TwistDx™ blog

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Detection: a multitude of options

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TwistDx Insights

Many RPA users will want to be able to detect that DNA amplification has occurred and monitor this over the course of a reaction. When we first developed the technology, we needed to create RPA-specific methods for achieving this. Resolving the product of your reaction on an agarose or polyacrylamide gel is the most common method for post-PCR detection, yet this is out of the question for high throughput activities or point-of-care applications, and undermines our aim of simplifying the equipment and protocols of molecular diagnostic techniques.

A quick glance at our website reveals a number of products named exo, fpg and nfo, relating to three primary methods of detection that we will discuss in this blog post: fluorescence signal generation with exonuclease III (exo) or formamidopyrimidine glycosylase (fpg), and lateral flow sandwich assays with endonuclease IV (nfo).

Translating the amplification event into a fluorescent signal is an obvious choice. The use of TaqMan probes – in which Taq polymerase cleaves a probe, releasing the fluorescent reporter – cannot simply be adopted from PCR, as RPA polymerase is strand displacing. Consequently, we have developed an alternative probe structure, containing a matched pair of fluorophore and quencher labels, separated by an abasic site – a typical product of cellular DNA damage – which is recognised by exonuclease III. This leads to the probe being digested, releasing the fluorophore quencher and increasing fluorescence, which can be detected by any available method. Other enzymes, such as fpg, can also be used with a slight modification to the probe structure.

Not everyone wants to detect fluorescence, however, and a second, enzymatic system uses nfo to cleave the probe – rather than releasing a reporter label – essentially turning it into a primer. The probe is synthesised to contain antigenic labels, such as biotin, digoxigenin or carboxyfluorescein, which are incorporated into the amplified genetic material and can be detected on a lateral flow strip using a sandwich assay; the appearance of a visible line indicates the presence of the labelled amplicon.