Developing plasmid vectors with cultivar specific U6 promoter to enhance CRISPR/Cas9 genome editing efficiency in potato (*Solanum tuberosum*)

Larichev K.T., Sergeeva E.M., Karetnikov D.I., Afonnikov D.A., Salina E.A., Kochetov A.V.

Kurchatov Genomic Center of the Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia
E-mail: klarichev@bionet.nsc.ru

Genetic engineering could bypass complications associated with potato breeding. Our goal was to develop a plasmid vector for efficient potato CRISPR/Cas9 genome editing via utilizing potato small nuclear U6 RNA promoter for single guide RNA (sgRNA) expression. Cultivar-specific StU6 promoter sequences were acquired from genome assemblies of Nevsky and Udacha using BLASTn algorithm and GenBank potato U6 promoter reference sequences Z17290, Z17292, Z17293, Z17301. Identified promoter variants (regions 400 bp upstream of U6 gene) were generated by PCR-amplification. The sgRNA was previously used to knock-out potato GBSS gene by another group. We generated several sgRNA expression cassettes with various U6 promoters from potato cultivars Nevsky and Udacha. These cassettes were ligated into a plasmid vector which encodes Cas9 protein (pHDE-35S-Cas9-mCherry). Next, generated vectors will be introduced into protoplasts of Nevsky and Udacha to evaluate frequency of target sequence editing events.

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Take-home message:

We develop a plasmid-vector for efficient potato genome editing by utilizing potato U6 RNA promoter for sgRNA expression.

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