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CytoChip™ Quality Control Summary

Key quality control tests performed on CytoChip clone libraries and shipped arrays.

Introduction

CytoChips are engineered and undergo quality control (QC) to the highest standards in order to satisfy the requirements of routine clinical use. Illumina has developed an extensive QC process to check both lot-independent effects (clone quality and mapping) and lot-specific effects (print-related). This technical note summarizes the QC process, and explains the QC reports provided by Illumina for each lot.

Clone Quality and Mapping

Illumina uses a subset of the well-validated Roswell Park Cancer Institute bacterial artificial chromosome (BAC) library. Illumina strategy to ensure high quality and accurate mapping of clones is ongoing and multi-fold:

- The Illumina clone set has already been used in the production of thousands of arrays, and has been carefully optimized on the basis of these data. Clone performance is routinely checked as part of the QC process for each lot.
- All clones known to be associated with genetic disease have been end-sequenced by Illumina and checked against Build 36 of the human genome in NCBI.
- The majority of non-disease backbone clones have been endsequenced, either by Illumina or other institutions.

Print-Related Parameters

Each lot of CytoChip arrays is rigorously tested to ensure that they meet the high standards essential for routine clinical use. Testing is designed to assess:

- Print quality, including spot morphology, consistency of gridding, and other effects related to the printing process
- Performance of arrays under hybridization, including individual clone performance, signal intensity, and overall consistency of ratios

Assessment of these quantities is performed on the basis of four commercial normal-normal sex-mismatched hybridizations, all carried out using standard CytoChip protocols and analyzed using BlueFuse® software for microarrays. A series of tests is then performed using internally developed, automated QC scripts. Finally, all QC arrays are checked by a trained operator.

If overall performance of any of the QC arrays in the lot does not meet strict criteria, whether measured by print quality or performance under hybridization, the lot is failed for external deployment. In some instances, despite excellent overall performance, a small percentage of spots may fail to print to a sufficiently high standard. Any such spots are removed from the lot-specific GAL file (e.g., lot20_ cytochip.gal) ensuring that customer analysis is not compromised. Where all spots corresponding to a clone have failed, the clone itself is failed and reported in the QC report.

The QC tests are comprehensive and detailed. The key stages, however, are:

- Assessment of overall print quality
- Detection of failed spots
- Detection of failed clones
- Assessment of hybridization performance

Assessment of Overall Print Quality

Overall print quality is assessed on the basis of all QC slides. QC scripts highlight any anomalies in spot radius, grid consistency, and spot morphology. Manual inspection of the TIFF images both confirms the finding of QC scripts, and allows more detailed assessment by an expert operator. If significant printing issues are observed, then the batch is failed.

Detection of Failed Spots

Failed spots are detected using all QC slides, which are spaced evenly over the entire lot to ensure complete representation of any effects in the lot. Signal intensities and quality metrics are used to determine whether each spot is of sufficient amplitude for robust analysis. Any spots with a weak signal are considered to have failed to print consistently across the entire lot and are failed.

A unique layout file (GAL file) is created for each lot, which results from all the failed spots being removed from the generic layout file. This process ensures that only validated probes are used in customer analyses and that results are not compromised by any printing artefacts.

Detection of Failed Clones

Following the detection of failed spots, any clone not represented at least two times on the CytoChip (including both hybridization areas) is failed. If the total number of failed clones exceeds 2%, then the batch is failed. Any failed clones are reported in the QC report.

Assessment of Hybridization Performance

The four male-female QC slides are used to assess the quality of the array as a complete test under hybridization conditions. Commercial DNA is used for these experiments, in order to minimize any variability associated with DNA quality and labeling that can occur using DNA extracted from blood.

The results from the hybridizations allow the assessment of three key quantities:

- The standard deviation of the autosome. This is a simple but powerful metric to assess hybridization quality.
- The median log₂ ratios for the X and Y chromosomes. This provides information on dynamic range of the experiment, and is a robust measure of clone and hybridization quality.

 In addition, any clones that depart significantly from their ideal log₂ ratios across the hybridizations are detected and removed from the lot-specific GAL file. These clones are subsequently checked individually before any use on future lots.

The results of the tests summarized above are provided to users in the form of a QC report, which can be downloaded from www. cambridgebluegnome.com. Figure 1 illustrates an artificial example report showing a disease clone failure. Figure 1: QC Report for a Failed Clone



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