Protocol

# ChIP–Quantitative Polymerase Chain Reaction (ChIP-qPCR)

Tae Hoon Kim and Job Dekker

It is critical to determine if the ChIP actually enriched the DNA sequences that are associated with the target protein. If there are known genomic binding sites, primers can be designed for quantitative PCR (qPCR) to determine if the known sites are specifically enriched by immunoprecipitation. If there are no known sites but candidate target genes are available, one can consider designing qPCR primers along the length of potential regulatory regions such as promoters and conserved noncoding sequences within intergenic and genic regions. If candidate target genes or potential sites are not available, ChIP-chip or ChIP-seq should be considered instead. Because real-time PCR can be performed in either a 96- or 384-well format in a minimal reaction volume and primers can be synthesized with minimal cost, ChIP-qPCR is an attractive strategy to interrogate target genes and potential regulatory regions across a large number of experimental conditions and different cell types.



## MATERIALS

**It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.**

### Reagents

PCR master mix reagent with SYBR Green dye

*Other detection chemistries can be used instead.*

PCR primers

Template DNA (ChIP DNA, total input DNA, or negative-control DNA)

*See Protocol:* ***ChIP*** *(Kim and Dekker 2018a).*

Equipment

Fluorometer

Real-time PCR machine

## METHOD

1. Assemble the real-time PCR using commercially available master mix (usually provided as 2× concentrate containing all the nucleotides and enzymes), primers (at 0.15 μM concentration of each primer), and 500 pg of template DNA.

   *Primer concentrations need to be optimized to achieve efficient amplification of the target DNA. The template will be either the ChIP DNA, total input DNA, or negative-control DNA obtained from the beads alone. 500 pg of DNA corresponds to about 150 copies of the genome.*

From the *Molecular Cloning* collection, edited by Michael R. Green and Joseph Sambrook.


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2. Perform real-time PCR using conditions appropriate for the melting temperature of the primers and the expected length of the PCR product. Follow the PCR parameters that are appropriate for the machine and the reagents:

| | |
|---|---|
| Step 1 | 10 min at 95°C |
| Step 2 | 15 sec at 95°C |
| Step 3 | 45 sec at 60°C |
| Step 4 | Go to Step 2, 39 times |
| Step 5 (optional) | Perform melt curve |

3. Calculate the enrichment by comparing the threshold cycle number ($C_T$) for the input and ChIP DNA templates, as measured by the real-time PCR machine. Use appropriate methods for determining the $C_T$ values (see Chapter 9 in Green and Sambrook 2012).

*The $\Delta C_T$ value represents the relative $\log_2$ enrichment by ChIP, assuming near 100% amplification efficiency.*

## RELATED INFORMATION

If candidate target genes or potential sites are not available, ChIP-chip or ChIP-seq should be considered. See Protocol: **ChIP-chip** (Kim and Dekker 2018b) and Protocol: **ChIP-seq** (Kim and Dekker 2018c).

## REFERENCES

Green MR, Sambrook J. 2012. *Molecular Cloning: A Laboratory Manual*, 4th ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Kim TH, Dekker J. 2018a. ChIP. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot082610.

Kim TH, Dekker J. 2018b. ChIP-chip. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot082636.

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