ChIP-qPCR and qbase^{PLUS} jointly identify a MYCN activated miRNA cluster in cancer

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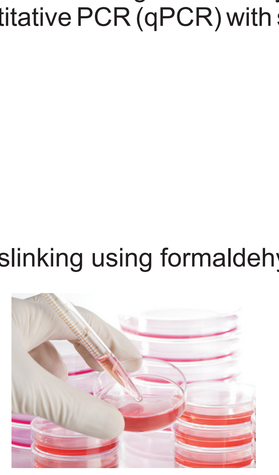
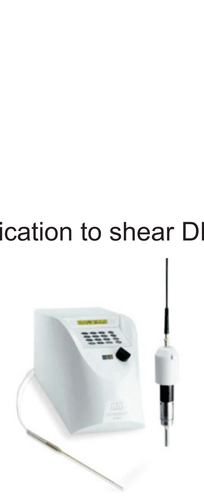
ChIP-qPCR

ChIP-qPCR starts with the treatment of intact nuclei with formaldehyde for the induction of protein-DNA crosslinks, trapping chromatin segments interacting with specific proteins (e.g. transcription factors). The crosslinked DNA undergoes sonication to obtain small DNA fragments. A fraction of this sheared DNA is put aside ('the input sample') and will later be used for rescaling. The remaining fraction is used for immuno precipitation using an antibody targeting the protein of interest. After reversing the crosslinks, a collection of DNA fragments is obtained. The presence and abundance of specific DNA fragments is determined using quantitative PCR (qPCR) with specific primers.



Crosslinking using formaldehyde → Sonication to shear DNA



Sonication to shear DNA → Input sample / Sample for ChIP

Input sample

Sample for ChIP → Chromatin Immuno Precipitation

Chromatin Immuno Precipitation → qPCR

qPCR → Data-analysis

qbase PLUS

ChIP-qPCR data-analysis using qbase^{PLUS}

Chromatin immunoprecipitation quantitative PCR (ChIP-qPCR) is very well suited to assess and quantify direct binding of specific regulatory proteins to genomic DNA sequences. Unfortunately, data-normalization and accurate quantification appear to be a major challenge for many users.

ChIP-qPCR data need to be normalized for sources of variability, including amount of chromatin, efficiency of immunoprecipitation, and DNA recovery. Here, we present the so-called 'fold enrichment method' in which ChIP-qPCR data are analysed relative to the input. The qPCR signals are normalized based on the average abundance of multiple non-specific genomic regions in the ChIP samples using the qbase^{PLUS} multiple reference gene normalization technology (Hellemans et al., 2007).

Perform qPCR reactions
* Sample maximization approach recommended (Hellemans et al., 2007)

Perform quality control
* Inspect melting curves
* Inspect amplification curves

Determine Cq values with qPCR instrument software

Import qPCR instrument export file in qbase^{PLUS}
* qbase^{PLUS} supports most export files

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Define calculation parameters
* Multiple reference gene normalization
  - Use assays for non-specific genomic regions
* Scale to 'input' sample
  - Not precipitated sample

Define quality control criteria
* Threshold for negative control
* Threshold for replicate variability
* Threshold for reference target stability measures
  - geNorm M and CV values

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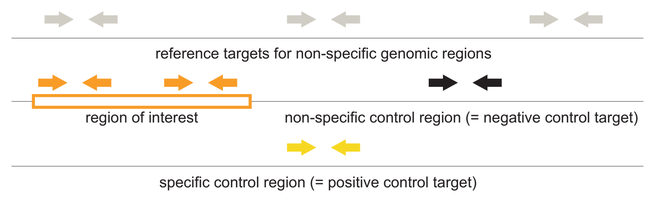
Appoint reference targets
* Use assays for non-specific genomic regions
  - For normalization
* Use >1 reference target for more accurate results



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Perform quality control using qbase^{PLUS} (D'haene and Hellemans, 2010)
* Evaluation of standard curves
* Determination of reference gene expression stability
* Control on PCR replicate variation
* Assessment of positive and negative controls
* Evaluation of deviating sample normalization factors
* Quality control on inter-run calibration

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Interpret fold enrichment for specific region in the precipitated samples
* Relative to 'input' sample
  - Not precipitated sample
* Compare to fold enrichment in non-specific region

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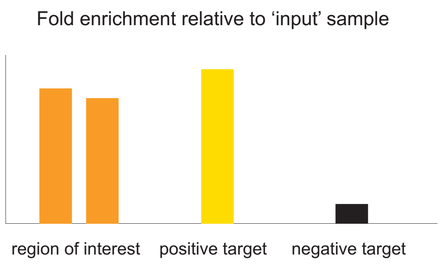


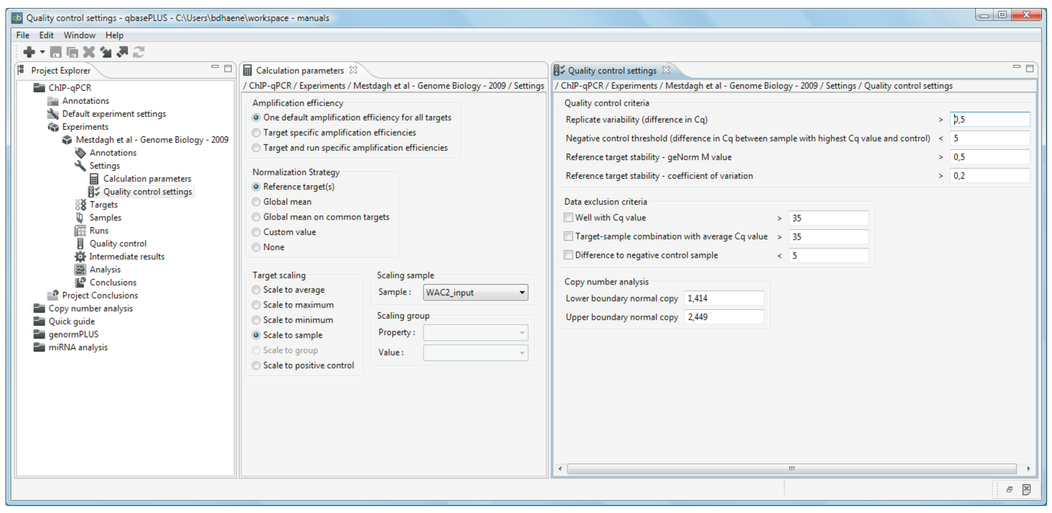
Example application: ChIP-qPCR to assess binding of transcription factor MYCN to miRNA cluster 17-92

miRNAs belonging to the oncogenic mir-17-92 cluster (miR-17, miR-18a, miR-19a, miR-20a, miR-19b and miR-92) are known to be upregulated in neuroblastoma tumors with MYCN amplification in comparison to samples with a normal MYCN copy number.

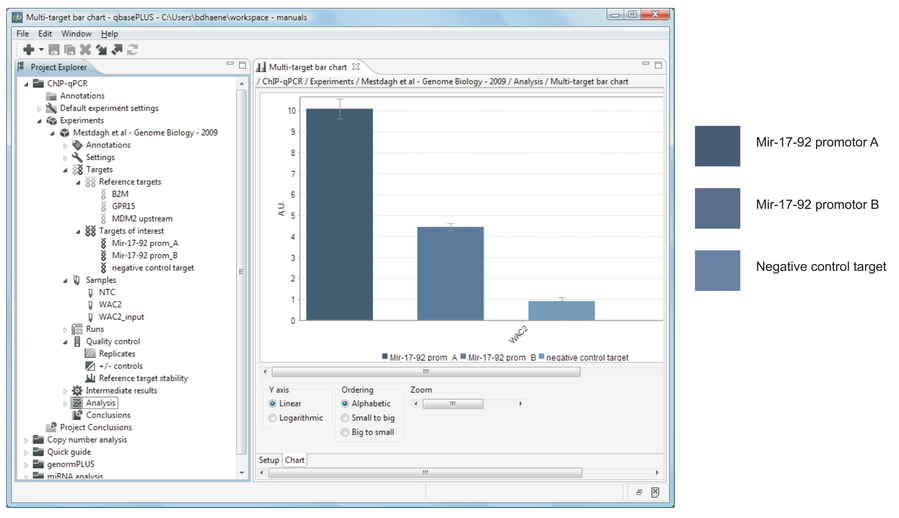
We applied ChIP-qPCR to assess binding of transcription factor MYCN to miRNA cluster 17-92, to positive control target MDM2, and to a negative control target region. ChIP-qPCR was performed in two MYCN overexpressing neuroblastoma cell lines (IMR5 and WAC2) using SYBR Green I detection chemistry in a 384-well plate and signals were normalized based on the average abundance of three non-specific genomic regions in the ChIP samples using the qbase^{PLUS} multiple reference gene normalization technology. Fold enrichment was calculated relative to the input sample (non-precipitated) and compared to that of a fourth non-specific region (negative control target).

Using this approach we were able to demonstrate strong MYCN binding to the positive control and to the miR-17-92 cluster. In keeping with this, the expression level of the miR-17-92 cluster is substantially increased in primary neuroblastoma tumor samples in which the MYCN gene is amplified and overexpressed.

Calculation parameters and quality control criteria



Fold enrichment in WAC2 relative to 'input' sample



Mir-17-92 promotor A

Mir-17-92 promotor B

Negative control target

* Hellemans et al., Genome Biology, 2007
* Westermann et al., Genome Biology, 2008
* Mestdagh et al., Genome Biology, 2009
* D'haene and Hellemans, International Drug Discovery, 2010

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