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Feedback on tGBS with Allegro : Does it hit the target ?

Unit EPGV US1279

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EPGV tGBS projects.

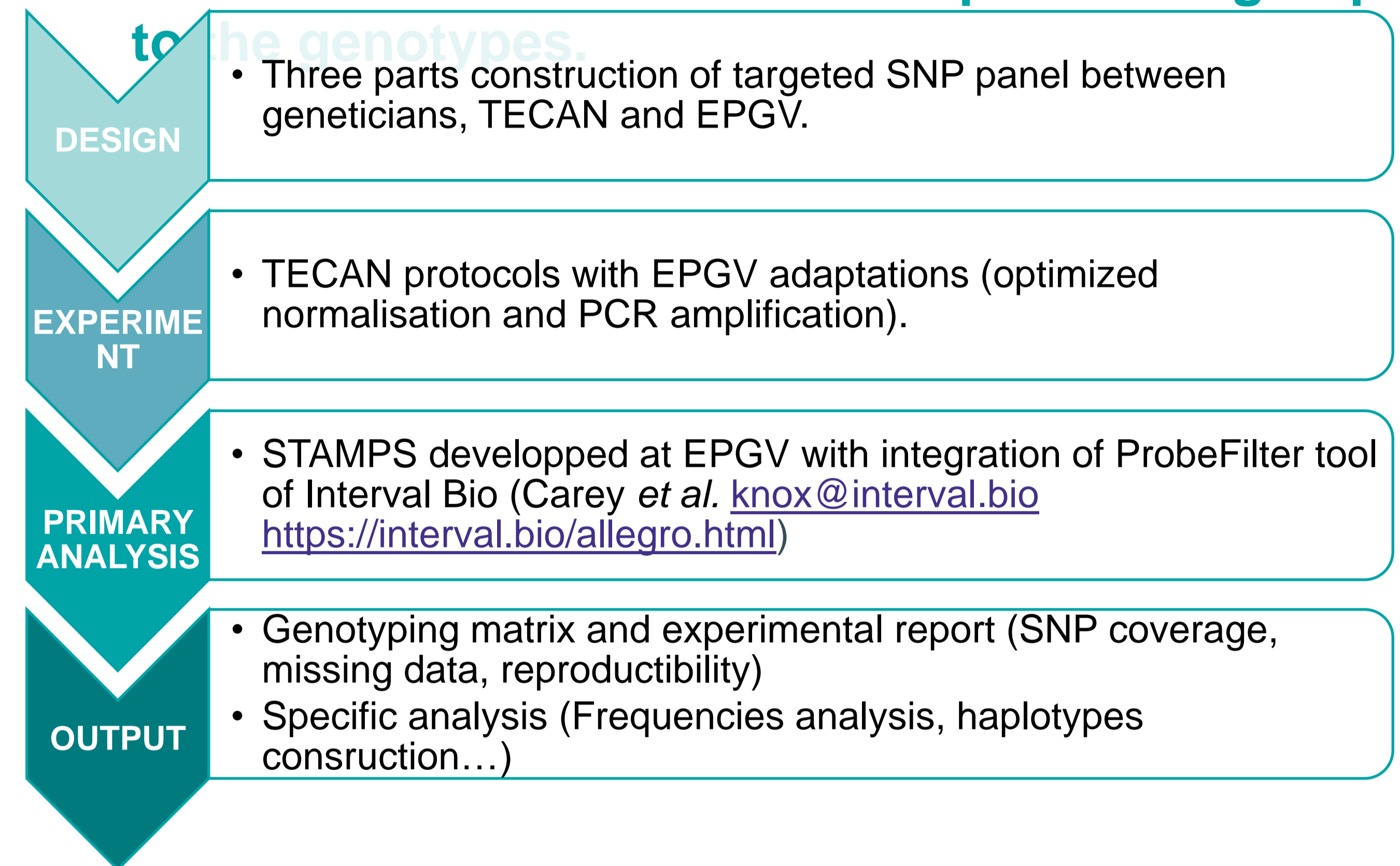
Organisms	Reference	Number of SNP Target	Number of samples	Studied samples
<i>Zea mais</i>	Genome v2.0 (2n=20, 2300 Mb) ¹	2 592	192	Lines, hybrids, pools
<i>Lens sp.</i>	Targeted regions (2n=14, ~25 Mb)	25 000	336	Varieties, wild accessions
<i>Solanum lycopersicum</i>	Genome v4.0 (2n=24, 950 Mb) ²	19 998	384	Varieties
<i>Plasmopara viticola</i>	Genome (2n=34, 93 Mb) ³	5 000	2x192	F1 populations

¹. https://download.maizegdb.org/B73_RefGen_v2/

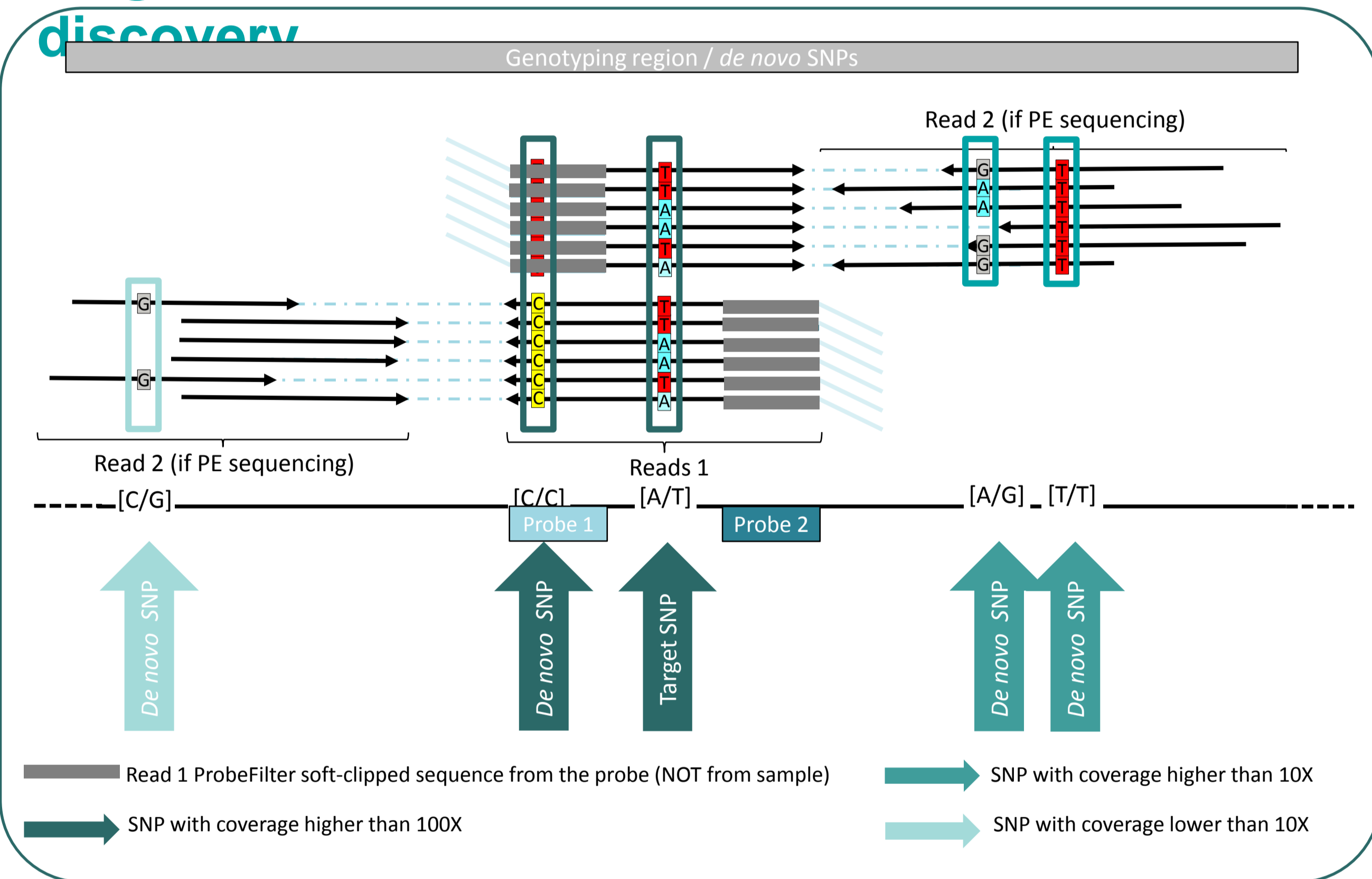
². Hosmani et al. 2019. https://solgenomics.net/organism/Solanum_lycopersicum/genome/

³. Dussert et al 2018. <https://entrepot.recherche.data.gouv.fr/dataset.xhtml?persistentId=doi:10.15454/4NYHD6>

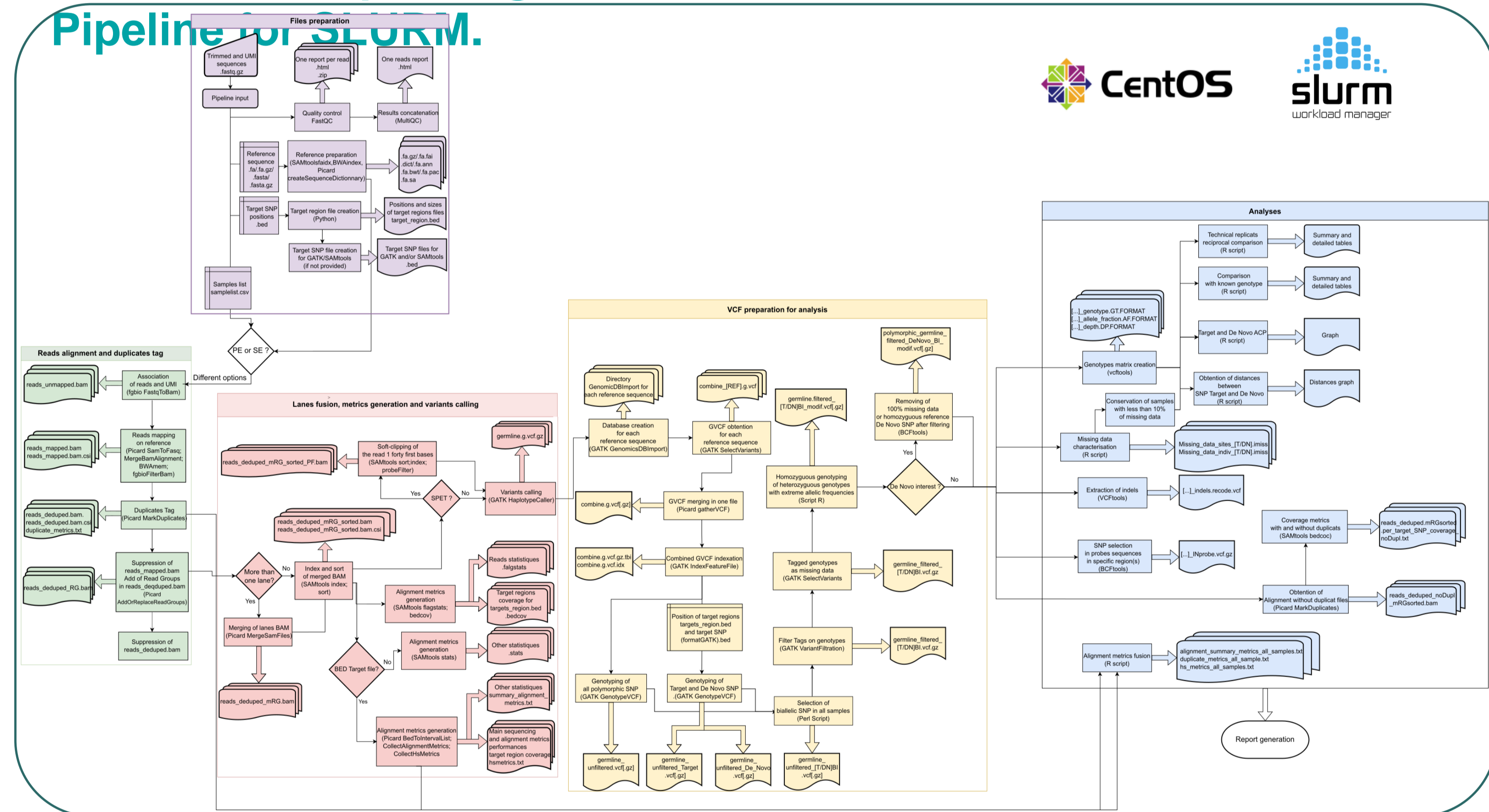
tGBS Workflow : from the SNP panel design up to the genotypes



Target and de novo SNP discovery



STAMPS - Simple Targeted GBS Automated and Modular Pipeline for SLURM.



Conclusions

SPET is a powerful flexible genotyping solution (from 1000 SNP up to 100 000, and 192 to 3 072 samples in one experiment) for organisms with reference genome or not.

The collaboration between geneticists, TECAN and EPGV leads to specific probes design illustrated by the high percent of bases alignment on the targeted regions. The *P. viticola* exception is potentially due to the lower specificity of SE mapping regarding to the PE mapping.

The targeted SNP coverage variability obtained, had no impact on the genotyping success rate. And a good sample repeatability was observed within the same experiment. According to our comparison results between genotypes from tGBS and available SNP array considered as golden standard, we can infer that tGBS could be an effective alternative to SNP array.

Moreover, the SNP *de novo* discovery allows haplotype reconstruction that could be useful to limit ascertainment bias. Additionally, the tGBS workflow can be adapted to genotype structural variations.

Result

Organism	SE/PE	Design Success Rate	On Target Bases Percent	Median, Min, Max coverage of Target SNP	Percent of genotype Target SNP after filtering ^a	Target SNP Repeatability Percent ^b	SPET tGBS vs Array Validation	De novo SNP Number	X-times Target SNP Number
<i>Z. Mais</i>	PE	99.4% (2 571/2 587)	89	238X, 10X, >450X	99.9	96.5	98.8 ^c	60 269	~23x
<i>L. species</i>	PE	100% (25 000/25 000)	98.7	75X, 31X, 167X	95.9	99.0	-	188 159	~7.5x
<i>S. lycopersicum</i>	SE	99.9% (19 998/20 000)	82.2	77X, 10X, 139X	94.1	99.9	-	23 522	~2x
<i>P. viticola</i>	PE	100% (5 000/5 000)	98.8	226X, 10X, >450X	99.9	98.7	-	122 647	~24.5x

^a Filter: DP > 10X, GQ > 20, heterozygous sites frequency (0.3 < AF < 0.8).
^b Filtered Target SNP genotypes comparison of technical repetitions (*Z. mais*: 4 rep. of B73; *L. sp.*: 6 rep. of Anicia; *S. lycopersicum*: 8 rep. of CR001; *P. viticola*: 4 replicats of PV1419 and PV412).
^c Comparison on 26 samples and 2 599 Target SNP with exclusion of missing data points. Results are validated in case of genotypes identity between both technologies.

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