Omixon Holotype HLA™

EAP Update, Whole Gene Consensus

April 27, 2015

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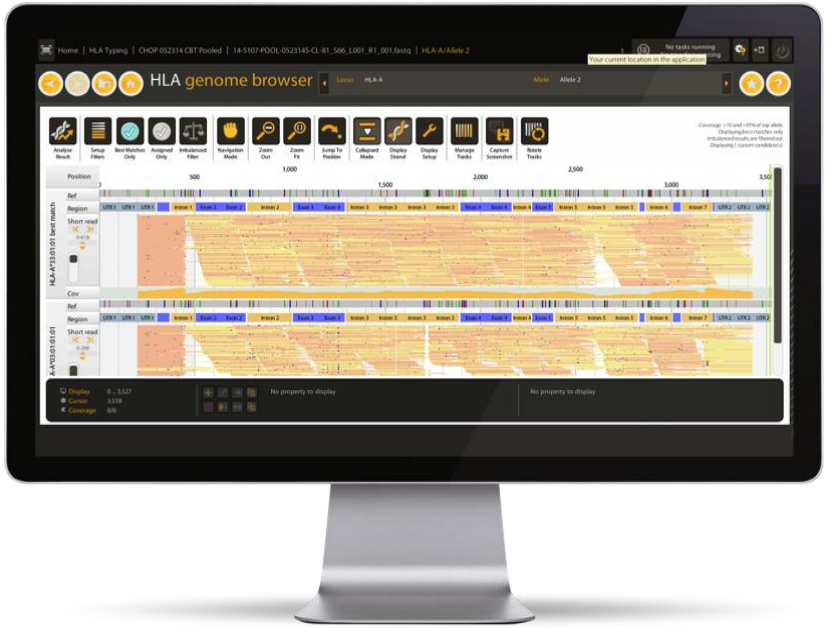
Agenda

- Introducing... **Holotype HLA**
- Early Access Program (EAP) Update
  - Talks from EAP Participants
- Whole Gene Consensus
- What's next for Holotype HLA?
- Q&A

Introduction to Holotype HLA™

Holotype HLA is a combination Assay and Software product for the comprehensive gene amplification of multiple HLA loci, and sequencing on the Illumina MiSeq.

NGS Assay + NGS Software



Omixon Holotype HLA – 7 loci (Assay)

7 loci – HLA-A, B, C, DRB1, DPB1, DQA1 and DQB1

- **X2 – 24/7**
- 24 Indexes

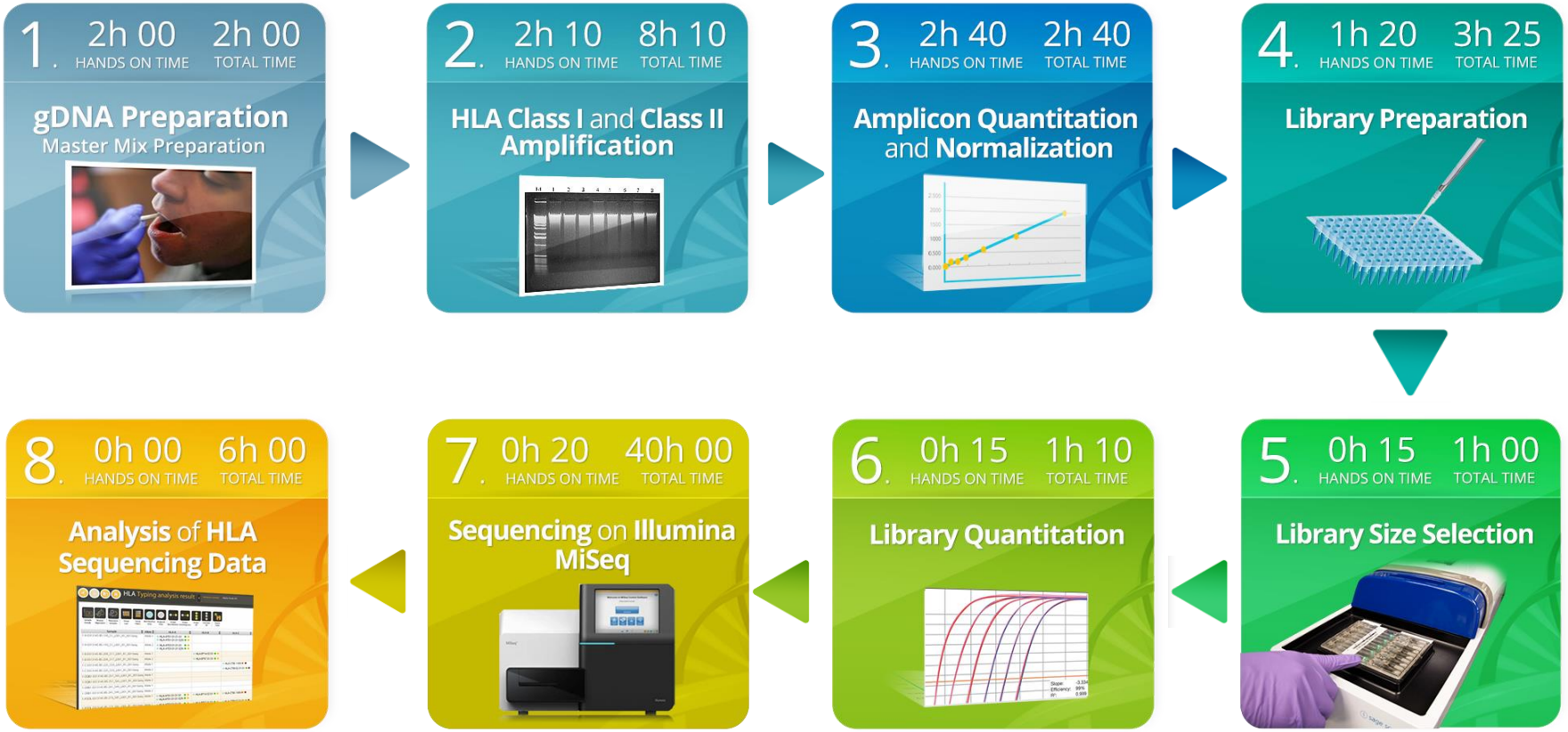
- **X2 – 96/7**
- 96 Indexes

Fully pooled (per sample indexing)

2 genotyping algorithms

Steps in Holotype HLA (7 Loci)

About 9 hours hands-on time for 96 samples
(About 4 hours hands-on time for 24 samples)



Believe The Data

- ASHI validation study at CHOP – 253 samples
- Double blind alpha study – 16 samples, 6 labs each
- Clinical routine – 1000+ clinical samples at CHOP
- Product evaluation study – 200 samples
- Worldwide early access program – 1500+ samples, 25 labs

Early Access Program

- Worldwide Early Access Program (EAP)
- Launched at ASHI 2014 in Denver, CO
- Runs until end of June 2015
- Full results will be presented at ASHI 2015 in Savannah, GA

Early Access Program

- Goals:
  - Reproducibility
  - Feedback and fine tuning
- Scope:
  - 25 participants
  - 13 countries
  - 1500+ samples

EAP – Guest Presenters

- Lydie Brunet
  - Geneva University Hospital

Geneva University Hospital

Overview of the Two Runs

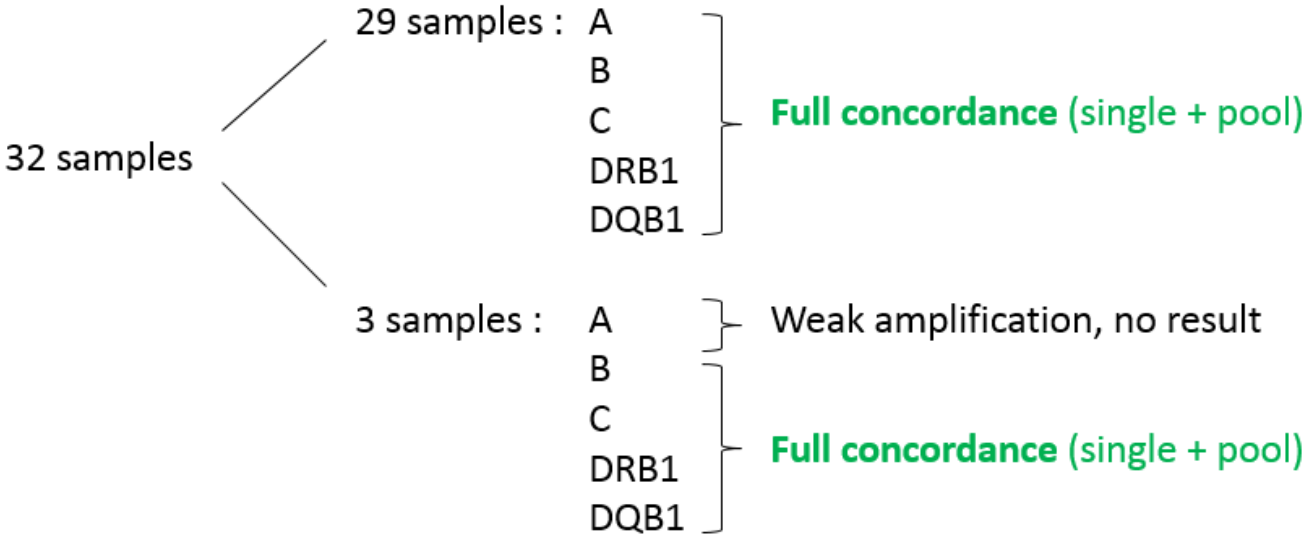
- Holotype HLA X4 configuration ? Single locus analysis + pool analysis
- 5 loci typing (HLA-A, B, C, DQB1, DRB1)
- 16 samples per run ? 32 samples
- Sotware Twin with HLA database v3.17

Geneva University Hospital

Results (1)
Concordance with previous methods

The original results were obtained using molecular typing methods (Luminex SSO, PCR-SSP, mono allelic SBT) → 2nd-field typing results

2nd field typing concordance with NGS (Omixon – Holotype kit):



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Results (2)

Concordance between single locus analysis and pool analysis

With the X4-holotype kit, we obtained sequences from each locus separately and sequences from the pool.

The amount of the processed reads between the locus and the pool are thus different. This leads to some differences in results.

Concordance between single locus analysis and pool analysis according to the allele assignment in Twin:

14 samples : 100% concordance at any field

7 samples : differences at the 3rd-field level

11 samples: differences at the 4th-field level

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Discrepancies (single versus pool)

Specific cases	Locus	Description	Nb of samples
Discrepancy at 3rd-field level (single versus pool)	DRB1	Single: warning for discordant algorithms, DRB1*01:01:05. In the pool: no warning, DRB1*01:01:01	1
Novel allele discrepancies (single versus pool)	DRB1	Discrepancies because of low coverage depth in some exonic regions; in all 3 cases these exonic regions are very similar for the two alleles (e.g. 01:01:01,16:01:01[new])	3
Novel allele discrepancies (single versus pool)	DQB1	Discrepancies because of no or low coverage depth in some exonic regions; in one case the exonic region is very similar for the two alleles	3
Intronic discrepancies (single versus pool)	DRB1	Single : DRB1*15:01:01:01/02/03/04 ; pool is less ambiguous	8
Intronic discrepancies (single versus pool)	DQB1	Single : DQB1*03:01:01:01/03 ; pool is less ambiguous	3

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Conclusions

- 100 % concordance with previous results (2nd-field level).
- Some discrepancies between single and pool (3rd and 4th field level).
- Ongoing analyses with X2 – 7 loci.
- Can be adapted to a clinical lab.

EAP – Guest Presenters

- Amalia Dinou
  - Athens Hellenic Cord Blood Bank, Biomedical Research Foundation of Academy of Athens

EAP – Guest Presenters

- Mette Christiansen
  - Aarhus University Hospital

Aarhus University Hospital

HLA typing by NGS in Aarhus, DK

Tissue typing lab at the Department of Clinical Immunology

- Performs tissue typing in relation to stem cell and organ transplantation.
- Maintains the Danish Bone Marrow Donor Registry

Why try Omixon Holotype HLA X4 ...

- Increasing workload of SBT High-resolution HLA typing
- Alpha-study participation at CHOP
- Easy assay
- High accuracy among 5 different labs

Our experience

- Easy to implement in a lab with no prior NGS
- Hands-on time quite low
- Few equipments needed
- Automation of analysis – time-saving
- Quick trouble-shooting response

Aarhus University Hospital

Accuracy of the assay in our lab

HLA-locus / Run#	1	2	3	4	5*	Average
HLA-A Single	94	100	100	100	100	99
HLA-A Pool	100	100	100	100	100	100
HLA-B Single	100	81	88	100	94	93
HLA-B Pool	100	94	88	100	100	96
HLA-C Single	100	88	56	100	100	89
HLA-C Pool	100	94	94	100	100	98
HLA-DQB1 Single	100	94	81	94	94	93
HLA-DQB1 Pool	100	94	88	94	94	94
HLA-DRB1 Single	100	88	94	100	94	95
HLA-DRB1 Pool	100	94	94	100	94	96
Loci with no typing result	0	1	4¤	0	1	1,2
Loci with discordant typing result	0	0	1	0	1	0,2

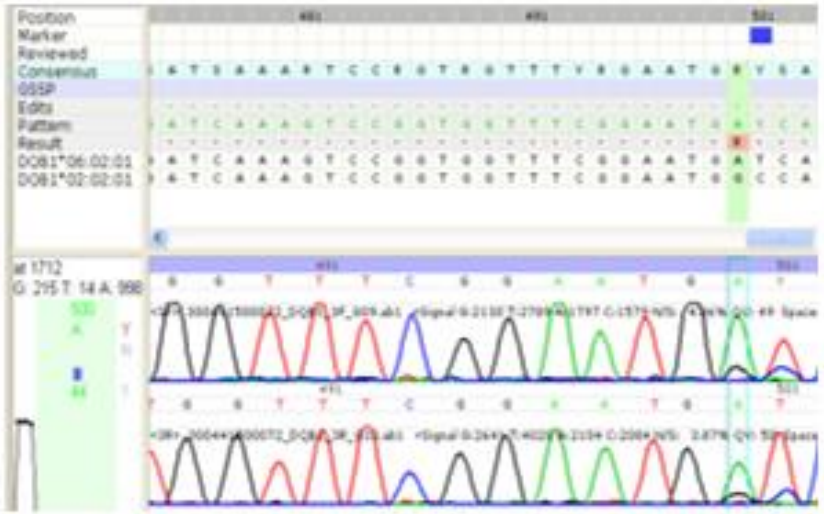
¤ MiSeq run not passed QC. * Repetition of run #3.

sensitivity is 99.4%

specificity is 99.7%.

Aarhus University Hospital

Correct assignment NGS vs. SBT



SBT initially called an A

DQB1*02:01:01, DQB1*06:02:01

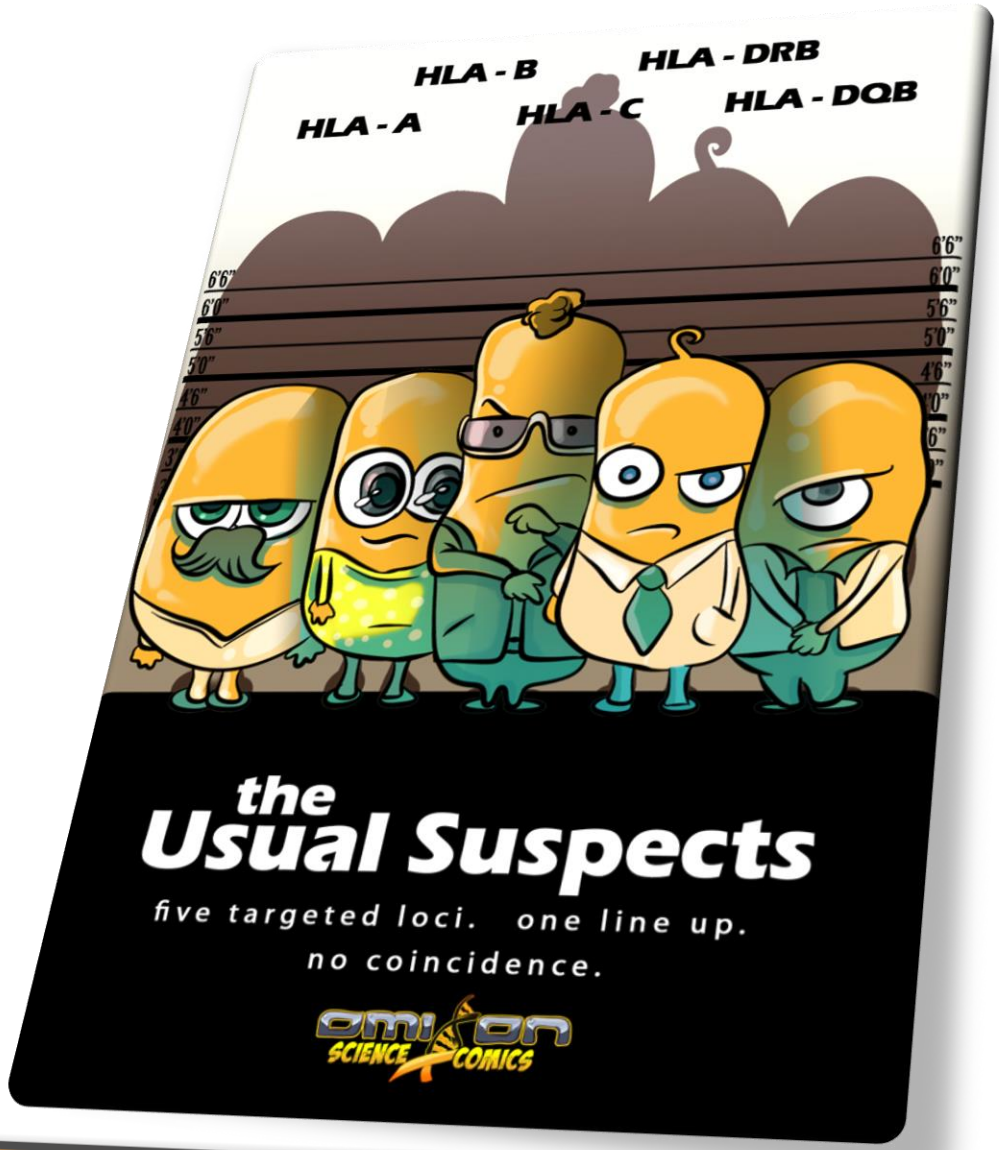
NGS finds 145 A's and 102 G's

DQB1*02:02:01, DQB1*06:02:01

Early Access Program - Update

- Main changes to Holotype HLA during EAP:
  - 2 more loci added
  - Full pooling of all loci by default
  - HLA Twin Software updated, new features added
- HLA Twin – new features
  - Locus-based subsampling
  - HML export
  - Whole gene consensus

The Usual Suspects



Justice League

EAP – Preliminary Results

- Most of the labs involved in the EAP had never seen the Holotype HLA protocol before
- All of the labs involved have been asked to use known samples, with known HLA types
- The results are for 5 locus data - we will also present 7 locus data and data from all 25 labs at ASHI
- These results are for 193 samples, from 3 labs
- Results are from fully pooled data only

EAP – Preliminary Results

- 193 samples, 1930 alleles
- 1.7% locus drop-out (no result for either allele)
  - Usual cause: FTA (Failure To Amplify)

Locus	Total alleles (with known types)*	Unique alleles
HLA-A	381	46
HLA-B	383	63
HLA-C	361	34
HLA-DQB1	337	18
HLA-DRB1	347	40

- * = some known types were missing

Concordance

Set	HLA-A	HLA-B	HLA-C	HLA-DQB1	HLA-DRB1
a_1	100% (1)	100%	100%	93.75%*	100%
a_2	100%	100%	100%	100%	100%
a_3	98.4%*	100%	100%	100%	100%
b_1	100% (1)	100%	100%	100%	92.8%
b_2	100%	100%	100%	100%	100%
b_3	100%	98.8% (1)	100%	100%	99.4%*
c_1	100%	100%	100%	93.75%* (2)	100% (1)
c_2	100%	100%	100%	100% (2)	100% (1)
Total	99.7%	99.7%	100%	98.9%	99.4%

- Concordance = 2 field concordance
- * = incorrect 'known' typing, (x) = number of novel alleles
- Red = genuine discordance

Ambiguity

Number of loci	HLA-A	HLA-B	HLA-C	HLA-DQB1	HLA-DRB1
Unambiguous	115	175	160	167	111
Cis/trans phase ambiguity	1 (1)	0	2 (0)	2 (1)	0
4th field ambiguity	74	17	23	24	69
3rd field ambiguity	0	0	0	0	6 [a]
2nd field ambiguity	0	1 [b]	0	0	2 [c]
1st field ambiguity	0	0	0	0	0

- a = alleles only have 3 field definition and differ in off-target exon sequence
- b = known issue with the software
- c = difference between the two alleles is in off-target exon 1
- (x) = number of cis/trans ambiguities remaining after reanalysis with more data

Result Statistics

Locus	HLA-A	HLA-B	HLA-C	HLA-DQB1	HLA-DRB1
Typed alleles	381	383	361	337	347
Sensitivity	100%	99.74%	100%	100%	100%
Specificity	100%	100%	100%	100%	99.98%
PPV	100%	99.74%	100%	100%	99.28%
NPV	100%	100%	100%	100%	100%
TCC	100%	99.9%	100%	100%	99.98%
Incorrect known typings	1	-	-	3	1

- PPV = Positive Predictive Value
- NPV = Negative Predictive Value
- TCC = Type Correctly Classified (TP + TN / N)

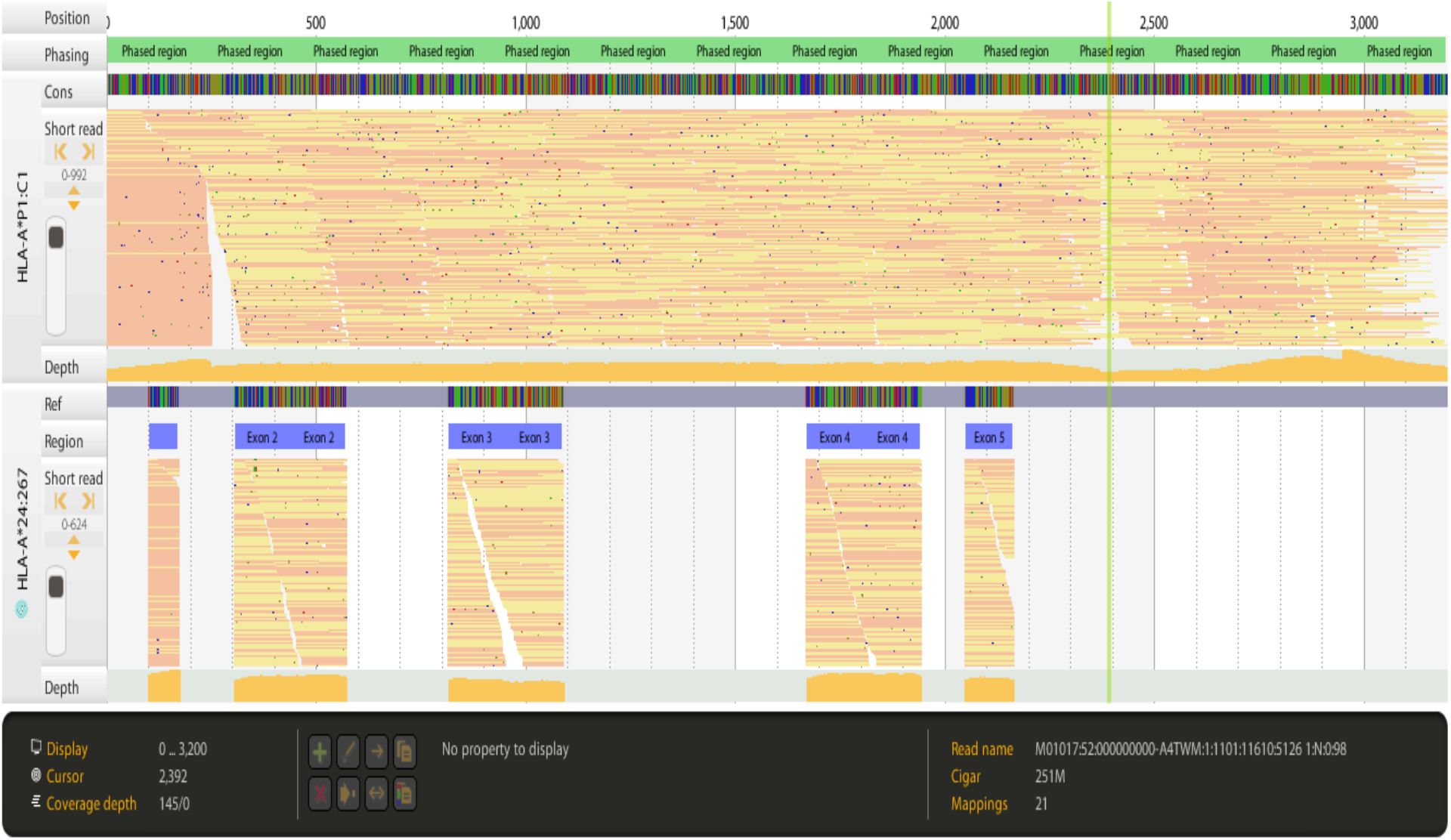
EAP – “Real Life”

- Whole uncovered plate dropped on the floor just before sequencing = 1
- Lab tech turned off thermal cycler on way out of the lab = 1
- Nano flow cell used by mistake = 1
- Lab director nominated themselves for the first ever hands-on run through of the protocol = 1
- Omixon's CEO visits lab during training = 2
  - Protocol fails completely = 2

Whole Gene Consensus

- HLA Twin generates consensus sequences for both alleles across the whole region covered
  - Independent of IMGT/HLA database
  - Whole gene for HLA-A, B, C, DQB1, DQA1
- Complete picture
- Unparalleled novel allele detection
- Future proof

Whole Gene Consensus – Example



The Other Allele

Holotype HLA – Next Steps

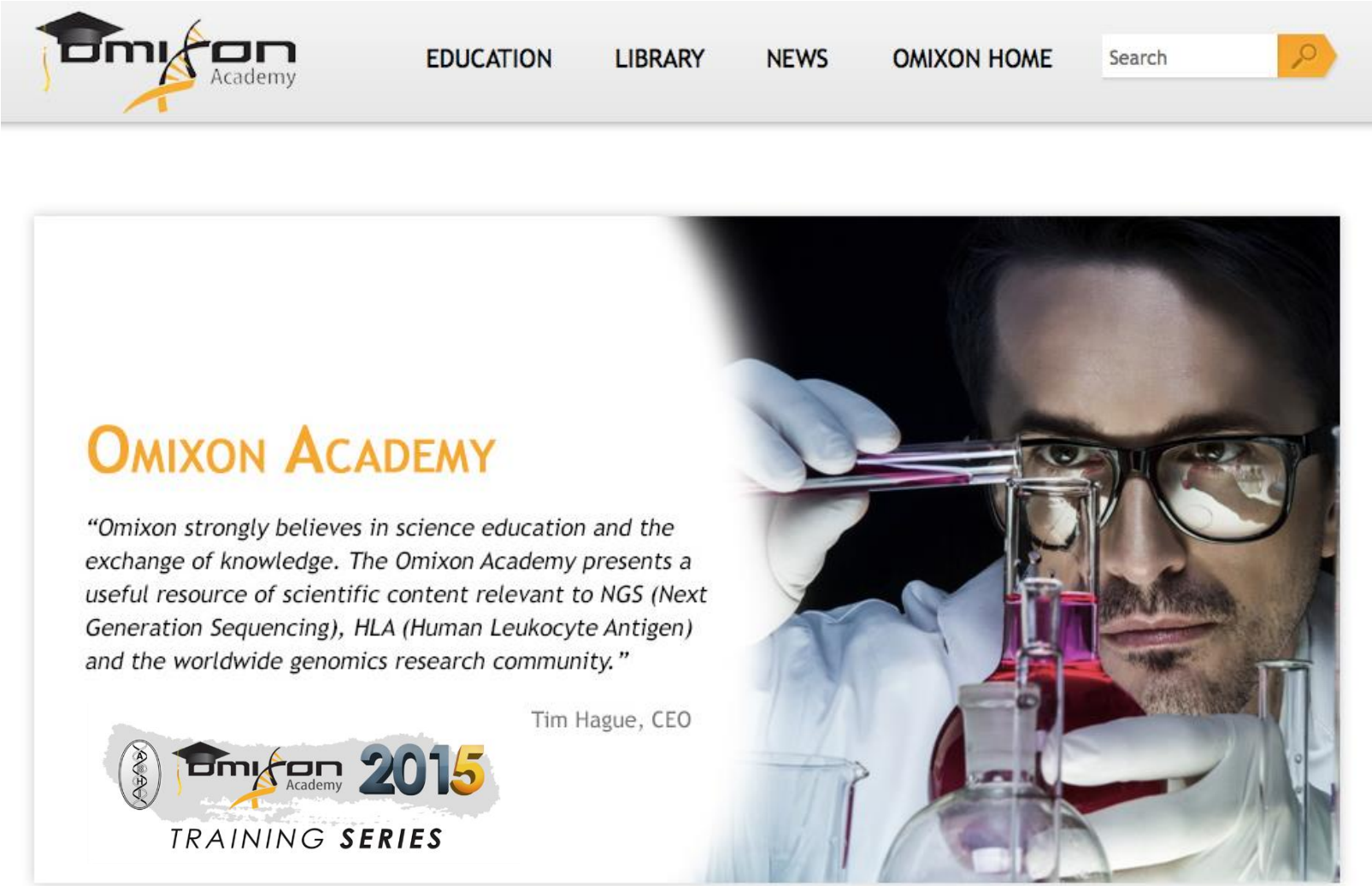
- DRB3/4/5
- European CE mark
- More optimizations to the assay protocol
- More optimizations to the software
- Automation
- 288 samples per MiSeq run

Announcements

- Competitor Special
  - Free HLA Twin analysis for all evaluations of competing kit products
  - No strings attached
- 2-4-1 Special
  - Two X2 kits (either 24 or 96 sample) for the price of one, until the end of May

The Omixon Website

The Omixon Academy



Questions?

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OMIXON.COM

LR-PCR Amplification Strategy

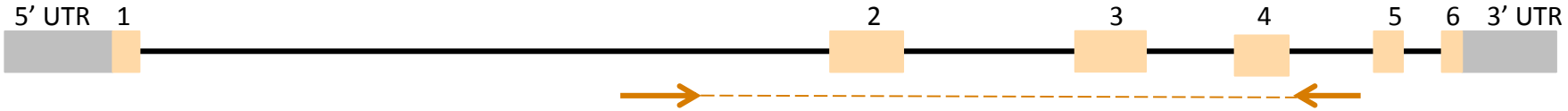
Full gene characterization

HLA-A, B, C, and DQA1 (Example is HLA-B)

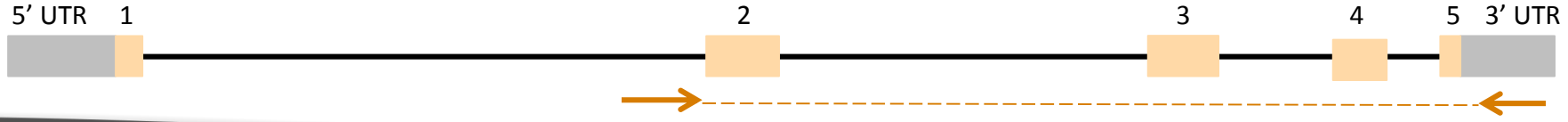
HLA-DQB1

Key region characterization

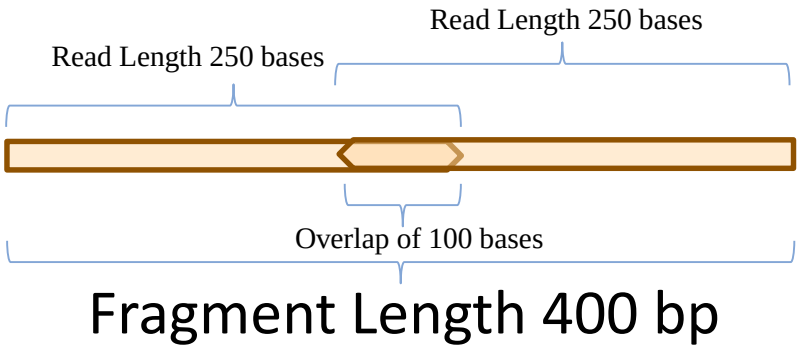
HLA-DRB1

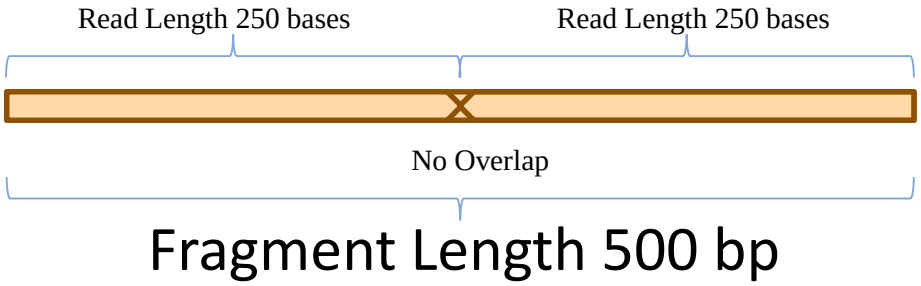


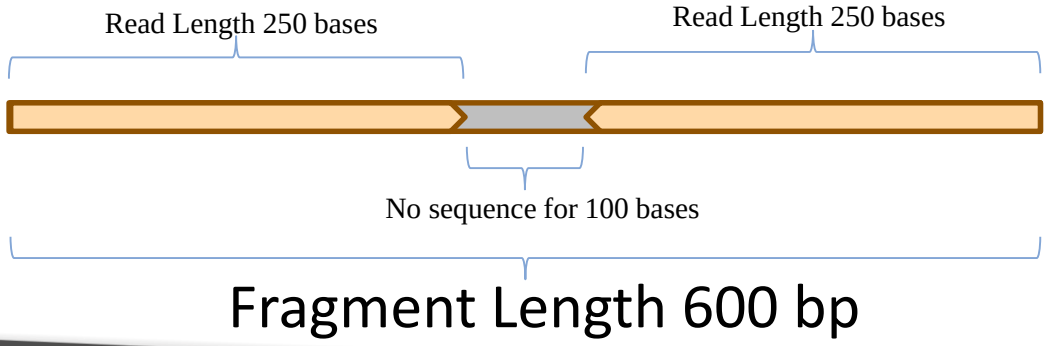
HLA-DPB1



Effect of Varying Insert Sizes among Paired Reads



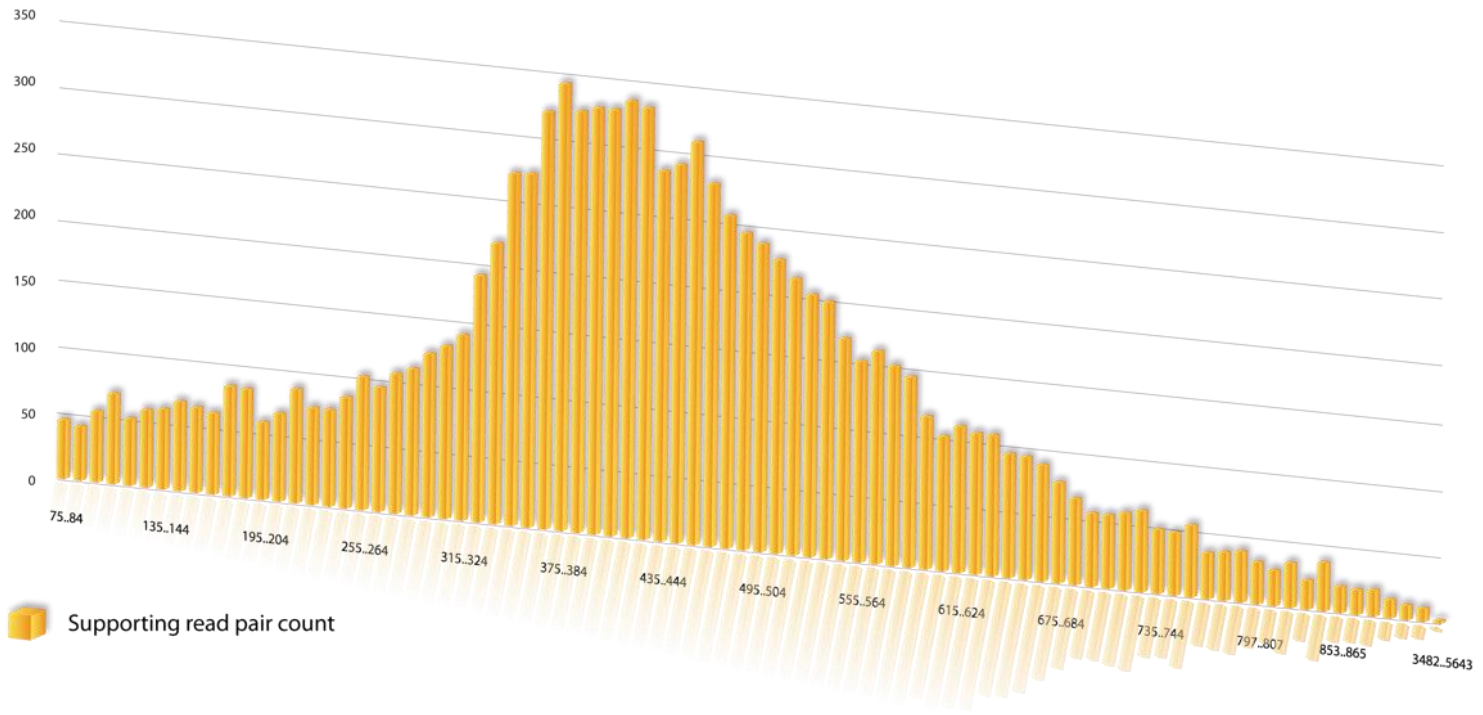




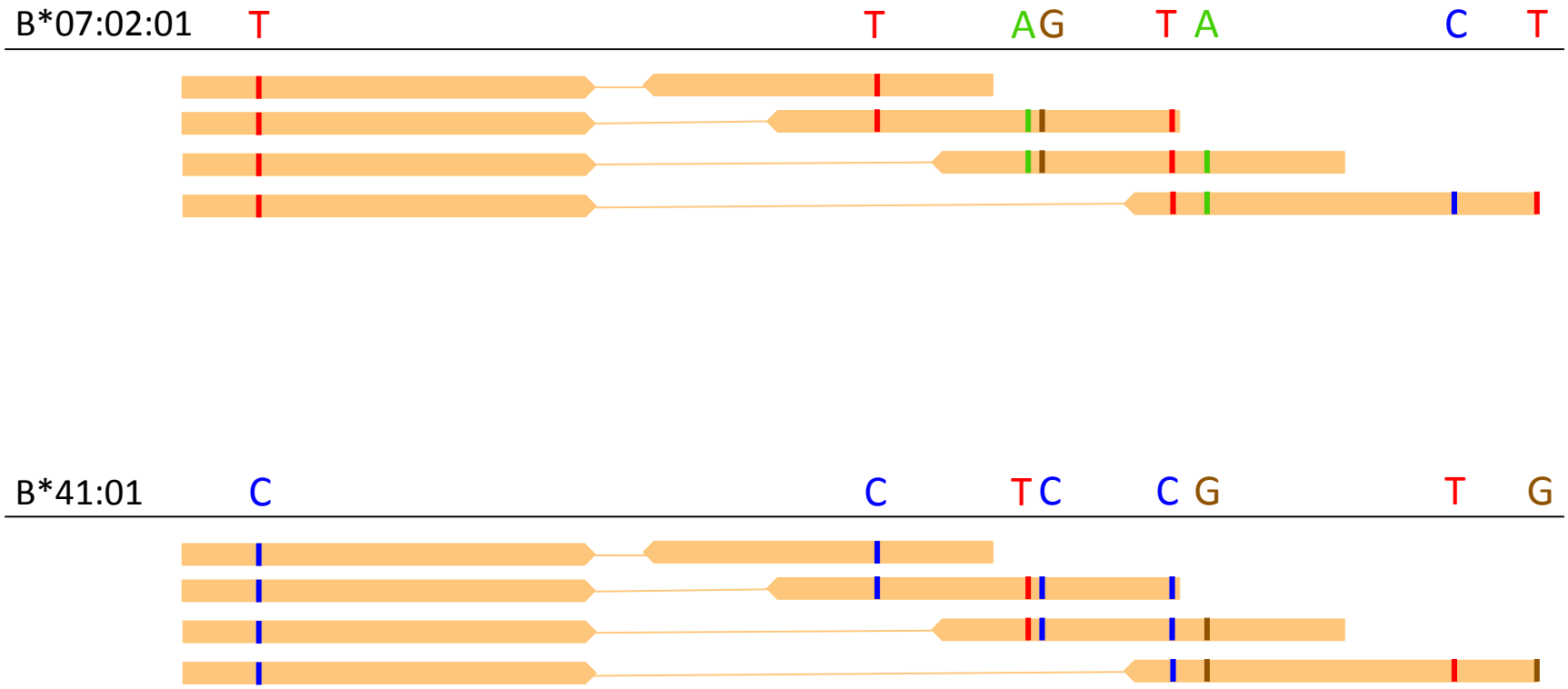
Optimized Size Selection

Fragment Size

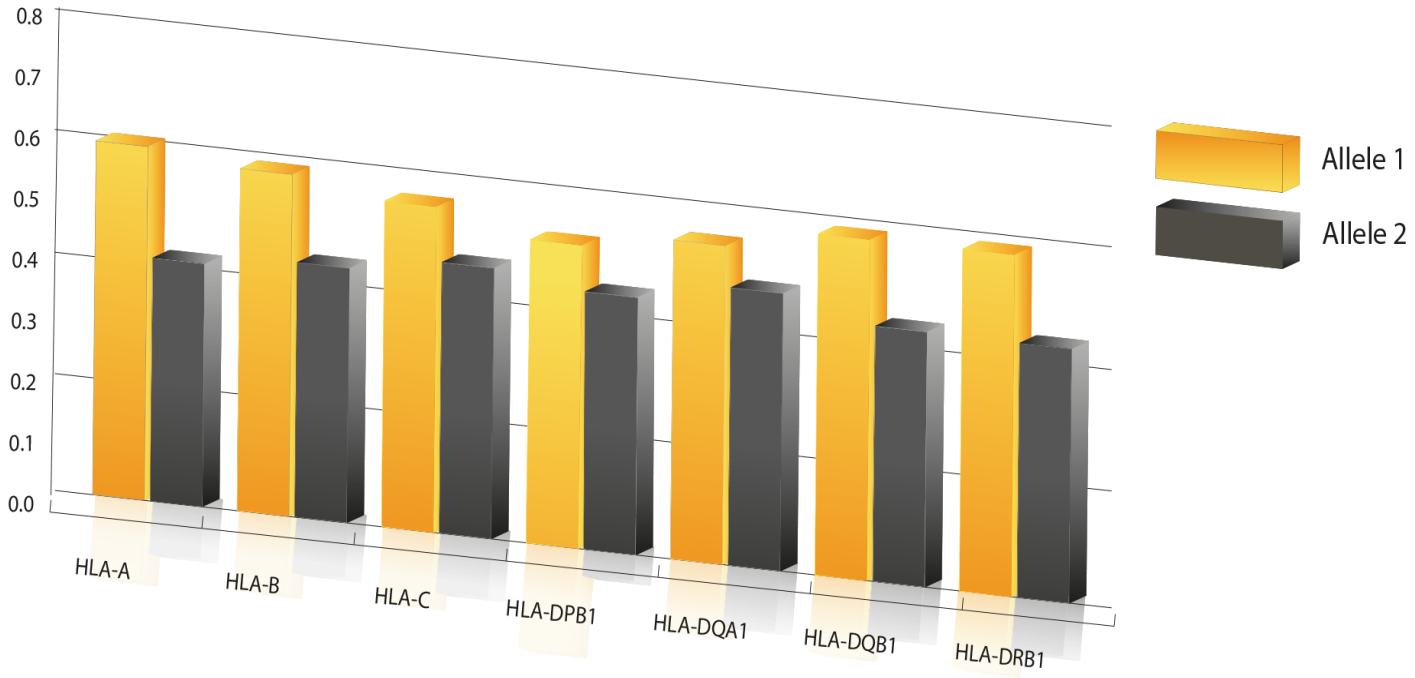
Number of read pairs referring to fragments within the specified length interval



Long Distance Phasing



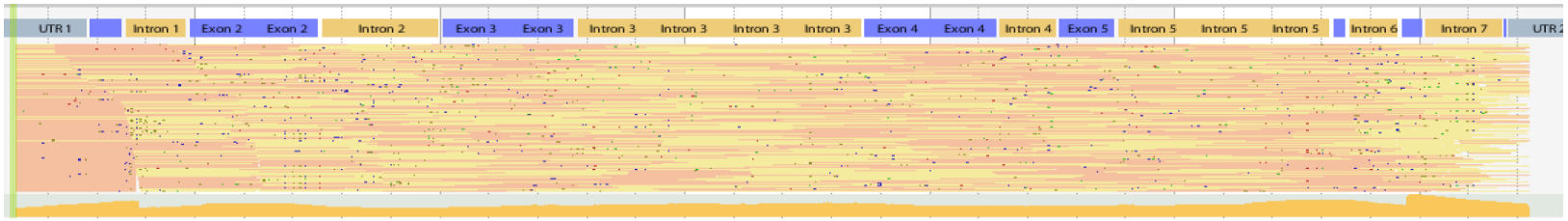
Allelic Balance



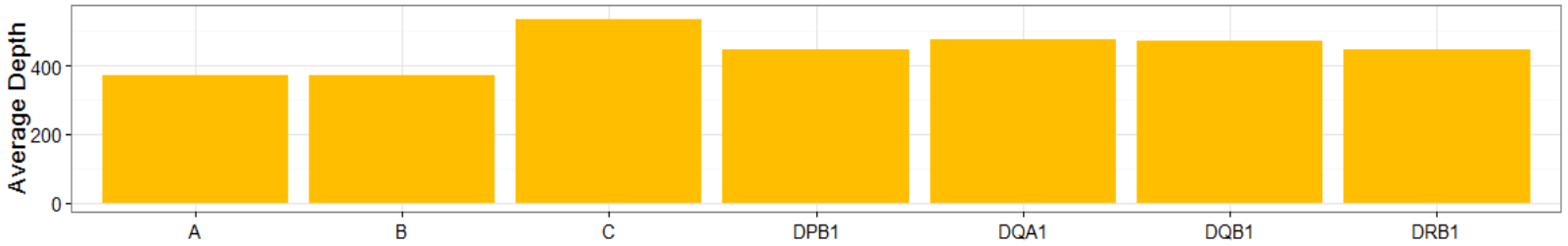
The optimized assay design achieves allelic balance across all loci for accurate HLA allele determination without bias towards easy-to-sequence genomic regions.

Even Coverage

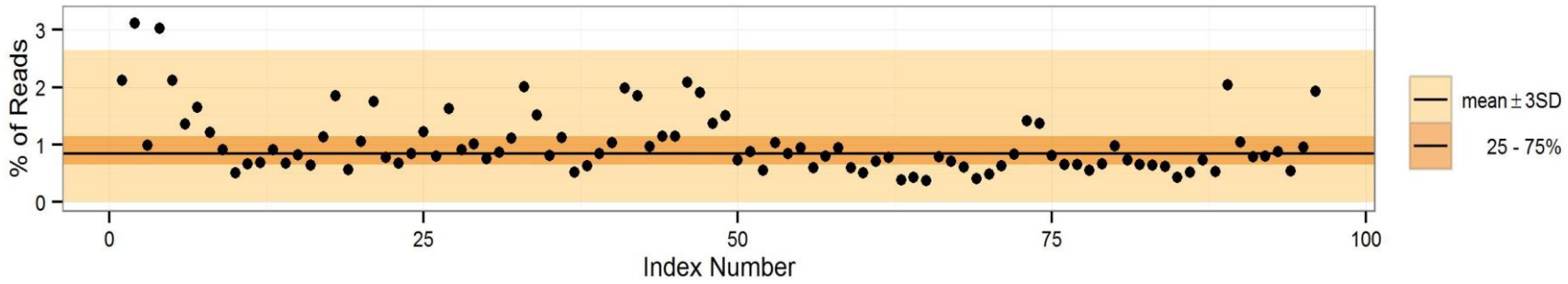
Across a locus



Across loci within a sample



Between samples



Omixon HLA Twin™ (Software)

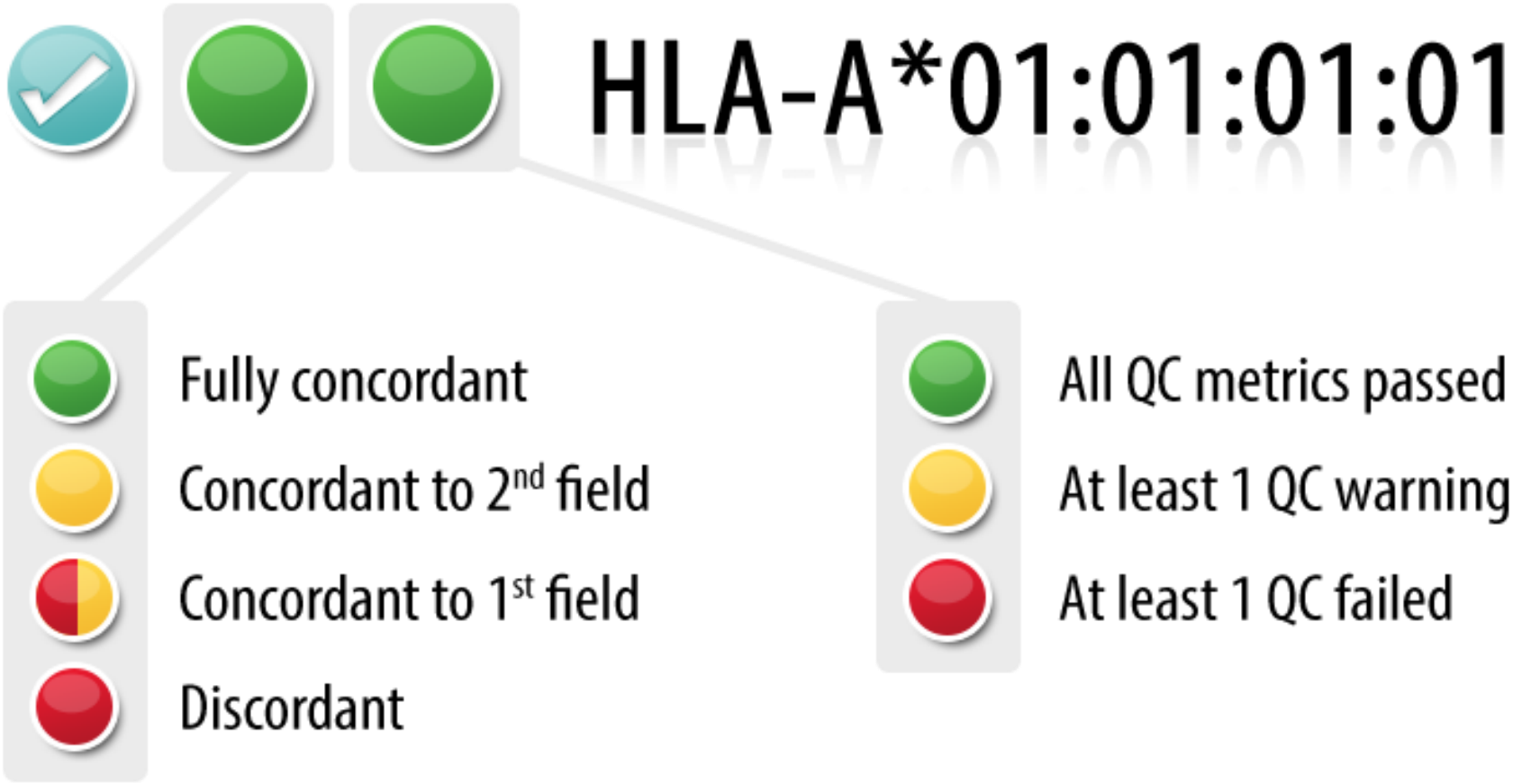
- Preconfigured Holotype HLA-specific settings
- Automated genotyping after MiSeq run
- Two algorithms for determining HLA genotypes
  - Consensus Genotyping (Assembly)
  - Statistical Genotyping (Alignment to IMGT/HLA)
- Traffic light system for data interpretation and workflow management

PASSED

WARNING

FAILED

The Traffic Light System



Benefits of Holotype HLA Assay

- LR-PCR for fully characterized loci
- Easy Library Preparation
- Variable-insert paired-end sequencing for phasing
- High-throughput NGS on a MiSeq
- Unambiguous genotyping
- No reflexive testing

Benefits of HLA Twin Software

- Accurate genotyping with two orthogonal algorithms
- No manual intervention for good quality data
- Informative quality control metrics for confidence in genotyping results
- Traffic Light System for easy interpretation
  - Novel allele detection
  - Null alleles always resolved (splice variant and intronic)
- Export genotypes or consensus sequence for reporting

Holotype HLA vs Competitors

Competing Products	Holotype HLA
Occasional allele dropout	Very rare allele dropout
6+ hours hands on time	About 4 hours hands on time (24 samples)
Up to 24 samples per run	Up to 96 samples per run
Uneven coverage, dips in coverage at key exons	Deep & even coverage across every exon for each locus
Allele imbalance	Balanced coverage depth across loci; and for both alleles at each locus
One algorithm, requires technician for every allele call	Fully automated genotyping with two algorithms
High rates of ambiguity	0.2% ambiguity (based on 5 loci)