Al-Farabi Kazakh National University (KazNU)

Faculty of Biology and Biotechnology

Department of Molecular Biology and Genetics

Discipline: «Modern Problems of Plant Genetics»

Lecture 10

CRISPR/Cas Genome Editing and Precision Plant Breeding

From bacterial adaptive immunity and SpCas9 structural mechanism to base editing, prime editing, RNP delivery, and de novo crop domestication

Author: Amirova Aigul Kuzembayevna, Candidate of Biological Sciences, Associate Professor
E-mail: aigul_amir@mail.ru

Aim and Structure of the Lecture

Aim: master molecular mechanisms of CRISPR/Cas nucleases, repair pathways, next-gen editors, and agricultural applications

KEY FOCUS

Lecture Plan & Modules

Key sections:
1. History & Nobel Discovery
2. Bacterial Adaptive Immunity
3. Cas Systems & SpCas9 Structure
4. DNA Repair & Nuclease Matrix
5. Precision Editing Toolkit
6. Delivery & Landmark Crops

- **History & Nobel Discovery:** Evolution from mysterious bacterial repeats (1987) to Charpentier & Doudna's 2012 sgRNA breakthrough
- **Bacterial Adaptive Immunity:** Three-stage prokaryotic viral defense: Cas1–Cas2 adaptation, crRNA biogenesis, and targeted interference
- **Cas Systems & SpCas9 Structure:** Class 1 vs Class 2 architectures; SpCas9 REC/NUC lobes, RuvC/HNH active sites, and 5'-NGG-3' PAM
- **DNA Repair & Nuclease Matrix:** Competing NHEJ vs HDR repair outcomes and benchmarking ZFN, TALEN, SpCas9, and Cas12a
- **Precision Editing Toolkit:** DSB-free Cytosine/Adenine Base Editors (CBE/ABE), Prime Editing (pegRNA), and dCas9 CRISPRa/i
- **Delivery & Landmark Crops:** DNA-free RNP delivery, morphogenic genes (BBM/WUS, GRF4-GIF1), wheat TaMLO, rice OsSWEET & tomato

Milestones from Prokaryotic Repeats to Plant Genome Design

Curiosity-driven microbiology transformed into the most versatile tool in modern life sciences

1987–2005

Discovery of CRISPR

- Ishino (1987) spots 29-nt repeats in E. coli IAP gene
- Jansen & Mojica (2002–2005) coin CRISPR & link spacers to phages

2007–2011

Immunity & tracrRNA

- Barrangou proves viral spacer immunity in S. thermophilus
- Charpentier (2011) identifies essential tracrRNA in S. pyogenes

2012–2013

Programmable sgRNA

- Jinek, Charpentier & Doudna fuse crRNA + tracrRNA into sgRNA
- First edited rice, wheat, tobacco, and Arabidopsis plants (2013)

2016–2026

Precision & Nobel Era

- Base Editing (2016), Prime Editing (2019), Nobel Prize (2020)
- Commercial high-GABA tomato & de novo domesticated crops

The 2020 Nobel Breakthrough in Programmable RNA-Guided Editing

Emmanuelle Charpentier and Jennifer Doudna unified two bacterial RNAs into a single engineered guide RNA (Science, 2012)



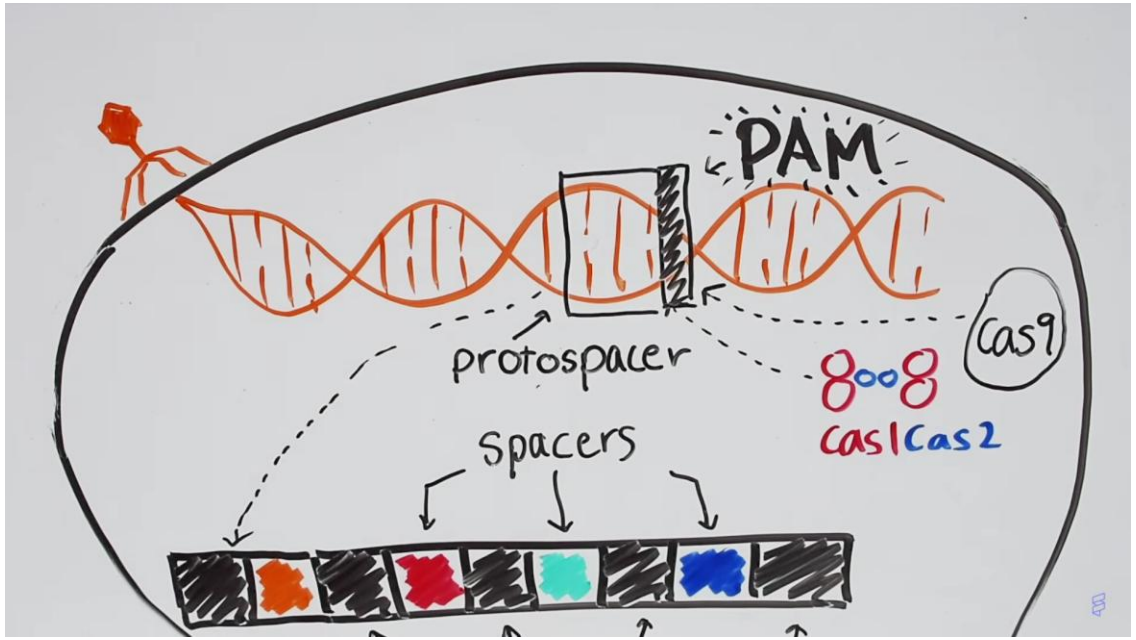
Nobel Laureates Emmanuelle Charpentier (left) & Jennifer Doudna (right) — 2020 Nobel Prize in Chemistry

- **From Two RNAs to One Chimeric sgRNA:** In Streptococcus pyogenes, Cas9 naturally requires duplexed crRNA (target spacer) + tracrRNA (scaffold)
- Jinek et al. fused crRNA and tracrRNA with a tetraloop linker into a single 100-nt guide RNA (sgRNA)
- Changing only the 20-nt 5' spacer directs Cas9 to virtually any genomic target adjacent to a PAM
- **Why This Revolutionized Plant Genetics:** Prior ZFN and TALEN nucleases required slow custom protein engineering for every single gene locus
- CRISPR sgRNAs allow effortless multiplex editing of polyploid plant genomes (e.g., 6 wheat homeologs simultaneously)
- Dramatically lowers time and cost for academic breeding labs worldwide

Biological Origin: Three-Stage Bacterial CRISPR/Cas Immunity

Prokaryotes retain heritable molecular memories of past bacteriophage and plasmid infections

- **1. Adaptation (Immunization):** Cas1–Cas2 integrase recognizes foreign viral protospacer adjacent to a PAM motif; Inserts new 30-bp viral DNA spacer at the leader end of the chromosomal CRISPR repeat-spacer array
- **2. Expression & Processing:** CRISPR array is transcribed into a long precursor pre-crRNA; tracrRNA hybridizes to repeats; host RNase III cleaves duplex into mature individual crRNAs
- **3. Targeted Interference:** Cas9–crRNA–tracrRNA surveillance complex scans invading phage DNA for matching PAM + spacer; Exact RNA–DNA base pairing activates cleavage, neutralizing viral infection without self-attack (no host PAM)



Adaptive Immunity Steps: (1) Adaptation/Spacer Acquisition, (2) Expression/Processing, (3) Interference

Classification of CRISPR/Cas Systems (Class 1 vs Class 2)

Evolutionary diversity (2 Classes, 6 Types) expands target PAM flexibility, DNA/RNA targeting, and deletion profiles

Class 1 Systems (Types I, III, IV)

- Represent ~90% of prokaryotic CRISPR loci; utilize large multi-subunit effector complexes (Cascade, Cmr/Csm)
- **Type I (Cas3 helicase-nuclease):** generates large unidirectional chromosomal deletions (10–100+ kb)
- **Type III:** co-transcriptionally cleaves both target RNA and nascent single-stranded DNA
- **Emerging plant tool:** Type I-E/I-D Cascade deletes tandem gene clusters in rice, maize, and trees

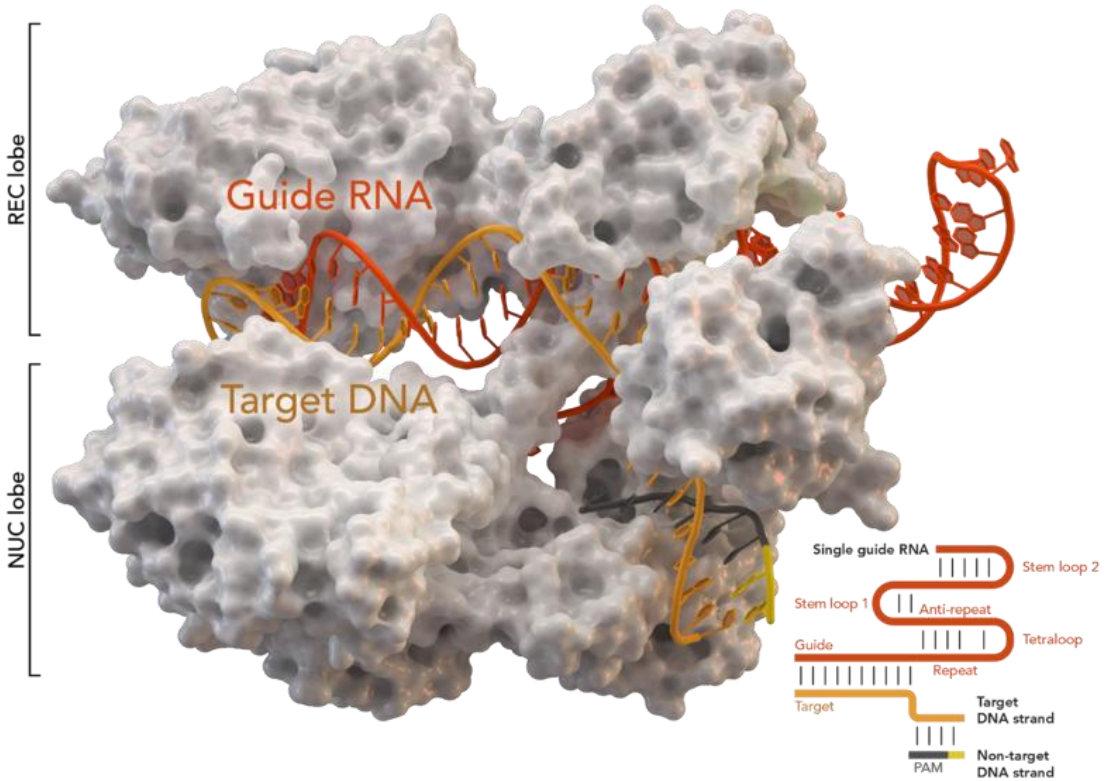
Class 2 Systems (Types II, V, VI)

- Single multi-domain effector protein simplifies cloning, delivery, and RNP complex assembly
- **Type II (SpCas9):** recognizes 5'-NGG-3' PAM; produces blunt DSB via RuvC & HNH domains
- **Type V (Cas12a / Cpf1):** recognizes AT-rich 5'-TTTV-3' PAM; creates 5' staggered cuts & self-processes arrays
- **Type VI (Cas13a/b/d):** RNA-guided RNase targeting plant RNA viruses (PVY, TMV) without altering DNA

Molecular Architecture of SpCas9 and sgRNA Target Recognition

Conformational proofreading between REC and NUC lobes ensures high cleavage fidelity

- **Bilobed Domain Organization:** REC (Recognition) Lobe binds the sgRNA–DNA heteroduplex inside a positively charged channel
- NUC (Nuclease) Lobe houses HNH and split RuvC catalytic domains plus C-terminal PAM-interacting (PI) domain
- **PAM Scanning & Dual Cleavage:** PI domain first interrogates 5'-NGG-3' PAM in dsDNA before unwinding strands directionally from PAM-proximal seed
- **Full 20-bp R-loop pairing flips HNH into active conformation:** HNH cuts target strand; RuvC cuts non-target strand 3 bp upstream of PAM



SpCas9–sgRNA–DNA Ternary Complex: REC Lobe, NUC Lobe (HNH & RuvC), PAM-Interacting Domain & sgRNA Scaffold

Cellular DNA Repair Pathways: NHEJ vs HDR in Plants

Cas9 creates the break; plant endogenous repair enzymes decide the final genomic outcome

Non-Homologous End Joining (NHEJ)

- Predominant pathway across G1, S, G2 somatic plant cells (~90–99% of DSB repairs)
- Directly ligates broken ends via Ku70/Ku80 & DNA Ligase IV, often introducing 1–20 bp indels
- Produces frameshift loss-of-function alleles, promoter deletions, or stop codons (SDN-1)
- Multiplex sgRNAs trigger precise chromosomal inversions or large genomic deletions

Homology-Directed Repair (HDR)

- Restricted largely to S/G2 cell-cycle phases; requires a homologous dsDNA or ssODN donor template
- Enables exact allele replacement (SDN-2) or site-specific gene knock-in (SDN-3)
- Native plant HDR efficiency is typically very low (<0.5–5%) due to poor nuclear template availability
- Boosted using geminivirus replicons, Cas9–VirD2 donor tethering, or high-efficiency Prime Editing

Comparative Analysis of Programmable Plant Genome Editors

Transitioning from protein–DNA recognition (ZFN/TALEN) to modular RNA–DNA hybridization (CRISPR)

Feature	ZFN	TALEN	SpCas9 (Type II)	Cas12a / Cpf1 (Type V)
Recognition Module	Protein zinc-finger triplets	Protein 34-aa TALE repeats	20-nt sgRNA (RNA–DNA)	20–24-nt single crRNA
Target Constraint	G-rich triplets preferred	5'-T preceding binding site	5'-NGG-3' PAM (GC-rich)	5'-TTTV-3' PAM (AT-rich)
DSB Cleavage Cut	5' overhangs (FokI dimer)	5' overhangs (FokI dimer)	Blunt ends (3 bp from PAM)	Staggered 4–5 nt overhang
Multiplexing Ease	Very difficult (protein dim.)	Moderate (Golden Gate)	High (tRNA/ribozyme array)	Highest (self-processing)
Off-Target Profile	Moderate to high cytotoxicity	Low off-target activity	Low with high-fidelity SpCas9-HF	Very low + temperature-tuned
Cloning & Cost	Months; high proprietary cost	Weeks; large repetitive DNA	Days; inexpensive oligos	Days; single short crRNA

DSB-Free Precision: Base Editing and Prime Editing

Writing single-nucleotide substitutions and targeted small edits cleanly without double-strand DNA breaks

Cytosine (CBE) & Adenine (ABE) Base Editors

- Fuse catalytically impaired Cas9 nickase (nCas9 D10A) to cytidine or evolved TadA adenosine deaminase
- CBE converts C·G → T·A inside a 4–8 nt R-loop window (uracil glycosylase inhibitor UGI prevents reversal)
- ABE converts A·T → G·C cleanly without DNA breaks, virtually eliminating chromosomal translocations
- **Plant use:** herbicide-resistant ALS alleles, splice-site modulation, and premature stop induction (iSTOP)

Prime Editing («Search-and-Replace», PE2–ePPE)

- Fuses nCas9 (H840A) to an engineered M-MLV Reverse Transcriptase guided by a pegRNA
- pegRNA encodes both target spacer and an RNA RT template carrying the desired edit (Primer Binding Site)
- Executes all 12 possible base conversions plus targeted insertions & deletions without donor dsDNA
- Plant optimizations (ePPEplus, viral pegRNA, paired pegRNAs) raise crop prime editing from <5% to 20–45%+

Beyond Sequence Editing: The dCas9 Transcriptional & Epigenetic Toolkit

Catalytically dead Cas9 (dCas9, D10A + H840A) acts as a programmable genomic GPS carrying effector cargos

01

01 — CRISPRa Activation: dCas9 fused to VP64, SunTag, or plant-optimized dCas9-TV activates silent promoters up to 50–100×; Fine-tunes quantitative yield or disease-resistance gene dosage without foreign coding regions

02

02 — CRISPRi Repression: dCas9-SRDX sterically blocks RNA Pol II elongation or recruits histone deacetylases at TSS; Enables reversible gene knockdown without permanent genomic mutations

03

03 — Epigenome Editing: dCas9-TET1cd demethylates cytosines (reactivating imprinted genes e.g. FWA in Arabidopsis); dCas9-DRM2 writes heritable CG methylation for transgene-free phenotypic stability

04

04 — CRISPR-Combo Systems: Simultaneous sgRNA lengths (20-nt cut vs 14-nt truncated scaffold) combine gene KO with CRISPRa in one plant; Activates regeneration genes while editing target traits in a single construct

Overcoming Plant Delivery and Regeneration Bottlenecks

Getting CRISPR machinery past the cell wall and regenerating intact plants across recalcitrant elite cultivars

DNA vs DNA-Free RNP Delivery

- **Agrobacterium tumefaciens T-DNA:** highest efficiency in transformable lines; T1 Mendelian segregation removes Cas9
- **Biolistics (gene gun):** delivers plasmids or pre-assembled Cas9–sgRNA ribonucleoproteins (RNPs) into embryos
- **DNA-Free RNP (PEG-protoplasts or particle bombardment):** degrades within 24–48 h—zero foreign DNA ever enters genome
- Viral vectors (PVX, TRV, BSMV with mobile FT/tRNA guides) achieve heritable germline editing without tissue culture

Breaking Genotype Recalcitrance

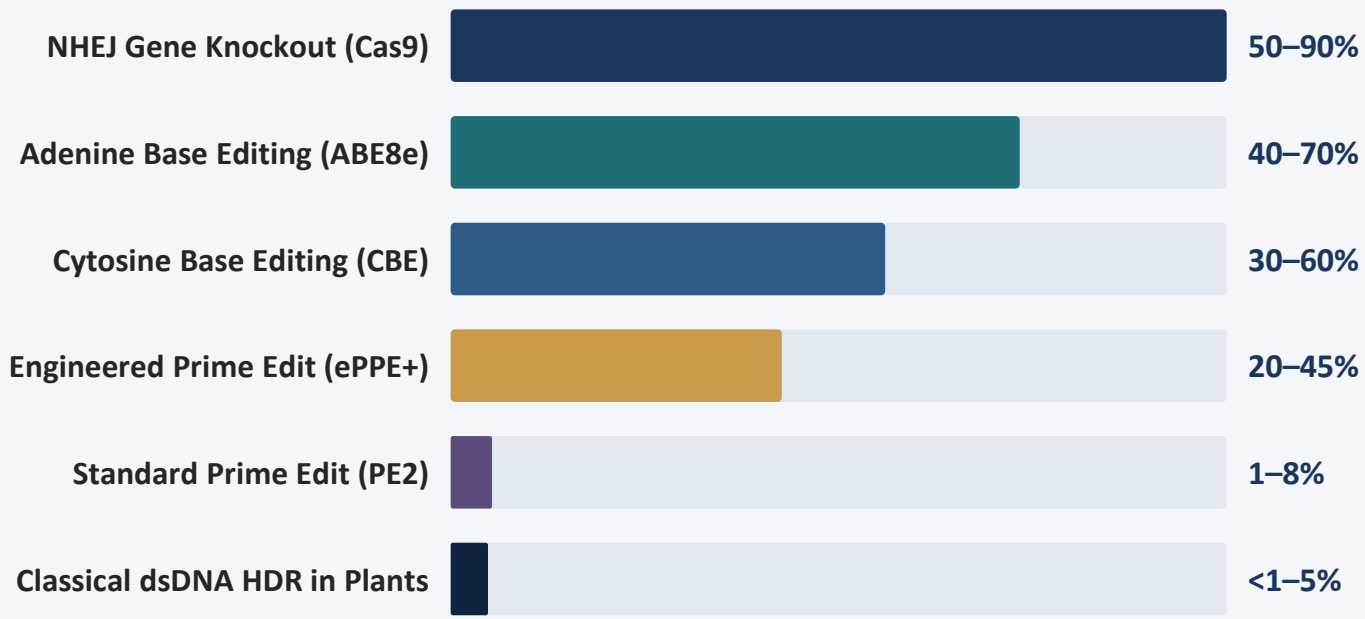
- Most elite wheat, maize, sorghum, and legume cultivars regenerate poorly from callus in vitro
- **Morphogenic Regulators:** Baby Boom (BBM) + Wuschel2 (WUS2) trigger rapid somatic embryogenesis in elite maize/sorghum
- GRF4–GIF1 fusion chimera boosts wheat, rice, and citrus transformation efficiency without developmental abnormalities
- De novo meristem induction & Cut-Dip-Budding bypass sterile tissue culture entirely in woody & root crops

Benchmarking Editing Efficiency and Breeding Speed in Crops

Rapid technological maturation (2015–2026) shifts plant CRISPR from single knockouts to complex precision alleles

~100%	20–45%	0% DNA	1–2 yrs
Multiplex Homeolog KO	Optimized Plant ePPE	RNP / Segregated SDN-1	Edit-to-Line Timeline

Representative Editing Efficiencies in Major Crops (%)



Breeding Cycle Compression

- Simultaneous knockout of all 3 homeologs (A, B, D) in hexaploid wheat avoids slow backcrossing
- Pairing CRISPR RNP editing with speed breeding cuts cultivar development from 8–12 yrs to 2–3 yrs
- Off-target frequency in edited plants sits below natural spontaneous mutation rate per generation

Benchmark ranges synthesized from Gao (2021) Cell, Zong et al. (2022) Nature Biotechnology (ePPE), and Chen et al. crop editing reviews.

Landmark CRISPR-Edited Crops and Precision Breeding Breakthroughs

From monogenic disease resistance to promoter engineering and de novo wild crop domestication

Crop Species	Target Locus & Edit	Precision Tool	Agronomic / Consumer Breakthrough
Bread Wheat (6x)	Simultaneous KO of 3 TaMLO-A/B/D alleles or Tamlo-R32	Multiplex Cas9 / TALEN	Broad-spectrum powdery mildew resistance; Tamlo-R32 retains full yield
Rice (Oryza sativa)	Promoter EBE edits in OsSWEET11, OsSWEET13, OsSWEET14	Cas9 promoter editing	Broad resistance to Xanthomonas bacterial blight (all 3 strains blocked)
Sicilian Rouge Tomato	Deletion of C-terminal autoinhibitory domain of SlGAD3	CRISPR-Cas9 KO	4–5× elevated GABA; world's 1st commercial CRISPR food (Japan, 2021)
Soybean (Glycine max)	Targeted KO of FAD2-1A and FAD2-1B desaturases	TALEN / Cas9 SDN-1	~80% heart-healthy oleic acid; zero trans fats in frying oil (Calyno)
Maize & Barley	Matrilineal (MTL / ZmPLA1) haploid induction + promoter edits	CRISPR KO + cis-edit	Accelerated doubled-haploid production & quantitative ear-row tuning
Wild Tomato / Physalis	Multiplex domestication of SP, SP5G, CLV3, WUS, SlGGP1	Multiplex CRISPR-Cas9	De novo domestication in 1–2 generations keeping wild stress & nutrient traits

★ Key Takeaway

- From monogenic disease resistance to promoter engineering and de novo wild crop domestication
- **Comparison scope (6):** Bread Wheat (6x); Rice (Oryza sativa); Sicilian Rouge Tomato; Soybean (Glycine max); Maize & Barley; Wild Tomato / Physalis
- **Priority synthesis:** Wild Tomato / Physalis — De novo domestication in 1–2 generations keeping wild stress & nutrient traits

Control Questions and Key Takeaways

Mastering CRISPR/Cas biology, tools, and applications in modern plant genetics

? Control & Review Questions

- 1. Describe the three stages of prokaryotic CRISPR/Cas immunity and why Cas9 avoids cleaving the host's own CRISPR array.
- 2. Trace the domain roles of REC, NUC (HNH & RuvC), PAM-interacting domain, and sgRNA during SpCas9 target cleavage.
- 3. Compare NHEJ, HDR, Cytosine/Adenine Base Editing (CBE/ABE), and Prime Editing in plant precision breeding.
- 4. How do RNP delivery and morphogenic regulators (BBM/WUS2, GRF4–GIF1) solve transgene and regeneration hurdles?
- 5. Explain how simultaneous editing of homeologous TaMLO genes or OsSWEET promoters illustrates polyploid & cis-regulatory editing.

✓ Key Takeaways

- Class 2 single effectors (SpCas9, Cas12a) require only a 20-nt guide change adjacent to a PAM to target new loci.
- Base editing and plant-optimized Prime Editing (ePPE) achieve clean single-bp and short edits without lethal DSBs.
- DNA-free RNP editing coupled with morphogenic regeneration unlocks transgene-free SDN-1 crops in elite lines.
- Realized milestones—wheat TaMLO, rice OsSWEET, high-GABA tomato, and de novo domestication—define Breeding 4.0.

Recommended Literature and Genome-Editing Tools

Foundational discovery papers, plant editing reviews, and sgRNA design servers

1 Main Literature

- 1. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337: 816–821.
- 2. Gao, C. (2021). Genome engineering for crop improvement and future agriculture. Cell 184(6): 1621–1635.
- 3. Zhu, H., Li, C., Gao, C. (2020). Applications of CRISPR–Cas in agriculture and plant biotechnology. Nature Reviews Molecular Cell Biology 21: 661–677.
- 4. Makarova, K.S. et al. (2020). Evolutionary classification of CRISPR–Cas systems: a burst of class 2 and derived variants. Nature Reviews Microbiology 18: 67–83.

2 Additional Literature & Databases

- 1. Zong, Y. et al. (2022). An engineered prime editor with enhanced editing efficiency in plants (ePPE). Nature Biotechnology 40: 1394–1402.
- 2. Wang, Y. et al. (2014) & Li, S. et al. (2022). Simultaneous editing of wheat TaMLO / Tamlo-R32 for durable powdery mildew resistance. Nature Biotechnology / Nature.
- 3. Zsögön, A. et al. (2018). De novo domestication of wild tomato using genome editing. Nature Biotechnology 36: 1211–1216.
- **4. Plant sgRNA & Off-Target Design Portals:** CRISPR-P 2.0, CRISPOR (crispor.gi.ucsc.edu), pegFinder, Benchling.

Thank you for your attention! Department of Molecular Biology and Genetics, KazNU · aigul_amir@mail.ru