Information in noncoding DNA

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Large scale sequencing consortia

International Cancer Genome Consortium

Cancer genome sequencing

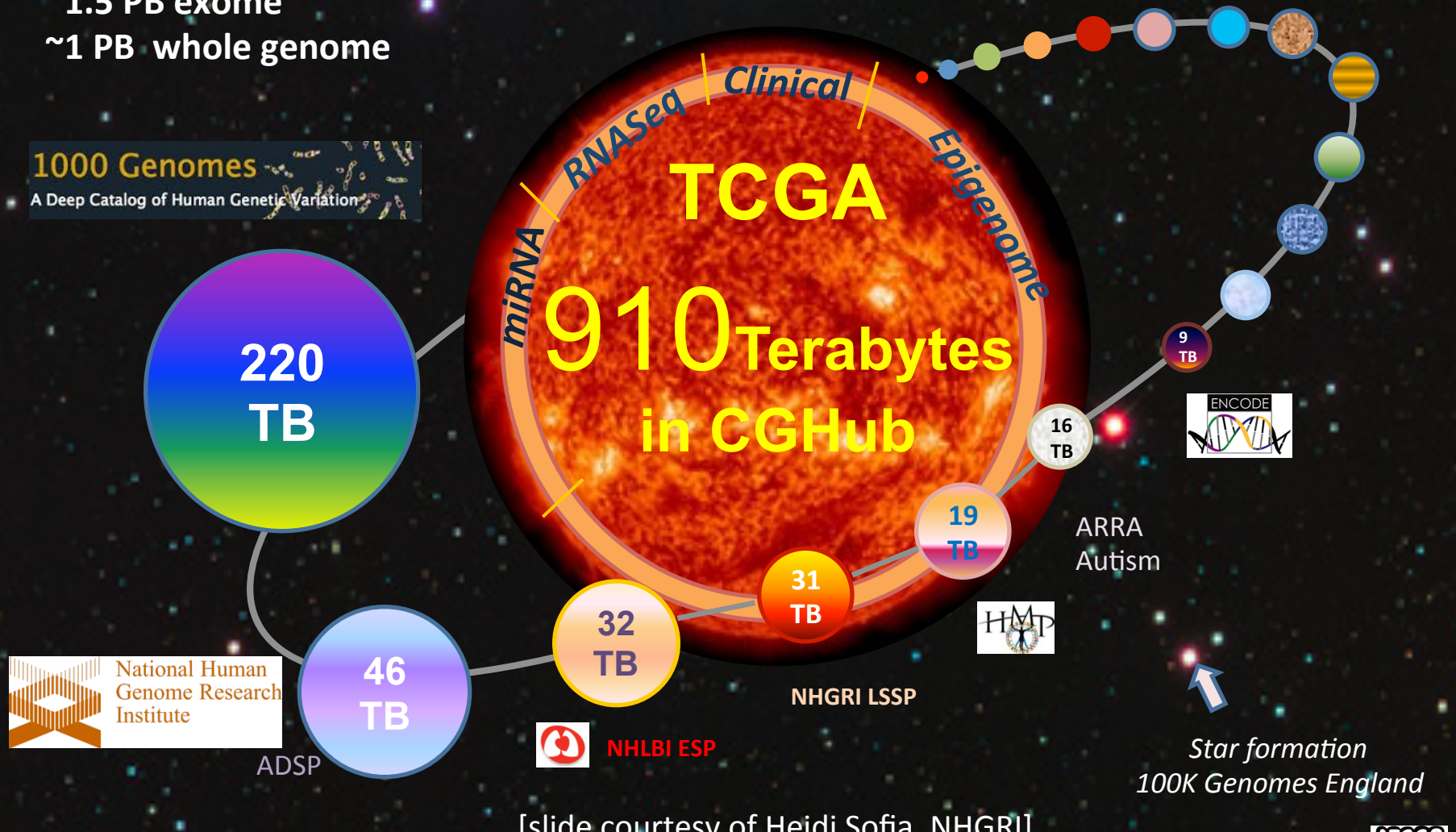
Population scale sequencing of healthy humans

Encyclopedia of DNA Elements, Genome annotation

Seq Universe

SRA >1 petabyte

TCGA endpoint: ~2.5 Petabytes
- **~1.5 PB exome**
- **~1 PB whole genome**



[slide courtesy of Heidi Sofia, NHGRI]

JESS3

Outline

Noncoding regions play an important role in gene regulation. Tumor genome contains thousands of somatic mutations in noncoding regions: how do we identify the functionally important ones

- Noncoding annotations
- Role of noncoding regions in cancer
- Our method for identifying noncoding candidate drivers

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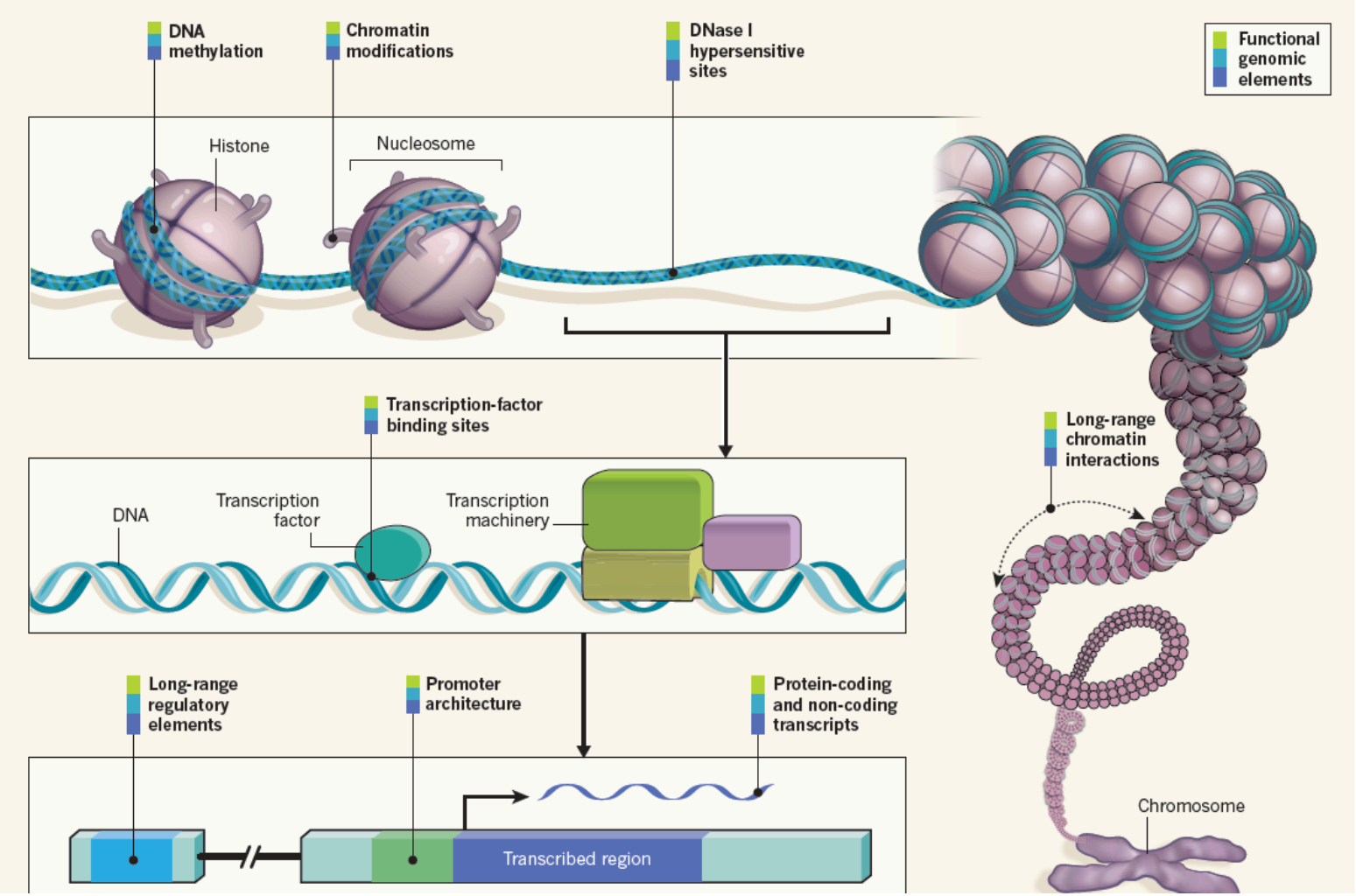
Functional elements in the genome



Enhancers,
Insulators, Silencers

Ecker, *Nature* – News and Views, 2012

ChIP-Seq (Chromatin immunoprecipitation followed by sequencing) to identify TF (transcription factor) binding sites

Crosslink proteins and DNA

Sample fragmentation
- Sonication
- Endonuclease (ChIP–exo)

Immunoprecipitate and then purify DNA

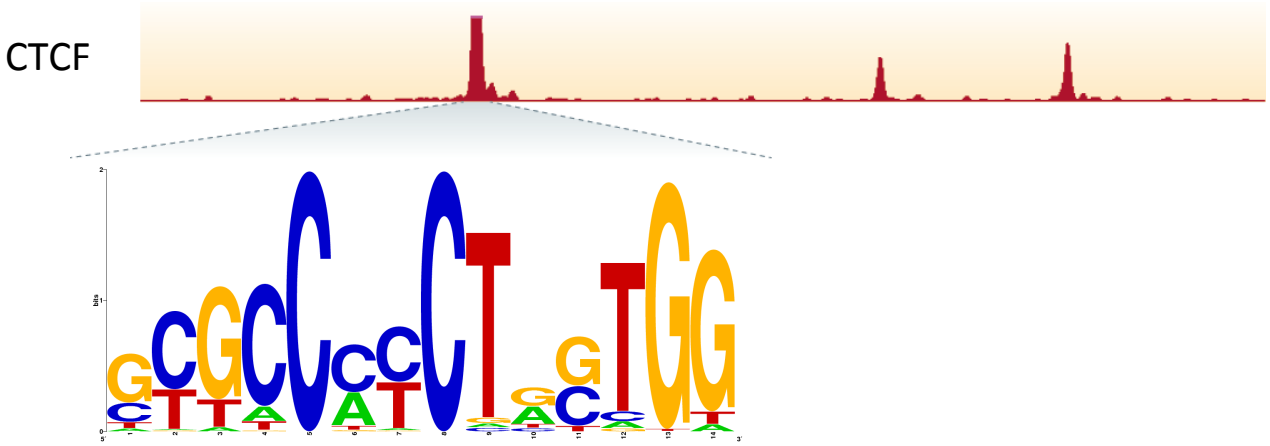
Amplify, if few cells
- LinDA

DNA library creation and sequencing →

→ Map reads to the genome

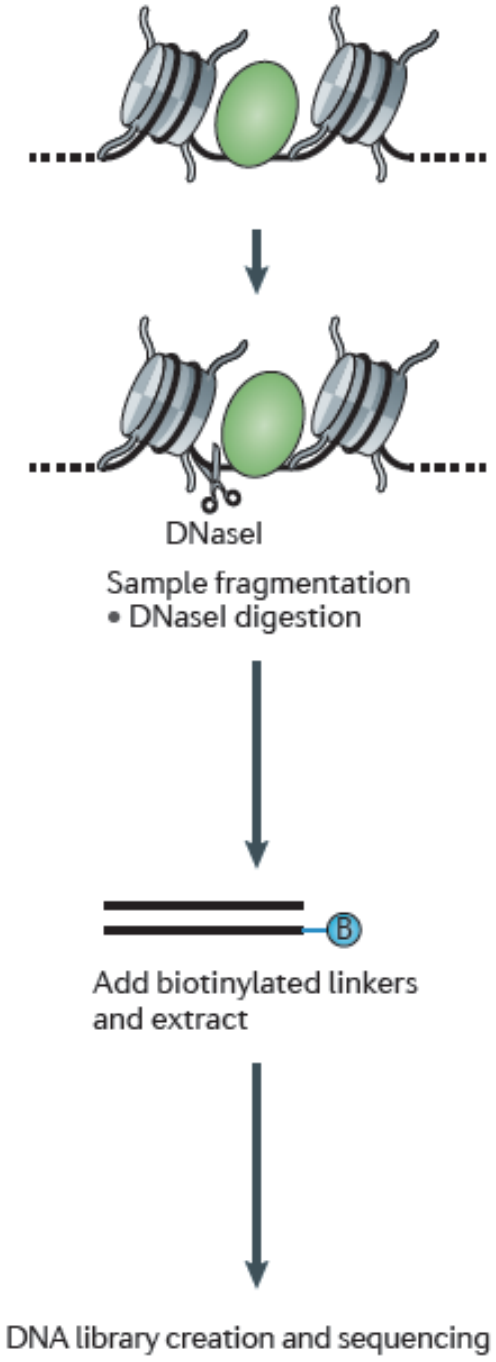
TF binding peak

CTCF



TF binding motif

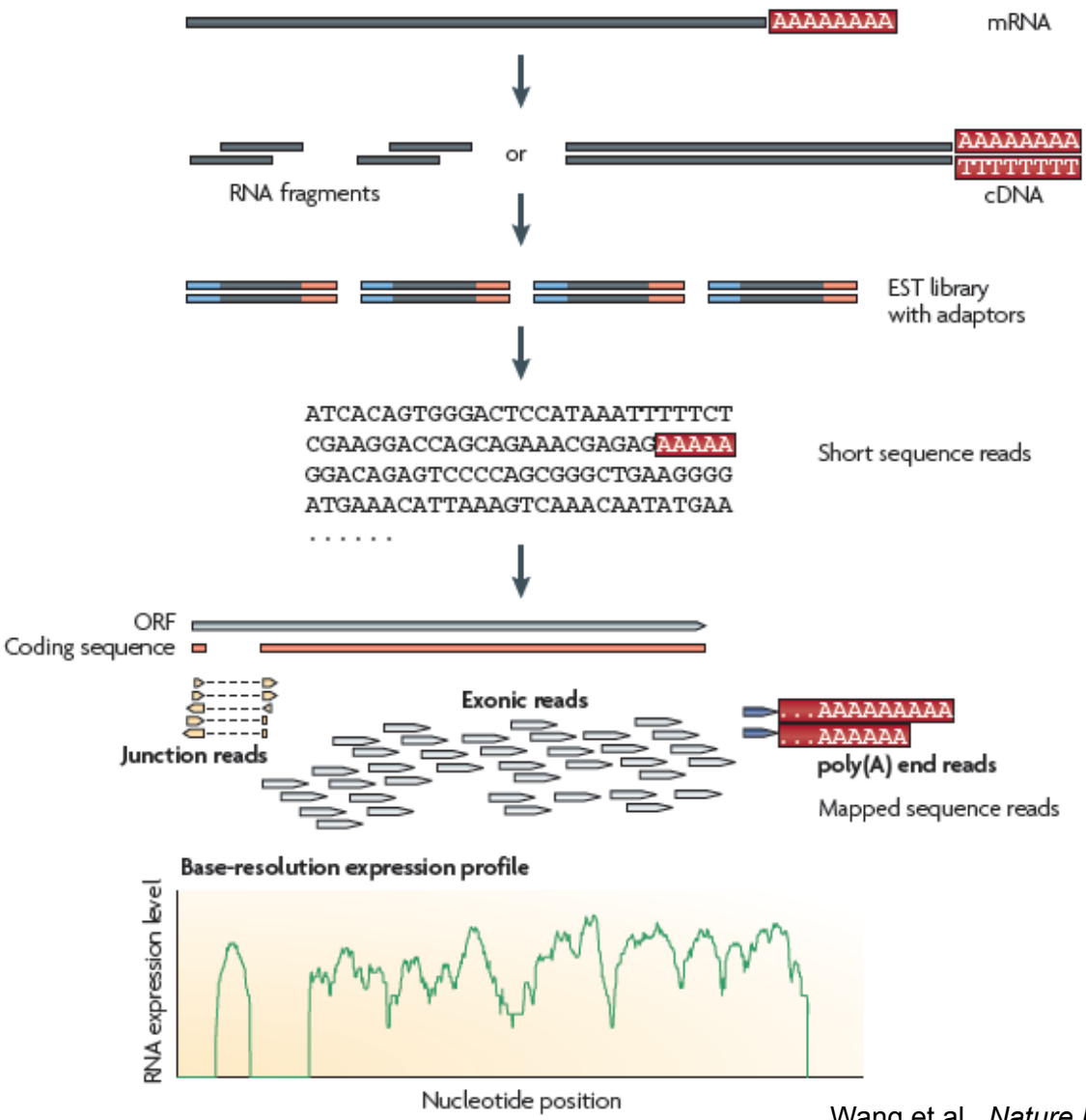
Furey, *Nature Rev. Genet.*, 2012



DNase-Seq to identify regions of open chromatin where any TF could be bound

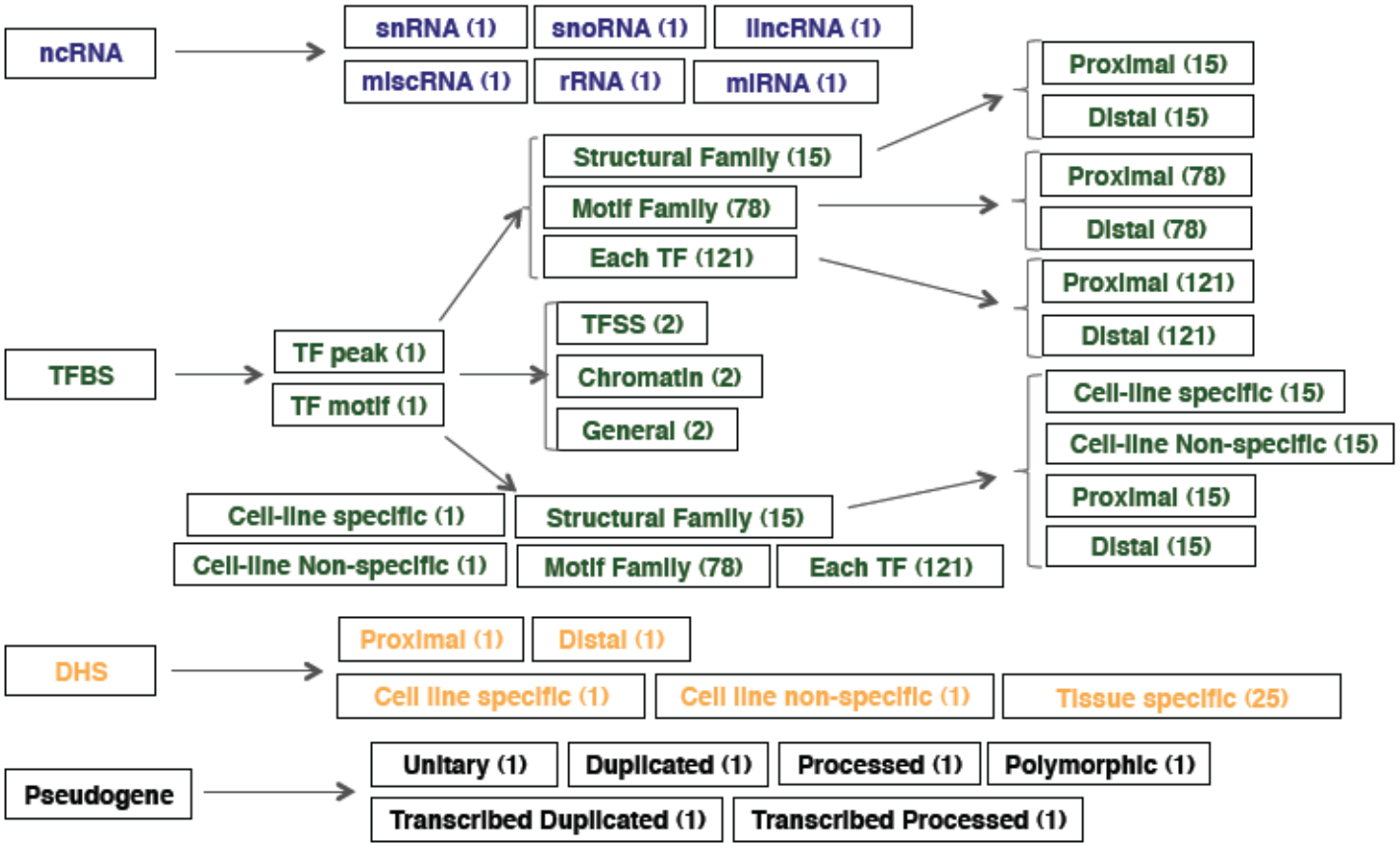
Furey, *Nature Rev. Genet.*, 2012

RNA-Seq to find transcribed regions

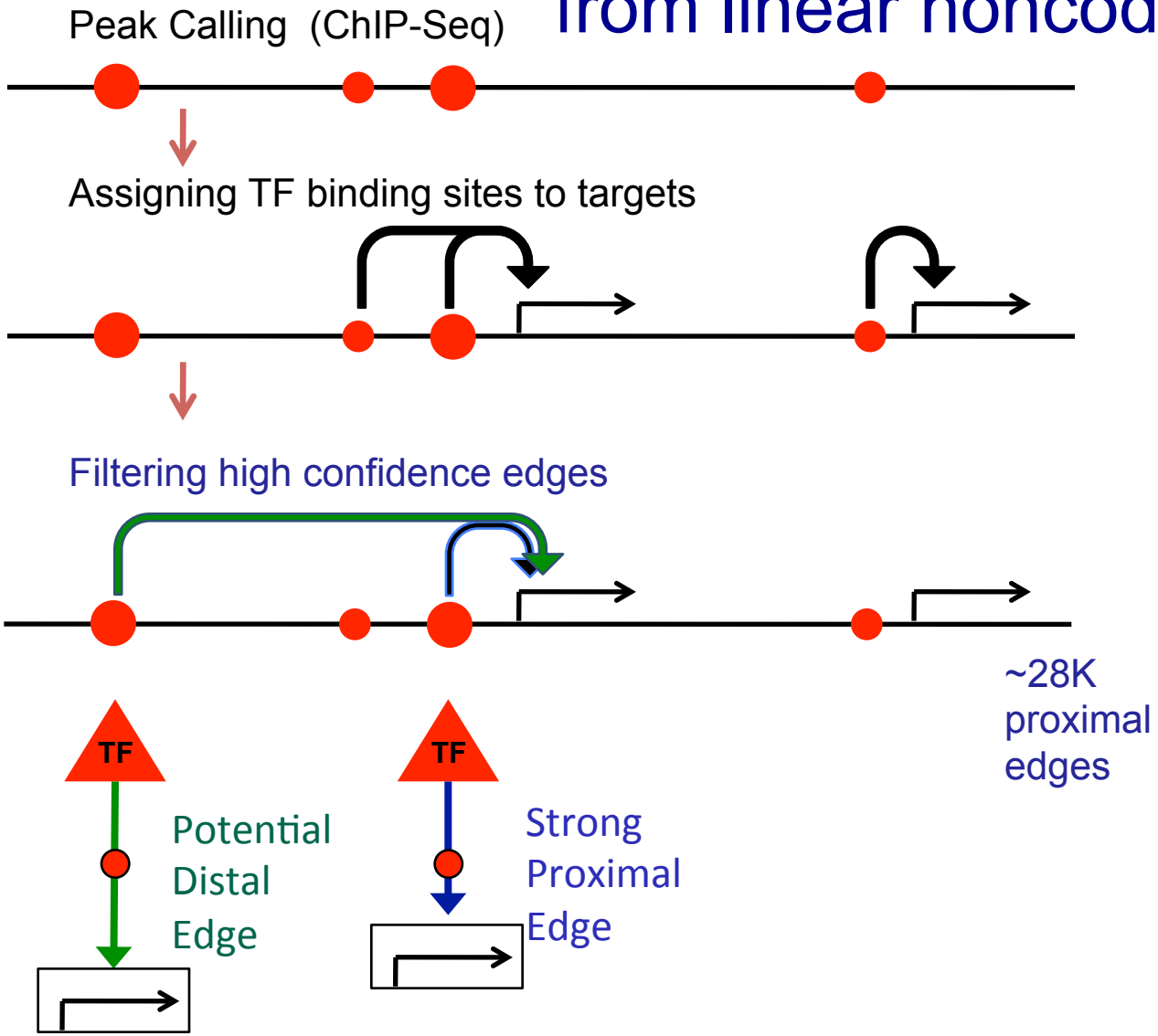


Wang et al., *Nature Rev. Genet.*, 2009

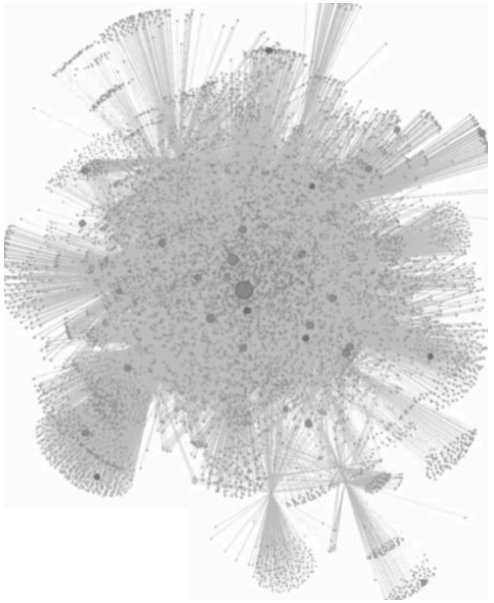
Complexity in levels of noncoding annotations



Building human regulatory network from linear noncoding annotations



Nodes
119 TFs, and ~9000 target genes
Edges
28,000 interactions



Using correlation with expression data

Gerstein¶…..Khurana¶…., *Nature*, 2012 (¶ co-first authors)
Yip et al, *Genome Res*, 2012

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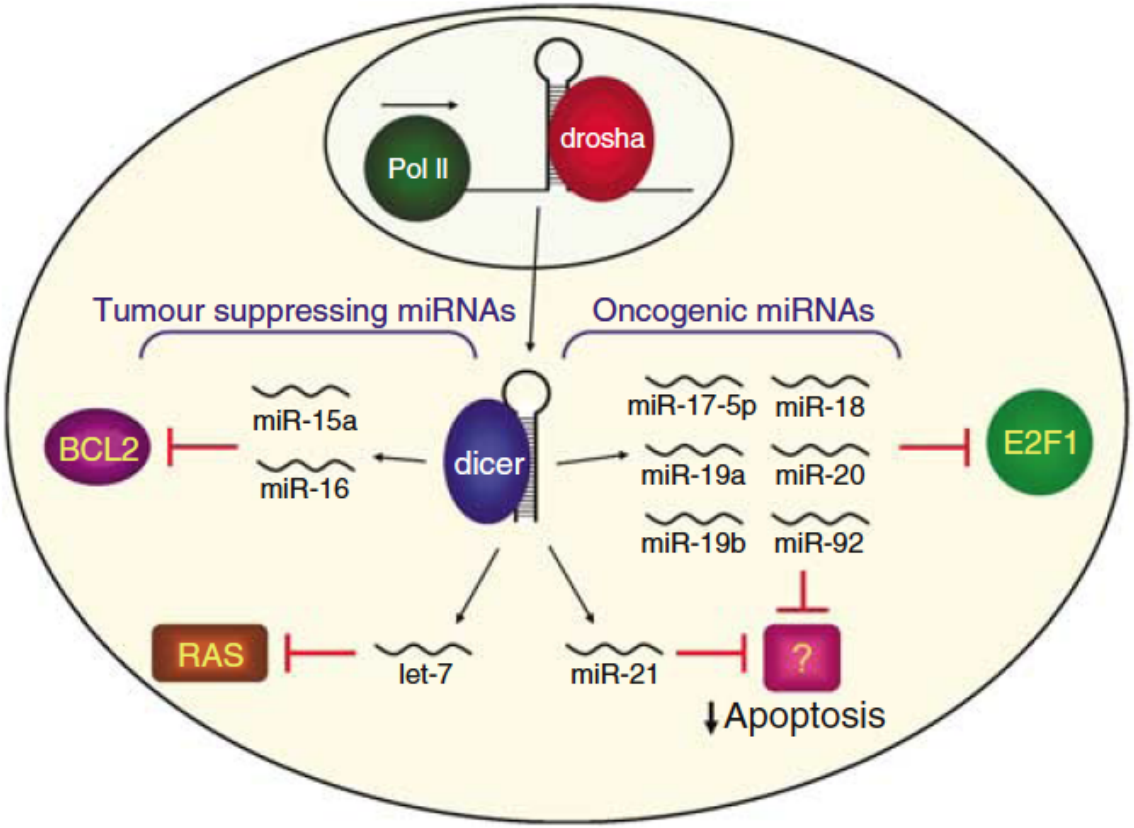
miRNAs can act as oncogenes or tumor-suppressor genes



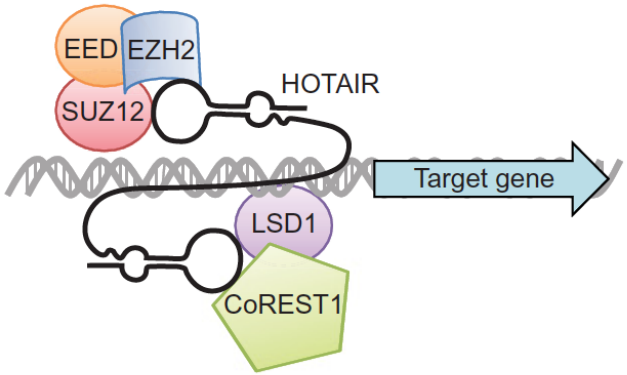
miRNA

- **~22 nt long**
- **Regulate ~30% of mRNAs**
- **Negative regulation of gene expression**

Hwang & Mendell, *British Journal of Cancer*, 2006

Some lncRNA mechanisms

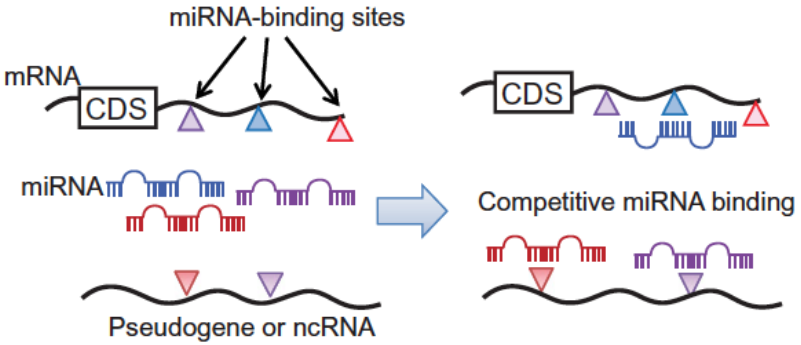
Flexible scaffold for chromatin-modifying complexes



HOTAIR
HOX antisense intergenic RNA

lncRNA
- **>200bp**
- **Include lincRNA and pesudogene derived transcripts**

miRNA sequestration



***PTEN* pseudogene loss in cancer related to reduced *PTEN* expression**

[Other mechanisms: Enhancer RNAs, Tumor suppressor signaling, RNA processing, RNA-RNA interactions]

Prensner & Chinnaiyan, *Cancer Discovery*, 2011
Poliseno et al., *Nature*, 2010
Gupta et al, *Nature*, 2010

Mutations affecting TF binding

Huang et al, *Science*, 2013

Highly Recurrent *TERT* Promoter Mutations in Human Melanoma

Horn et al., *Science*, 2013

***TERT* Promoter Mutations in Familial and Sporadic Melanoma**

***TERT* (Telomerase Reverse Transcriptase) promoter mutation frequency**

Tumor type*	No. tumors	No. tumors mutated (%)
Chondrosarcoma	2	1 (50)
Dysembryoplastic neuroepithelial tumor	3	1 (33.3)
Endometrial cancer	19	2 (10.5)
Ependymoma	36	1 (2.7)
Fibrosarcoma	3	1 (33.3)
Glioma†	223	114 (51.1)
Hepatocellular carcinoma	61	27 (44.2)
Medulloblastoma	91	19 (20.8)
Myxofibrosarcoma	10	1 (10.0)
Myxoid liposarcoma	24	19 (79.1)
Neuroblastoma	22	2 (9)
Osteosarcoma	23	1 (4.3)
Ovarian, clear cell carcinoma	12	2 (16.6)
Ovarian, low grade serous	8	1 (12.5)
Solitary fibrous tumor (SFT)	10	2 (20.0)
Squamous cell carcinoma of head and neck	70	12 (17.1)
Squamous cell carcinoma of the cervix	22	1 (4.5)
Squamous cell carcinoma of the skin	5	1 (20)
Urothelial carcinoma of bladder	21	14 (66.6)
Urothelial carcinoma of upper urinary epithelium	19	9 (47.3)

Killela et al, *PNAS*, 2013

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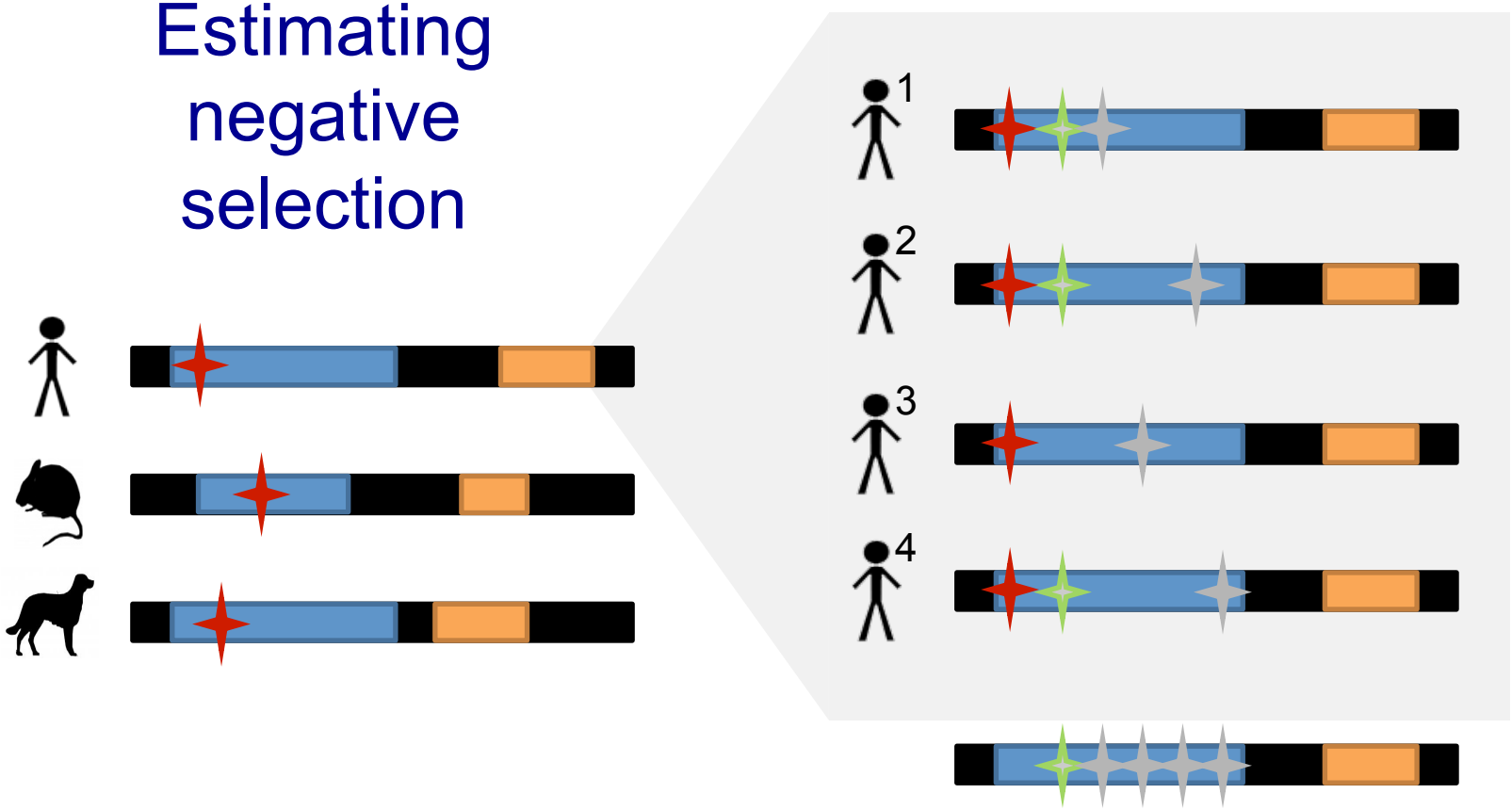
Estimating negative selection



Evolutionary conservation

- Typically defined by comparison across species

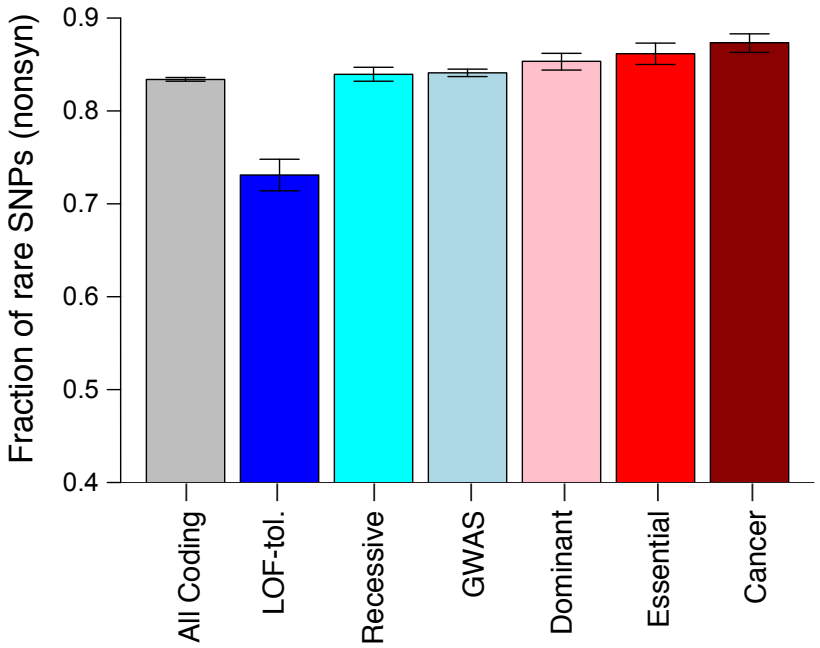
Conservation among humans

- Depletion of common variants/**Enrichment** of rare variants

Common variant Rare variants

Fraction of rare variants = (Num of rare variants/ Total num of variants)

Enrichment of rare SNPs as a metric for negative selection in protein-coding genes



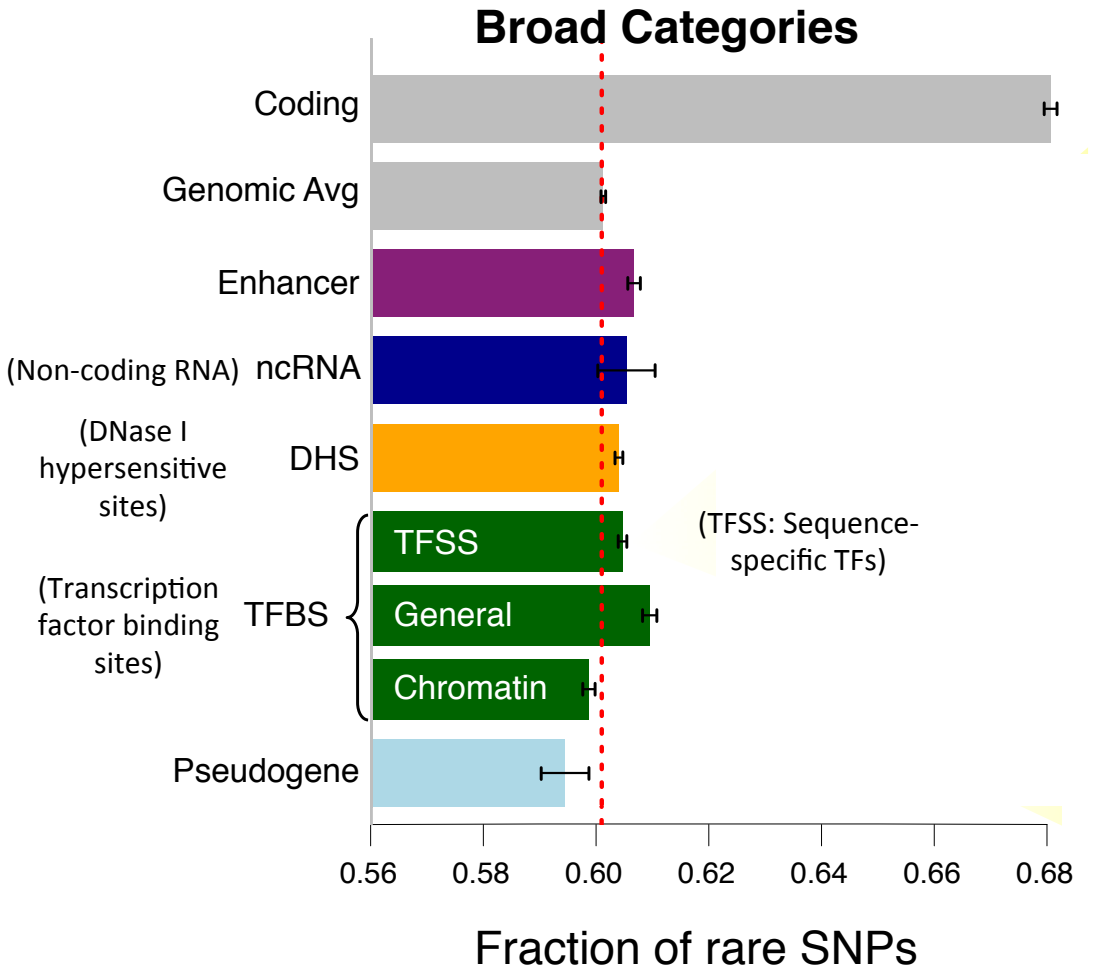
(rare=derived allele freq < 0.5%)

- Depletion of common polymorphisms in regions under selection
  Negative selection restricts the allele frequency of deleterious mutations.
- Results for coding genes consistent with known phenotypic impacts
- Cancer drivers under strongest negative selection

LOF-tol (Loss-of-function tolerant): least negative selection
Cancer: most selection

Khurana et al., *Science*, 2013

Negative selection in noncoding elements

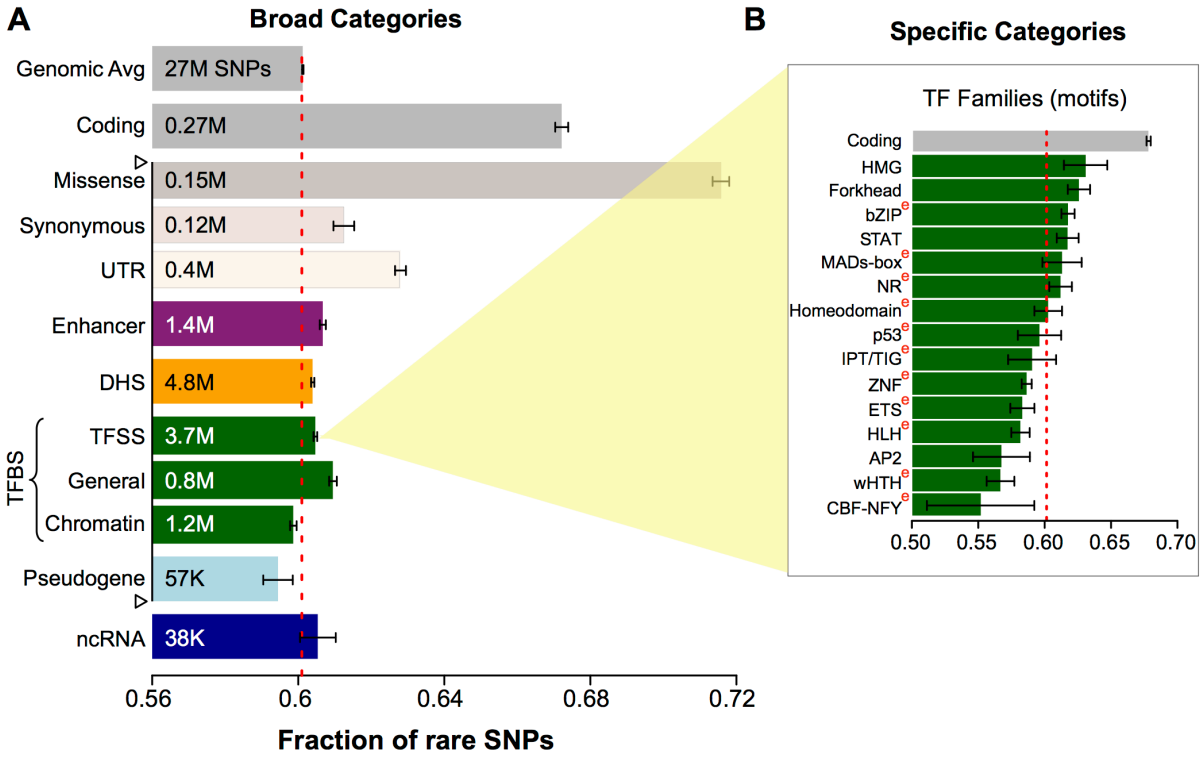


- Broad categories of regulatory regions under negative selection
- Consistent with previous studies

ENCODE, *Nature*, 2012

Khurana et al., *Science*, 2013

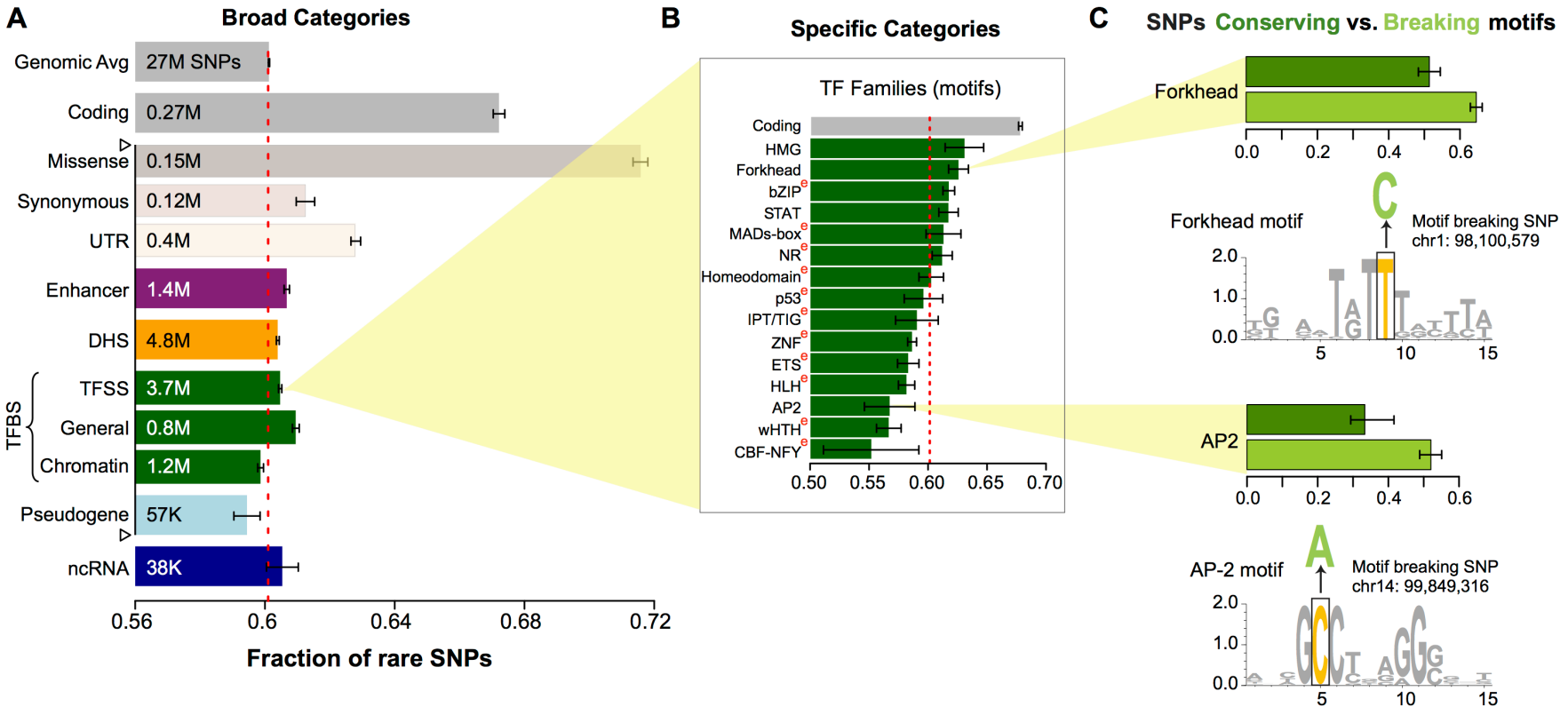
Differential selective constraints among sub-categories



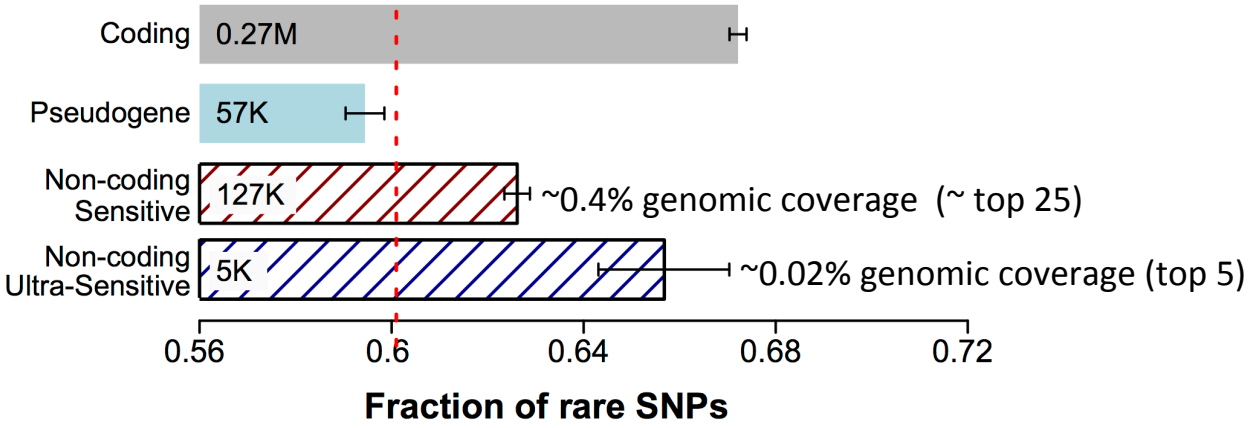
- Divide broad categories
  - ncRNA: snRNA, snoRNA, miRNA, lincRNA
  - Motifs & binding sites of different TF families
  - TFBSs divide into proximal vs distal and cell-line–specific vs – non-specific

Khurana et al., *Science*, 2013

SNPs which break TF motifs are under stronger selection



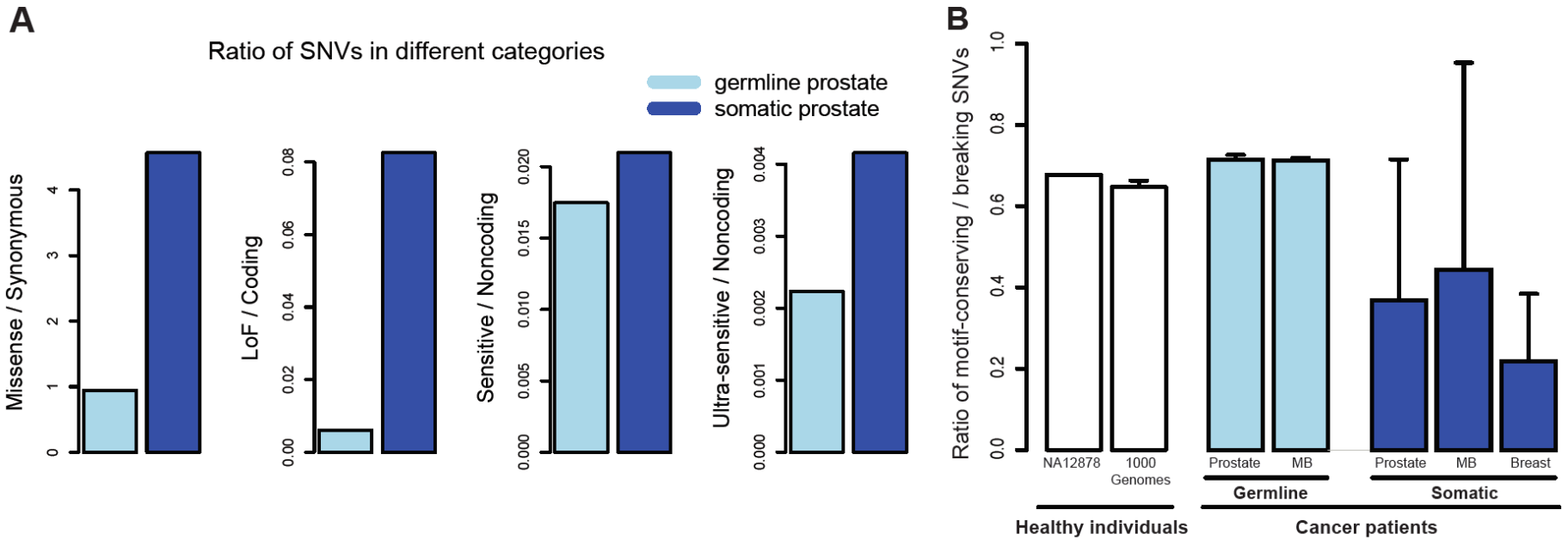
Which noncoding categories are under very strong “coding-like” selection ?



- Identify the top categories under strong negative selection
- Binding peaks of some general TFs (eg *FAM48A*)
- Core motifs of some TF families (eg *JUN*, *GATA*)
- DHS sites in spinal cord and connective tissue

Khurana et al., *Science*, 2013

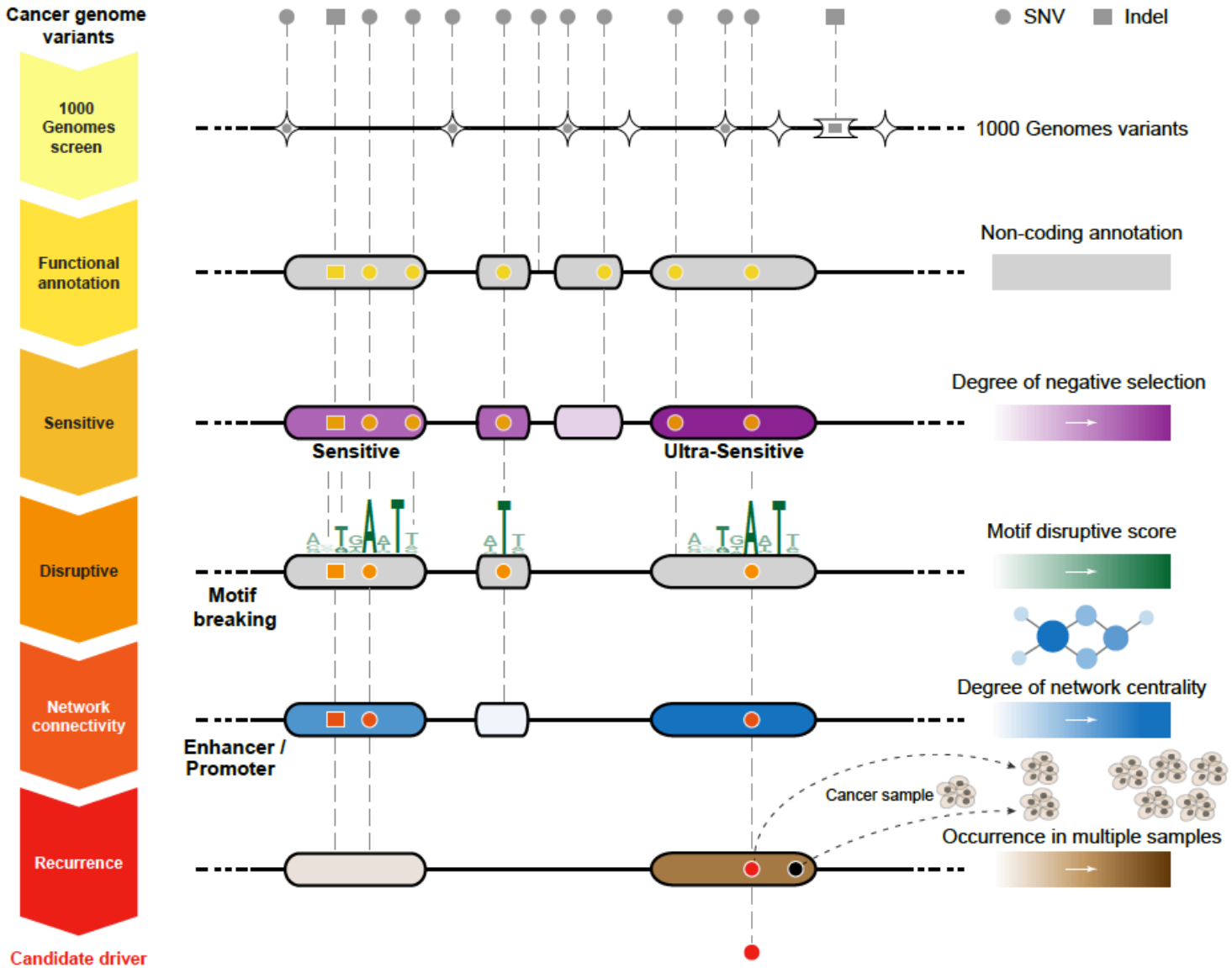
Germline vs somatic variants



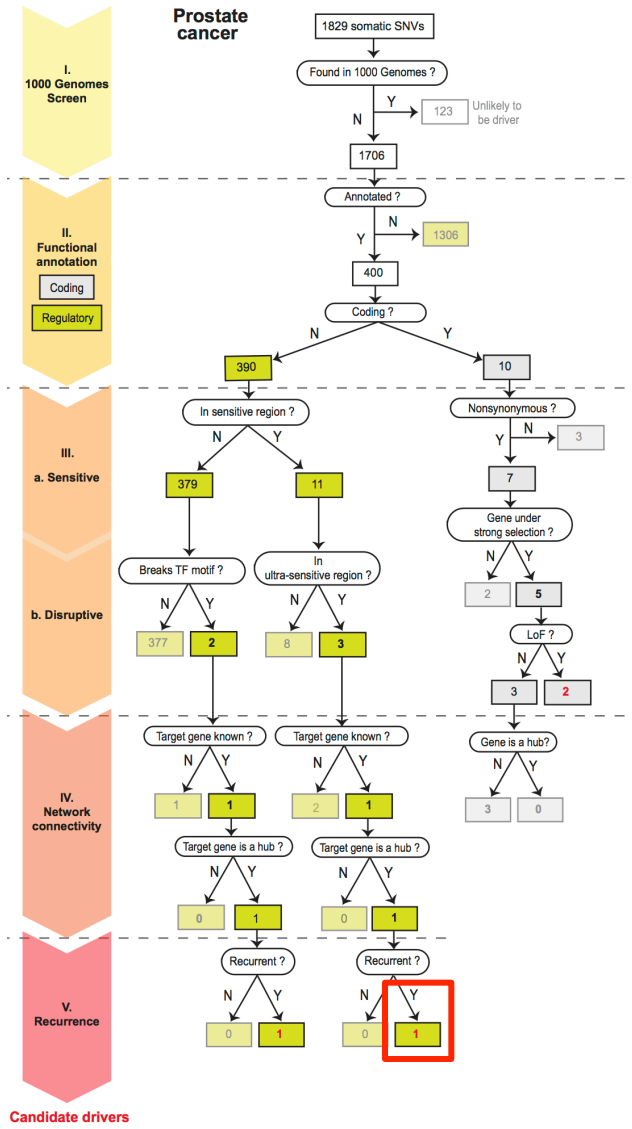
- Somatic mutations do not follow patterns of natural polymorphisms
- Those deviating the most from these patterns are most likely to be cancer drivers providing selective advantage to the tumor cells (confirmed for protein-coding genes)
- Look for mutations in elements under strong negative selection

Khurana et al., *Science*, 2013

Identification of noncoding candidate drivers among somatic variants: Scheme

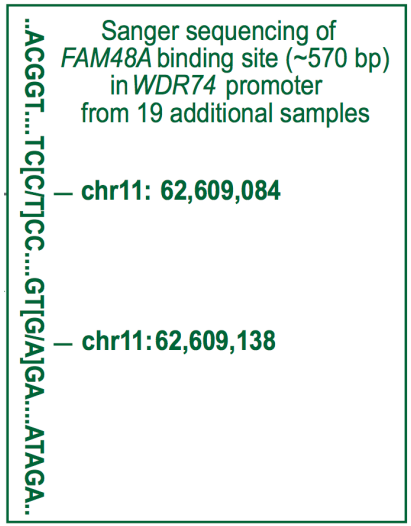


Identification of noncoding candidate drivers among somatic variants: Example

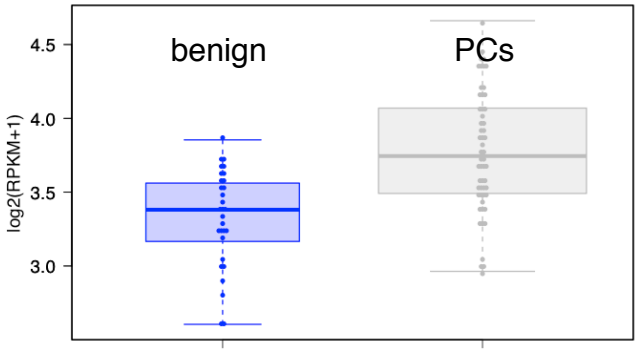


Validation of a candidate driver identified in prostate cancer sample in *WDR74* gene promoter

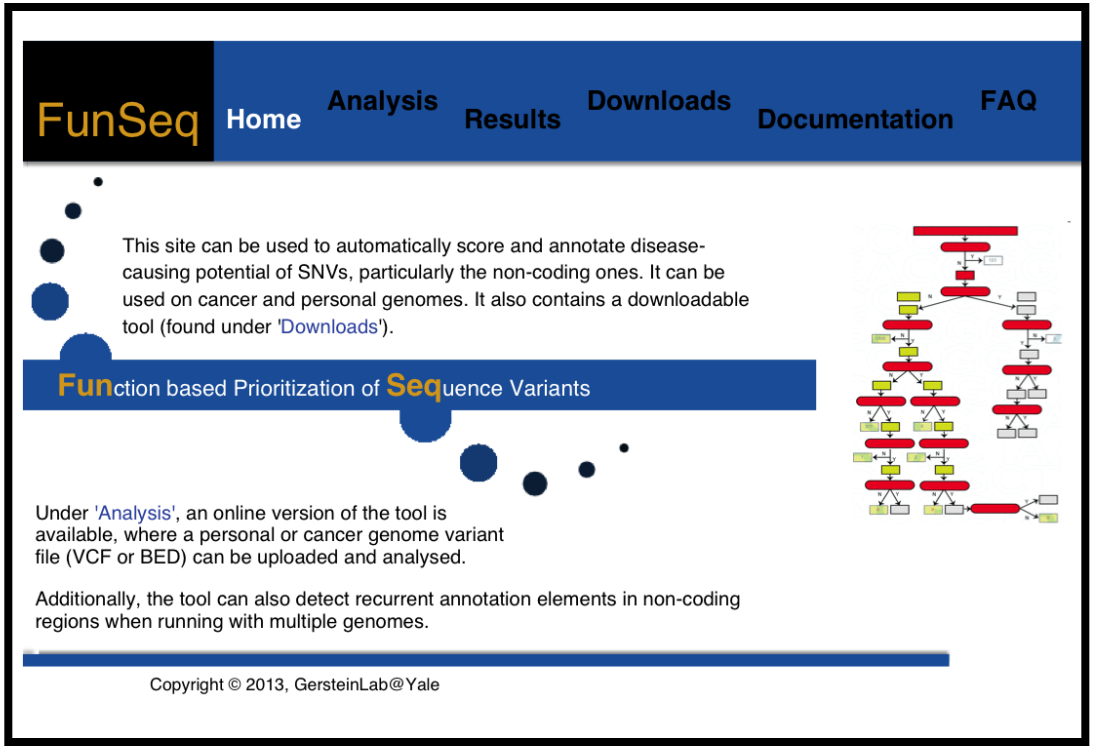
- Sanger sequencing in 19 additional samples confirms the recurrence in one more sample



- *WDR74* shows increased expression in tumor samples



FunSeq.GersteinLab.org : webserver & code download



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Functional Interpretation Group

~50 participants

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