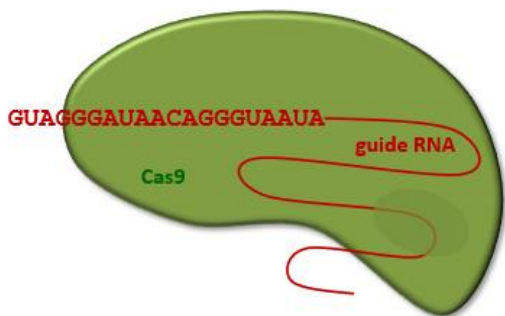


N\*

# Targeted modification of regulatory genes associated with barley grain color formation

Egorova A.\*<sup>1,2</sup>, Strygina K.<sup>4</sup>, Shoeva O.<sup>1</sup>, Hertig C.<sup>3</sup>, Gerasimova S.<sup>1,2</sup>, Koeppl I.<sup>3</sup>, Hiekel S.<sup>3</sup>, Vikhorev A.<sup>1,2</sup>, Kumlehn J.<sup>3</sup>, Khlestkina E.<sup>4</sup>



Cas9/gRNA-mediated site-directed mutagenesis

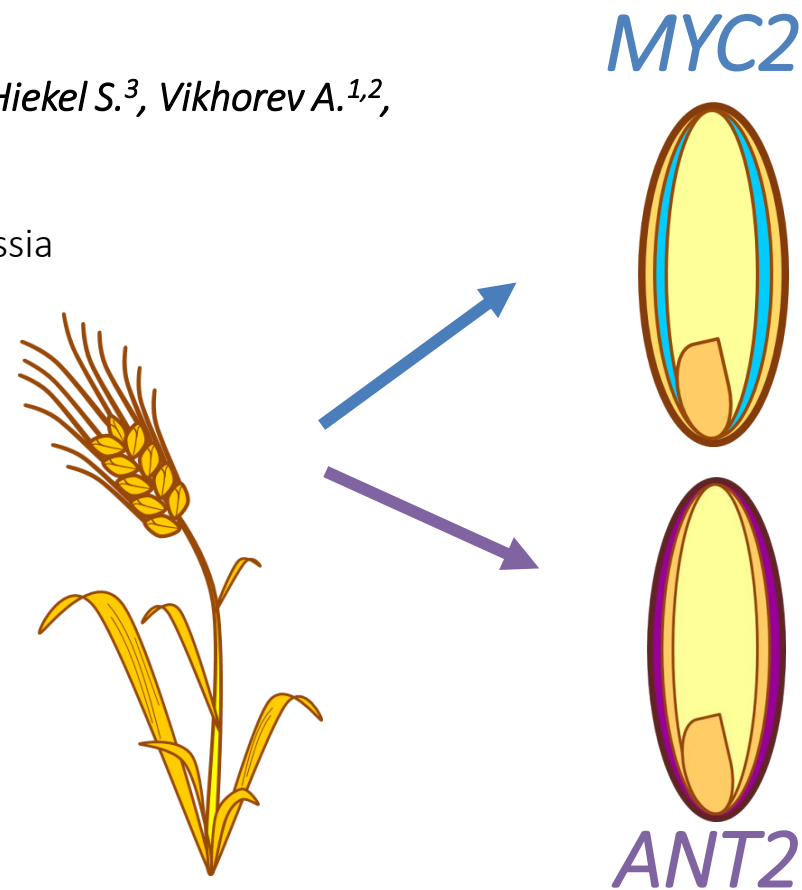
<sup>1</sup> Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia

<sup>2</sup> NSU, Novosibirsk, Russia

<sup>3</sup> IPK, Gatersleben, Germany

<sup>4</sup> VIR, St. Petersburg, Russia

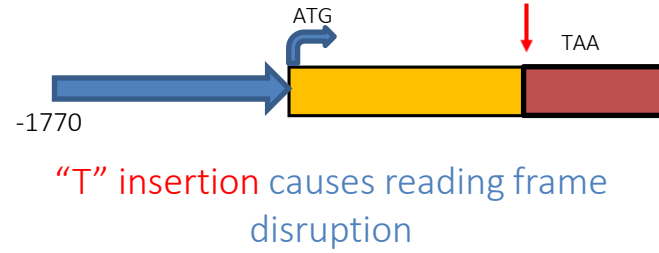
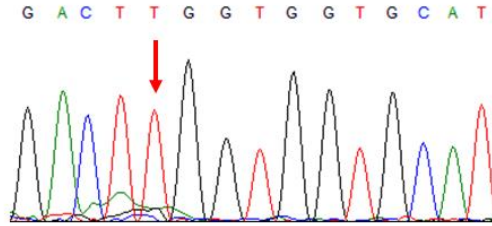
Blue and purple colors of barley (*Hordeum vulgare* L.) grain are caused by anthocyanin accumulation in aleurone and pericarp, respectively. Anthocyanins are known for their human health benefits. It is thus desirable to breed and grow barley with colored grains. In our previous experiments, the bHLH transcription factors MYC2 and ANT2 have been shown to be implicated in the control of barley grain pigmentation. In the present investigation, the *MYC2* and *ANT2* genes of barley were subjected to Cas9/gRNA-mediated site-directed mutagenesis aiming to further elucidate the roles of these genes in grain color formation.



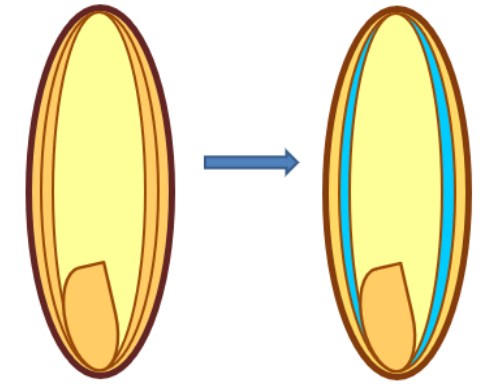
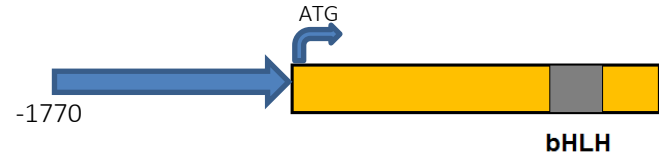
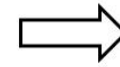
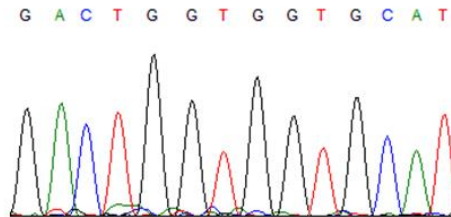
*MYC2* and *ANT2* are candidate genes for barley grain color formation

# MYC2

Uncoloured barley



Blue barley



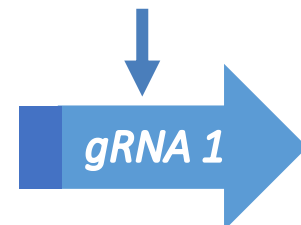
Aim: To confirm MYC2 function and turn white grain color to blue

It was previously shown that the loss-of-function of *HvMYC2* is associated with non-colored compared to blue-grained barley. To provide compelling evidence of MYC2 function in regard to blue aleurone formation, an attempt was made to restore the reading frame of the mutated allele in non-colored barley by deleting the spontaneously inserted nucleotide.

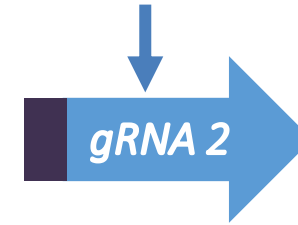
## Target motif selection

TGGGGAGTCACAGAAGTTGCTGAATAAAGTTGTGAC**T**TGGTGGTGCATGGGCGAGCAATTATGG**CGGT**TGGAACCATGGTGAGAGCTCC

Target motif 1

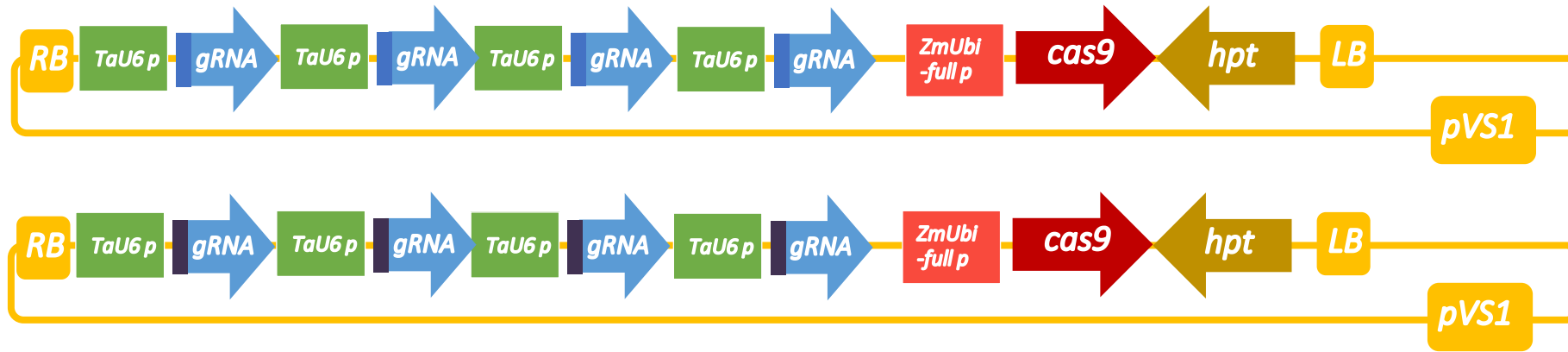


Target motif 2



gRNAs were chosen to restore the reading frame of the mutated allele

# MYC2



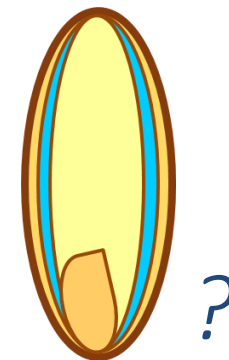
'Golden Promise' with uncolored grains was chosen as donor material for *Agrobacterium*-mediated transformation

Cas9/gRNA plasmids were constructed for two target sites in the *HvMYC2* gene

## Stable transformation results

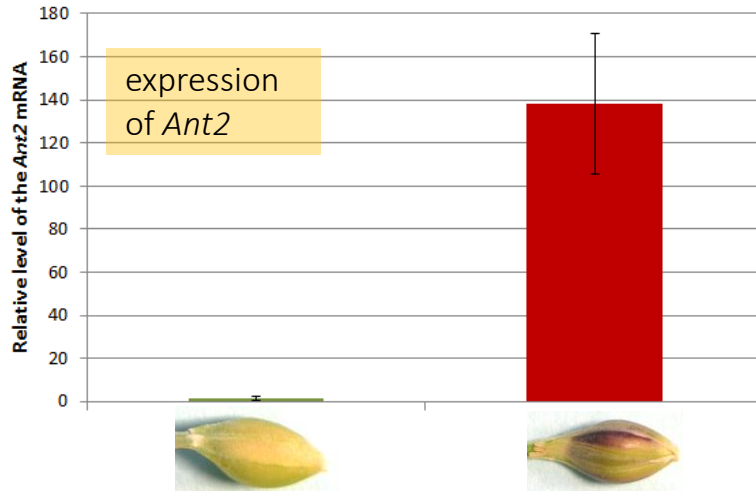
	Number of embryos	Number of regenerants	Mutant plants (T0)	Plants with required mutation ( -1, -4)
gRNA 1 (4x)	200	27	23	18
gRNA 2 (4x)	200	28	26	1

After *Agrobacterium*-mediated transformation, 49 primary mutant plants were obtained, out of which 19 featured the desired loss of a single nucleotide. The phenotypic analysis of the generated mutants is in progress.

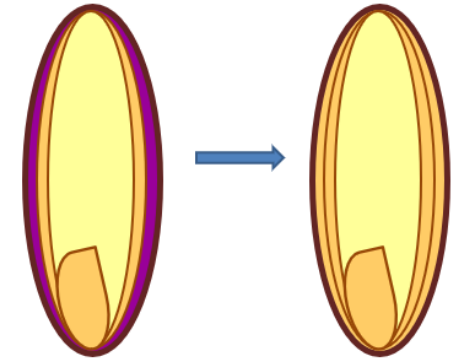


We are expecting blue color

# ANT2



*ANT2* is candidate gene for purple grain color formation



Aim: To confirm *ANT2* function via gene knockout in purple grain barley

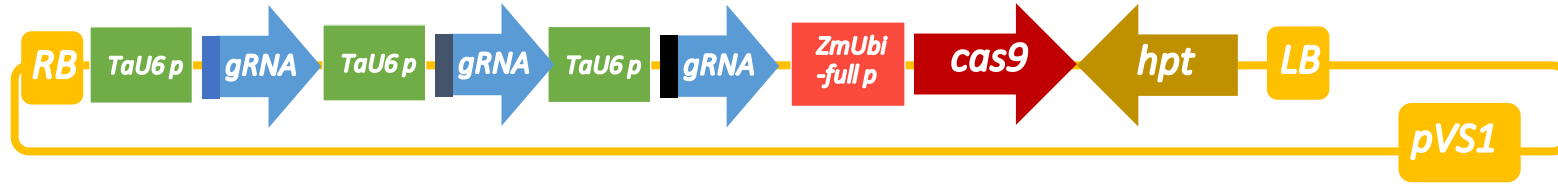
To confirm the role of *ANT2* in purple pericarp formation, a targeted knockout of the respective gene was required in purple-grained barley. To generate experimental lines amenable to genetic transformation, the purple PLP accession was crossed with 'Golden Promise', which was followed by the use of resulting hybrids for the knockout approach.

## Target motif selection



Target motifs and corresponding gRNAs were chosen to *ANT2* knockout

# ANT2



A cas9/gRNA construct was generated, containing three gRNAs for different target sites in the *HvANT2* gene

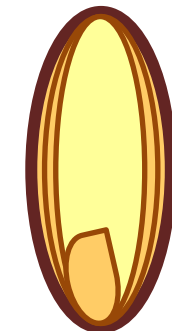


Purple barleys from GPxPLP (F2) were chosen as donor material for *Agrobacterium*-mediated transformation

## Stable transformation results

Number of embryos	Number of regenerants	Mutant plants (T0)
400	71	67

*Agrobacterium*-mediated transformation resulted in 67 primary mutant plants. The phenotypic analysis of the generated mutants is in progress.



?

We are expecting white color





### *IPK*

Dr. Jochen Kumlehn  
Christian Hertig  
Iris Koeppel  
Stefan Hiekel  
Sabine Sommerfeld  
Ingrid Otto  
Carola Bollmann

### *ICG&VIR*

Dr. Elena Khlestkina  
Dr. Alexey Kochetov  
Dr. Olesya Shoeva  
Dr. Ksenia Strygina  
Dr. Sophia Gerasimova  
**Anastasiya Egorova**  
Alexander Vikhorev

*Thanks for your attention!*



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