APPLICATION NOTE

MIPs: MOLECULAR INVERSION PROBES

HIGH QUALITY TARGET ENRICHMENT SOLUTION

An Introduction to MIPs

The advances in DNA analysis made a great leap forward with the emergence of Next Generation Sequencing (NGS). With these advances different target enrichment techniques have been developed to select the regions of interest for NGS analysis in a sensitive and cost-effective way. Amongst these techniques a solution phase “capture by circularization” method using “Molecular Inversion Probes” (MIPs) has gained increasing interest. Extensively used for research in Single Nucleotide Polymorphisms (SNPs) and Copy Number Variation (CNV), now MIPs have shown multiple advantages as a Genomic partitioning technique allowing enrichment for regions of interest at a scale that is matched by Next Generation Sequencing platforms.

At Biolegio we have closely collaborated with customers to develop, optimize and validate our target enrichment solutions to introduce MIP assays of the highest quality and performance.

WE ♥ YOUR DNA

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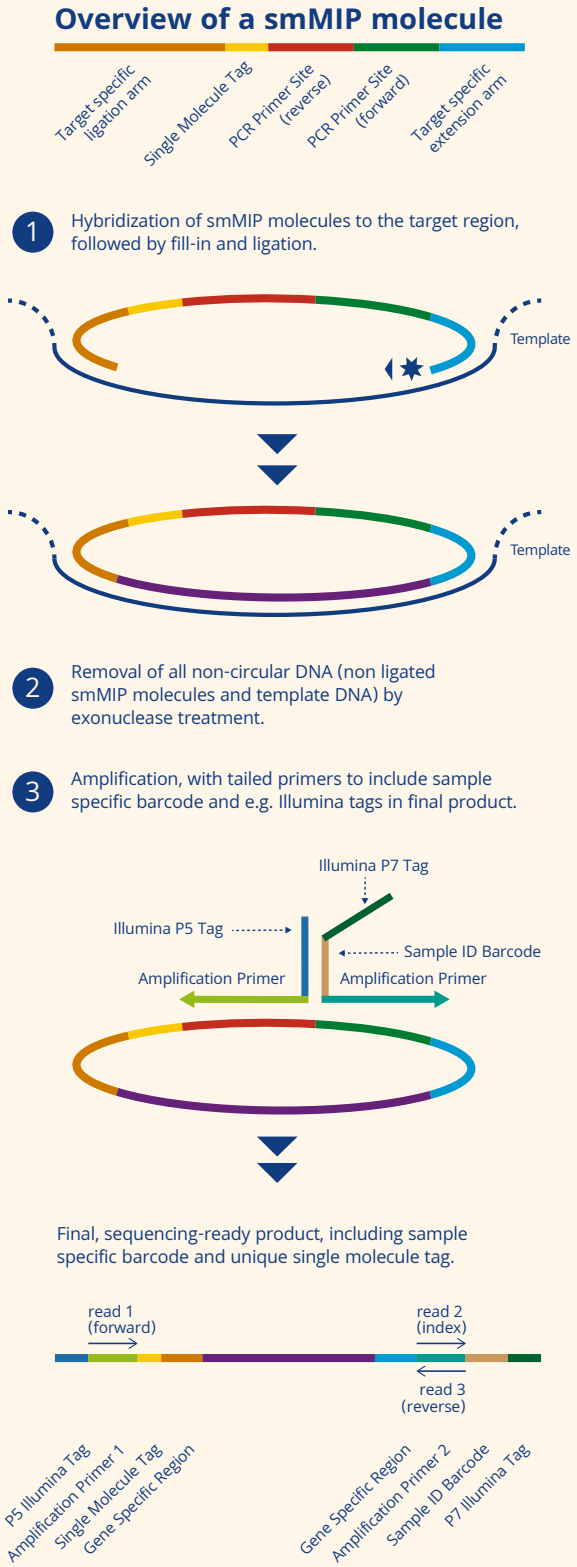
What is a MIP

A Molecular Inversion Probe (fig. 1) is a single stranded oligonucleotide containing two annealing arms complimentary to the target of interest with a sequence gap in between. This sequence gap can target a SNP or a larger region of interest. In between the annealing arms of the MIP binding sites, Universal primers are included and other functionalities like index sequences or digestion sites can be incorporated depending on the experimental setup.

The MIP technology has been combined with the "single molecule tagging" approach [7] resulting in "Single Molecule Molecular Inversion Probes" or "smMIPs" [8]. These probes incorporate a stretch of random degenerate nucleotides creating probes with unique molecular tags enabling detection of low-frequency and sub clonal genetic variation. This resulted in an ultra-sensitive targeted sequencing method exhibiting the specificity and multiplexing advantage of the MIPs and the quantitation ability of the "single molecule tagging" approach.

These advantages combined with the practical workflow, flexible probe sequence / quantity adjustment and low per-sample costs, give science a valuable tool for future research.

Figure 1 | Molecular Inversion Probe (MIP)



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Advantages

The MIP technology exhibits some major advantages in a range of different applications.

Specificity

The two annealing arms are held in each other's proximity by the MIP backbone increasing hybridization if sequences are complimentary. The backbone also suppresses PCR cross-reactions when multiplexing. The circulation step is performed by two enzymes and needs two errors to introduce bias before non-matching sequences will be digested during the exonuclease step. Compared to other target enrichment strategies MIPs exhibit an extraordinary high specificity [3, 4].

No Library prep

Since the MIPs are hybridized directly to the target DNA [3], no fragmentation and PCR is necessary before SNP/ target of interest detection, with as little as 200 ng of input DNA [1]. This avoids time-consuming procedures and the introduction of fragmentation-breakpoint bias as well as PCR related bias like preferential amplification and substitution errors. Additionally barcodes for the NGS platform of choice can be incorporated during the final amplification step of the circularized probes [4].

Multiplexing

Due to the high specificity the technique is highly scalable and suitable for multiplexing; it appears to be suitable for thousands of probes in a single reaction [5] and up to 10,000 probes have been used . Molecular tags or (dual) indices that uniquely mark reads can be introduced between the annealing arms of the MIPs to filter out PCR duplicates and/or pooled samples [3].

Reproducibility

A balanced pool of MIPs exhibits high capture reproducibility for a low amount of input DNA.
As coverage uniformity can be a limitation in this technique a MIP pool usually requires balancing to optimize the uniformity of the capture efficiency.

Ease of use

No expensive instrumentation is needed, standard laboratory equipment is sufficient. The workflow is practical, straight forward, easily automatable and scalable for high throughput [2].

Biolegio's unrivalled synthesis of long oligonucleotides assures the best quality of MIPs available.
In combination with our optimized synthesis protocol, Biolegio achieves an exceptionally high coupling efficiency of over 99.5%, resulting in the ability to generate highly specific probes for long genomic targets.

Custom designed for your experimental needs

From individual probes to custom panels Biolegio is ready to partner in the analysis of your genomic regions of interest.

With access to nearly all commercially available modifications virtually any design requirement can be achieved.
The highest quality product, easy ordering and fast turnaround makes Biolegio your collaborator of choice for target enrichment strategies.

Order your MIPs at Biolegio to Experience the following Advantages

MIPs Key Features		
Specifity	High specificity compared to other genome partitioning techniques.	Biolegio offers high quality MIPs produced with robust and sublime coupling efficiency.
Multiplexing	Due to the high specificity MIPs are ideal for multiplexing reactions.	MIPs are produced in the NGS workflow where cross-contamination is eliminated.
Reproducability	Multiple experimental repeats with a balanced pool of MIPs exhibits high reproducability.	Order Biolegio MIPs at any custom concentration to facilitate your workflow.
ibrary prep	No need for fragmentation or PCR reducing bias.	Isolate your DNA, add the MIP pool and you are ready to go! Use our flexible dispense service to receive your oligo's in any concentration, pooled combination and any tube/plate format to optimize and standardize your workflow.
Easy of use	Straight forward and automatable-workflow without the need of specialized instrumentation.	See above.

More modifications available, please contact us for additional information: www.biolegio.com

References

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