

# Sequencing and deletion/duplication analysis of exons 12–15 of *PMS2* using next-generation sequencing

Lynch syndrome, also known as hereditary non-polyposis colorectal cancer (HNPCC), is characterized by familial predisposition to cancers of the colon, endometrium, ovary, stomach, and urinary tract.<sup>1</sup> Most cases of Lynch syndrome are caused by variants in *MLH1*, *MSH2*, and *MSH6*, but 4–11 percent of cases are caused by variants in *PMS2*.<sup>2–4</sup>

Testing for inherited variants in *PMS2* is hampered by the presence of a pseudogene, *PMS2CL*, which has nearly identical homology to *PMS2* in the final four exons of the gene (exons 12–15). Thus, sequence reads derived from hybridization capture in next-generation sequencing (NGS) methods cannot be unambiguously aligned to *PMS2* or *PMS2CL*. Gene conversion between exons 12 and 15 of *PMS2* and *PMS2CL* further complicates this issue.<sup>5</sup>

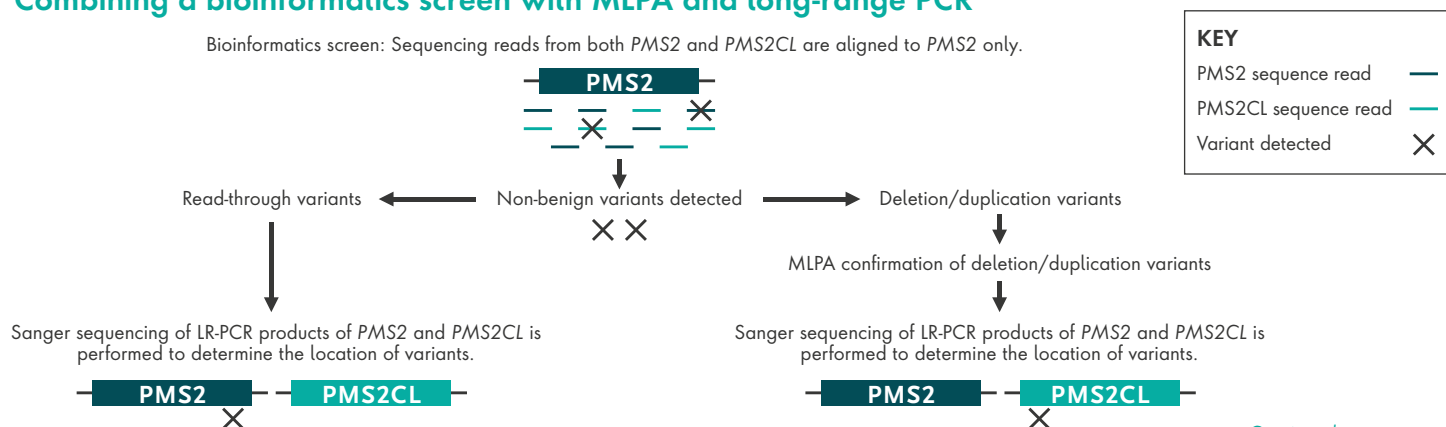
## Why develop an NGS method?

Invitae is committed to making high-quality genetic testing affordable and accessible. Most laboratories perform multiplex ligation-dependent probe amplification (MLPA) to identify deletion/duplication variants, and use long-range PCR (LR-PCR) before sequencing to identify read-through variants and avoid interference from the *PMS2CL* pseudogene. This is a highly customized and resource-intensive approach to the analysis of a single gene in every sample. Having developed an approach that maximizes the use of our established workflows and capabilities, we are able to offer sequencing of this difficult but important region of *PMS2* while maintaining our commitment to affordability.

## Invitae's approach to *PMS2*

Invitae's approach to the evaluation of exons 12–15 of *PMS2* is a two-step process for read-through variants and a three-step process for deletions and duplications (Figure 1). The first step for both types of variants is a bioinformatics screen in which sequence reads derived from both *PMS2* and the paralogous *PMS2CL* gene are analyzed for the presence of variants using *PMS2* as the reference sequence. For read-through variants, non-benign variants identified in the screen are definitively assigned to *PMS2* or *PMS2CL* using Sanger sequencing of LR-PCR products of *PMS2* (exons 12–15) and *PMS2CL* (exons 3–6). For deletion/duplication variants, the second step is to confirm the bioinformatics screen call with MLPA, and to account for the possibility of gene conversion, a final step with LR-PCR is used to disambiguate the location of the variant.<sup>6</sup>

**Figure 1. Invitae's method of *PMS2* sequencing and deletion/duplication analysis: Combining a bioinformatics screen with MLPA and long-range PCR**



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## Validation data

This approach was validated with samples known to have specific variants in these exons for both genes (reference set). For validation of the read-through method, we analyzed 32 unique samples carrying 205 true positive and 34,876 true negative variants in *PMS2* or *PMS2CL* and demonstrated an accuracy, reproducibility, and analytical sensitivity and specificity of 100% (Table 1). For validation of the deletion/duplication method, we analyzed 28 unique samples carrying 90 true positive and 50 true negative individual exon variants in *PMS2* or *PMS2CL* and demonstrated an accuracy, reproducibility, and analytical sensitivity and specificity of 100% (Table 2).

**Table 1: Analytical sensitivity and specificity of 32 samples with read-through variants in *PMS2* and *PMS2CL*, as tested by Invitae**

Results		Details	Conclusions
NGS false negatives	0	<b>205 of 205</b> previously known sequence variants in exons 12–15 of <i>PMS2</i> or exons 3–6 of <i>PMS2CL</i> were detected by Invitae's NGS/LR-PCR approach.	<b>100% sensitivity</b>
NGS false positives	0	All <b>34,876</b> true negative (reference matching) sites were correctly identified by the bioinformatics screen, and no false positive variants were identified.	<b>100% specificity</b>

**Table 2: Analytical sensitivity and specificity of 28 samples with deletion/duplication variants in *PMS2* and *PMS2CL*, as tested by Invitae**

Results		Details	Conclusions
NGS false negatives	0	<b>90 of 90</b> previously known individual exon deletion/duplication variants in exons 12–15 of <i>PMS2</i> or exons 3–6 of <i>PMS2CL</i> were detected by Invitae's NGS/MLPA/LR-PCR approach.	<b>100% sensitivity</b>
NGS false positives	0	All <b>50</b> true negative (reference matching) sites were correctly identified by the bioinformatics screen and confirmed by MLPA, and no false positive variants were identified.	<b>100% specificity</b>

### References

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