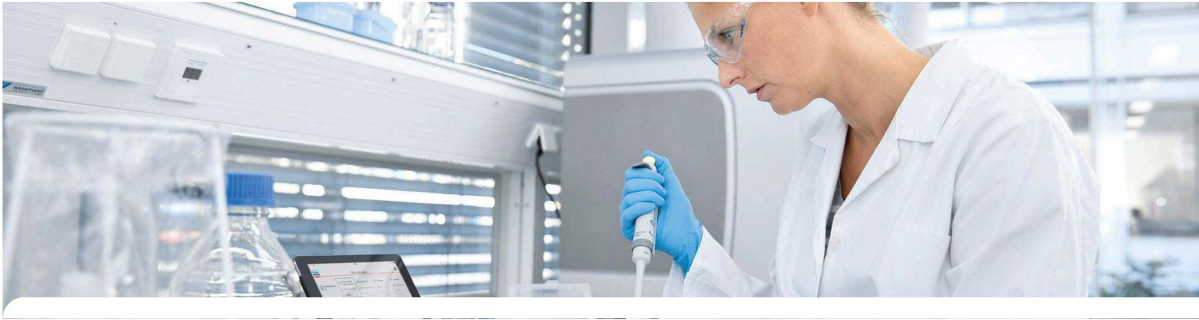




June 7, 2018 | Genomics

Issues with primer dropouts

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Having issues with primer dropouts in your 2-primer amplicon-based panel? You should!

Don't you hate seeing coverage gaps in your NGS panel after you've run your precious samples? Especially when the reason for the gap is as simple as primer dropouts? What's even worse is that filling this gap might require a complete re-design of your panel!

Well, this is not an uncommon problem for panels that utilize a 2-primer amplicon approach for enrichment. Examples of non-coverage due to primer dropouts can be seen in this paper, and the table below as a summary.

2-primer amplicon		
Gene	Region	Coverage
<i>Akt1</i>	Chr14: 105246446–105246583	0
<i>FGFR3</i>	Chr4: 1806082–1806187	0
<i>NOTCH1</i>	Chr9: 139399338–139399447	0
<i>STK11</i>	Chr19: 1220481–1220603	0
<i>TP53</i>	Chr17: 7579351–7579485	0
	Primer dropout	

The reason for primer dropouts in 2-primer amplicon designs is the interaction between the forward and reverse primers targeting a specific region to be enriched. If these primers share common sequences, there is a chance they bind to each other, thereby failing to enrich the targeted region, and resulting in coverage gaps (see figure).

2-primer amplicon design



Two primers are required to define a targeted region

Outcomes:

- Increased risk of primer dimers and dropouts
- Lower uniformity
- Multiple pools of primers might be required
- Low library complexity
- Increasing panel content might require primer redesign

The solution to this problem is simple – eliminate the need for 2 region-specific primers by using only one region-specific primer that targets one end of the targeted region. The other primer is a universal primer that can be shared by all the region-specific primers by binding to universal sequences introduced through library adapters, an approach known as Single Primer Extension (SPE).

SPE amplicon design – QIAseq



Only one primer is required to define a targeted region

Outcomes:

- Lower risk of primer dimers and dropouts
- Higher uniformity
- Only a single pool of primers required
- Higher library complexity
- Increasing panel content does not require primer redesign

SPE-based amplicon designs alleviate the restrictions of 2-primer-based amplicon designs, resulting in enhanced coverage (see table below), and provide extraordinary flexibility, allowing additional targets to be included without the need for a panel re-design.

Gene	2-primer amplicon		SPE in QIAseq		
	Region	Coverage	Region	Coverage	LOD
<i>Akt1</i>	Chr14: 105246446–105246583	0	Chr14: 105246245–105246553 NM_001014431	5734	0.4%
<i>FGFR3</i>	Chr4: 1806082–1806187	0	Chr4: 1806057–1806247 NM_000142	4406	0.5%
<i>NOTCH1</i>	Chr9: 139399338–139399447	0	Chr9: 139399125–139399556 NM_017617	5406	0.4%
<i>STK11</i>	Chr19: 1220481–1220603	0	Chr19: 1220372–1220504 NM_000455	5286	0.4%
<i>TP53</i>	Chr17: 7579351–7579485	0	Chr17: 7579312–7579590 NM_001276761	5740	0.4%
	Primer dropout		No primer dropout		

Is your 2-primer amplicon panel giving you trouble with some regions? Let us show you how SPE-based designs cover those regions. [Click here to design your own panel.](#)



Raed Samara

Raed Samara, PhD is Associate Director of Global Product Management for NGS technologies at QIAGEN. Prior to joining QIAGEN, he was a postdoctoral fellow at the National Cancer Institute conducting research in the field of cancer immunology with emphasis on identifying strategies to boost the efficacy of cancer vaccines. He received his Ph.D. degree from Georgetown University in tumor biology.



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