Certificate of Analysis of the Holotype Primer Component Box 24/11 v2

Product name	Holotype Primer Component Box 24/11 v2
Reference number	P7.1
LOT number	P7.1/014-E1/012
Expiration data	2020.06.25

1 Quality control application overview

Reagents from Holotype Kits (HLA locus specific primers and PCR enhancers) are combined with Qiagen Long Range PCR Kit reagents for amplification of HLA-A, B, C, DRB1/3/4/5, DQB1, DQA1, DPA1 and DPB1 genes for next-generation sequencing. Amplicons for all loci from each sample are combined in a roughly equimolar amount. Library preparation reagents (Fragmentation, End repair, and Ligation enzymes and buffers) are used to create libraries for sequencing from the pools of amplicons. Sample HLA typings are derived from the sequencing data. Indexed adaptor plates are tested for contamination and variability in a separate set of experiment.

1.1 Primer Component Box

The primer component provides specific ready to use primer solutions for targeted Long Range PCR amplification of HLA genes A, B, C, DPA1, DPB1, DQA1 and DQB1, and DRB1/3/4/5. Additionally, it also contains two types of PCR additives (Enhancer 1 and Enhancer 2).

Primer mix	REF #	Rxns	Vol/tube	# Tubes	Color code
HLA-A	*P013*	*24*	*60 µL*	*1*	*Yellow*
HLA-B	*P023*	*24*	*60 µL*	*1*	*Red*
HLA-C	*P033*	*24*	*60 µL*	*1*	*Orange*
HLA-DRB1/DRB3	*P043.1*	*24*	*60 µL*	*1*	*Green*
HLA-DRB4	*P103*	*24*	*60 µL*	*1*	*White*
HLA-DRB5	*P113*	*24*	*60 µL*	*1*	*Pink*
HLA-DQB1 set 3	*P123*	*24*	*60 µL*	*1*	*Blue*
HLA-DPA1	*P133*	*24*	*60 µL*	*1*	*Black*
HLA-DPB1	*P073*	*24*	*60 µL*	*1*	*Purple*

HLA-DQA1	*P083*	*24*	*60 µL*	*1*	*Brown*
Enhancer 1	*E01*	*96*	*1100 µL*	*1*	*Clear*
Enhancer 2	*E02*	*96*	*300 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 and shipping.

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 Amplification

Amplification primers and enhancer reagents are tested on a selected panel of cell lines that have known typing information for the tested 11 HLA loci. Primers were used to amplify 12 samples in duplicate. All samples are assessed by gel electrophoresis for presence of amplicon at each loci and also through quantitative assessment of amplicon amount by QuantiFluor

dsDNA System.

2.2.1.1 Results of amplification

Criteria for acceptability	Pass/Fail
Quantitative assessment of amplification by QuantiFluor dsDNA System : > 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 HLA 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

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

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 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). Therefore, a set of performance measures was calculated for each locus independently to measure the performance of genotyping:

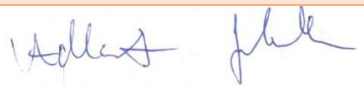
- Sensitivity
- Specificity
- Precision/Positive Predictive Value (PPV)
- Negative Predictive Value (NPV)
- Accuracy/Type Correctly Classified (TCC)
- Repeatability
- Reproducibility

Performance statistics were calculated on a three field level. Genotypes derived from both replicates in the pooled configuration were taken into account for the aforementioned calculations and were recorded separately.

2.2.4.1 Results for genotyping performance assessment

Criteria for acceptability	Pass/Fail
Sensitivity: ***Pass:*** **Sensitivity is 100% for all loci.** ***Fail:*** **Sensitivity is less than 100% for one or more loci.**	Pass
Specificity: ***Pass:*** **Specificity is 100% for all loci.** ***Fail:*** **Specificity is less than 100% for one or more loci.**	Pass
Precision/PPV: ***Pass:*** **Positive predictive value is 100% for all loci.** ***Fail:*** **Positive predictive value is less than 100% for one or more loci.**	Pass
NPV: ***Pass:*** **Negative predictive value is 100% for all loci.**	Pass

Fail: **Negative predictive value is less than 100% for one or more loci.**	
Accuracy/TTC: ***Pass:*** **Type correctly classified is 100% for all loci.** ***Fail:*** **Type correctly classified is less than 100% for one or more loci.**	Pass
Repeatability: ***Pass:*** **Results are identical between repeats within a QC run.** ***Fail:*** **There is at least one difference between repeats within a QC run.**	Pass
Reproducibility: ***Pass:*** **Results of the current QC run and the QC run of the previous manufacturing lot are identical.** ***Fail:*** **Results of the current QC run and the QC run of the previous manufacturing lot are not identical.**	Pass

Authorization for release			
Name	**Zoltán Simon - Omixon**	**Function**	**COO**
Signature		**Sign date**	**2019.04.09**
Name	**Gabriella Adlovits - Omixon**	**Function**	**RAQS Manager**
Signature		**Sign date**	**2019.04.09**