CDF1 gene editing strategy in potato wild species within *de novo* domestication concept

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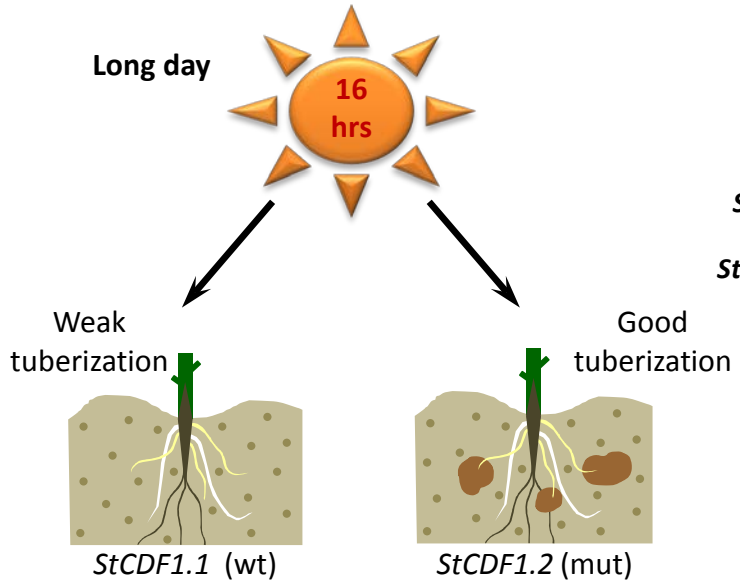
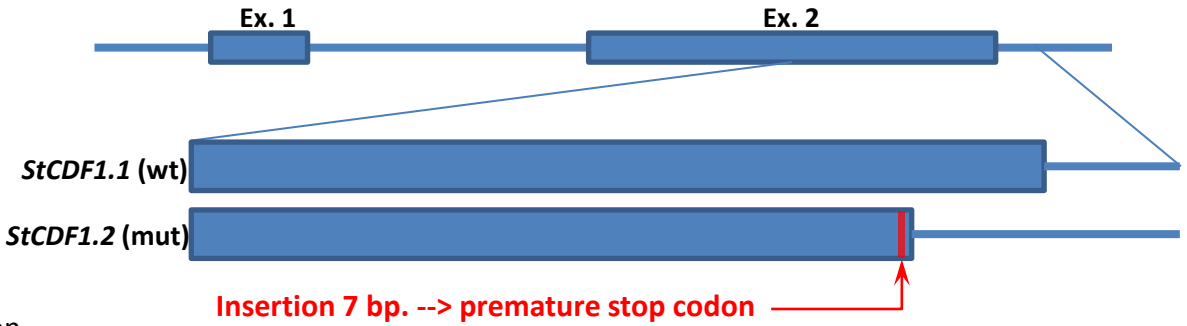
Wild potato species as trait donors

- During long selection process modern potato cultivars have lost their ability to resist various negative environmental factors, while many wild species have retained these traits.
- As part of the *de novo* domestication concept, we are looking for genes with modification potential supposed to accelerate the production of new trait donors for their introduction into breeding.
- One of such genes is *StCDF1*, which is involved in the regulation of tuberization.



Kloosterman, B. *et al* (2013), Nature
https://doi.org/10.1038/nature11912

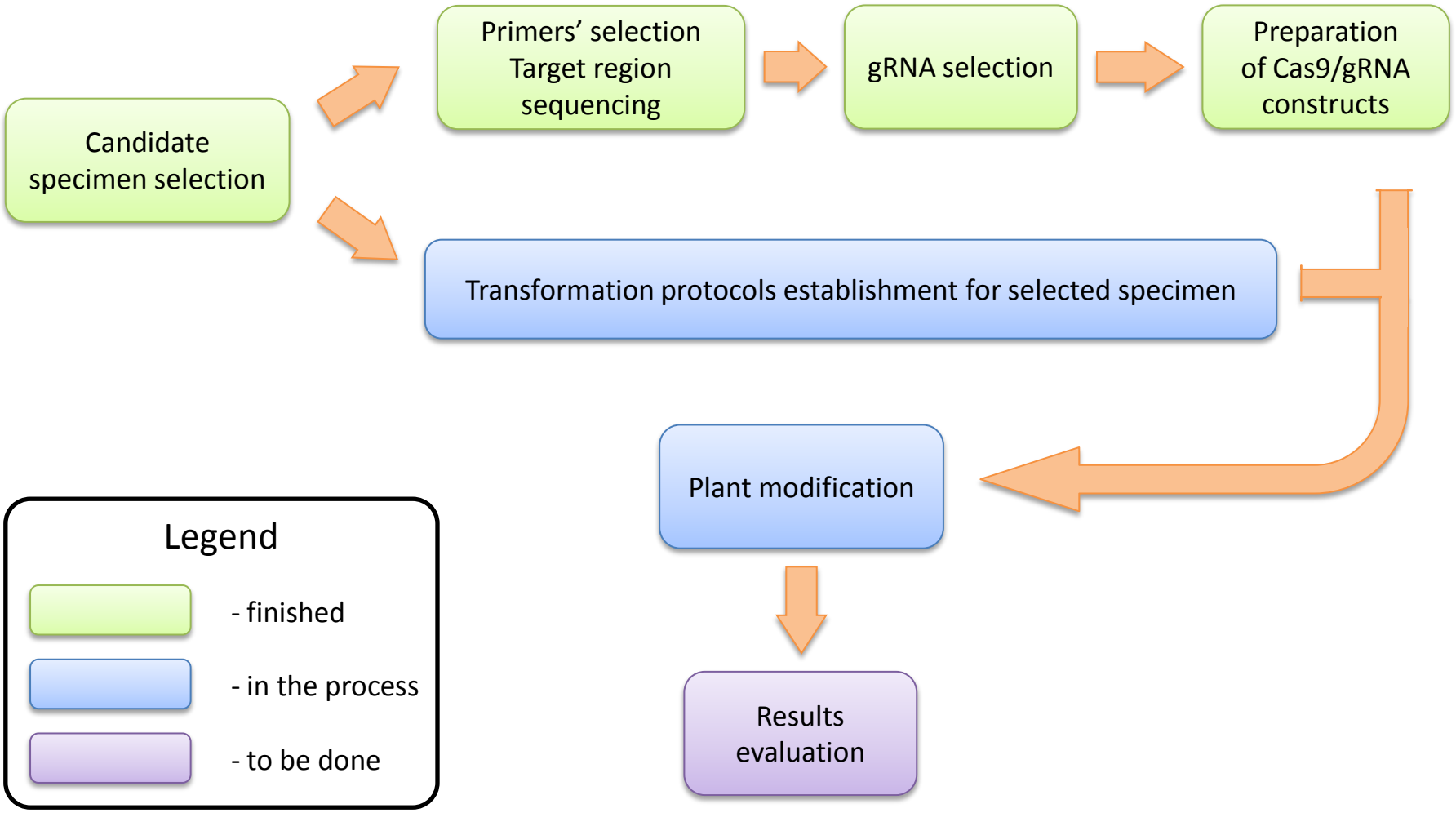
StCDF1 exon-intron structure and allele difference



The hypothesis:

- The modification of the *CDF1* gene in wild potato via Cas9/gRNA system will induce tuberization under long-day conditions

Project pipeline



S.polyadenium was chosen for modification

- During last 3 years 19 specimen of wild potato from VIR collection were cultivated and phenotyped in long-day conditions (http://wildpetotadb.biores.cytogen.ru/ru)
- *S.polyadenium* having some good traits doesn't tuberize under long-day conditions. Therefore it was selected for modification of the *CDF1* gene.

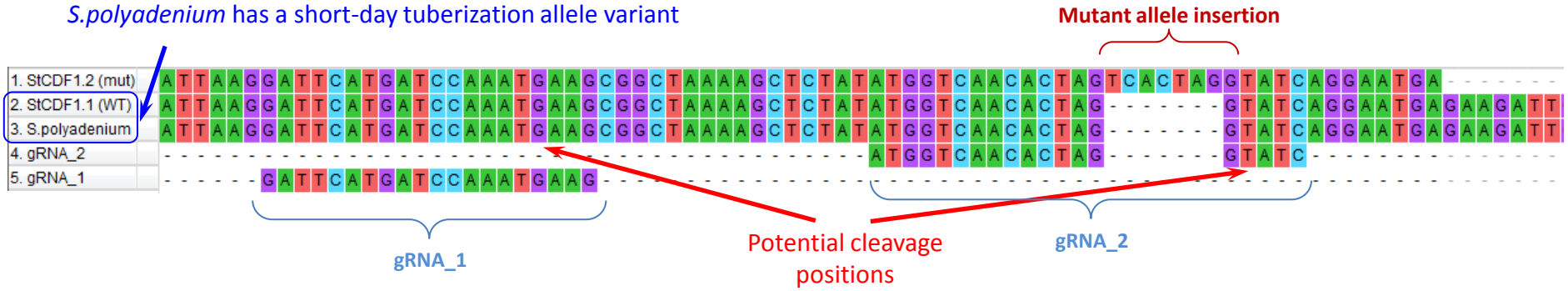
№ in collection	Specimen	Tuberization*	Steroidal glycoalkaloids**	Virus infection (virus type)	Resistance to Colorado potato beetle	*in vitro* regeneration	Ploidy, n
1	*S. verrucosum*	1		X		not established	2
2	*S. demissum*	0	-	Y	100%	established	6
3	*S. stoloniferum*	1	++	not found		not established	4
4	*S.polyadenium*	1	-	X	100%	established	2
5	*S. pinnatisectum*	2	-	X		not established	2
6	*S. ehrenbergii*	2	-	X		not established	2
12	*S. stoloniferum*	1	+	not found	65%	not established	4
16	*S. jamesii*	2	-	not found		not established	2
17	*S. tarijence*	2	-	Y		not established	2
19	*S. cardiophyllum*	2	-	X	100%	not established	2
24	*S. pinnatisectum*	2	-	X		established	2
27	*S. dolichostigma*	3	+++	not found	0%	established	2
28	*S. commersonii*	1	-	X, Y, S		not established	2
30	*S. fendleri*	2	-	X	15%	established	4
31	*S. kurtzianum*	2	++	not found			2
32	*S. vernei*	1	-	not found			2
33	*S. chacoense*	3	+++	not found	100%	established	2
34	*S. chacoense*	1	-	X, Y		established	2
36	*S. demissum*	0	-	X, Y		established	6

* - Tuberization scale: 0 – no tubers; 1 - zero or few tubers during all three years; 2 – steady tuberization all three years; 3 – lots of tubers all three years

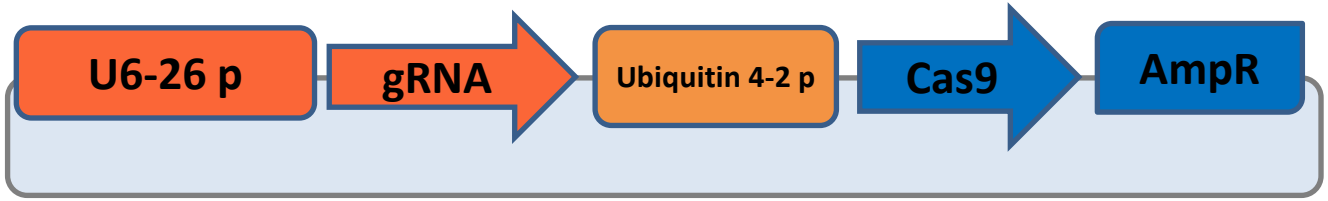
** - Concentration of SGA: "-" – below 1mg/g of dry weight, "+" – between 1mg/g and 10mg/g, "++" – between 10mg/g and 20mg/g, "+++" – more then 20mg/g

Target site selection and gRNA design

Target sites were chosen in a region of natural mutation which leads to long-day tuberization allele



Vector structure



We expect a frameshift mutation leading to conversion from a short-day tuberization to a long-day tuberization allele

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