The Pillar Post

Stem-Loop Inhibition Mediated Amplification (SLIMamp™): A multiplexed, single-vial overlapping PCR technology

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The underlying technology for Pillar Biosciences

The clinical oncology laboratory faces a challenge in implementing new technology, and with the advent of next-generation sequencing, that level of complexity has expanded on both the wet-laboratory side as well as the data-processing side.

Several problems to solve: complexity, robustness and cost

There are many methods of next-generation sequencing target enrichment available on the market today, based upon hybridization or multiplex PCR technologies refined over the past eight years. The reason Pillar Biosciences developed a new method of multiplex PCR is threefold: to simplify the complexity of target enrichment, to increase the robustness of the process, and to make clinical NGS available more broadly by making it more affordable.

By reducing the complexity of the process to a single-well assay (available multiplexing schemes today have to physically separate overlapping amplicons, thus requiring the splitting of the sample into two or more wells), the chance of sample switching is eliminated, along with cutting the amount of handling in at least one-half.

In addition, without any lengthy hybridization step (which typically takes 10 or 16 hours and performed overnight) and without any ligation step (which also is time consuming, often involves expensive reagents to increase the reaction efficiency), the overall process is fast and streamlined. Utilizing two standard magnetic-bead cleanup steps, the workflow is easy to automate, although one scientist has remarked "why would you need to automate this, when what you have done with this assay makes it so easy to do."

Another problem to solve is one of assay robustness, or in other words, resistance to failure. A dreaded "DNS" (DNA Not Sufficient) indication is avoided with the SLIMamp technology only requiring as little as 10 ng of input DNA (or even lower). Other assays have a narrow range of DNA input requirements – too little DNA is as bad for their assays as too much. Pillar Biosciences' SLIMamp can use as little as 5 ng of input DNA, or as much as 100 ng.

Robustness also speaks to the overall completeness of the assay coverage. It is not unusual for very G-C rich (and alternatively G-C poor) regions to have inadequate coverage, so that those amplicons' regions are covered poorly. With >99% on-target and coverage uniformity close to 100% at only 20% of the mean, there can be sensitivity and specificity measurements of nearly 100%.

Lastly, regarding affordability, the Pillar Biosciences available assays are attractively priced, making a simplified and robust technology accessible for all laboratories.

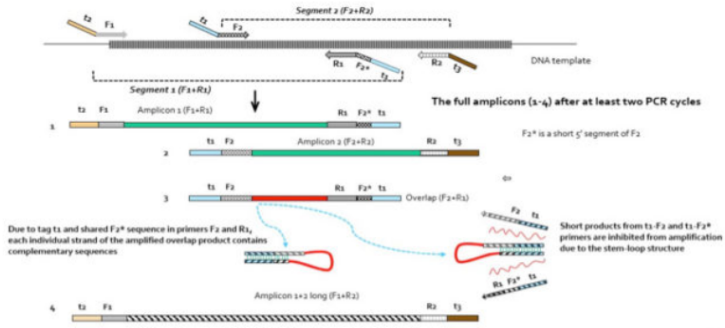
How the SLIMamp technology works

In traditional PCR, you need a single opposing primer pair to amplify a single target region. In a multiplex reaction, if you want to enrich overlapping target regions you need to separate them into different physical vials, because with a total of four primer pairs in a single region, you can come up with a total of four potential amplicons.

all the other products

By using a novel stem-loop complementary between the two unmatched primers for the smallest PCR amplicon, the Pillar Biosciences technology enables overlapping multiplex PCR amplification. In Figure 2, the DNA template (top) has two sets of overlapping primers designed to the region of interest, labeled F1/R1 and F2/R2. Contiguous to the gene-specific primer sequences are tag sequences labeled t1, t2 and t3. Due to tag t1 and partial F2 sequence (labeled F2*) in primers t1-F2 and t1-F2*-R1, each individual strand of the amplified overlap product contains complementary sequences, thus forming a stem-loop. Short products from t1-F2 and t1-F2*-R1 primers are inhibited from amplification due to the stem-loop structure.

Figure 2



The illustration shows how the excess primers t1-F2 and t1-F2*-R1 will be suppressed ('inhibited') from amplifying the short product in excess. These primers are available to produce the desired t1-F2 / t3-R2 product and the desired t1-F2*-R1 / t2-F1 product. In addition, this process can do multiple overlapping products, so that you can basically tile amplicons across any genomic region of interest desired.

How the Pillar Biosciences SLIMamp Technology scales

At present, the highest level of multiplexing is the ONCO/Reveal Solid Tumor Cancer Panel with 251 amplicons; the Lung & Colon Cancer Panel has 103 amplicons and the germline BRCA1 & BRCA2 panel has 91. As the goal for panel development is the clinical actionability of particular genes and mutations, the need for large-scale panels is obviated.

Additional mutational types, not only Single Nucleotide Variants (SNVs) and insertion-deletion mutations (indels), but also copy number variants (CNV) and structural variants such as fusion transcripts, are currently under development. Recently, a group from Dartmouth-Hitchcock Cancer Center (Lebanon NH) presented some CNV data based upon Pillar Biosciences SLIMamp technology at the Association for Molecular Pathology conference in Salt Lake City UT in November 2017. This poster is available for download here.

If you are interested in accessing a paper that shows more detail about this assay's development and approach to enrich for the BRCA1 & BRCA2 genes, a PLoS One article can be accessed here called "Amplification of overlapping DNA amplicons in a single-tube multiplex PCR for targeted next-generation sequencing of BRCA1 and BRCA2".

Another method of looking at scalabilty is sample type, and an assay and product around cell-free DNA is also under development. This would require unique molecular identifiers (UMIs) and higher sample input (20 ng of cell-free DNA is recommended).

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