Introduction to genome tiling microarray analysis

Biological motivations

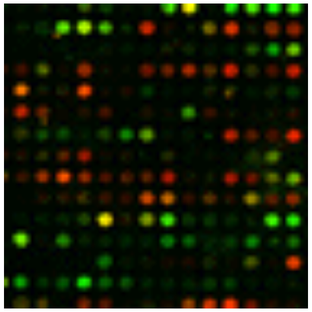
- There are many types of “events” happen at different locations on the genome. For example, protein bindings, epigenetic modifications (DNA methylation and histone modifications), copy number variations, etc.
- It is often of great interests to detect the genomic locations where a specific event happens, or quantify the events along the genome.
- The locations of these events provide explanations for many biological processes.

An example: transcription factor(TF) binding

- Transcription factors (TF): proteins that binds to specific DNA sequences and control the transcription from DNA to mRNA.
- There are many different types of TFs, each recognize different DNA sequences (motifs).
- The functions of the TFs are important for understanding gene regulatory mechanisms.
- The first step toward the understanding is to detect the TF binding sites (TFBS).

Traditional Method for Understanding Transcription Regulation

Gene expression microarray analysis



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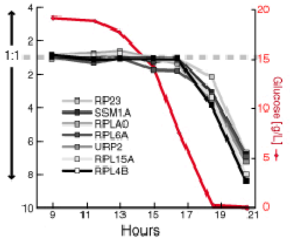
Clustering genes by expression profile



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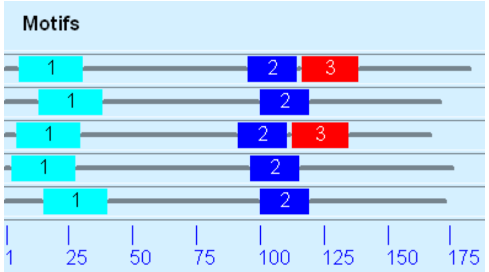
Search conserved sequence motifs in cluster promoters



Very challenging for mammalian genomes!

By Hongkai Ji at Hopkins

Use tiling array (ChIP-chip) to detect TFBS

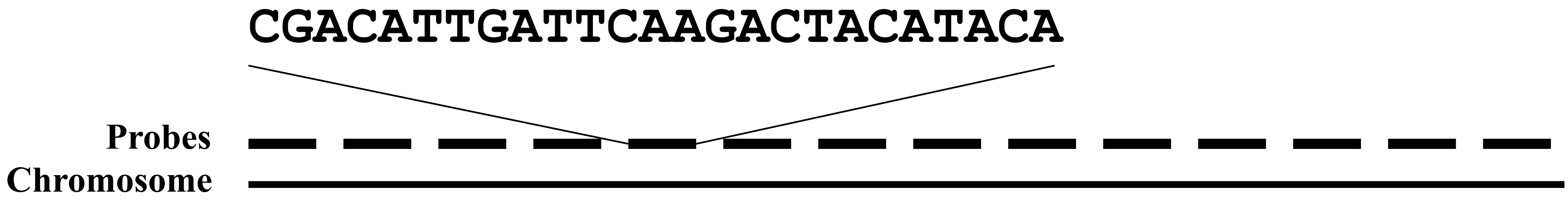
- Detect genome-wide *in vivo* location of TF and other DNA-binding proteins.
- Can learn the regulatory mechanism of a transcription factor or DNA-binding protein much better and faster.

Another example: DNA methylation and histone modification

- DNA methylation and histone modifications are chemical modification of DNA molecule.
- The strengths of such modifications varies along the genome, and they are related to gene regulatory and many diseases.
- The methylation or modification strengths can be measured using ChIP-chip or MeDIP-chip.

Tiling arrays

- The goal is to quantify the events of interests along the genomes, and/or detect the genomic coordinates for the events.
- Work the same as gene expression array (hybridization based), except that the probes are designed to tile up the genome at non-repeat regions.
- Data for probes in the location of interest often behave differently from backgrounds (e.g., bigger intensities).



Types of tiling arrays

- ChIP-chip: Chromatin ImmunoPrecipitation (ChIP) + tiling array (chip) for detecting transcription factor binding sites or measuring histone modification levels.
- MeDIP-chip: Methyl-DNA ImmunoPrecipitation (MeDIP) + tiling array (chip) for measuring DNA methylation level.
- ArrayCGH (Comparative Genomic Hybridization) for detecting copy number variations.

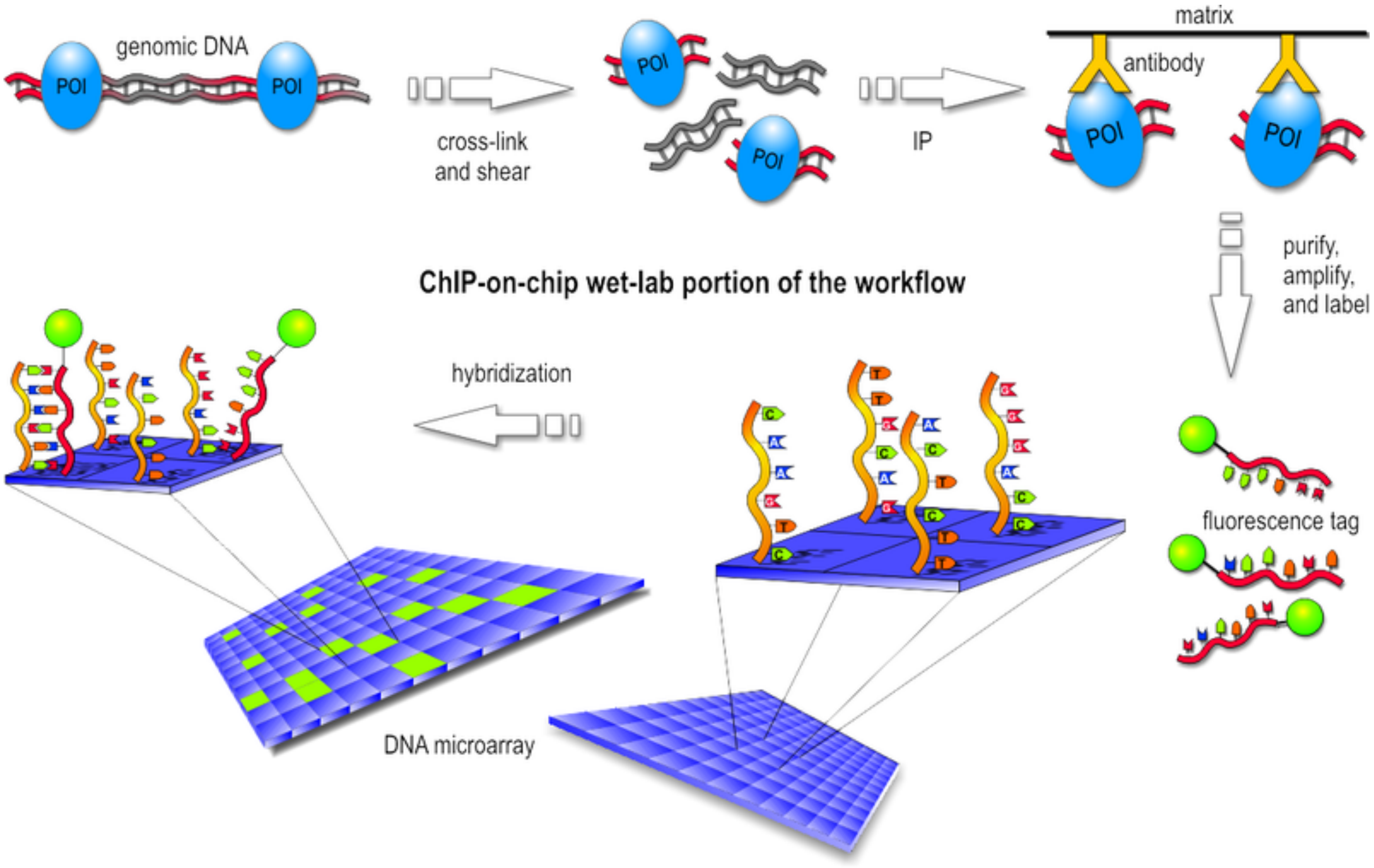
There's no major differences in array designs. Difference are the ways to prepare biological samples.

Available platforms for ChIP-chip

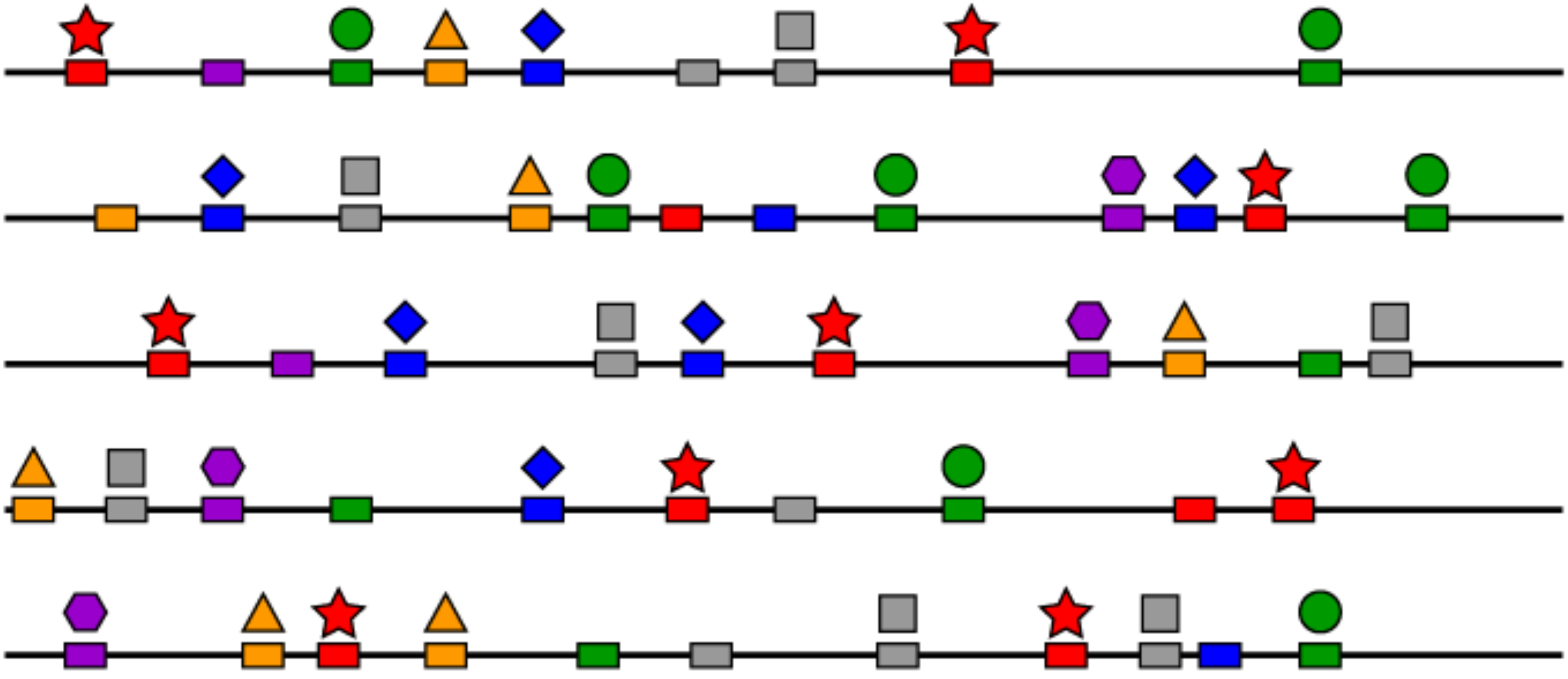
	# Arrays human genome	# Probes / Array	# Total Probes	Probe Length	Probe Resolution
Affymetrix	7	6M	42.0M	25mer	35 bp
Nimblegen	10	2.1M	21M	50mer	100 bp
Agilent	21	244K	5.1M	60mer	300 bp in genes; 500 bp in intergenic

By Xiaole Shirley Liu at Harvard

ChIP-chip procedures

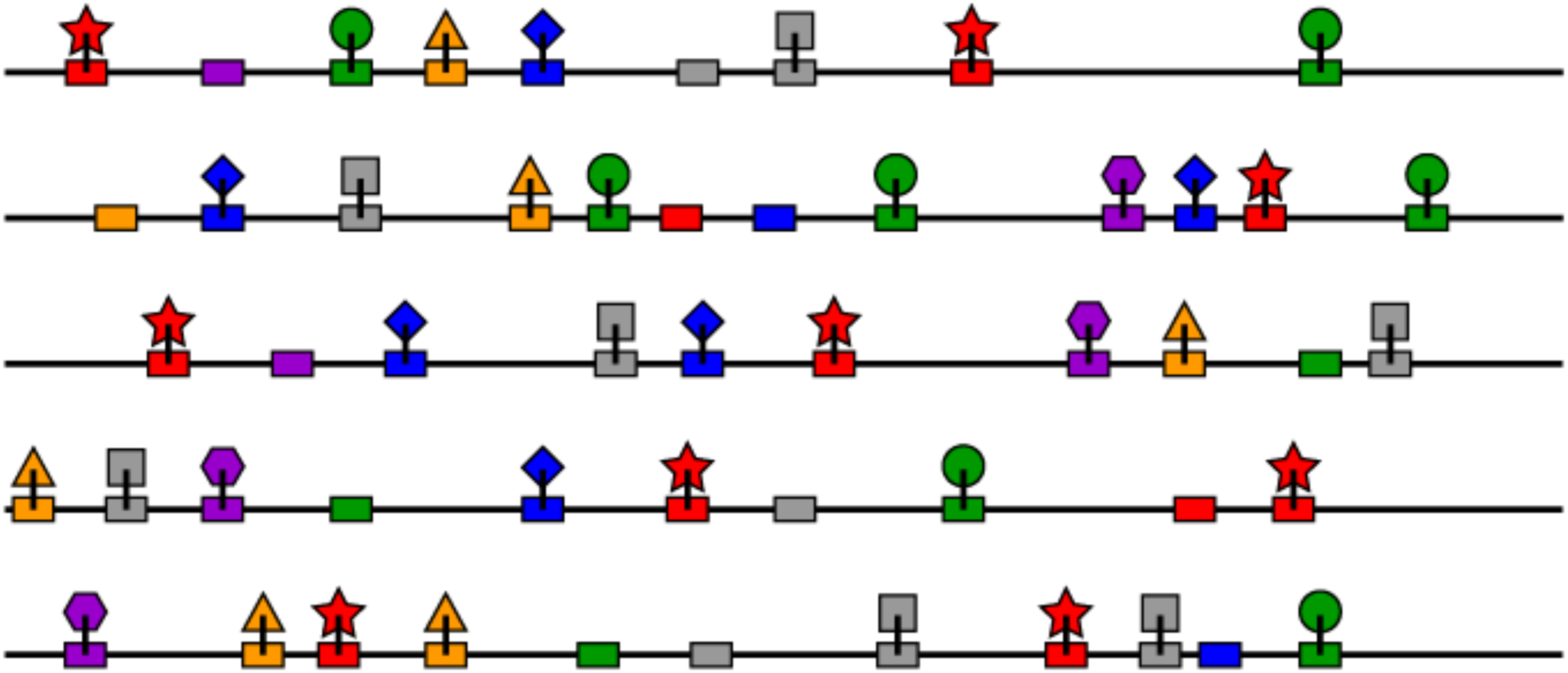


Chromatin ImmunoPrecipitation (ChIP)



By Richard Bourgon at UC Berkley

TF/DNA Crosslinking *in vivo*



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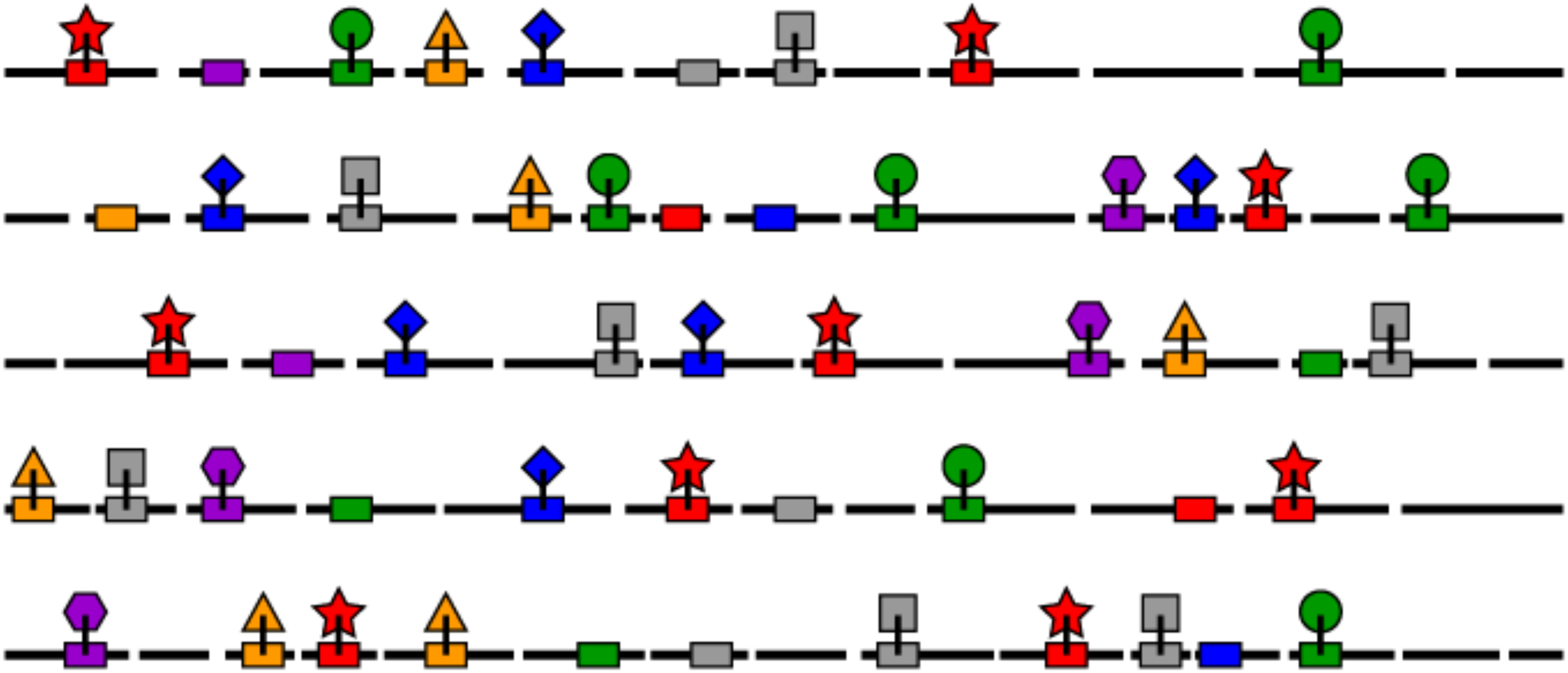
Sonication (~500bp)



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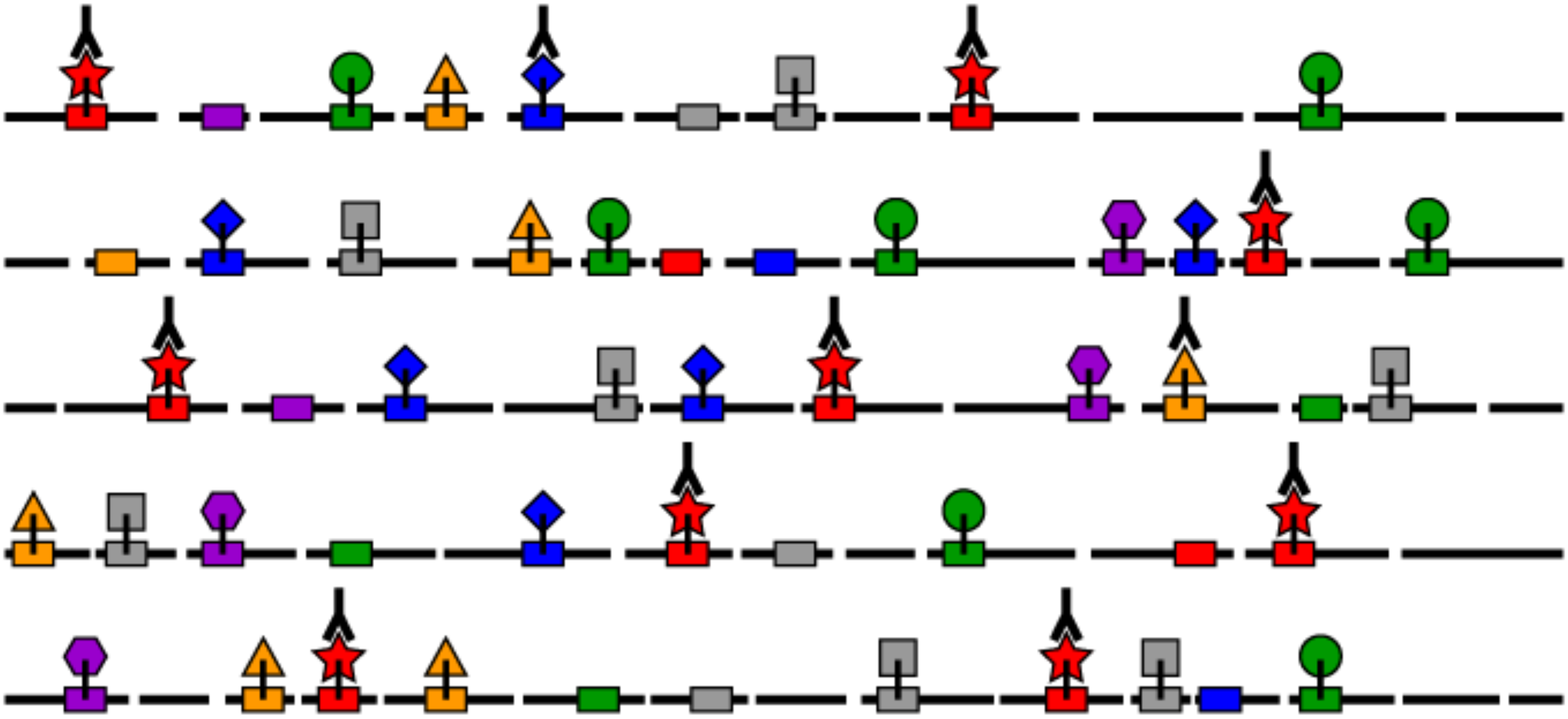
TF-specific Antibody



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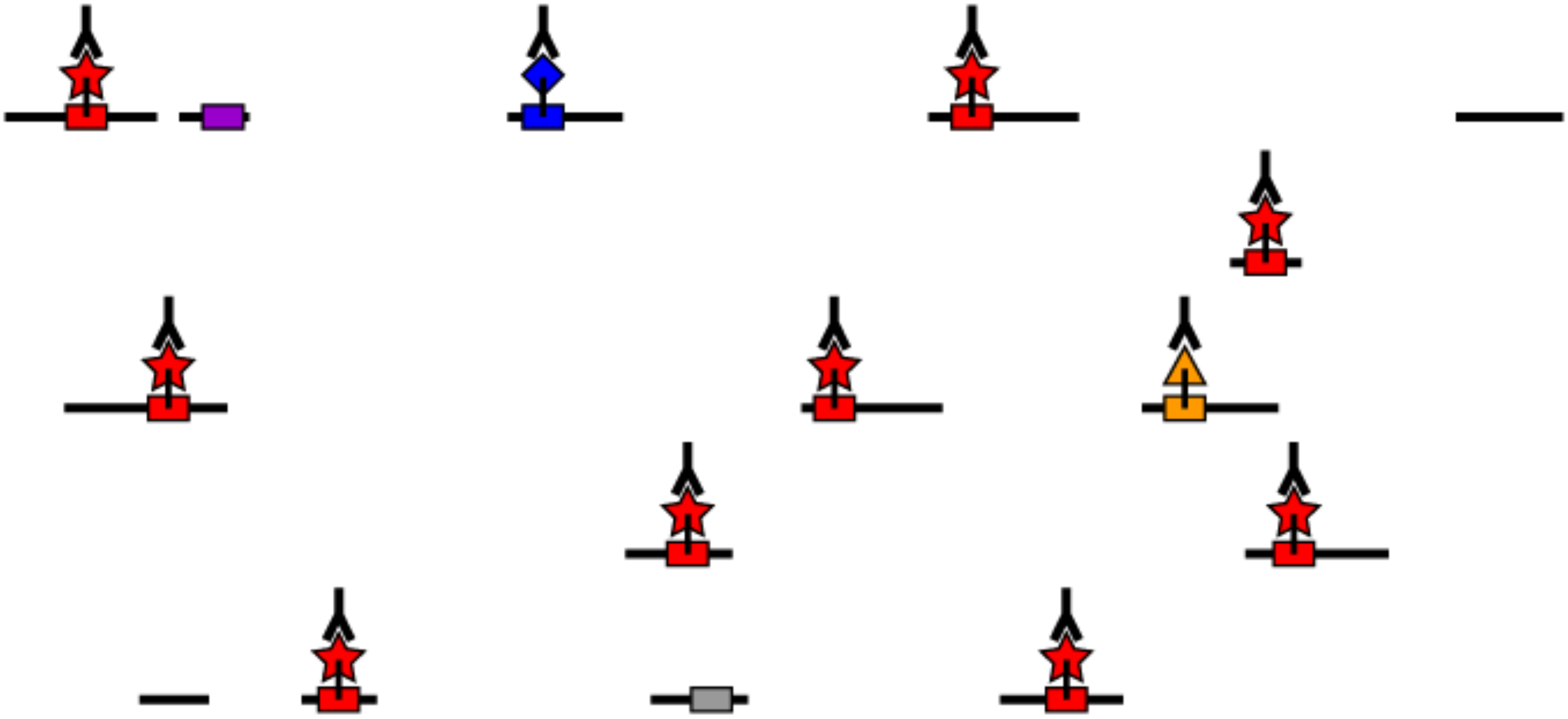
Immunoprecipitation (IP)



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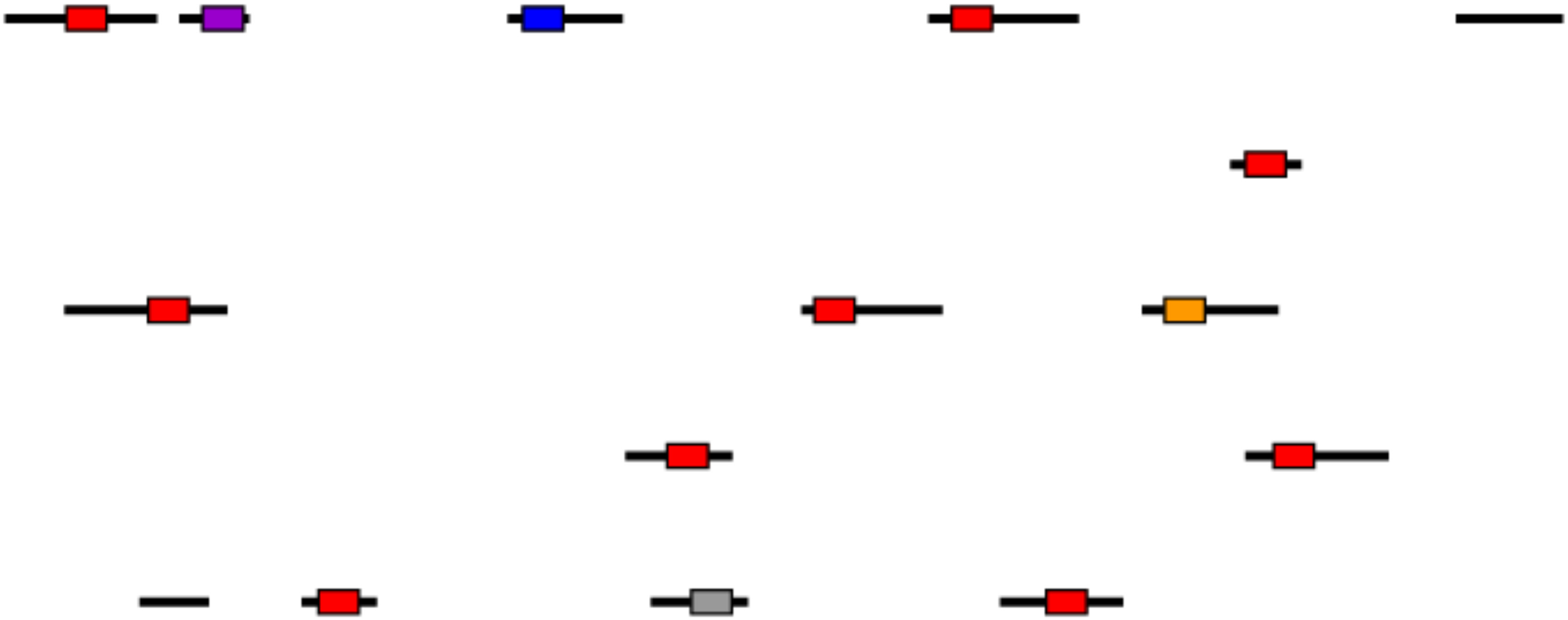
Reverse Crosslink and DNA Purification



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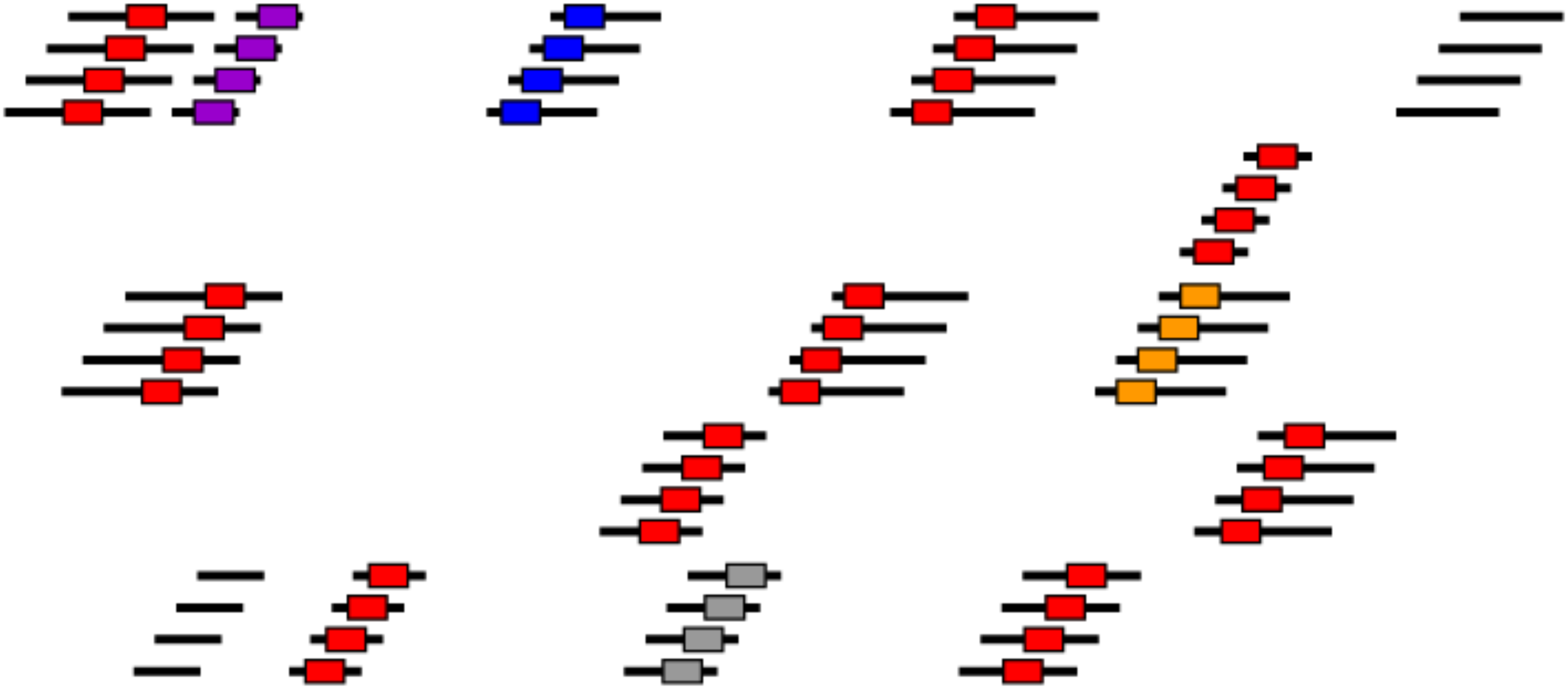
Amplification



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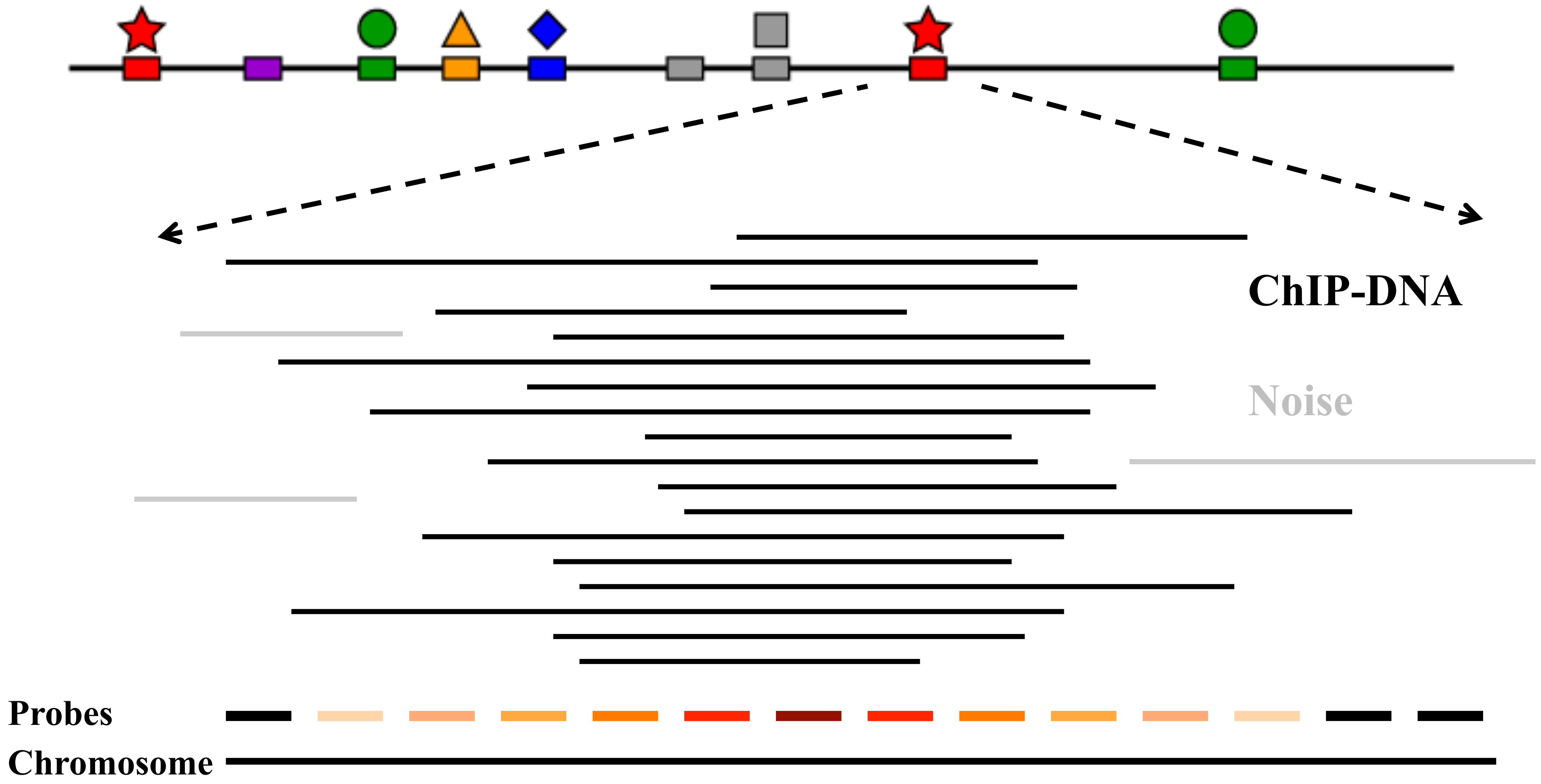
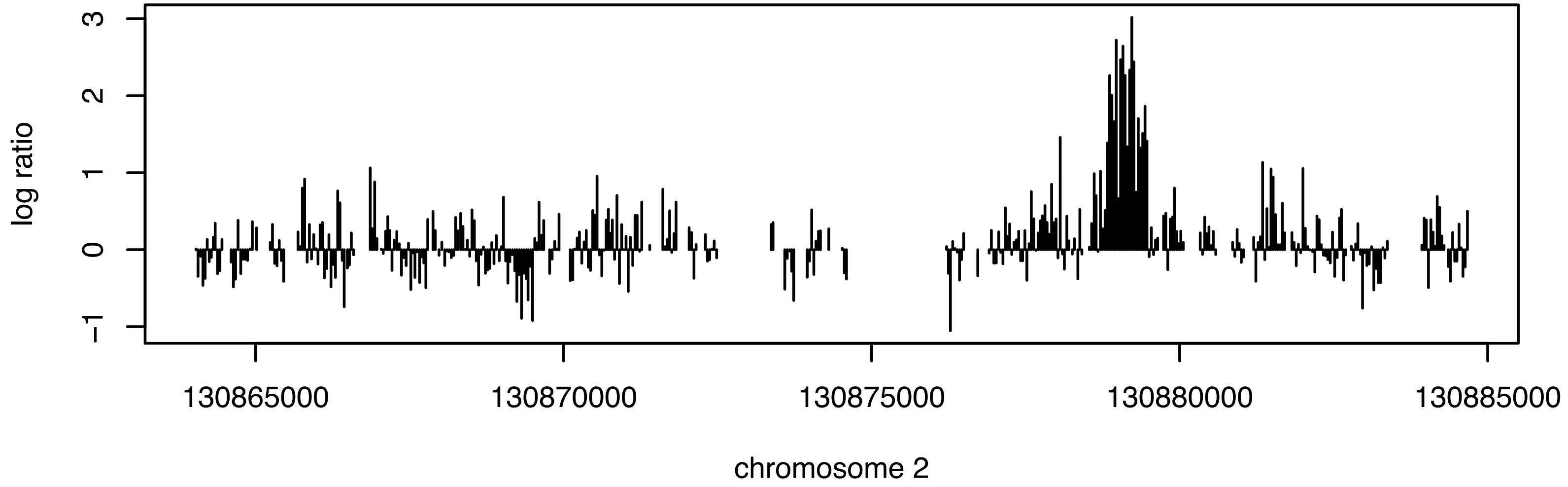
ChIP-chip Hybridization



Based on Xiaole Shirley Liu at Harvard

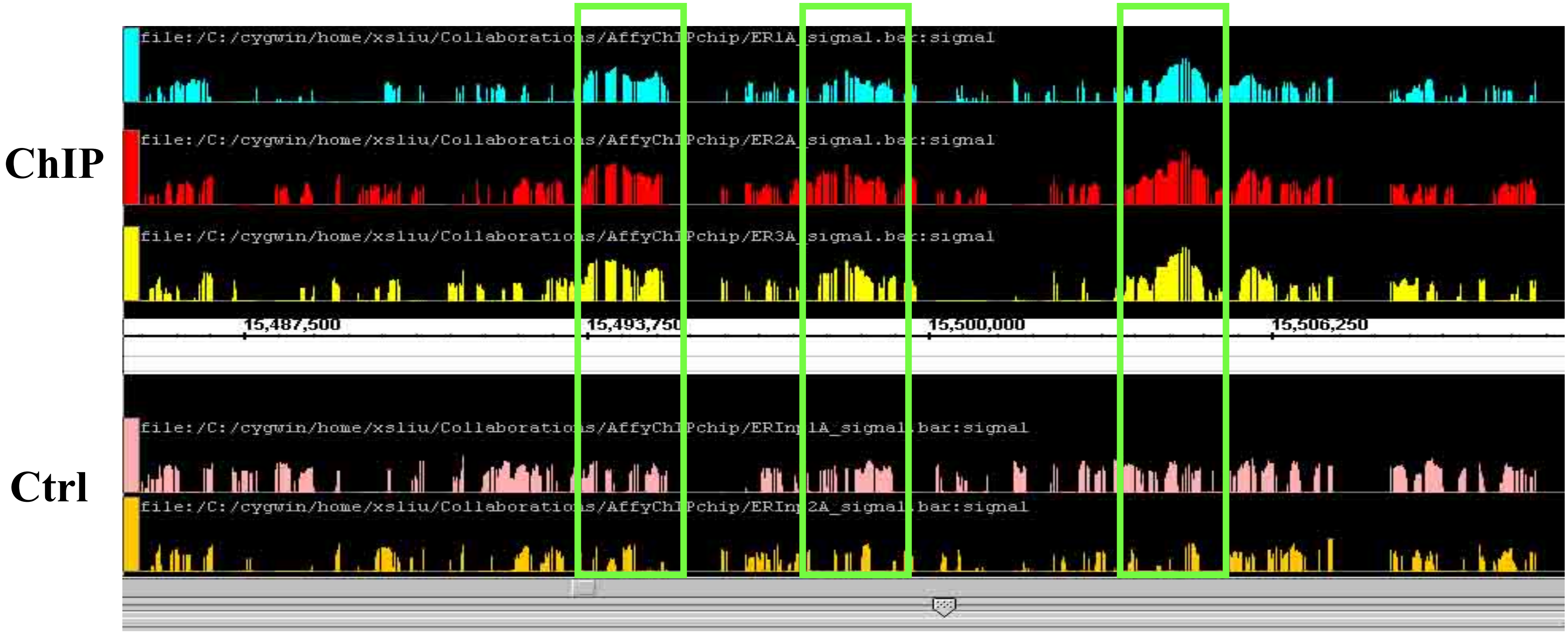
Data from ChIP-chip

- Can be thought as a file with millions of rows and three columns.
  - Each row is for a probe.
  - Columns are chromosome number, probe location on the genome, and signal (intensity values or log fold change).
- To visualize: plot the probe signals against probe locations.



Identify ChIP-enriched Region

- Controls: sonicated genomic input DNA (non-treated).
- Often 3 ChIP, 3 Ctrl replicates are needed


ChIP
file:/C:/cygwin/home/xsliu/Collaborations/AffyChIPchip/ER1A_signal.bar:signal
file:/C:/cygwin/home/xsliu/Collaborations/AffyChIPchip/ER2A_signal.bar:signal
file:/C:/cygwin/home/xsliu/Collaborations/AffyChIPchip/ER3A_signal.bar:signal
15,487,500
15,493,750
15,500,000
15,506,250
Ctrl
file:/C:/cygwin/home/xsliu/Collaborations/AffyChIPchip/ERInp1A_signal.bar:signal
file:/C:/cygwin/home/xsliu/Collaborations/AffyChIPchip/ERInp2A_signal.bar:signal

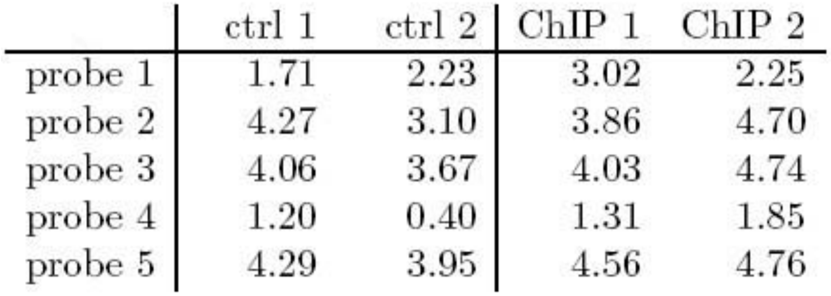
By Xiaole Shirley Liu at Harvard

ChIP-chip data analysis

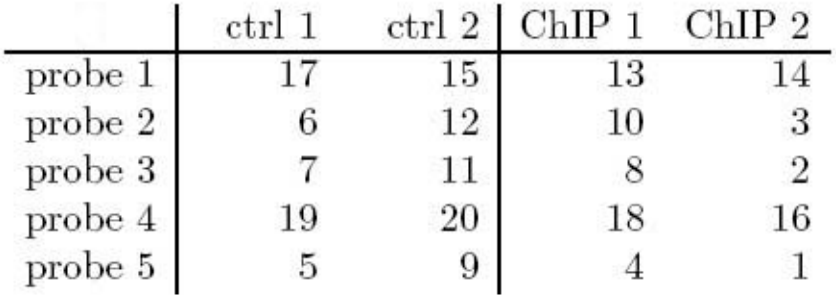
- Goal: detect locations of interests (e.g., binding sites, also called “peaks”) based on probe locations and signals.
- Normalization: remove technical artifacts.
- Detection for regions of interests:
  - Many different methods. Fundamentally data from neighboring probes need to be combined to make inference, because the regions of interests often overlap many probes.
  - Easiest method: moving average, then use an arbitrary cutoff.

Mann-Whitney U-test

- Affy TAS, Cawley et al (*Cell* 2004):
  - Each probe: rank probes signals within [-500bp, +500bp] window.
  - Check whether sum of ChIP ranks is much smaller



	ctrl 1	ctrl 2	ChIP 1	ChIP 2
probe 1	1.71	2.23	3.02	2.25
probe 2	4.27	3.10	3.86	4.70
probe 3	4.06	3.67	4.03	4.74
probe 4	1.20	0.40	1.31	1.85
probe 5	4.29	3.95	4.56	4.76



	ctrl 1	ctrl 2	ChIP 1	ChIP 2
probe 1	17	15	13	14
probe 2	6	12	10	3
probe 3	7	11	8	2
probe 4	19	20	18	16
probe 5	5	9	4	1

By Xiaole Shirley Liu at Harvard

TileMap (Ji and Wong, Bioinformatics 2005)

STEP 1:
Compute a test statistic for each probe to summarize probe level information

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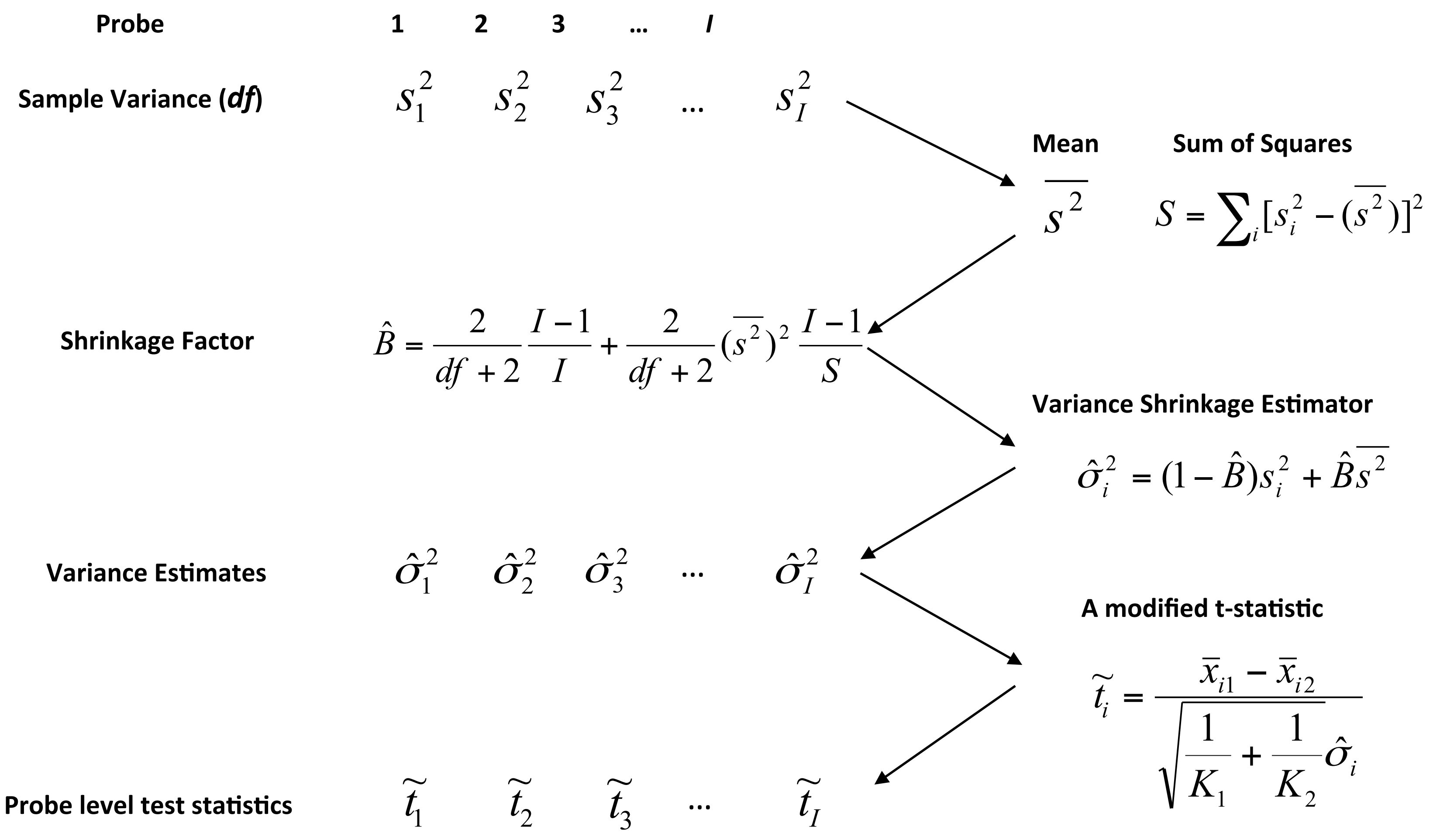
STEP 2:
Combine probe level test statistics of neighboring probes to help infer binding regions

By Hongkai Ji at Hopkins

Probe level test statistic: empirical Bayes approach



Probe **1** **2** **3** **…** ***I***

Sample Variance (*df*) s_1^2 s_2^2 s_3^2 … s_I^2

Mean

$$\overline{s^2}$$

Sum of Squares

$$S = \sum_i [s_i^2 - (\overline{s^2})]^2$$

Shrinkage Factor

$$\hat{B} = \frac{2}{df+2}\frac{I-1}{I} + \frac{2}{df+2}(\overline{s^2})^2\frac{I-1}{S}$$

Variance Shrinkage Estimator

$$\hat{\sigma}_i^2 = (1-\hat{B})s_i^2 + \hat{B}\overline{s^2}$$

Variance Estimates $\hat{\sigma}_1^2$ $\hat{\sigma}_2^2$ $\hat{\sigma}_3^2$ … $\hat{\sigma}_I^2$

A modified t-statistic

$$\tilde{t}_i = \frac{\bar{x}_{i1} - \bar{x}_{i2}}{\sqrt{\frac{1}{K_1} + \frac{1}{K_2}}\hat{\sigma}_i}$$

Probe level test statistics $\tilde{t}_1$ $\tilde{t}_2$ $\tilde{t}_3$ … $\tilde{t}_I$

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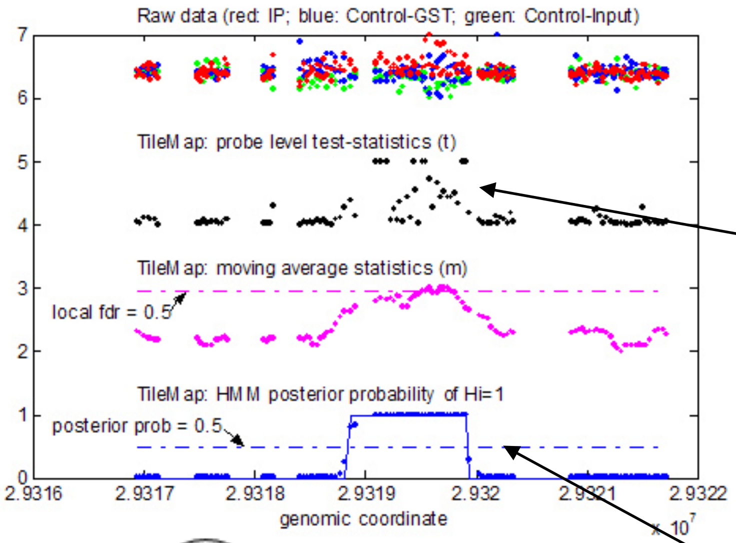
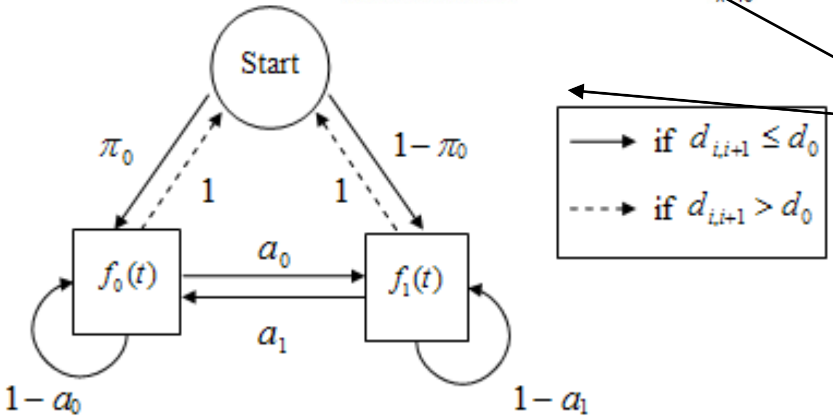
Combining neighboring probes





TileMap (MA)

1. Compute the probe level test statistic *t* for each probe;
2. Compute a moving average statistic to measure enrichment;
3. Estimate FDR.

TileMap (HMM)

1. Compute the probe level test statistic *t* for each probe;
2. Estimate the distribution of *t* under H_0 and H_1;
3. Model *t* by a Hidden Markov Model, and decode the HMM.

By Hongkai Ji at Hopkins

TileMap summary

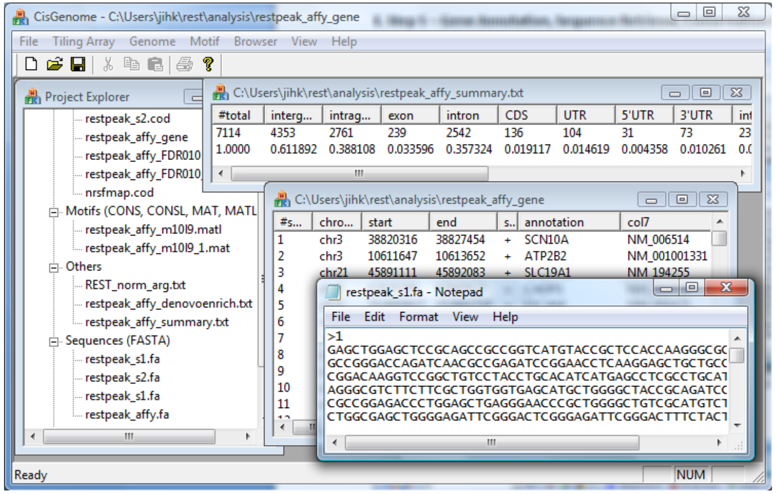
- Now a part of a software suite CisGenome.
- Windows based GUI.
- Command line version available for Mac and Linux.
- Freely available from:

  `http://www.biostat.jhsph.edu/~hji/cisgenome/`

CisGenome

(Ji H. et al. Nature Biotechnology, 2008)

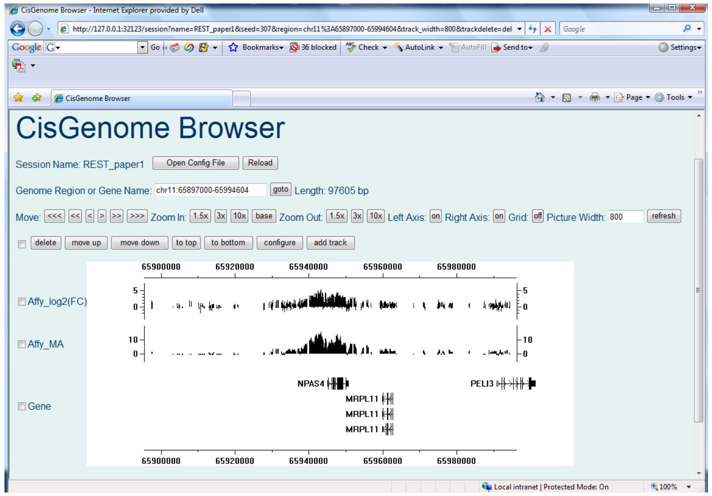
Graphic User Interface



#total	interg...	intrag...	exon	intron	CDS	UTR	5'UTR	3'UTR	int
7114	4353	2761	239	2542	136	104	31	73	23
1.0000	0.611892	0.388108	0.033596	0.357324	0.019117	0.014619	0.004358	0.010261	0.(

#s...	chro...	start	end	s..	annotation	col7
1	chr3	38820316	38827454	+	SCN10A	NM_006514
2	chr3	10611647	10613652	+	ATP2B2	NM_001001331
3	chr21	45891111	45892083	+	SLC19A1	NM 194255
4						
5						
6						
7						
8						
9						
10						
11						

```
>1
GAGCTGGAGCTCCGCAGCCGCCGGTCATGTACCGCTCCACCAAGGGCGC
GCCGGGACCAGATCAACGCCGAGATCCGGAACCTCAAGGAGCTGCTGCC
CGGACAAGGTCCGGCTGTCCTACCTGCACATCATGAGCCTCGCCTGCAT
AGGGCGTCTTCTTCGCTGGTGGTGAGCATGCTGGGGCTACCGCAGATCC
CGCCGGAGACCCTGGAGCTGAGGGAACCCGCTGGGGCTGTCGCATGTCT
CTGGCGAGCTGGGGAGATTCGGGACTCGGGAGATTCGGGACTTTCTACT
```



http://127.0.0.1:32123/session?name=REST_paper1&seed=307&region=chr11%3A65897000-65994604&track_width=800&trackdelete=del

CisGenome Browser

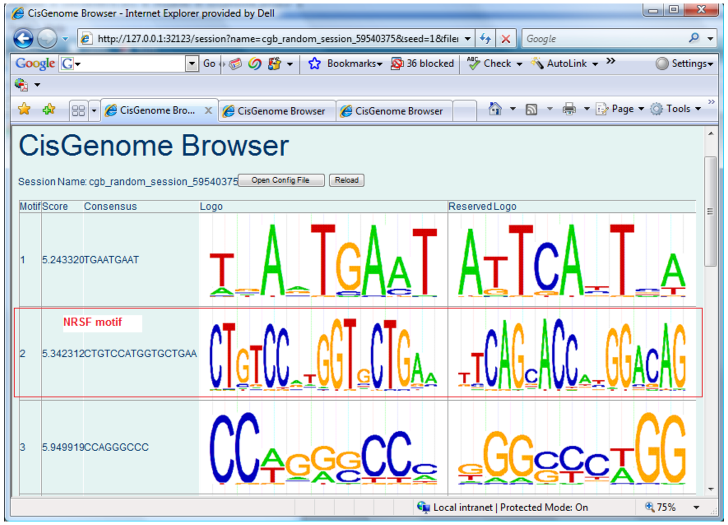
Session Name: REST_paper1

Genome Region or Gene Name: chr11:65897000-65994604 Length: 97605 bp

Move: Zoom In: Zoom Out: Left Axis: on Right Axis: on Grid: off Picture Width: 800

Core Data Analysis Programs

CisGenome Browser



http://127.0.0.1:32123/session?name=cgb_random_session_59540375&seed=1&file

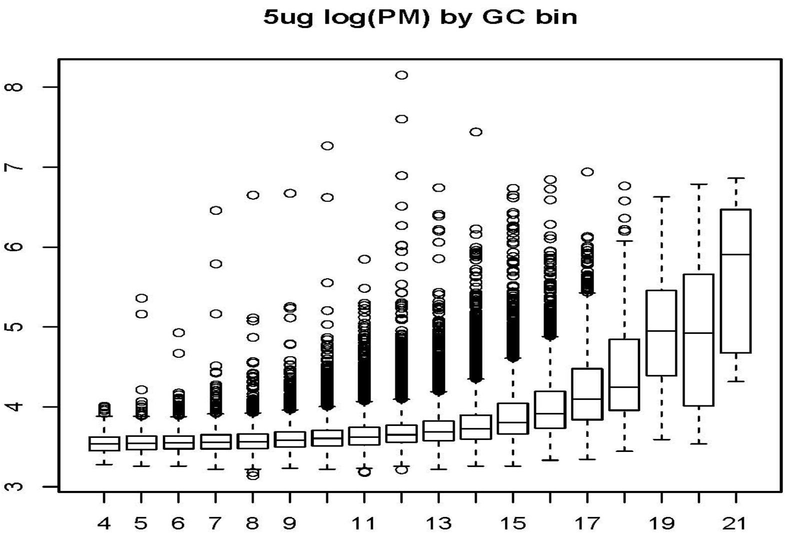
CisGenome Browser

Session Name: cgb_random_session_59540375

Motif	Score	Consensus	Logo	Reserved Logo
1	5.243320	TGAATGAAT		
2	5.342312	CTGTCCATGGTGCTGAA		
3	5.949919	CCAGGGCCC		

MAT: Model-based Analysis of Tiling arrays (Johnson W.E. et al. *PNAS*, 2006)

- Estimate probe behavior by checking other probes with similar sequence on the same array
- Probe sequence plays a big role in signal value.
- Most of the probes in ChIP-chip measures non-specific hybridization.



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Probe Behavior Model

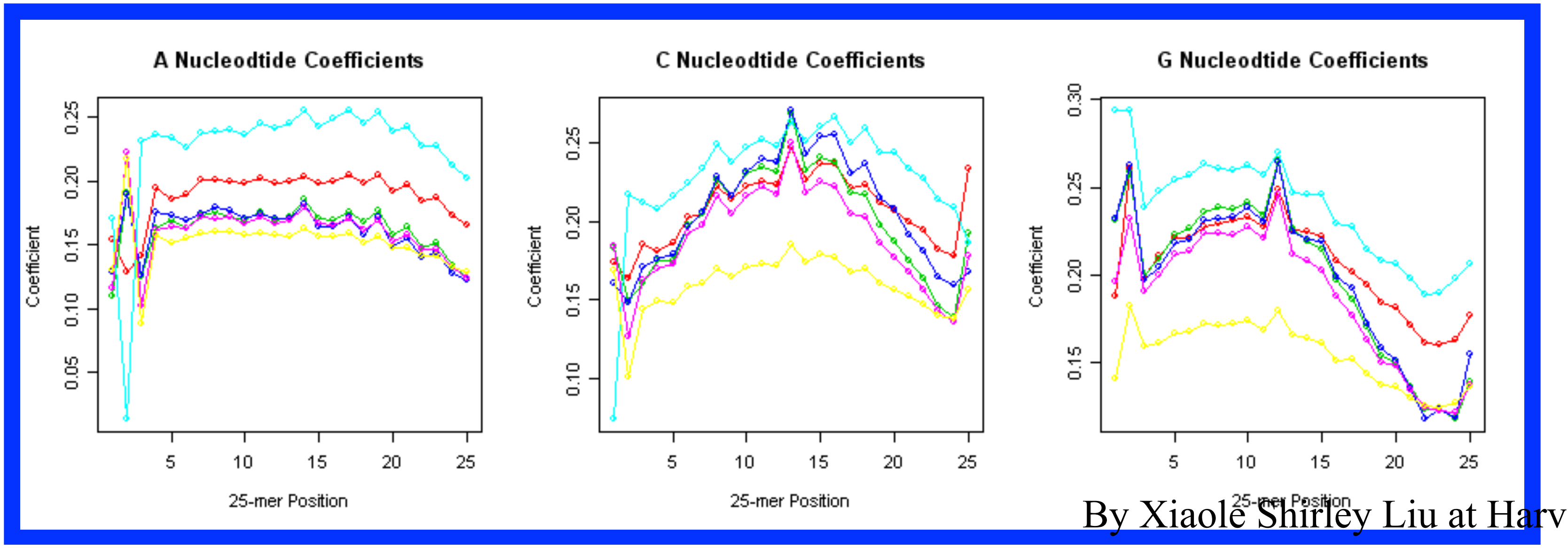
$$Log(PM_i) = \alpha n_{iT} + \sum_{j=1}^{25} \sum_{k=A,C,G} \beta_{jk} I_{ijk} + \sum_{l=A,C,G,T} \gamma_l n_{il}^2 + \delta Log(c_i) + \varepsilon_i$$

Baseline on number of Ts

A,C,G at each position of the 25mer

A,C,G,T Count Square

25mer Copy Number along the Genome



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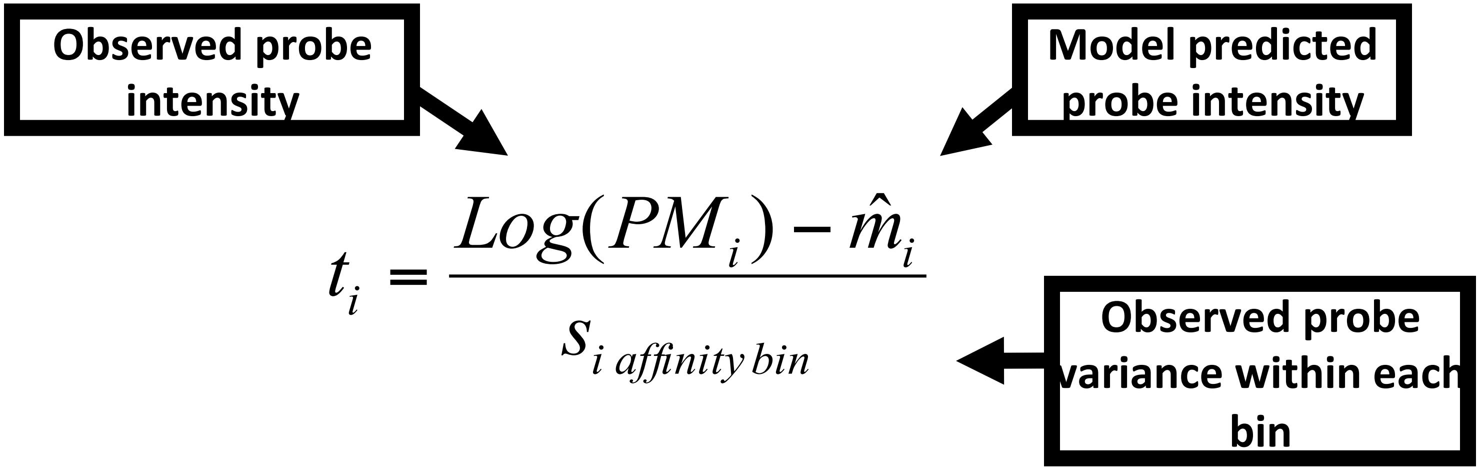
Probe Standardization

- Fit the probe model array by array
- Divide array probes to bins (3k probes/bin)
- Background-subtraction and standardization (normalization) on a single array;



Observed probe intensity

Model predicted probe intensity

$$t_i = \frac{Log(PM_i) - \hat{m}_i}{s_{i\ affinity\ bin}}$$

Observed probe variance within each bin

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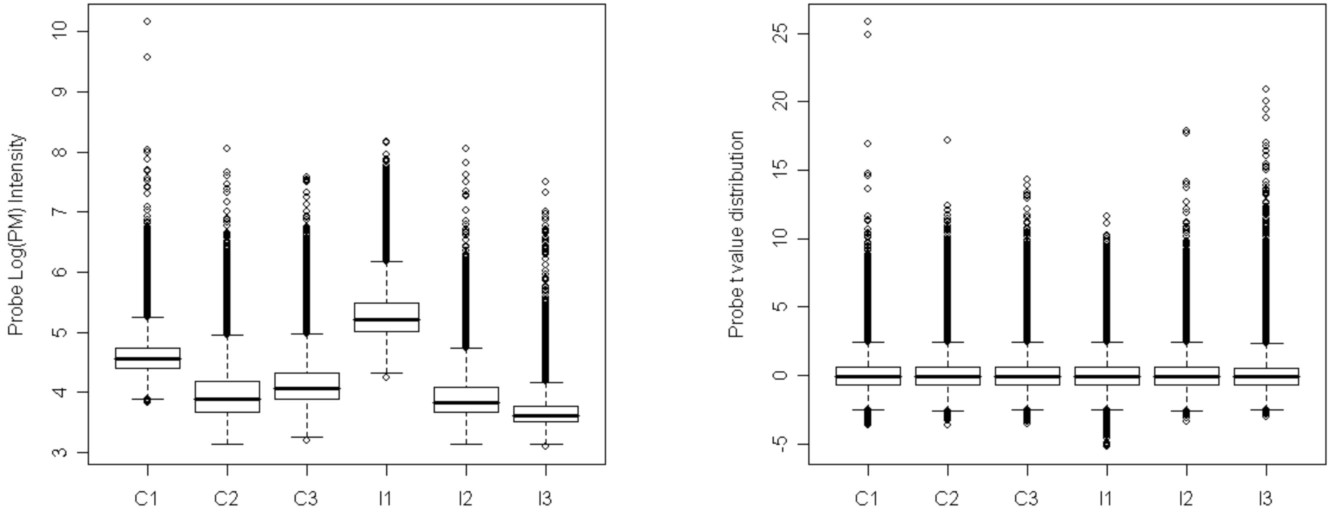
- Probe signals before and after standardization



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Binding region detection

- Window-based MATscore
  - ChIP without Ctrl

$$MAT(region) = TM(t's\ in\ region)\sqrt{n_{probe}}$$

  - TM: trimmed mean
  - Multiple ChIP with multiple Ctrl

$$MAT(region) = \frac{TM(t's\ in\ ChIP) - TM(t's\ in\ Input)}{\sigma_{Input}}\sqrt{n_{probe}}$$

  - More probes, higher t values in ChIP, less variance (fluctuation) → more confident

By Xiaole Shirley Liu at Harvard

To use MAT

- Create a text configuration file (config.txt):

```
[data]
BpmapFolder = /home/bst/student/hwu/Project/Ji/MVHMM/DREAM/rawdata/
CelFolder = /home/bst/student/hwu/Project/Ji/MVHMM/DREAM/rawdata
GenomeGrp =Hs
Group = 111000
[bpmap]
1=Hs_PromPR_v02-3_NCBIv36.bpmap
[cel]
1=IP1.CEL IP2.CEL IP3.CEL CT1.CEL CT2.CEL CT3.CEL
[intensity analysis]
BandWidth =     300
MaxGap =     300
MinProbe  =     10
[interval analysis]
Pvalue = 1e-3
```

- Then run "`MAT config.txt`" at command line.

MAT summary

- Open source, written in python at http://chip.dfci.harvard.edu/~wli/MAT/
- Installation could be tricky.
- Good computational performance.
- Can work with single ChIP, multiple ChIP, and multiple ChIP with controls with increasing accuracy.

Bioconductor packages for analyzing ChIP-chip data

- Most of the ChIP-chip analysis are done using MAT or CisGenome, so there are relatively fewer R packages.
- Useful ones:
  - rMAT: R implementation of MAT model. Works for Affy ChIP-chip.
  - Ringo (R Investigation of NimbleGen Oligoarrays): works for NimbleGen two-color tiling arrrays.
  - Starr: an extension of Ringo, works for Affymetrix arrays.
  - ChIPpeakAnno: annotation of peaks, e.g., find closeby genes, GO terms, DNA sequences, etc.

rMAT

- R implementation of MAT.
- Works for Affymetrix arrays only.
- Needs bpmap file (factory provided file to probe annotations), and raw data file in CEL format.

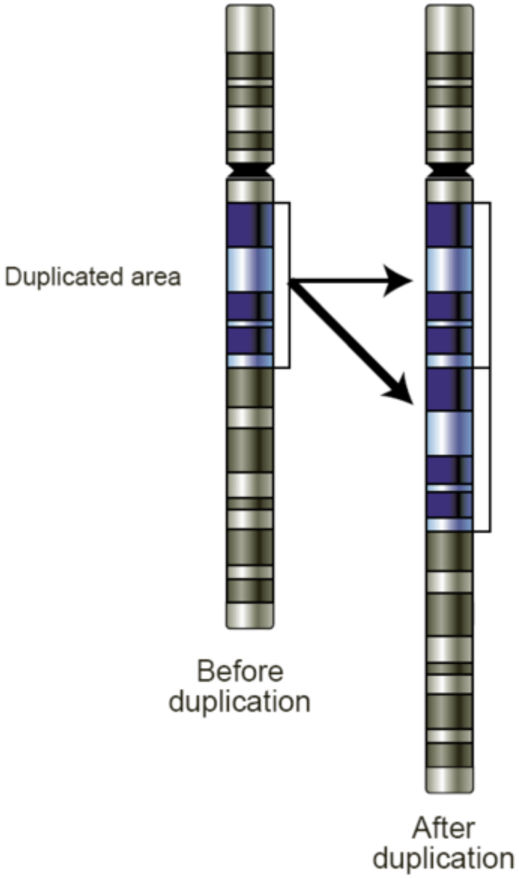
```
library(rMAT)
bpmapFile=“Hs.bpmap”
### read in data
arrayFile=c(“IP.CEL”, “Control.CEL”)
rawdata=BPMAPCelParser(bpmapFile, arrayFile, groupName="Sc")

## normalization - run MAT model
normdata=NormalizeProbes(rawdata,method="MAT”)

## compute MAT scores and find peaks
RD=computeMATScore(normdata,cName=“Control”, dMax=600)
Enrich=callEnrichedRegions(RD, dMax=600, dMerge=300,
   nProbesMin=8, method="score", threshold=2)
```

Copy number variation arrays

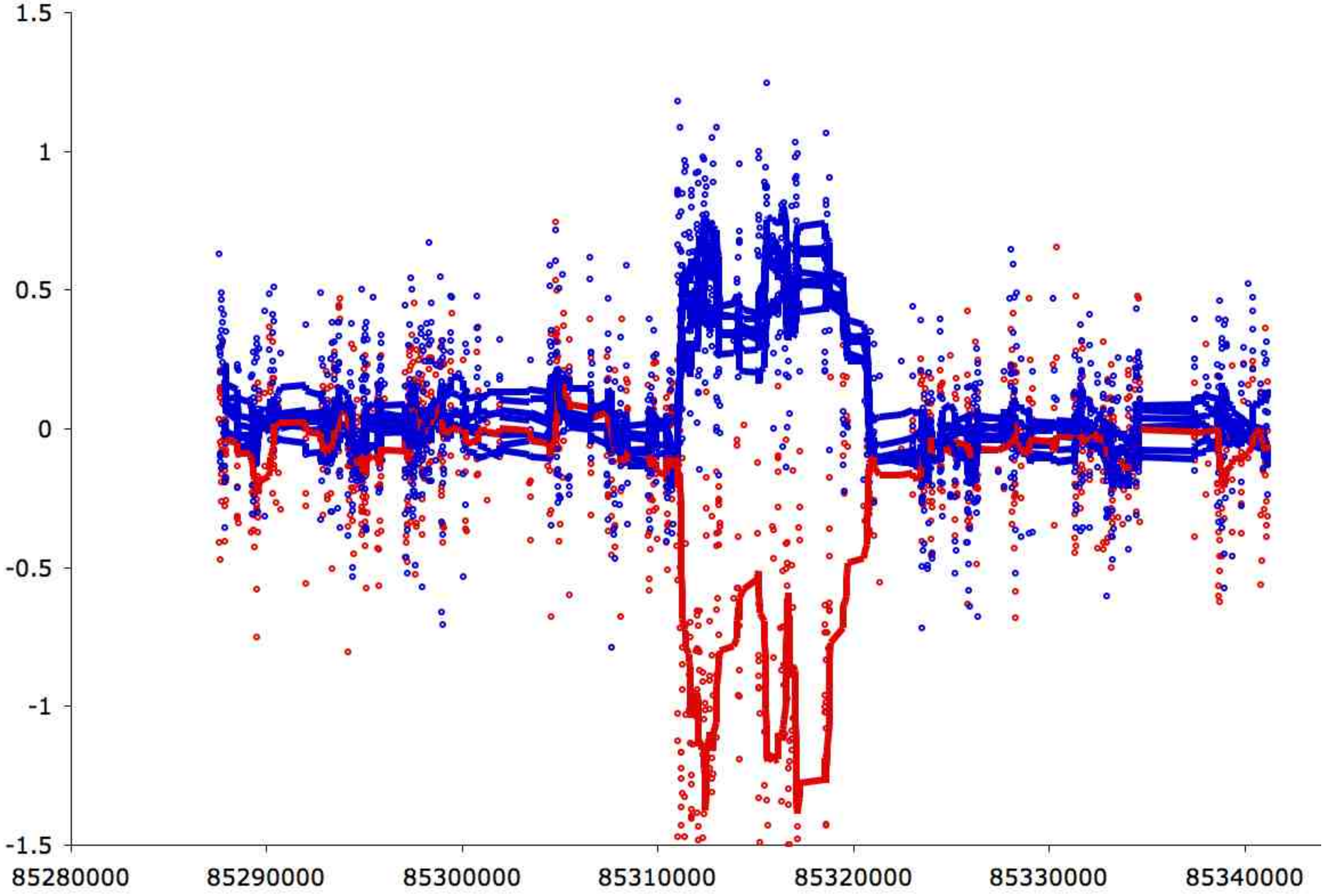
- CNV: phenomenon that sections of DNA have abnormal number of copies (deviate from 2).
- Can be detected by SNP arrays (for one sample) or arrayCGH (comparing two samples case vs. control).



Data from CNV arrays

- Data format are the same, e.g., probe locations and signal, but characteristics are different:
  - No peaks, but long, flat-topped "plateaus".
  - Heights of the plateaus are discrete, corresponding to different number of copies (integers: 1, 2, 3, …)

Example data from arrayCGH



Analysis of CNV arrays

- Methods are different from ChIP-chip, but still smoothing based to combine neighboring probe information, for example, Hidden Markov Model.

A list of CNV array software

- Affymetrix:
  - APT: uses a hidden Markov model
  - R package VanillaICE: HMM base. R. Scharpf *et al.* (2008) AOAS
  - R package DNAcopy: Circular Binary Segmentation. Olshen *et al.* (2004) Biostatistics
- Illumina:
  - QuantiSNP: S. Colella *et al.* (2007), NAR
  - PennCNV: K. Wang *et al.* (2008), NAR

Review

- Tiling arrays are DNA microarrays for detecting locational modifications of genome.
- Probes tile up a part of whole genome.
- Still hybridization based (DNA segments stick to probes), same as gene expression arrays.
- Data need to be visualized along genome.
- Location of interests shows some patterns: peaks for TFBS, or plateau for CNV.
- Need to combine data from neighboring probes to make calls.