Advanced copy number variant analysis with qbase^{PLUS} 2

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accelerating your analysis

qPCR based copy number analysis

Copy number changes under the form of deletions and duplications are known to be involved in numerous human genetic disorders. Moreover, each individual's genome embodies several copy number polymorphisms of various sizes which are thought to contribute to normal phenotypic variation and susceptibility to multifactorial disease.

A wide spectrum of laboratory methods has been developed to identify these copy number changes. qPCR has many advantages over alternative methods, such as its low consumable and instrumentation costs, fast turnaround and assay development time, high sensitivity and open format (independent of a single supplier). To date, qPCR is the golden standard for gene expression analysis. For copy number determination, qPCR has been less frequently used, but recent developments hold the promise of taking this application to the next level.

Copy number analysis module in qbase^{PLUS} 2

* More than one reference sample can be used for accurate copy number calling
* Calibrator samples with varying copy numbers can be used to provide flexibility
* User defined thresholds for the lower/upper boundary for normal copy
* Thorough quality control to ensure trustworthy results
* Copy numbers visualized on a per sample basis
* Conditional bar coloring for easy detection of copy number alterations

qbase PLUS

Workflow for accurate and reliable copy number calling

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

Perform quality control
* Inspect melting curves
* Inspect amplification curves

Calculate Cq values with qPCR instrument software

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

Define calculation parameters
* Multiple reference gene normalization
* Scale to positive control (= calibrator samples)

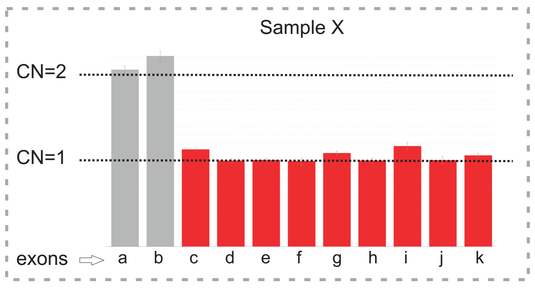
Define quality control criteria
* Threshold for negative control
* Threshold for replicate variability
* Threshold for reference target stability measures
  - geNorm M and CV values
* Threshold for upper/lower boundary for normal copy number

Appoint calibrator samples
* Samples with known copy number
  - For copy number calling
* Use >1 calibrator for more accurate results

Appoint reference genes
* Genes/targets with normal copy number (CN=2) in all samples
  - For normalization
* Use >1 reference gene for more reliable results

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

Interpret copy number results
* Inspect copy number calling in calibrator samples
  - Positive controls
* Inspect copy number calling in samples of interest



Example application: copy number analysis of the *SHOX* region

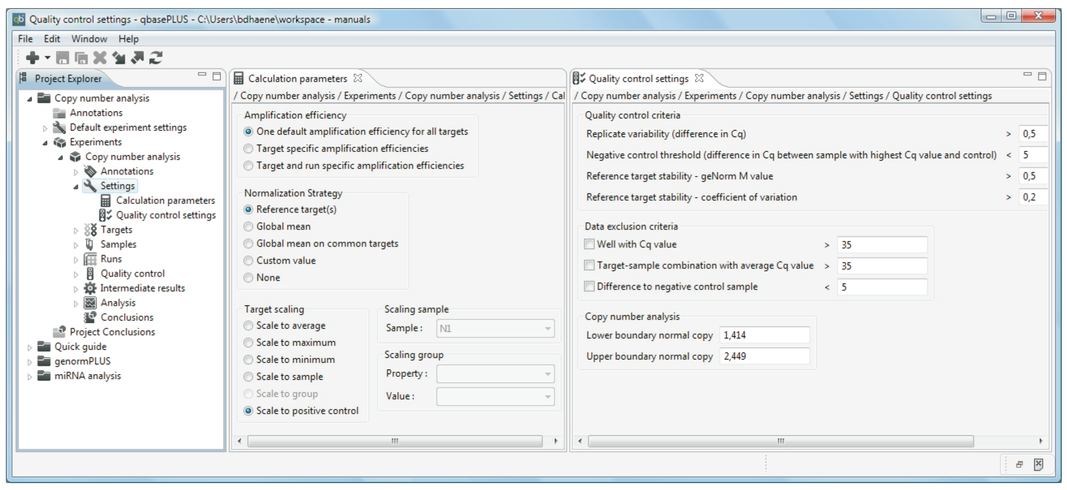
Short stature has an incidence of 3 in 100 in children. Reliable molecular genetic testing may be crucial in the context of beneficial disease management. Deletions spanning or surrounding the *SHOX* gene account for a significant proportion of patients with idiopathic short stature (ISS) and allied disorders, such as Leri-Weill dyschondrosteosis (LWD). To allow fast and reliable molecular testing, D'haene et al. (JCEM, 2010) introduced a quantitative PCR (qPCR) based copy number profiling test, consisting of 11 amplicons targeting clinically relevant regions (i.e. the *SHOX* gene and regulatory regions).

Here, we present the data-analysis of a small *SHOX* copy number experiment for 8 patients with ISS or LWD.

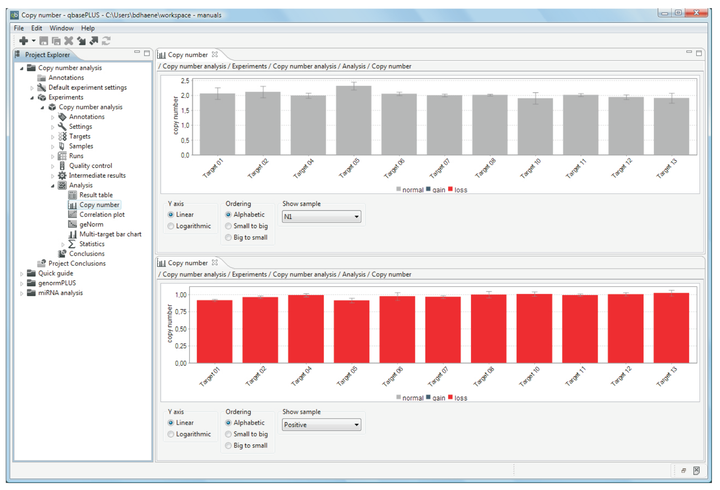
qPCR was carried out in a white 384 well plate (Roche) using the qPCR Core kit for SYBR Green I (Eurogentec) on the LightCycler 480 (Roche). The run consisted of 8 patient samples with unknown copy number (S1-S8), 3 calibrator samples with known copy number (N1, N2 and Positive) and 1 no template control (NTC). The 3 calibrator samples included 2 positive controls with normal copy number (CN=2) and 1 positive control with a deletion (CN=1). Eleven assays targeting the *SHOX* region and 2 reference assays for normalization were tested.

Data-analysis was done using qbase^{PLUS} 2. Post-PCR quality control was performed to ensure trustworthy results. Accurate copy number calling and objective interpretation was conducted with the advanced module for copy number analysis.

Calculation parameters and quality control criteria



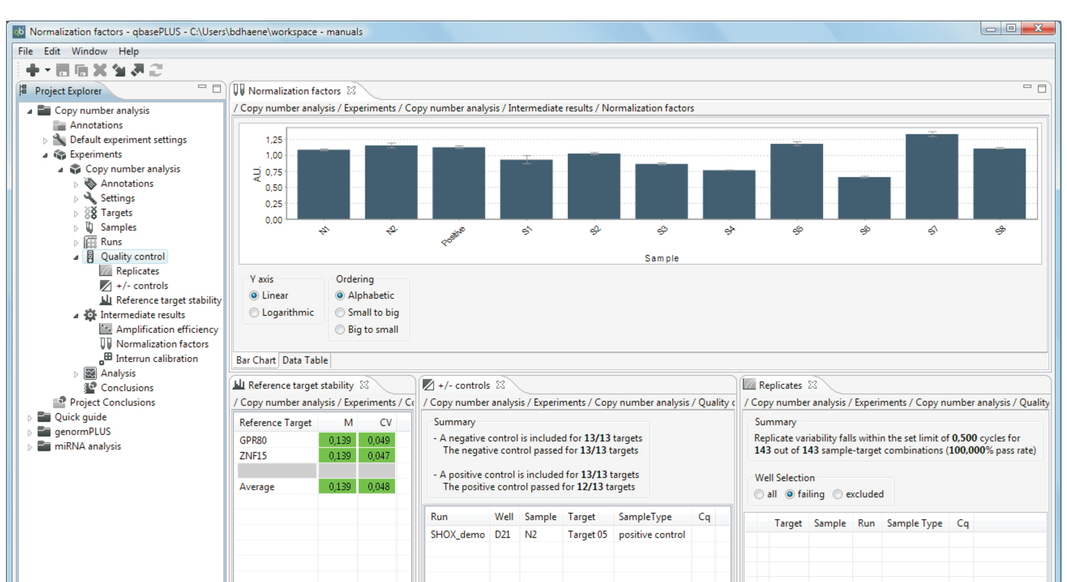
Evaluation of the calibrator samples



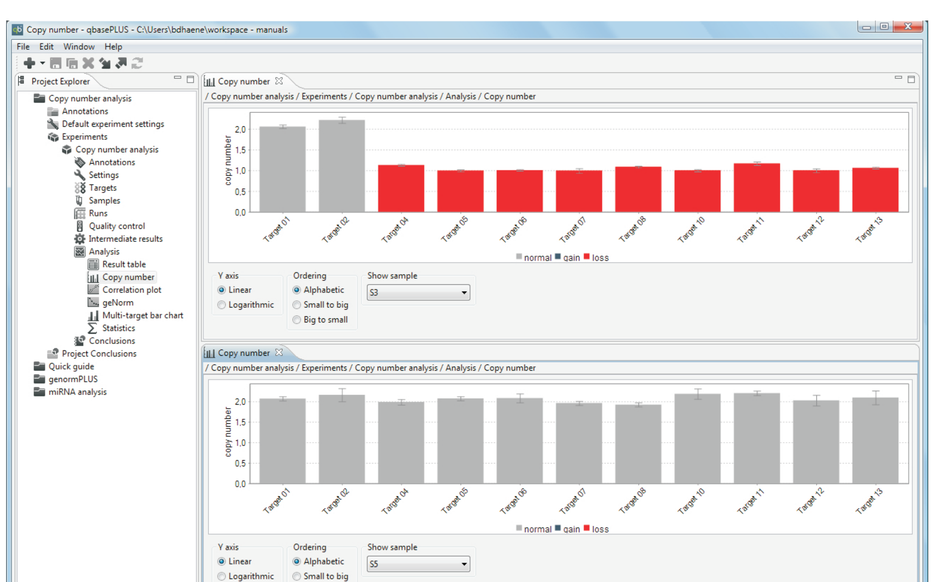
Calibrator sample' N1' with normal copy number (CN=2)

Calibrator sample 'Postive' with deletion (CN=1)

PCR quality control



Copy number calling in patient samples



* Hellemans et al., Genome Biology, 2007
* D'haene et al., Journal of Clinical Endocrinology and Metabolism, 2010
* D'haene et al., Methods, 2010
* D'haene and Hellemans, International Drug Discovery, 2010

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