How HT SNP genotyping can help timber tracking

P. Faivre Rampant
INRA-EPGV

EPGV Etude du Polymorphisme des Génomes Végétaux

Plant Genome Polymorphism Unit

Directrice de l'Unité
Expertise technologique
Marie-Christine Le Paslier

Expertise scientifique

Génomique végétale
Dominique Brunel

Génomique des arbres forestiers
Patricia Faivre Rampant

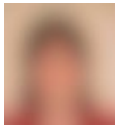
Développement technologique et Production

Aurélie Bérard, AI
Coordination opérationnelle

Aurélie Chauveau, TR, 80%

-:-:-:-:-:-:-:-

Isabelle Le Clainche



Informatique & Bio-Informatique

Elodie Marquand
Coordination informatique

Sandrine Contenot

Aurélie Canaguier

Gestion Administrative et Financière

Mélanie Atlan

Facilities at the François Jacob Institute

Genoscope

Sequencing

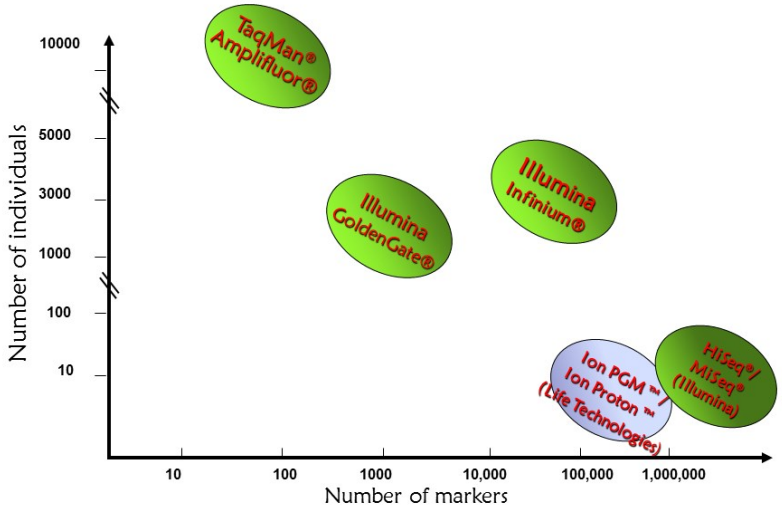
HiseqX5
Hiseq 4000
Hiseq 2500
NextSeq500
Miseq
Proton
PGM

Genotyping

TaqMan_Ampifluor
Amplicon sequencing
Infinium iSelect

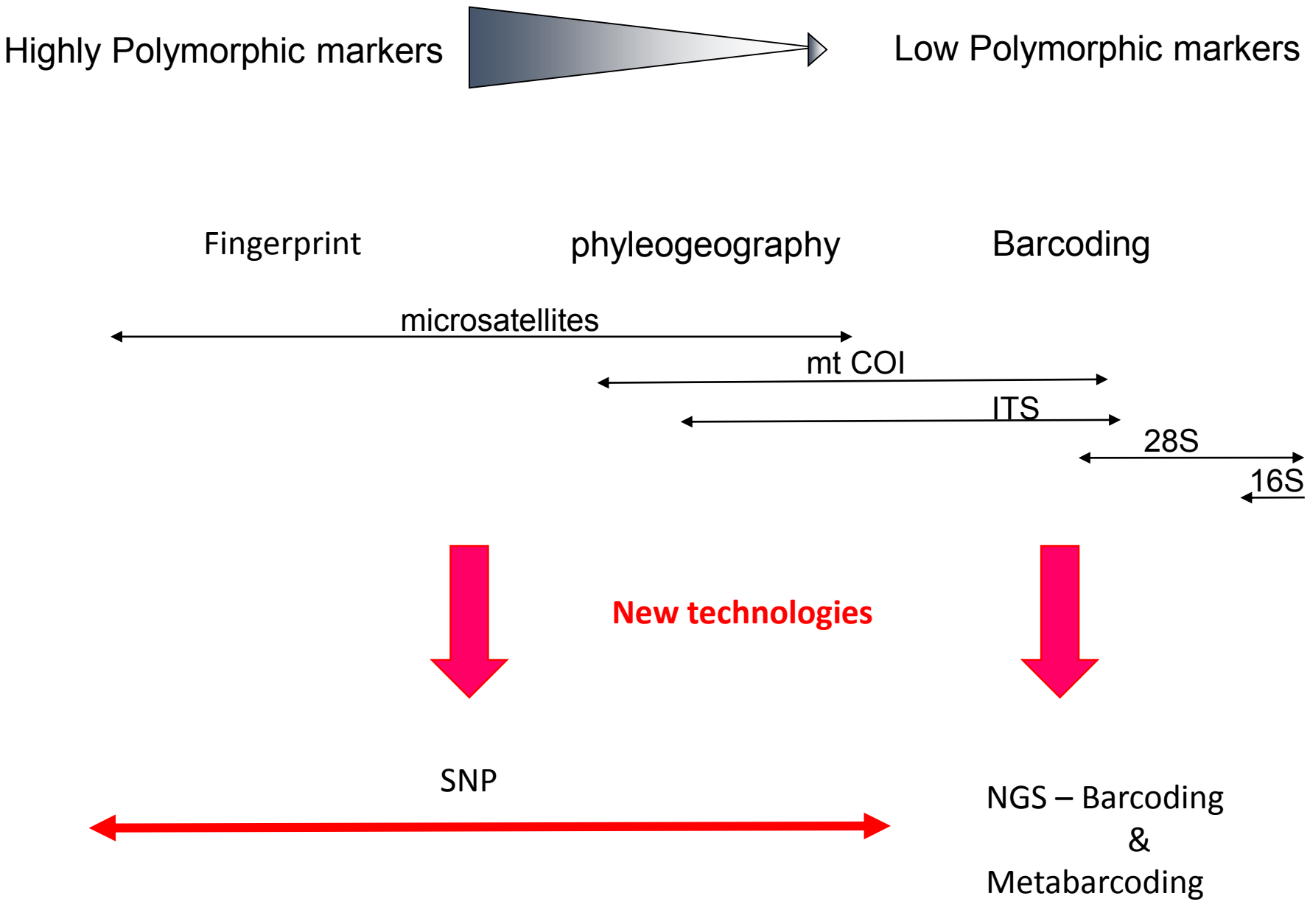
HT technologies

New technologies



http://www.cng.fr/ http://www.cns.fr/

DNA polymorphism



- **Metabarcoding** → Genus and species identification
- **How to start from scratch** → SNP discovery
- **Genotyping tools**

Tracking flower-foraging insects and their pollen loads in French mountain permanent grasslands

'Who visits what and…. Who transports what?'

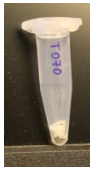
INRA_EcoServ_Polipré_@ JN Galliot, A. Farrugia, Galliot et al, 2017

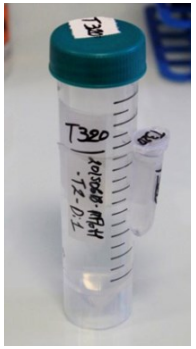
Sampling protocol

Flower-foraging insects, collecting actively pollen and/or nectar

Add alcool and shake

Visual identification

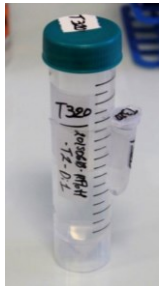




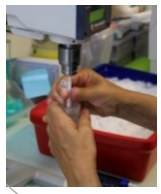
Metabarcoding

Galliot et al, 2017 J Insect Conserv DOI 10.1007/s10841-017-0022-z

Methods



Amplicon sequencing



Pollen collection

DNA extraction

PCR

Séquencing

Primers :
- ITS2
- COI

bio-informatic

- rDNA-ITS2
- COI

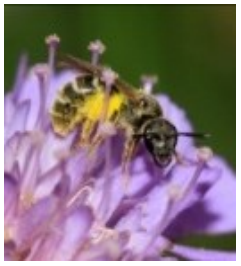
BOLD
GenBank
Database

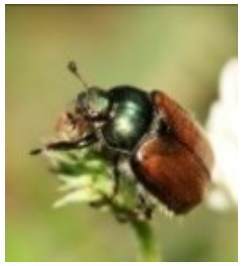
KCl buffer
9’ 95°c
Dilution

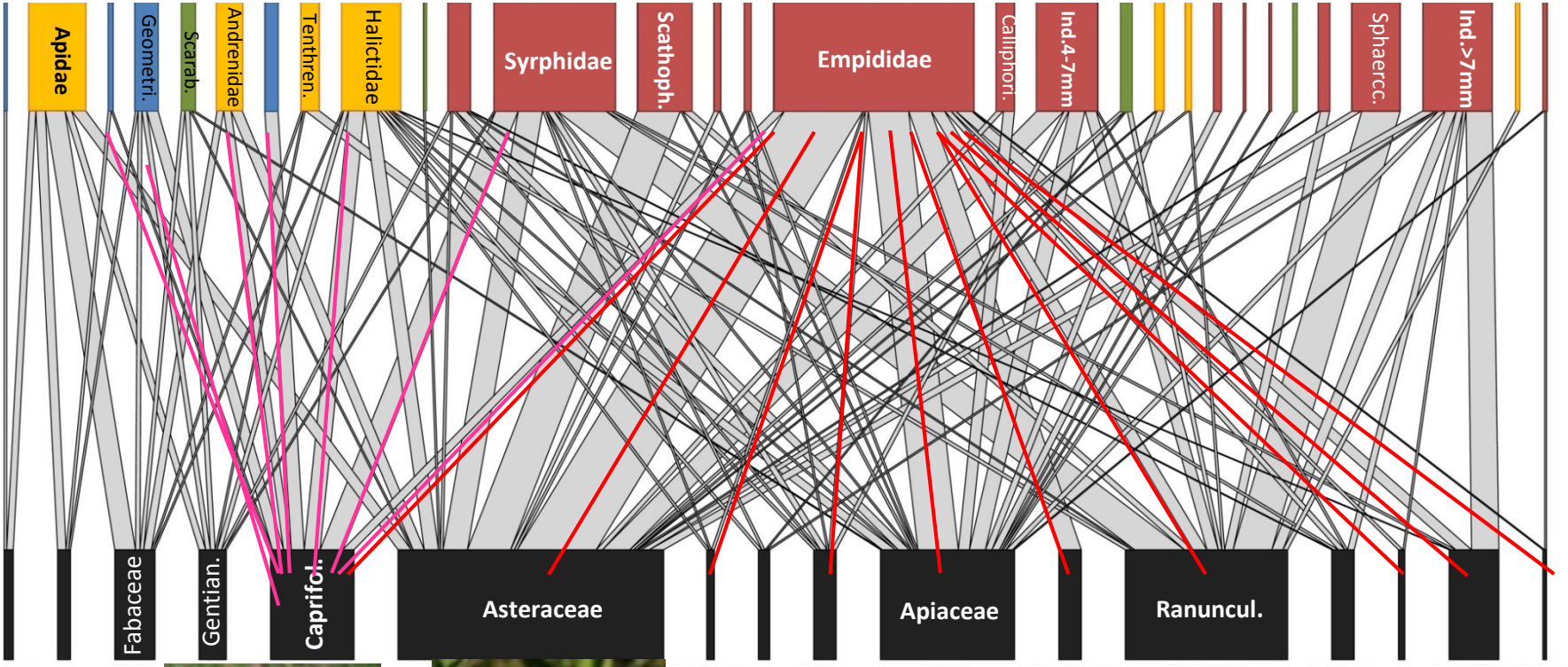
Identification

Galliot et al, 2017 J Insect Conserv DOI 10.1007/s10841-017-0022-z

The flower-foragers network








Galliot et al, 2017 J Insect Conserv DOI 10.1007/s10841-017-0022-z

Numech	Name 1	al read cou	1%	Name 2	read cc	2%	Name 3	read courcent		Name 4	al read cou	Pourcent 4
Mi266	Hypochaeris_radicata_ITS2-NCBI	3084	0,61	Alopecurus_myosuroides_ITS2-NCBI	1027	0,20						
Mi267	Hypochaeris_radicata_ITS2-NCBI	364	0,43	Festuca_arundinacea_ITS2-NCBI	123	0,15	Centaurea_nigrescens_ITS2-NCBI	117	0,14			
Mi268	Centaurea_nigrescens_ITS2-NCBI	3150	0,87									
Mi269	Centaurea_nigrescens_ITS2-NCBI	82	0,42	Poa_trivialis_ITS2-NCBI	25	0,13	Ranunculus_acris_ITS2-NCBI	25	0,13			
Mi270	Hypochaeris_radicata_ITS2-NCBI	14442	0,99									
Mi271	Centaurea_nigrescens_ITS2-NCBI	4268	0,75									
Mi272	Hypochaeris_radicata_ITS2-NCBI	4860	0,90									
Mi273	Hypochaeris_radicata_ITS2-NCBI	2808	0,88									
Mi274	Plantago_lanceolata_ITS2-NCBI	926	0,54	Plantago_leiopetala_ITS2-NCBI	462	0,27						
Mi275	Centaurea_nigrescens_ITS2-NCBI	6981	0,83									
Mi276	Olea_europaea_ITS2-NCBI	211	0,29	Centaurea_nigrescens_ITS2-NCBI	132	0,18	Cucurbita_pepo_ITS2-NCBI	101	0,14	ris_radicata_	99	0,13
Mi277	Centaurea_nigrescens_ITS2-NCBI	3397	0,39	Sonchus_kirkii_ITS2-NCBI	2810	0,32	Sonchus_arvensis_ITS2-NCBI	883	0,10			
Mi278	Centaurea_nigrescens_ITS2-NCBI	8518	0,80									
Mi279	Alopecurus_myosuroides_ITS2-NCBI	1201	0,75	Dupontia_fisheri_ITS2-NCBI	262	0,16						
Mi281	Lapsana_communis_ITS2-NCBI	1154	0,30	Medicago_lupulina_ITS2-NCBI	1102	0,29	Centaurea_nigrescens_ITS2-NCBI	1039	0,27			
Mi282	Centaurea_nigrescens_ITS2-NCBI	3214	0,44	Spinacia_oleracea_ITS2-NCBI	1150	0,16						
Mi283	Centaurea_nigrescens_ITS2-NCBI	14359	0,82									
Mi285	Dupontia_fisheri_ITS2-NCBI	910	0,71									
Mi286	Centaurea_nigrescens_ITS2-NCBI	3601	0,78									
Mi287	Centaurea_nigrescens_ITS2-NCBI	4364	0,69									
Mi292	Ranunculus_acris_ITS2-NCBI	269	0,55	Hypochaeris_radicata_ITS2-NCBI	181	0,37						
Mi293	Centaurea_nigrescens_ITS2-NCBI	6240	0,84									
Mi295	Petroselinum_crispum_ITS2-NCBI	336	0,23	Centaurea_nigrescens_ITS2-NCBI	333	0,23	Anthemis_cotula_ITS2-NCBI	317	0,22			
Mi296	Centaurea_nigrescens_ITS2-NCBI	7469	0,64									
Mi297	Crepis_capillaris_ITS2-NCBI	460	0,31	Papaver_rhoeas_ITS2-NCBI	239	0,16	Bellis_perennis_ITS2-NCBI	204	0,14	_vulgaris_IT	180	0,12
Mi298	Centaurea_nigrescens_ITS2-NCBI	25	0,54									
Mi301	Centaurea_nigrescens_ITS2-NCBI	695	0,44	Urtica_dioica_ITS2-NCBI	170							
Mi302	Ranunculus_acris_ITS2-NCBI	12421	0,76	Ranunculus_macounii_ITS2-NCBI	174							
Mi303	Dioscorea_polystachya_ITS2-NCBI	16	0,21	Matricaria_chamomilla_ITS2-NCBI	16			11	0,14			
Mi305	Ranunculus_acris_ITS2-NCBI	27	0,27	Centaurea_nigrescens_ITS2-NCBI	21	0,21						
Mi307	Lolium_perenne_ITS2-NCBI	258	0,27	Urtica_dioica_ITS2-NCBI	142	0,15						
Mi308	Knautia_longifolia_ITS2-NCBI	595	0,15	Knautia_subscaposa_ITS2-NCBI	591	0,15	Centaurea_nigrescens_ITS2-NCBI	467	0,12			
Mi310	Moringa_oleifera_ITS2-NCBI	248	0,20	Urtica_dioica_ITS2-NCBI	128	0,11	Moringa_peregrina_ITS2-NCBI	125	0,10			
Mi311	Lotus_tenuis_ITS2-NCBI	2158	0,24	Lotus_corniculatus_ITS2-NCBI	2085	0,24	Lotus_environmental_ITS2-NCBI	1014	0,11			
Mi315	Campanula_lambertiana_ITS2-NCBI	2397	0,68	Campanula_rapunculus_ITS2-NCBI	854	0,24						
Mi316	Plantago_media_ITS2-NCBI	3519	0,48	Plantago_lanceolata_ITS2-NCBI	1796	0,25	Urtica_dioica_ITS2-NCBI	776	0,11	_leiopetala_	738	0,10
Mi318	Centaurea_nigrescens_ITS2-NCBI	2409	0,68									
Mi319	Centaurea_nigrescens_ITS2-NCBI	324	0,31	Lolium_perenne_ITS2-NCBI	136	0,13						
Mi320	Centaurea_nigrescens_ITS2-NCBI	239	0,36	Anthemis_cotula_ITS2-NCBI	139	0,21	Ranunculus_acris_ITS2-NCBI	78	0,12			
Mi322	Centaurea_nigrescens_ITS2-NCBI	649	0,35	Plantago_lanceolata_ITS2-NCBI	193	0,10						
Mi325	Centaurea_nigrescens_ITS2-NCBI	8075	0,70									

Knautia arvensis

1st Conclusion

1- A crude DNA extraction is working

2-Amplicon sequencing _ Universal primer

3-Pool sequencing_barcode

4-Bioinformatics for Biologist

5-Data bases need to be curated/completed

How to identify SNP in orphean species ex *lavandula*

No genomic ressources
Chromosome number?
Genome size 1Gb?

How to obtain a reference sequence (low cost & quickly)

Reconstruction of the gene space

RNASeq

Gene 1 Gene2 Gene 3

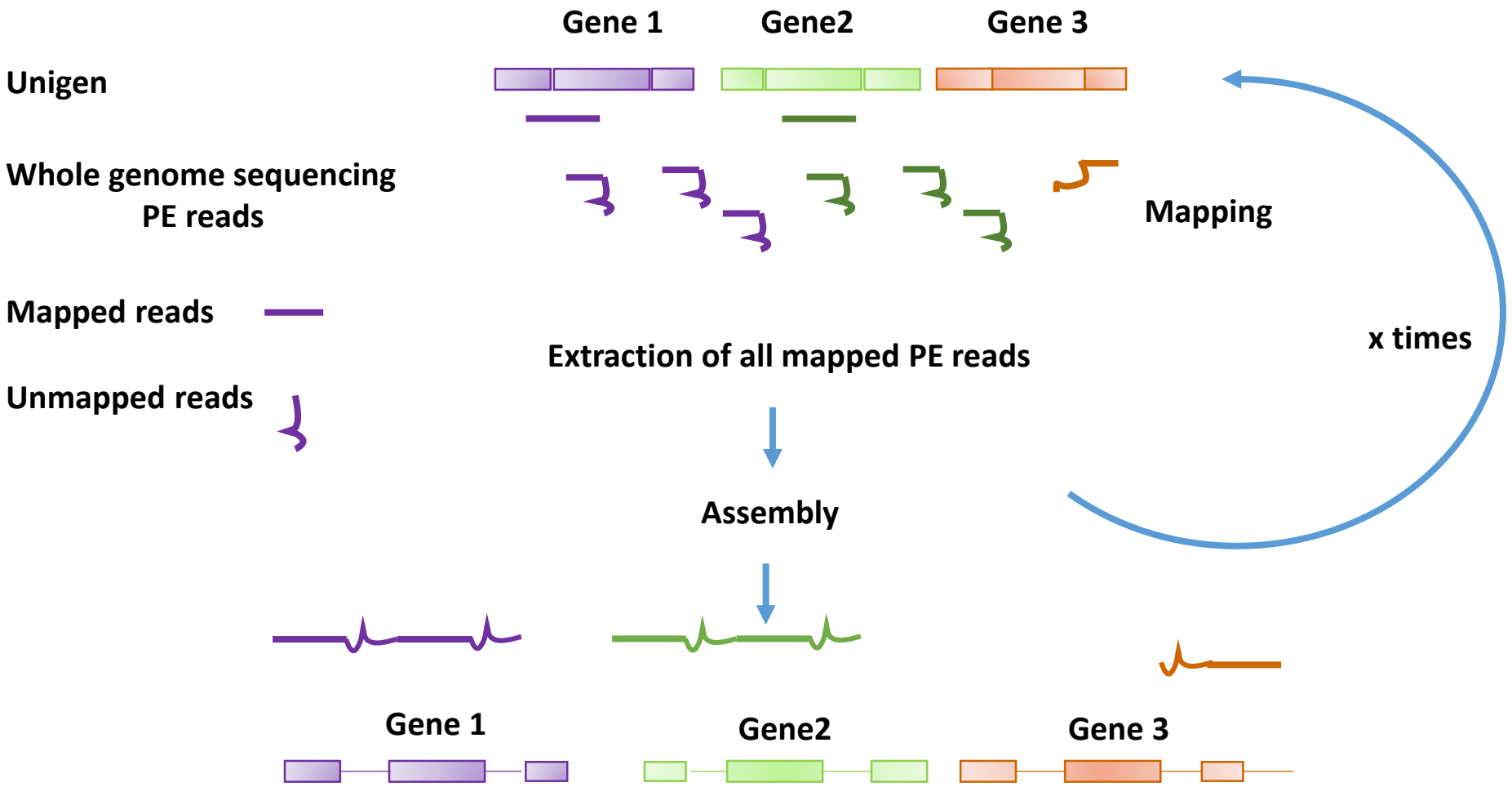
Short reads

CDS

DNASeq

Gene/exon + intron

iterative Process of Extraction and Assembly of NGS reads



Aluome et al, 2016

Illumina PE

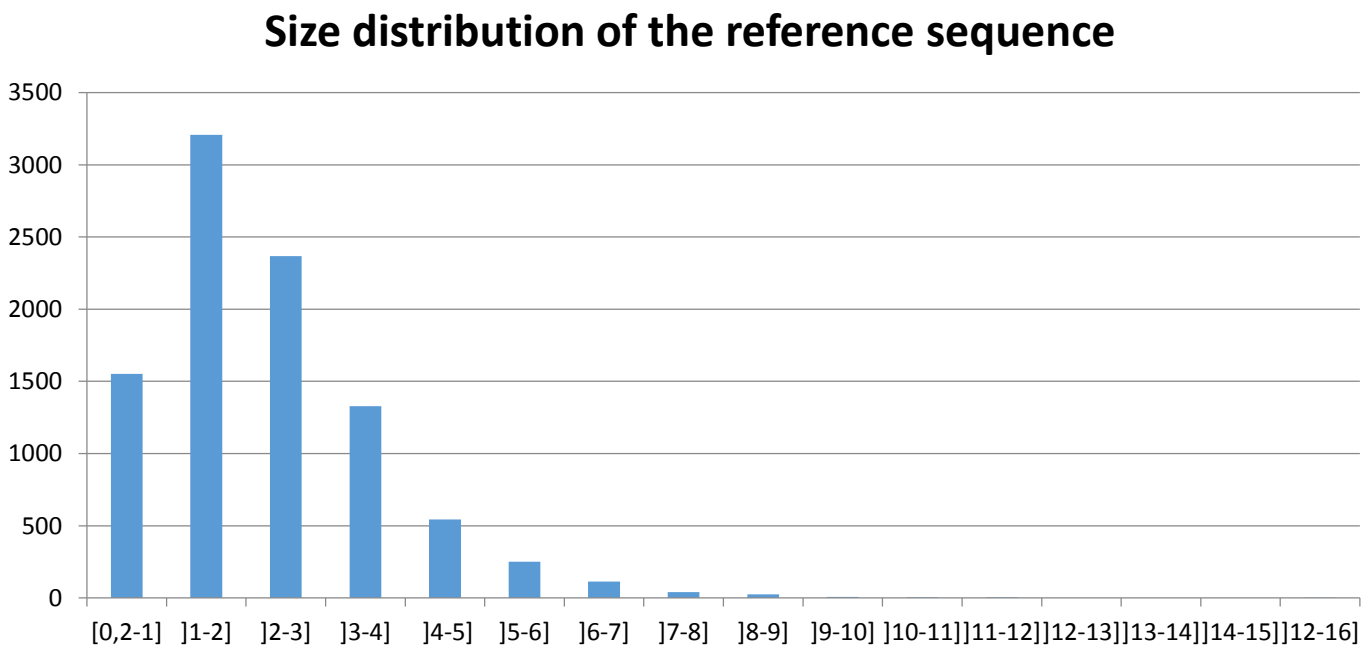
Quality trimmig

denovo assembly

Functionnal Annotation

	Diva	Maillette
PE reads	62 430 893	66 107 230
Nb séquences with a similarity >70%)	8 714	10 060

7928 common sequences



Fopa-Fomeju et al, in prep

SNP detection

15 cultivars

↓

DNA extraction

↓

Sequencing 5X-38x
HiSeq2500 2*150 pb

↓

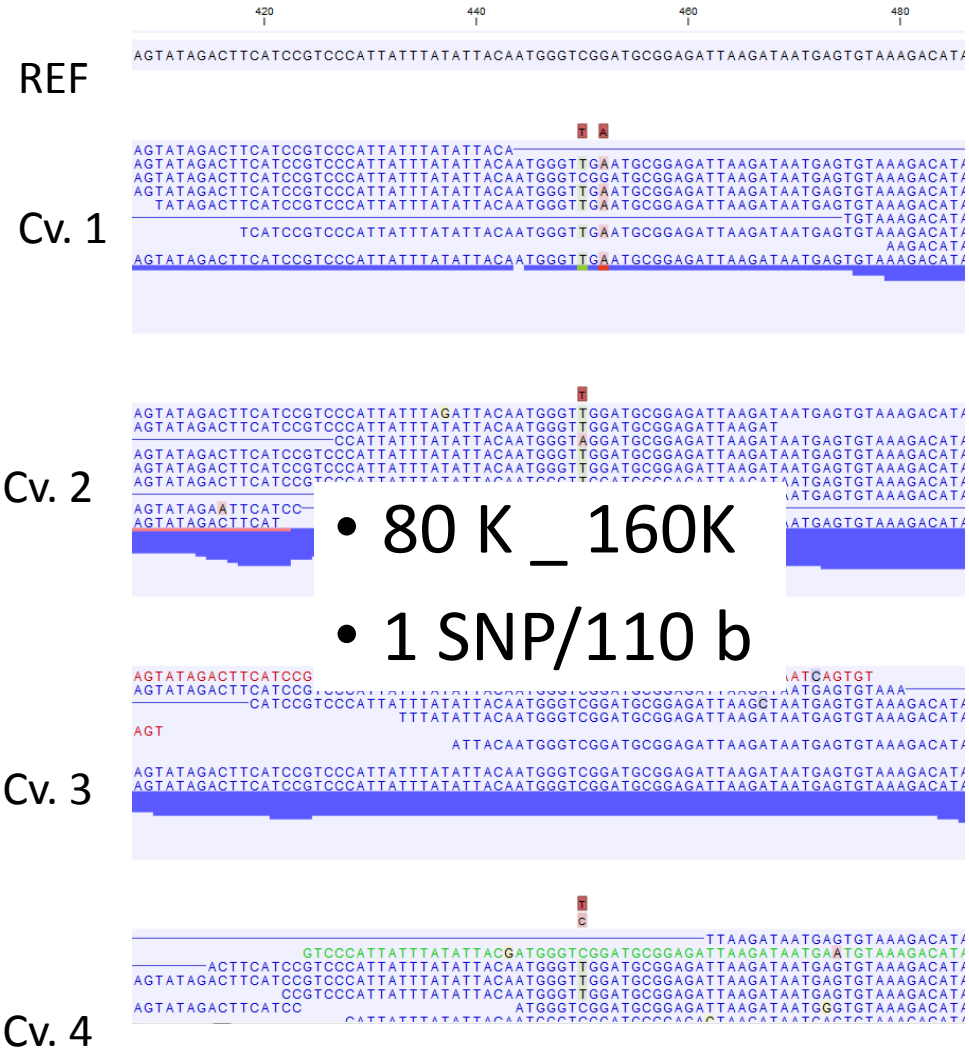
Q trimming

↓

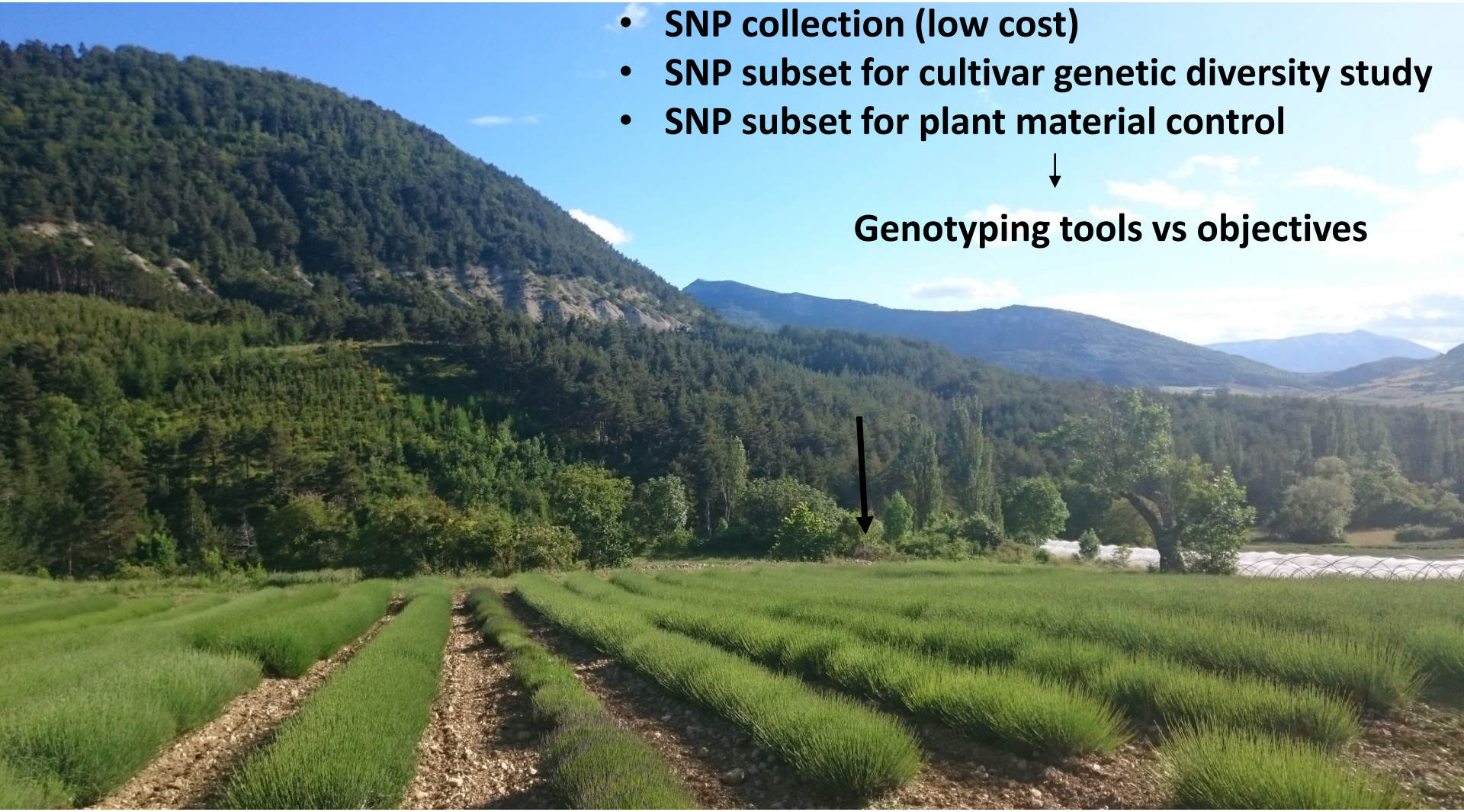
Mapping on reference

↓

SNP
detection



Fopa-Fomeju et al, in prep



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PÔLE DE COMPÉTITIVITÉ

CRIEPPAM

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SCIENCE & IMPACT

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CASDAR

Genotyping = genotyping technology + SNP calling

GBS vs SNP arrays

	Prior knowledge	Template	Develop -pement	Sample number	Bioinformatics Capacities, skills, time	Experimental Cost including Analysis/sample
Amplicon sequencing	***+***	***DNA***	***+++***	***+_+++***	***++ (+)***	***$$***
Chip array	**+**	***DNA***	**+**	**+++**	**+**	***$-$$***

SNP/samples

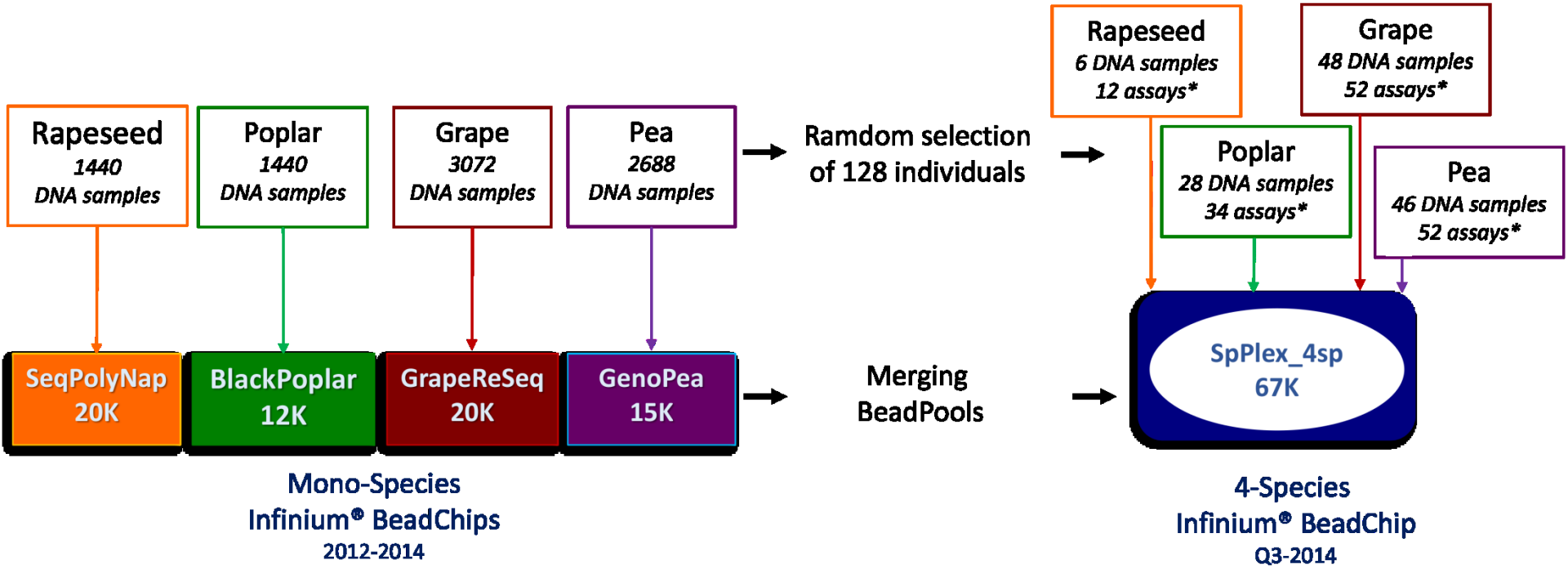
$$$$

Sequencing machines
384 barcodes

Multispecies SNP array
A solution

SNP ↗ samples ↗ cost ↘

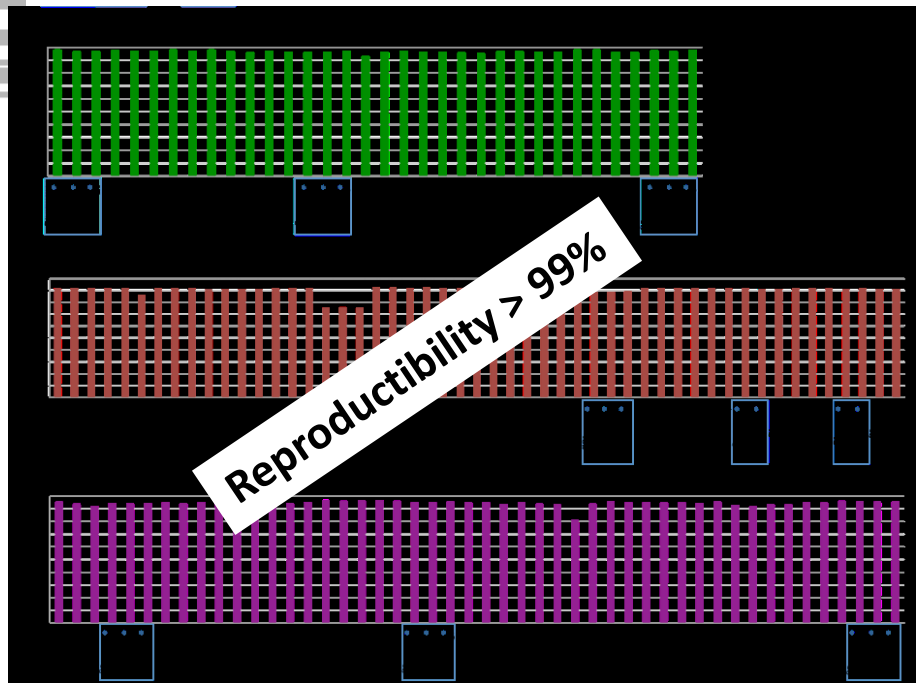
Multi Plant Species Illumina bead chip



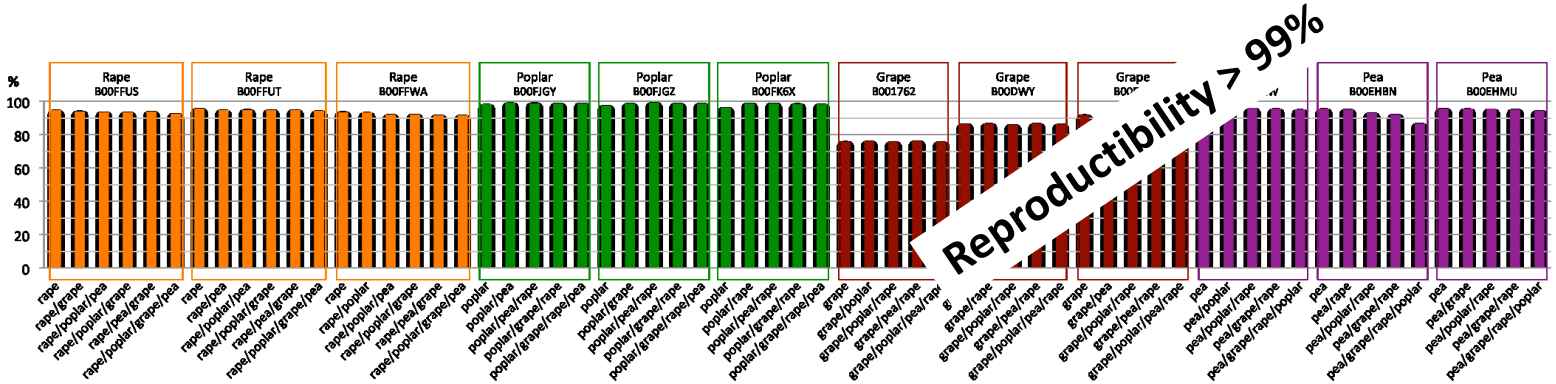
**Assays : total number of genotyped samples (singletons, duplicates, triplicates)*

Performance of a multi-plant-species Illumina Infinium BeadChip array
Le Paslier et al, PAG XXIV, San Diego, Ca, Janvier 2016, Berard et al, in preparation

Multi Plant Species Illumina bead chip success rate



Mix of DNA



Personnal comments

SNP discovery → **Strategy available** → **Low cost and fast**

Genus and species → **Barcoding**
Universal primers → **Standard protocol** → **Good quality**
Public database

Phyleogegraphy/assignation to population → Multispecies SNP array

- How much time? Barcoding could be quick, SNP array depending on the facilities
- Cost? → DNA extraction → How much SNP is needed?

Future / sequencing on your smartphone
Real time

Thank you for your attention