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QIAseq and Single Primer Extension Chemistry

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QIAseq and Single Primer Extension Chemistry: Redefining Amplicon-Based NGS

Targeted NGS has been widely used for the detection and confirmation of genetic changes, yet there are technical challenges that undermine precise quantification and

confound analyses. Multiplex PCR and nested PCR-based library preparation approaches present primer design and specificity challenges inherent to these methods.

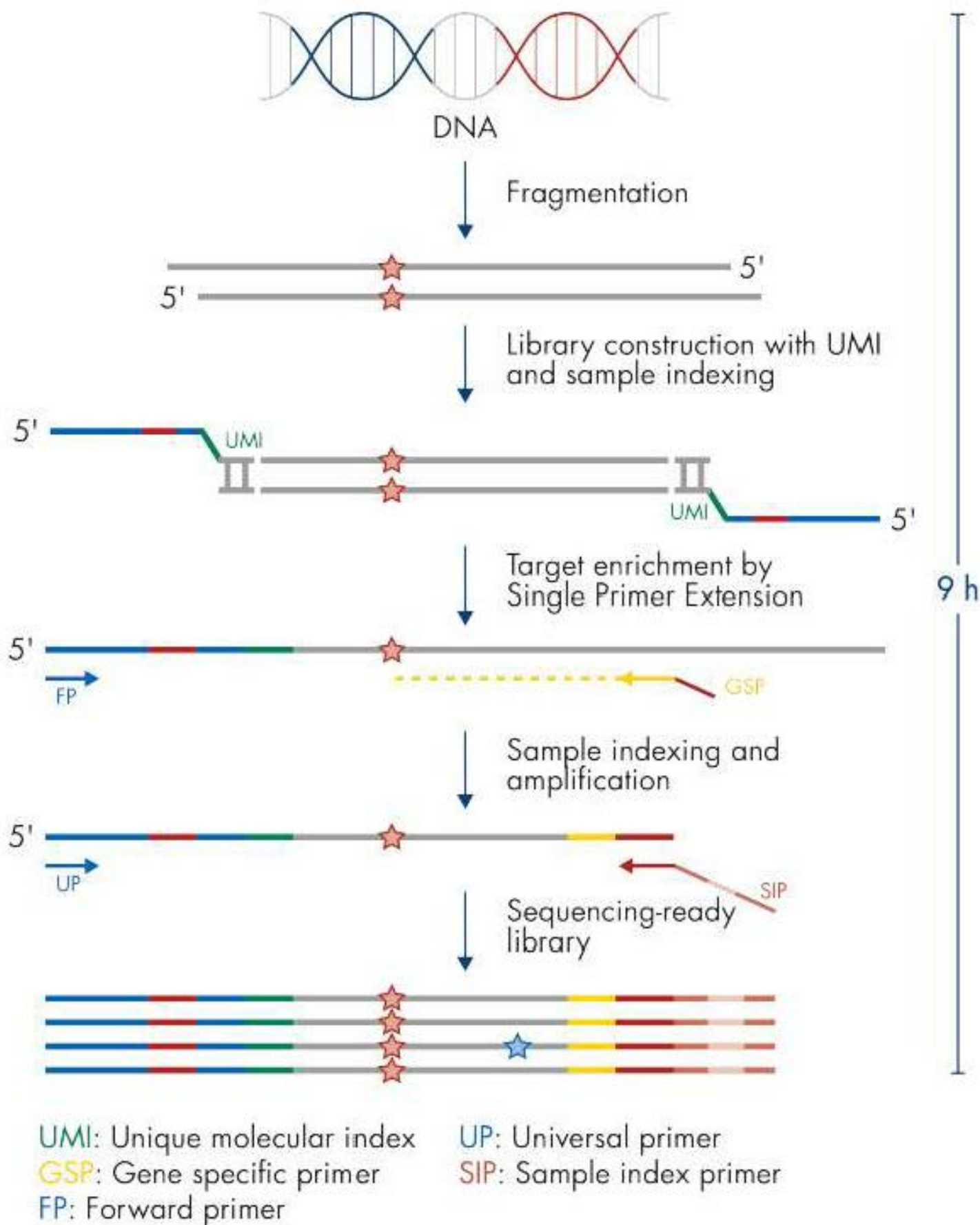
Amplicon-based next-generation sequencing (NGS) assays offer many advantages for targeted enrichment. However, typical approaches have suffered due to restraints in multiplexing capacity and introduction of additional bias. As primer multiplexing and PCR cycle numbers are increased, so does the potential for bias and error.

To overcome these challenges, QIAseq NGS panels employ unique molecular indices (UMI's) to correct for PCR amplification bias and use single primer extension (SPE) technology which provides design flexibility and highly-specific target enrichment. The concept of UMI's is that prior to any amplification, each original target molecule is 'tagged' by a unique barcode sequence. This DNA sequence must be long enough to provide sufficient permutations to assign each founder molecule a unique barcode. In its current form, a 12-base random sequence provides 412 or 16,777,216 UMI's for each target molecule in the sample.

A single-primer extension approach without the predefined amplicon size constraint

After removing unused adapters, a limited number of PCR cycles is conducted using an Illumina adapter primer and a pool of single primers, each carrying a gene specific

sequence and a 5' universal sequence. During this process, each single primer repeatedly samples the same target locus from different DNA templates. Afterwards, additional PCR cycles are conducted using universal primers to attach complete Illumina adapter sequences and to amplify the library to the desired quantity (see figure below).



Compared to existing targeted enrichment approaches, the SPE method relies on single end adapter ligation, which inherently has a much higher efficiency than requiring adapters to ligate to both ends of the dsDNA fragment. More DNA molecules will be available for the downstream PCR enrichment step. PCR enrichment efficiency using one primer is also better than conventional two primer approach, due to the absence of an efficiency constraint from a second primer. During the initial PCR cycles, primers have repeated opportunities to convert (i.e. capture) maximal amount of original DNA molecules into amplicons.

All three features help to increase the efficiency of capturing rare mutations in the sample. In addition, incorporated UMI's within the amplicon are the key to estimating the number of DNA molecules captured and to greatly reduce sequencing errors in downstream analysis. Single primer extension also permits discovery of unknown structural variants, such as gene fusions.

Explore our QIAseq applications [here](#) and learn more about the path forward to:

- Less primer dropouts
- UMI's and minimal bias
- High uniformity
- Streamlined workflow

[Download the brochure](#) and explore new frontiers with QIAseq NGS.



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