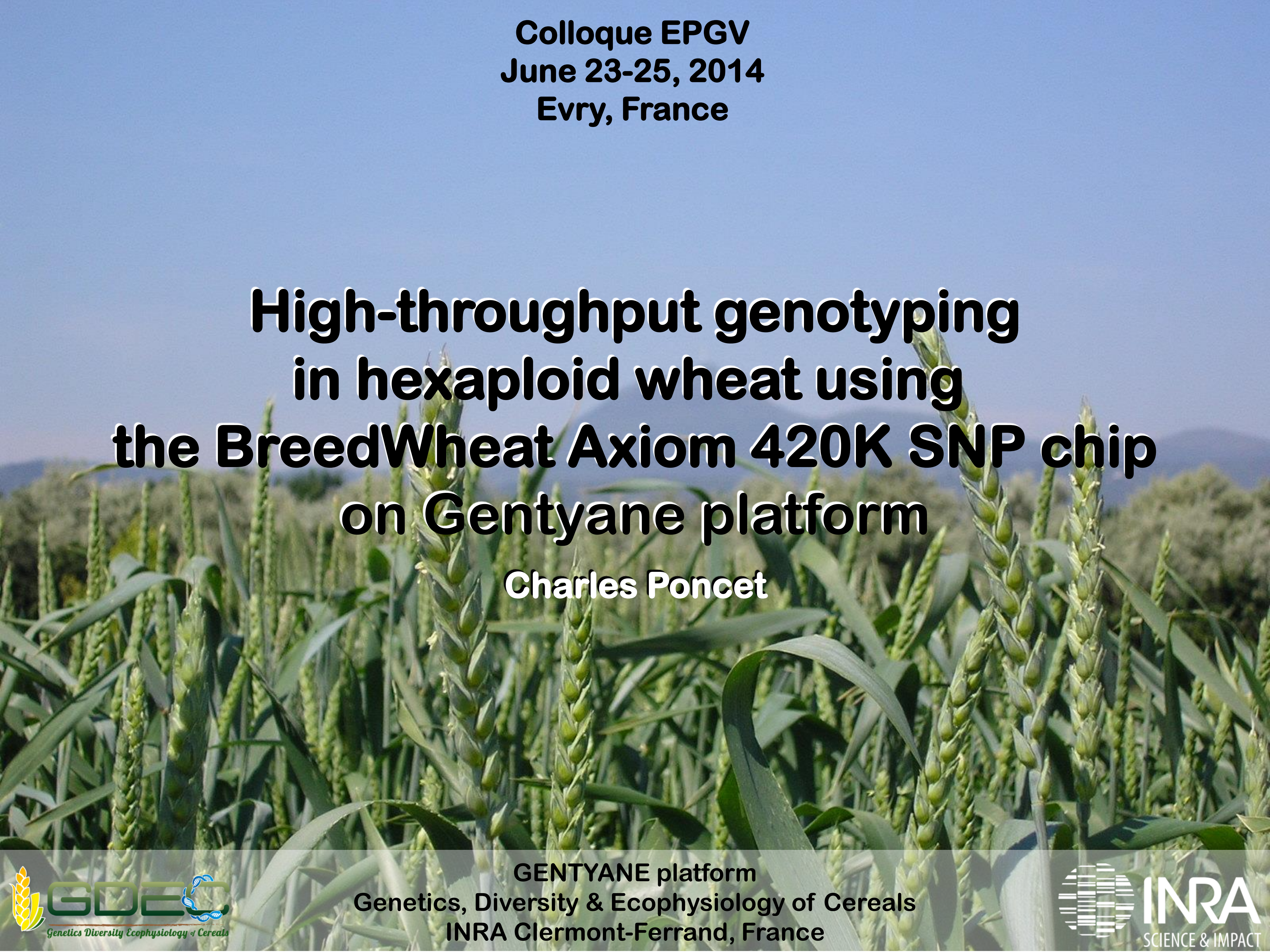


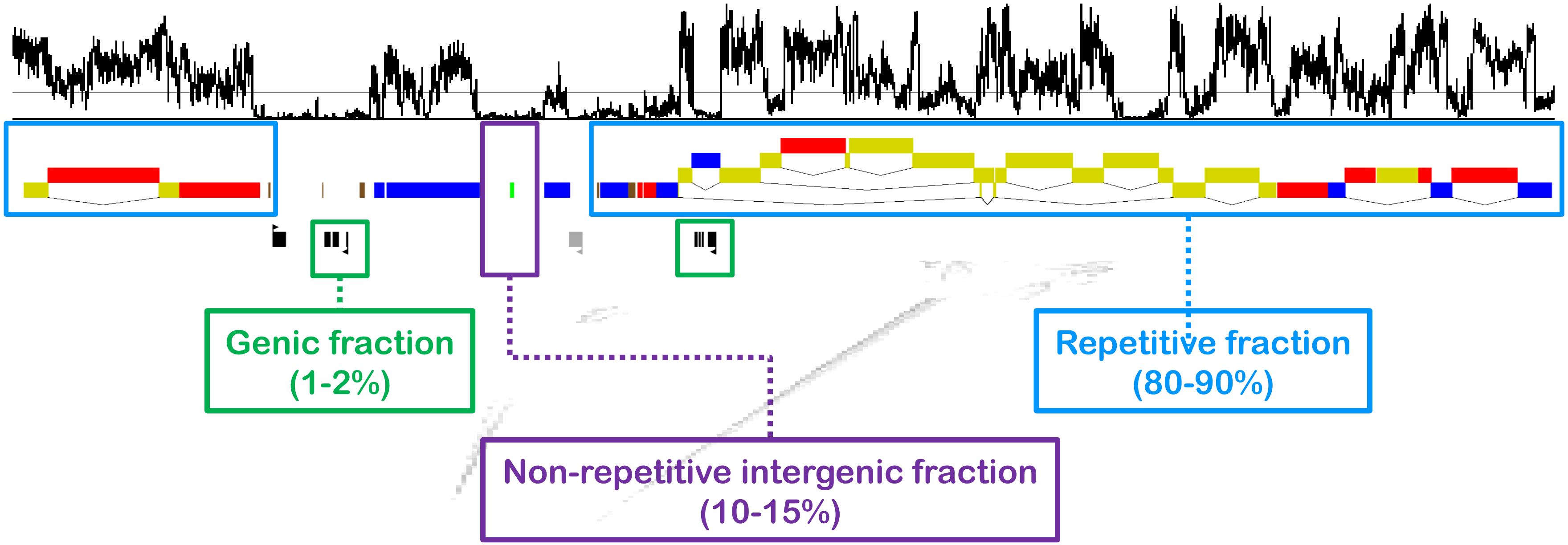
Colloque EPGV
June 23-25, 2014
Evry, France

High-throughput genotyping in hexaploid wheat using the BreedWheat Axiom 420K SNP chip on Gentyane platform

Charles Poncet

GENTYANE platform
Genetics, Diversity & Ecophysiology of Cereals
INRA Clermont-Ferrand, France

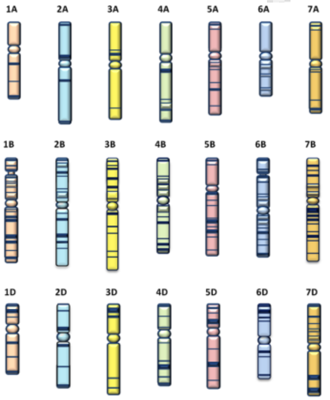
Combining different sources of SNPs



Genic fraction (1-2%)

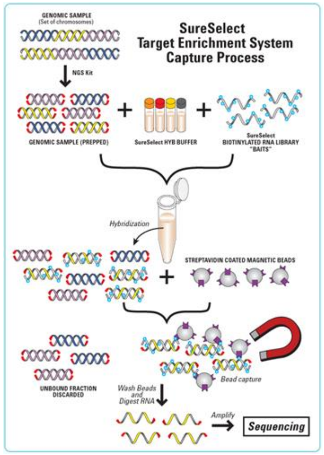
Non-repetitive intergenic fraction (10-15%)

Repetitive fraction (80-90%)



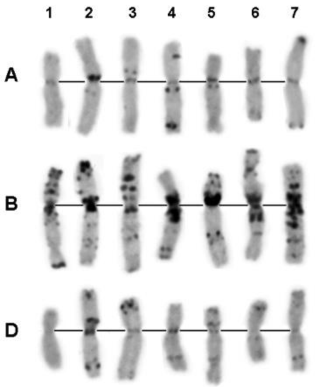
Chromosome-based draft sequence of the hexaploid bread wheat genome

x



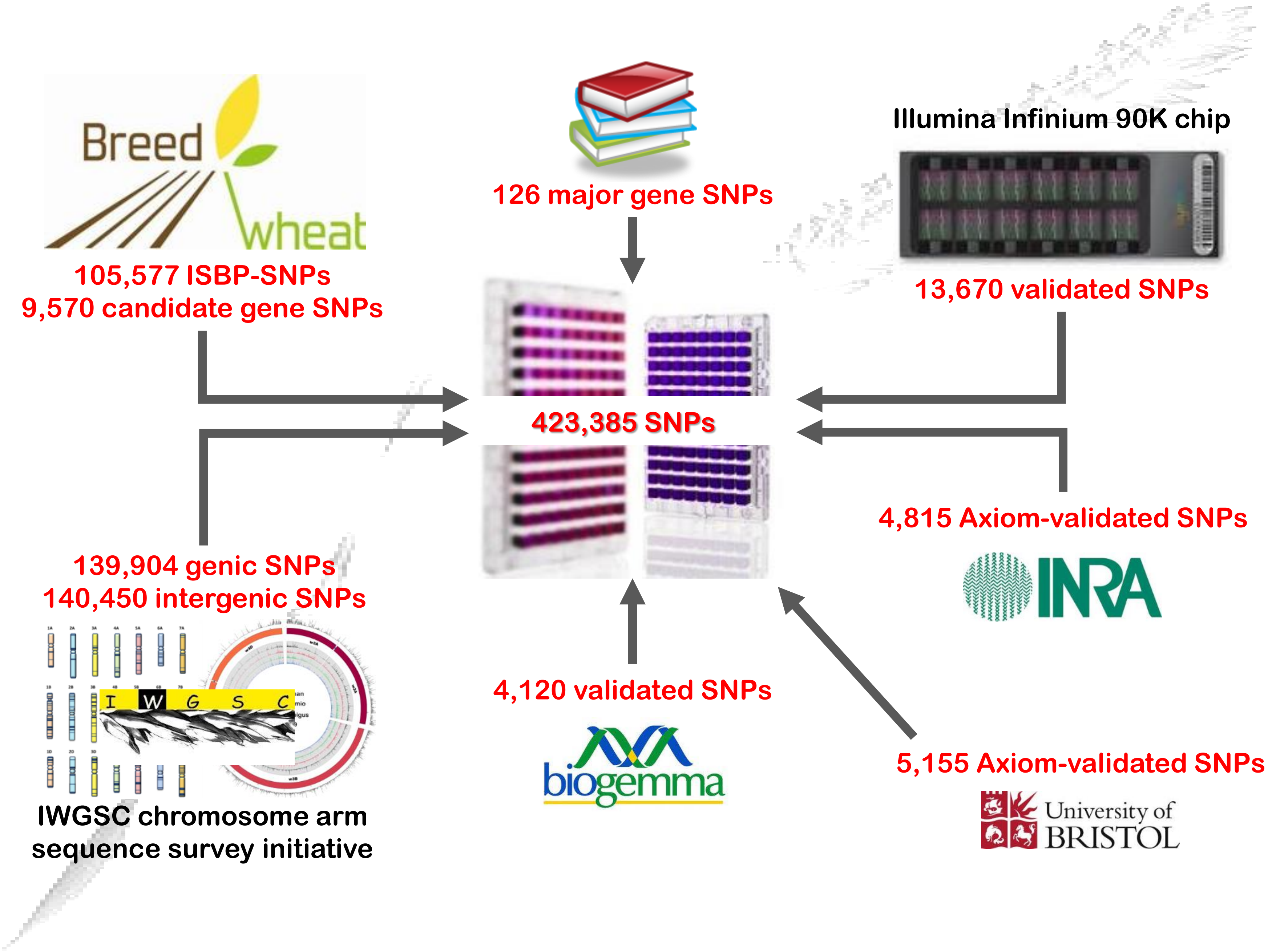
Sequence capture

or



Whole-genome resequencing

The BreedWheat 420K SNP chip



126 major gene SNPs

Illumina Infinium 90K chip

13,670 validated SNPs

105,577 ISBP-SNPs
9,570 candidate gene SNPs

423,385 SNPs

4,815 Axiom-validated SNPs

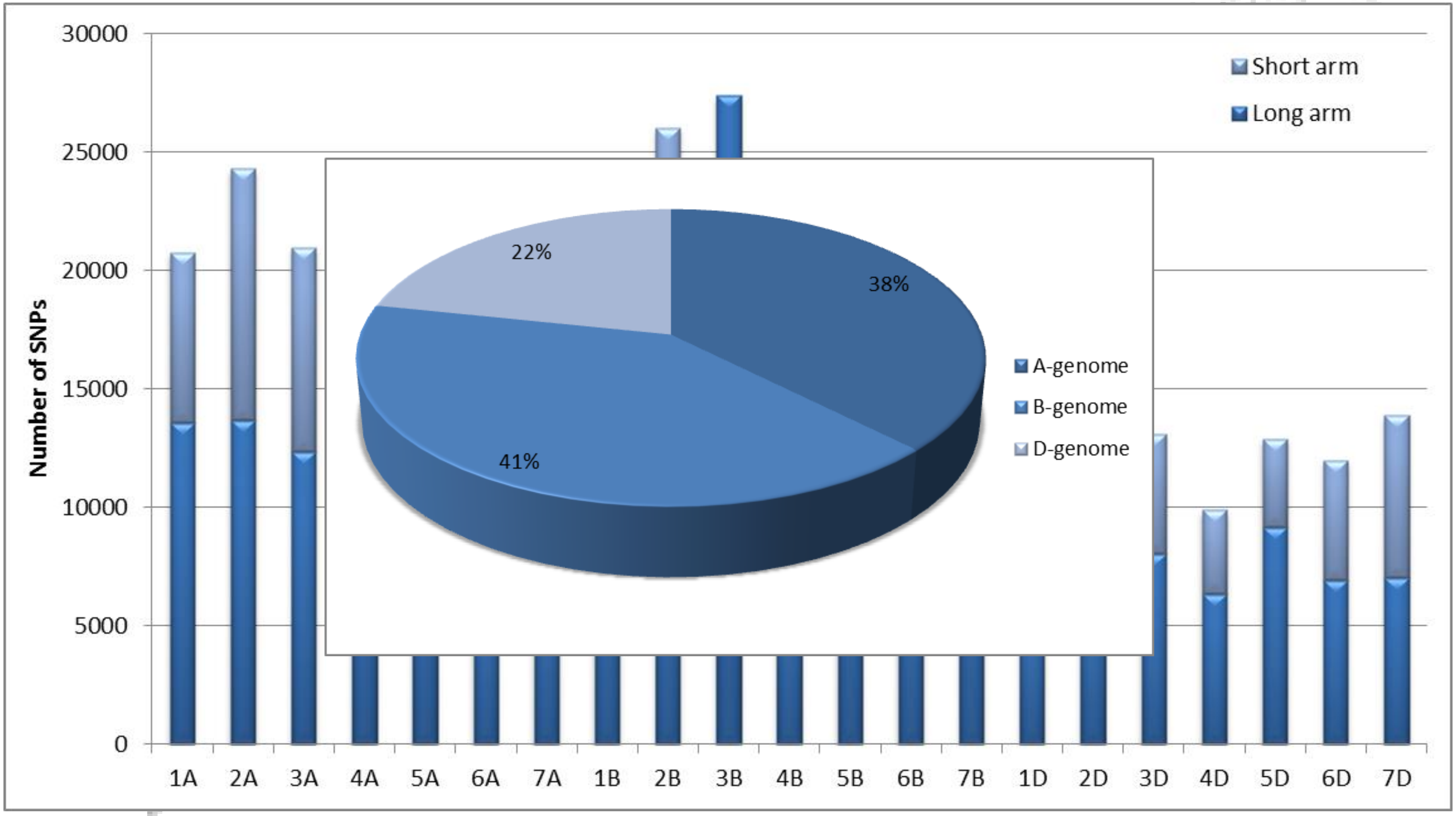
139,904 genic SNPs
140,450 intergenic SNPs

IWGSC chromosome arm sequence survey initiative

4,120 validated SNPs

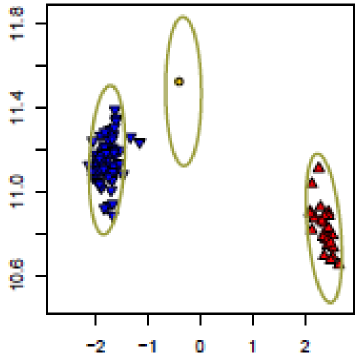
5,155 Axiom-validated SNPs

The BreedWheat 420K SNP chip: *in silico* mapping



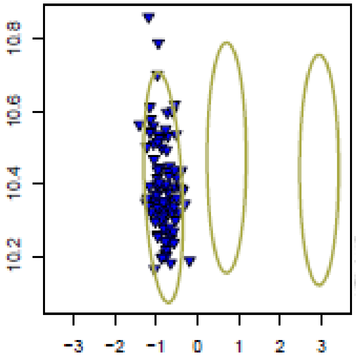
Affymetrix SNP classification

PolyHighResolution



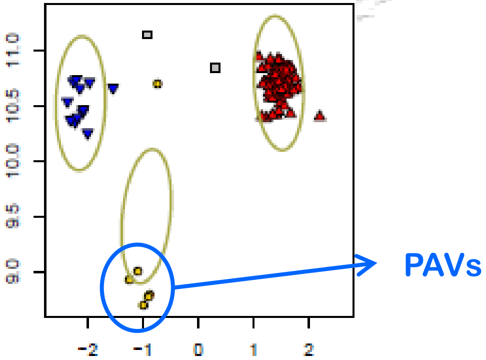
Good cluster resolution, and at least 2 example of the minor allele.

MonoHighResolution



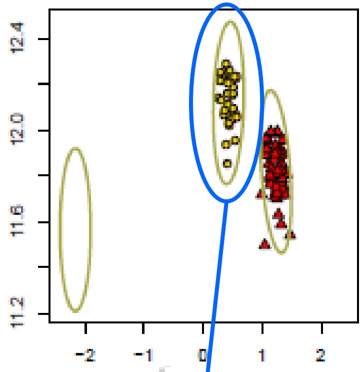
Less than 2 examples of the minor allele usually due to low MAF samples, but possible cluster fusion / compression

OTV : Off Target Variants (SNP en présence / absence?)



OTV's (discussed above) can be assayed by OTV genotyping

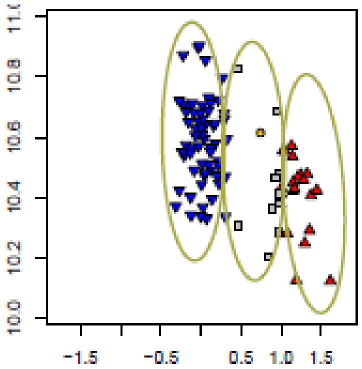
No Minor Hom (sur polyploïdes nuages homozygotes?)



Two clusters with no examples of the minor homozygous genotypes. Het cluster not in OTV location

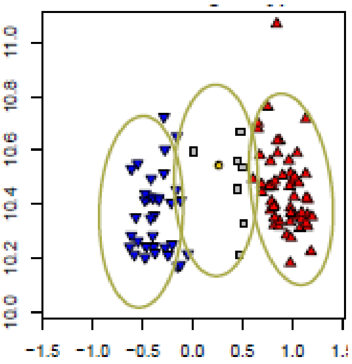
Heterozygous = homozygous

CallRate Below Thresh.



SNP callrate is below threshold, but other cluster properties are above threshold.

Other



One or more cluster properties are below threshold.
Expect lower quality genotypes

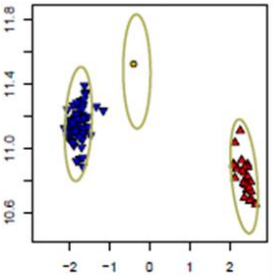
SNP conversion rate

BreedWheat 420K SNP chip

X

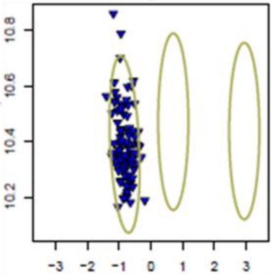
96 wheat lines
(13 European elite varieties
+ 83 world-wide diversity lines)

PolyHighResolution



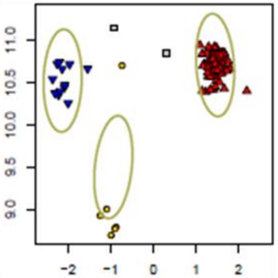
168,573
(39.8%)

MonoHighResolution



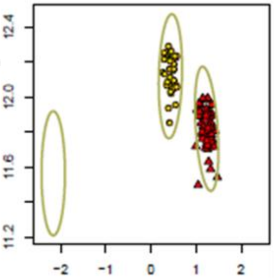
31,799
(7.5%)

OTV



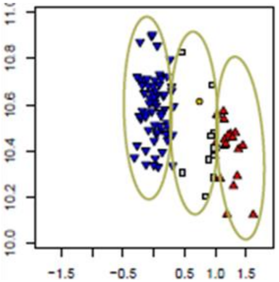
37,147
(8.8%)

No Minor Hom



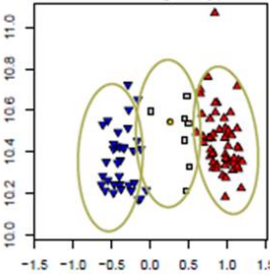
49,338
(11.7%)

CallRate Below Thresh.



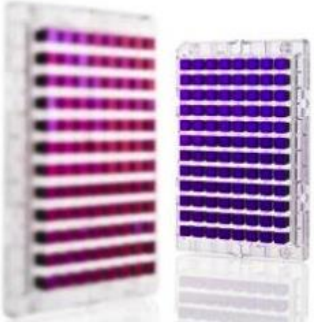
21,809
(5.2%)

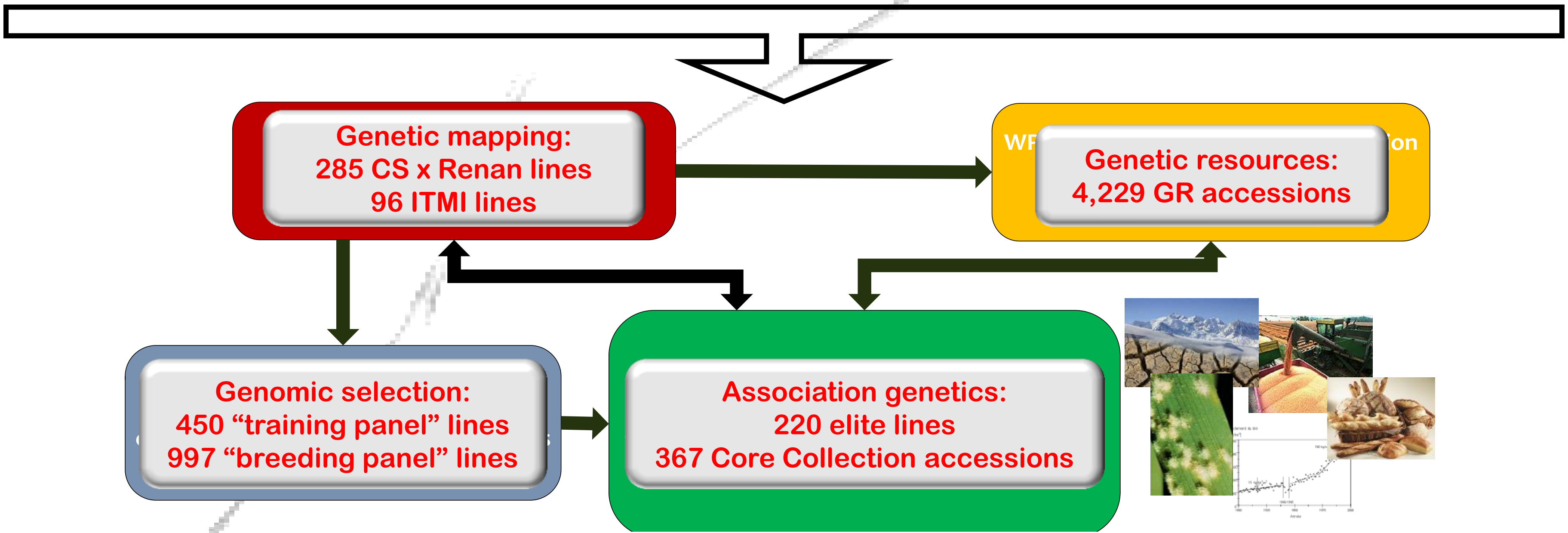
Other



114,719
(27.1%)

The BreedWheat genotyping panels





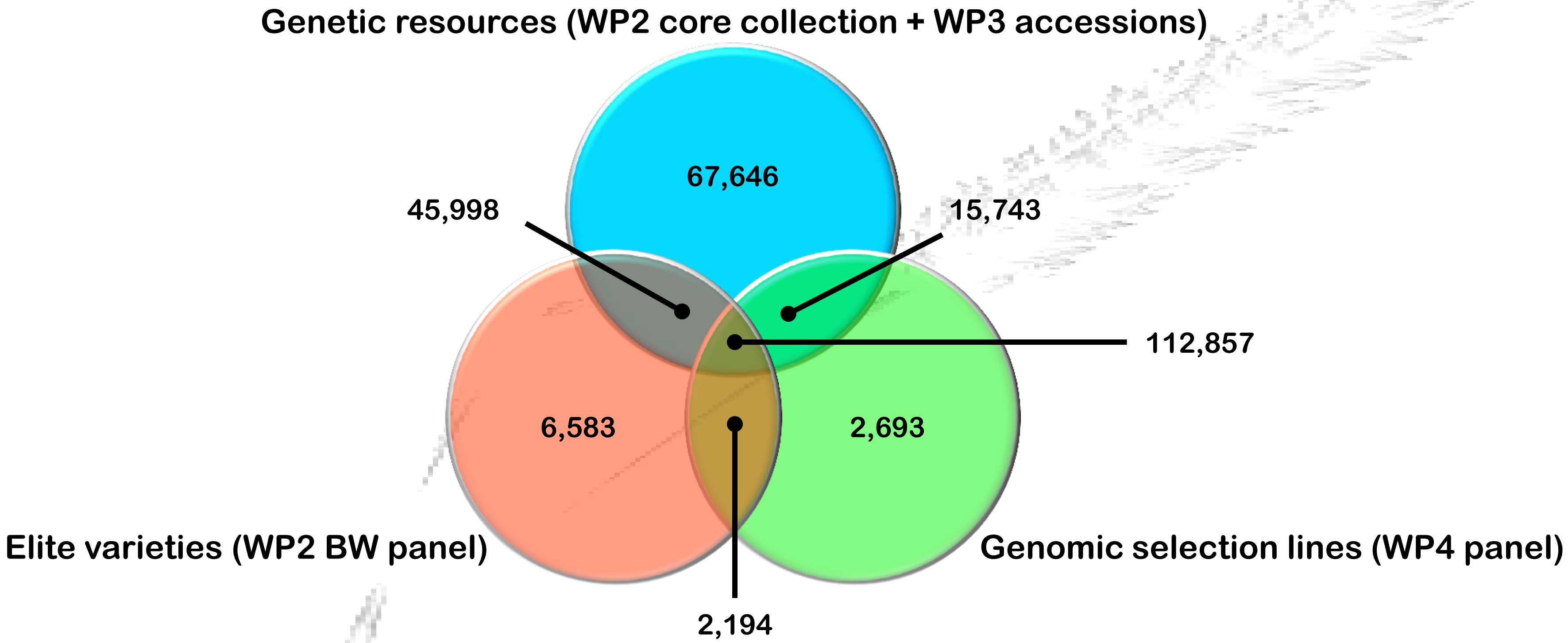
Genetic mapping:
285 CS x Renan lines
96 ITMI lines

Genetic resources:
4,229 GR accessions

Genomic selection:
450 “training panel” lines
997 “breeding panel” lines

Association genetics:
220 elite lines
367 Core Collection accessions

Comparison of the different panels



☺ 275,661 SNPs (65% of the whole chip)

☹ But incomplete overlap

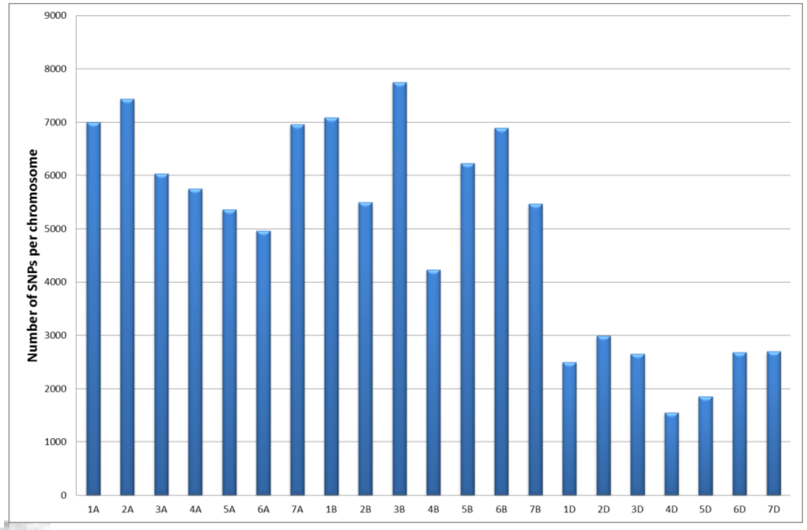
A 100K SNP genetic map

BreedWheat 420K SNP chip

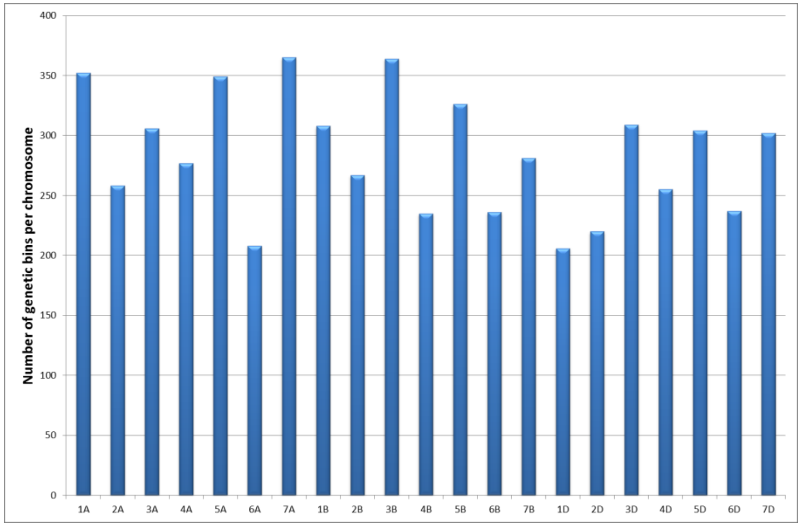
x

285 Chinese Spring x Renan F7 lines

103,581 mapped SNPs

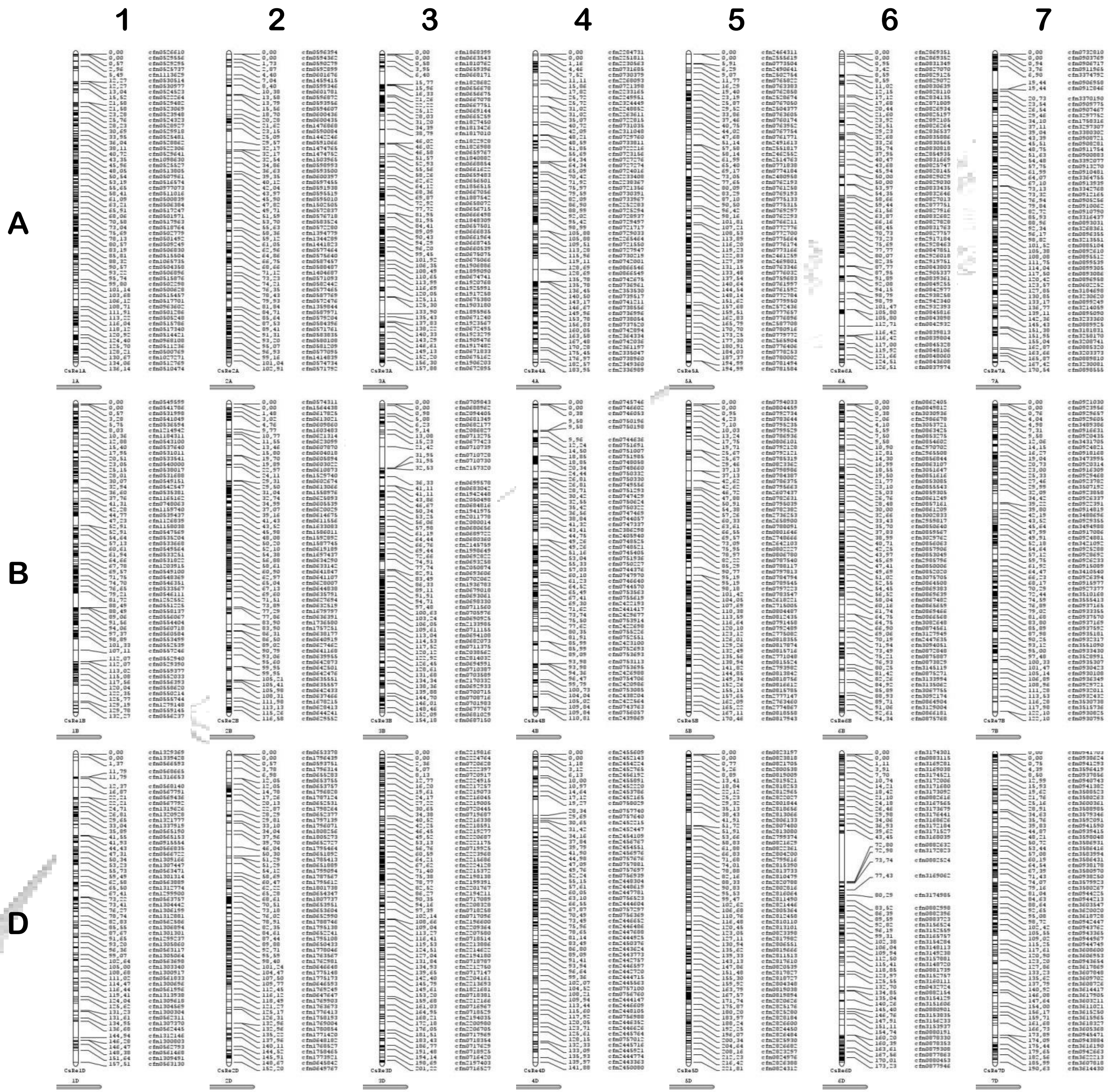


Number of SNPs per chromosome



Number of genetic bins per chromosome

A 100K SNP genetic map



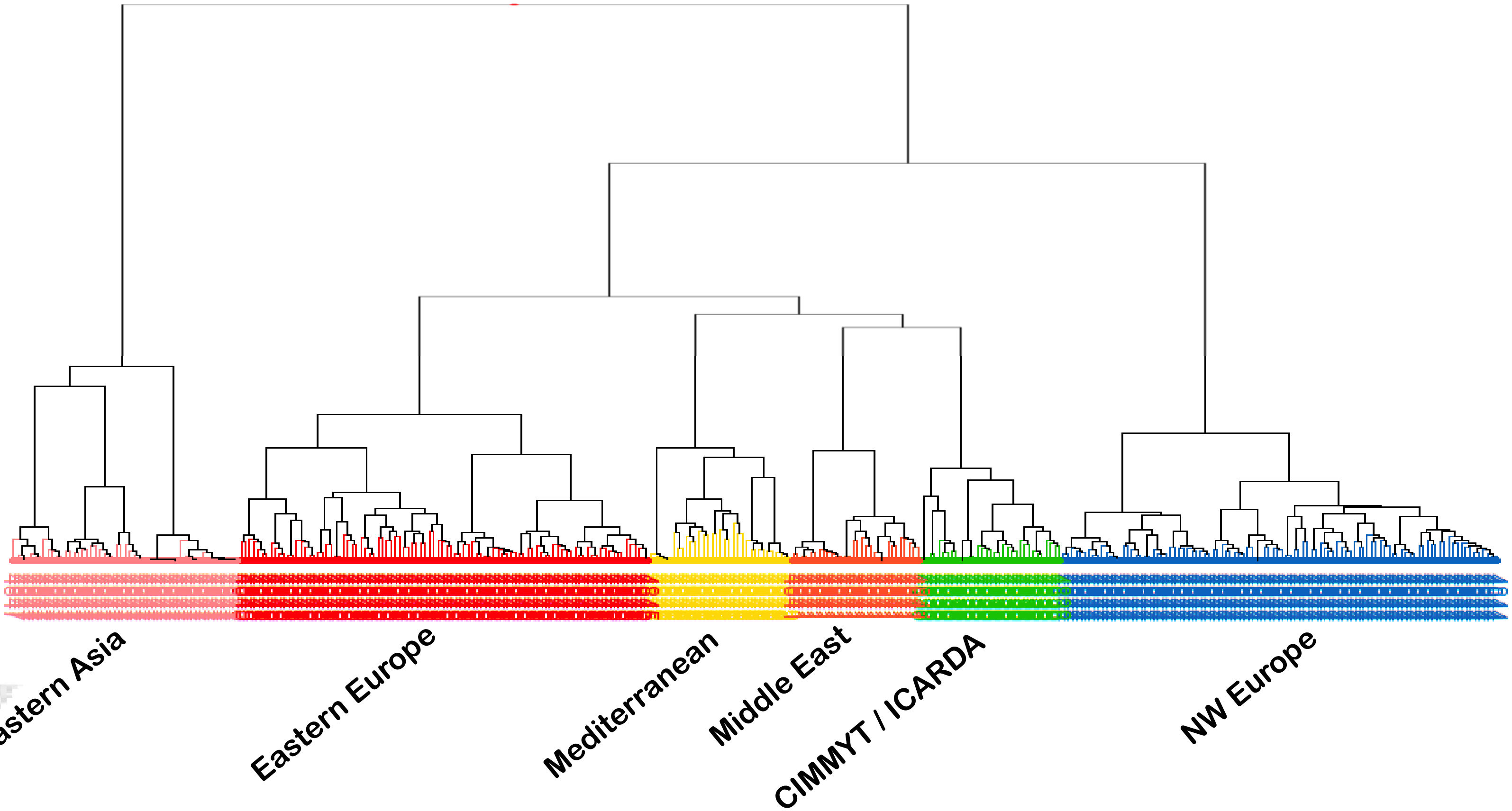
Characterizing the worldwide diversity

BreedWheat 420K SNP chip

X

367 accessions representing the worldwide diversity

67,699 polymorphic SNPs

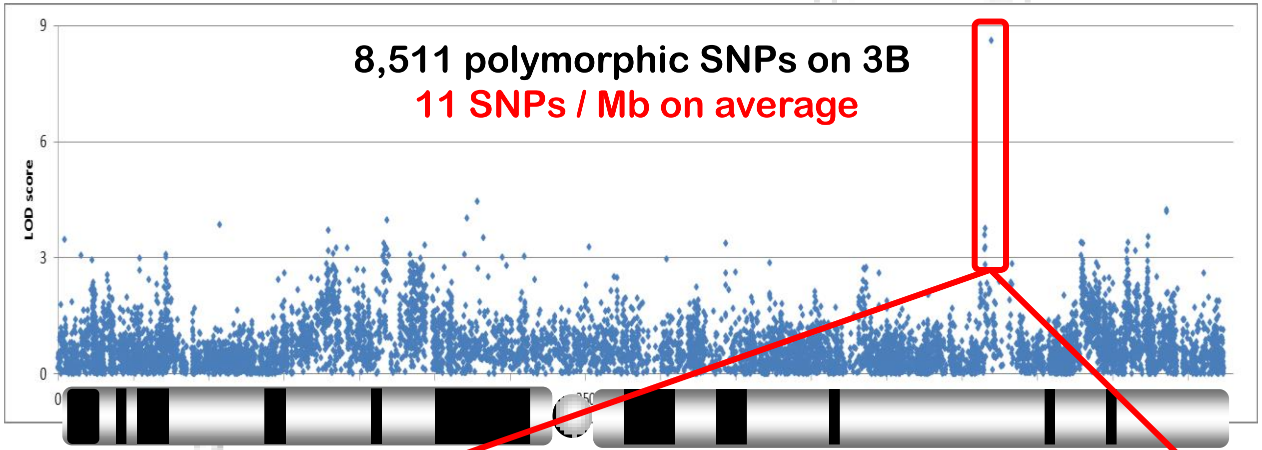


Genome-wide association study for pre-harvest sprouting

BreedWheat 420K
SNP chip

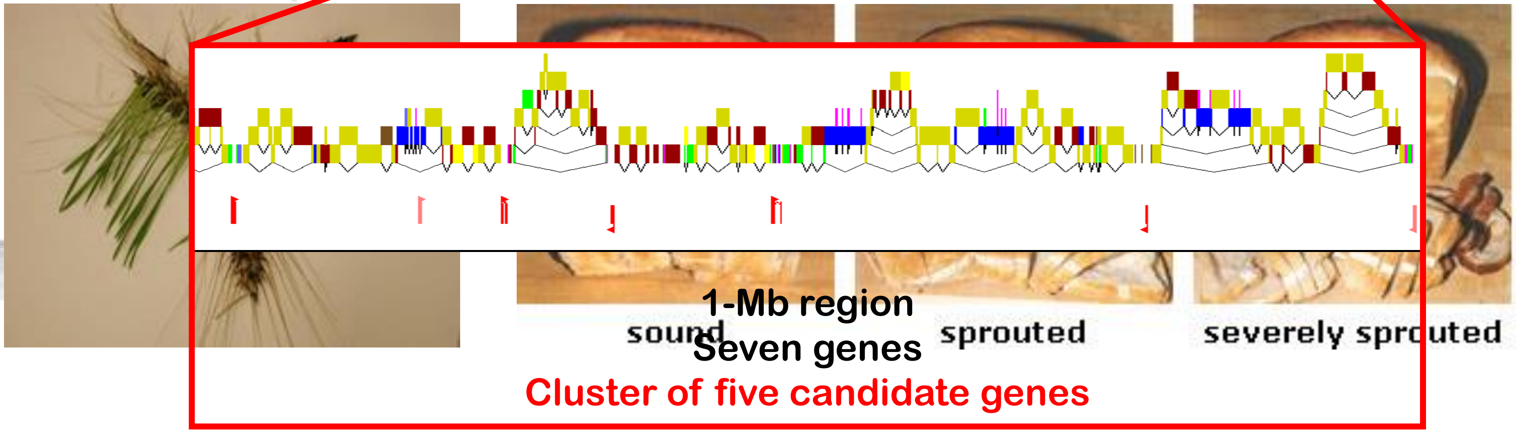
X

367 accessions
representing the
worldwide diversity



8,511 polymorphic SNPs on 3B

11 SNPs / Mb on average



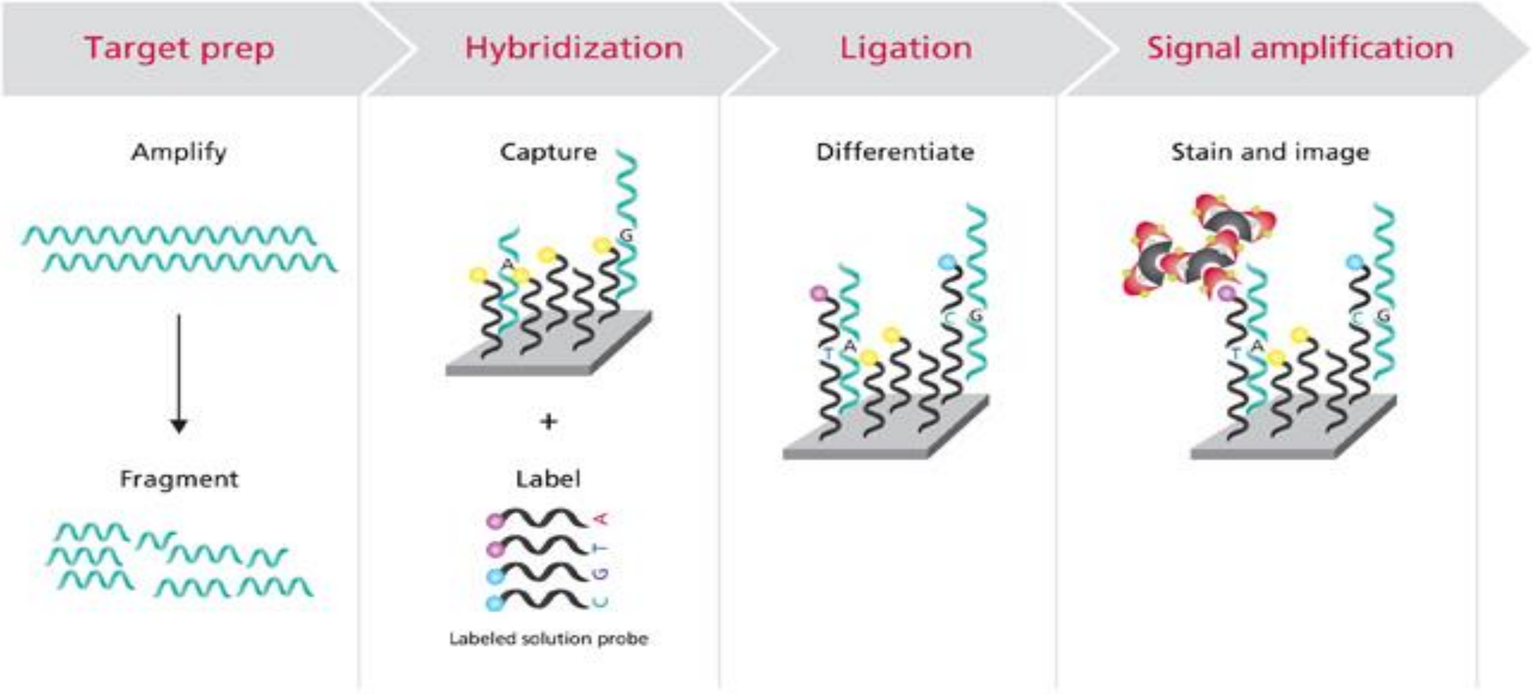
1-Mb region

Seven genes

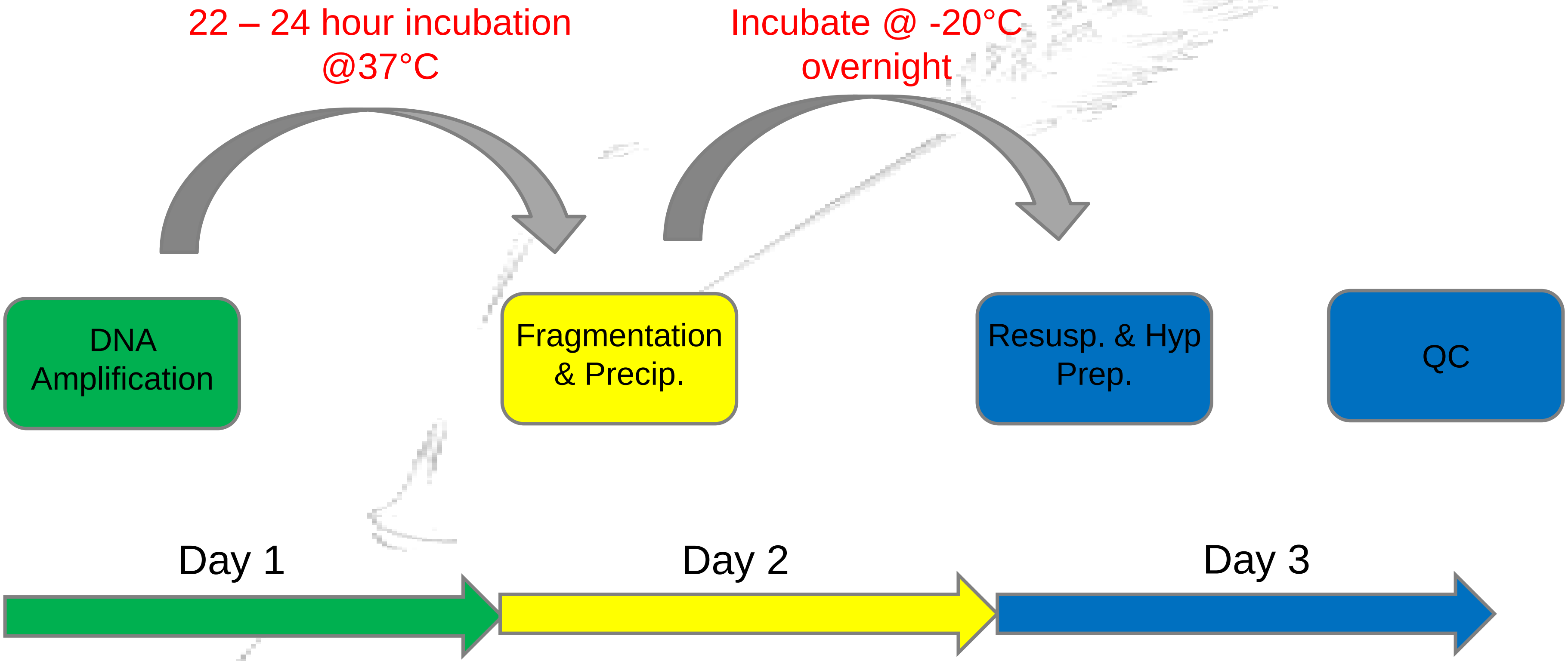
Cluster of five candidate genes

AXIOM Automated Solution

Fully automated on the Biomek FXp and GeneTitan



Target Preparation with Biomeck FxP



22 – 24 hour incubation @37°C

Incubate @ -20°C overnight

DNA Amplification

Fragmentation & Precip.

Resusp. & Hyp Prep.

QC

Day 1

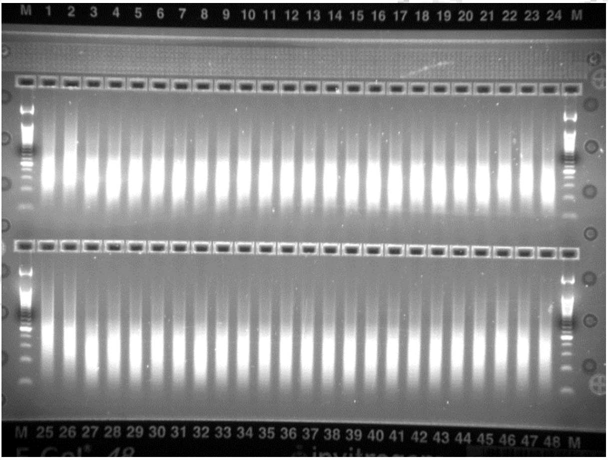
Day 2

Day 3

AXIOM Assay QC Checkpoints

✓ DNA Fragmentation

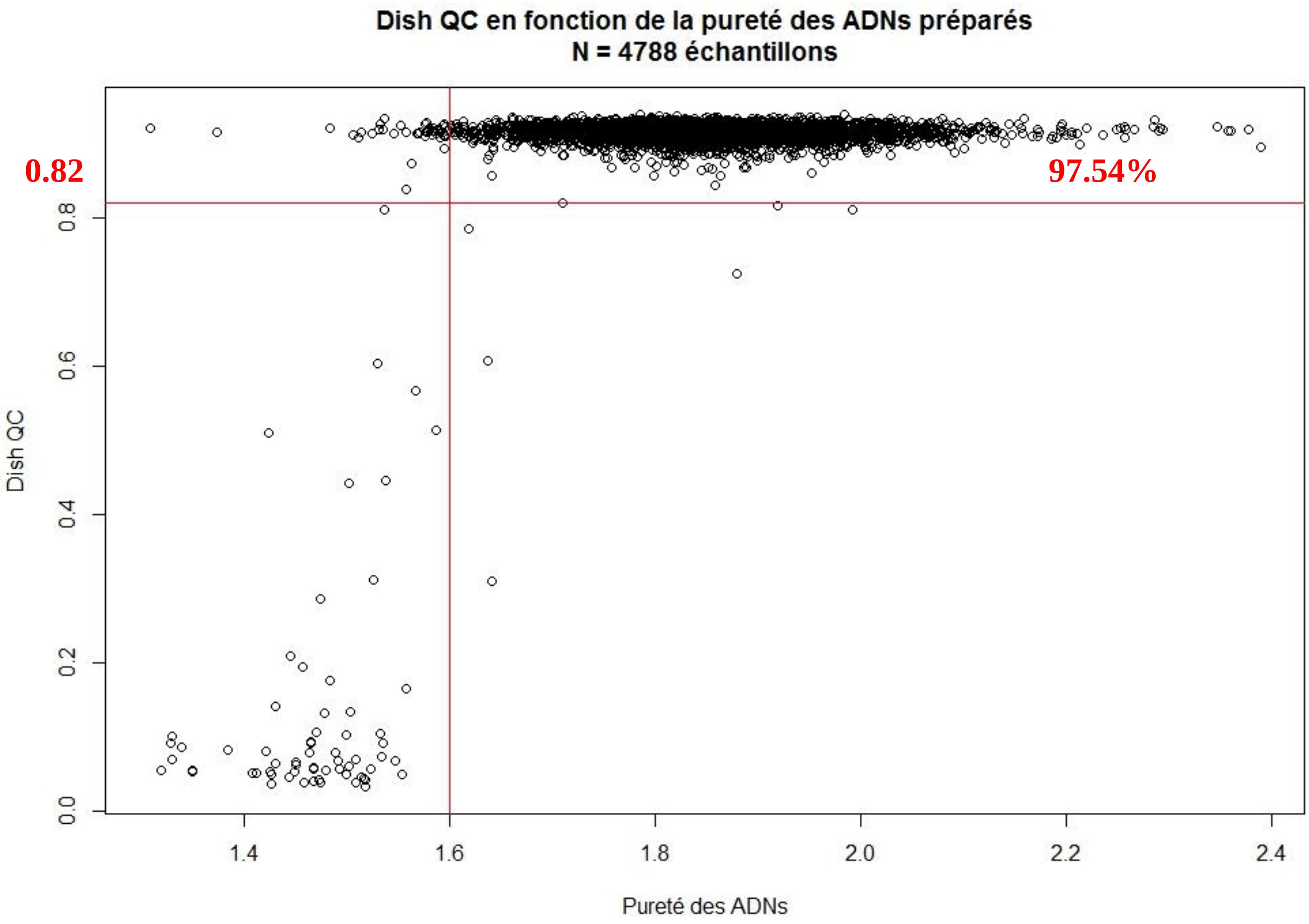
Use 4% E-gel, DNA should be between 25bp – 125 bp



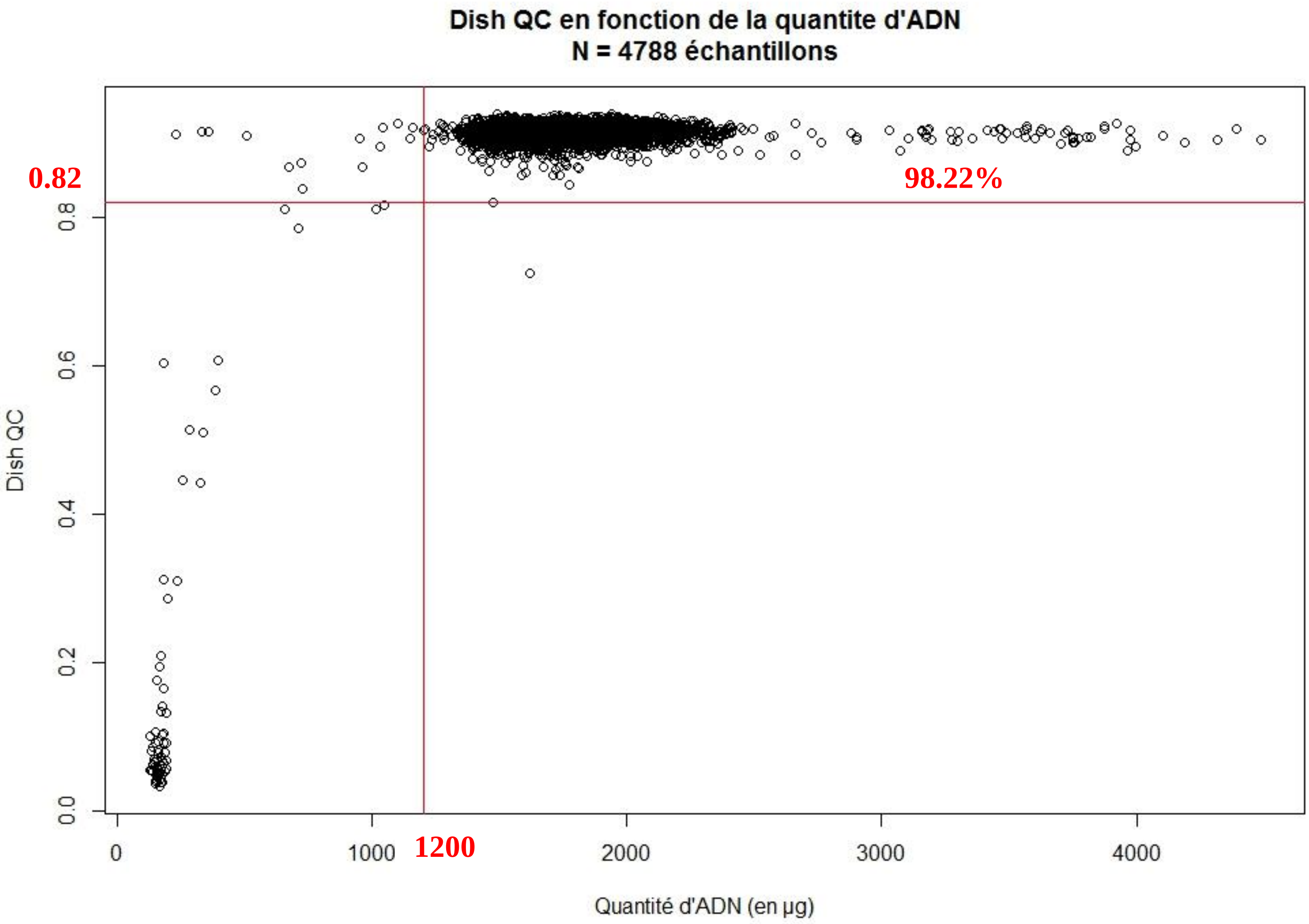
✓ DNA Yield

OD 260 nm, 280 nm and 340 nm on Tecan Infinite 1000
The yield must be > 1200µg

Dish QC / DNA purity

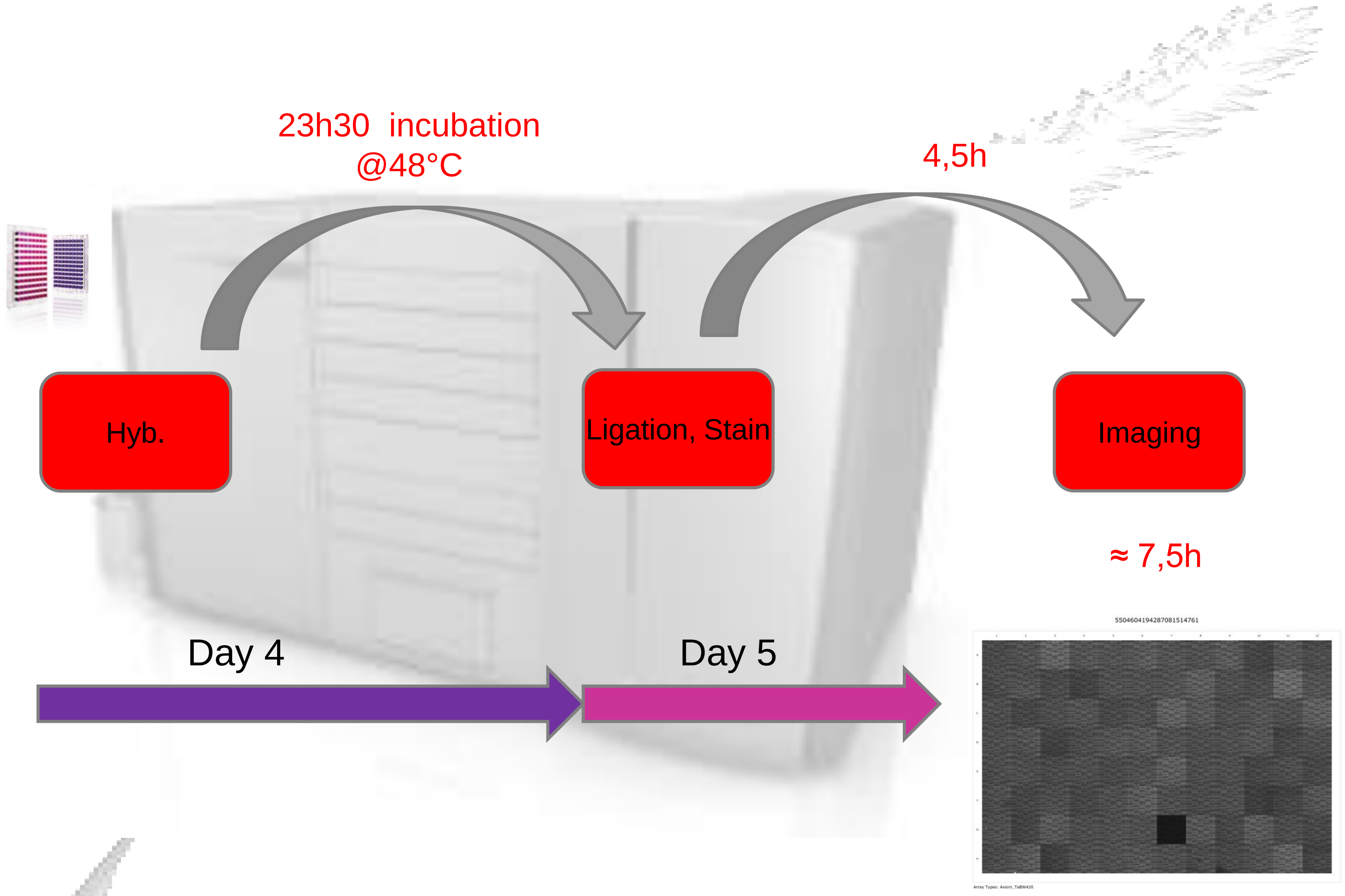


Dish QC / ADN quantity



Dish QC en fonction de la quantite d'ADN
N = 4788 échantillons

Array processing with GeneTitan



23h30 incubation
@48°C

4,5h

Hyb.

Ligation, Stain

Imaging

≈ 7,5h

Day 4

Day 5

Acknowledgements

GDEC
Genetics Diversity Ecophysiology of Cereals

- Nelly Cubizolles
- Elodie Rey
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- Charles Poncet
- Cora Boedo
- Benoît Darrier
- Pierre Sourdille
- François Balfourier
- Jacques Le Gouis
- Catherine Feuillet…

biogemma

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Magalie Leveugle
Jorge Duarte
Nathalie Rivière

INRA

Marie-Christine Le Paslier
Dominique Brunel

Bioplatforms Australia

BioPlatforms Australia

IWGSC

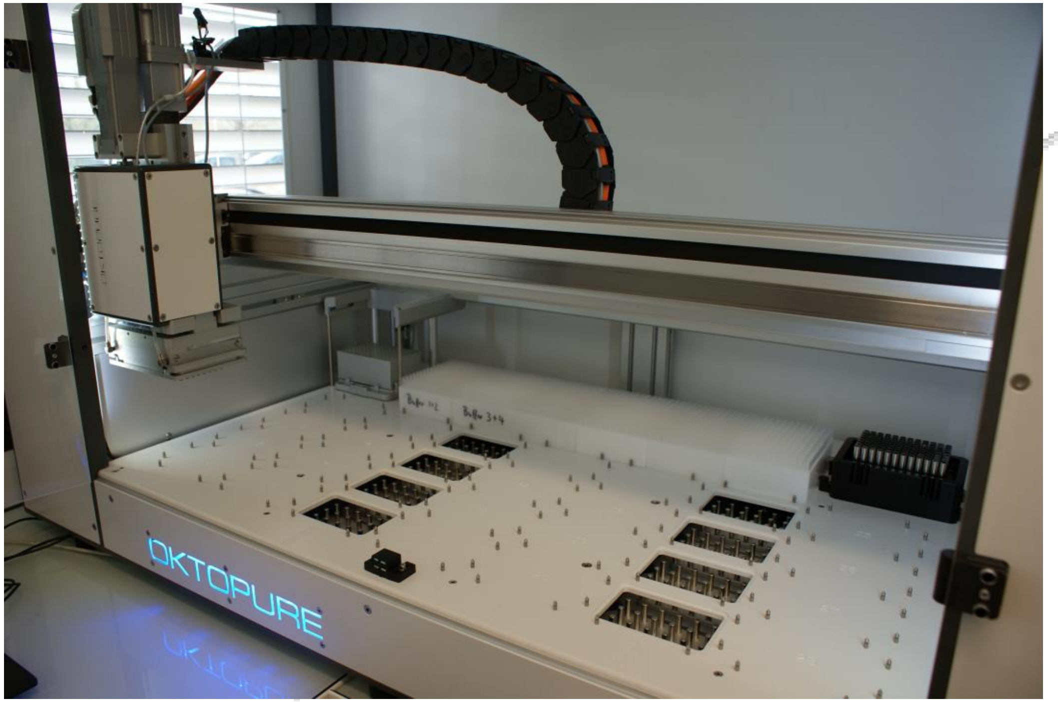
Breed Wheat

ANR FranceAgriMer FSOV GIS bV

The research leading to these results has received funding from the INRA, the French Government managed by the Research National Agency (ANR) under the Investments for the Future call 2010, as well as from FranceAgriMer

Nouveautés GENTYANE 2014

- Oktopure LGC Genomics

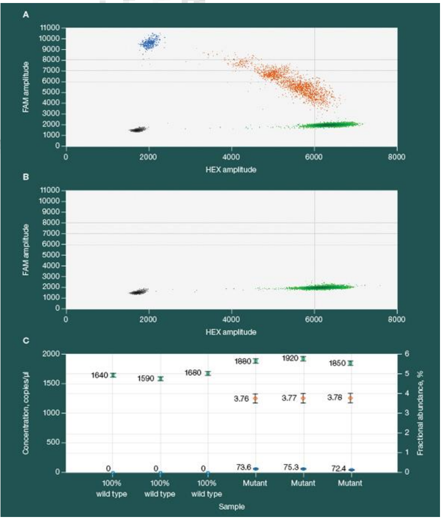


Lyse → Bind 1 → Wash 1 → Bind 2 → Wash 2 → Elute

Extraction d’ADN automatisée billes magnétiques sbeadex 8*96 extractions / 2h

Développements GENTYANE 2014-2015

- Développement CNV sur Droplet PCR QX200 Biorad et/ou sur puce Fluidigm



Développements GENTYANE 2014-2015

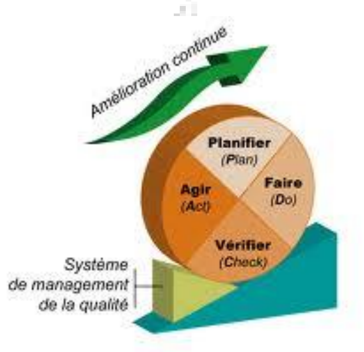
- Développement GBS méthode AmpliSeq / Ion Proton

Figure 1. Ion AmpliSeq™ custom workflow. Typical assay design results targeting a 10 kb region are available in as little as 2 hours and require only minutes of hands-on time. It takes just 10 hours to go from DNA to variants.

Politique Qualité GENTYANE 2014

- Site pilote Certification AFNOR NF X 50-900

Exigences pour les Plateformes Technologiques de Recherche en Sciences du Vivant - Systèmes de management intégrant un système de management de la qualité ISO 9001 :2008



LLOYD'S REGISTER QUALITY ASSURANCE · ISO9001

GENTYANE
PLATE-FORME de
GENOTYPAGE et SÉQUENÇAGE en AUVERGNE

Merci de votre attention