

OpenArray® PCR Platform

PCR technology, including sample preparation, has improved dramatically beyond the introduction of gel-based PCR in the 1980s. At Charles River, it is our goal to identify and incorporate the most analytically sensitive and specific technologies available in order to provide the most accurate and reproducible results. Building on our 20 years of PCR experience, we provide an approach unparalleled within the industry. Presented in this document is our test and process development summary, which defines our services and our commitment to excellence.

Overview

The OpenArray® provides an efficient and cost-effective PCR platform to test a sample for a large panel of agents. On a chip the size of a standard microscope slide, the OpenArray® contains individual PCR assays in a high-density format so that there is no need to combine assays; therefore, the PCR amplification will be optimal. This platform is in contrast to the multiplex PCR used by some laboratories. Assay multiplexing can confound sensitivity due to competitive inhibition among the multiple assays, which can lead to false-negative results.

The OpenArray® platform gives us the opportunity to run each infectious agent assay and the controls in triplicate. The OpenArray® PCR platform, combined with TaqMan® technology, allows us to provide the most accurate results possible, all while streamlining our internal processes to minimize results turnaround time. At this time, we have integrated the use of a PCR OpenArray® platform for the rodent virus panels we use to screen research biologics as well as the Prevalent Rodent Infectious Agent (PRIA) Panel.

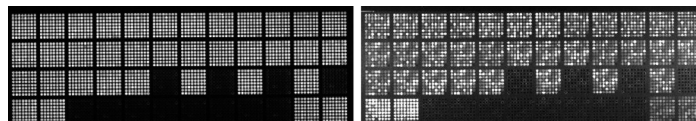
Proven Well-to-Well Sensitivity

The OpenArray® maintains the same well-to-well sensitivity as the 96-well format. Sensitivity was determined by performing a process development study in which 20 different assays were tested in triplicate and analyzed on both the OpenArray® and 96-well platforms using ten-fold dilutions of plasmid templates with and without background nucleic acid; previous studies at Charles River have shown that adding background nucleic acid will improve plasmid detection at the lower copy levels. We demonstrated that the OpenArray® provides comparable sensitivity to the 96-well platform and that the concentration step, in which sample nucleic acid is concentrated at the last step prior to addition to account for the smaller OpenArray® PCR chamber volume, may slightly improve sensitivity over the 96-well format.

Visual Confirmation of Sample Loading

The OpenArray® allows for visual confirmation of sample loading. The OpenArray® platform is loaded with one homogenous mixture that contains both sample nucleic acid and PCR master mix, which is distributed across a single sub-array with simple automation. Unlike a 96-well, a visual pre-check can easily be performed to assure that the sample was loaded into each PCR chamber. The same is done at the end of the process via fluorescent image of the chip (Figure 1).

Figure 1: Post-Amplification OpenArray® CCD Scans



Each sample/master mix homogeneous mixture is loaded in an 8 x 8 sub-array. **At left:** Post-PCR fluorescence image for sample loading assurance. **Note:** Samples are loaded in all reaction holes for each loaded sub-array. **At right:** Post-PCR fluorescence image for control and agent assay interpretation. **Note:** All reaction holes except for bottom right of each sub-array contain an assay as indicated by lack of any fluorescence signal (no fluorescence probe present).

Sensitivity through TaqMan® Technology

TaqMan® technology is ten to one hundred times more sensitive than traditional gel-based qualitative PCR, and the use of an internal probe provides incomparable specificity. This technology allows samples to be analyzed without opening reaction tubes, which prevents the release of potentially contaminating PCR products, a common downfall associated with gel-based PCR assays.

Sample- and System-Suitability Controls Verify Results Accuracy

Unique to Charles River's infectious agent PCR testing, we have taken extra measures to prevent false-negative results by incorporating Spike and Nucleic Acid Recovery Controls, which help to determine sample suitability; positive and negative template controls are also included to help verify system suitability. A negative result for the Spike Control, in which a known amount of exogenous template is added to the sample and tested, indicates the presence of PCR inhibitors, requiring dilution or re-extraction of the sample prior to retesting. The Nucleic Acid Recovery Control (NARC) is included to monitor the nucleic acid extraction procedure and reverse-transcription.

Contact Us

For further details about the OpenArray® PCR platform, TaqMan® technology or our available assays, our professional and technical staff are available to consult. Please visit www.criver.com/info/dx or contact us at 1.800.338.9680 or comments@crl.com.

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