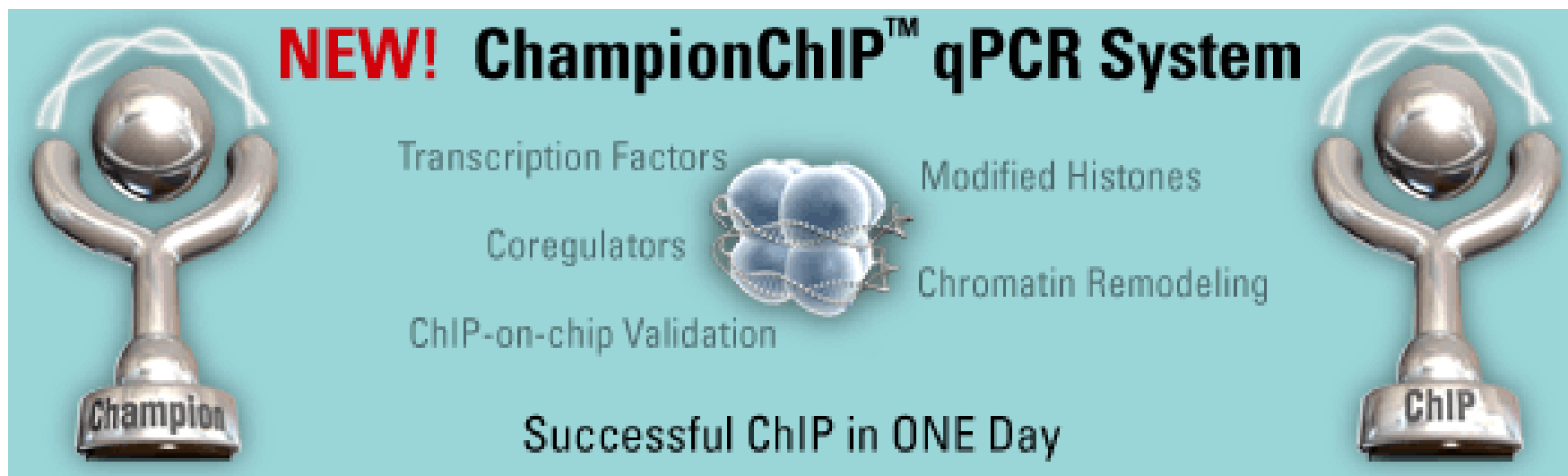


# ChampionChIP™

Quick, High Throughput Chromatin Immunoprecipitation Assay System



**NEW! ChampionChIP™ qPCR System**

Transcription Factors  
Coregulators  
ChIP-on-chip Validation

Modified Histones  
Chromatin Remodeling

Successful ChIP in ONE Day

The graphic features two silver trophies. The left trophy has a base labeled 'Champion' and the right trophy has a base labeled 'ChIP'. In the center, there is a 3D model of a nucleosome, which is a DNA molecule wrapped around a core of histone proteins.

Liyan Pang, Ph.D.  
Application Scientist

# Topics to be Covered

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- **Introduction**
  - What is ChIP-qPCR?
  - Challenges Facing Biological Researchers
  - Solutions
- **How the ChampionChIP System Works**
  - Technology & Protocol Overview
  - Benefits of ChampionChIP System
  - ChIP-qPCR Performance Data
- **Application Examples**
  - p53 & Histone Regulation of CDKN1A Expression



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# ChIP-qPCR

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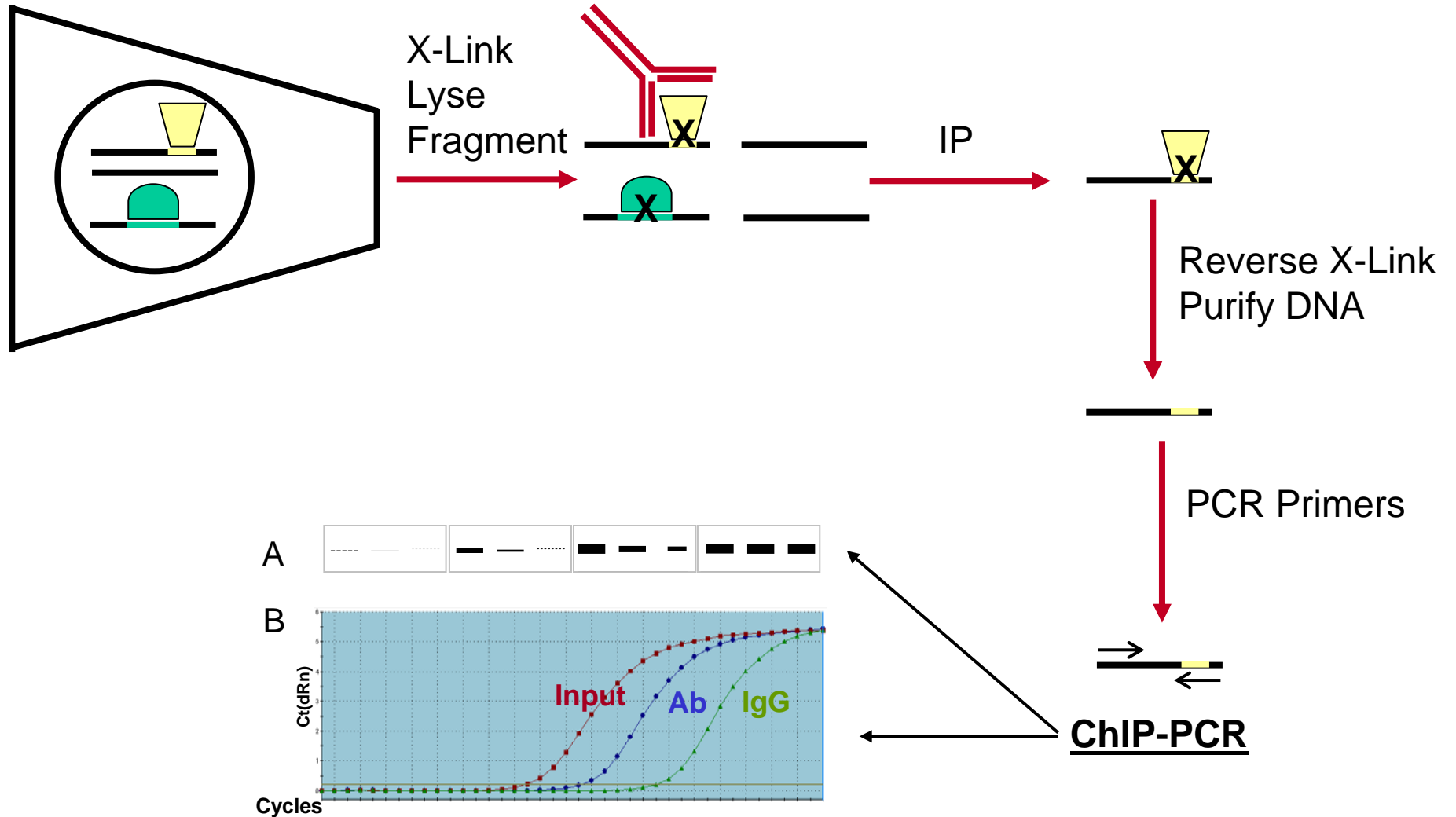
## What is ChIP?

- **Chromatin Immuno-Precipitation**
- Method to examine *in vivo* protein-DNA interactions
  - Transcription Factors, Modified Histones, and other nuclear factors

## What is qPCR?

- qPCR = Quantitative Real-Time PCR
- Faster, Higher Throughput than agarose gel electrophoresis
- Gold-Standard Method to Detect DNA

# Chromatin Immunoprecipitation



# Challenges Facing Biological Researchers

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- **Procedure Related:**
  - Very timing consuming (5-6 days)
  - Multiple optimization steps involved
- **Antibody Related:**
  - Antibody may not be suitable for ChIP
  - Tedious & frustrating ChIP-grade antibody screening
- **ChIP DNA Analysis Related:**
  - Low throughput ChIP analysis with agarose gel
  - Have no experience in design ChIP appropriate qPCR primers
  - Lack of positive & negative control primers
  - Tedious data analysis step

# What are the Solutions?

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## ChampionChIP-qPCR System from SA Biosciences

# Topics to be Covered

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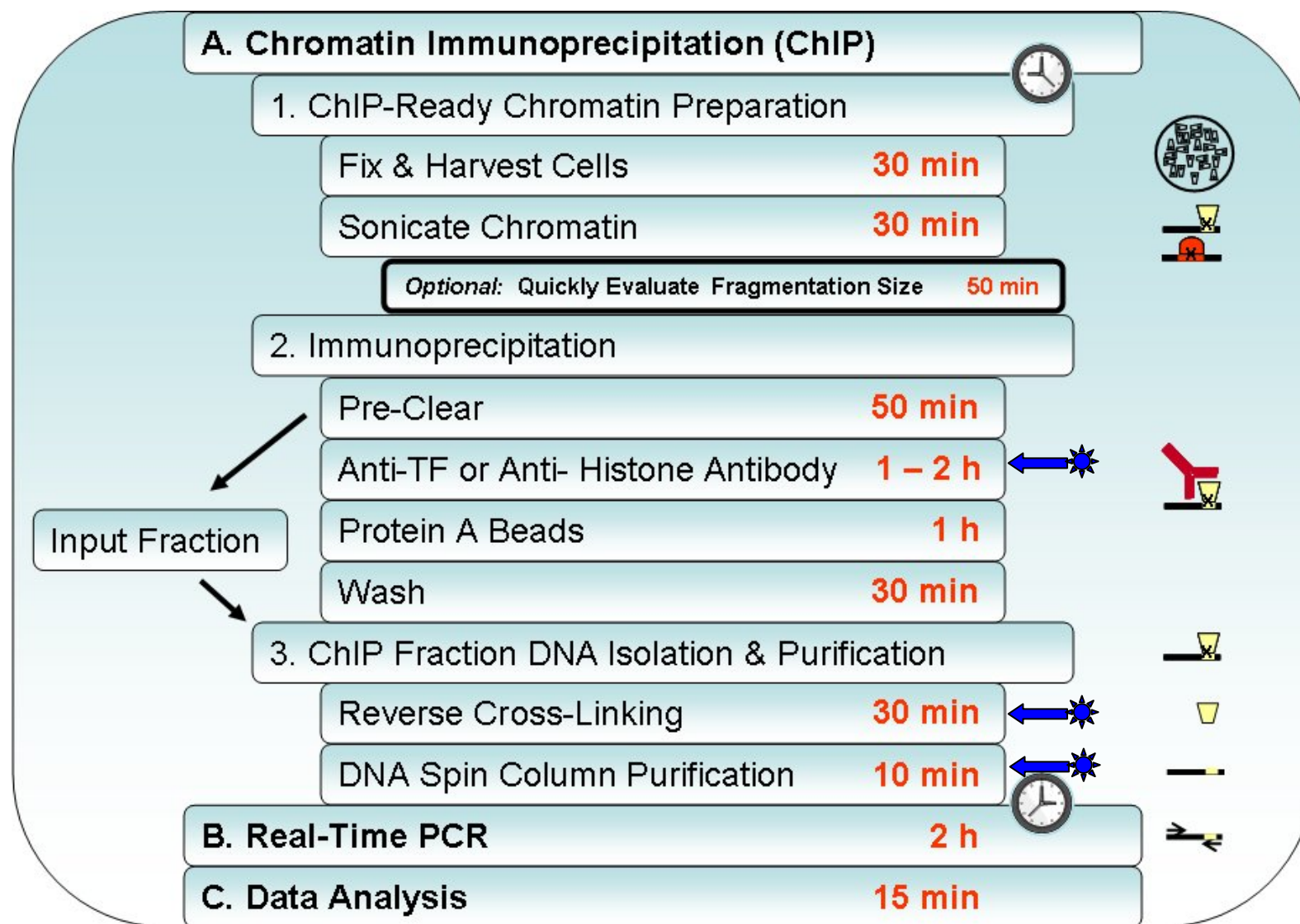
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# ChampionChIP System

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- **One-Day Kit**
  - One-Day Protocol, Higher DNA Yield and Quality
  - Universal Kit Compatible with ANY ChIP-Grade Antibody
- **ChIP-Grade Antibody Kits**
  - Validated ChIP-Grade Antibodies
  - Both Positive & Negative Control Primers are included.
  - Available for Transcriptional Factors & Histones
- **ChIP-qPCR Primers & Arrays**
  - EVERY Site on EVERY human, mouse, or rat promoter
  - Custom ChIP-qPCR Arrays: Arrange Multiple Assays and Controls in one PCR Plate
- **Free Data Analysis Software**

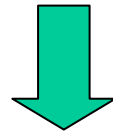
# ChampionChIP System: One-Day kit



# What ChampionChIP One-Day Kits Provides?

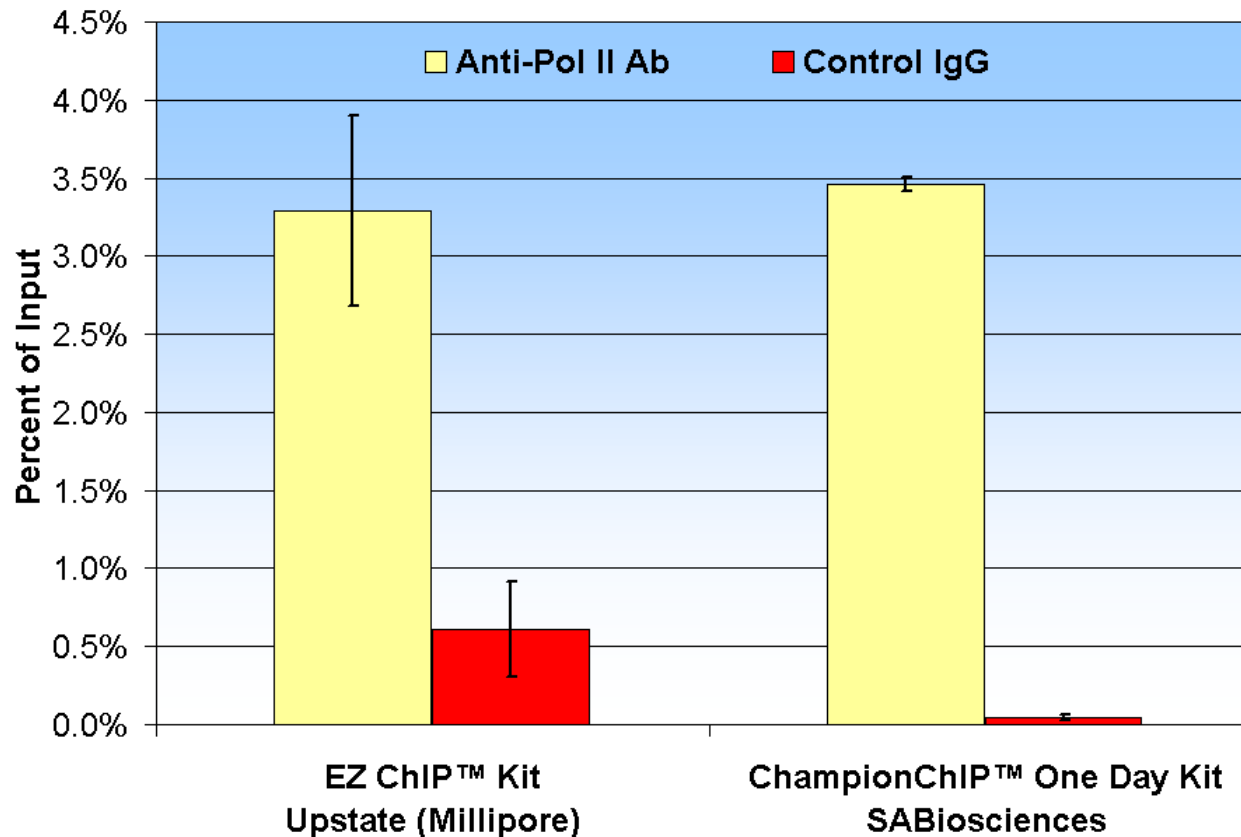
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- IP in 2 hours
- Reverse cross link in 30 minutes
- DNA purification in 10 minutes



- **Speed & Ease:** Simplified protocol easily obtains ChIP DNA from cells in just 6 hours & minimum optimization steps
- **Universality:** General kit compatible with ANY ChIP-Grade antibody
- **Reliability & Consistency:** High-yield and high-quality DNA for real-time PCR & highly reproducible enrichment

# Competitive ChIP Performance



**EZ ChIP Kit:** 100  $\mu$ L ChIP DNA  
**One Day Kit:** 200  $\mu$ L ChIP DNA  
2  $\mu$ L ChIP DNA per each PCR  
**Same  $C_t$  values (PCR sensitivity)**

**ChampionChIP™ One-Day Kit Provides:**

- Better Reproducibility
- Greater Signal-to-Noise Ratios
- Higher Yield

# ChampionChIP System: Antibody Kits

## ChIP-Grade Histone Antibody Kits:

Antibody Name	Modifications
H3ac	Acetylation
H3K9ac	
H4ac	
H3K4me1	Methylation
H3K4me2	
H3K4me3	
H3K9me1	
H3K9me2	
H3K9me3	
H3K27me3	
H3K36me3	
H3K79me3	
H4K20me3	
H3	

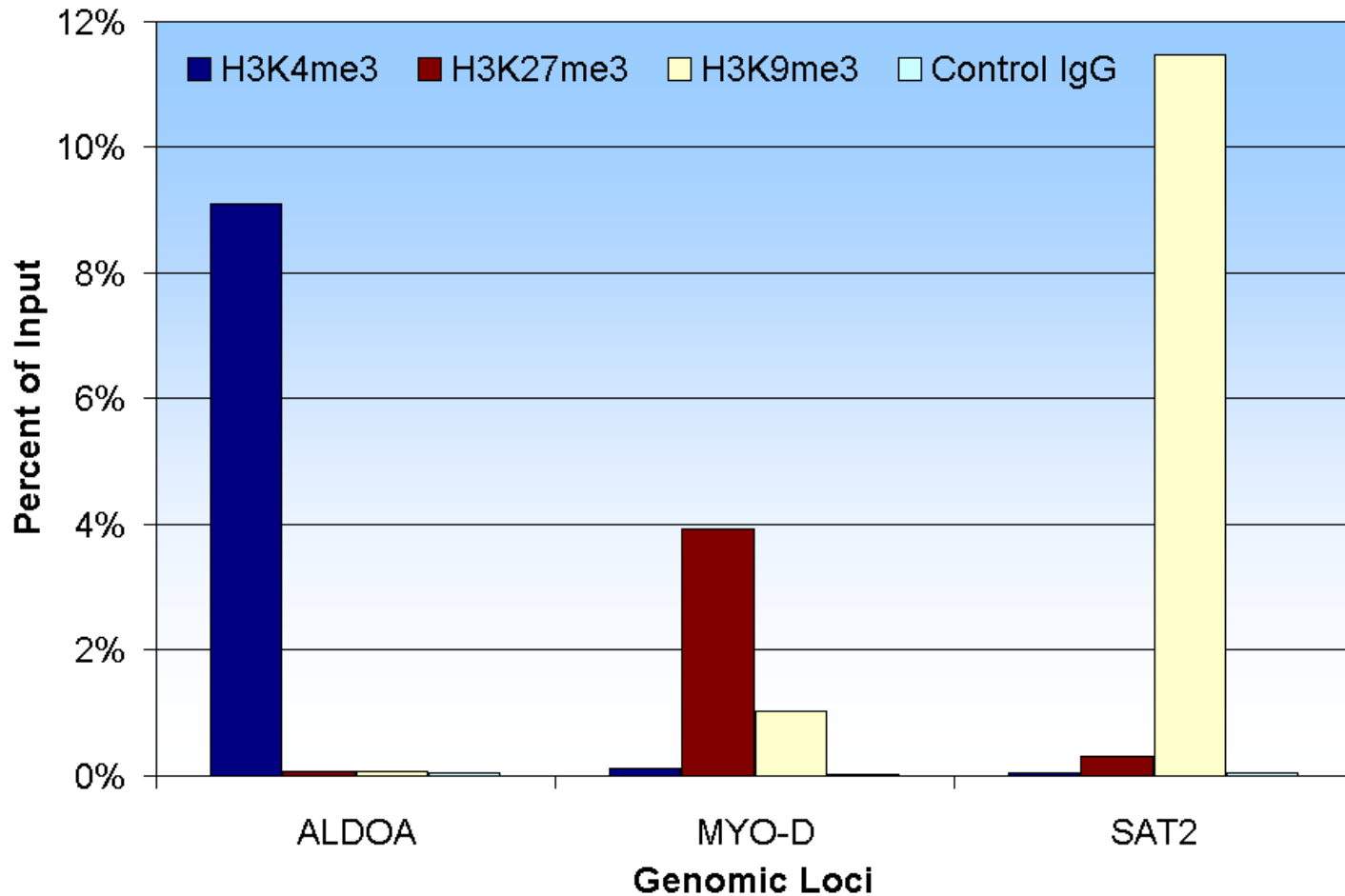
## ChIP-Grade TF Antibody Kits:

RNA Pol II
p53

### Benefits:

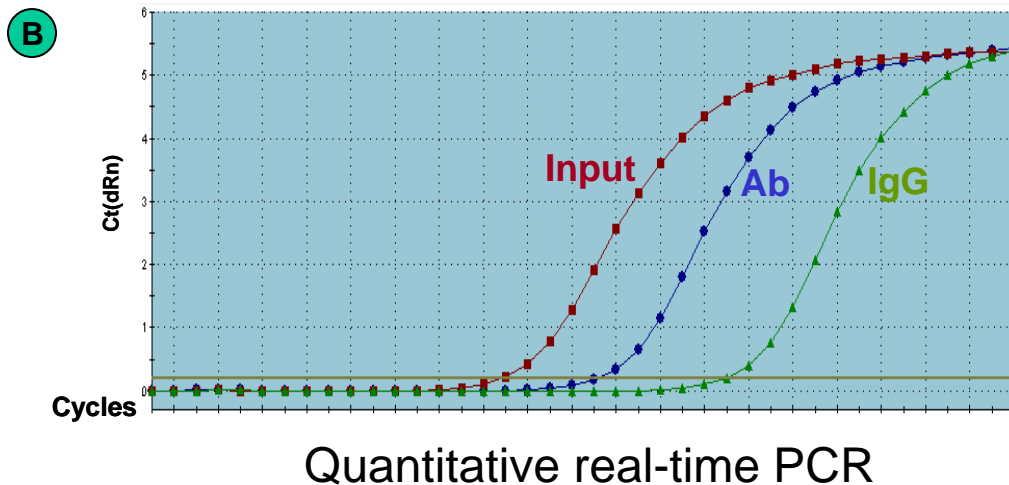
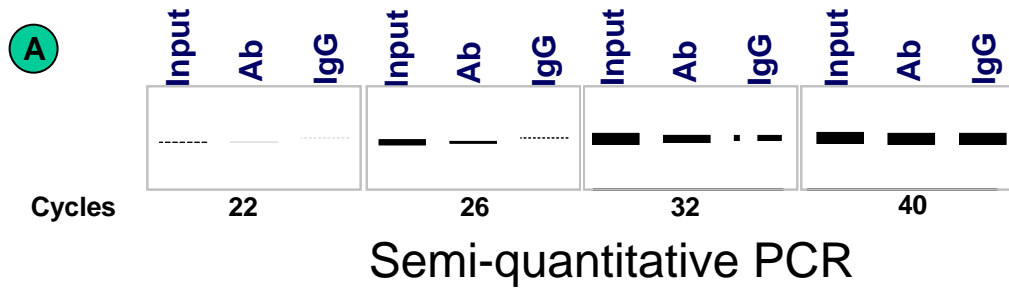
- Experimental validated, ready-to-use ChIP-grade antibodies & control IgGs
- High efficiency and low background
- Including appropriate positive and negative control real-time qPCR primers

# Specificity & Efficiency of Antibody Kits



ALDOA: Transcriptionally Active Euchromatin  
MYO-D: Transcriptionally Inactive Euchromatin  
SAT2: Heterochromatin

# ChampionChIP System: qPCR Primers

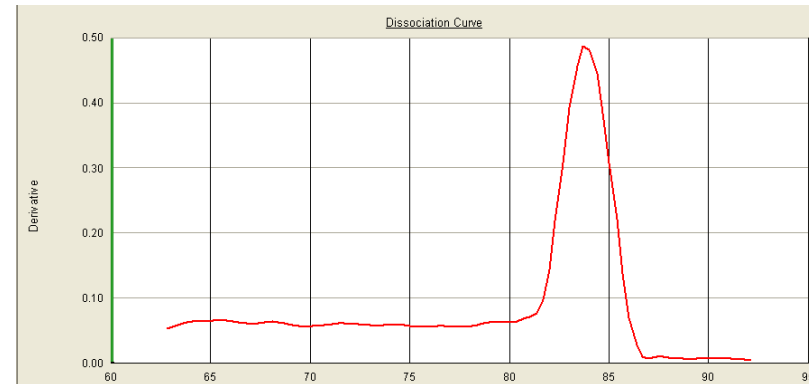
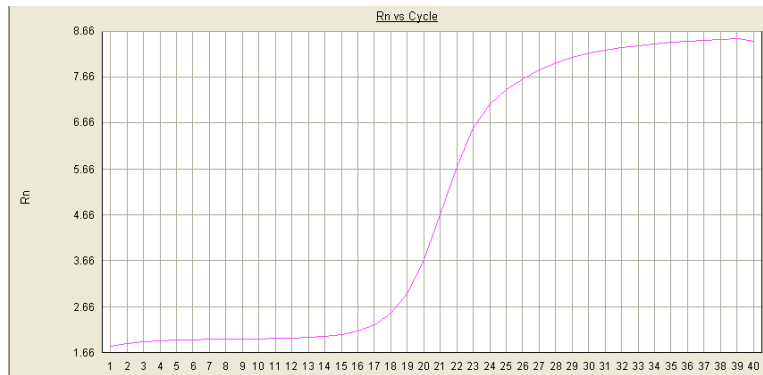


## ChIP-qPCR Primers:

- for EVERY site on EVERY human, mouse, or rat promoter
- Specially designed for genomic DNA enriched from ChIP
- Validated primer specificity & high amplification efficiency
- Similar T<sub>m</sub> & amplification efficiency for all ChIP-qPCR primers

# Guaranteed Performance

## Amplification Efficiency & Specificity



Consistently High Amplification Efficiencies  
(> 90 %, DART Method)

Single Dissociation Curve Peak  
Single Band by Agarose Gel

Each assay design is experimentally validated on the bench before shipment.  
Certificate of Analysis provided with each assay.

# Finding Your ChIP-qPCR Assays

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**EVERY TF binding site on EVERY human, mouse, or rat promoter**

- <http://www.sabiosciences.com/chipqpcrsearch.php>
- Search by Gene
  - Find primers for known binding site relative to TSS
- Search by Transcription Factor and/or Gene
  - Find primers for predicted binding sites for factor in gene(s) of interest
- Search by Chromosomal Position
  - Find primers for specific position identified by ChIP-on-chip

# Custom ChIP-qPCR Arrays

- Consistent performance of assays under same cycling conditions
- Assays and controls conveniently arranged
- Same promoter regions, multiple ChIP experiments
- Same ChIP experiment, multiple promoter regions
- ChIP-on-chip validation
- Histone Modification Promoter Tiling Experiments

Fraction:	INPUT				NIS / Mock IP				Nuclear Factor-Specific IP			
	1	2	3	4	1	2	3	4	1	2	3	4
	5	6	7	8	5	6	7	8	5	6	7	8
	9	10	11	12	9	10	11	12	9	10	11	12
	13	14	15	16	13	14	15	16	13	14	15	16
	17	18	19	20	17	18	19	20	17	18	19	20
	21	22	23	24	21	22	23	24	21	22	23	24
	25	26	27	28	25	26	27	28	25	26	27	28
	29	30	Negative Control	Positive Control	29	30	Negative Control	Positive Control	29	30	Negative Control	Positive Control

# Data Analysis

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## Data Analysis Principles:

- Normalize CHIP Fraction  $C_t$  to Input DNA Fraction  $C_t$  Values
- Percent of Input (or fraction enrichment) =  $100 * 2^{-\Delta C_t}$
- Fold-Changes in DNA Binding =  $2^{-\Delta\Delta C_t}$

## Free Data Analysis Excel Template:

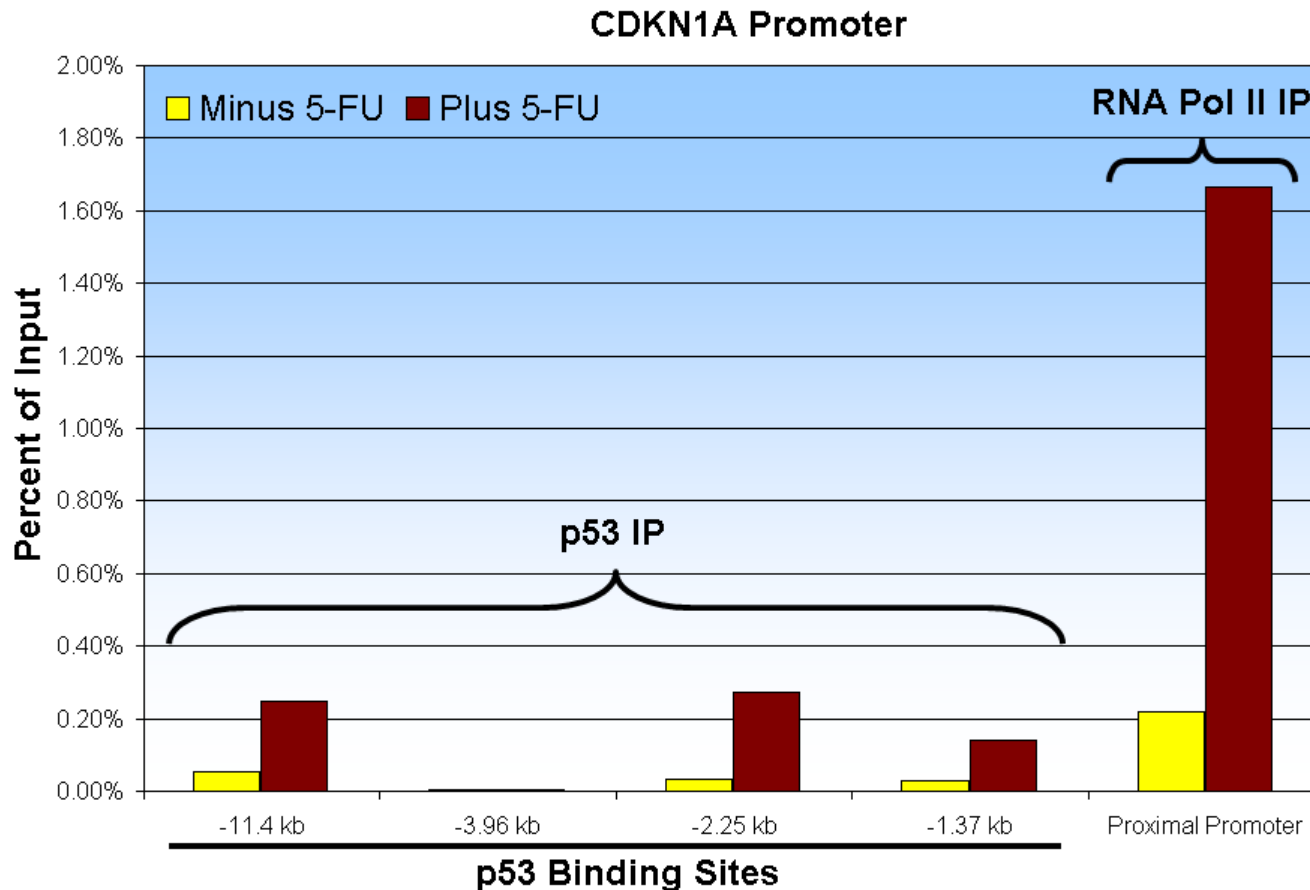
<http://www.sabiosciences.com/manuals/chipqpcranalysis.xls>

# Topics to be Covered

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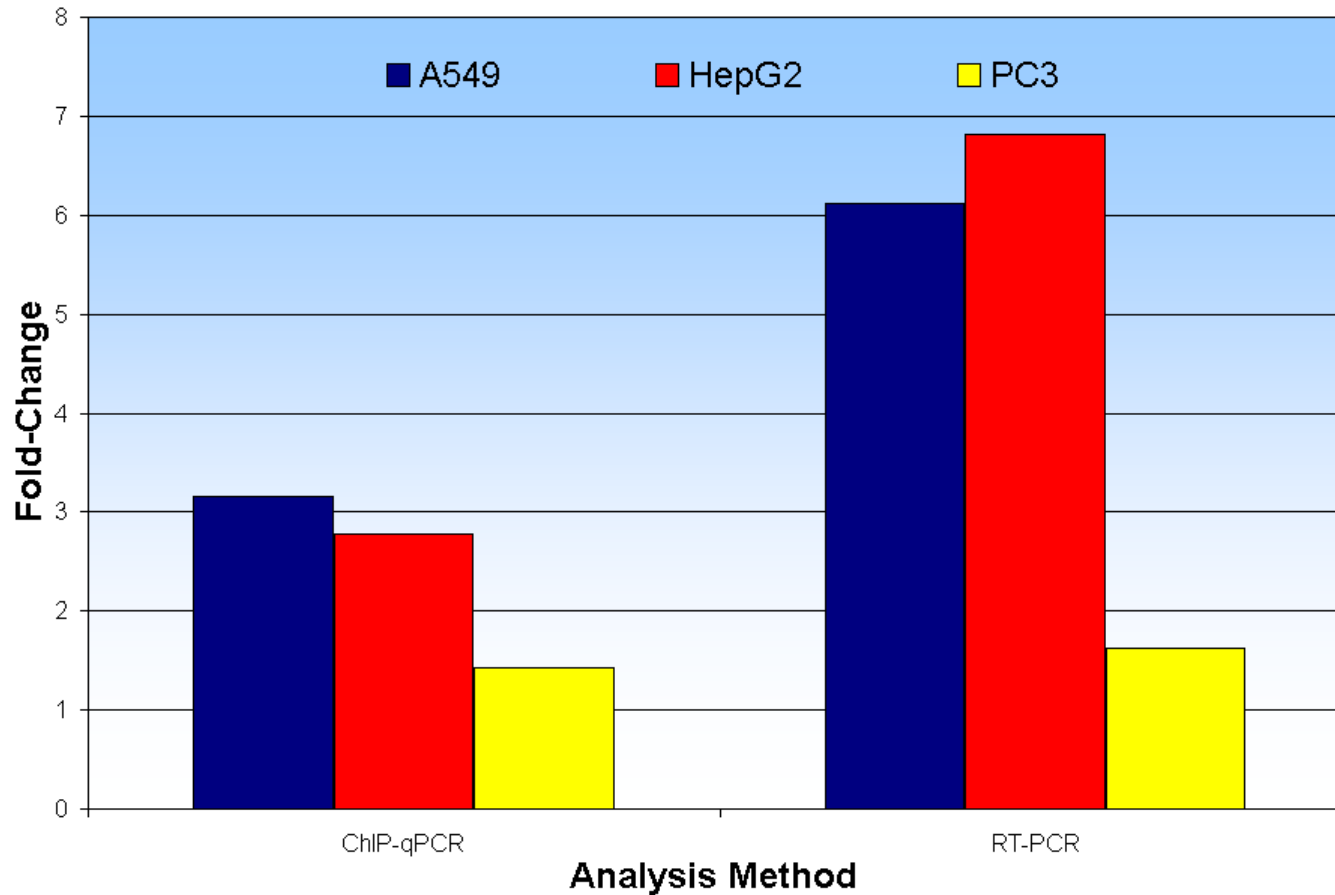
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# Application Example: p53 ChIP & CDKN1A qPCR (1)



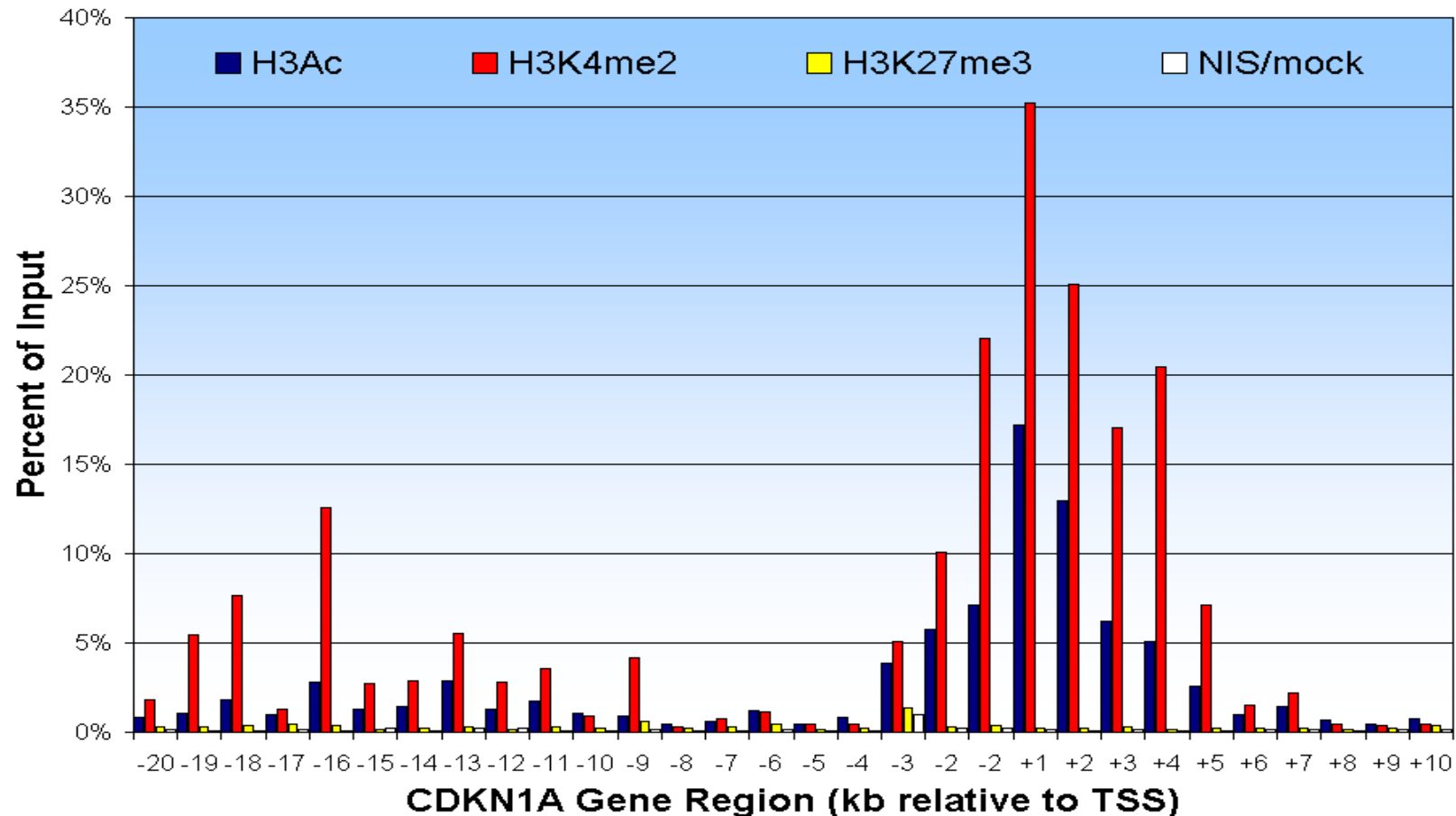
Treatment with 5-FU increases p53 Binding to CDKN1A Promoter by 5- to 9-fold  
It also increases RNA Pol II binding to CDKN1A Promoter by 7.5-fold.  
Results are consistent with observed increase in mRNA levels ...

## Application Example: p53 ChIP & CDKN1A qPCR (2)



Increased p53 binding correlates with increased mRNA expression in wild-type (A549 and HepG2) but not mutant p53 cell lines (PC3).

# Application Example: Modified Histone ChIP



Histone markers for actively transcribed genes (H3Ac and H3K4me2) but not for transcriptionally inactive genes (H3K27me3) surround the CDKN1A gene making it available to p53 regulation.

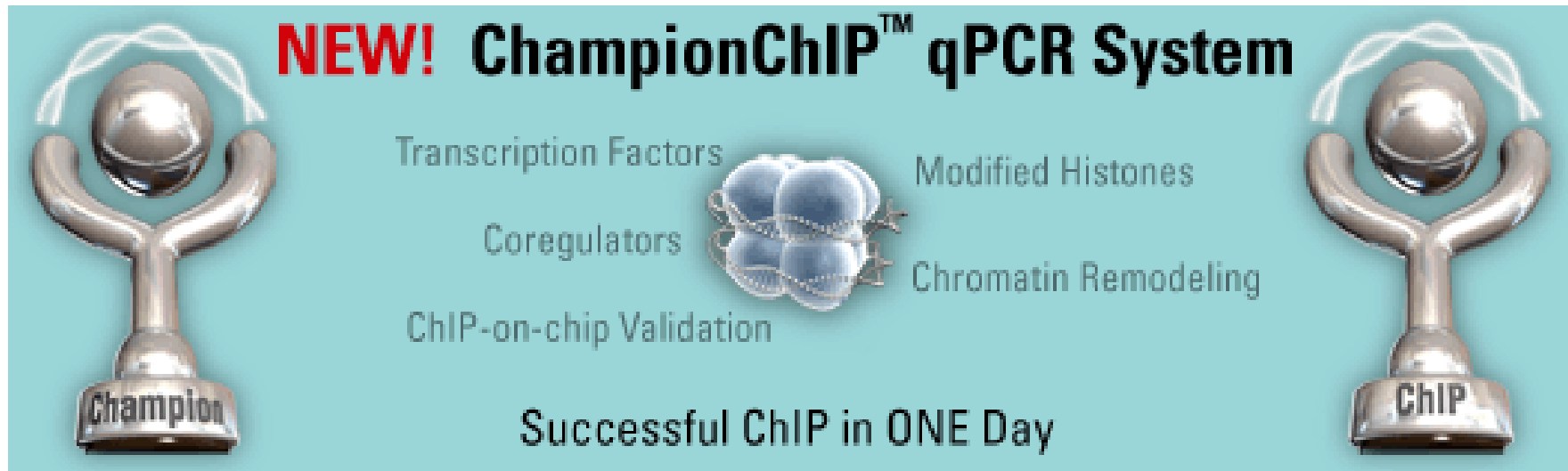
# Summary of ChampionChIP System

SABiosciences = ONLY source for COMPLETE ChIP System

- **One-Day Kit - Solutions to the Procedure Related Challenges:**
  - Very timing consuming (5-6 days) ➡ **One Day**
  - Multiple optimization steps involved ➡ **Minimal Optimization Steps**
- **ChIP-Grade Antibody Kit – Solutions to the Antibody Related Challenges:**
  - Antibody may not be suitable for ChIP
  - Tedious & frustrating ChIP-grade antibody screening } **Bench Validated ChIP-Grade Antibody**
- **ChIP-qPCR Primers & Arrays – Solutions to the ChIP DNA Analysis Related Challenges:**
  - Low throughput ChIP analysis with agarose gel ➡ **Real-Time PCR**
  - Have no experience in design ChIP appropriate primers
  - Lack of positive & negative control primers } **Genome-Wide ChIP qPCR**
- **Free Data Analysis Software**
  - Tedious data analysis step ➡ **No More! A Simple Copy & Paste**


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Modified Histones  
Chromatin Remodeling

Successful ChIP in ONE Day

The graphic features two silver trophies. The left trophy has a base labeled 'Champion' and the right trophy has a base labeled 'ChIP'. The background is light blue with a central image of a DNA double helix with several blue protein complexes bound to it.

## Questions?

Ask now or Contact Technical Support M – F 9 AM – 6 PM Eastern  
Telephone: 888-503-3187 OR Email: [support@SABiosciences.com](mailto:support@SABiosciences.com)