# ChIP-on-chip

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# Background

- Chromatin immunoprecipitation-on-chip
  - ChIP + micoarrays
- ChIP-on-chip developed by Dr. Richard Young, Dr. David Gifford, Dr. Heidi Wyle[1]
- Purpose: looks at how protein regulators interact with DNA[1]

# Uses of ChIP-on-chip

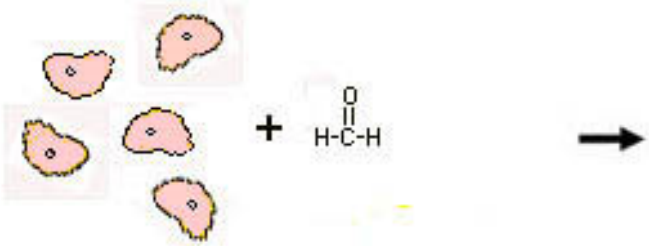
- Determines where a regulator binds to DNA
  - Helps elucidate transcription binding and mechanisms of repression and activation
  - Helps understanding of "methylation, histone modification, as well as DNA replication, modification, and repair"[1]
  - Increases understanding of diseases and cell processes[1]
- Significance: Target genes to alter pathways[1]

# ChIP-on-chip: How Does It Work?

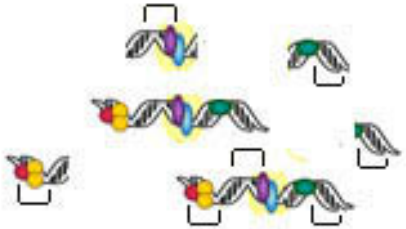
- There are six main steps involved in ChIP-on-chip
  1. Prepare protein-DNA complex
  2. Hybridize DNA to microarray

# Mechanism

1. Cross-link the protein-DNA complexes

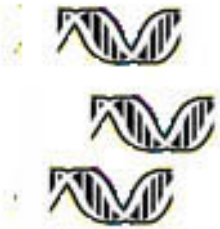



2. Lyse cells and sonicate DNA



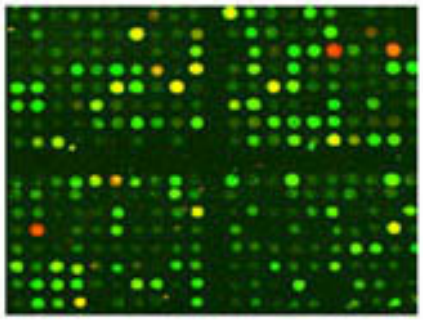
3. IP chromatin to capture and purify bound DNA

4. Release and amplify DNA fragments

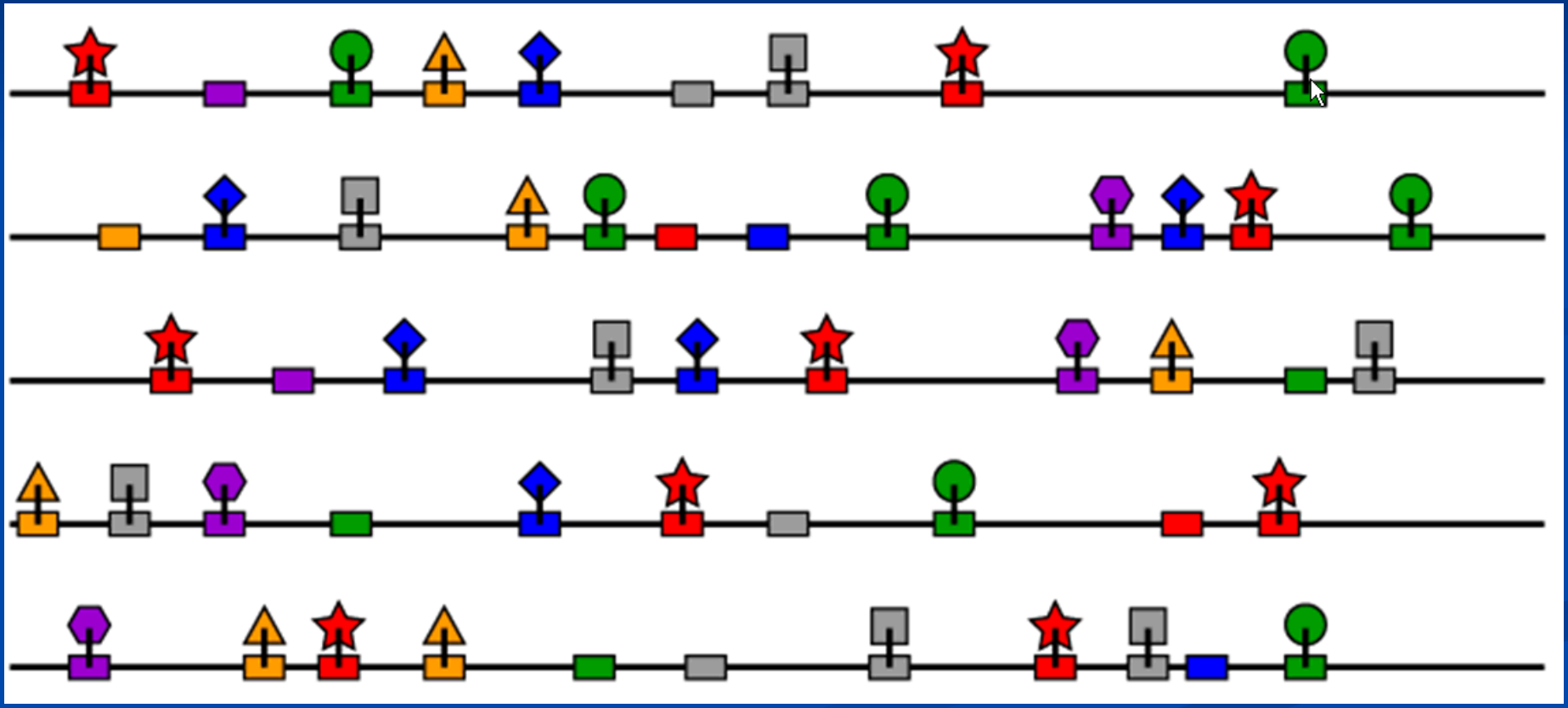


5. Labeled enriched pool of fragments

6. Hybridize to microarray for detection



Source:
http://www.chem.agilent.com/en-us/products/instruments/dnamicroarrays/pages/gp37461.aspx



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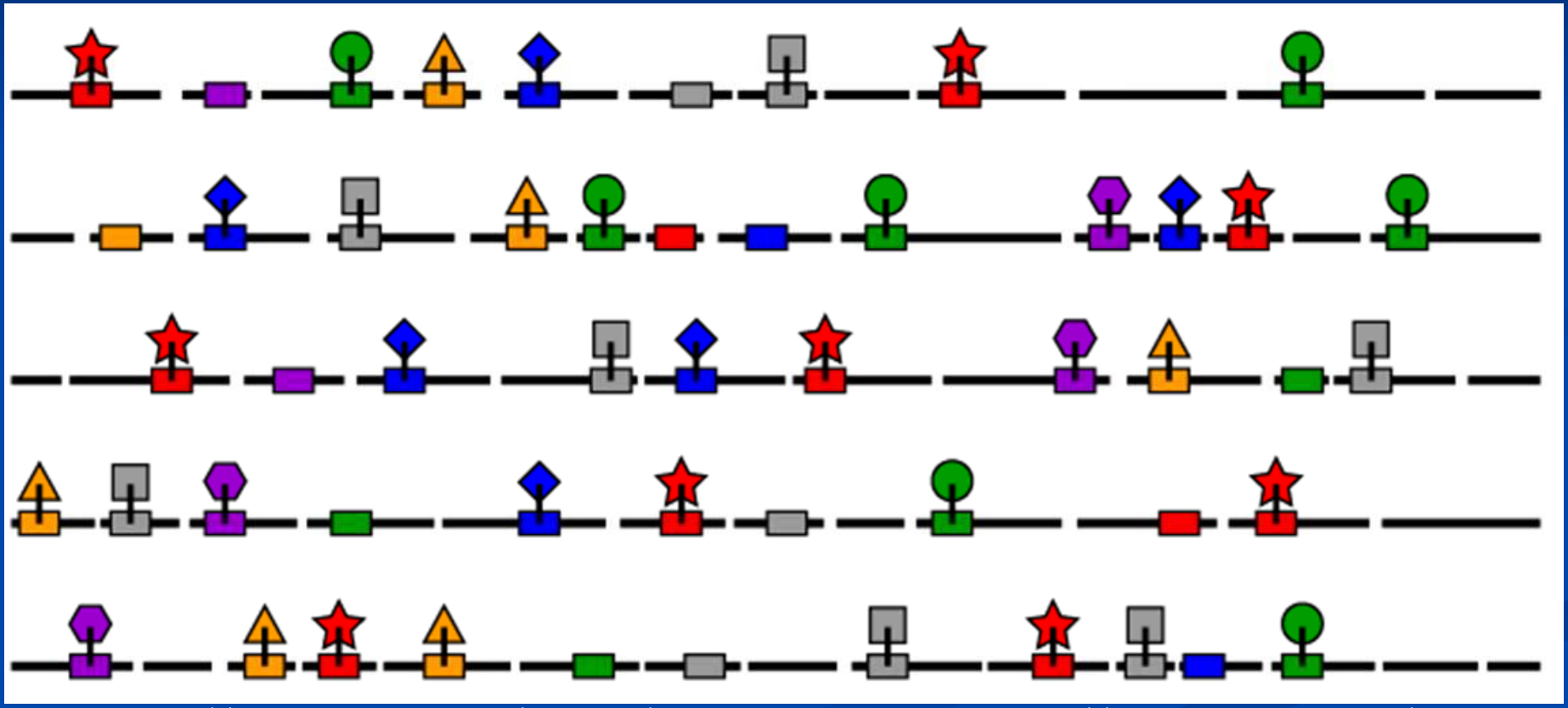

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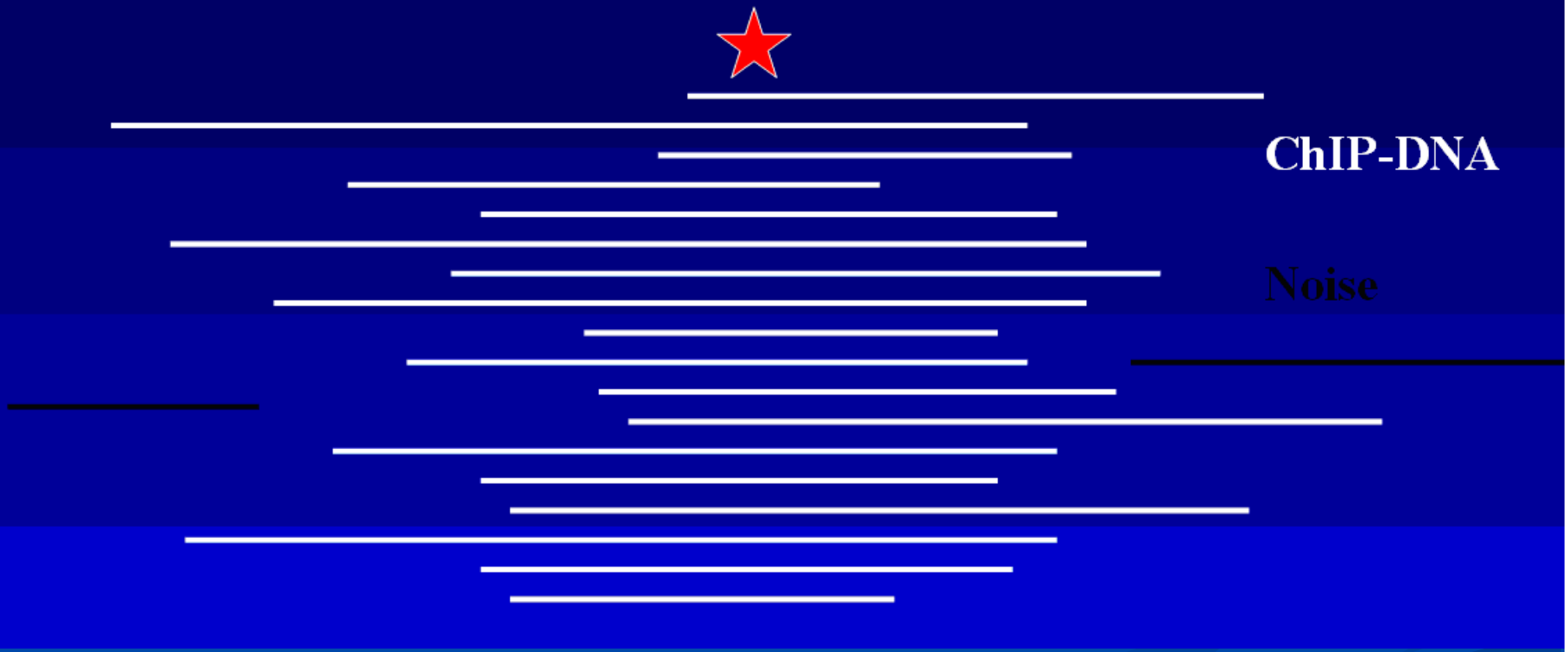
# ChIP-chip Array Hybridization

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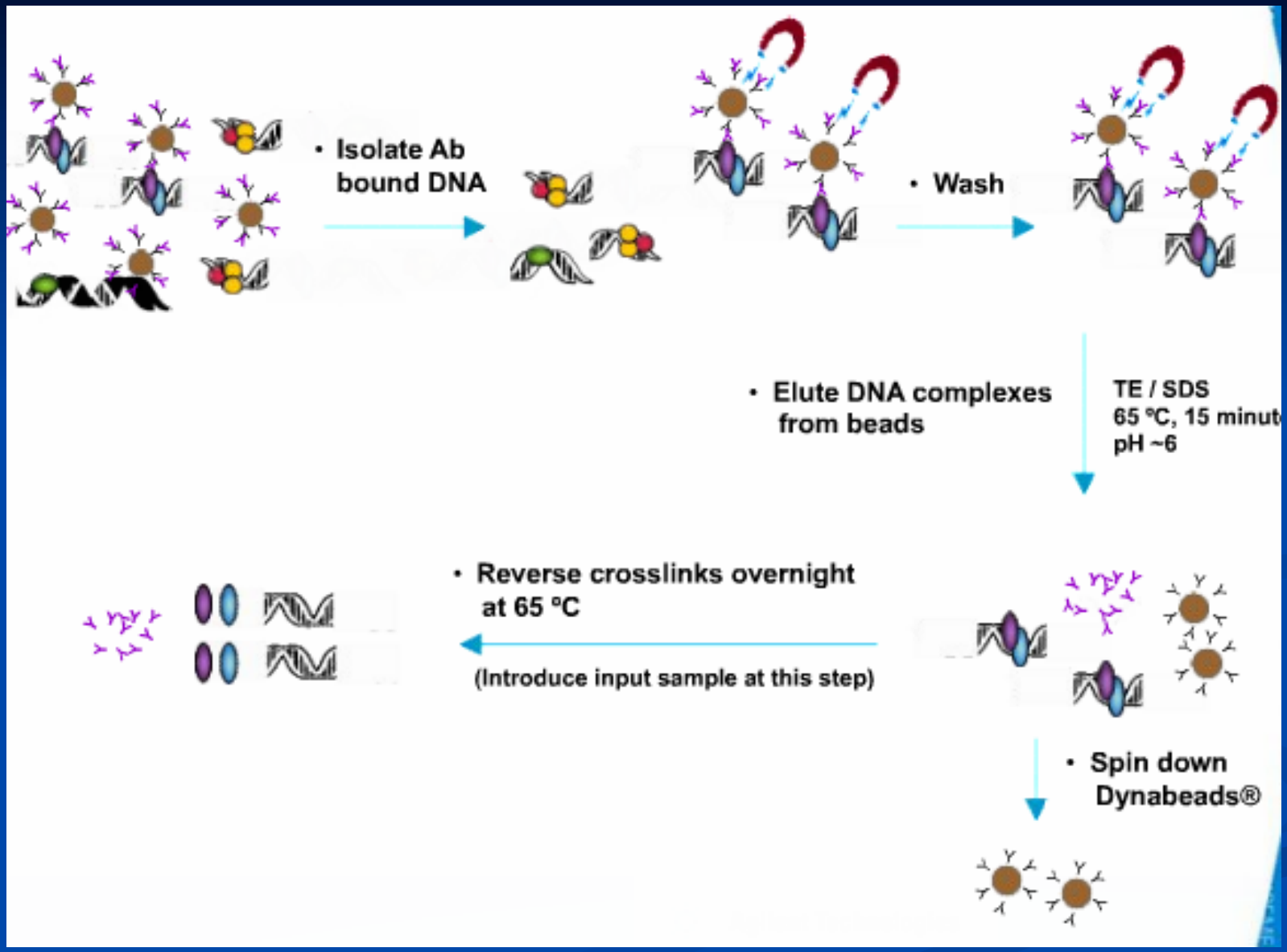


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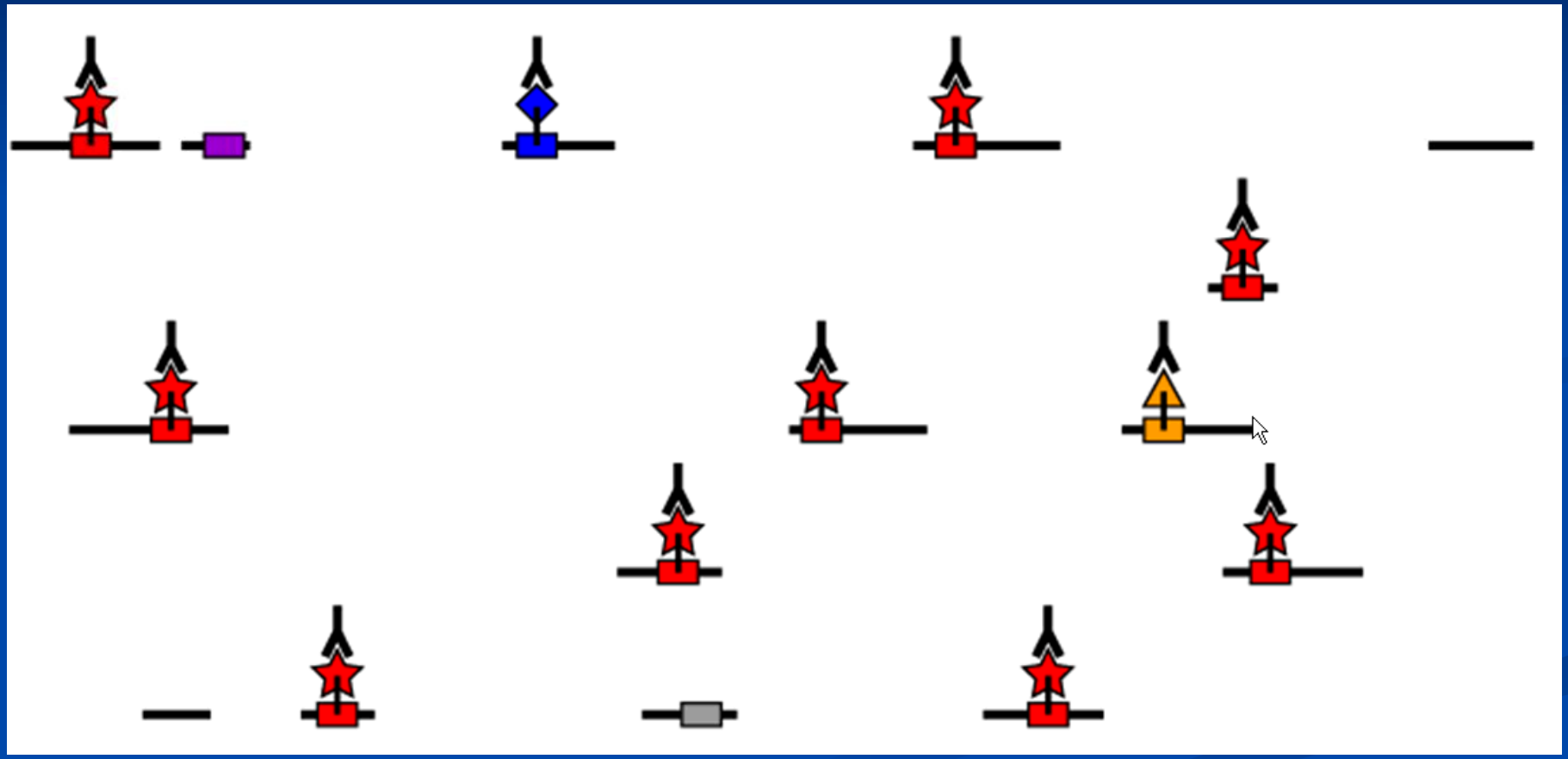

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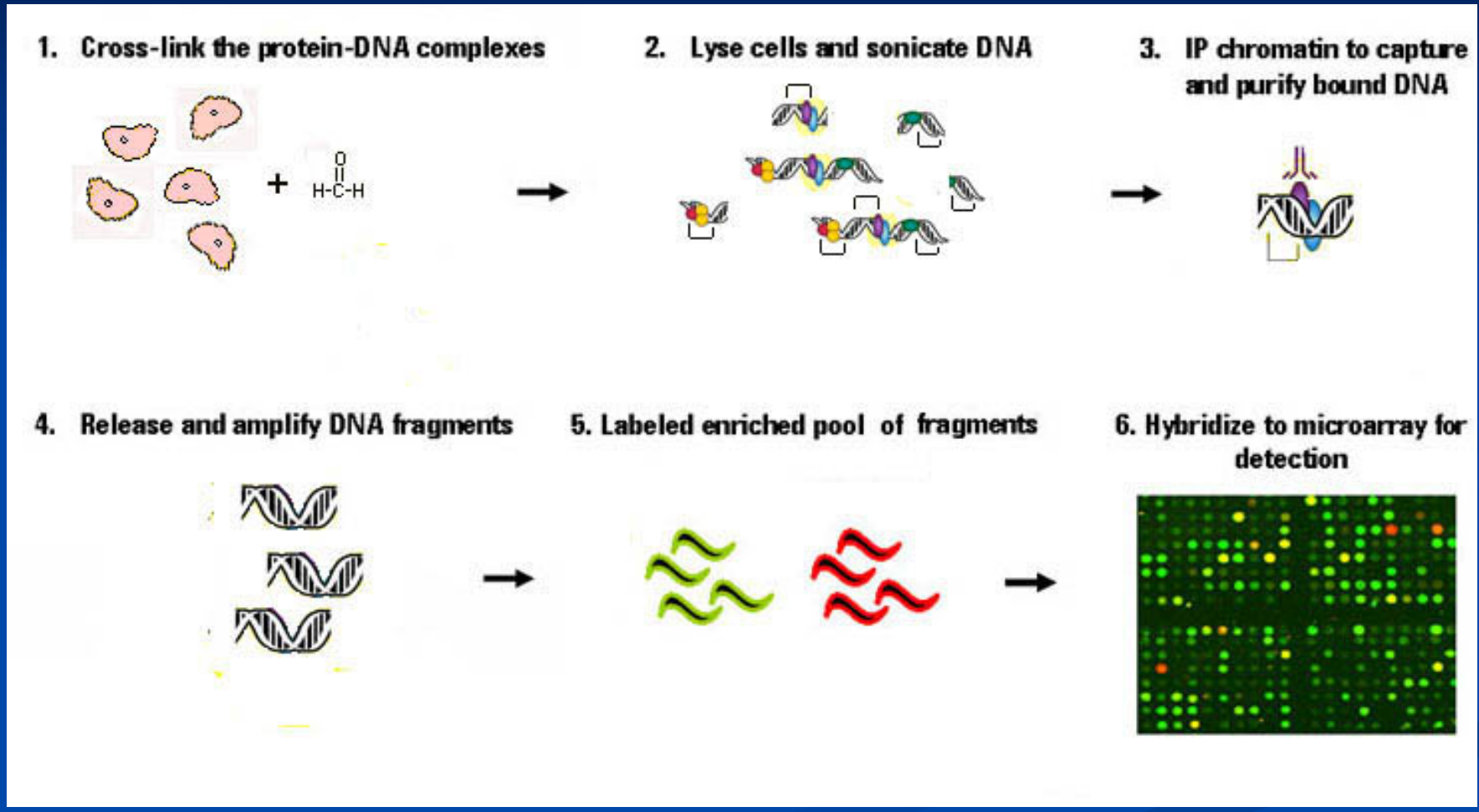
# Mechanism

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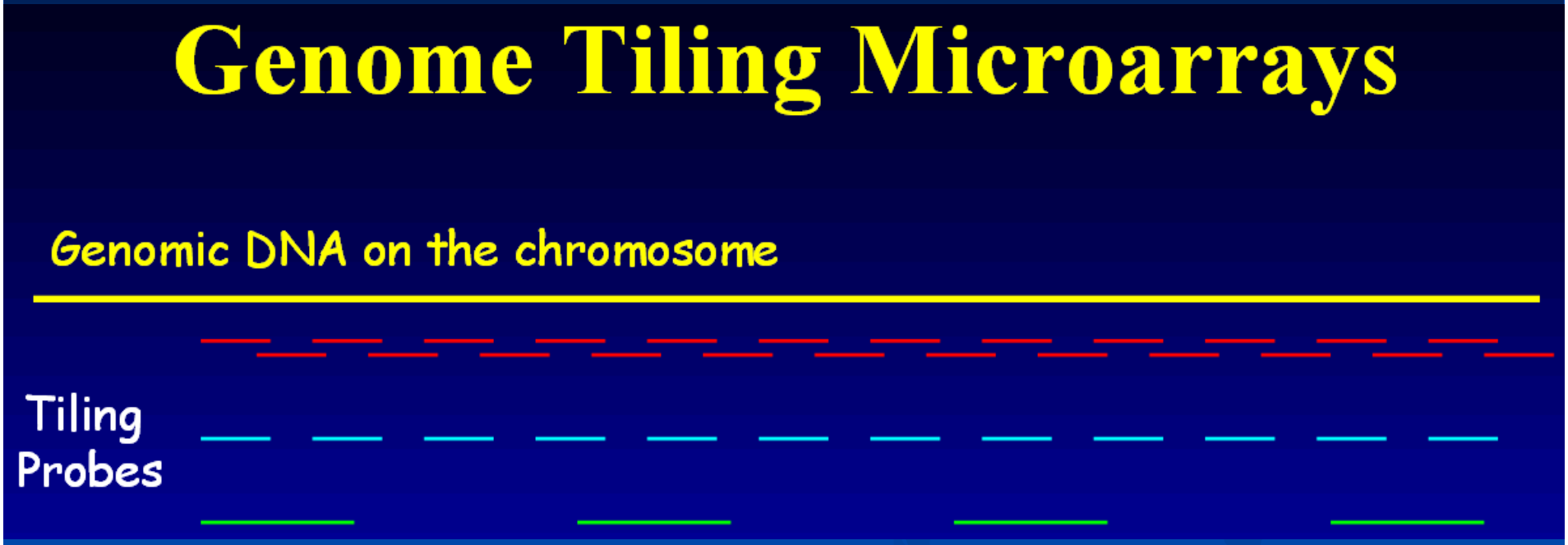
http://www.chem.agilent.com/en-us/products/instruments/dnamicroarrays/pages/gp37461.aspx

# Genome Tiling Microarrays




**7 arrays × 6 million probes = 42 million data points**

Source: http://intercall.webex.com/intercall/playback.php?FileName=http://www.affymetrix.com/about_affymetrix/media/events/archived_events/liu.wrf

# Affymetrix Tiling Analysis Software Mann-Whitney Rank Sum Test

| | 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 |

**Rank Sum** **121** **89**

Source: http://intercall.webex.com/intercall/playback.php?FileName=http://www.affymetrix.com/about_affymetrix/media/events/archived_events/liu.wrf

# Model-based Analysis of Tiling Arrays

- Deals with the problems in TAS
  - Works with just one ChIP sample
    - Generates a lot less data
  - More ChIPs, more controls mean greater sensitivity
  - Ability to measure data quality[3]
- Most algorithms checks probes against many samples
  - MAT looks in array
    - Assumption: Most probes are not pointing to presence regulator binding site

# MAT models effect of probe sequence on probe signal

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

- Probe signal
- # of T's intercept
- Position-specific A, C, G effect
- A,C,G,T count squared
- 25-mer copy number

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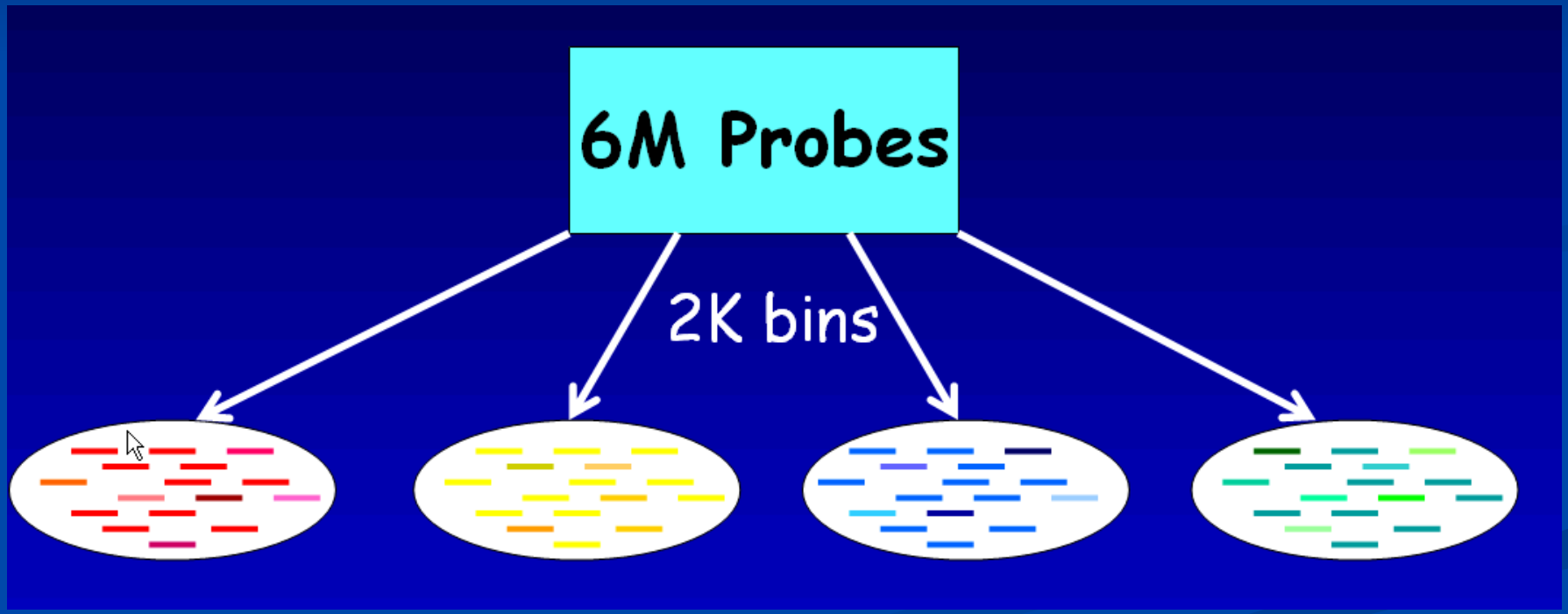


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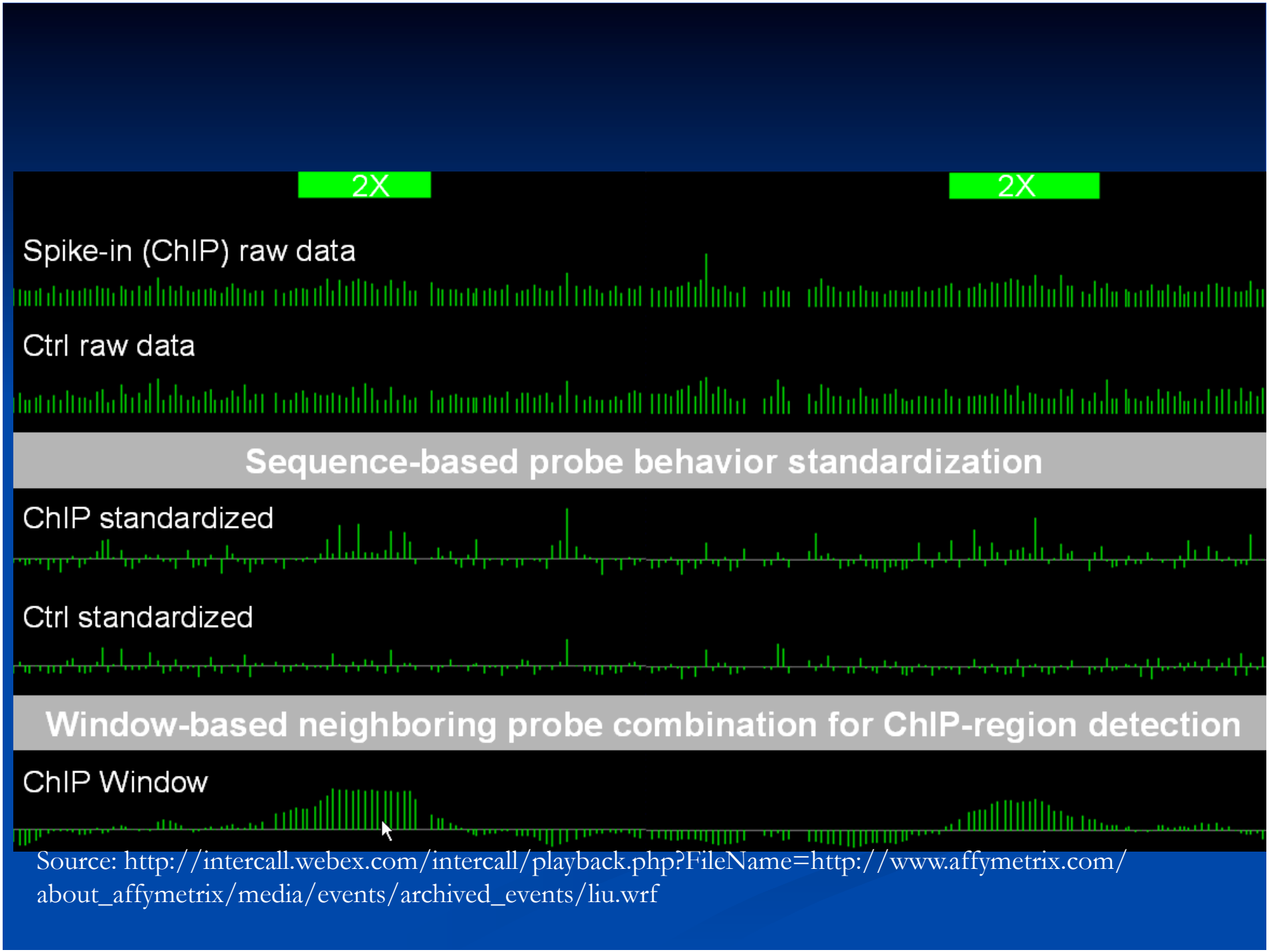


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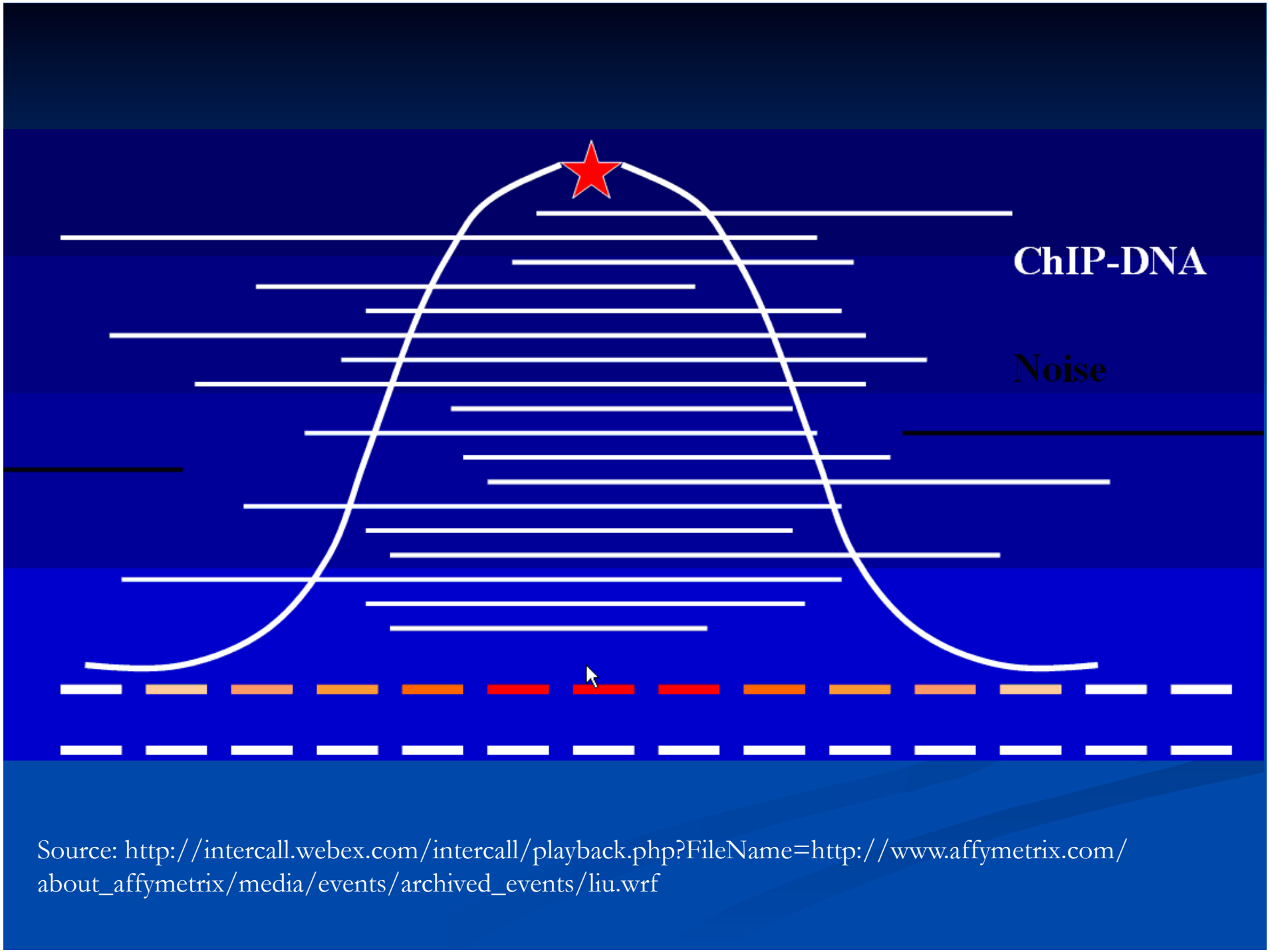


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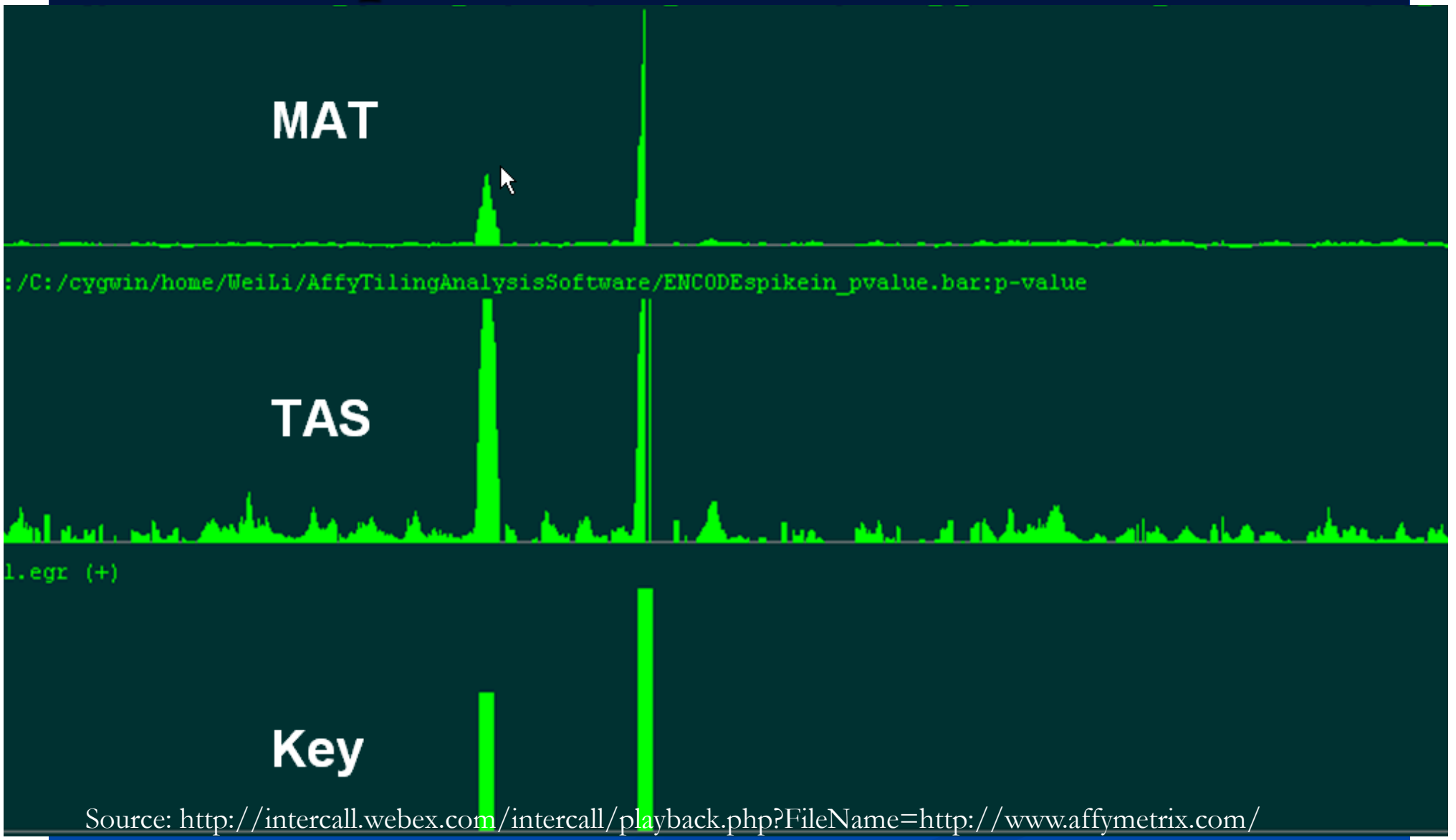
# Comparison Between the Tools

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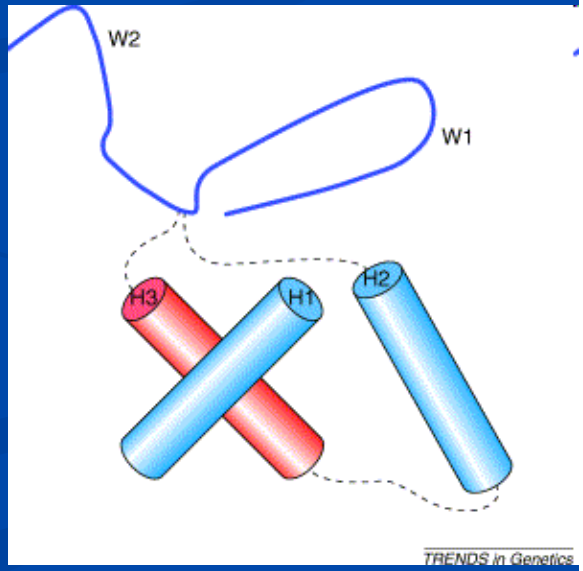
# FOXA1 Transcription Factor

- Forkhead Box (Fox) proteins
  - Transcription factors
  - Important for "cellular proliferation, differentiation, transformation, longevity, and metabolic homeostasis."[5]
- Forkhead genes characterized by:
  - loops/wings
  - Three alpha helices
  - Beta chain




http://www.sciencedirect.com/cache/MiamiImageURL/B6TCY-48H2WC4-4-1/0?wchp=dGLzVzz-zSkzV

# FOXA1 Transcription Factor

- Important in causing cancers, development of some organs
- Brings other transcription factors

http://www.nature.com/nrg/journal/v9/n5/full/nrg2378.html#references-links

# Impact of ChIP-on-chip

- Used ChIP-on-chip to find FOXA1 binding sites
  - Breast cancer cells
  - Prostate cancer cells
- Conclusions: Binding sites mainly in enhancer instead of promoter regions

# Epigenetic Marks?

- Epigenetics: Changes made on genes instead of to the DNA itself
  - Methylation
  - Histone acetylation
- Researchers found that –
  - Methylated histones were found near the binding sites
    - Demethylase – enzyme that removes methyl groups
    - Result: reduced FOXA1 binding
    - Conclusion: FOXA1 transcription dependent on methylation of histones
  - FOXA1 improves DNase I sensitivity
    - Conclusion: FOXA1 involved in regulating chromatin structure

## Sources


1http://www.chem.agilent.com/en-us/products/instruments/dnamicroarrays/pages/gp37461.aspx
2http://agilent.cnpg.com/lsca/webinar/chevalier/20051121/
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