

AIBT

Associazione Italiana
di Immunogenetica
e Biologia dei Trapianti

Roma 19 novembre 2019

Giornata di formazione AIBT: NGS nel laboratorio di Istocompatibilità

ACCREDITAMENTO EFI E NGS

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Considerazioni preliminari

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E5	CLINICAL APPLICATIONS
E5.1	Renal and/or Pancreas Transplantation
E5.2	Other Organ Transplantation
E5.3	Haematopoietic Stem Cell Transplantation
E5.4	Transfusion
E5.5	Disease Association

Livello risoluzione dei risultati

Valutazione economica

Valutazione attività

Valutazioni strutturali

Apparecchiature

Considerazioni preliminari

C1.3.2.1 Validation/verification, before introduction into routine use, of all new tests, by systematic comparative evaluation of results obtained in parallel with the new and the standard system

Per la realizzazione del progetto è necessario una valutazione complessiva

D1.4	EPT samples must be
D1.4.1	Tested by the same techniques as routinely employed for clinical samples, either individually or in combination
D1.4.2	Interpreted in a manner comparable to routine clinical samples

E4.5	Nucleic Acid Analysis
E4.5.1	Nucleic acid extraction
E4.5.1.1	The method used for nucleic acid extraction:
E4.5.1.1.1	Must be published and documented
E4.5.1.1.2	Must be validated in the laboratory
E4.5.1.2	Purity and concentration of Nucleic Acids:
E4.5.1.2.1	Must be sufficient to ensure reliable test results



B5.1	The Director/Co-Director or designee must:
B5.1.1	Evaluate the competence of each technologist to accurately perform tests. This must be done at least yearly for each technique the technologist performs and must be based on a defined process
B5.1.2	Maintain records of these evaluations for each individual

B5.2 The Laboratory Director and the technical staff must participate in continuing education relating to each category for which EFL accreditation is sought



Considerazioni preliminari

Per la realizzazione del progetto è necessario una valutazione complessiva

C2.1.3	Laboratories performing amplification of nucleic acids must use:
C2.1.3.1	A dedicated work area with restricted traffic flow
C2.1.3.2	Physical and/or biochemical barriers to prevent DNA contamination, including the use of dedicated
C2.1.3.2.1	Equipment
C2.1.3.2.2	Laboratory coats
C2.1.3.2.3	Disposable supplies
C2.1.4	Pre-amplification procedures must be performed in an area which excludes amplified DNA that has the potential to serve as a template for amplification in any of the genetic systems tested in the laboratory
C2.1.5	All activities occurring from and including thermal cycling must take place in the post-amplification area

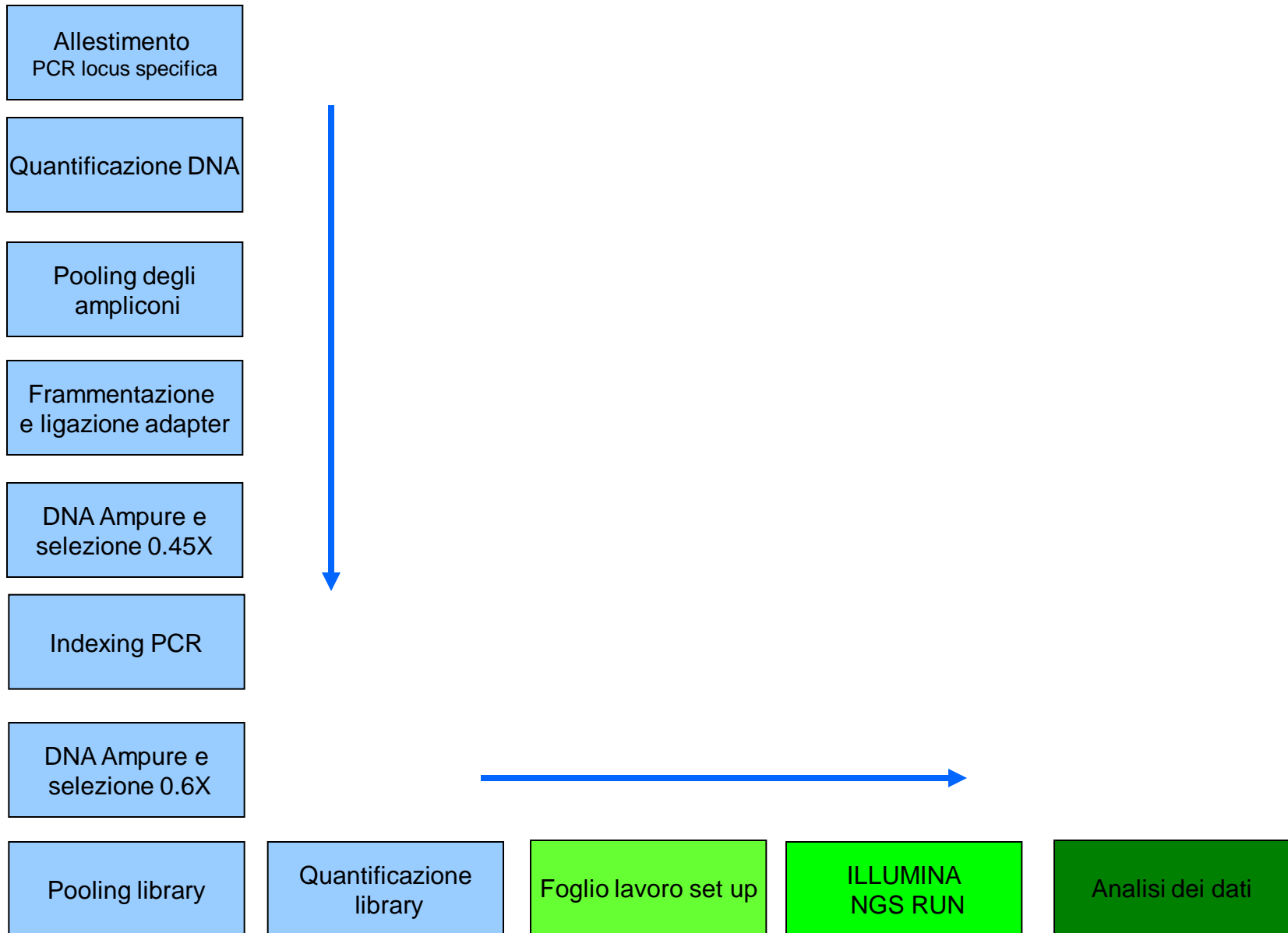
Considerazioni preliminari

Per la realizzazione del progetto è necessario una valutazione complessiva per stabilire le probabilità di successo

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C2.2	Equipment
C2.2.1	The laboratory must establish and employ policies and procedures for the proper maintenance of equipment, instruments and test systems by:
C2.2.1.1	Defining its preventive maintenance programme for each instrument and piece of equipment at least once a year
C2.2.1.2	Performing and documenting function checks on equipment with at least the frequency specified by the manufacturer
C2.2.1.3	The laboratory must use calibrated dispensing instruments (e.g. pipettes, etc.) to perform assays
C2.2.1.3.1	Calibration of dispensing instruments must be performed at least once a year
C2.2.1.3.2	Calibration must be documented

Processo di tipizzazione in NGS



Processo di tipizzazione in NGS e Standard EFI

Allestimento
PCR locus specifica

Quantificazione DNA

E4.10 Next Generation Sequencing

E4.10.1 Sequencing Templates:

E4.10.1.1 Must have sufficient purity, specificity, quantity and quality to provide interpretable sequencing data

Dna Ampure e

E4.10.4 PCR artefacts must be documented

E4.10.4.1 The information must be used in the routine interpretation of data following established policies. (i.e. PCR cross-over and/or artefact)

Dna Ampure e
selezione 06X

Pooling library

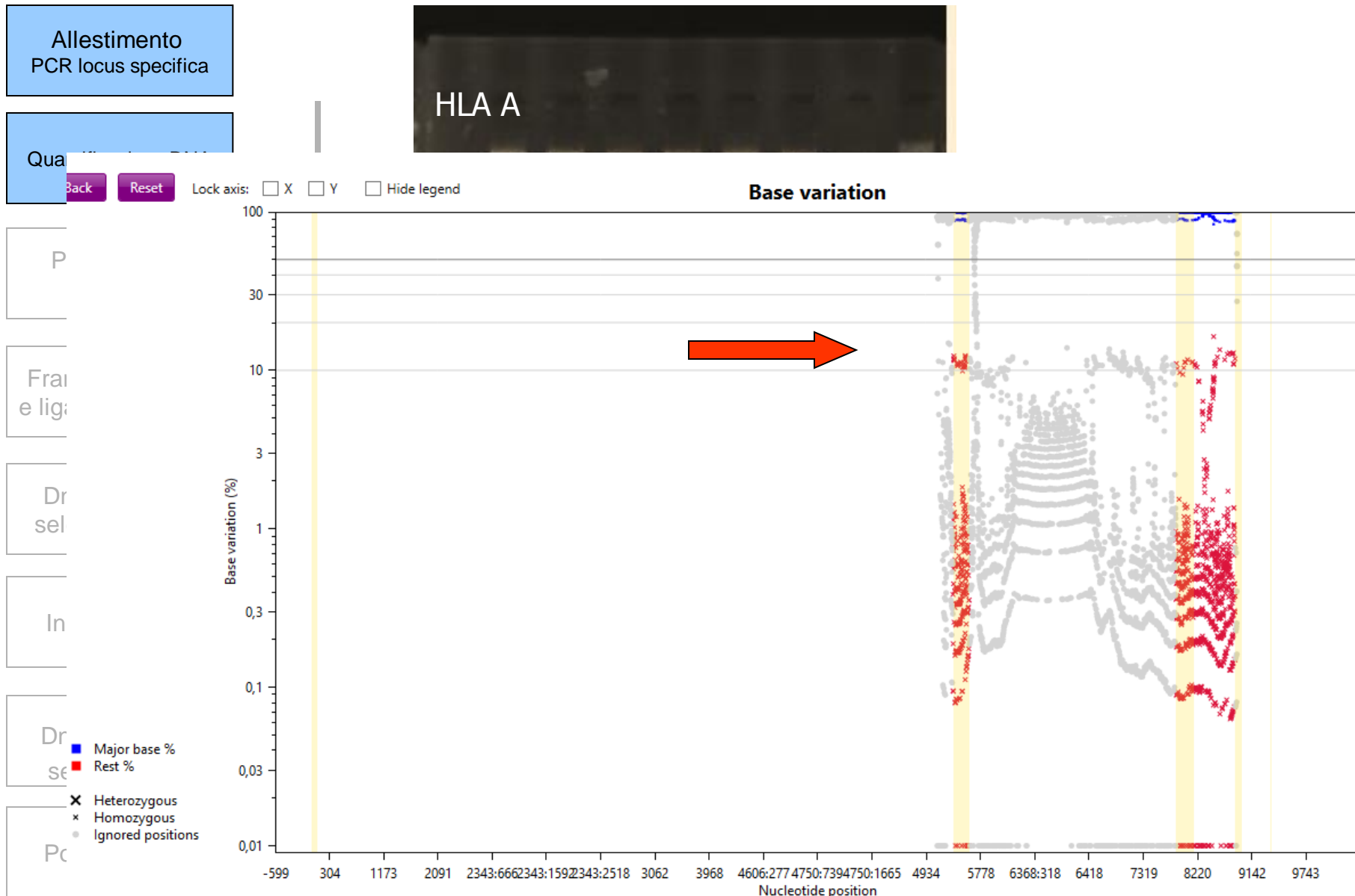
Quantificazione
library

Foglio lavoro set up

ILLUMINA
NGS RUN

Analisi dei dati

Processo di tipizzazione in NGS e Standard EFI





Processo di tipizzazione in NGS e Standard EFI

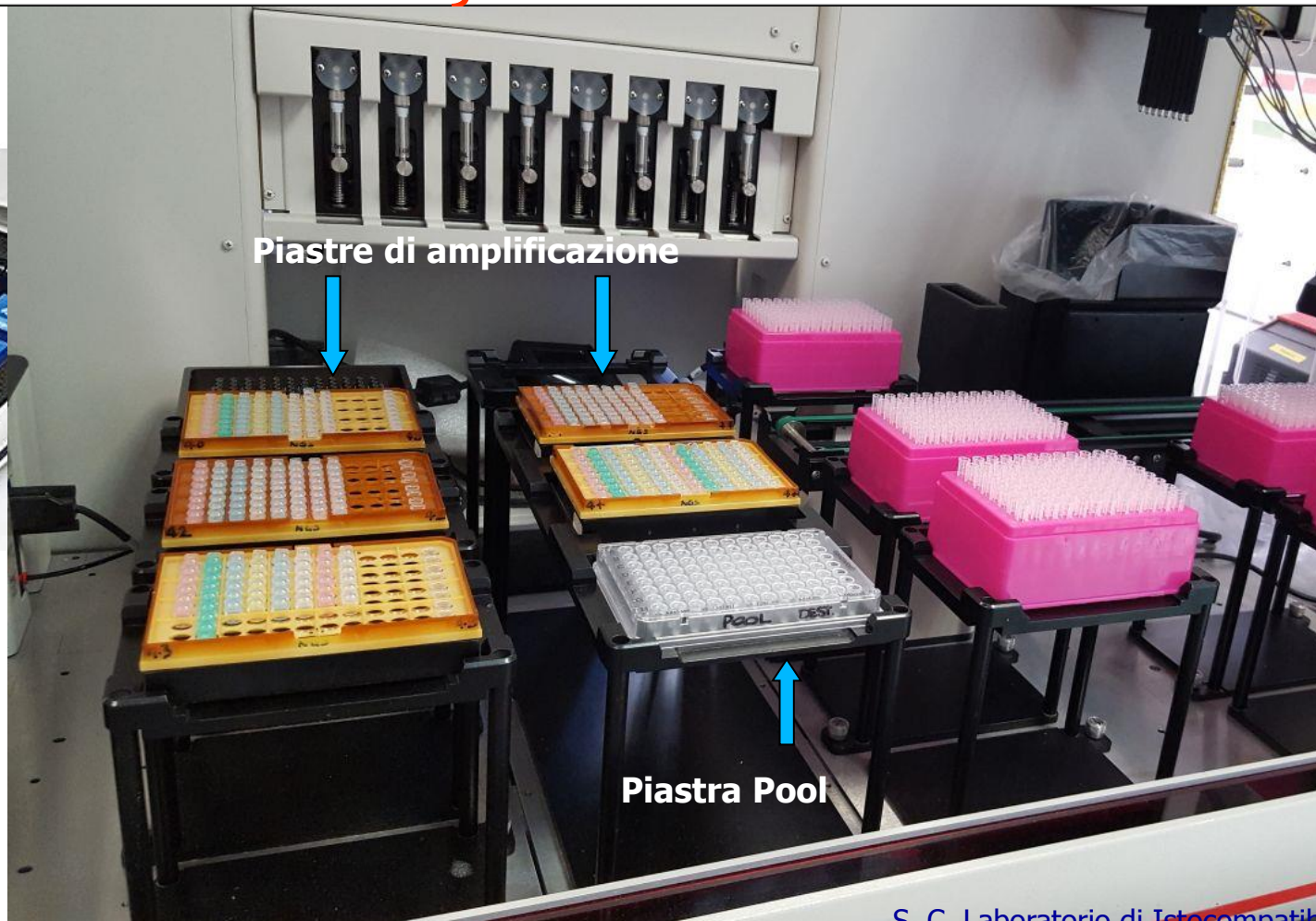
E4.10.2	The following must be documented:
E4.10.2.1	Sample tagging
E4.10.2.2	Purification
E4.10.2.3	Normalization
E4.10.2.4	Pooling methods

Pooling degli



Dna Ampure e
selezione 06X

Pooling library



Processo di tipizzazione in NGS e Standard EFI

E4.10.6	If shotgun sequencing is used
E4.10.6.1	Method of fragmentation must be specified
E4.10.6.2	For each run the size of fragments must be documented and the selection must be specified

Quantificazione DNA

Pooling degli

Le dimensioni dei frammenti sono critici per il successo della sequenza NGS, la frammentazione può essere

C1.1

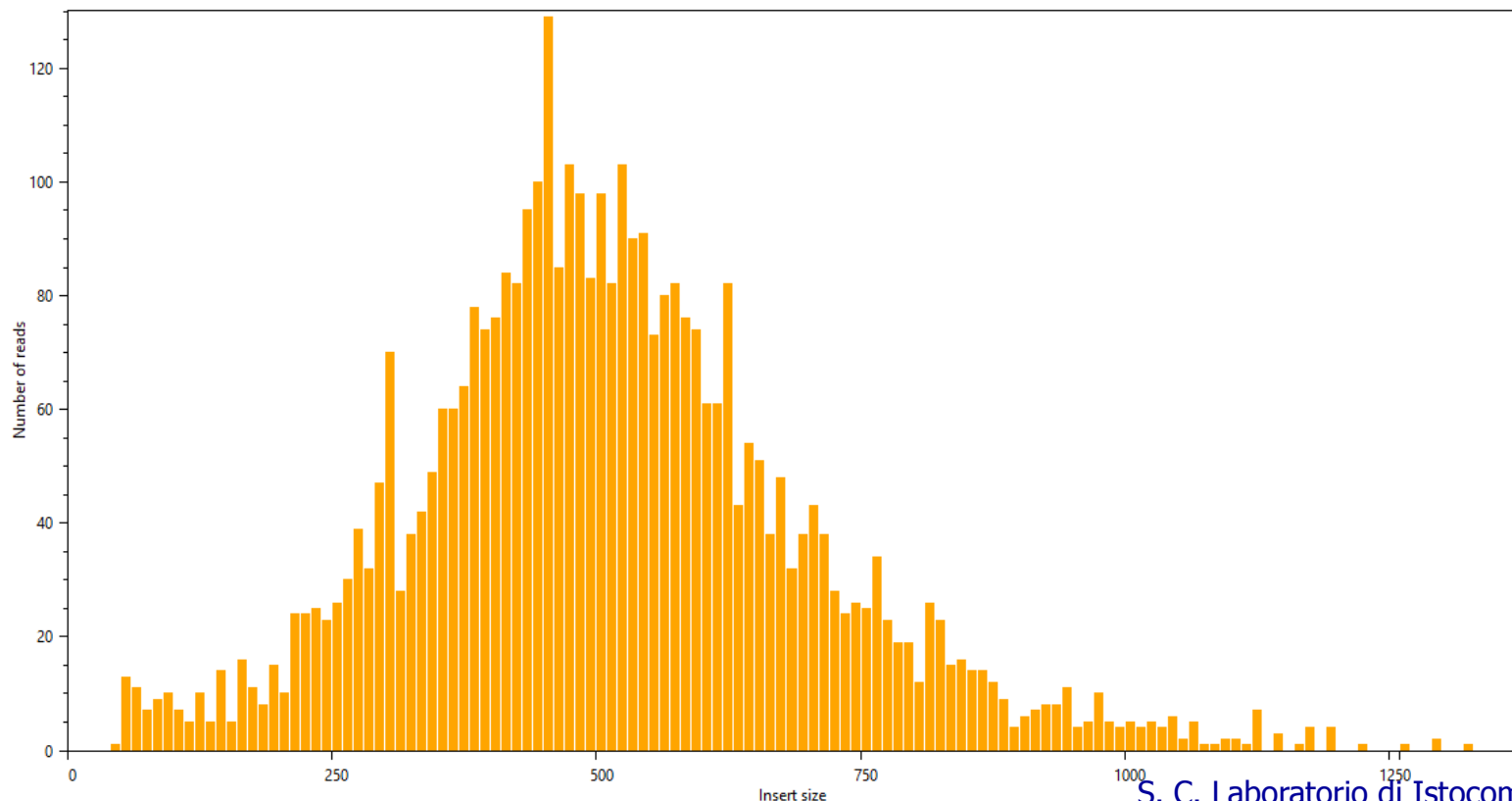
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Insert sizes



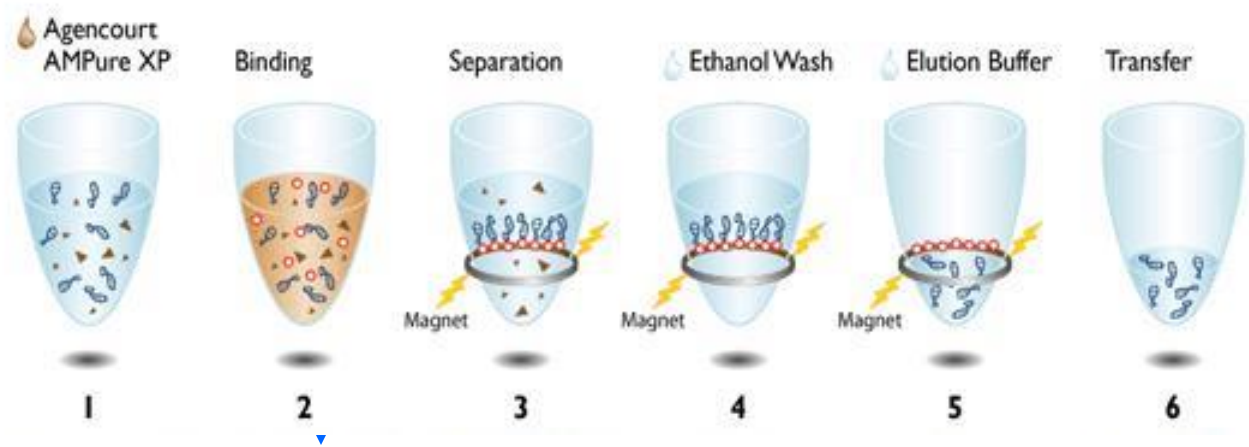
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Processo di tipizzazione in NGS e Standard EFI

Allestimento
PCR locus specifici

E4.10.2	The following must be documented:
E4.10.2.1	Sample tagging
E4.10.2.2	Purification ←
E4.10	
E4.10	



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Indexing PCR

Dna Ampure e
selezione 06X

Pooling library

Quantificazione
library

Foglio lavoro set up



Processo di tipizzazione in NGS e Standard EFI

Allestimento
PCR locus specifica

E4.10.5 Controls and procedures must be established to ensure sample tracking during pooling and barcoding

E4.10.5.1 Periodic barcode rotation is recommended to detect contamination. If contamination is detected Standard E4.5.4 must be followed

Data: 12/09/19

Nome piastra: 29M

Tecnico: CI

INDICI		706	707	708	709	710	712	711					
		1	2	3	4	5	6	7	8	9	10	11	12
501	A	201902559	201902577	201902572	201902590	201902585	201902604	201902554	0	0	0	0	0
502	B	201902567	201902562	201902580	201902599	201902593	201902588	201902556	0	0	0	0	0
503	C	201902575	201902570	201902565	201902583	201902602	201902596	201902557	0	0	0	0	0
504	D	201902560	201902578	201902573	201902591	201902586	201902605	201902629	0	0	0	0	0
505	E	201902568	201902563	201902581	201902600	201902594	201902589	201902630	0	0	0	0	0
506	F	201902576	201902571	201902566	201902584	201902603	201902598	0	0	0	0	0	0
507	G	201902561	201902579	201902574	201902592	201902587	201902551	0	0	0	0	0	0
508	H	201902569	201902564	201902582	201902601	201902595	201902552	0	0	0	0	0	0

Dna Ampure e
selezione 06X



Pooling library

Quantificazione
library

Foglio lavoro set up

ILLUMINA
NGS RUN

Analisi dei dati

Processo di tipizzazione in NGS

E4.10.9.8

Each sample processed must be traceable through the whole process including data analysis and reporting

Experiment Name	2021_29M				
Date	09/12/2019				
Workflow	GenerateFASTQ				
Application	FASTQ Only				
Chemistry	Amplicon				
Sample_ID		I7_Index_ID	index	I5_Index_ID	index2
2019/2559		IN-IL-706	CTGCGTAG	IN-IL-501	ATCGTACG
2019/2567		IN-IL-706	CTGCGTAG	IN-IL-502	ACTATCTG
2019/2575		IN-IL-706	CTGCGTAG	IN-IL-503	TAGCGAGT
2019/2560		IN-IL-706	CTGCGTAG	IN-IL-504	CTGCGTGT
2019/2568		IN-IL-706	CTGCGTAG	IN-IL-505	TCATCGAG
2019/2576		IN-IL-706	CTGCGTAG	IN-IL-506	CGTGAGTG
2019/2561		IN-IL-706	CTGCGTAG	IN-IL-507	GGATATCT
2019/2569		IN-IL-706	CTGCGTAG	IN-IL-508	GACACCGT
2019/2577		IN-IL-707	TAGTCTCC	IN-IL-501	ATCGTACG
2019/2562		IN-IL-707	TAGTCTCC	IN-IL-502	ACTATCTG
2019/2570		IN-IL-707	TAGTCTCC	IN-IL-503	TAGCGAGT
2019/2578		IN-IL-707	TAGTCTCC	IN-IL-504	CTGCGTGT
2019/2563		IN-IL-707	TAGTCTCC	IN-IL-505	TCATCGAG
2019/2571		IN-IL-707	TAGTCTCC	IN-IL-506	CGTGAGTG

library

NGS RUN

Processo di tipizzazione in NGS e Standard EFI

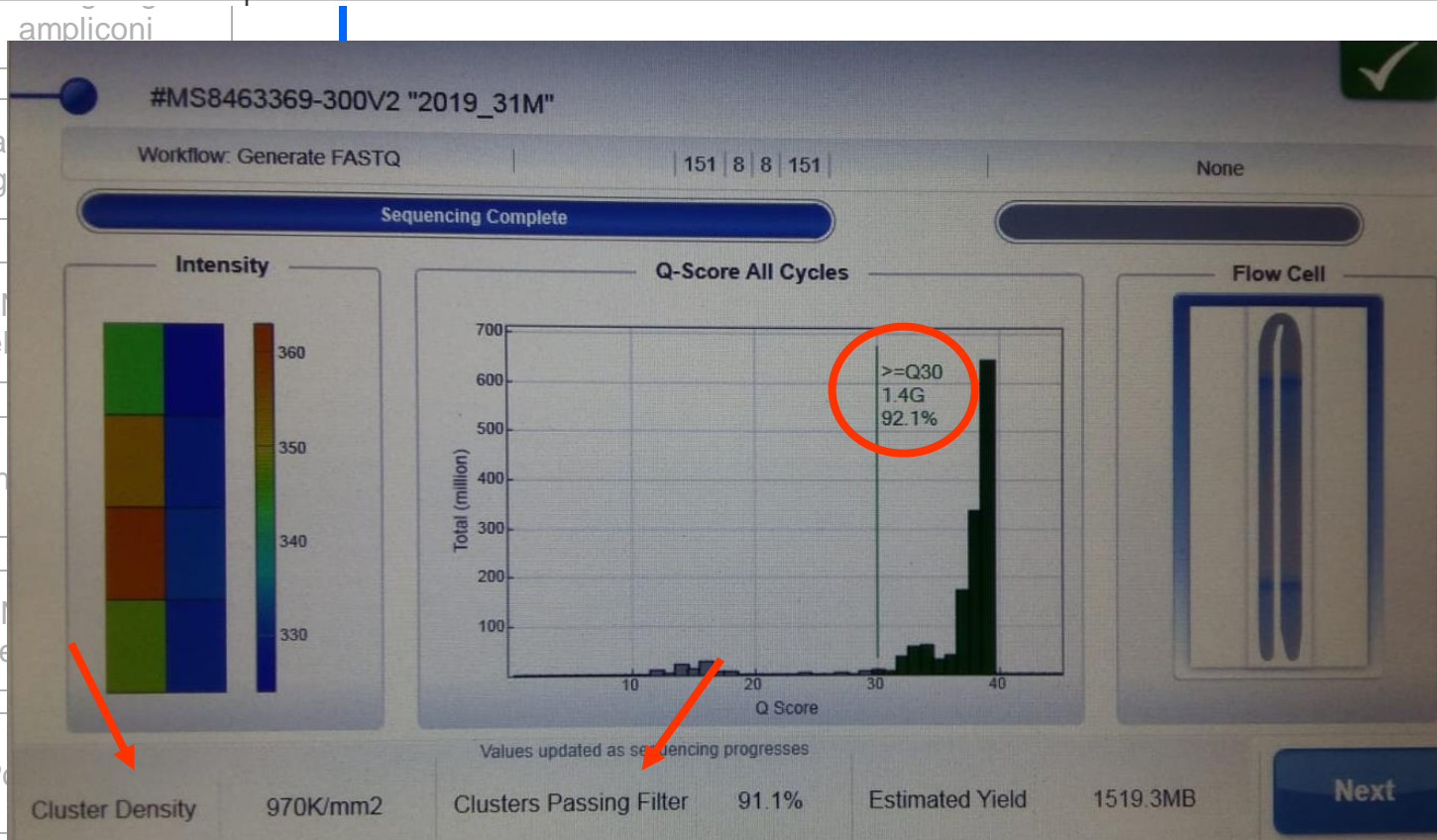
E4.10.2	The following must be documented:
E4.10.2.1	Sample tagging
E4.10.2.2	Purification
E4.10.2.3	Normalization
E4.10.2.4	Pooling methods





Processo di tipizzazione in NGS e Standard EFI

E4.10.9	Bioinformatics
E4.10.9.1	Sequencing metrics and QC parameters for optimal performance must be documented, specified and in range
E4.10.9.2	Each deviation from the standard operation procedure must be documented
E4.10.9.3	Detailed documentation and validation of the bioinformatics process supporting the analysis, interpretation and reporting results must be established



Processo di tipizzazione in NGS e Standard EFI

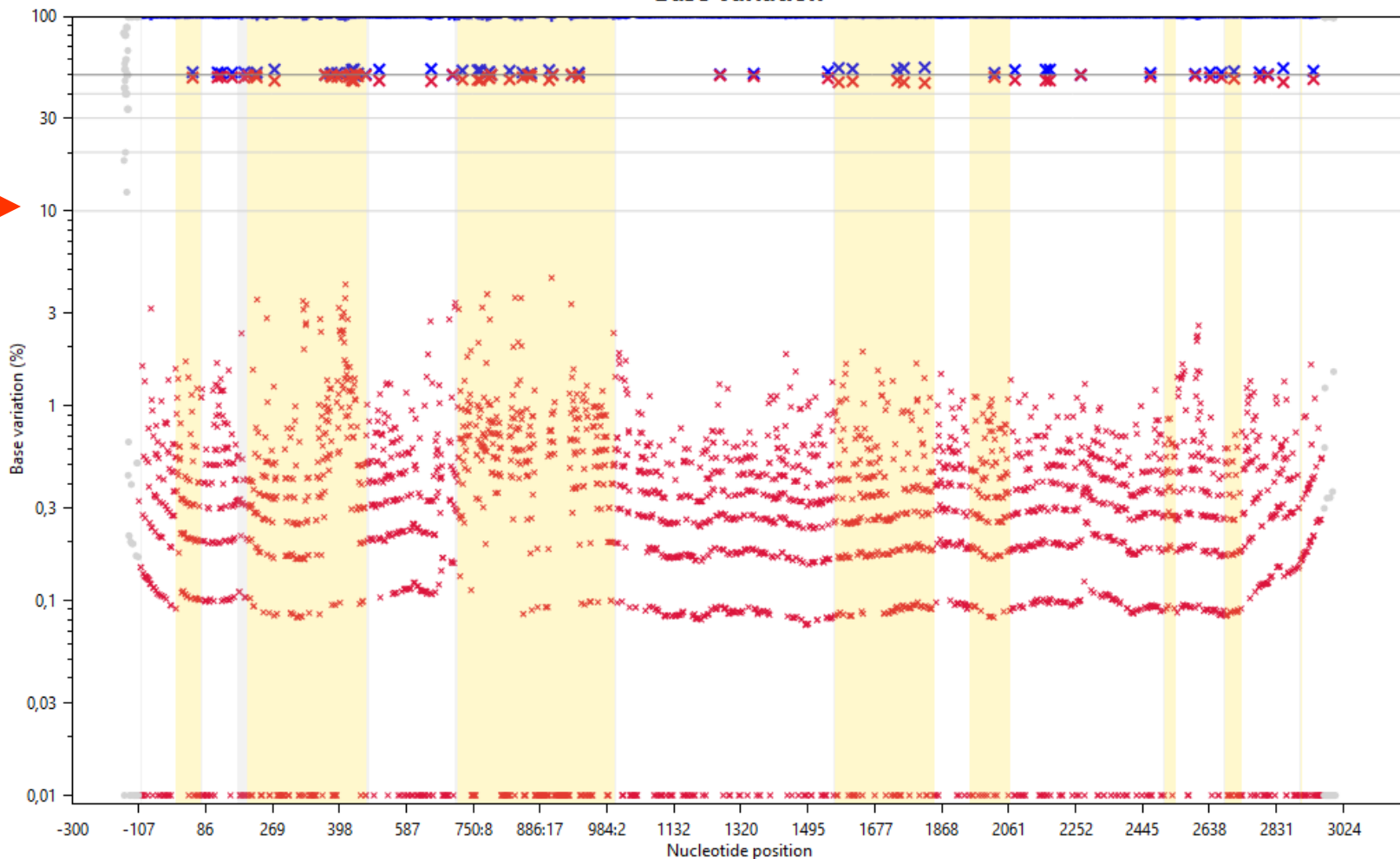
Alignment Statistics Genotype ranking XML Report SNP Calling Approval Quality metrics

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Base variation



Processo di tipizzazione in NGS e Standard EFI

PC	E4.10.7	Nucle
	E4.10.7.1	
Qua	E4.10.7.2	
	E4.10.7.3	
	E4.10.7.4	

Frammentazione
e ligazione adapter

DNA Ampure e
selezione 0.45X

Indexing PCR

DNA Ampure e
selezione 0.6X

Pooling library

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Overview

> 201902860

HLA-B

Alignment

Statistics

Genotype ranking

XML

Report

SNP Calling

Approval

Quality metrics

Data quality metrics

Mappability perct. [accepted / total reads] 97% [7811 / 8030]

Read length (median)	150		
	Core+	Exon+	Amplicon
Read depth			
Median	589	570	528
Minimum	345	300	200
Coverage	100 %	100 %	100 %
OV (median)	36	36	36
Noise			
Median	0.3%	0.3%	0.3%
Maximum	8.5%	8.5%	8.5%

Analysis quality metrics

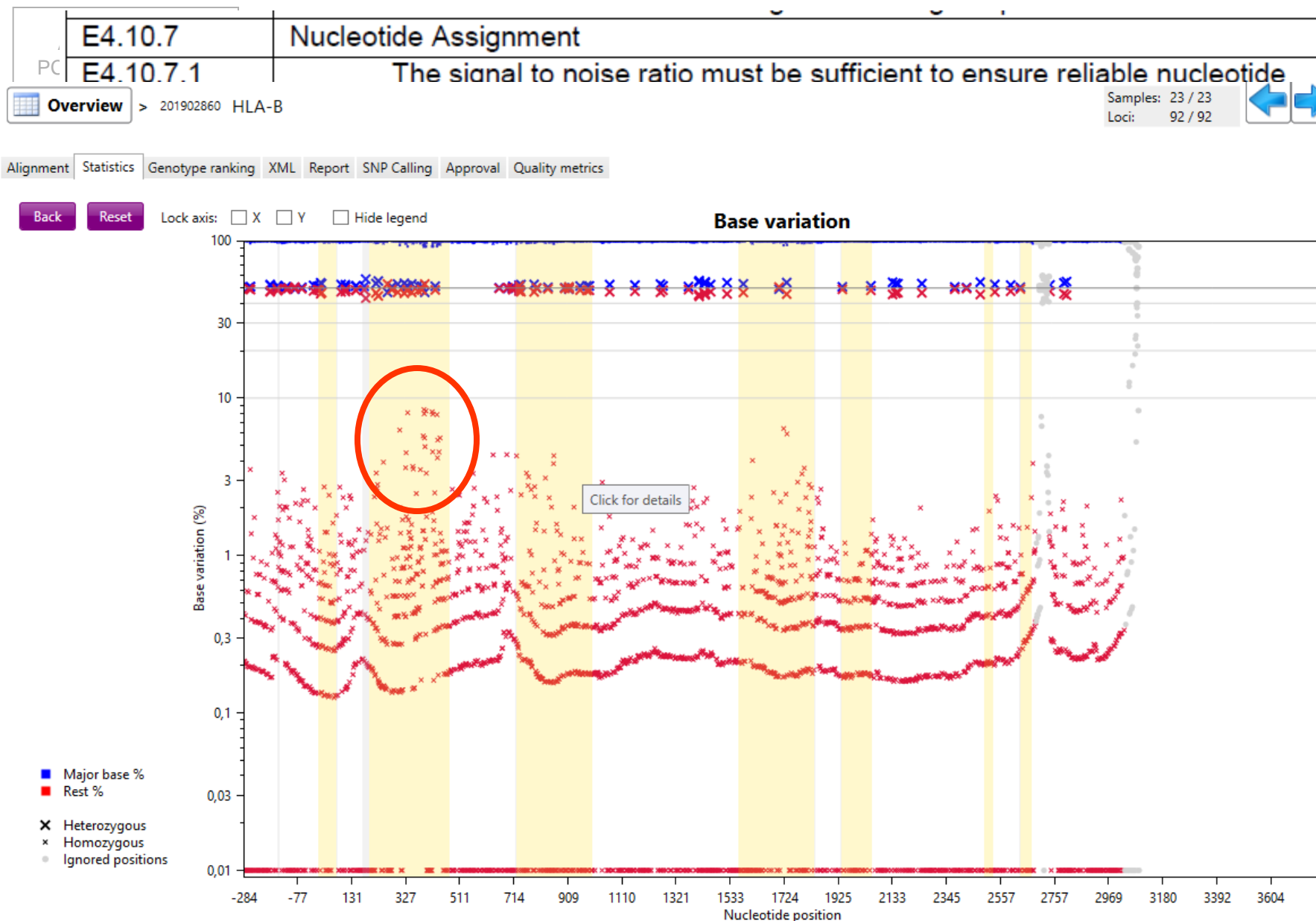
Analyzed	100 %	100 %	98.5 %
Ignored positions count	0	0	52
Heterozygous positions count	31	40	87
Delta signal to noise	34.4%	34.4%	34.4%
Second allele			
Median	48.0%	47.9%	47.7%
Minimum	42.8%	42.8%	42.8%
Phased regions	-	-	1
Mismatches	0	0	0
Question mark positions	-	-	-

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Processo di tipizzazione in NGS e Standard EFI



Processo di tipizzazione in NGS e Standard EFI

PC	E4.10.7	N
	E4.10.7.1	
Qua	E4.10.7.2	
	E4.10.7.3	
	E4.10.7.4	


Frammentazione
e ligazione adapter

DNA Ampure e
selezione 0.45X

Indexing PCR






DNA Ampure e
selezione 0.6X

Pooling library






 **Overview** > 201902863 DRB1

Alignment Statistics Genotype ranking XML Report SNP Calling Approval Quality metrics

Data quality metrics

Mappability perct. [accepted / total reads]	86% [24395 / 28216]		
 Read length (median)	150		
	<i>Core+</i>	<i>Exon+</i>	<i>Amplicon</i>
 Read depth			
Median	2286	2348	1087
Minimum	1312	1312	262
 Coverage	100 %	100 %	100 %
 QV (median)	37	37	37
 Noise			
Median	0.4%	0.4%	0.4%
Maximum	3.1%	3.1%	4.6%

Analysis quality metrics

 Analyzed	100 %	100 %	26.5 %
Ignored positions count	0	0	3613
Heterozygous positions count	17	18	19
 Delta signal to noise	28.0%	28.0%	26.4%
 Second allele			
Median	32.4%	32.4%	32.4%
Minimum	31.1%	31.1%	31.1%
 Phased regions	-	-	3
Mismatches	0	0	0
 Question mark positions	-	-	-

nucleotide

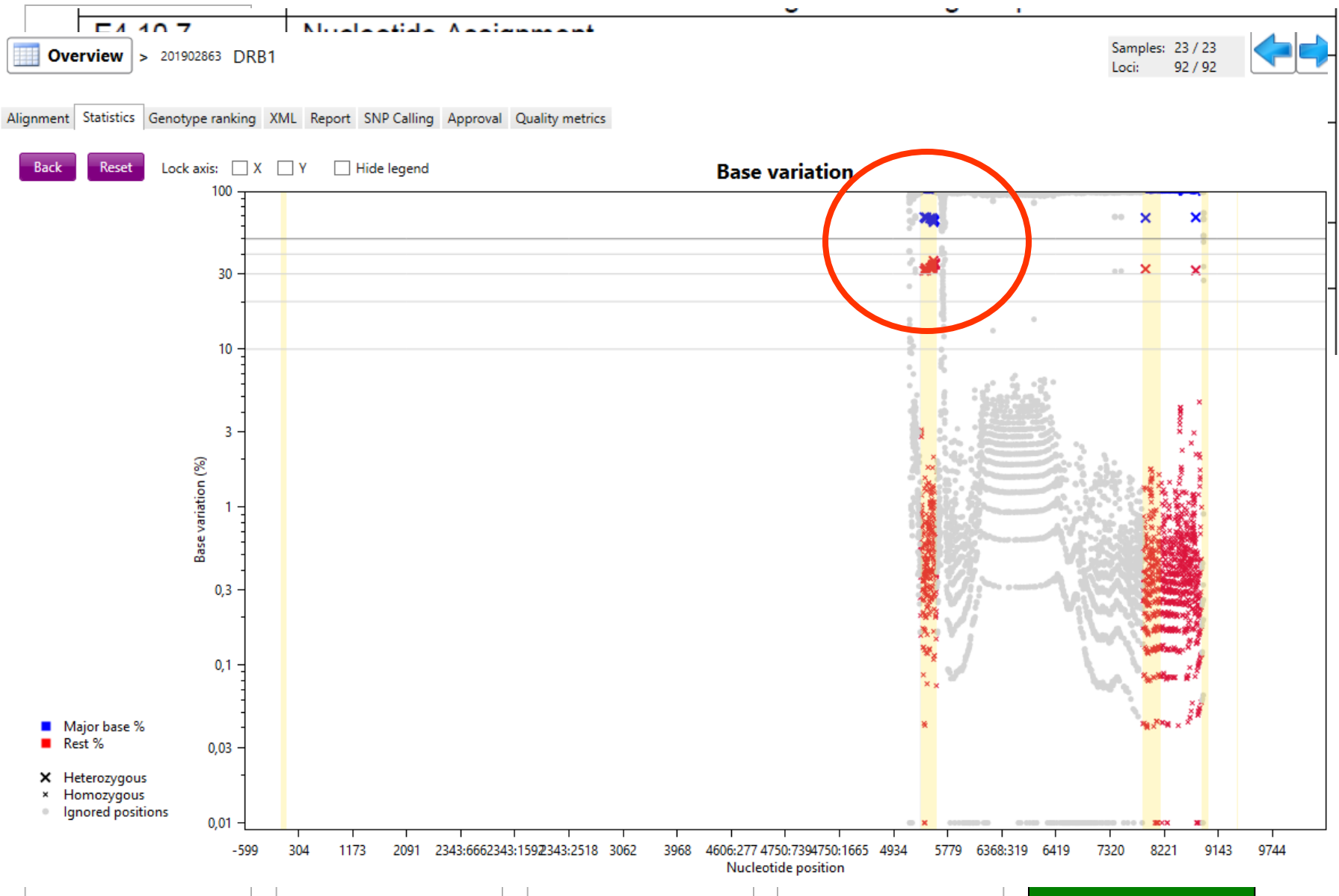
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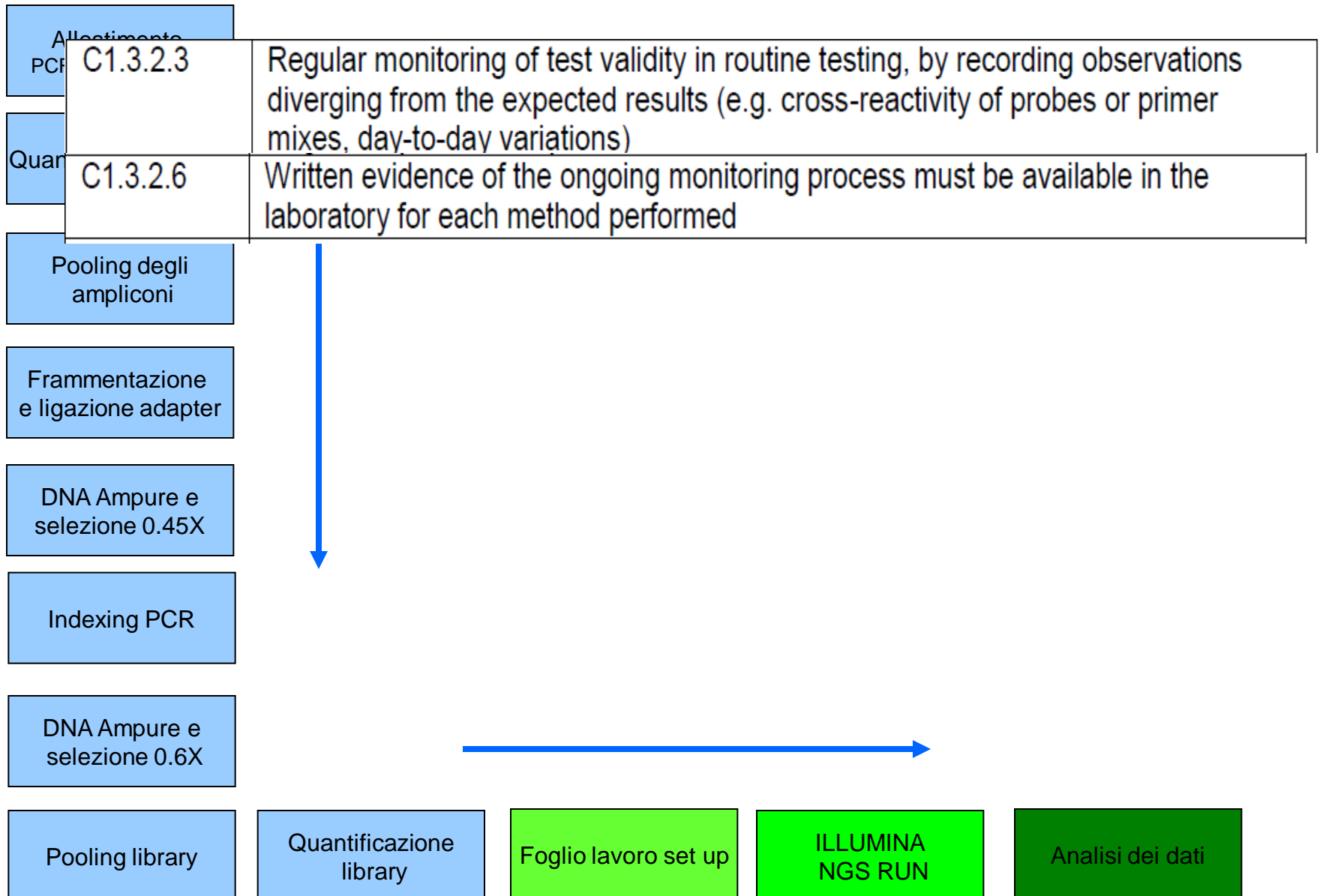
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Processo di tipizzazione in NGS e Standard EFI



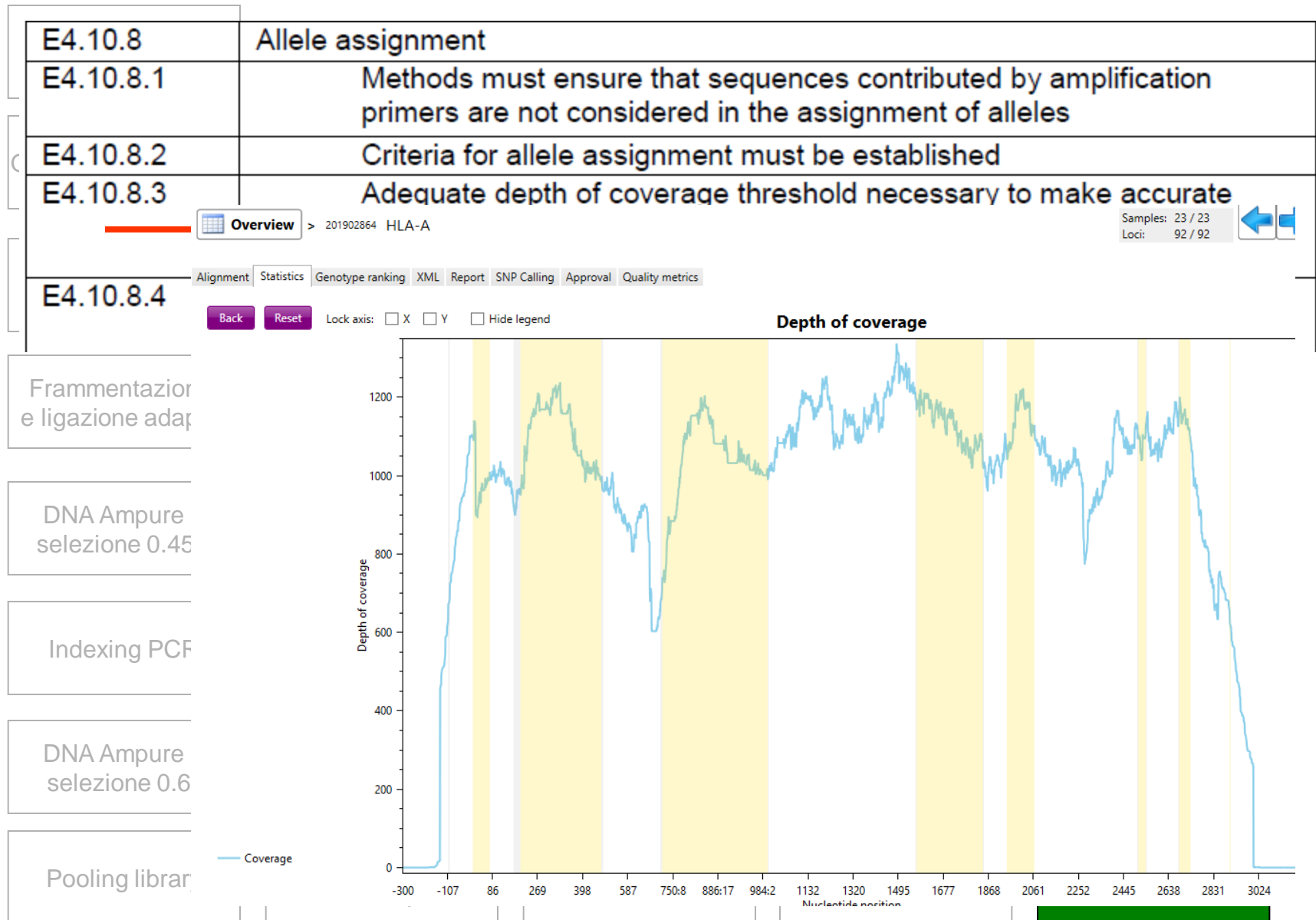
Processo di tipizzazione in NGS



Processo di tipizzazione in NGS e Standard EFI

		Alignment	Statistics	Genotype ranking	XML	Report	SNP Calling	Approval	Quality metrics
E4.10.8	Allele assignment	Data quality metrics							
E4.10.8.1	Methylation	Mappability perct. [accepted / total reads]							97% [13922 / 14345]
E4.10.8.2	Criteri	Read length (median)							150
E4.10.8.3	Adequacy of allele validation	Read depth							
		Median							1056
		Minimum							681
E4.10.8.4	Over	Coverage							100 %
		QV (median)							36
		Noise							
		Median							0.4%
		Maximum							4.5%
Frammentazione e ligazione adapter		Analysis quality metrics							
DNA Ampure e selezione 0.45X		Analyzed							100 %
Indexing PCR		Ignored positions count							0
DNA Ampure e selezione 0.6X		Heterozygous positions count							37
Pooling library		Delta signal to noise							39.9%
Quantificazione library		Second allele							
		Median							48.3%
		Minimum							44.5%
		Phased regions							-
		Mismatches							0
		Question mark positions							-

Processo di tipizzazione in NGS e Standard EFI



Processo di tipizzazione in NGS e Standard EFI

E4.10.8	Allele assignment
E4.10.8.1	Methods must ensure that sequences contributed by amplification primers are not considered in the assignment of alleles



G T C C R A G A G G G A G C C G C G G G M G C C G G T A A G T C C T G R A C A G T G C C Y T A A C Y G C T A C

G T C C A G A G G G G A G C C G C G G G A G C

C C G A G A G G G G A G C C G C G G G C G C C

G G G A G C C G G T A A G T C C T G A C A G T G

G C G G G C G C C G G T A A G T C C T G A A C A

G T C C T G A C A G T G C C T T A A

C G G T A A G T C C T G A A C A G T G C C T A A C T G C T A C

A C A G T G C C C T A A C C G C T A C

Selezione 0.5X

Pooling library

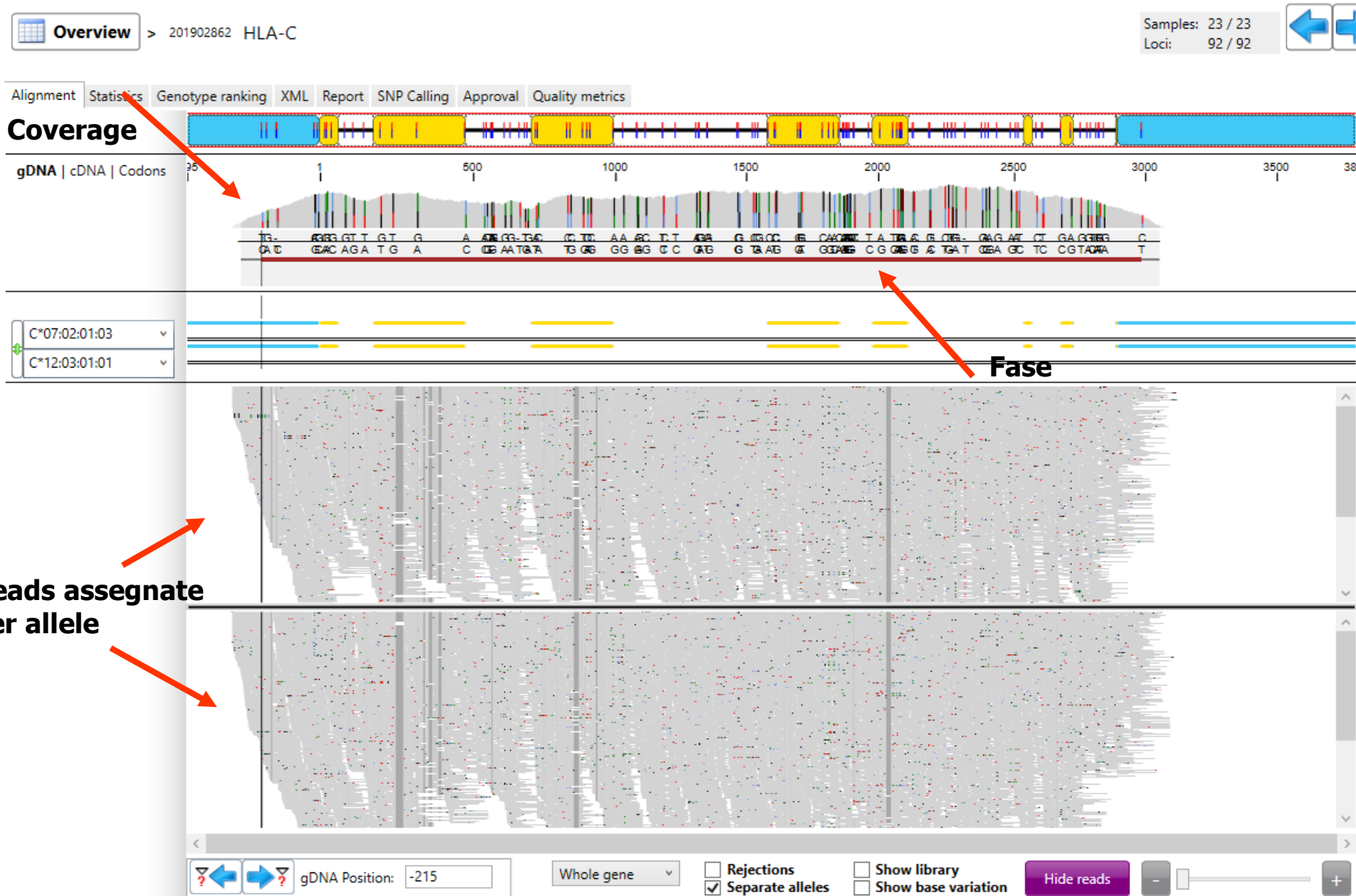
Quantificazione
library

Foglio lavoro set up

ILLUMINA
NGS RUN

Analisi dei dati

Processo di tipizzazione in NGS e Standard EFI





Processo di tipizzazione in NGS e Standard EFI

E4.10.9.8

Each sample processed must be traceable through the whole process including data analysis and reporting

Quantificazione DNA

Pooling degli
ampliconi

Frammentazione
e ligazione adapter

DNA Ampure e
selezione 0.45X

Indexing PCR

DNA Ampure e
selezione 0.6X

Pooling library

Quantificazione
library

Foglio lavoro set up

ILLUMINA
NGS RUN

Analisi dei dati



Processo di tipizzazione in NGS e Standard EFI

E3	COMPUTER ASSISTED ANALYSES
E3.1	The Laboratory Director and/or the Supervisor must
E3.1.1	Review
E3.1.2	Verify
E3.1.3	Sign computer assisted analyses before issue
E3.2	The computer software programme used for analyses must be:
E3.2.1	Identified
E3.2.2	Validated/Verified before use
E4.5.3.3	The allele database must be:
E4.5.3.3.1	Documented
E4.5.3.3.2	Updated at least once a year with the most current version of the IPD-IMGT/HLA database



Processo di tipizzazione in NGS e Standard EFI

E4.5.4	Contamination control ("wipe-test")
E4.5.4.1	Contamination must be monitored for amplification products produced in the laboratory
E4.5.4.2	Routine wipe-tests must:
E4.5.4.2.1	Include pre-amplification work areas
E4.5.4.2.2	Include pre-amplification equipment
E4.5.4.2.3	Be performed at least every two months
E4.5.4.2.4	Be performed using a method that is at least as sensitive as routine test methods
E4.5.4.2.5	Include positive controls to assure proper performance of monitoring
E4.5.4.2.6	Include inhibition controls to assure proper performance of monitoring
E4.5.4.2.6.1	Actions needs to be taken if inhibition control is weaker than positive control
E4.5.4.2.7	Include other areas of the laboratory as relevant
E4.5.4.3	If amplified product is detected, there must be:
E4.5.4.3.1	Written description of how to eliminate the contamination
E4.5.4.3.2	Measures taken to prevent future contamination
E4.5.4.3.3	Evidence of elimination of the contamination



Grazie per l'attenzione