Can I assess my digitalMLPA reactions or library using a TapeStation?

The digitalMLPA™ technique has been designed in such a way that library preparation can be done quickly and easily without the need for purification, quantification or assessment before sequencing. However, we understand that some customers still like to check that there was successful amplification of the ligated digitalMLPA probes. Analysis using traditional NGS library assessment methods such as a TapeStation can be used, but with some additional guidelines.

Important

We do not consider checking digitalMLPA reactions or libraries on a TapeStation a necessary step in the protocol, and do not do this ourselves. Checking digitalMLPA reactions or libraries prior to sequencing will only reveal serious issues, such as the complete absence of digitalMLPA PCR products, but does not guarantee that the quality of the reaction is sufficient for analysis. Coffalyser digitalMLPA provides more detailed information about the quality of individual reactions using a large set of control probes present in every probemix. The following information is only supplied for the convenience of our customers, and may require some optimization.

Note

The quality of digitalMLPA reactions can also be assessed using capillary electrophoresis on an ABI device.

Using a TapeStation

If it is desired to visualise a digitalMLPA library before loading on an Illumina sequencer this can be done using an Agilent TapeStation. The TapeStation is a non-denaturing electrophoresis system that is commonly used for the assessment of NGS libraries. This system must be used with caution, as sample DNA and carrier DNA will be visible along with digitalMLPA amplicons. The majority of DNA present (80–90%) will be digitalMLPA amplicons visible in the 190–220 bp range.

After the digitalMLPA reaction, all amplicons are single-stranded or part of heterodimer complexes. For correct visualisation, the TapeStation sample should first be incubated for ≥3 hours at 60ºC. This allows for all amplicons to become double stranded (forward and reverse strand of the same probe), enabling the correct fragment visualisation at ~200 bp.

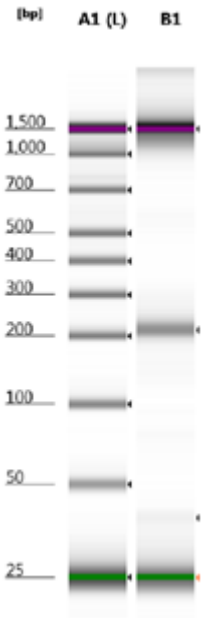

Figure 1. TapeStation run of a digitalMLPA library incubated at 60ºC for 3 hours before loading (right). A dominate band is seen at around 200 bp. This indicates that the digitalMLPA reaction was successful and there were predominately digitalMLPA amplicons present in the library. *Important*: your library may migrate slightly differently depending on the probemix and on conditions; this image is only meant to serve as an example.

Artifacts without incubation at 60°C

If the additional incubation period at 60ºC is not performed, larger fragments than expected may be visible. This is due to the formation of mixed hybrids: products formed from the forward strand of one probe and the reverse strand of a different probe. Mixed hybrids are formed quickly after the PCR due to the homology between the sequences at both ends of all probes (e.g. primer sequences, digitalMLPA read identifier). These mixed hybrids still have the same fragment length but migrate at a much slower rate due to the non-hybridising “bubble” between the double stranded ends. It is also possible that more complex hybrid structures form, further reducing fragment mobility.

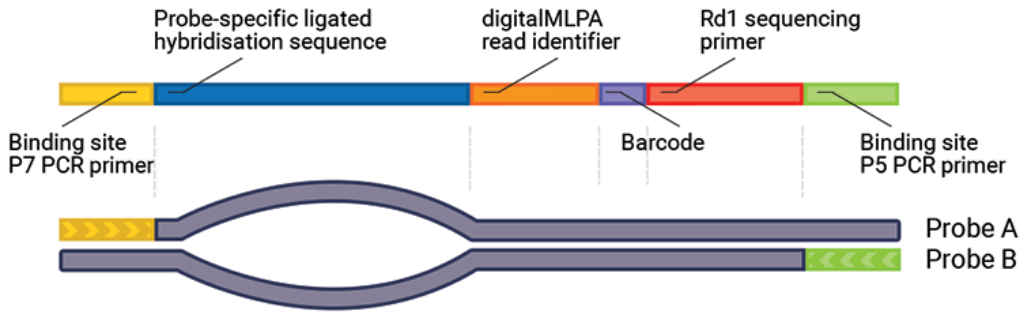

Figure 2. Schematic of a digitalMLPA probe highlighting the different parts of the probe sequence. Below the probe an example of a mixed hybrid is shown where the forward strand amplicon of probe A has formed a heterodimer complex with the reverse strand amplicon of probe B.

Tags

digitalMLPA

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