COA Version 01; Issued 23 March 2020

Certificate of Analysis
SALSA® MS-MLPA® Probemix ME033 TNDM

Catalogue #	**ME033-025R, ME033-050R, ME033-100R**
Product name	**Probemix ME033 TNDM**
LOT	**A1-0220**

Σ	25, 50, or 100 reactions.	
Shipping conditions	Dry ice or cooling elements.	
	Store upon arrival between -25°C and -15°C.	
	Expiration date: February 2025, when stored at recommended conditions. This product should not be frozen/thawed more than 25 times.	
Use	This product has been developed to determine the methylation status of an alternative *PLAGL1* Transcription Start Site that is shared with the *HYMAI* gene, as well as copy number detection of the 6q24 and 11q15 region, as described in table 1 and 2 of the product description. This probemix is designed for use only in combination with SALSA MLPA reagent kits, SALSA HhaI, and Coffalyser.Net as described in the MS-MLPA General Protocol.	
Quality control specifications	- Sufficient distance between peaks, absence of extra or shoulder peaks, and completeness of hybridisation and HhaI digestion of each individual probe, as tested on Applied Biosystems 3130 and Beckman/SCIEX GeXP sequencers. - Standard deviation of each individual probe ≤0.10, when tested on 23 different DNA samples of healthy individuals, extracted by various methods. - Each individual probe meets reaction-specific criterial when tested on a single DNA sample under various experimental conditions. - No DNA controls result in only five major peaks shorter than 121 nucleotides (nt): four Q fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 121 nt AND with a height <25% of the median of the four Q fragments are not expected to affect MLPA reactions when sufficient (50-250 ng) sample DNA is used. Note: We observed five prominent peaks with lengths of approximately 114, 145, 202, 207, and 271 nt in a No DNA control. These peaks appeared very sporadically and are not expected to affect MLPA reactions when sufficient (50-250 ng) sample DNA is used. MRC Holland has not yet been able to establish the cause. However, we found that the amount and height of these peaks is greatly reduced by not spinning down your MLPA reactions in between the ligation and PCR reaction. The non-specific peaks are not expected to influence results. Please notify us if you still regularly observe these peaks: info@mrcholland.com.	Test result PASS

None of the ingredients are derived from humans, animals, or pathogenic bacteria. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. **A Safety Data Sheet (SDS) is not required for these products**: none of the preparations contain dangerous substances (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and amendments) at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments). If spills occur, clean with water and follow appropriate site procedures.

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More information: www.mrcholland.com	
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SALSA MS-MLPA Probemix ME033-A1 TNDM sample pictures

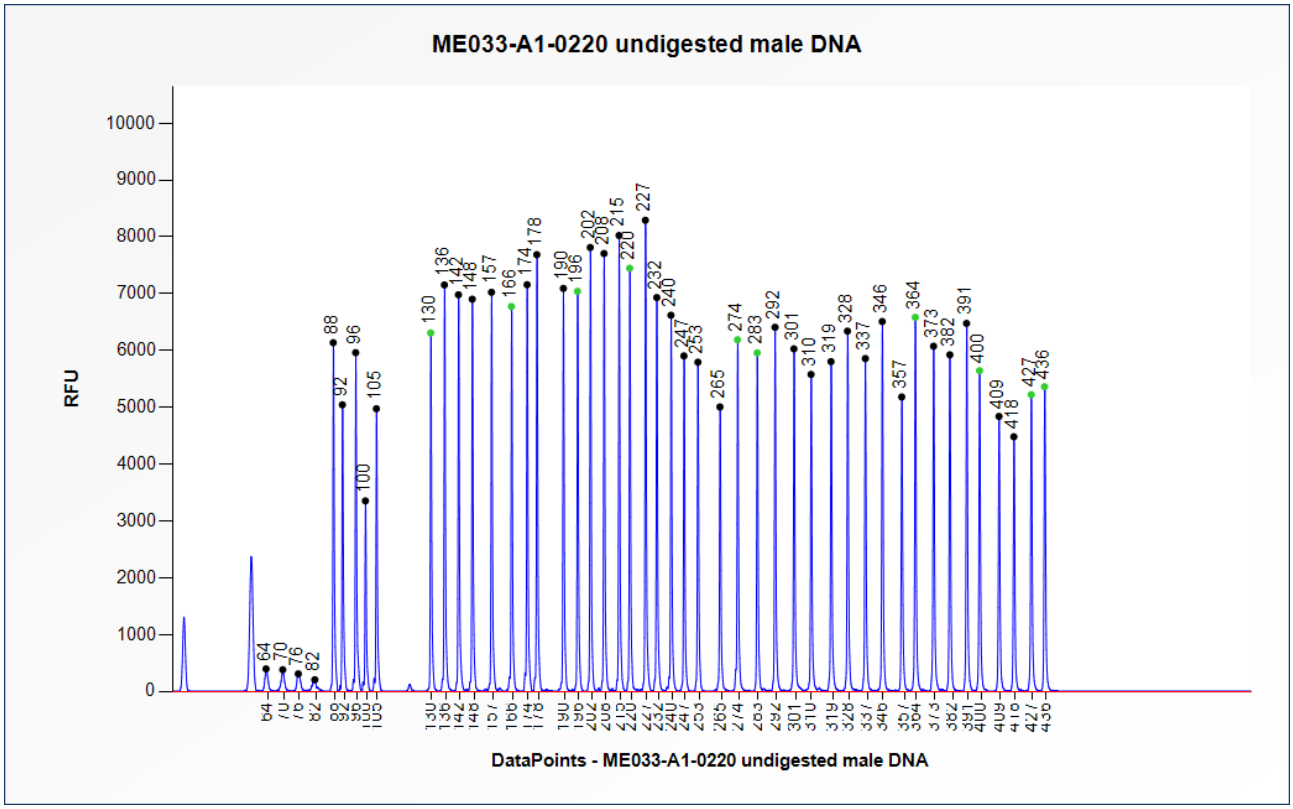

Figure 1. Capillary electrophoresis pattern from a sample of approximately 50 ng undigested human male control DNA analysed with SALSA MS-MLPA Probemix ME033 TNDM (A1-0220) for the quantification of copy numbers.

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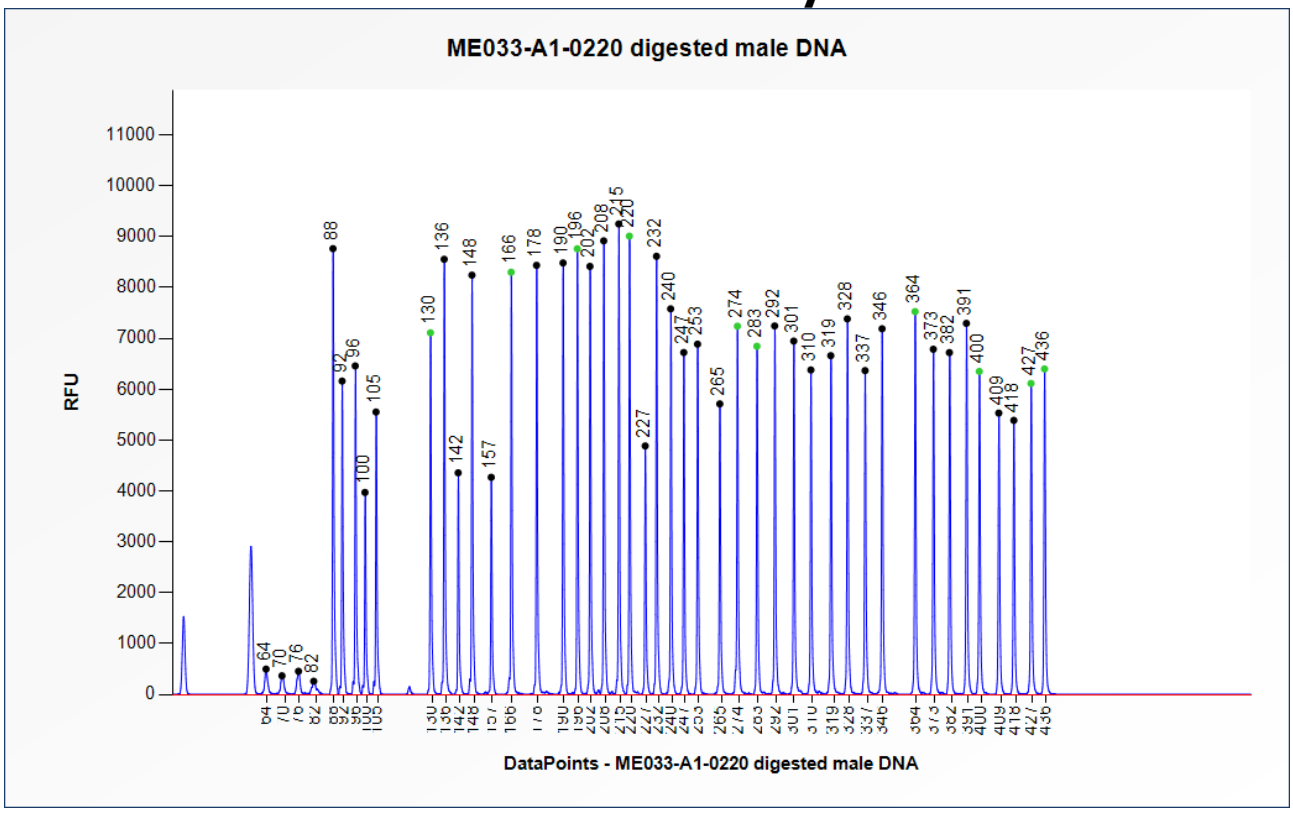

Figure 2. Capillary electrophoresis pattern from a sample of approximately 50 ng digested human male control DNA analysed with SALSA MS-MLPA Probemix ME033 TNDM (A1-0220) to determine the methylation status.

This lot was certified by MRC Holland on 23 March 2020.
This certificate is a declaration of analysis at the time of the manufacturing process. All assays were run in compliance with manufacturer's instructions for use.

Implemented changes in the COA
Version 01 – 23 March 2020 (02) - Not applicable, new document.