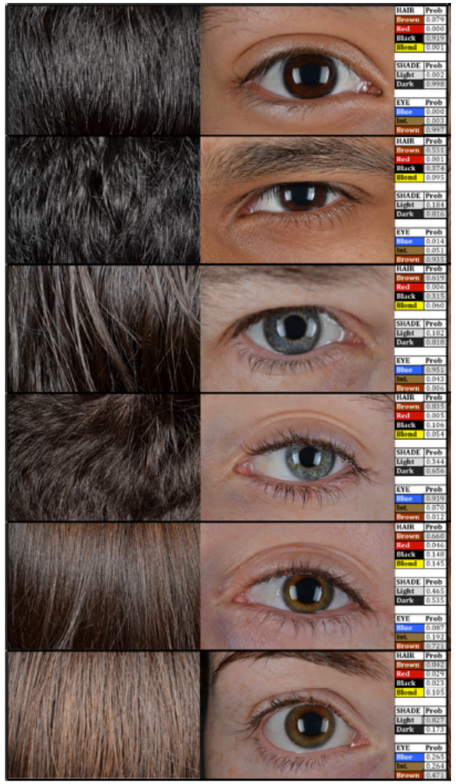
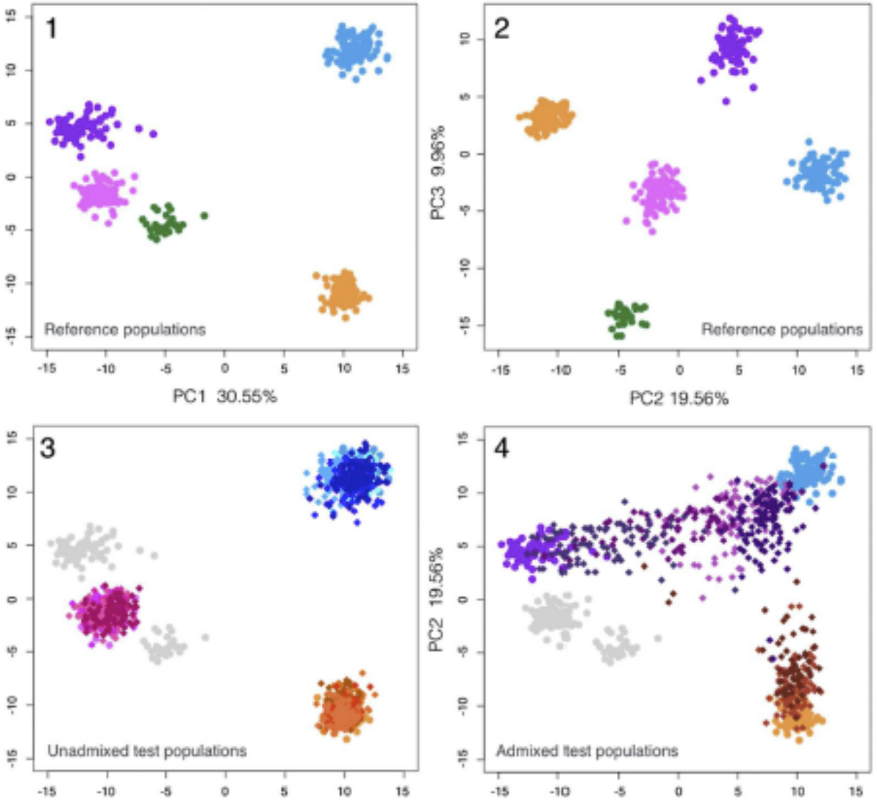
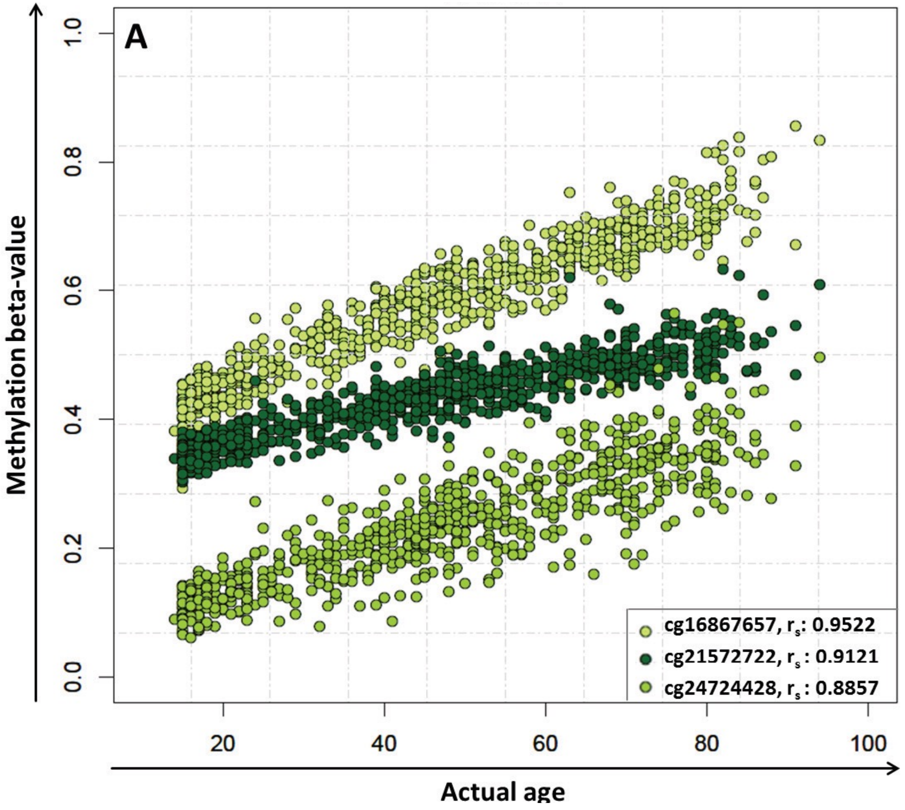
HIDS Meeting, Kobe, Japan, June 17-19 2019

VISAGE - Visual Attributes through Genomics

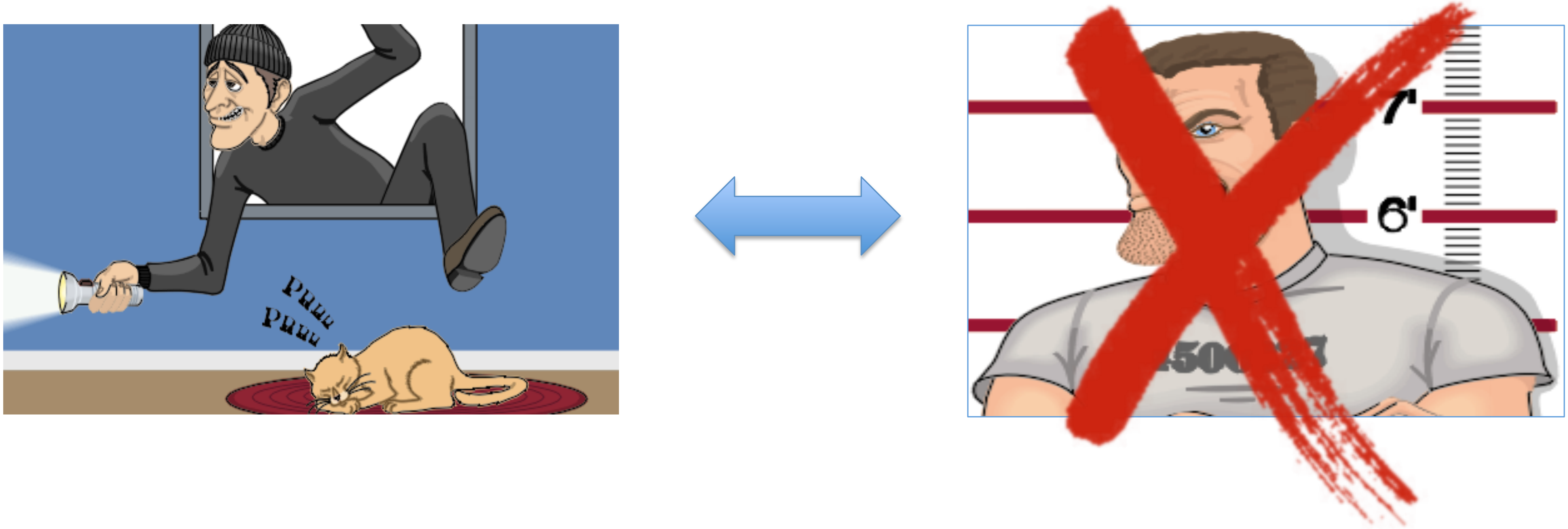






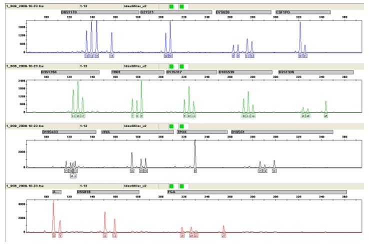
Catarina Xavier[1], Antonia Heidegger[1], Harald Niederstätter[1], Maria de la Puente[1,2], The VISAGE Consortium[3], Christopher Phillips[2], Wojciech Branicki[4], Manfred Kayser[5], Walther Parson[1,6]

Cases without suspects



Forensic DNA Phenotyping

DNA profilling



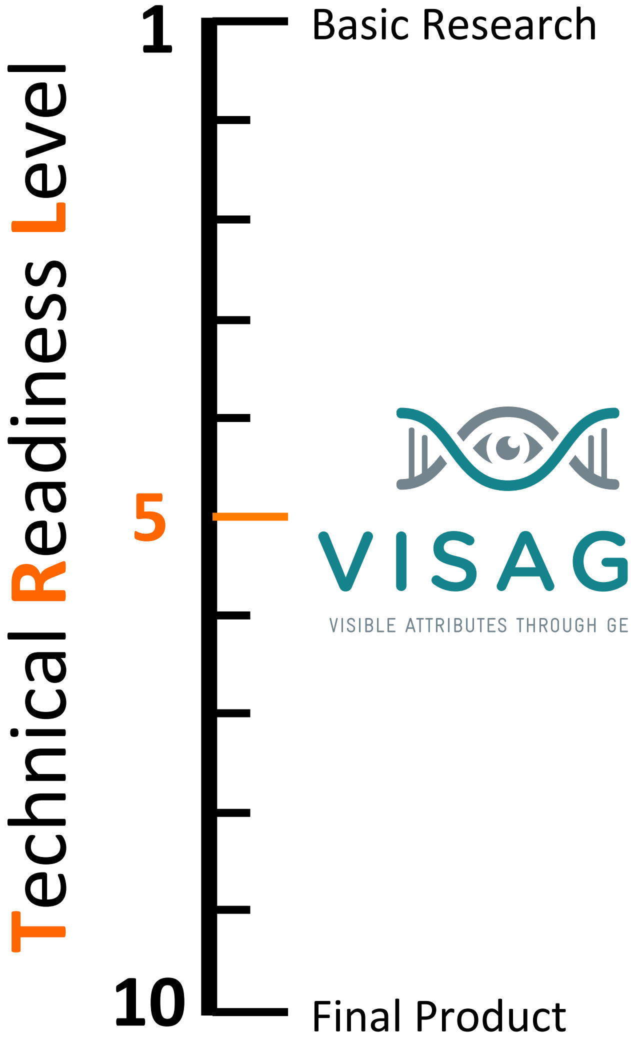
Database

NO MATCH

DNA intelligence

appearance
ancestry
age

VISAGE OBJECTIVES



VISAGE

VISIBLE ATTRIBUTES THROUGH GENOMICS

The **VISAGE** - Consortium is developing genotyping and statistical prototype tools, forensically validate and implement them into forensic practice for predicting **appearance**, **age**, and **ancestry** from DNA traces and study its ethical, societal & regulatory dimensions (period: 05/2017-04/2021).

Tool 1: Appearance & Ancestry (SNP multiplex)

Tool 2: Age (quantitative methylation)

VISAGE - Visual Attributes Through Genomics

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www.visage-h2020.eu

VISAGE PROGRESS

- WP1 MANAGEMENT (EMC)
- WP2 MARKER DISCOVERY (EMC, JU, USC)
- WP3 PROTOTYPE ANALYSIS TOOL DEVELOPMENT AND VALIDATION (MUI)
- WP4 STATISTICAL PREDICTION MODELLING AND SOFTWARE DEVELOPMENT (UoK)
- WP5 ETHICAL, SOCIETAL AND REGULATORY DIMENSION MAPPING (KCL)
- WP6 IMPLEMENTATION OF PROTOTYPE TOOLS IN RELEVANT ENVIRONMENT (BKA)
- WP7 EDUCATION AND TRAINING

WP2 MARKER DISCOVERY

D2.1 Markers for **Basic** Prototype Tool (M3)

Appearance - 41 SNPs (EMC)

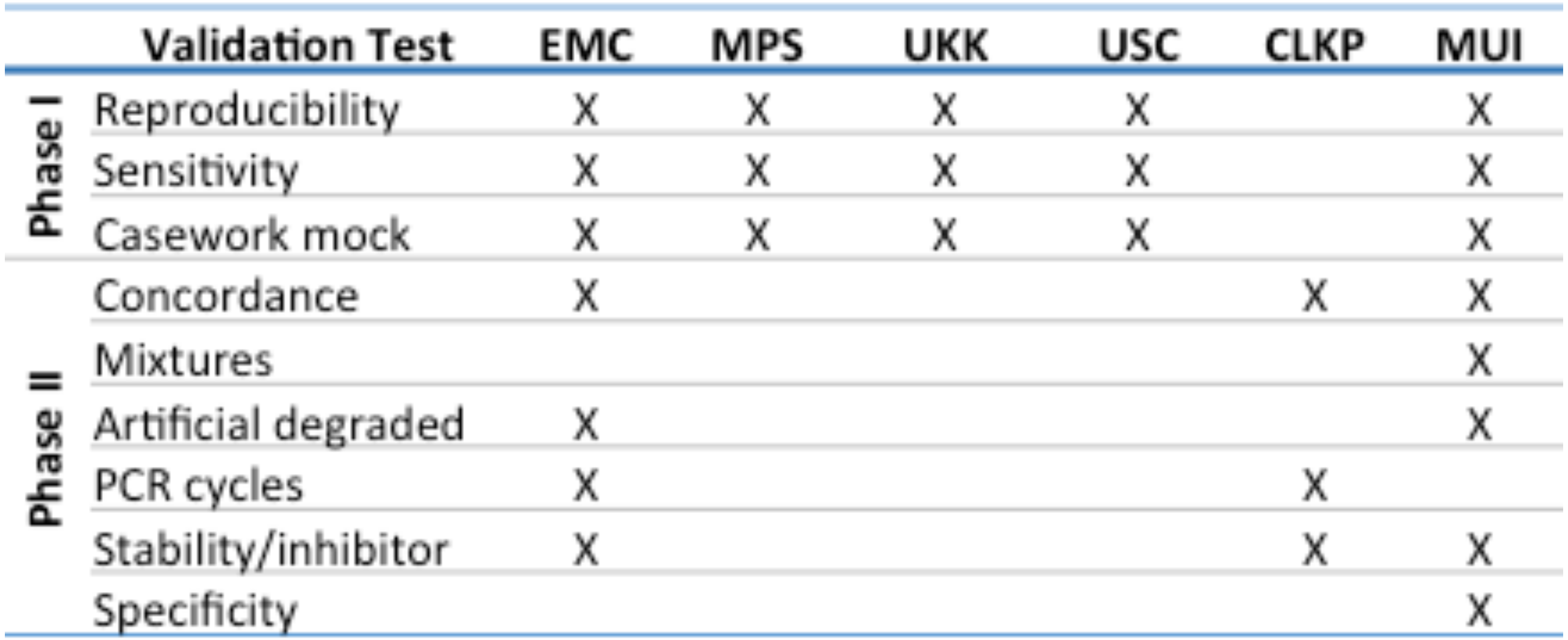
Ancestry - 116 SNPs (USC)

154 SNPs

Age (blood/saliva) - 5 genes / 32 CpG sites (JU)

Appearance
Hair
Eye
Skin

Ancestry
Continental

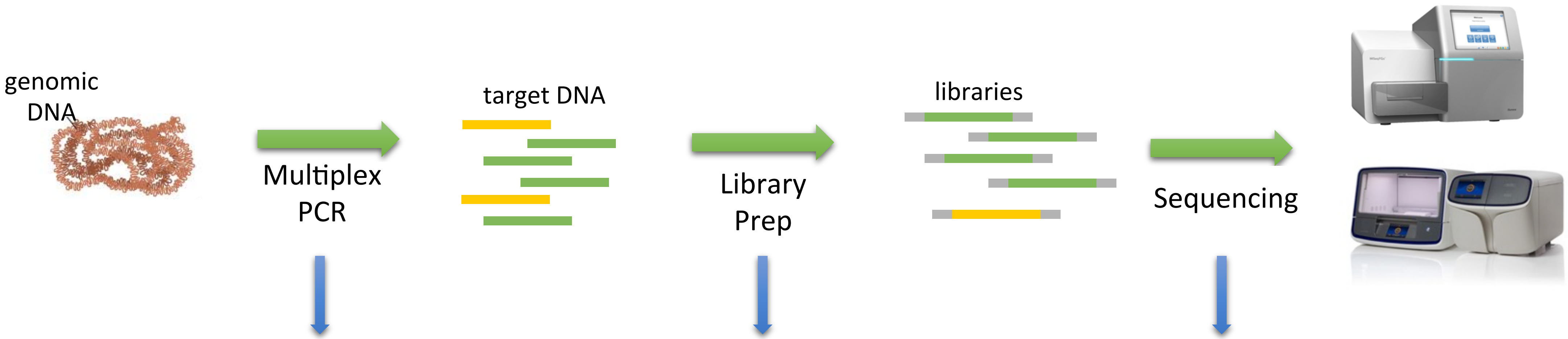


	Validation Test	EMC	MPS	UKK	USC	CLKP	MUI
Phase I	Reproducibility	X	X	X	X		X
	Sensitivity	X	X	X	X		X
	Casework mock	X	X	X	X		X
Phase II	Concordance	X				X	X
	Mixtures						X
	Artificial degraded	X					X
	PCR cycles	X				X	
	Stability/inhibitor	X				X	X
	Specificity						X

VISAGE Basic Tool

Tool 1: Appearance & Ancestry (SNP multiplex)

Design, develop and validate prototype tools


genomic DNA

Multiplex PCR

target DNA

Library Prep

libraries

Sequencing

Multiplex design/ optimization
primer pools
how many reactions
optimize PCR

Adapt to different library prep commercial products

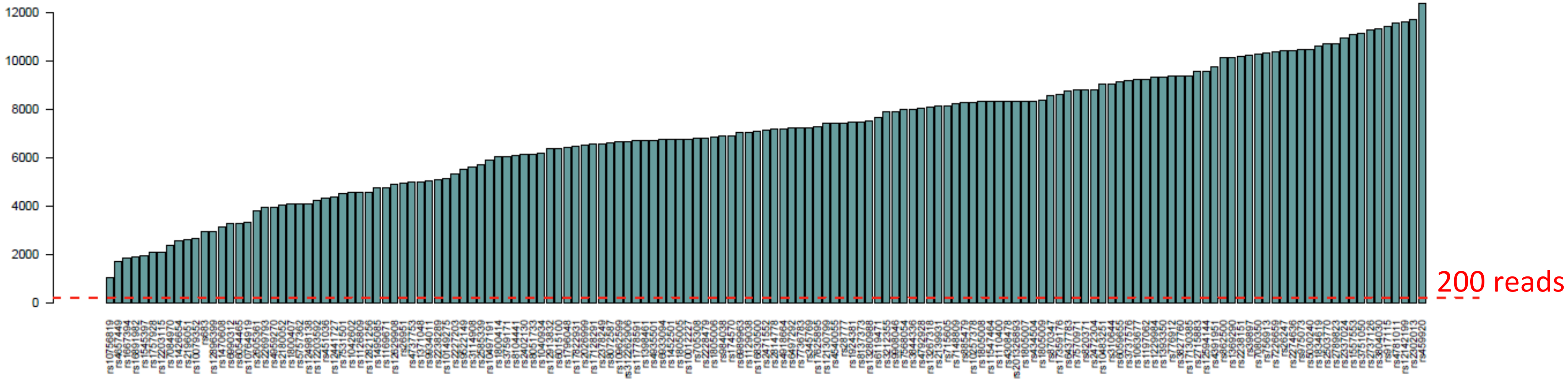
How many samples per chip
Overall performance
Coverage requirements

ThermoFisher SCIENTIFIC | Ion AmpliSeq Designer

VISAGE WP3 – MS9/D3.2

Developmental validation BASIC Tools

BASIC A&A Tool – Mean read depth per SNP

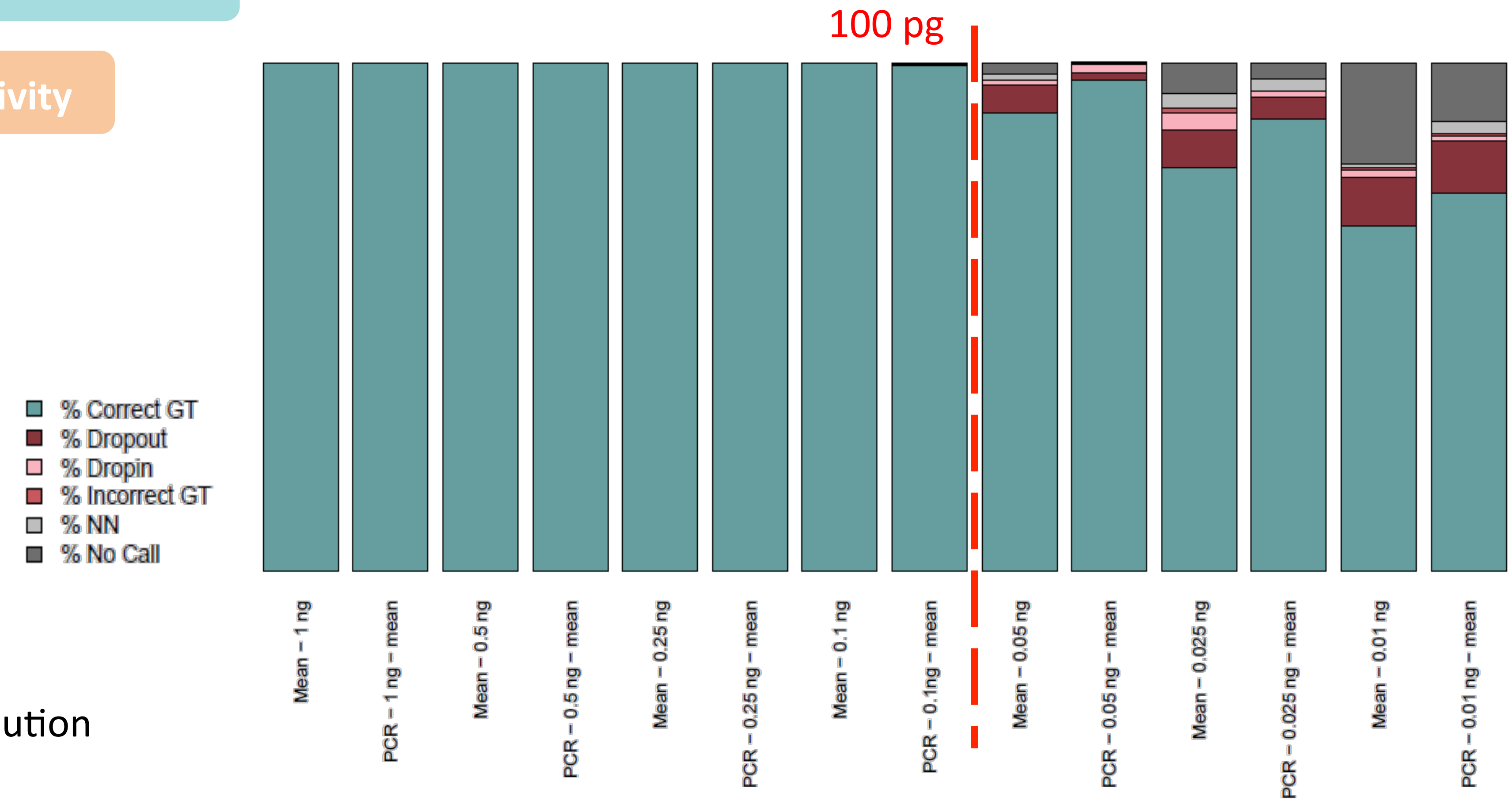


5 labs; DNA input 1 ng; 25 replicates, 16 samples/530 chip; Ion S5

VISAGE WP3 – MS9/D3.2

Developmental validation BASIC Tools

BASIC A&A Tool – sensitivity



<u>SENSITIVITY:</u>

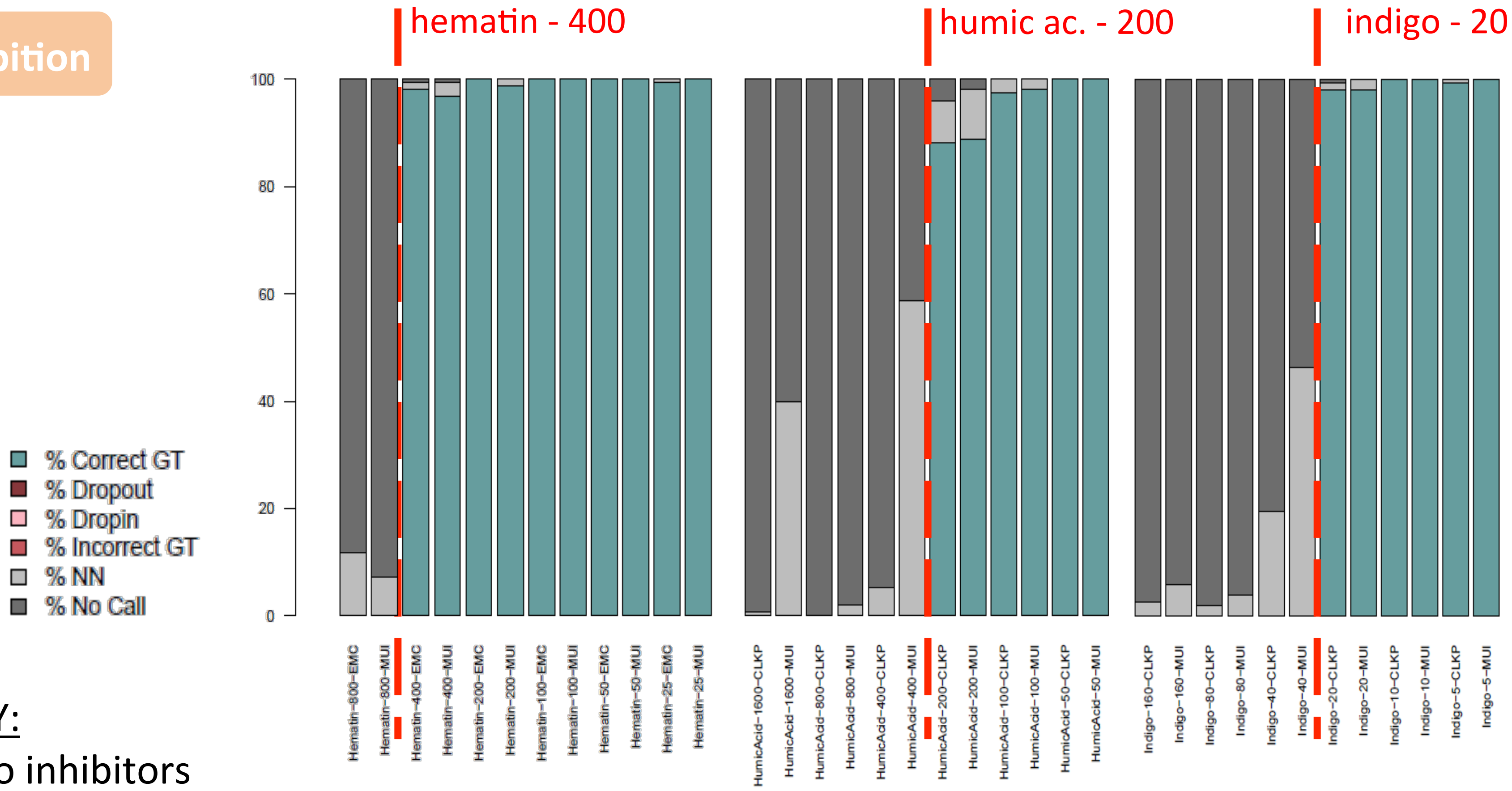
- ✓ full profiles down to 0.1 ng
- ✓ increased PCR cycles → good solution for challenging samples

5 labs; DNA input 1 - 0.01 ng; 10 replicates per dilution; 16 samples/530 chip; Ion S5

VISAGE WP3 – MS9/D3.2

Developmental validation BASIC Tools

BASIC A&A Tool – inhibition



INHIBITOR TOLERANCE/ STABILITY:

approx. 15 times more sensitive to inhibitors compared to STR kits

5 labs; DNA input 1ng; duplicates, 16 samples/530 chip; Ion S5

VISAGE WP3 – MS9/D3.2

Developmental validation BASIC Tools

BASIC A&A Tool – results

CONCORDANCE - CORIELLS:

✓ 99.83% concordant

    ✓ SGDP and 1K genomes data

„MOCK“ CASEWORK - GEDNAP:

✓ 5,355 genotype comparisons

    11 disparities

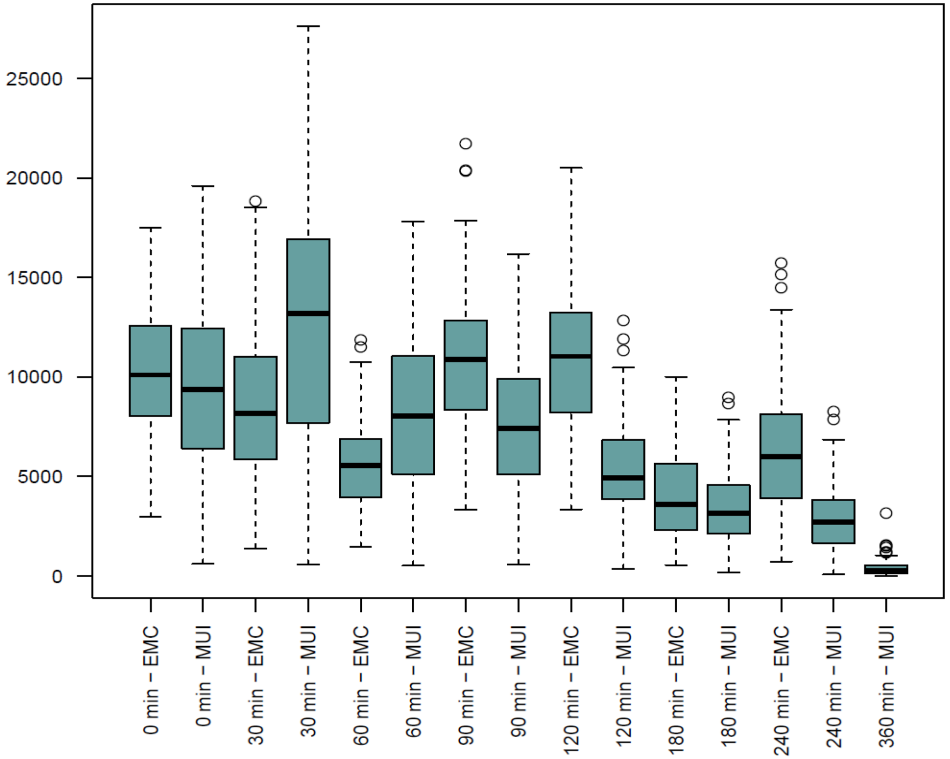
        9 no calls

        2 allele dropouts

DEGRADED DNA:

✓ full profiles down to 240 min sonication

✓ 360 min – one replicate failed



2 labs; DNA input ~1 ng degraded; duplicates; 16 samples/530 chip; Ion S5

VISAGE WP3 – MS9/D3.2

Developmental validation BASIC Tools

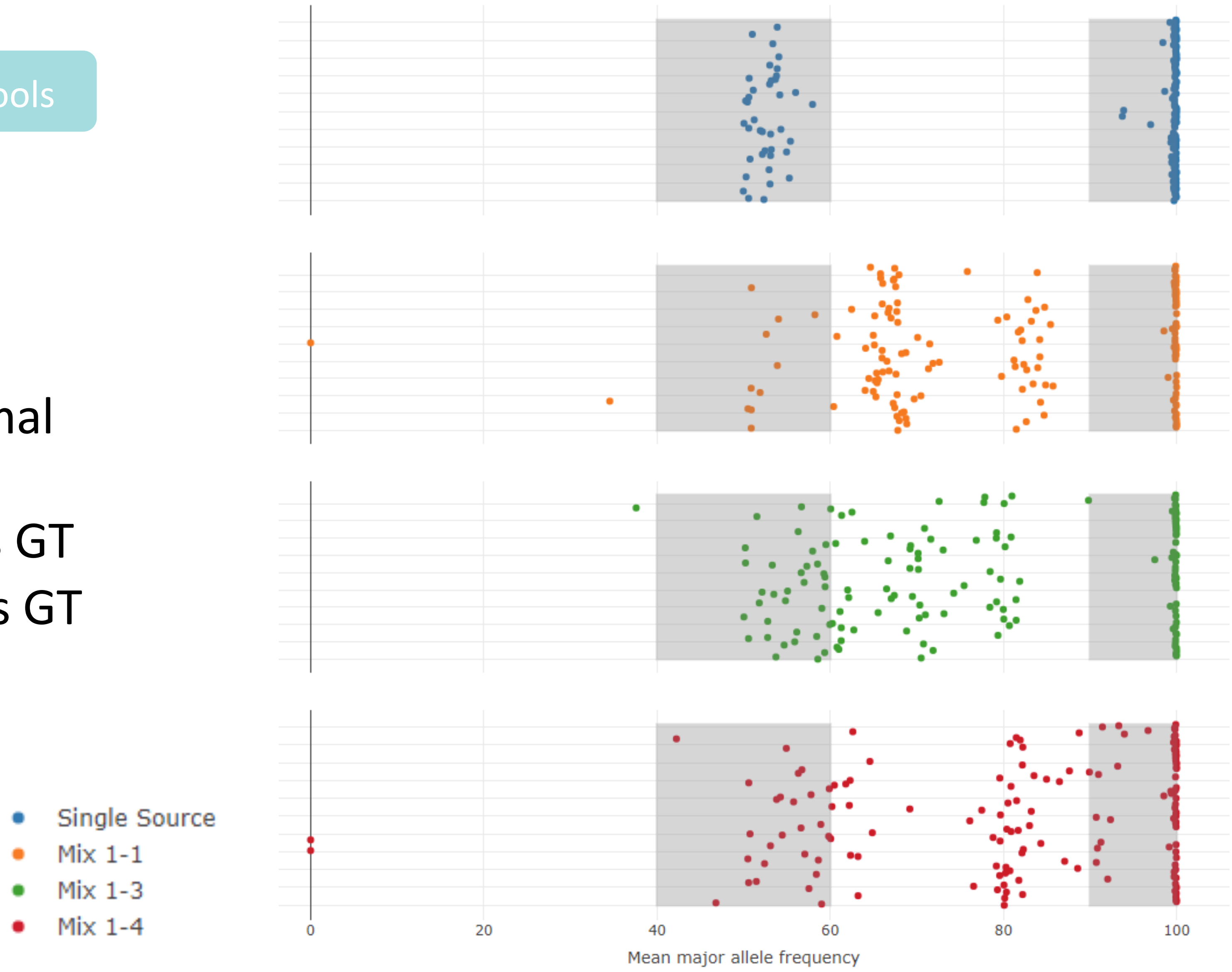
BASIC A&A Tool – mixtures

MIXTURES:

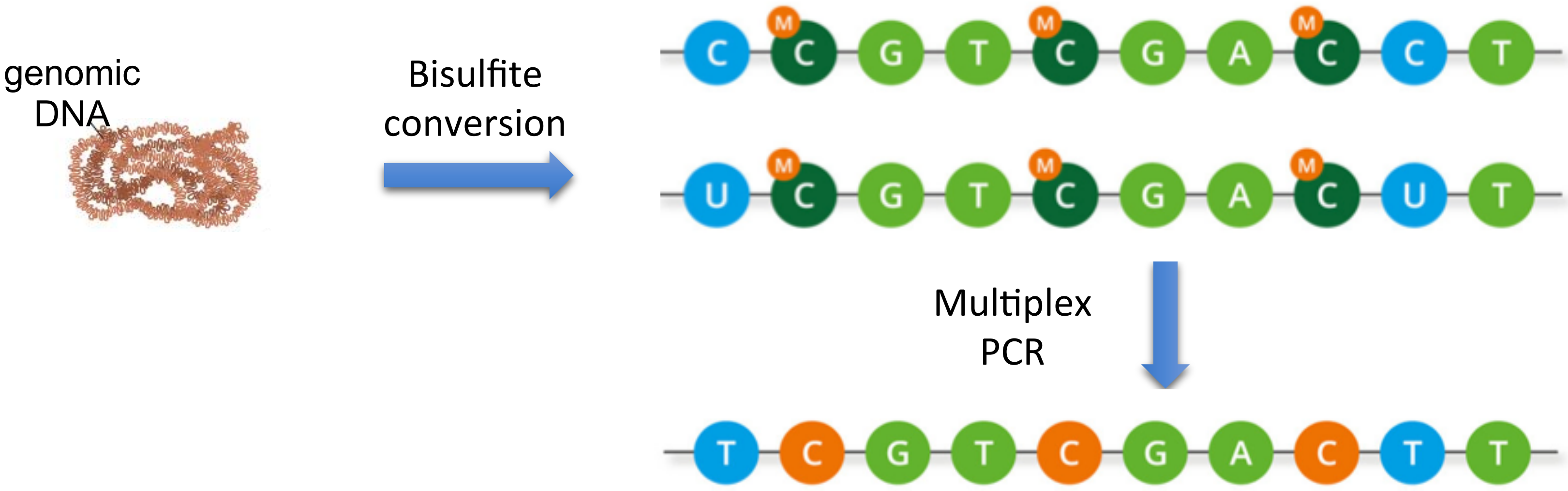
MAF flags → outside of normal intervals:

40-60% for heterozygous GT

90-100% for homozygous GT



Age estimation by quantitative methylation

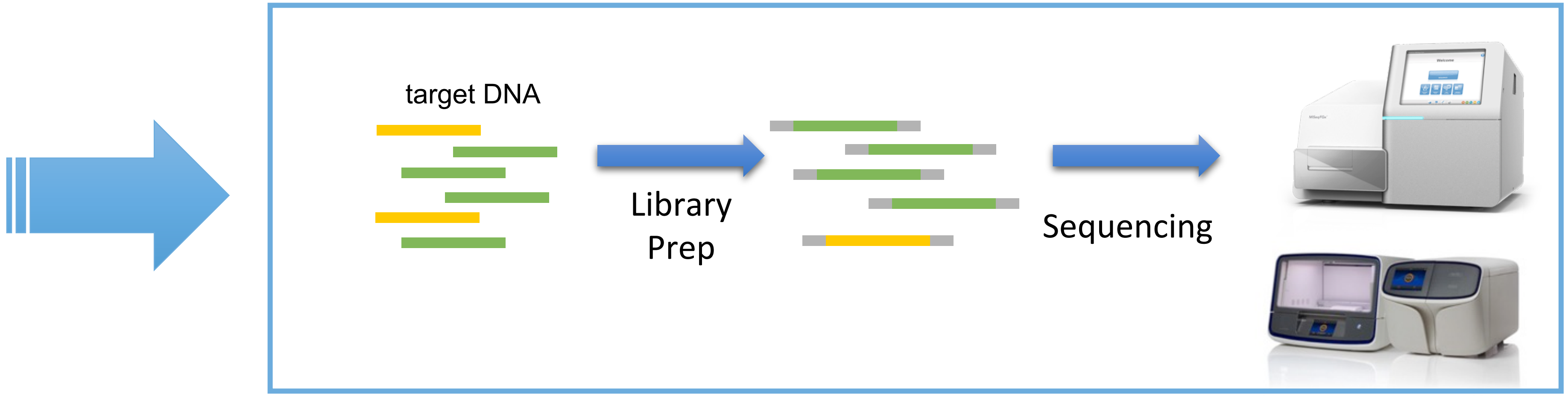


genomic DNA

Bisulfite conversion

Multiplex PCR

https://www.gatc-biotech.com



target DNA

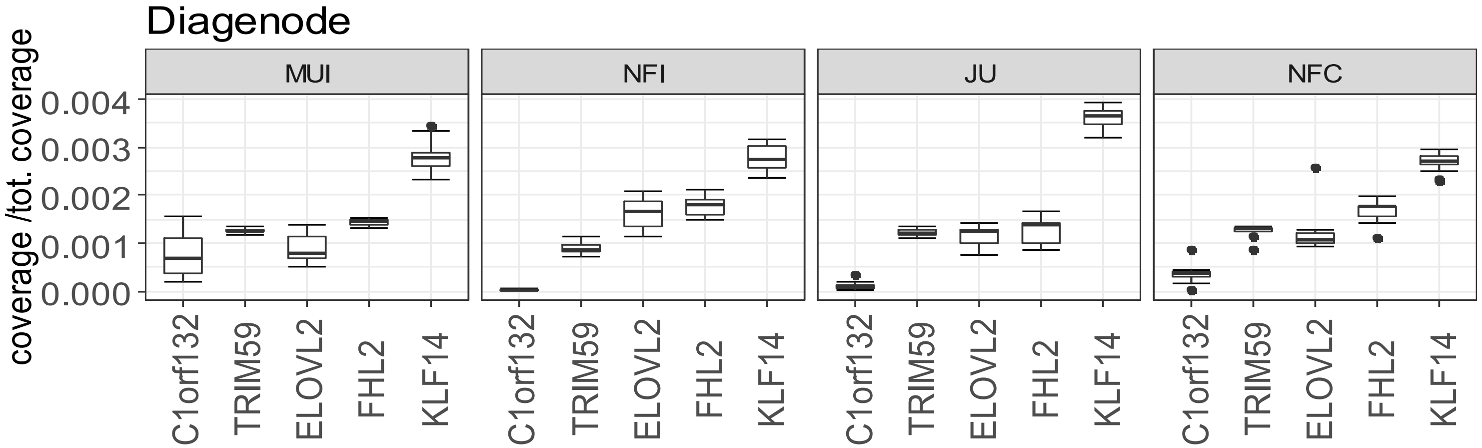
Library Prep

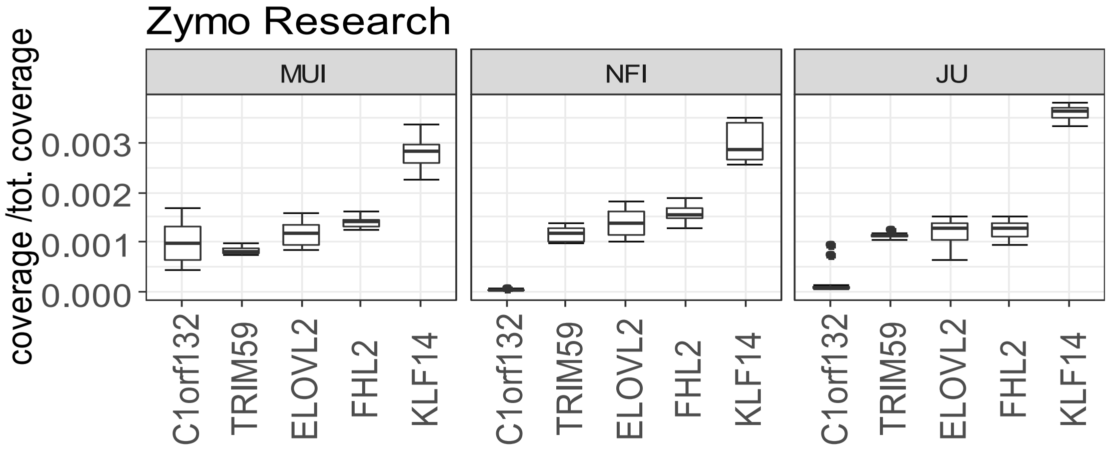
Sequencing

VISAGE WP3 – MS9/D3.2

Developmental validation BASIC Tools

BASIC AGE Tool – results





Reproducibility, normalized coverage; 200 ng; MiSeq FGx; Contributing labs: JU, MUI, NFC, NFI

WP2 MARKER DISCOVERY

D2.1 Markers for **Basic** Prototype Tool (M3)

Appearance - 41 SNPs (EMC)
Ancestry - 116 SNPs (USC)
} **154 SNPs**

Age (blood/saliva) - 5 genes / 32 CpG sites (JU)

Appearance
Hair
Eye
Skin

Ancestry
Continental

D2.2 Markers for **Enhanced** Prototype Tool (M18)

Appearance - 211 SNPs (EMC)
Ancestry - 206 SNPs + 22MHTs (USC)
} **524 SNPs**

Age (blood/saliva) - 8 genes / 42 CpG sites (JU)

Age (semen) - 13 genes / 13 CpG sites (JU)

Appearance
Hair
Eye
Skin
Eyebrow
Hair shape
Freckles
MPB

Ancestry
Continental
Middle East
North Africa
X and Y-SNPs

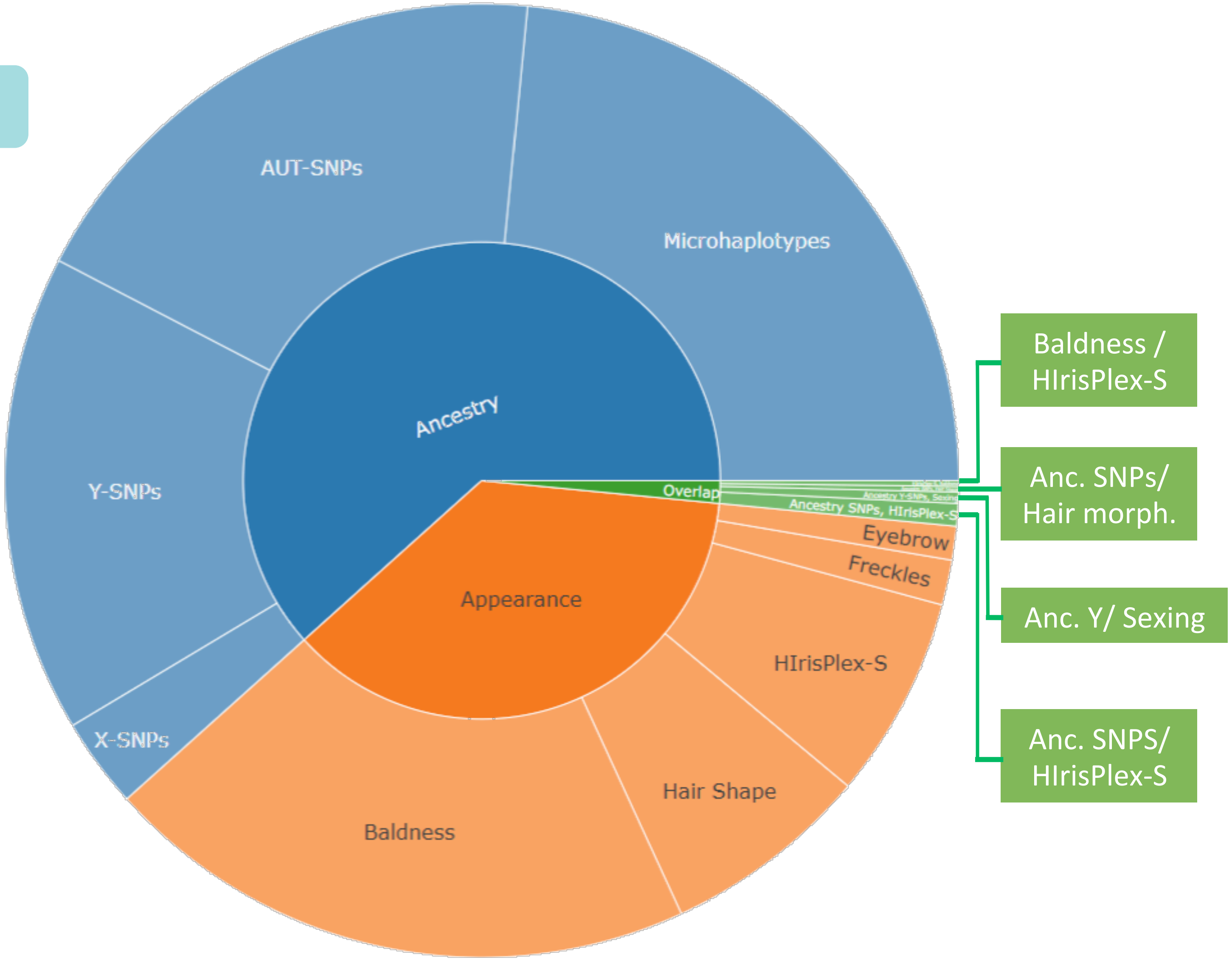
VISAGE WP3 – MS10/D3.3

Design and optimization ENHANCED Tools

VISAGE En. A&A Tool

524 MARKERS succesfully designed:
- ✓ 323 ancestry
- ✓ 193 appearance
- ✓ 8 overlap

<u>INTERNAL TESTS so far:</u>
- ✓ reproducibility
- ✓ sensitivity
- ✓ casework „mock“ samples



Baldness / HIrisPlex-S

Anc. SNPs/ Hair morph.

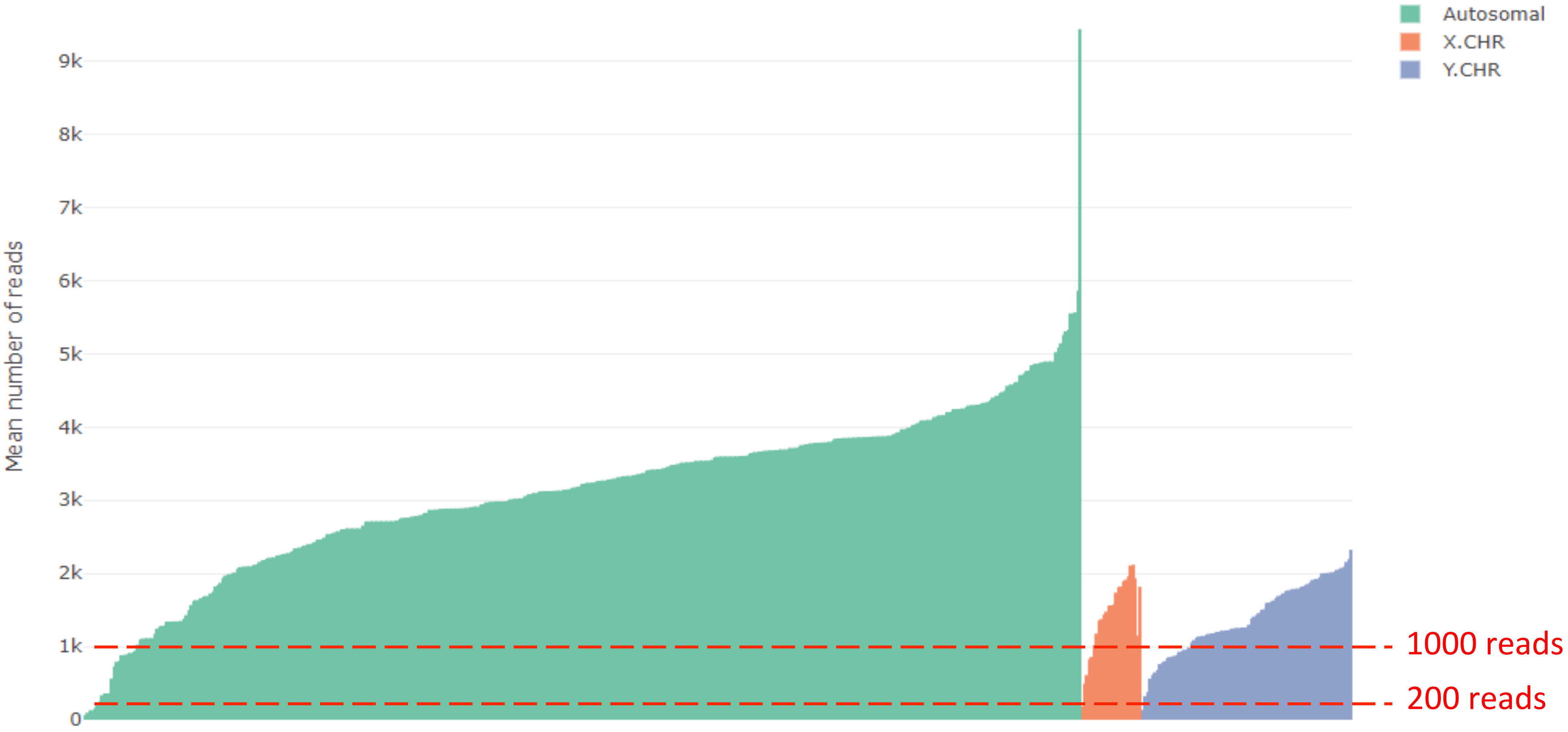
Anc. Y/ Sexing

Anc. SNPS/ HIrisPlex-S

Thermo Fisher SCIENTIFIC | Ion AmpliSeq Designer

VISAGE WP3 – MS10/D3.3

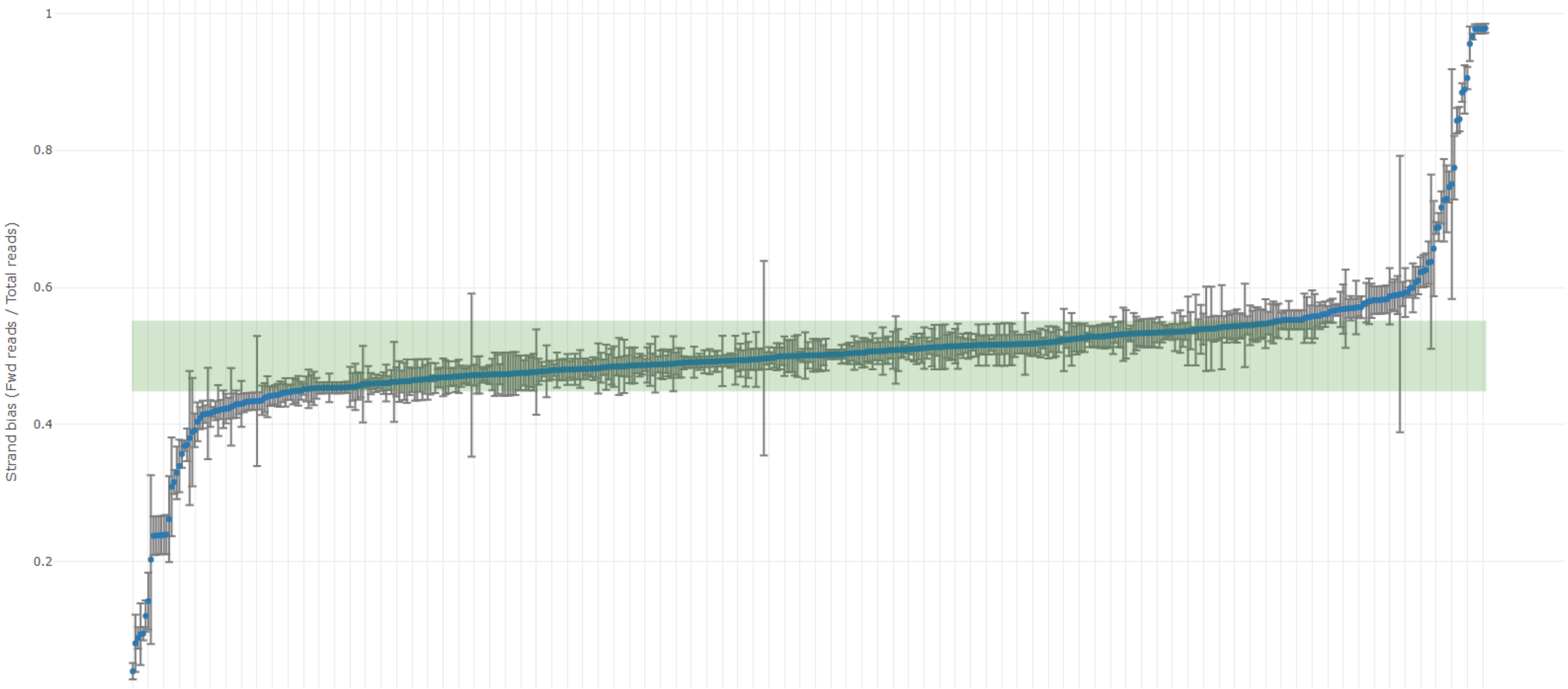
Design and optimization ENHANCED Tools



DNA input 1 ng; 5 replicates; 16 samples/530 chip; Ion S5

VISAGE WP3 – MS10/D3.3

Design and optimization ENHANCED Tools



DNA input 1 ng; 5 replicates; 16 samples/530 chip; Ion S5

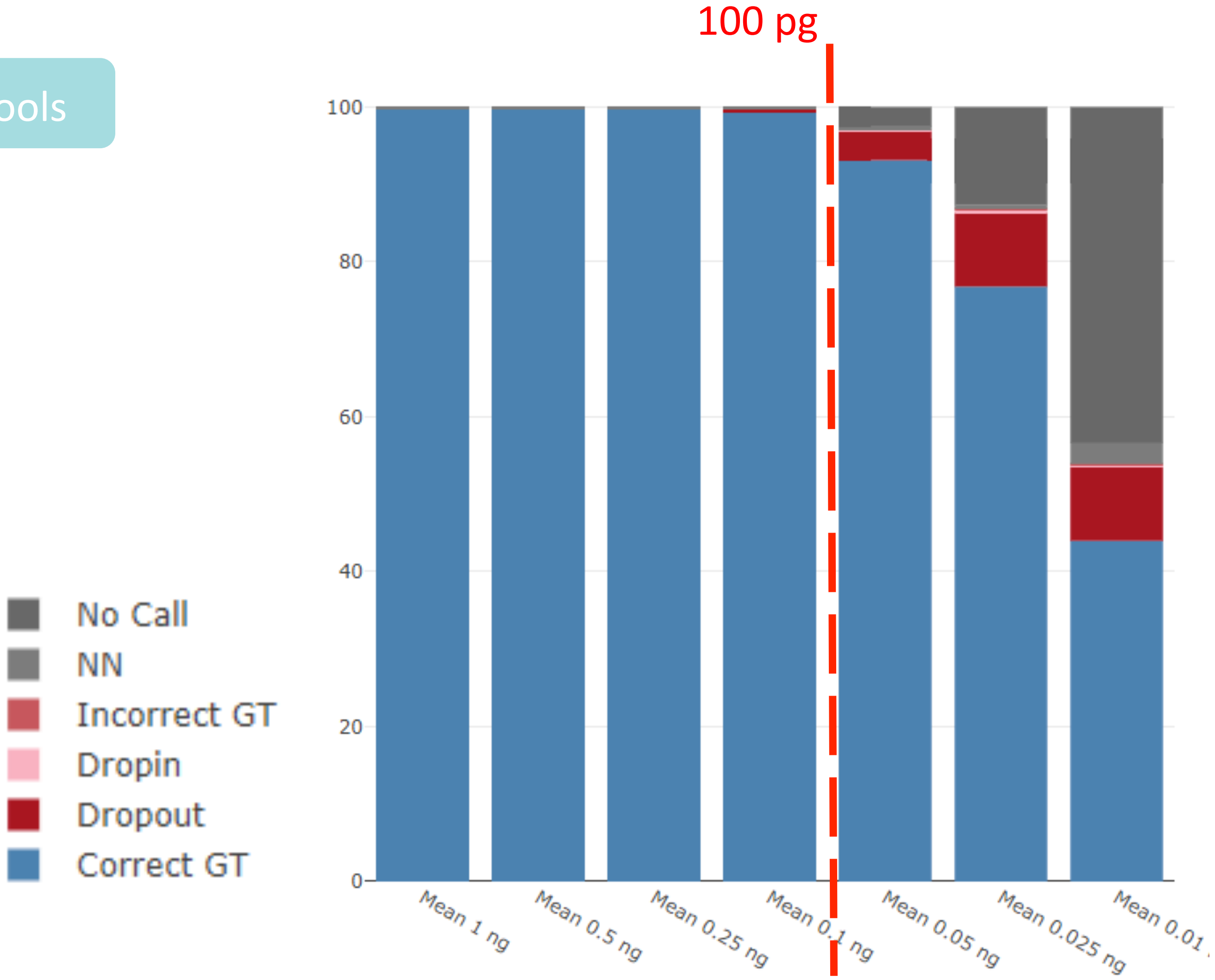
VISAGE WP3 – MS10/D3.3

Design and optimization ENHANCED Tools

→ 1 marker **dropout** in all reps:

✓ 100 pg – 99.43% correct GT

✓ 10 pg – 43.99 % correct GT



DNA input 1 - 0.01 ng; duplicates; 16 samples/530 chip; Ion S5

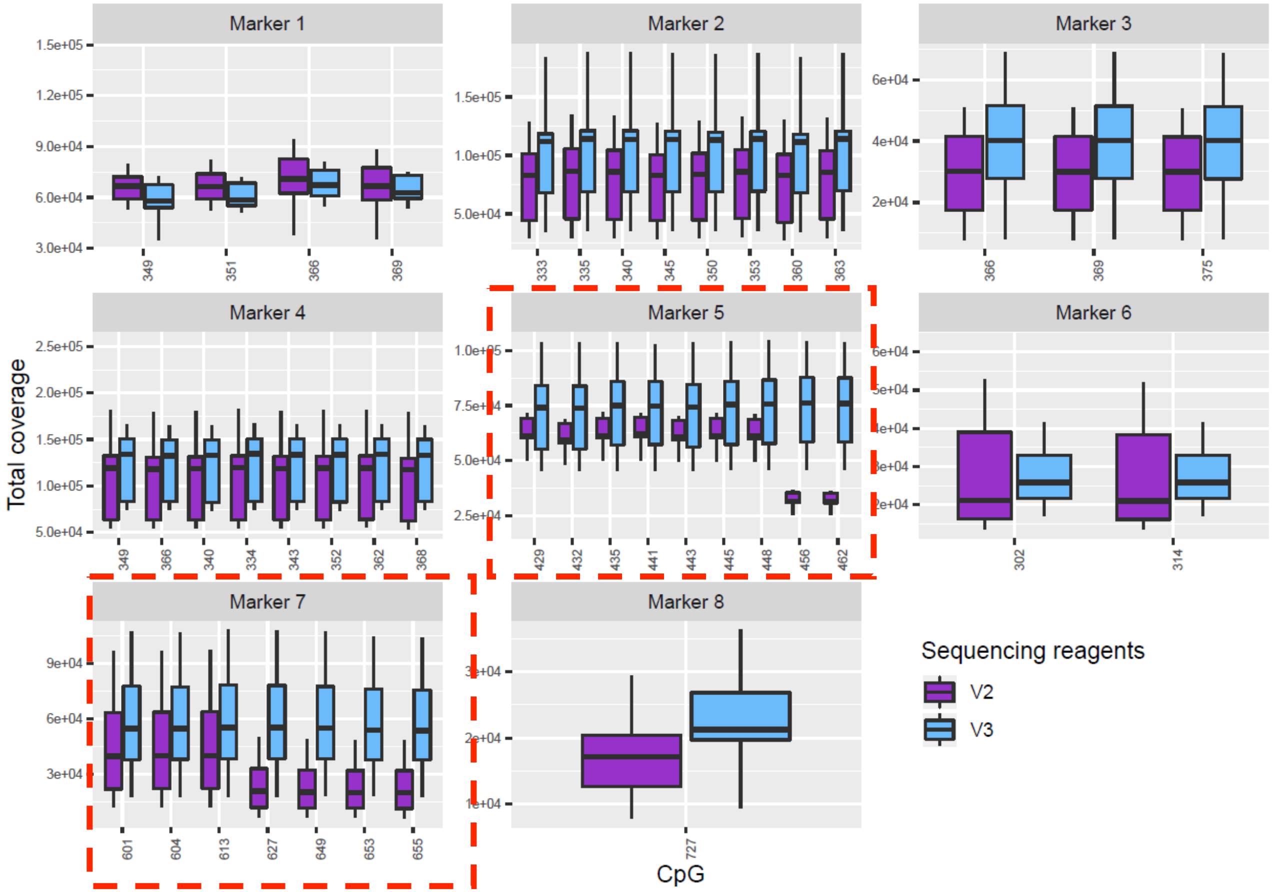
VISAGE WP3 – MS10/D3.3

Design and optimization ENHANCED Tools

Blood/Saliva

<u>V2 vs. ForenSeq V3:</u>

✓ Uniform coverage throughout amplicon

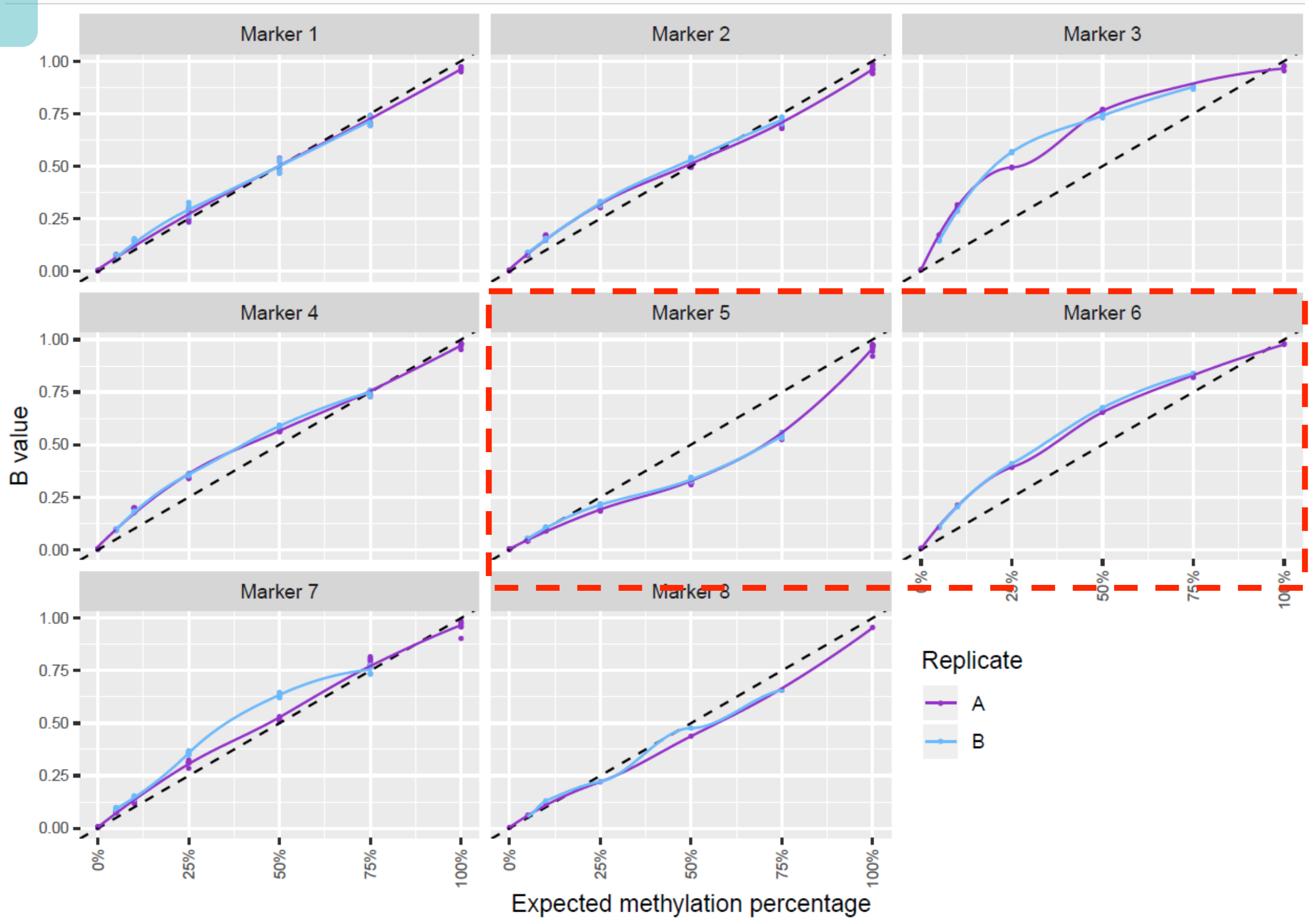


DNA input 200 ng; 24 samples/flow cell; MiSeq FGx

VISAGE WP3 – MS10/D3.3

Design and optimization ENHANCED Tools

Blood/Saliva



METH. quantitation @ optimum input:

- ✓ good performance from most markers
- ✓ 3 and 6 meth is overrepresented
- ✓ 5 is under represented.

VALIDATION:

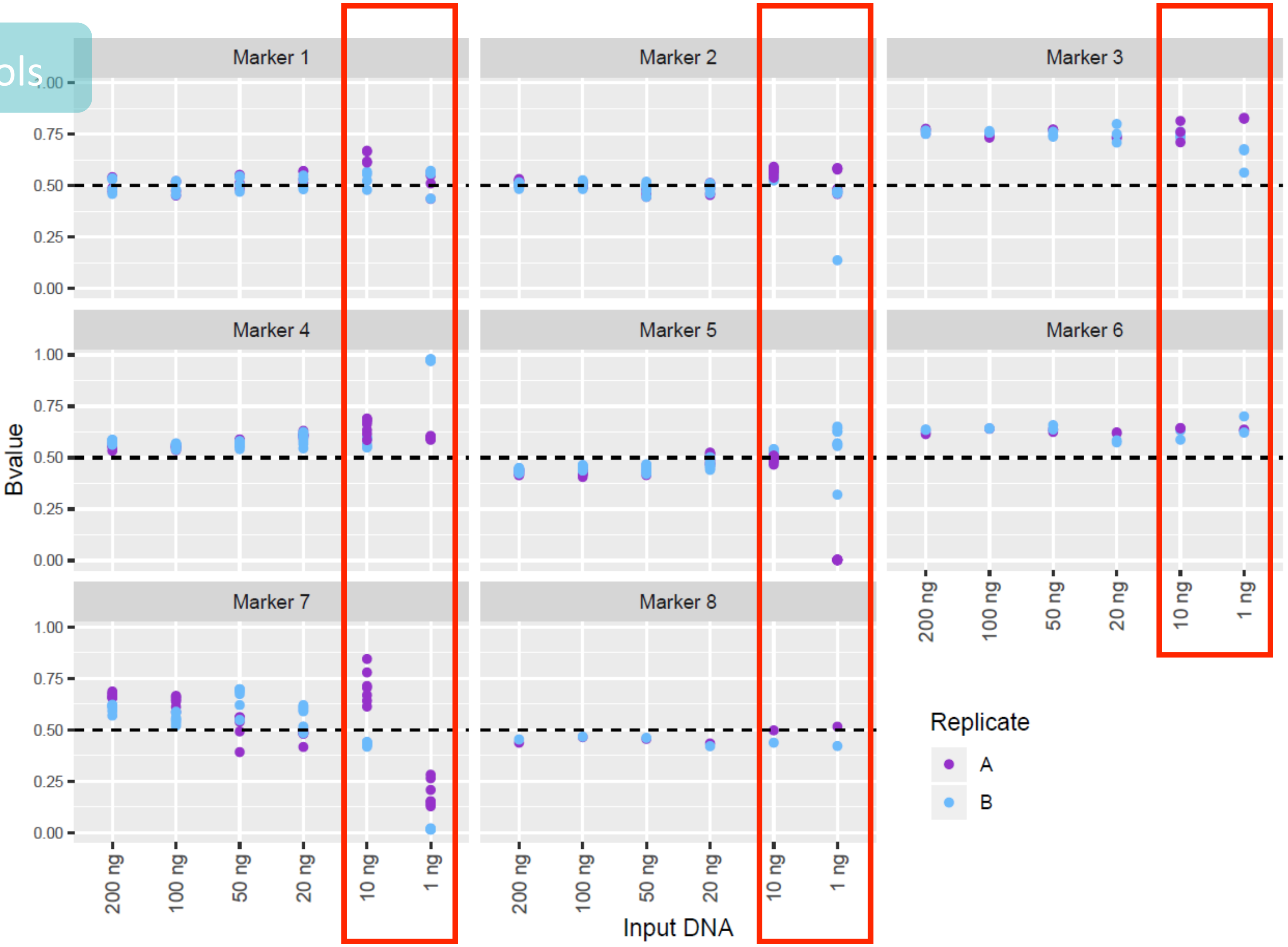
- ✓ more replicates
- ✓ deeper performance analysis
- ✓ modelling

DNA input 200 ng; duplicates; 24 samples/flow cell; MiSeq FGx

VISAGE WP3 – MS10/D3.3

Design and optimization ENHANCED Tools

Blood/Saliva



<u>METH. quantitation @ lower inputs:</u>

- ✓ 50% only tested so far
- ✓ variation among replicates increases
  - ✓ below 20 ng

DNA input 200 - 1 ng; duplicates; 24 samples/flow cell; MiSeq FGx

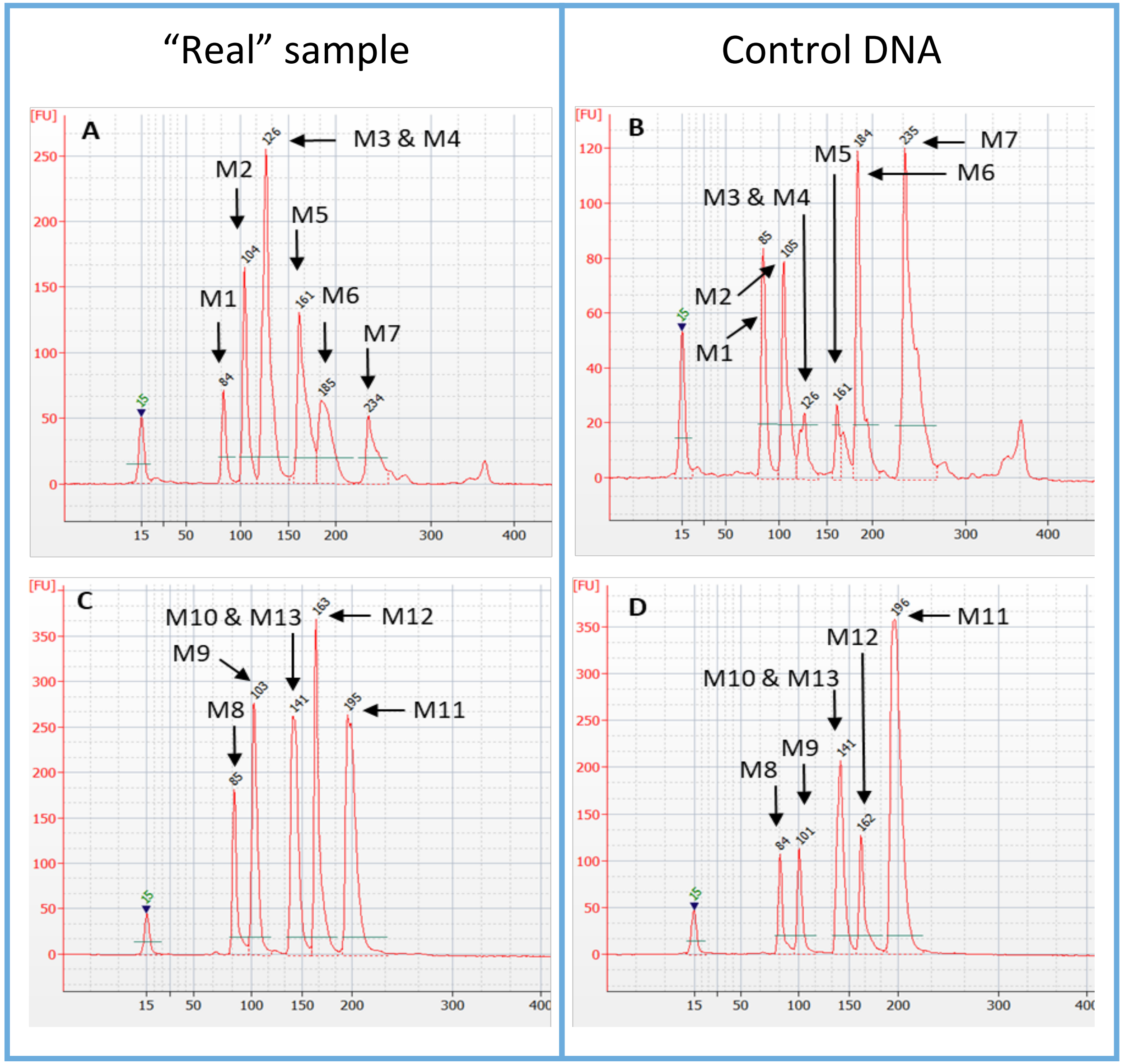
VISAGE WP3 – MS10/D3.3

Design and optimization ENHANCED Tools

Semen

OPTIMIZATION:

- ✓ 2 multiplex assay
- ✓ **no extra sample needed** → use half eluate from BC for each rxn.
- ✓ assay was optimized **for real samples**



DNA input 200 ng; 24 samples/flow cell; MiSeq FGx

Acknowledgements

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Catarina Xavier (MUI)
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Aleksandra Pisarek (JU)

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Ana Freire Aradas

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INPS
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