

Contamination study

EFS Rhone-Alpes

HLA laboratory

Dr D Masson

Design

- We prepared 4 samples :
- Contamination5 : 95%DNA1+5%DNA2
- Contamination10: 90%DNA1+10%DNA2
- Contamination20 : 80%DNA1+20%DNA2
- Contamination50 : 50%DNA1+50%DNA2
- DNA1 is our internal control

DNA1	A*32:01	B*40:01	C*03:04	DPB1*04:02	DQA1*01:02	DQB1*03:01	DRB1*04:01	
	A*68:01	B*44:02	C*05:01	DPB1*13:01	DQA1*03:03	DQB1*05:02	DRB1*16:01	
	A*01:03	B*18:01	C*07:01	DPB1*04:01	DQA1*02:01	DQB1*02:02	DRB1*07:01	DRB3*02:02
DNA2	A*11:01	B*73:01	C*15:05	DPB1*09:01	DQA1*05:05	DQB1*03:01	DRB1*11:04	

Tests on Omixon kit V2, 96/7

- 48 samples were tested with kit V2 , A, B, C, DRB1, DQB1, DQA1, DPB1, DRB3 for the validation of this test.



- Standard Flow cell 500



- Miseq Illumina



Analysis using omixon twin software

The screenshot shows the omixon twin software interface. At the top is a toolbar with icons for various functions: Sample Details, Browse Alignment, Setup Loci, Setup Filters, Best Matches Only, Assigned Only, Approved Only, Assign Best Matches, Assign Unambiguous Concordants, Assign QC Passed, Assign All, Unassign All, LIMS Export, Export Table, Sample Comment, Approve Result, Cancel Approval, and Show LD details. Below the toolbar is a table displaying HLA genotypes for two alleles.

Allele	HLA-A	HLA-B	HLA-C	HLA-DPB1	HLA-DQA1	HLA-DQ
Allele 1	HLA-A*24:02:01:01	HLA-B*07:02:01	HLA-C*03:04:01:01 HLA-C*03:04:01:02	HLA-DPB1*04:01:01:01	HLA-DQA1*01:02:01:01	HLA-DQB1*
Allele 2	HLA-A*32:01:01	HLA-B*40:01:02	HLA-C*07:02:01:03	HLA-DPB1*04:01:01:01 HLA-DPB1*04:01:01:02	HLA-DQA1*02:01	HLA-DQB1*

Up to two algorithms for determining HLA genotypes

- Consensus Genotyping (Assembly)
- Statistical Genotyping (Alignment to IMGT/HLA)

Traffic Light #1 - Algorithm Concordance Check

Evaluates the concordance between the results of the two algorithms.

Traffic Light #2 - Quality Check

Evaluates 23 different quality metrics such as read length, read quality, noise ratio, fragment size, consensus coverage depth, allele balance, phasing and more for every locus of every sample.

The diagram shows the traffic light status for HLA-A*01:01:01:01. The status is represented by three green circles, indicating a fully concordant and high-quality result. A legend explains the meaning of the traffic light colors:

- Fully concordant** (Green circle)
- Concordant to 2nd field** (Yellow circle)
- Concordant to 1st field** (Red circle)
- Discordant** (Red circle)
- All QC Metrics Passed** (Green circle)
- Passed with extra info** (Yellow circle)
- Inspect result** (Yellow circle)
- Investigate result** (Red circle)
- Failed. Rerun or reflexive test** (Red circle)

Results

Omixon HLA Twin CE - using 'spt-auragregns1'

Genotyping | Result of 6 analyses

dominique.masson@hla 2 task(s) running (41%)

Genotyping analysis result

Analysis name(s): CIQ30-yyy01dd5-4_S4_L001_R1_001_2018-12-04_16-34-43, CIQ5-yyy01dd5-1_S1_L001_R1_001_2018-12-04_16-47-02, Contamination10-yyy01dd5-6_S6_L001_R1_001_2018-12-04_17-10-57, Contamination20-yyy01dd5-7_S7_L001_R1_001_2018-12-04_17-39-20, Contamination5-yyy01dd5-5_S5_L001_R1_001_2018-12-04_18-10-10, Contamination50-yyy01dd5-9_S9_L001_R1_001_2018-12-04_19-11-16

Displaying 8 loci out of 8.
Displaying best matches only.

State	Sample	Allele	HLA-A	HLA-B	HLA-C	HLA-DPB1	HLA-DQA1
Ready	CIQ30-yyy01dd5-4_S4_L001_R1_001_2018-12-04_16-34-43	Allele 1	HLA-A*32:01:01:01	HLA-B*40:01:02:02 HLA-B*40:01:02:06 HLA-B*40:01:02:01 HLA-B*40:01:02:04	HLA-C*03:04:01:02 HLA-C*03:04:01:01	HLA-DPB1*04:02:01:04	HLA-DQA1*01:02:02
Ready	CIQ30-yyy01dd5-4_S4_L001_R1_001_2018-12-04_16-34-43	Allele 2	HLA-A*68:01:02:02	HLA-B*44:02:01:01	HLA-C*05:01:01:03 HLA-C*05:01:01:02	HLA-DPB1*13:01:01 HLA-DPB1*107:01	HLA-DQA1*03:03:01:0
Ready	CIQ5-yyy01dd5-1_S1_L001_R1_001_2018-12-04_16-47-02	Allele 1	HLA-A*32:01:01:01	HLA-B*40:01:02:02 HLA-B*40:01:02:06 HLA-B*40:01:02:01 HLA-B*40:01:02:04	HLA-C*03:04:01:02 HLA-C*03:04:01:01	HLA-DPB1*04:02:01:04	HLA-DQA1*01:02:02
Ready	CIQ5-yyy01dd5-1_S1_L001_R1_001_2018-12-04_16-47-02	Allele 2	HLA-A*68:01:02:02	HLA-B*44:02:01:01	HLA-C*05:01:01:03 HLA-C*05:01:01:02	HLA-DPB1*13:01:01 HLA-DPB1*107:01	HLA-DQA1*03:03:01:0
Ready	Contamination10-yyy01dd5-6_S6_L001_R1_001_2018-12-04_17-10-57	Allele 1	HLA-A*32:26:02#1	HLA-B*40:47	HLA-C*03:04:01:01#1	HLA-DPB1*04:02:01:04	HLA-DQA1*01:02:02
Ready	Contamination10-yyy01dd5-6_S6_L001_R1_001_2018-12-04_17-10-57	Allele 2	HLA-A*68:01:02:02#1	HLA-B*83:01#1	HLA-C*05:01:01:02#1	HLA-DPB1*13:01:01 HLA-DPB1*107:01	HLA-DQA1*03:03:01
Ready	Contamination20-yyy01dd5-7_S7_L001_R1_001_2018-12-04_17-39-20	Allele 1	HLA-A*32:01:01:01 HLA-A*32:54	HLA-B*40:47#1	HLA-C*03:04:03 HLA-C*03:04:19 HLA-C*03:314	HLA-DPB1*13:01:01 HLA-DPB1*107:01	HLA-DQA1*01:02:02
Ready	Contamination20-yyy01dd5-7_S7_L001_R1_001_2018-12-04_17-39-20	Allele 2	HLA-A*68:65#1	HLA-B*83:01#1	HLA-C*05:01:01:03 HLA-C*05:01:01:05 HLA-C*05:01:01:02 HLA-C*05:01:15 HLA-C*05:01:17 HLA-C*05:09:02	HLA-DPB1*80:01#1	HLA-DQA1*03:03:01
Ready	Contamination5-yyy01dd5-5_S5_L001_R1_001_2018-12-04_18-10-10	Allele 1	HLA-A*32:01:01:01	HLA-B*40:01:02:02 HLA-B*40:01:02:06 HLA-B*40:01:02:04 HLA-B*40:01:02:01	HLA-C*03:04:01:01#1	HLA-DPB1*04:02:01:04	HLA-DQA1*01:02:02
Ready	Contamination5-yyy01dd5-5_S5_L001_R1_001_2018-12-04_18-10-10	Allele 2	HLA-A*68:65#1	HLA-B*44:12#1	HLA-C*05:01:01:03 HLA-C*05:01:01:02	HLA-DPB1*13:01:01 HLA-DPB1*107:01	HLA-DQA1*03:03:01
Ready	Contamination50-yyy01dd5-9_S9_L001_R1_001_2018-12-04_19-11-16	Allele 1	HLA-A*01:25#1	HLA-B*44:31#1	HLA-C*05:58:01#1	HLA-DPB1*35:01:01 HLA-DPB1*65:01:01	HLA-DQA1*01:02:02
Ready	Contamination50-yyy01dd5-9_S9_L001_R1_001_2018-12-04_19-11-16	Allele 2	HLA-A*68:01:28#1	HLA-B*73:01#1	HLA-C*07:196#1	HLA-DPB1*86:01 HLA-DPB1*474:01	HLA-DQA1*03:03:01

Démarrer Page vierge - Interne... hla Ordinateur Omixon HLA Twin C...

FR 09:50 03/05/2019

Results

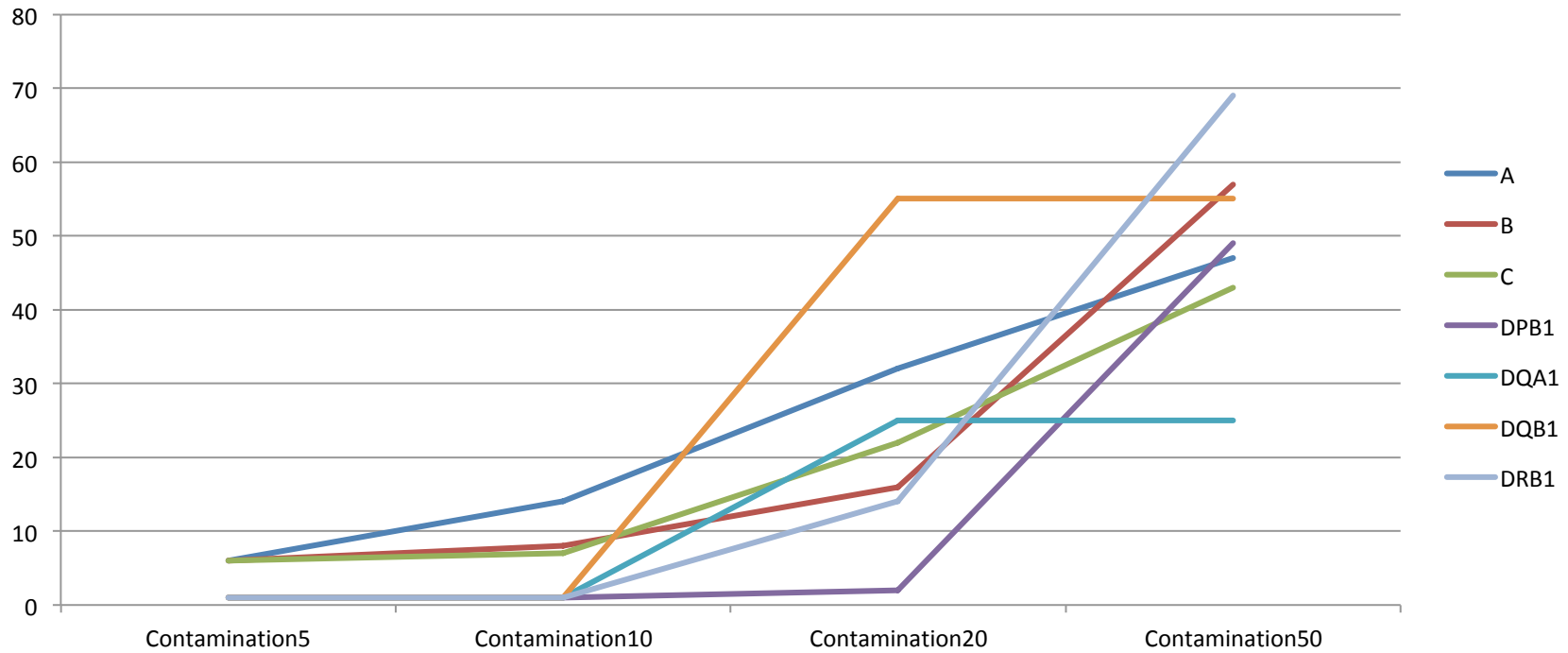
DNA1	A*32:01	B*40:01	C*03:04	DPB1*04:02	DQA1*01:02	DQB1*03:01	DRB1*04:01	
	A*68:01	B*44:02	C*05:01	DPB1*13:01	DQA1*03:03	DQB1*05:02	DRB1*16:01	
	A*01:03	B*18:01	C*07:01	DPB1*04:01	DQA1*02:01	DQB1*02:02	DRB1*07:01	DRB3*02:02
DNA2	A*11:01	B*73:01	C*15:05	DPB1*09:01	DQA1*05:05	DQB1*03:01	DRB1*11:04	

Results kit V2									
Sample	Allele	HLA-A	HLA-B	HLA-C	HLA-DPB1	HLA-DQA1	HLA-DQB1	HLA-DRB1	HLA-DRB3
0%	ALLELE_1	A*32:01:01:01	B*40:01:02:02	C*03:04:01:02	DPB1*04:02:01:04	DQA1*01:02:02	DQB1*03:01:01:10	DRB1*04:01:01:01	
	ALLELE_2	A*68:01:02:02	B*44:02:01:01	C*05:01:01:03	DPB1*13:01:01	DQA1*03:03:01:01	DQB1*05:02:01:01	DRB1*16:01:01	
5%	ALLELE_1	A*32:01:01:01	B*40:01:02:02	C*03:04:01:01#1	DPB1*04:02:01:04	DQA1*01:02:02	DQB1*03:01:01:10	DRB1*04:01:01:01	DRB3*02:02
	ALLELE_2	A*68:65#1	B*44:12#1	C*05:01:01:03	DPB1*13:01:01	DQA1*03:03:01:01	DQB1*05:02:01:01	DRB1*16:01:01	
10%	ALLELE_1	A*32:26:02:01#1	B*40:47	C*03:04:01:01#1	DPB1*04:02:01:04	DQA1*01:02:02	DQB1*03:01:01:10	DRB1*04:01:01:01#1	DRB3*02:02
	ALLELE_2	A*68:01:02:02#1	B*83:01#1	C*05:01:01:02#1	DPB1*13:01:01	DQA1*03:03:01:01	DQB1*05:02:01:01	DRB1*16:01:01	
20%	ALLELE_1	A*32:01:01:01/32:54	B*40:47	C*03:04:03:03:314	DPB1*80:01#1	DQA1*01:02:02	DQB1*03:01:01:10	DRB1*04:01:01:01#1	DRB3*02:02
	ALLELE_2	A*68:65#1	B*83:01#1	C*05:01:01:03	DPB1*13:01:01	DQA1*03:03:01:01	DQB1*05:02:01:01	DRB1*16:01:01#1	
50%	ALLELE_1	A*01:25#1	B*44:31#1	C*05:58:01#1	DPB1*31:01/65:01	DQA1*01:02:02#1	DQB1*03:01:01:3	DRB1*04:01:01:01#1	DRB3*02:02
	ALLELE_2	A*68:01:28#1	B*73:01#1	C*07:196#1	DPB1*474:01	DQA1*05:46#1	DQB1*05:46#1	DRB1*11:04:01#1	

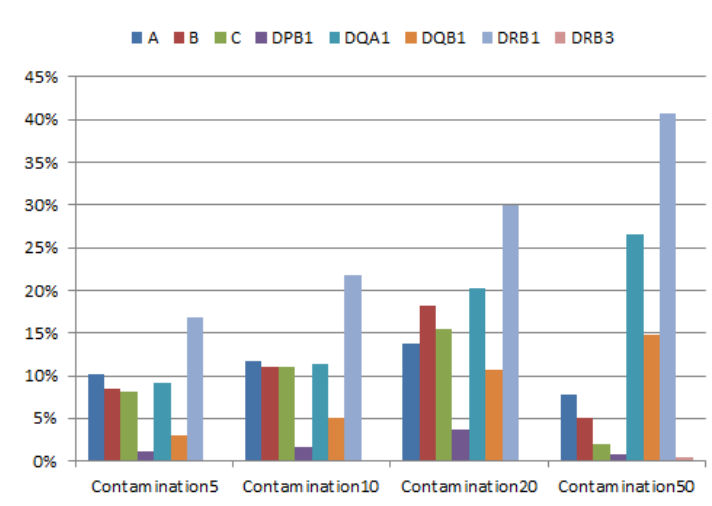
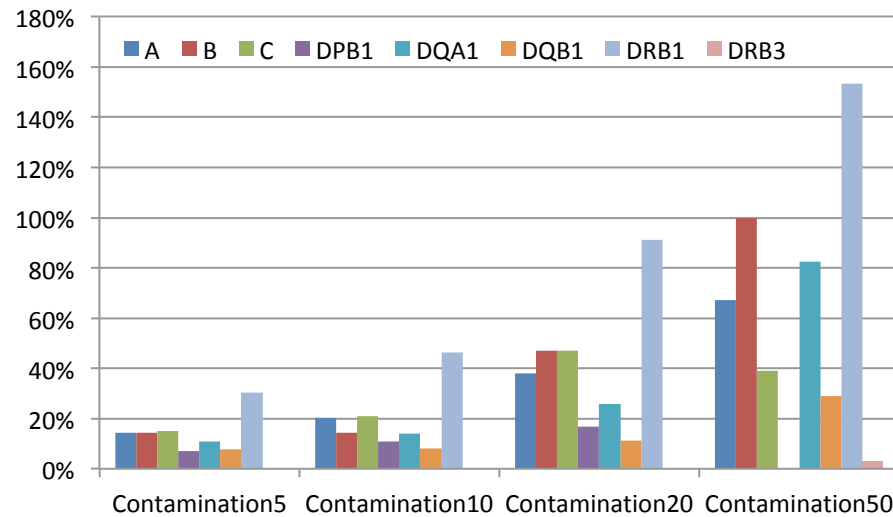
Locus A at 10%



Phases



Key exon spot noise ratio/ Noise ratio



To conclude

- Twin is able to detect DNA contamination at level of 5% and **most of the loci have failed criteria**.
- This is **not true** in case of homozygoty or DRB3, 4 or 5 if only one is expected.
- You can also observe « false » new or rare alleles.
- To be sure of this kind of alleles , check them and control them.
- The alerts of the software are useful but can be insufficient if only one allele is contaminated.

Acknowledgments

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