Certificate of Analysis of the MICA Primer Component Box 96

Product name	MICA Primer Component Box 96
Reference number	P12.2
LOT number	P12.2/014 - E1/010
Expiration data	2020.06.03

1 Quality control application overview

1.1 Primer Component Box

The primer component provides specific ready to use primer solutions for targeted Long Range PCR amplification. Additionally, it also contains PCR additive (Enhancer 1).

Primer mix	REF #	Rxns	Vol/tube	# Tubes	Color code
MICA	*P152*	*96*	*220 µL*	*1*	*Black*
Enhancer 1	*E01*	*96*	*1100 µL*	*1*	*Clear*

2 Summary of Quality Control testing

Evaluation/Assessment	Pass/Fail
Physical inspection	Pass
Qualitative assessment of amplification by gel electrophoresis	Pass
Quantitative assessment of amplification by picogreen	Pass
Assessment of mappability of sequences	Pass
Assessment of amplification bias	Pass
Performance specifications: accuracy, precision, sensitivity, specificity	Pass

2.1 Physical inspection

All contents of the kit are inspected for proper components, volumes and labeling. The condition of all primers and enhancers were inspected after packaging.

2.1.1 Results of physical inspection

Criteria for acceptability	Pass/Fail
Expected volumes in all tubes	Pass
Proper labeling	Pass
Proper shipping condition (on dry ice)	Pass
Reagents clear and not discolored	Pass

2.2 Amplification components quality control testing

2.2.1 Results of amplification

Criteria for acceptability	Pass/Fail
Quantitative assessment of amplification by picogreen : > 50 ng/µL for all amplicons (excluding FTA)	Pass
Qualitative assessment of amplification by gel electrophoresis	Pass

2.2.2 Assessment of amplification success and specificity

The primers used to amplify the MICA genes are intended to be specific to a given locus (or loci in the case of multiplexed primers). To assess the performance of the primers for specificity to the intended loci four measures are used:

- *Mapped read count per locus*: This measure estimates amplification success
- *Best quality mapped read count per locus*: This measure estimates amplification success
- Best quality mapped read count / Mapped read count per locus: This measure estimates amplification quality
- Reads mappable to the seven targeted loci / Processed read count for the sample: This measures estimates primer specificity

2.2.2.1 Results of amplification success and specificity

Criterion	Pass or Fail	Result
Amplification success	*Pass*: At least 6000 mappable reads AND at least 5000 best mapping reads found for each locus in all samples. *Fail*: Less than 6000 reads OR less than 5000 best mapping reads found for at least one locus.	PASSED
Amplification quality	*Pass:* At least 25% of mappable reads can be used for consensus generation.	PASSED

	Fail: Less than 25% of mappable reads can be used for consensus generation.	
Primer specificity	*Pass*: At least 60% of processed reads are mappable to the targeted loci. *Fail*: Less than 60% of processed reads are mappable to the targeted loci.	PASSED

2.2.3 Amplification balance assessment

The differences between the representations of each allele in the samples is evaluated for balance.

2.2.3.1 Results for amplification balance assessment

Criteria for acceptability	Pass/Fail
Amplification balance: ***Pass:*** **Minor allele should be no lower than 20% (i.e. the major allele shown in graph should be lower than 0.8)** ***Fail:*** **At least one minor allele goes lower than 20%.**	Pass

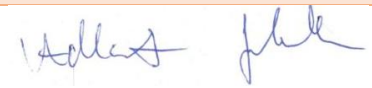
2.2.4 Genotyping performance assessment

Given a set of samples, it is expected that the Holotype MICA v1 kit that is being quality control tested produces the same genotyping results obtained using independent genotyping methods (e.g. SSO, SBT or previous NGS runs). Concordance statistics were calculated on a full resolution level. A genotyping result produced by Omixon HLA Twin was considered to be concordant with the reference typing information if the allele pair present in the reference typing was present in the Omixon result as well.

2.2.4.1 Results for genotyping performance assessment

Criteria for acceptability	Pass/Fail
Concordance: **Passed: Concordance with the reference typing information is over 95%.** **Failed: Concordance with the reference typing is less than 95%.**	Pass
Reproducibility: ***Pass:*** **Results of the current QC run and the QC run of the previous manufacturing lot are identical.**	Pass

Fail: **Results of the current QC run and the QC run of the previous manufacturing lot are not identical.**

Authorization for release			
Name	**Zoltán Simon - Omixon**	**Function**	**COO**
Signature		**Sign date**	**2019.05.03**
Name	**Gabriella Adlovits - Omixon**	**Function**	**RAQS Manager**
Signature		**Sign date**	**2019.05.03**