ChIP-IT® qPCR Analysis Kit

(version A2)

Catalog Nos. 53029

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Overview

Chromatin Immunoprecipitation (ChIP) is a powerful tool for studying protein/DNA interactions, including transcription factors, co-regulatory proteins, modified histones, chromatin-modifying enzymes and polymerases. Analysis of individual protein DNA interactions can be determined by combining ChIP with downstream qPCR analysis. However, there are multiple data normalization approaches that have been used and the widely used delta CT method, or percent input method of ChIP DNA qPCR analysis can be difficult to grasp. In order to simplify ChIP qPCR data analysis, Active Motif has developed a normalization strategy that enables accurate qPCR analysis across multiple samples and experiments.

The ChIP-IT® qPCR Analysis Kit contains standard curve DNA, a standard curve primer pair, positive and negative control PCR primer pairs for human and mouse samples and a qPCR analysis spreadsheet to perform the analysis calculations. The analysis strategy determines primer efficiency and utilizes this information in combination with input DNA values, the amount of chromatin used in the ChIP reaction and the ChIP DNA resuspension volumes to normalize the ChIP data.

The ChIP-IT qPCR Analysis Kit contains sufficient reagents to perform 10 standard curves and is recommended for use in combination with Active Motif's ChIP-IT® High Sensitivity Kit (Catalog No. 53040). This ChIP-IT qPCR Analysis Kit is designed for use with ChIP DNA from human or mouse samples. To learn about available ChIP-IT® Kits, species-specific qPCR primer sets or ChIP-validated antibodies, please visit our website at **www.activemotif.com/chip**.

product	format	catalog no.
ChIP-IT® qPCR Analysis Kit	10 rxns	53029

Kit Performance and Benefits

ChIP-IT qPCR Standard Kit Advantages:

- Simplifies qPCR data analysis to enable a faster, more reliable interpretation of data
- All calculations are included in a simple Excel sheet in which data from your qPCR machine can be entered directly resulting in automatic data normalization and graph generation
- Normalized data can be directly compared across multiple sample types and experiments
- Includes human and mouse positive control and negative control qPCR primer sets
- Recommendations for acceptable data values allows users to evaluate the quality of the ChIP reaction before proceeding with downstream applications such as ChIP-Seq

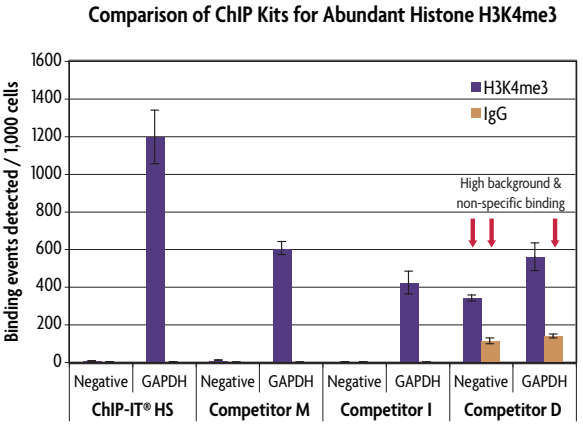

Figure 1: ChIP-IT qPCR Analysis Kit was used to normalize data across competitor ChIP Kits.

MCF-7 chromatin was prepared according to each manufacturer's recommendations for their ChIP assay from 1.5 x 10^6 cells. The optimal amount of chromatin for each kit was used in the immunoprecipitation reactions; for ChIP-IT® High Sensitivity (Catalog No. 53040), 10 µg chromatin was used. Following each manufacturer's protocol, the chromatin was incubated with Active Motif's Histone H3K4me3 antibody (Catalog No. 39915) and a negative control IgG. Following enrichment, qPCR analysis was performed using the ChIP-IT qPCR Analysis Kit in order to normalize the data and allow for direct comparison of the results. The included Human Negative Control PCR primer set was used as a negative control as it amplifies a gene desert on chromosome 12 that should not show any H3K4me3 enrichment, while GAPDH is an actively transcribed gene that is associated with the presence of H3K4me3. Data is expressed as binding events detected per 1,000 cells which represents the average of the raw data triplicates adjusted for the amount of chromatin in the reaction, the resuspension volume and the primer efficiency. Because the ChIP-IT qPCR Analysis Kit adjusts for the variation in chromatin amounts and resuspension volumes, this normalization method allows for direct comparison of data from the different competitor ChIP Kits. This calculation provides consistency in data analysis and allows direct comparison across samples and experiments. To convert this scale to the percent of ChIP input recovered, divide the values by 1,000. From this data it is apparent that the ChIP-IT High Sensitivity Kit provides at least 2-3 fold enrichment compared to Competitor M and Competitor I, while Competitor D has high background and non-specific DNA binding that is visible in the Negative control primer set and IgG results.

Kit Components and Storage

Please store each component at the temperature indicated in the table below.

Reagents	Quantity	Storage
DNA Standard AM1	100 µl	-20°C
DNA Standard AM2	100 µl	-20°C
DNA Standard AM3	100 µl	-20°C
Standard Curve Primer Pair (2 µM)	400 µl	-20°C
Human Negative Control Primer Set 1 (2.5 µM)	400 µl	-20°C
Human Positive Control Primer Set GAPDH-2 (2.5 µM)	400 µl	-20°C
Mouse Negative Control Primer Set 1 (2.5 µM)	400 µl	-20°C
Mouse Positive Control Primer Set Gapdh-2 (2.5 µM)	400 µl	-20°C
2 mM Tris-HCl, pH 8.0	2 x 1 ml	RT

Additional materials required

- Purified ChIP DNA and Input DNA for each sample to be tested
- qPCR Primer Pairs to study the gene locus of interest in the ChIP DNA. Positive control primers are recommended. Primers should be designed to work optimally at an annealing temperature of 58°C. See Reagent Information section for complete details on primer design.
- 2X SYBR Green qPCR Master Mix (*e.g.* Bio-Rad Catalog No. 170-8882)
- 96-well PCR plate or PCR tubes
- Thermocycler

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING!

Reagent Information

DNA Standards AM1, AM2 and AM3

The DNA Standards provided in the kit can be used to generate a standard curve. Each standard vial contains a known amount of genomic DNA. Since the DNA mass in each standard is known, the number of copies of the genome present in each standard can be calculated (assuming that each cell contains 6.6 pg of DNA). DNA Standards are provided ready to use. Duplicate qPCR reactions are prepared for each DNA Standard.

Standard Curve Primer Pair

The Standard Curve Primer Pair is designed to be used with the standard curve generated from the DNA Standard samples. The primer pair is provided at 2 µM.

ChIP DNA

qPCR reactions with ChIP DNA should be performed in triplicate for each primer pair used. For ChIP DNA that was eluted in a 100-200 µl volume, use 5 µl DNA per qPCR reaction. However, if the DNA was eluted in a smaller volume (*e.g.* ChIP-Seq DNA eluted in 36 µl volume) prepare a dilution by adding 6 µl ChIP DNA to 94 µl of 2 mM Tris-HCl, pH 8.0. Use 5 µl diluted ChIP DNA per qPCR reaction. It is necessary to run the included Negative Control Primer Pair (human or mouse) in triplicate with the ChIP DNA. This helps with data interpretation as deviation from the expected output may indicate potential problems in the ChIP reaction.

Input DNA

Input DNA for each sample type should be diluted to a final concentration of 2.5 ng/µl using 2 mM Tris-HCl pH 8.0. In experiments with multiple Input DNAs prepared from multiple samples, a DNA pool should be made and used for testing. Combine the various Input DNAs at their diluted 2.5 ng/µl concentration into a single tube. Input DNA should be tested in triplicate.

2 mM Tris-HCl, pH 8.0

2 mM Tris-HCl is provided ready to use.

Gene-specific qPCR Primer Pair

When designing PCR primer pairs for use in qPCR analysis of the ChIP DNA, it is recommended to design primers that generate amplicons 75-150 bp long. Primers should also be designed to perform optimally at an annealing temperature of 58°C with a recommended length of 18-22 bp each. The Primer3 program (Primer3 at http://frodo.wi.mit.edu/) is a good resource for designing primers to meet these criteria. We suggest preparing a mix of both primers at a concentration of 2.5 µM each for qPCR reactions. Gene-specific primer sets should be tested with 12.5 ng Input DNA in a qPCR reaction. Usable primer pairs will have CT values between 23-27. If primers have CT values greater than 27, the primers should be redesigned.

Human Negative Control Primer Set1

The negative control primer set is designed to serve as a universal negative ChIP control when performing chromatin immunoprecipitation with human samples. If your test ChIP sample is human, run triplicate qPCR reactions using the Human Negative Control Primer Set in addition to a positive gene-specific qPCR primer pair. When the Human Negative Control primer set is used in the analysis spreadsheet the signal should fall within an expected range. For more information on data interpretation, please refer to Section D of the manual. The primer pair is provided at 2.5 µM.

Note: The universal negative control primers could give a positive readout for repressive histone marks such as H3K27me3. In this case we recommend using the supplied positive control primer set for GAPDH-2 as the negative control.

Human Positive Control Primer Set GAPDH-2

The positive control primer set is designed to serve as a positive ChIP control when performing chromatin immunoprecipitation with human samples. The Human Positive Control Primer Set GAPDH-2 amplifies a region of the metabolic gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) associated with intron 1 and has been validated to work with a multitude of ChIP antibodies including: H3K9ac, H3K14ac, H3K4me2, H3K4me3, H4K5ac, H4K8ac, H4K12ac, H4K16ac, Total RNA pol II, and RNA pol II phospho Ser5. If your test ChIP sample is human, run triplicate qPCR reactions using the Human Positive Control Primer Set GAPDH-2 in addition to the gene-specific qPCR primer pair for the gene of interest. The primer pair is provided at 2.5 µM.

Mouse Negative Control Primer Set 1

The negative control primer set is designed to serve as a universal negative ChIP control when performing chromatin immunoprecipitation with mouse samples. If your test ChIP sample is mouse, run triplicate qPCR reactions using the Mouse Negative Control Primer Set in addition to a positive gene-specific qPCR primer pair. When the Mouse Negative Control primer set is used in the analysis spreadsheet the signal should fall within an expected range. For more information on data interpretation, please refer to Section D of the manual. The primer pair is provided at 2.5 µM.

Note: The universal negative control primers could give a positive readout for repressive histone marks such as H3K27me3. In this case we recommend using the supplied positive control primer set for Gapdh-2 as the negative control.

Mouse Positive Control Primer Set Gapdh-2

The positive control primer set is designed to serve as a positive ChIP control when performing chromatin immunoprecipitation with mouse samples. The Mouse Positive Control Primer Set Gapdh-2 amplifies the promoter region of the metabolic gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and has been validated to work with a multitude of ChIP antibodies including: H3K9ac, H3K14ac, H3K4me2, H3K4me3, H4K5ac, H4K8ac, H4K12ac, H4K16ac, Total RNA pol II, and RNA pol II phospho Ser5. If your test ChIP sample is mouse, run triplicate qPCR reactions using the Mouse Positive Control Primer Set Gapdh-2 in addition to the gene-specific qPCR primer pair for the gene of interest. The primer pair is provided at 2.5 µM.

Section A. qPCR Plate Set Up

We recommend using Active Motif's ChIP-IT® High Sensitivity Kit (Catalog No. 53040) to perform the chromatin immunoprecipitation. If using a different method to perform ChIP, the enriched ChIP DNA should be purified prior to use in qPCR analysis.

Following ChIP, qPCR reactions should be performed in triplicate for each primer pair used with the ChIP and Input samples. The DNA Standard curve should be performed in duplicate using the provided Standard Curve Primer Pair.

An example 96-well plate qPCR set up including the DNA Standards and standard curve primers along with six different ChIP DNA samples and four different PCR primer pairs is provided below. The top table shows the layout of the DNA samples and the bottom table shows the layout of the PCR primer pairs. Please adjust your plate layout based on the number of samples and primer sets to be analyzed.

	1	2	3	4	5	6	7	8	9	10	11	12
A	–	Std AM1	Std AM1	Std AM2	Std AM2	Std AM3	Std AM3	–	–	–	–	–
B	ChIP 1	ChIP 1	ChIP 1	ChIP 1	ChIP 1	ChIP 1	ChIP 1	ChIP 1	ChIP 1	ChIP 1	ChIP 1	ChIP 1
C	ChIP 2	ChIP 2	ChIP 2	ChIP 2	ChIP 2	ChIP 2	ChIP 2	ChIP 2	ChIP 2	ChIP 2	ChIP 2	ChIP 2
D	ChIP 3	ChIP 3	ChIP 3	ChIP 3	ChIP 3	ChIP 3	ChIP 3	ChIP 3	ChIP 3	ChIP 3	ChIP 3	ChIP 3
E	ChIP 4	ChIP 4	ChIP 4	ChIP 4	ChIP 4	ChIP 4	ChIP 4	ChIP 4	ChIP 4	ChIP 4	ChIP 4	ChIP 4
F	ChIP 5	ChIP 5	ChIP 5	ChIP 5	ChIP 5	ChIP 5	ChIP 5	ChIP 5	ChIP 5	ChIP 5	ChIP 5	ChIP 5
G	ChIP 6	ChIP 6	ChIP 6	ChIP 6	ChIP 6	ChIP 6	ChIP 6	ChIP 6	ChIP 6	ChIP 6	ChIP 6	ChIP 6
H	Input	Input	Input	Input	Input	Input	Input	Input	Input	Input	Input	Input

Note: Input DNA contains a mixture of Input material associated with all 6 ChIP reactions.

	1	2	3	4	5	6	7	8	9	10	11	12
A	–	Standard Curve PCR Primer Pair						–	–	–	–	–
B	Negative Control Primer Set			Positive Control Primer Set			Gene-specific PCR Primer Set 1			Gene-specific PCR Primer Set 2		
C												
D												
E												
F												
G												
H												

1. Prepare DNA for use in qPCR reactions.

   a. **DNA Standards:** Use 5 µl DNA Standard per qPCR reaction. Also use 5 µl of the provided 2 µM Standard Curve Primer Pair per qPCR reaction.

   b. **ChIP DNA:** For ChIP DNA that was eluted in a 100-200 µl volume, use 5 µl DNA per qPCR reaction. However, if the DNA was eluted in a smaller volume (*e.g.* ChIP-Seq DNA eluted in 36 µl volume) prepare a dilution by adding 6 µl ChIP DNA to 94 µl 2 mM Tris-HCl, pH 8.0. Use 5 µl diluted ChIP DNA per qPCR reaction.

   c. **Input DNA:** Input DNA should be adjusted to 2.5 ng/µl concentration. Use 5 µl per qPCR reaction.

2. Set up qPCR reactions as follows:

Reagent	20 µl PCR reactions
2X SYBR Green qPCR master mix	10 µl
PCR primer pair (2.5 µM each)	5 µl
DNA sample (ChIP or Input)	5 µl
Total volume	20 µl

3. Place the PCR plate in a real time PCR instrument. Using the software for your qPCR machine, assign a value of 3788 to DNA Standard AM1, a value of 378.8 to DNA Standard AM2 and a value of 37.88 to DNA Standard AM3. These numbers represent copy numbers for any unique genomic region of DNA contained in the Standard Curve qPCR reactions. These numbers are used to calculate copy numbers for the genomic regions detected in the test reactions on the qPCR plate.

4. If the gene-specific PCR primer pairs were designed with an optimal annealing temperature of 58°C, follow the amplification conditions listed below. The provided primers have been optimized for use with Bio-Rad SYBR Green qPCR Master Mix (Catalog No. 170-8882). Other master mixes could affect PCR performance and melt curves. If necessary, optimize conditions based on the SYBR Green master mix reagent and PCR instrument used.

   95°C for 2 minutes
   (95°C for 15 seconds, 58°C for 20 seconds, 72°C for 20 seconds) for 40 cycles

5. Include and inspect the melt curve based on the protocols recommended by the qPCR instrument manufacturer to ensure that primer pairs amplify only a single product.

Section B. Using the Analysis Spreadsheet

1. Download Active Motif's ChIP-IT® qPCR Analysis sheet from the Active Motif website at www.activemotif.com/qPCRanalysis. This file is an Excel template that contains formulas to perform all the calculations necessary to normalize sample data according to primer pair efficiency, the amount of chromatin in each reaction and the resuspension volume of the ChIP DNA. The spreadsheet template is designed to accommodate data from multiple 96-well PCR plates in a single analysis.
2. In column A of the template, fill in the primer name on the first line of each box. Then in columns B-I, copy the numbers calculated by the qPCR instrument software for Standard Quantity into the Analysis spreadsheet. In column J, enter the Standard Quantity values for the Input. (See example below)

	A	B	C	D	E	F	G	H	I	J
	Primer Names	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Input
10	Negative	Triplicate 1								Triplicate 1
11		Triplicate 2								Triplicate 2
12		Triplicate 3								Triplicate 3
13	Positive	Triplicate 1								Triplicate 1
14		Triplicate 2								Triplicate 2
15		Triplicate 3								Triplicate 3
16	GAPDH	Triplicate 1								Triplicate 1
17		Triplicate 2								Triplicate 2
18		Triplicate 3								Triplicate 3

3. Enter the amount of chromatin used in the ChIP reaction into the template worksheet in cell D2. This value should be recorded in micrograms (µg).
4. Enter the resuspension volume of the ChIP DNA in cell D4. This value should be recorded in microliters (µl).

   For qPCR elution: This is the final volume amount the ChIP DNA was resuspended in at the end of the ChIP reaction (*e.g.* 200 µl)

   For ChIP-Seq elution: For ChIP DNA that was diluted in Section A, Step 1, the final resuspension volume must be adjusted to account for the dilution factor. Using the volumes listed in Section A, Step 3 enter 600 µl into cell D4.

   (100 µl total dilution volume) / (6 µl ChIP DNA for dilution) = 16.67 dilution factor

   (16.67 dilution factor) x (36 µl elution volume) = 600 µl equivalent resuspension volume

   Note: This step is critical for accurate quantification of the DNA. If ChIP samples were not diluted in Section A, Step 1 do not make any calculation adjustments.

Section C. Data Calculations

The spreadsheet will perform the calculations to normalize the data based on Binding events detected per 1,000 cells as follows. The rationale behind the calculations is explained below.

Standard Curve

a. The qPCR instrument will generate a standard curve based on the known quantities of DNA that were assigned to each DNA standard. The standard curve primer set is assigned a primer efficiency value of 1.

b. Based on the values of the standard curve, the qPCR instrument will assign a Standard Quantity value to each of the ChIP and Input samples. These values are used for the normalization calculations.

Primer Efficiency

a. Primer efficiency ratios for the gene-specific primers and positive and negative control primers are calculated by dividing the average input value by the expected copy number in the input. The average input value is the average of the 3 qPCR values that are generated by the primer set when 12.5 ng of Input DNA are amplified. The expected copy number is calculated assuming 6.6 pg of DNA per cell.

Input DNA: (2.5 ng/µl) * (5 µl per PCR reaction) = 12.5 ng Input DNA amplified

$$\frac{\text{12,500 pg Input DNA}}{\text{6.6 pg DNA per cell}} * \text{2 DNA copies per cell} = \text{3,788 copies}$$

Data Normalization

a. The average of the 3 qPCR values generated by the primer set when each ChIP sample is amplified in triplicate is calculated. This value represents the number of copies in the 5 µl ChIP sample that was amplified.

b. The above value is then multiplied by the resuspension volume and divided by 5 to calculate the number of copies in the entire ChIP reaction.

c. To normalize the values per 1,000 cells, the value is then multiplied by the ratio of 1,000 divided by cell equivalents in the ChIP reaction.

d. Finally, the value is divided by the primer efficiency ratio.

e. The complete formula for normalization is:

$$\frac{\text{average qPCR value} * \left(\frac{\text{resuspension volume}}{5}\right) * \left(\frac{1000}{\text{cell equivalents in ChIP}}\right)}{\text{primer efficiency ratio}}$$

Section D. Data Interpretation

The following recommendations are provided to evaluate the success of the ChIP reactions and the quality of the ChIP DNA. These recommendations are based on the use of the ChIP-IT High Sensitivity Kit (Catalog No. 53040) to perform the chromatin immunoprecipitation reactions. If another method was used to perform the ChIP reactions, the interpretation results listed below may not apply. Use the values obtained for Binding events detected per 1,000 cells to perform the data interpretation. See Figure 2 for an example of good and poor results.

1. Evaluate the signal of the included Negative Control PCR primer set
    a. Values should be less than 2 Binding events detected per 1,000 cells. Values higher than 2 could indicate a poor quality ChIP reaction. The ChIP reaction should be repeated, especially if the ChIP DNA will be used for ChIP-Seq.
    b. High background levels can sometimes be associated with certain chromatin preparations. If high background persists in ChIP, we recommend making new chromatin.
    c. Some antibodies show signal as high as 1,000 Binding events detected per 1,000 cells. Antibodies that give extremely high signal may have negative control values above 2. In these cases, values above 2 are acceptable.
    d. The negative control primer may actually serve as a positive control for repressive histone marks such as H3K27me3. If testing a repressive histone, we recommend using the included GAPDH primer set as the negative control.
2. Signal strength of the positive control and gene-specific primer sets
    a. Antibodies for high abundance targets, such as histone antibodies, will routinely give signal between 100 and 1,000 Binding events detected per 1,000 cells.
    b. Antibodies for low abundance targets, such as transcription factor antibodies, will routinely give signal between 10 and 100 Binding events detected per 1,000 cells.
    c. ChIP reactions that give signals between 5 and 10 Binding events detected per 1,000 cells can still perform well in downstream applications such as ChIP-Seq, however, fold enrichment over the negative control primer set becomes the critical variable. If fold enrichments are greater than 5, the ChIP reactions may still be of high enough quality to perform ChIP-Seq.
    d. ChIP reactions that give signals of less than 5 Binding events detected per 1,000 cells are usually not of high quality. The ChIP should be repeated or a different antibody should be used.
3. Enrichment levels over the negative control primers
    a. The negative control primers serve as a measure of the background of the ChIP reactions and are an important indicator of the quality of the ChIP DNA. The negative control primers are an alternative to using an IgG or non-specific antibody as a control. The advantage is that the negative control primers measure the background in the ChIP reaction containing the actual antibody of interest while the IgG control is a separate reaction that may not have much relevance to what is occurring in the reaction of

interest. Fold enrichment refers to signal from positive control or gene-specific primers divided by signal from negative control primers.

b. A successful ChIP reaction will have a minimum of 5-fold higher signal with positive control primers as compared to negative control primers.

c. When comparing data from multi-sample ChIP experiments it is best not to express the data as fold enrichment since background levels (measured by the negative control primer sets) can fluctuate across multiple ChIP reactions. When data is expressed as fold enrichment, a two-fold increase in background could be interpreted as a 2-fold change in binding when comparing two samples. Instead, use background levels as a measure of the quality of the experiment. When comparing multiple samples, changes in actual binding can be evaluated by directly comparing the Binding events detected per 1,000 cells.

4. Conversion of Binding events detected per 1,000 cells to % input

a. To convert the calculated Binding events detected per 1,000 cells to the more recognizable "% input" scale, simply divide the values by 1,000.

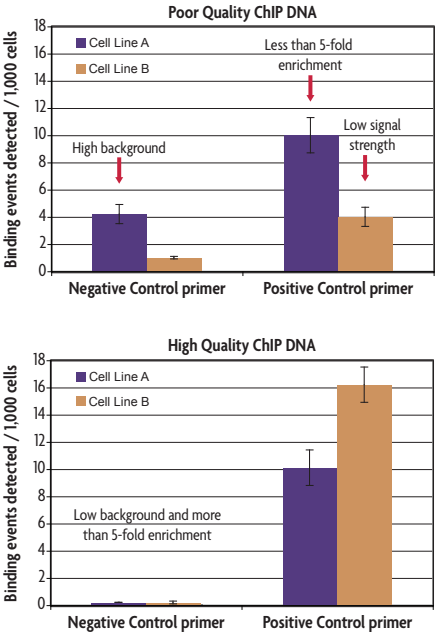

Figure 2: Comparison of qPCR results showing good versus poor enrichment over negative control primers.

Data shows qPCR results analyzed using the ChIP-IT qPCR Analysis Kit with the Human Negative Control Primer as a reference and a gene-specific positive control primer. In the top image the negative control primer set for Cell Line A gives high background levels with Binding events detected per 1,000 cells above a value of 2, while Cell Line B has positive control signal levels below 5 Binding events detected per 1,000 cells. The bottom image shows low background in the negative control primer set with Binding events detected per 1,000 cells below a value of 2. The fold enrichment of the positive control primer set exceeds 5-fold. Only the ChIP DNA from the bottom sample set is recommended for use in ChIP-Seq.

Appendix

Section E. Troubleshooting Guide

Problem/question	Recommendation
Do I need to run a standard curve for every primer pair I include on my qPCR plate?	No, the included standard curve and primer provide a mechanism by which the efficiencies of all other primers can be determined. The key is knowing how much DNA is in the standard curve and running qPCR with other primers using input DNA of a known concentration.
Do I have to perform triplicate reactions?	We recommend triplicate qPCR reactions for ChIP DNA and Input DNA in order to provide more accurate data. However, the ChIP-IT qPCR Analysis Spreadsheet is able to calculate accordingly if only single or duplicate values are entered.
When using the provided Negative Control Primer Set I saw a positive result.	The included Negative Control Primer Sets are intended to be as universal as possible and were designed to serve as negative controls for all activating histone marks and transcription factors. However, these primer sets can give positive results for repressive histone marks such as H3K27me3. If evaluating repressive histone marks, we recommend using the supplied Positive Control Primer Set in place of the negative control primer set to establish a negative result.
Why don't you recommend using an IgG control antibody?	In testing this method across thousands of samples we have found that using primers that target an unbound region is a more stringent control than IgG. The negative control primers serve as an internal control for the ChIP reaction as they are tested using the ChIP DNA generated from the antibody of interest, while IgG is a completely separate reaction using an antibody that may give different non-specific binding than the experimental reaction of interest.
Why am I detecting negative control primer Binding events detected per 1,000 cells values greater than 2?	In most cases this means that there is high non-specific binding in your reaction. Sometimes this can be rectified by repeating the ChIP, but usually it is associated with the chromatin preparation (for reasons that are unknown) and we recommend preparing new chromatin.
	Sometimes antibodies give very high signals and therefore can have correspondingly higher signal from the negative control primers. If you are achieving enrichment levels of the positive control primer set of 5-fold above the negative control primers then the higher background levels are not of critical importance. However, if the downstream application is for ChIP-Seq, then low background levels are critical. For ChIP-Seq you should strive to obtain less than 2 Binding event detected per 1,000 cells for negative control primers.
Can the Binding events detected per 1,000 cells data be converted to percent of input?	Yes, simply divide the output values by 1,000 to achieve percent of input.
Can I use this method with any ChIP reactions?	This method can be used with commercially available ChIP Kits or homebrew methods to offer the advantage of normalization based on the amount of starting chromatin used and resuspension volume for direct comparison across experiments. However, our recommendations for specific signal levels and data interpretation are unique for use in combination with Active Motif's ChIP-IT High Sensitivity Kit (Catalog No. 53040). If using a different method to perform ChIP, you will most likely not achieve the low background levels of ChIP-IT High Sensitivity and therefore our recommendations on data interpretation and cut-off signals will not apply.
Can I publish my data using this method?	Yes, data presented and normalized according to Active Motif's ChIP-IT qPCR Analysis Kit protocol has been published many times. To access references, please contact Active Motif.

Technical Services

If you need assistance at any time, please call or send an e-mail to Active Motif Technical Service at one of the locations listed below.

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