



## Real-time PCR

**Why/When:** The Biological Sciences Bureau (BSB) uses Polymerase Chain Reaction (PCR) technologies to target nucleic acids (RNA or DNA) in order to detect pathogens (viruses, bacteria & fungi) in clinical samples or to identify the species of culture isolates.

PCR was invented in the early 1980s and has revolutionized biology, forensics, and clinical diagnostics. PCR allows the amplification of a few molecules of DNA/RNA, which could never before be detected, into billions of copies that *can* be detected. The discovery of heat-resistant enzymes that could be used in PCR propelled the technology which now is used in every facet of biology. PCR can be more sensitive than culture and more specific than other types of assays.

PCR harnesses the same enzymes and mechanisms that cells use to copy their own genome, to amplify targeted sequences of DNA within a pathogen of interest. Targets are carefully chosen when designing a PCR assay to ensure specificity and sensitivity.

The amplification process doubles the number of copies of the target with every PCR cycle. A typical PCR assay has between 35 and 45 cycles, thus billions of copies are generated by the end of the assay. Visualizing the amplified material (amplicons) is performed in a variety of ways. Real-time PCR uses fluorophores attached to molecules that adhere to the amplicons and are detected by the instruments we use.

PCR has been implemented in most diagnostic laboratories and culture has been abandoned by many to save money. [Culture Independent Diagnostic](#) (CID) testing has had serious impacts on public health, both positive and negative. One positive is that PCR allows a rapid turnaround time, often producing results in hours rather than days or weeks. On the downside, CID testing eliminates having the isolates on-hand that may be needed to further characterize a pathogen, providing critical information during outbreak surveillance, to test for antibiotic resistance, or to understand why a vaccine is no longer protective. For these reasons BSB continues to culture many of the organisms that are tested by molecular methods. See the other sections of BSB for bacterial and viral culture information.