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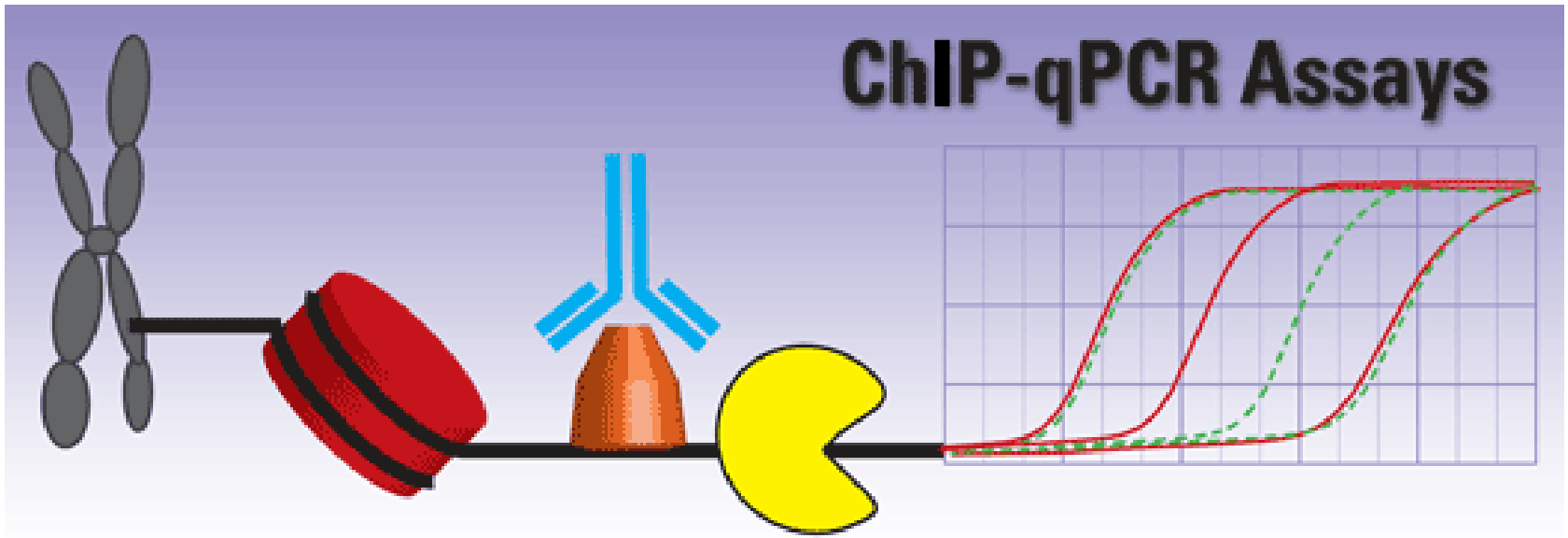
ChIP-qPCR Assays Technology Overview



Quantitative Real-Time PCR for Chromatin Immunoprecipitation Analysis

“A ChIP off the old (real-time PCR) Block”

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Topics to be Covered

- **Challenges Facing ChIP Researchers**
- **Solutions that the ChIP-qPCR Assays Provide**
- **How the ChIP-qPCR Assays Work**
  - Tiled Primer Assays for Every Human, Mouse, Rat Promoter
  - Quantitative Real-Time PCR Performance
  - Protocol Overview & Data Analysis
- **How YOU Can Use the ChIP-qPCR Assays**
  - Application Example:
  - p53 ChIP with CDKN1A and GADD45A qPCR

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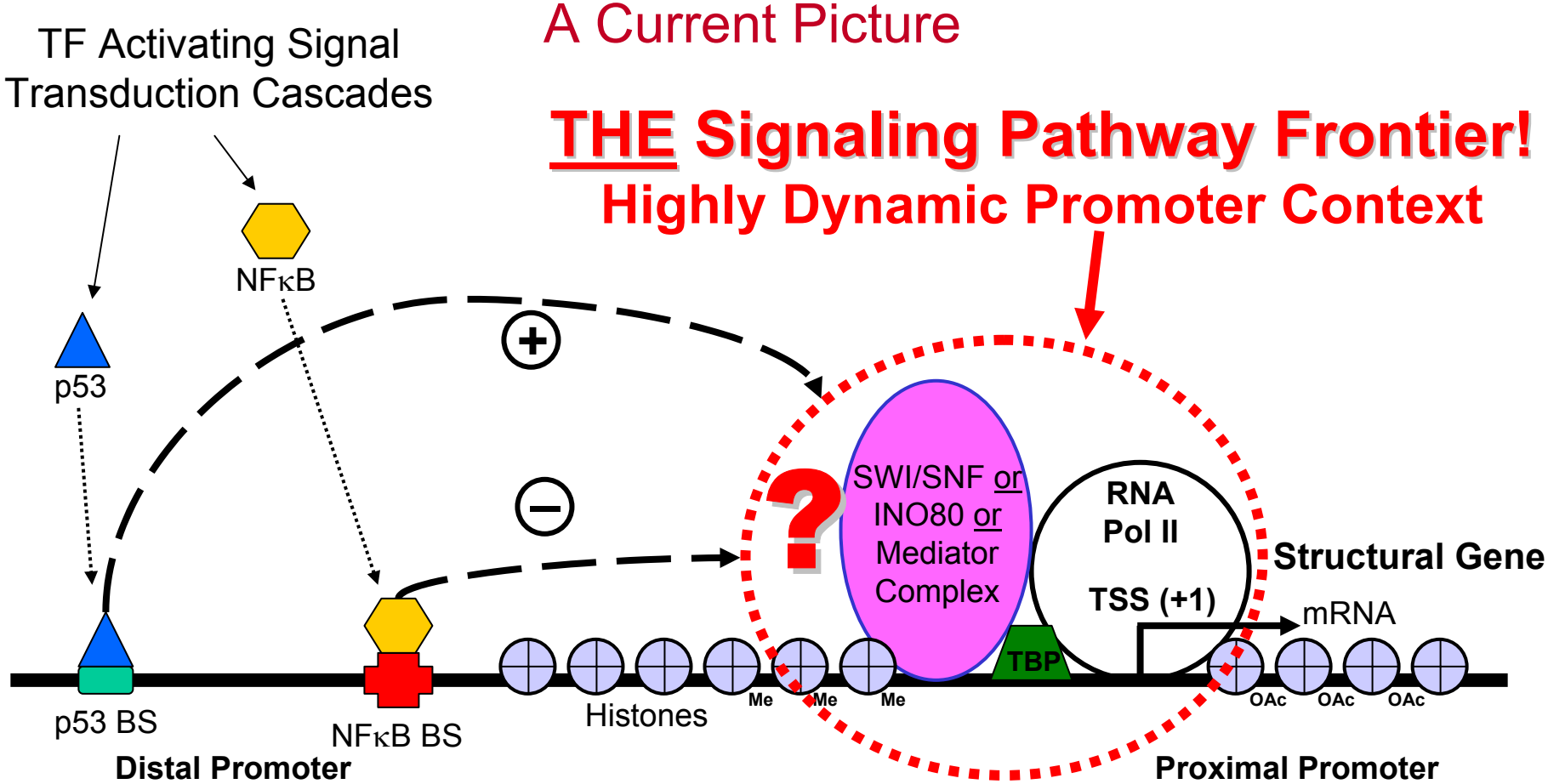
Pathway Signaling Directed Transcriptional Activation:
A Current Picture



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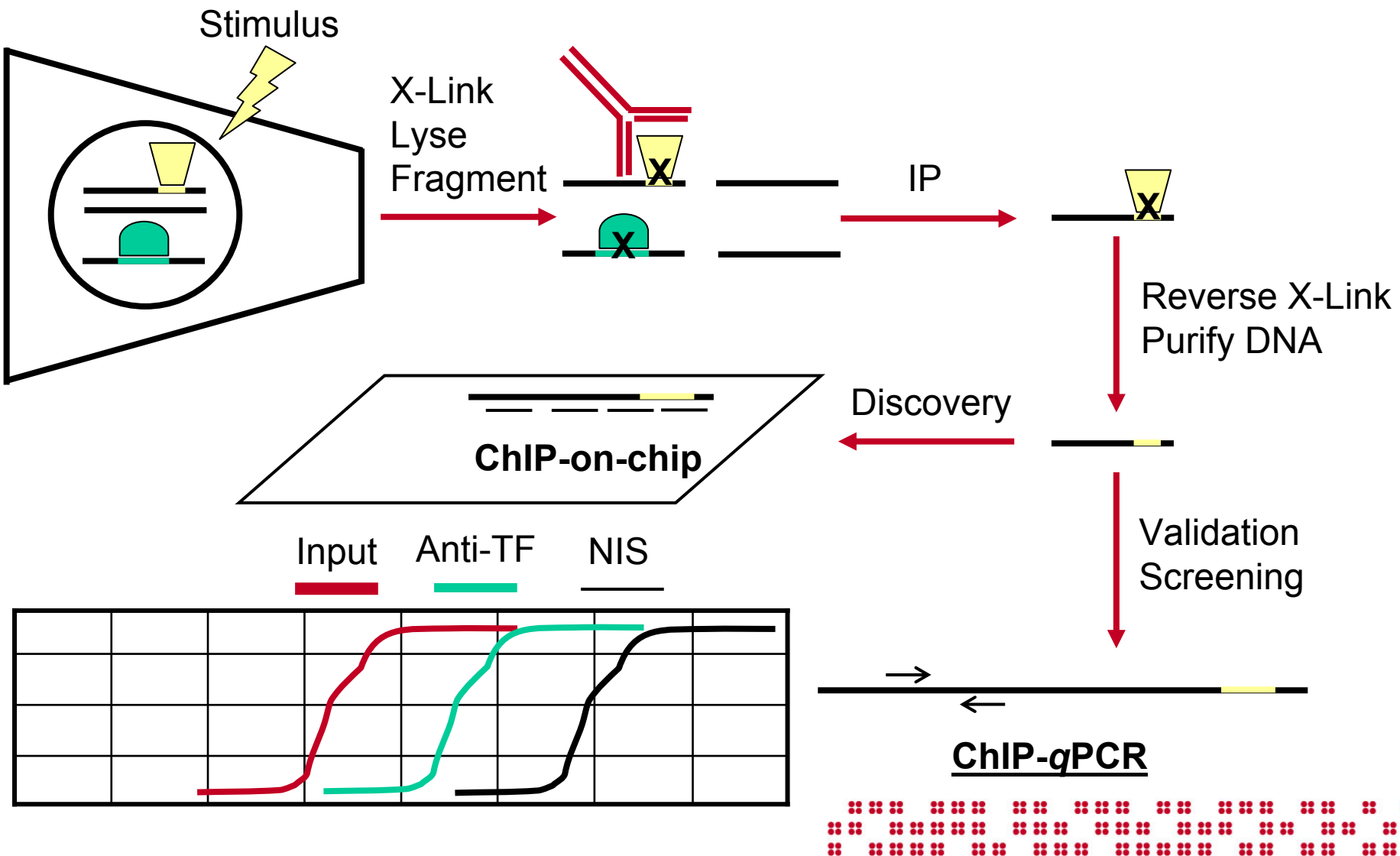
Chromatin Immunoprecipitation



Challenges Facing ChIP Researchers

- **Difficulty Designing PCR Primers for gDNA:**
  GC-rich, high sequence content variation & repetitive sequences
  Localizing amplicon near TF binding site can be a hindrance
  Multiple rounds of assay design & validation
- **Time-Consuming End-Point PCR:**
  Requires post-run gel electrophoresis & image analysis
  NOT quantitative, narrow dynamic range, low sensitivity
- **Detecting Other Target Promoter Sites:**
  Interesting data on one gene's promoter
  Test another genes' promoters with SAME precious ChIP material
- **ChIP-on-chip Requires PCR Validation:**
  Many enriched sites to validate
  Each involving above optimization difficulties

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What the ChIP-qPCR Assays Provide

- **Time and Money Savings:**
  - No more repeated fruitless rounds gDNA assay design & optimization
  - Ready-to-use, optimized assays; Standardized PCR conditions
- **Quick and Easy Use:**
  - No more post-run gel-based analysis
  - Quantitative & higher-throughput SYBR® Green real-time PCR
  - Measure enrichment at more promoter regions in same run & ChIP prep
- **Guaranteed Performance:**
  - High amplification efficiency and specificity guaranteed
  - More accurately quantify ChIP DNA fractions, including mock IP
- **Comprehensive Coverage:**
  - Tiled assays for every human, mouse or rat gene promoter
  - Customized genome-wide options also available

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What are ChIP-qPCR Assays?

- **Pre-designed and validated real-time PCR primer assays measuring genomic DNA promoter region sequence enrichment within chromatin immunoprecipitation samples**
- Accommodate every human, mouse and rat gene promoter with a ChIP-target independent approach
- Thirty (30) 1-kb tiles from -20 kb to +10 kb relative to each TSS
- When coupled with an average 1-kb fragmentation size, provides best possible sensitivity and resolution balance
- Quality Control: One product with high amplification efficiency
- Well-suited for functional screening assays
  - Fold-differences in fractional enrichment
  - ChIP-on-chip validation

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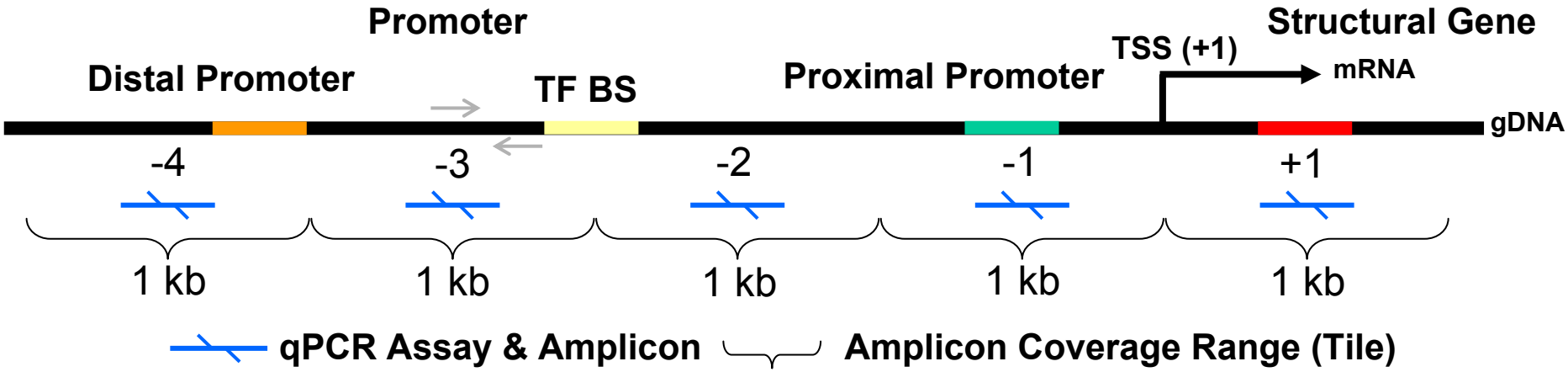
HOW IT WORKS: Tiling Scheme



- Many transcription factor binding sites (TF BS) per promoter
- Optimal qPCR primer design with stringent criteria needs room to find best amplicon sequence
- On average, amplicon is located within 0.5 to 1.0 kb of (and not necessarily at) TF binding site
- Appropriately long fragmentation size becomes critical to the success (sensitivity) of the ChIP-qPCR Assay …

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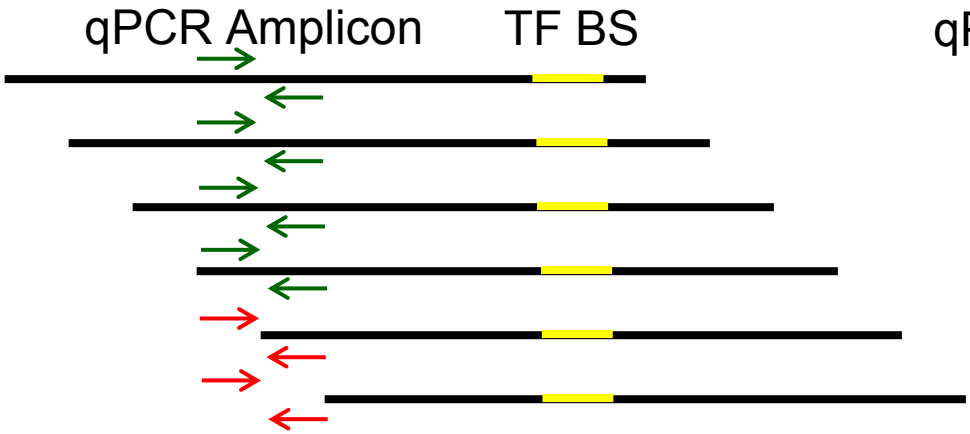
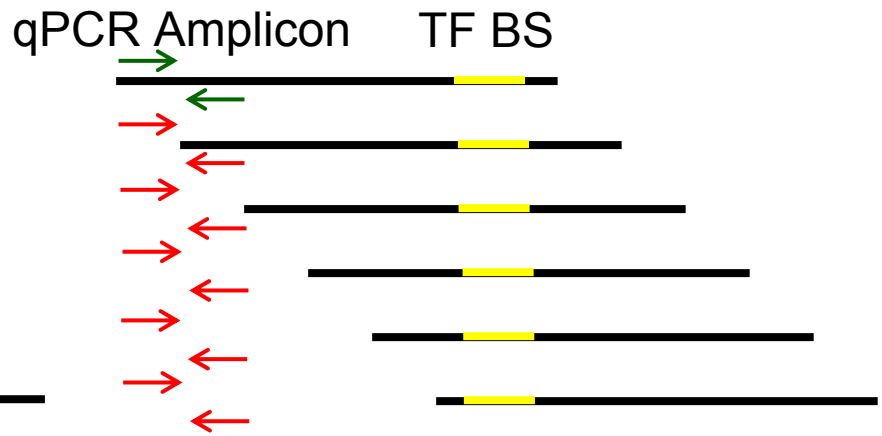
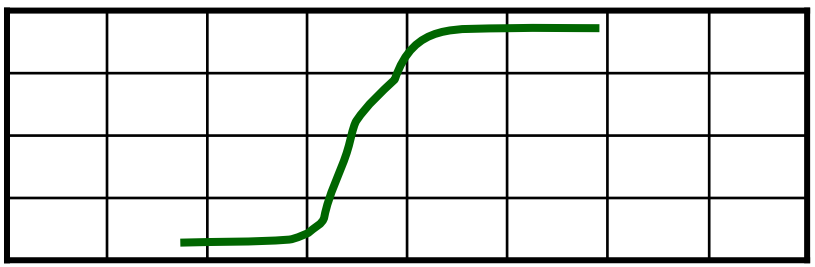
Tiling Scheme & Fragmentation Size: Resolution & Detection Sensitivity Balance



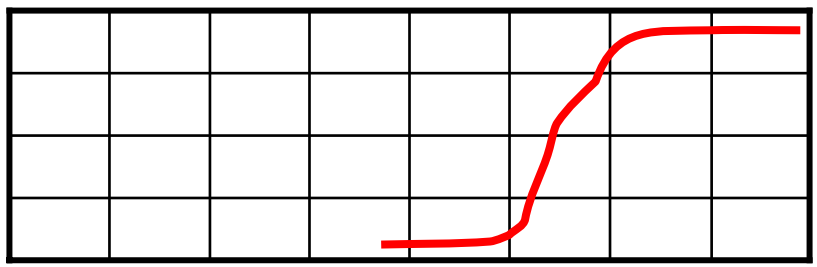
Longer Fragments



Shorter Fragments



Greater Sensitivity



Reduced Sensitivity

We recommend an average fragment size or 0.8 to 1.0 kb for ChIP-qCPR Assays.

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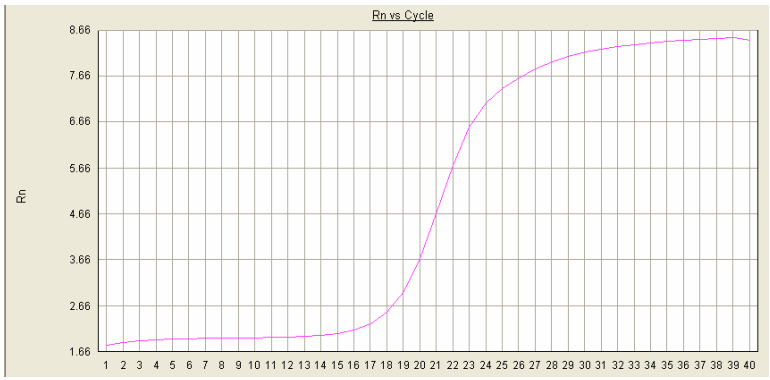
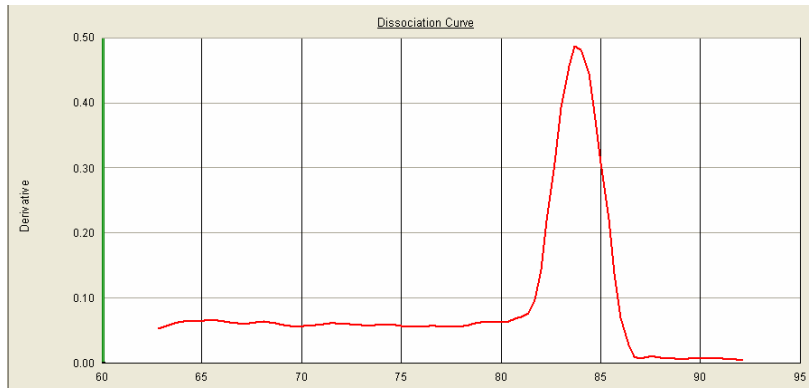
Performance: Amplification Efficiency & Specificity



Consistently High Amplification Efficiencies
DART Method



Single Dissociation Curve Peak

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HOW IT WORKS: Protocol Overview

- Grow & Treat Cells with Biological Replicates
- Cross-Link with Formaldehyde; Lyse Cells & Prepare Chromatin
- Fragment DNA by Sonication to **800-1,000-bp Average Size**
- **Divide Sample into Four Fractions:**
- Input gDNA (~1/100th of total)
- Immunoprecipitations:
  - Target TF = Experimental Inquiry
  - RNA Polymerase II = Positive Control for ChIP process
  - Non-Immune Serum = Negative Control for non-specific DNA IP
- Reverse Cross-Linking & Purify DNA

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HOW IT WORKS: Protocol Overview

- **Three Types of qPCR Assays:**
- Promoter Target of Interest
  - Experimental Inquiry
  - **ChIP-qPCR Primer Assays, Cataloged and Custom**
- Positive Control for ChIP Process
  - Assay proximal to constitutively active promoter of an abundantly expressed housekeeping gene
  - **ChIP-qPCR Positive Control Proximal HKG Promoter Assays**
- Negative Control for Non-Specific DNA co-IP
  - Specific gDNA sequence within ORF-free intergenic region
  - “Promoter Desert”
  - **ChIP-qPCR Negative Control IGX Assays**

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HOW IT WORKS: Protocol Overview

- **qPCR Analysis for each biological conditions' replicate:**

		qPCR Assay		
		Promoter Region	Positive Control	Negative Control
ChIP Fraction	Input	√	√	√
	Anti-TF	√	X	√
	Anti-Pol II	X*	√	√
	Non-immune serum	√	√	√

- Therefore, **10** reactions per biological conditions' replicate

* Unless querying proximal to promoter of interest

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HOW IT WORKS: Data Analysis

ChIP	qPCR	Reports On …
Input	Promoter Target	Total # TF Binding Sites
Anti-TF		# Binding Sites Bound by TF
Non-immune		# Binding Sites Non-Specifically co-IP
Input	HKG Proximal Promoter	Total # Pol II Binding Sites
Anti-RNA Pol II		# Binding Sites Bound by RNA Pol II
Non-immune		Background # RNA Pol II-binding sites
Input	IGX Negative Control	Amount gDNA Non-Specifically co-IP-ed with Each ChIP Antibody
Anti-TF		
Anti-RNA Pol II		
Non-immune		

HOW IT WORKS: Data Analysis

- Recommendations:
  - Normalize each IP Fraction C_t to Input DNA Fraction C_t (ΔC_t)
    - Standardizes # sites bound by TF to total # of sites
    - Percent of input binding sites (**% Input**) = 100 * 2 ^ (- ΔC_t)
  - **Fold Enrichment** Above Background = 2 ^ (- $\Delta\Delta C_t$)
    - $\Delta\Delta C_t$ = ΔC_t IP fraction - Δ C_t NIS/mock IP fraction
    - Sample specific background compensated measure
  - **Differential Site Occupancy** Across Samples = 2 ^ (- $\Delta\Delta C_t$)
    - $\Delta\Delta C_t$ = Experimental IP fraction ΔC_t – Control IP fraction Δ C_t
  - Positive Control for ChIP Process =

    RNA Pol II **% Input** @ HKG Proximal Promoter
  - Antibody Non-Specific DNA Binding Assessment =

    Negative Control qPCR Assay **% Input** for each IP Fraction matches NIS/mock IP fraction

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Application Example

- Treat HCT-116 cells with or without 5-flourouracil (5-FU)
- Save fraction of total or INPUT genomic DNA
- ChIP with anti-Pol II, anti-p53 and non-immune serum IgG (mock)
- Measure enrichment of:
  - p53 at CDKN1A Promoter
  - p53 at GADD45A Promoter
  - RNA Pol II at GAPDH Promoter
  - IGX1A negative control region
- Profile expression of p53 Signaling Pathway genes with RT^2 Profiler™ PCR Array

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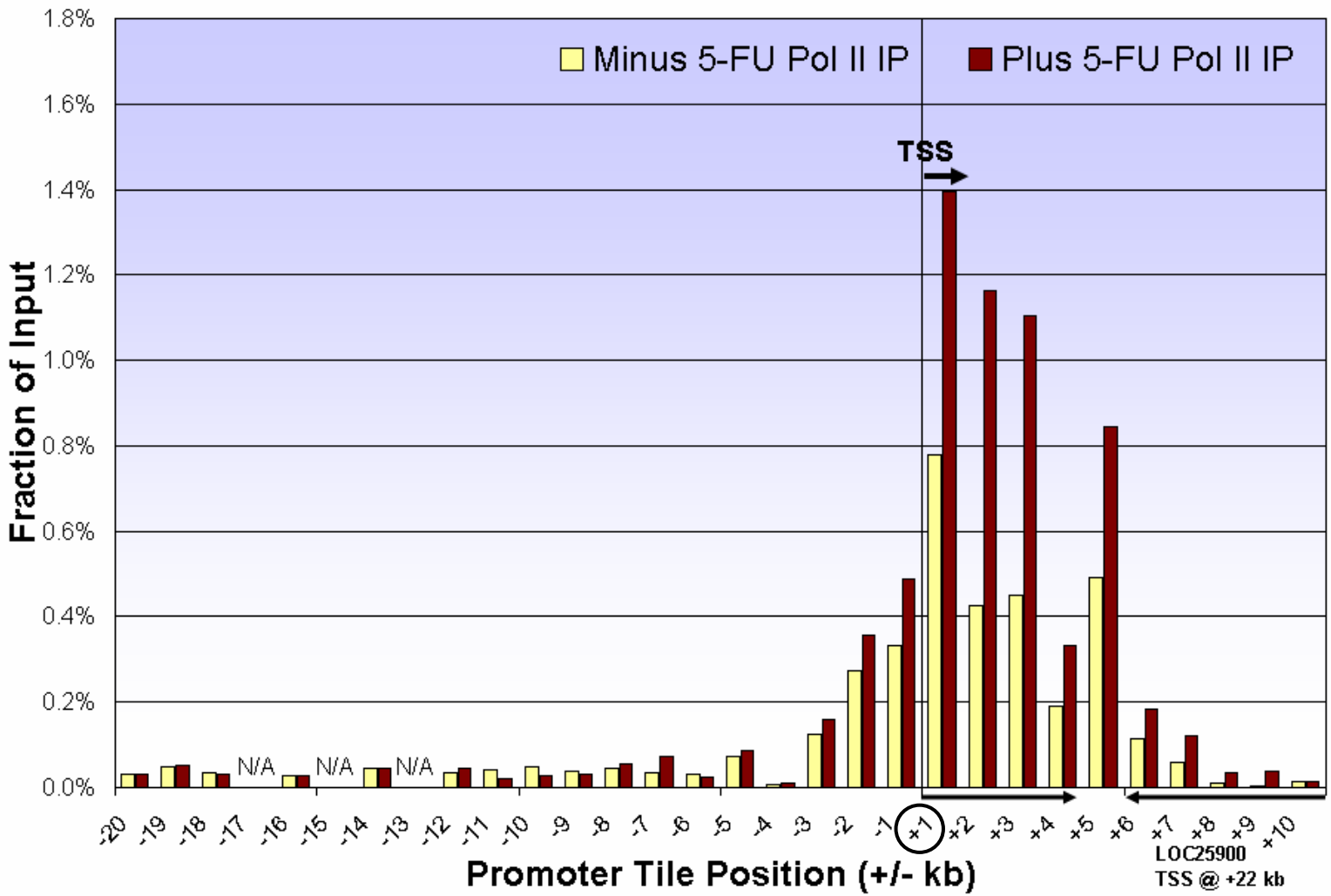
Application Example: POL II IP & GAPD qPCR Tiling



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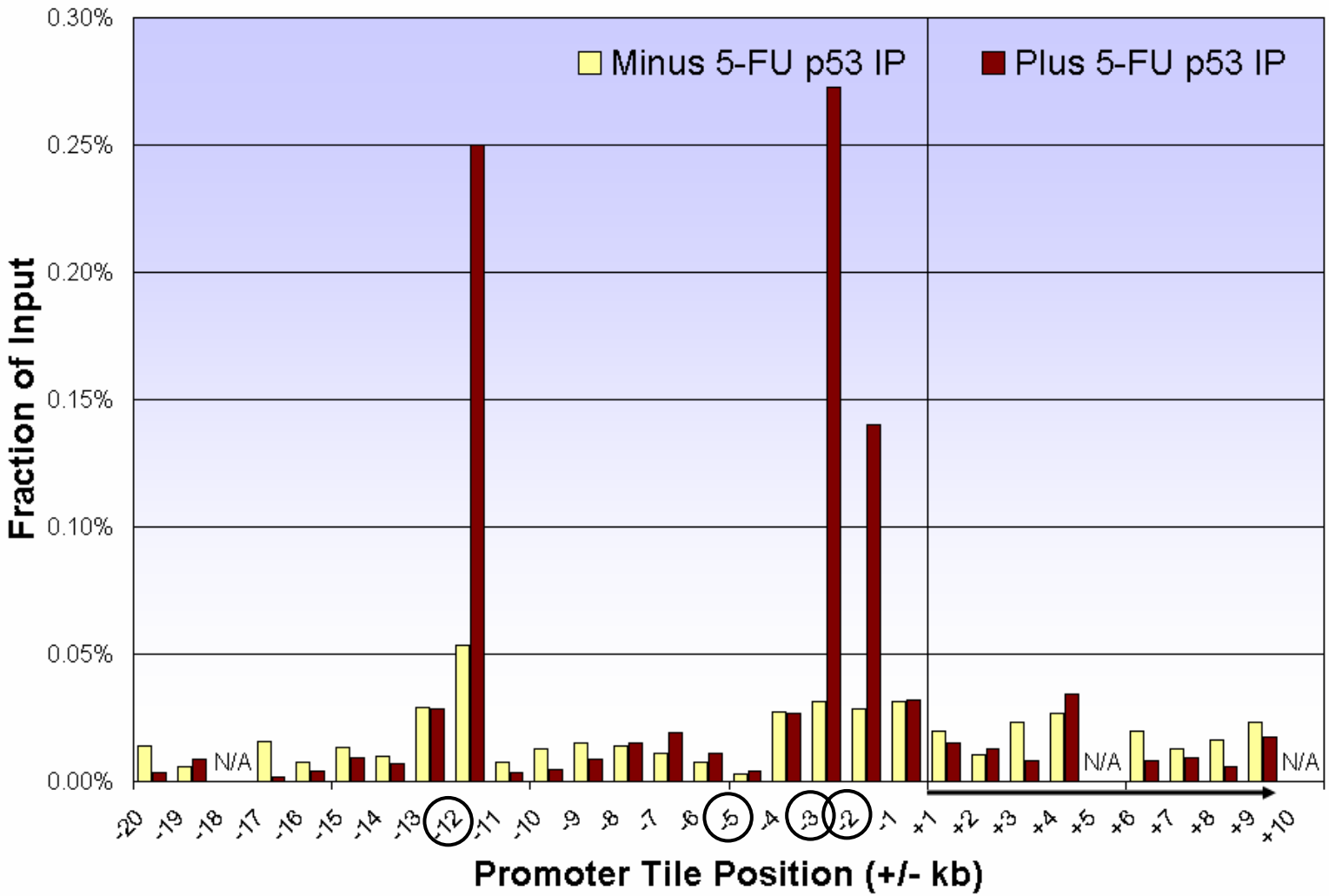
Application Example: p53 IP & CDKN1A qPCR Tiling



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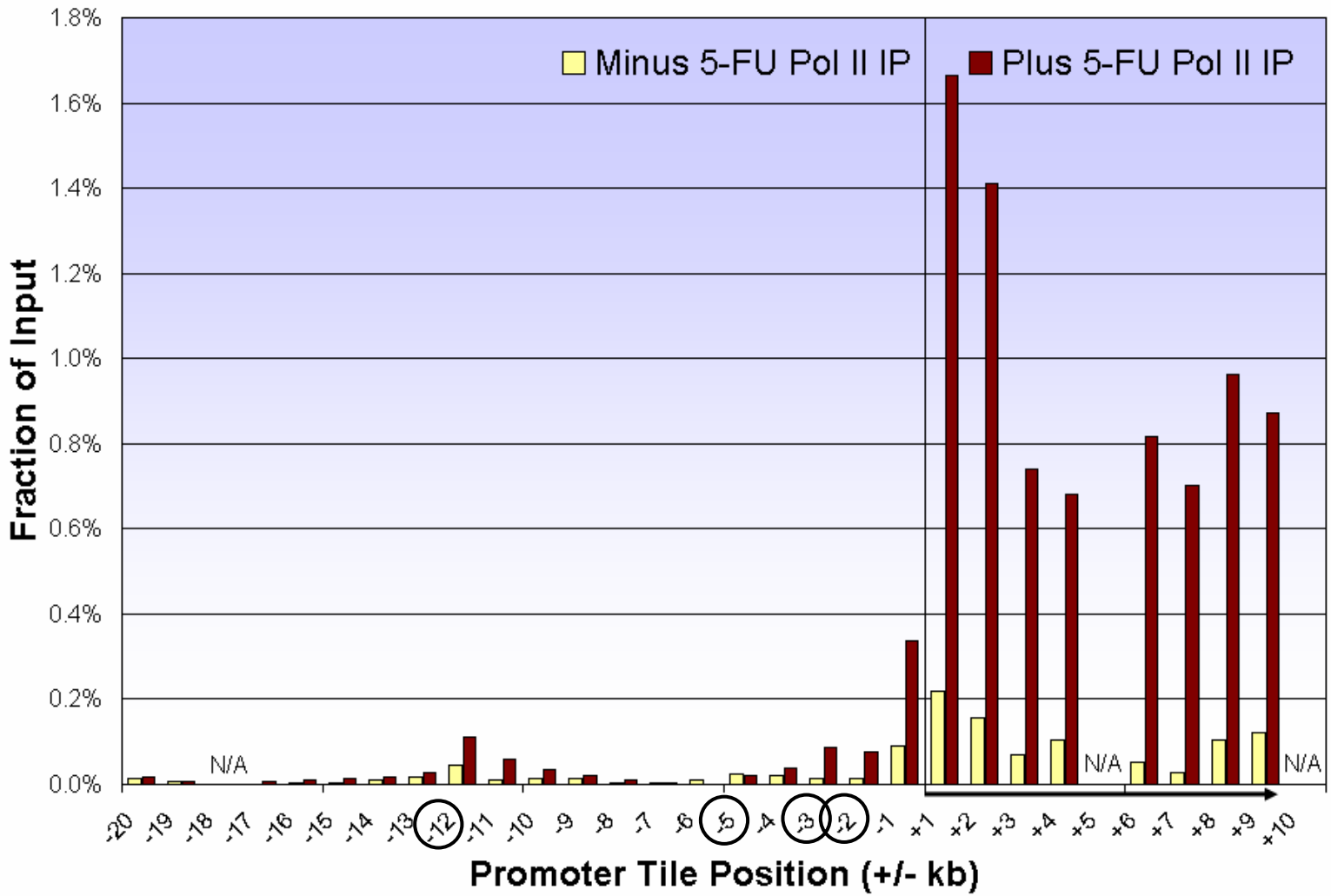
Application Example: Pol II IP & CDKN1A qPCR Tiling



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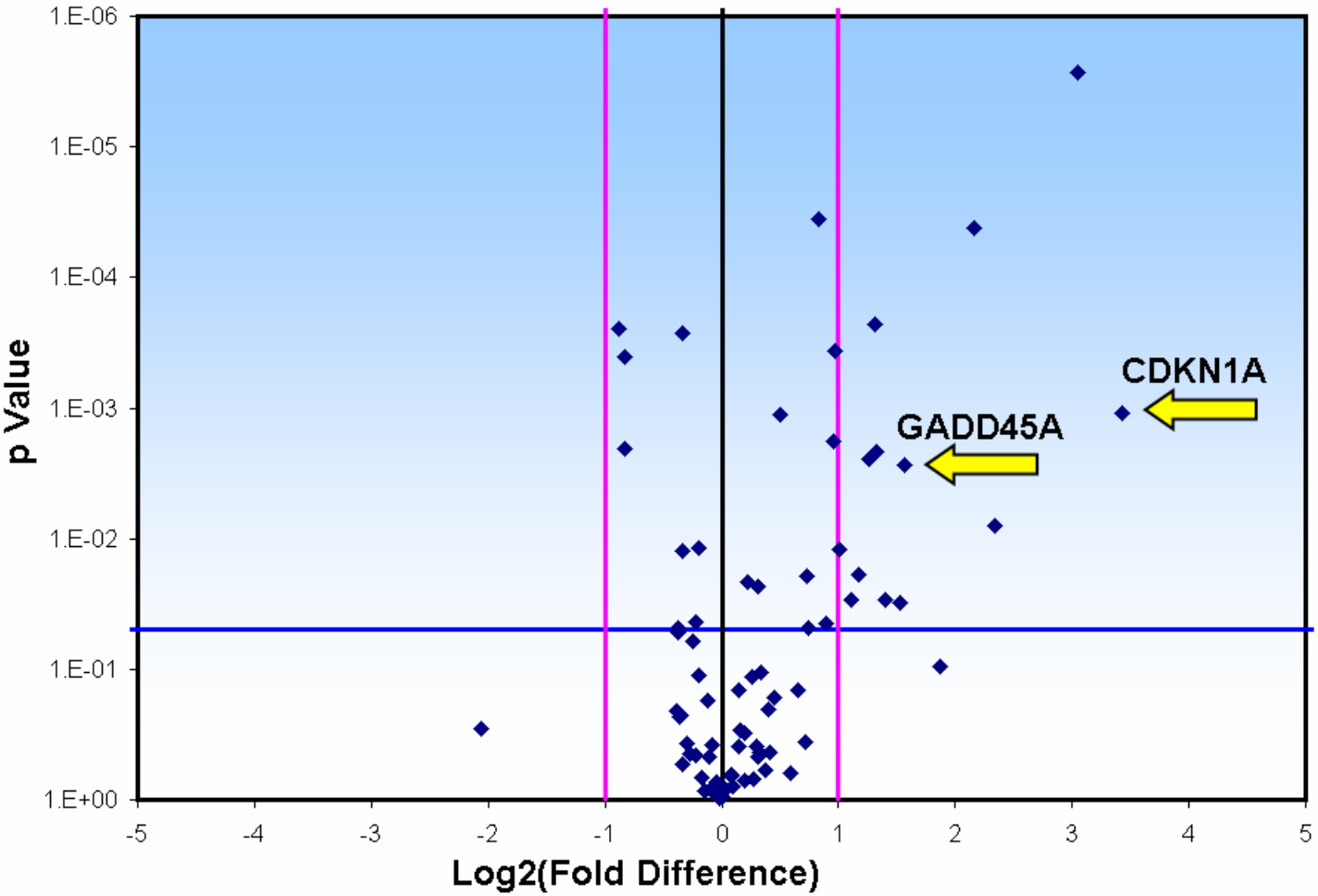
Application Example:
RT² Profiler Human p53 Signaling PCR Array



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Application Example: p53 IP & GADD45A qPCR Individual TF BS Assays

ChIP Fraction	qPCR Assay	C_t	ΔC_t	Fold Enrichment	$\Delta\Delta C_t$	Fold Change
Input	GADD45A +02 Tile -5FU	29.39	-	-	-	-
RNA Pol II		33.47	10.7	0.061 %	-	-
p53		36.31	13.5	0.009 %	-	-
NIS		39.05	16.3	0.001 %		
Input	GADD45A +02 Tile +5FU	31.24	-	-	-	-
RNA Pol II		32.68	8.0	0.380 %	2.64	6.2
p53		34.06	9.4	0.146 %	4.10	17.1
NIS		38.16	13.5	0.009 %		

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How to Order ChIP-qPCR Assays

- **http://www.superarray.com/chipqpcrsearch.php**
- Find the promoter region of interest
  - Species then RefSeq and Tile or Chromosome Location
- Growing set of pre-designed assays for popular TF target sites
- Other assays designed upon request - 2 business days
- Produced and QC on-demand with ~ one-week turnaround time
- May be necessary to contact you if design / QC problematic to find next-best tile

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Custom Assays and Plates

- **Custom ChIP-qPCR Assays**
  - Whole Genome / Whole Chromosome Tiling Array Validation
  - Non-promoter Regions
    - Chromatin Remodeling / Histone ChIP
    - Differentially Methylated Loci / 5-MeC ChIP, MB-PCR, MIRA
  - Narrower Tiling Interval, Wider Tile Range
  - *Saccharomyces cerevisiae*, *Drosophila melanogaster*
- **Custom ChIP-qPCR Arrays**
  - All assays and controls conveniently arranged
  - Same promoter regions, multiple ChIP experiments
  - Same ChIP experiment, multiple promoter regions
    - ChIP-on-chip validation
  - Promoter Tiling Experiments

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SUMMARY

- **Solutions to ChIP Research Challenges:**
- **Save Time and Money:**

  Don't design your own assays anymore – We can do that for you
- **Speedy and Easy Use:**

  Stop running gels – Capture all of the data during one PCR run
- **Guaranteed Performance:**

  Get accurate, quantitative results
- **Comprehensive Coverage:**

  Query ANY promoter or non-promoter region of interest
- **Expand your ChIP experiments to more IP targets or more promoter regions of interest with the ChIP-qPCR Assays.**

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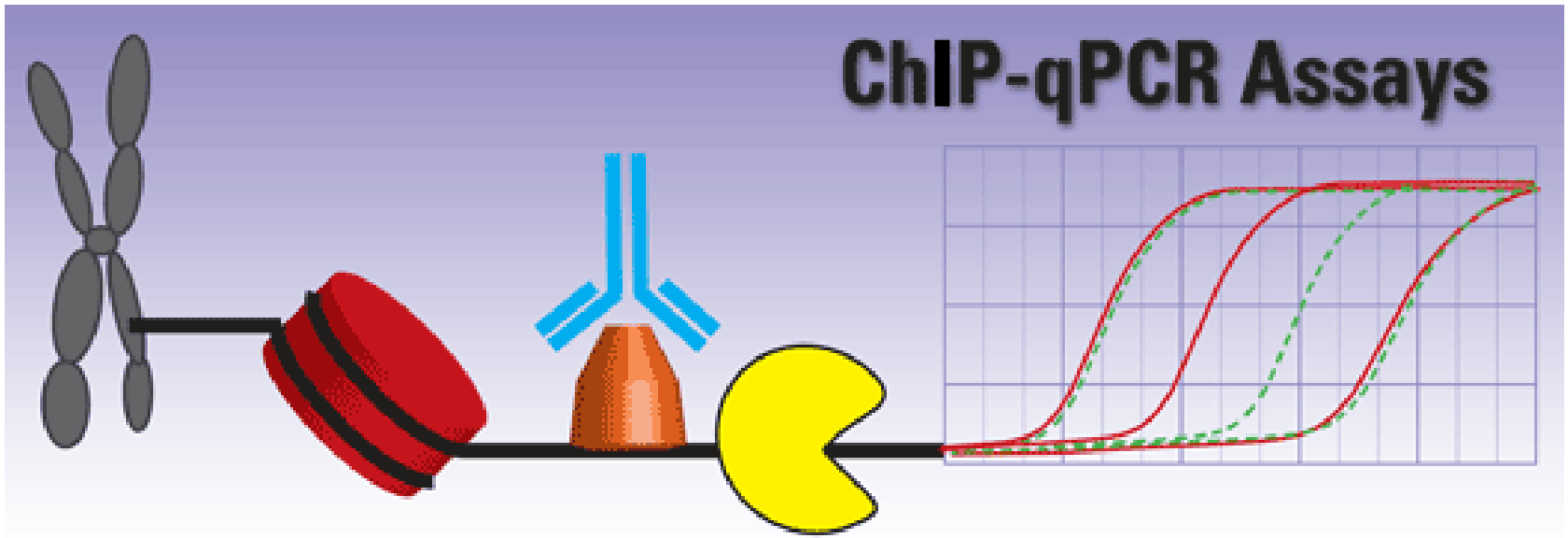
ChIP-qPCR Assays Technology Overview



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