The selection of efficient sgRNAs for *CRISPR-Cas9* genome editing of potato (*Solanum tuberosum* L.) aimed to obtain the cultivars with low-amylose starch properties

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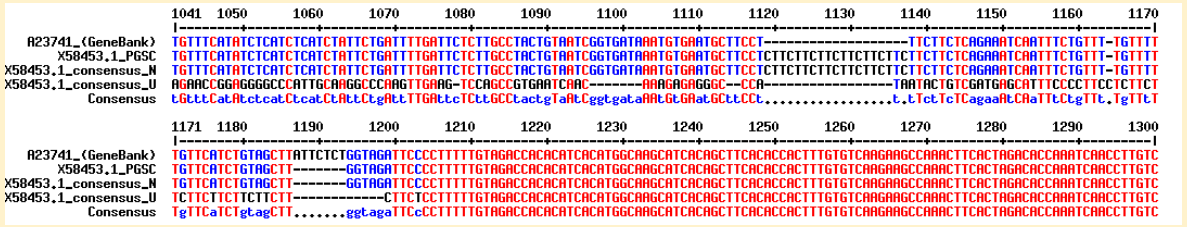
Potato (*Solanum tuberosum* L.) (2n = 4x = 48) is an important starch producing agricultural plant. Starch is the main storage carbohydrate widely used for industrial and nutritional needs. The two main components of starch are polysaccharides amylose and amylopectin, which account for approximately 20-25 and 75-80 % of native potato starch, respectively. The starch functional properties (swelling power, viscosity, gelatinization) are mainly defined by the ratio of amylose and amylopectin. The low-amylose starches known as “waxy” and have high gelatinization and pasting properties, whereby are widely used in food and non-food industry. The *GBSS1* gene is known to be responsible for amylose biosynthesis in potato tubers. There are several studies devoted to CRISPR/Cas9 knockout of *GBSS1* gene in potato cultivars Kuras, Sayaka, Desiree, Wotan, resulted in successful production of low- and no amylose transformants [1-4].

<u>The aim of the study</u> is development of Russian potato varieties with low- or no-amylose starch properties using the *CRISPR-Cas9* genome editing.

Methods and Results

The cultivars of choice for editing are popular Russian varieties **Nevskiy** and **Udacha**, which have good *in vitro* regeneration ability and good crop yield.

We used Illumina genomic sequencing data for **Nevskiy** and **Udacha** (obtained on NextSeq550 at the ICG SB RAS Core Genome Facility). The Illumina reads were aligned to the *GBSS1* from PGSC reference genomic sequence. Therefore we obtained the *GBSS1* locus consensus sequences for our cultivars.



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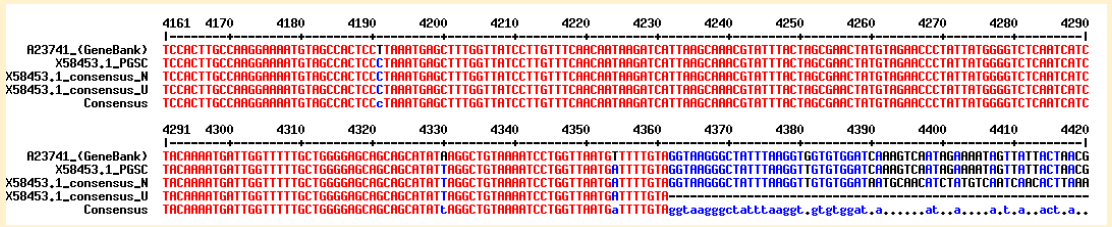
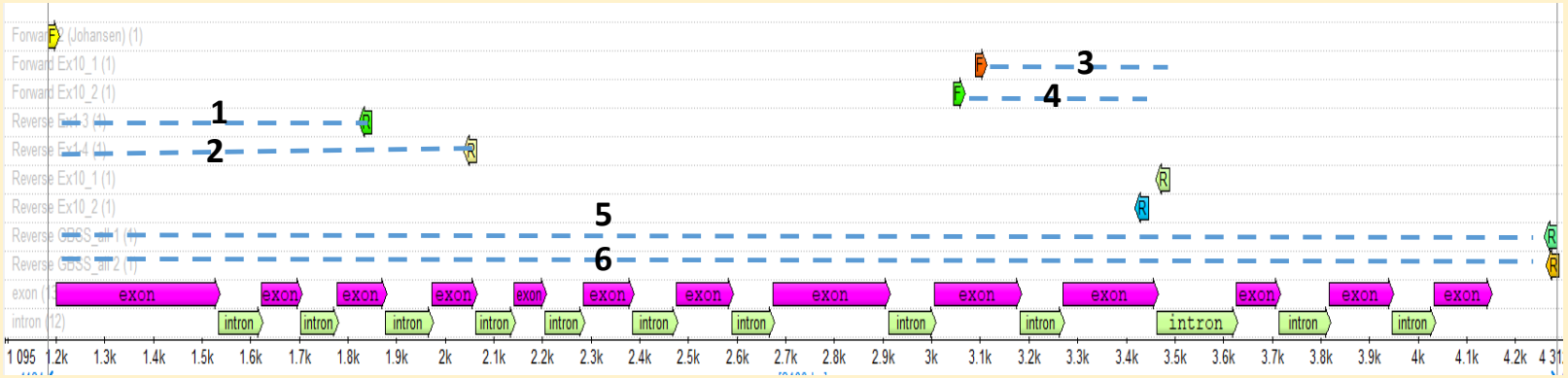
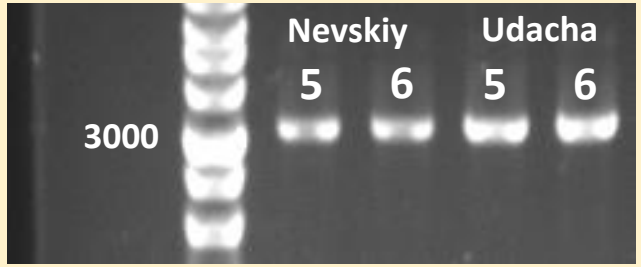


The 6 specific pairs of PCR primers were designed to *GBSS1* for **Nevskiy** and **Udacha** in order to amplify the target genomic sequences for *in vitro* CRISPR/Cas9 testing.

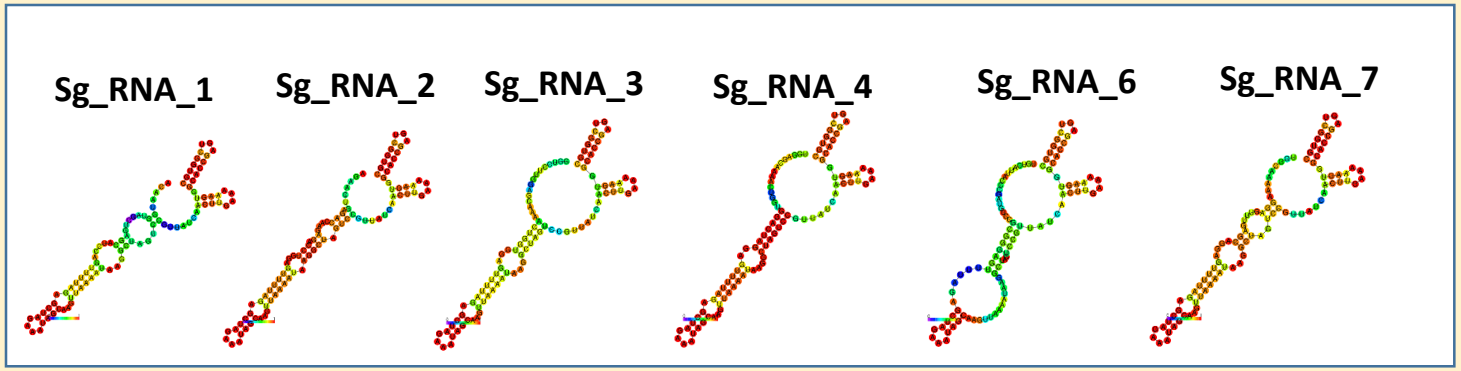




Design of sgRNA The obtained *GBSS1* consensus gene sequences were analyzed with CRISPOR, CRISPRdirect, CRISPR-P programmes to pick the candidate sgRNAs. From 109 total output oligos six were selected based on criteria of predicted specificity and secondary structure. The six selected sgRNAs are targeted to *GBSS1* exons 1, 2 and 10.

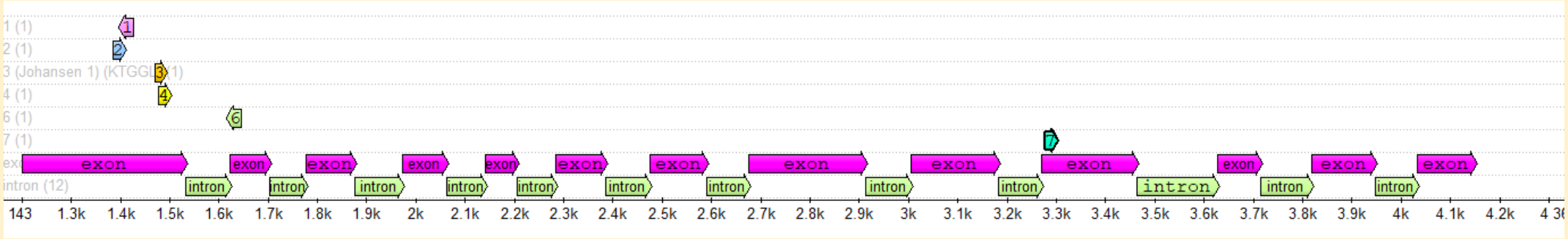
sgRNA	Exon number	Number of target sites		
		20 mer	12 mer	8 mer
Sg_RNA_1	1	1	1	204
Sg_RNA_2	1	1	2	469
Sg_RNA_3*	1	1	5	967
Sg_RNA_4	1	1	1	557
Sg_RNA_6	2	1	1	97
Sg_RNA_7	10	1	1	539

* The sgRNA 3 variant was already successfully used by Johansen et al., 2019 [3]



Verification of the secondary structure of selected sgRNAs

The sgRNA positions on *GBSS1* sequence

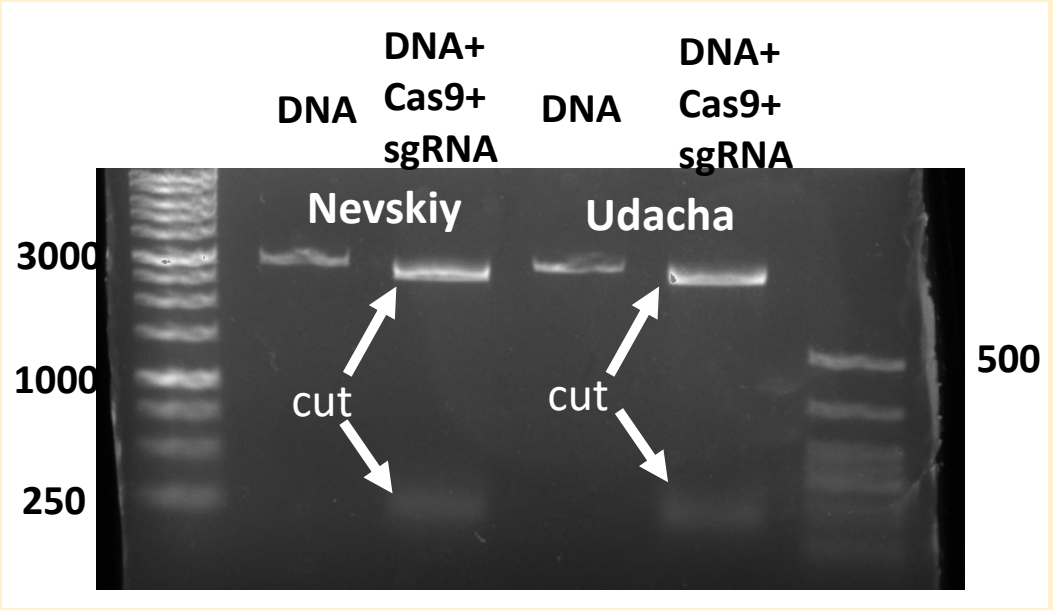


In vitro testing of sgRNA

The next step was the synthesis of designed sgRNAs and *in vitro* pre-validation as part of Cas9 ribonucleoprotein (RNP) complex.
Sg_RNA_3 and Sg_RNA_6 was synthesized with the aid of Precision gRNA Synthesis Kit (Invitrogen).
Sg_RNA_3 was successfully used to cleave the PCR-produced *GBSS1* sequences of Nevskiy and Udacha.
The remaining sgRNAs are ready for cloning to pUC57-sgRNA expression vector and will be *in vitro* tested in the near future.

Conclusion We designed 6 sgRNAs targeted to *GBSS1* gene of popular Russian potato cultivars Nevskiy and Udacha, one of sgRNAs was *in vitro* tested and demonstrated efficacy to cleave PCR-produced *GBSS1*. Further the *in vitro* effective sgRNAs will be used for DNA-free (RNP) biolistic transformation of potato protoplasts and explants.

In vitro testing of Sg_RNA_3



References
1) Andersson et al., Plant Cell Reports, 2017, v 36, p 117–128
2) Kusano et al., Scientific Reports, 2018, v 8, Article number: 13753
3) Johansen et al., Scientific Reports, 2019, v 9, Article number: 17715
4) Veillet et al., Plant Cell Reports, 2019, v 38, p 1065–1080

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