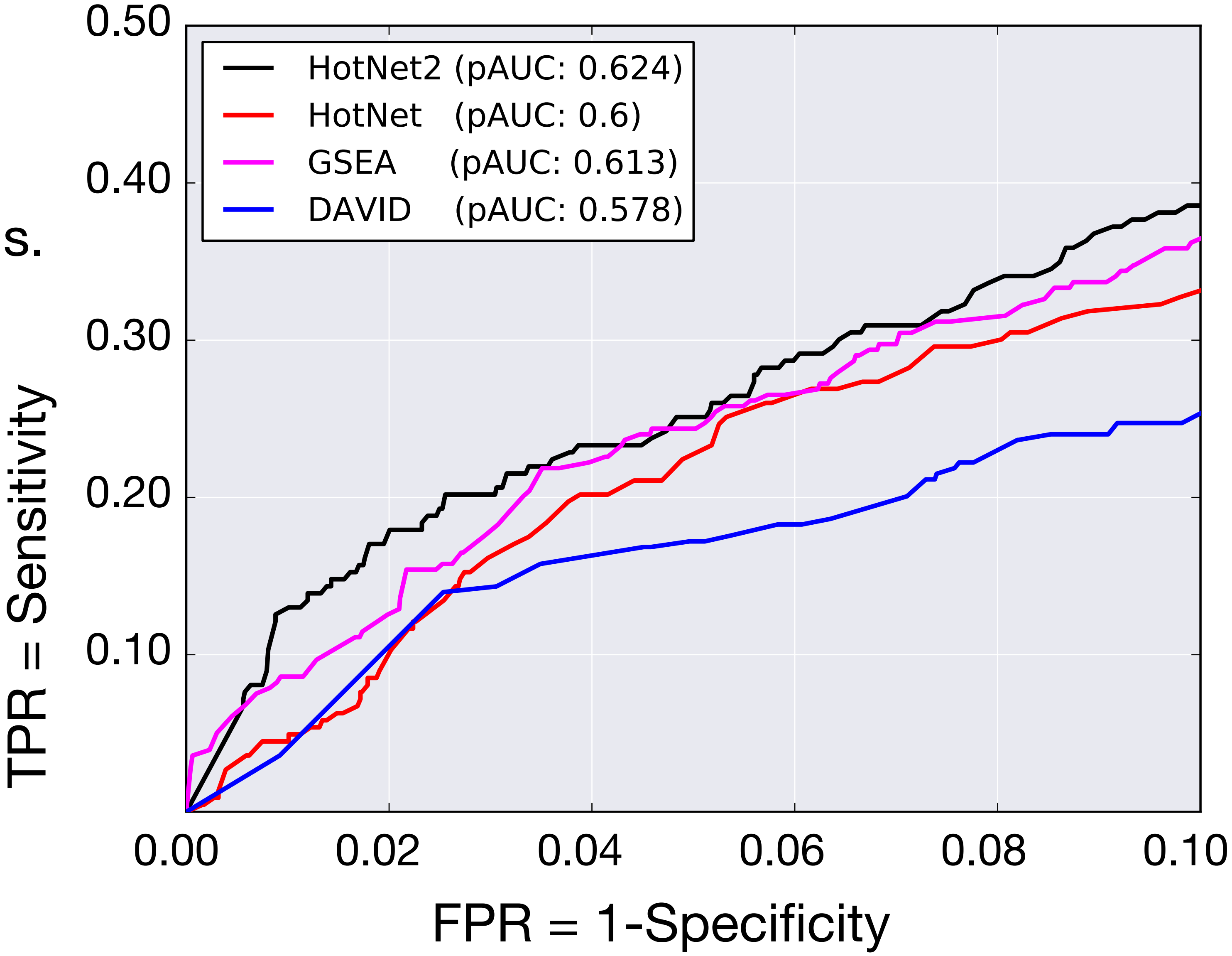
Dataset of *putative* cancer genes

Cancer genes have:

1. ≥ 20% truncating mutations; or,
2. ≥ 20% mutations clustered at a locus.



Vogelstein *et al*. (*Science*, 2013)

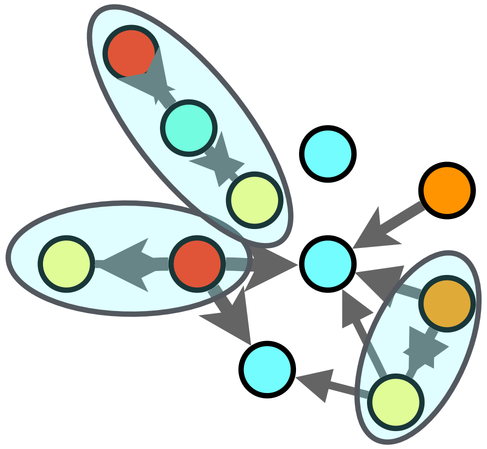


Summary

- HotNet2: Novel algorithm that analyzes topology and mutations simultaneously with asymmetric heat diffusion.

- Identifies known and novel pathways and complexes with frequently and rarely mutated genes on TCGA Pan-Cancer data.

- Future work:
  - Alternate graph partitioning algorithms?
  - Other applications: gene expression, GWAS, social networks, etc.



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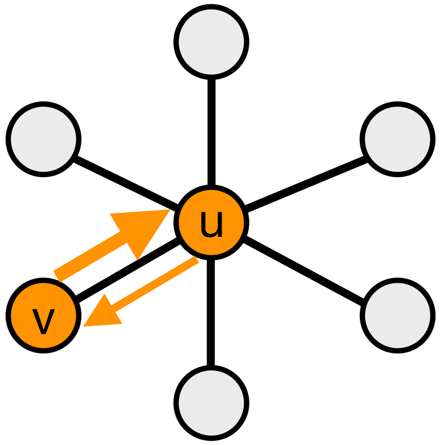

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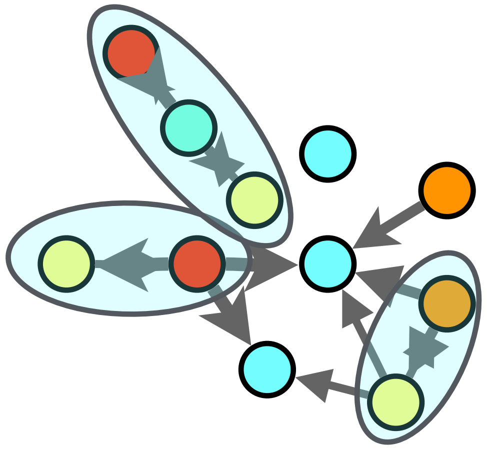

Acknowledgements

Research Group

Ben Raphael
Fabio Vandin
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Jason R. Dobson
Matt Reyna
Jonathan Eldridge
Alexandra Papoutsaki
Jacob Thomas
Younhun Kim

BROWN

CCMB

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BROAD INSTITUTE

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upf. Universitat Pompeu Fabra Barcelona

Yuwei Chang
Greg Ryslik

Yale University

LUX ET VERITAS

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NSF National Science Foundation
WHERE DISCOVERIES BEGIN

NATIONAL INSTITUTES OF HEALTH

The Cancer Genome Atlas