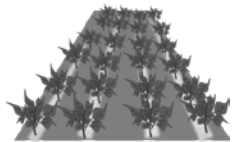




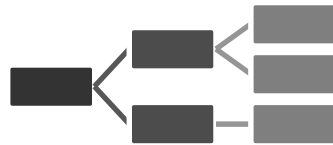
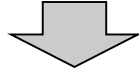
Deep sequencing for Quality Control of Real-time PCR diagnostic used for potato certification

**O. Schumpp, L. Farinelli, V. Golyaev, N. Gonzalez, P. Otten, M.
Pooggin, J.S. Reynard**

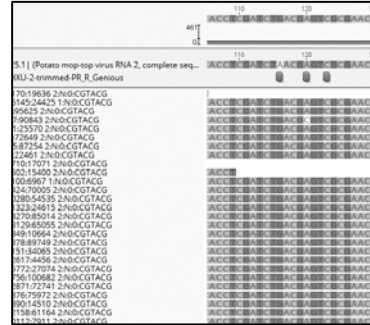
22.11.2017



Certification



Bioinformatics

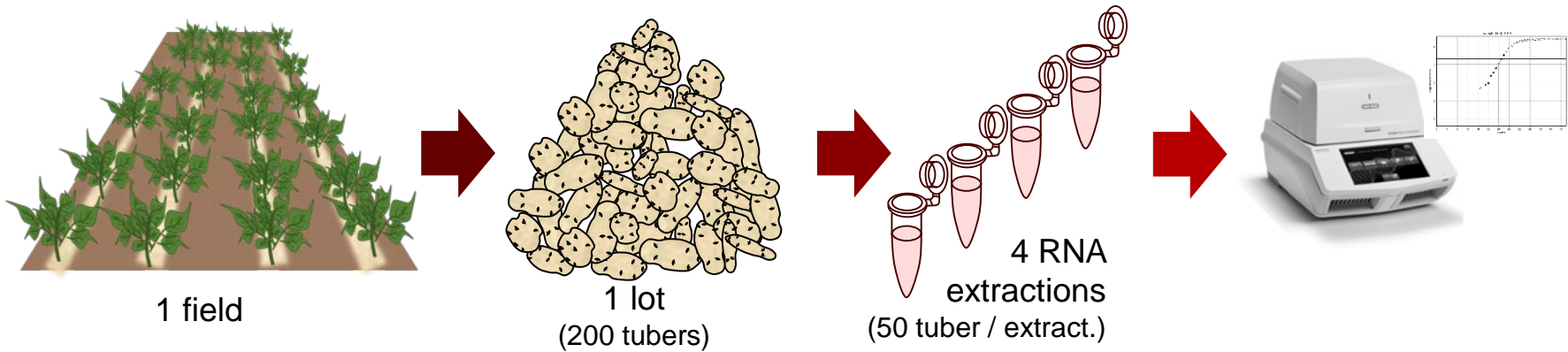


Mapping / SNPs

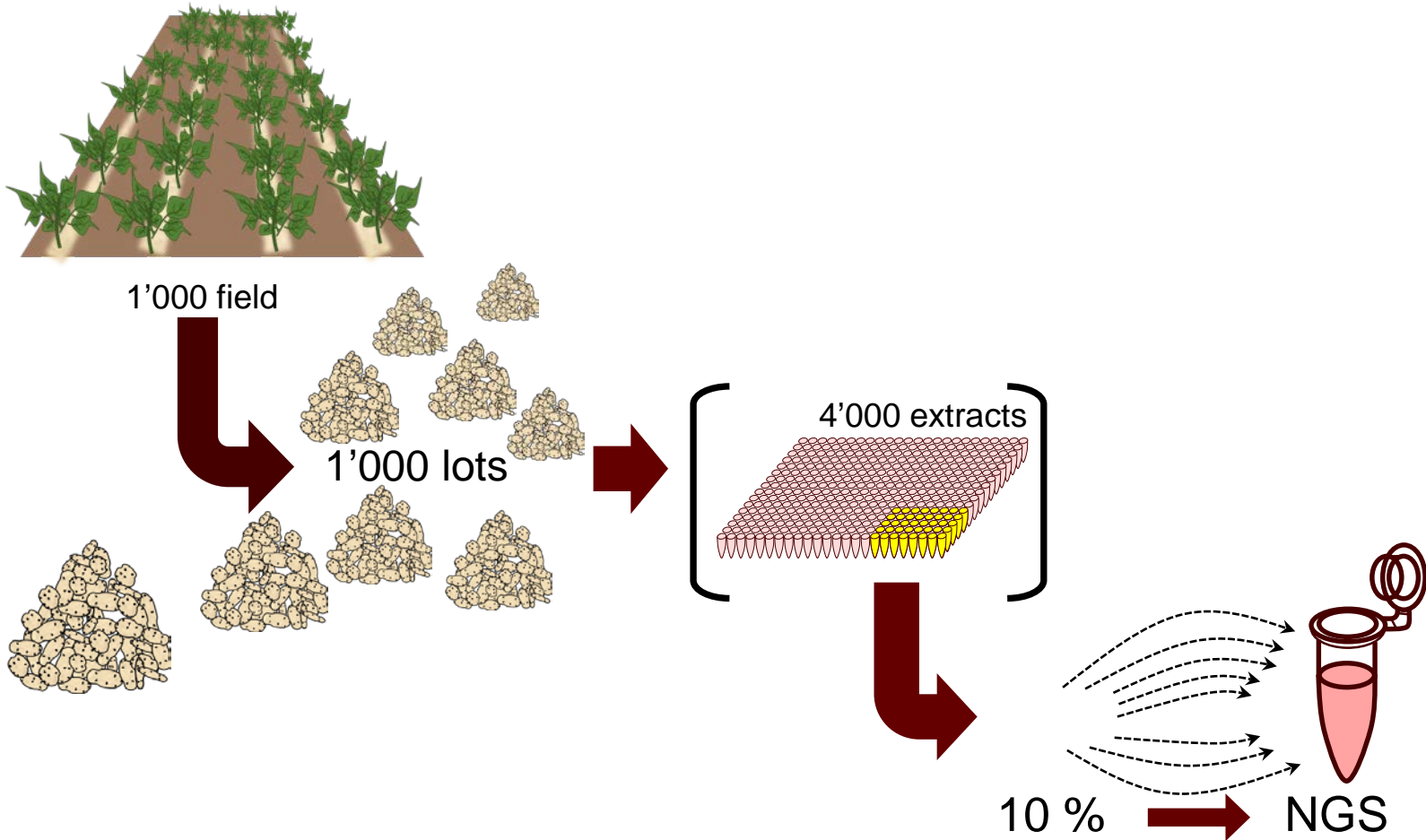


Primer mismatches

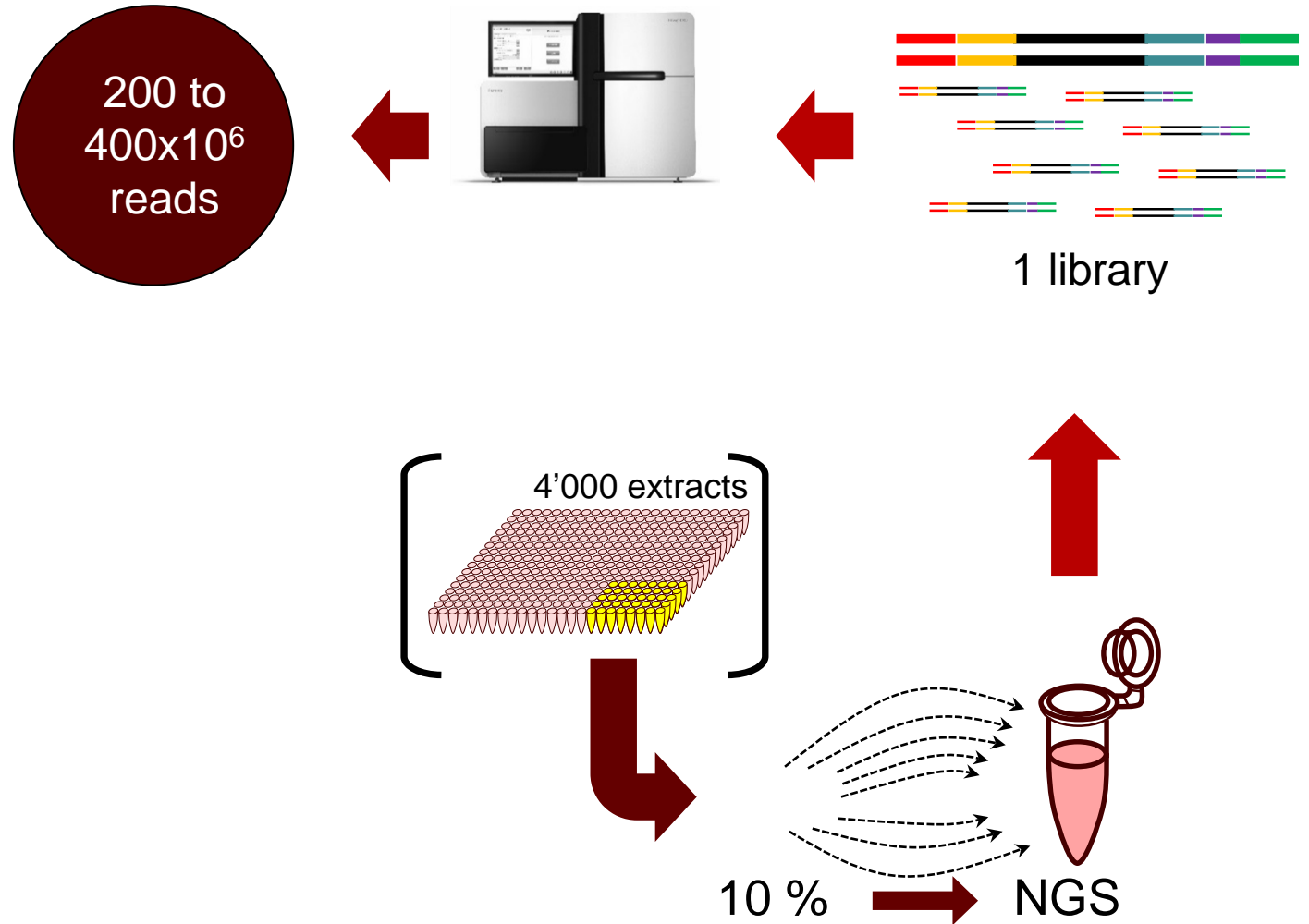
RT PCR-based diagnostics for certification of dormant seed potato tubers



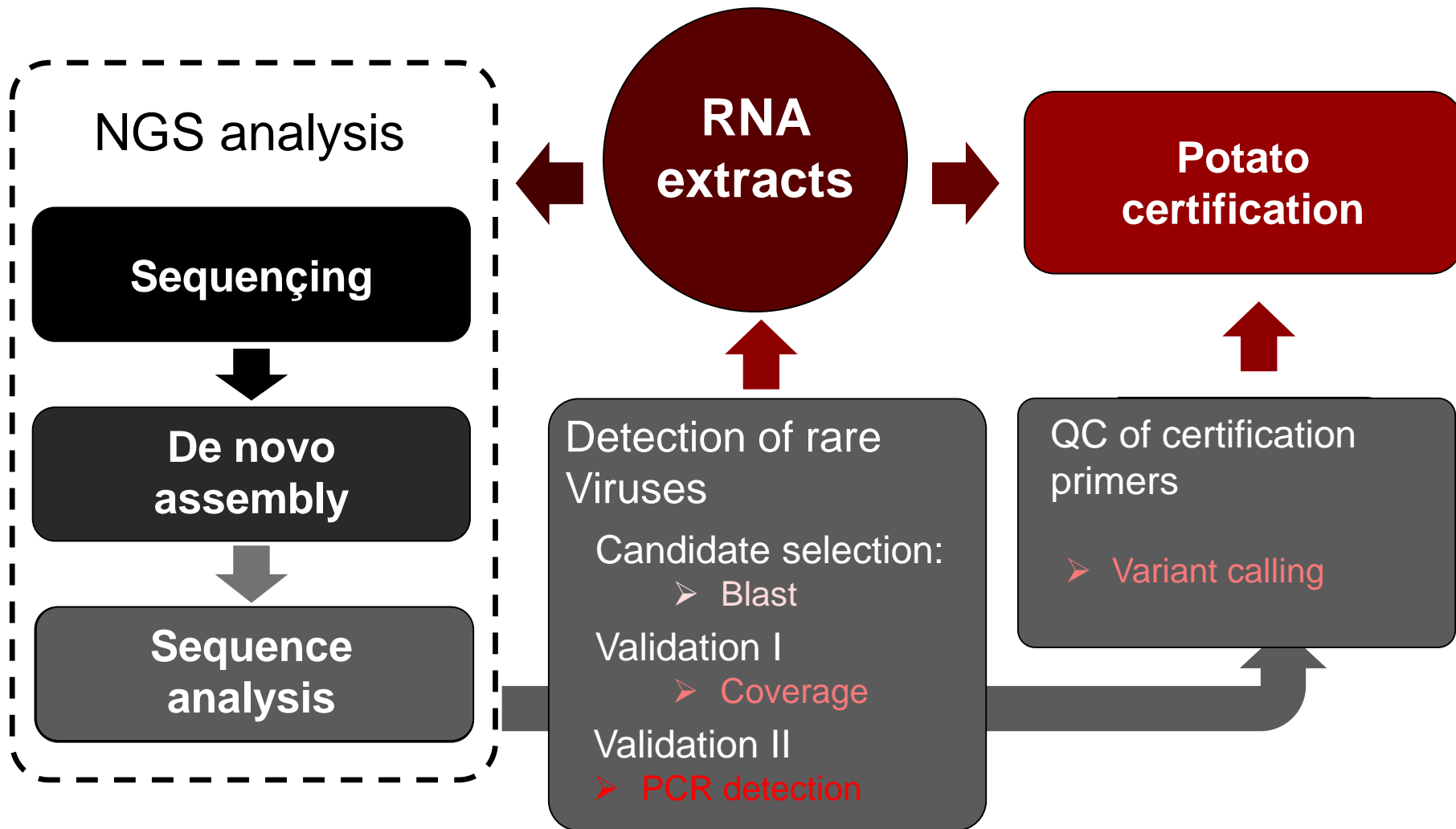
RT PCR-based diagnostics for certification of dormant seed potato tubers



RT PCR-based diagnostics for certification of dormant seed potato tubers



Process flow diagram





De novo assembly + Blast



Candidate selection

	Parameters (<i>kmer length</i>)	Contig number	Blast (Local DB)	
			Hits	# viruses
Paired reads (P)	113, 103, 93, 83, 73, <u>63</u>	40'569	145	12
Joined reads (J)	123, 113, 103, 93, 83, <u>73</u>	40'025	363	12
(P + J)	103, 93, 83, 73, <u>63</u> , 53	63'561	397	12
Subtraction of host and (P + J)	123, 113, 103, 93, <u>83</u> , 73, 63, 53	164	18	6



Mapping

Validation I

Validation II

Virus	% Cov	Estimated Infected Tubers
PVY	100.0	#NA
PVS	99.9	#NA
PLRV	99.8	10
PVX	99.7	2
PMTV -RNA 2	97.9	10
XXX-RNA 2	87.7	1
YYY-RNA 1	83.4	3
XXX-RNA1	68.4	1
PMTV -RNA 1	61.8	10
PMTV -RNA 3	51.8	10
YYY-RNA 2	32.1	3



SNPs



Primers control

Annotations	Mapping Method			SNPs commons to all
	Joined	Paired	Paired 25x	
PVY	996	1216	1428	28 %
PLRV	217	264	301	60 %
XXX-RNA2	410	338	824	29 %
YYY-RNA2	45	67	636	5 %

High quality SNPs (filters)

- Maximum variant P-value of 0.01
- Minimal depth of coverage of 10 variant calls
- Threshold of 10 x sequencing error rate (based on positional Q-scores)

Primer alterations

	Cq values		
	PVY-Univ	PVY-Univ Xa	PVY-Univ Xb
PVY-N 605	13	21	28
PVY-NTN 1317	18	26	32
PVY-O 803	19	28	32
PVY-Wi 1315	20	29	35

PVY-N 605		+8	+7
PVY-NTN 1317		+8	+7
PVY-O 803		+8	+4
PVY-Wi 1315		+9	+6

PVY-Univ Fw C A T A G G A G A A A C T G A G A T G C C A A C T

PVY-Univ Fw **Xa** C A T A G G A G A A A C T G A G A T G C C A A C **G**

PVY-Univ Fw **Xb** C A T A G G A G A A A C T G A G A T G C C A A **A G**



Conclusions

➔ NGS as a complement / control of the potato tuber certification process

➤ Methodology

- Genome subtraction reduces the sensitivity of virus detections
- Sensitivity is virus species dependent
- Mapping method is critical for SNP detection

➤ Quality Control

- Critical mutations have not been observed...

➤ Epidemiology

- New virus are detected every year....



Thank you for your attention

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