Craig Pierson et al., J Cancer Sci Ther 2016, 8:11(Suppl)
http://dx.doi.org/10.4172/1948-5956.C1.090

Comprehensive detection of expressed mutations by anchored multiplex PCR and next-generation sequencing

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The landscape of driver mutations in cancer encompasses many types of mutations occurring across many genes. These include copy number variants (CNVs), single nucleotide variants (SNVs), insertions and deletions (indels), exon skipping and gene fusions. Traditional molecular testing to detect each of these mutation types in clinical samples cannot be easily coupled to detect all possible mutation types in a single sample. Next-generation sequencing (NGS) on whole transcriptome (RNA-Seq) enables comprehensive profiling of expressed mutations. However, low sensitivity and high cost renders RNA-Seq impractical for routine clinical use. Target-enrichment strategies for NGS, such as Anchored Multiplex PCR (AMP™), increase read depth and enhance sensitivity. AMP uses unidirectional gene-specific primers and molecular barcoded adapters ligated to DNA ends for amplification. This enables amplification of both known and unknown mutations and increases coverage of target regions. We developed AMP-based Archer® FusionPlex® assays to detect the presence and expression of known and novel SNVs, indels, exon skipping and fusions from RNA transcripts. Here, we used FusionPlex assays to detect each mutation type in RNA extracted from clinical sample types, including: EGFR L858R SNV in a non-small cell lung cancer (NSCLC) FFPE sample; FLT3 internal tandem duplications (ITDs) in acute myeloid leukemia (AML) blood samples; MET exon 14 skipping in NSCLC FFPE samples and; EML4-ALK fusion in an NSCLC FFPE sample by breakpoint identification and expression imbalance. Together, these results demonstrate that FusionPlex assays enable comprehensive NGS-based detection of expressed mutations in RNA extracted from clinical sample types.

Biography

Craig Pierson earned his BSc in Zoology from the University of Canterbury, New Zealand. His work started with Ovine Linkage Mapping projects while at AgResearch in Dunedin and continued into genome sequencing while at Baylor College of Medicine in Houston. He has trained and supported customers in Sanger sequencing, qPCR, NGS, and gene expression applications while at Applied Biosystems, Illumina, HTG, VelaDx and ArcherDx. He joined the ArcherDX team as a Field Application Specialist in July, 2015.

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