**Transgenics and editing: Study guide and readings**

***Required readings:*** Ueta et al. 2017. Scientific Reports 7:507.

1. Full article
2. Explain why transgenic plants are created – considering both commercial and research applications.
3. What are the essential elements of a transgene construct?
4. Explain the advantages and disadvantages of different types of promoters in transgenic constructs.
5. Why are selectable markers used in transgenics? Give an example of a common selectable marker.
6. What are reporter genes and how do they differ from selectable markers?
7. What are two key differences between GUS and GFP?
8. What are the two main methods for introducing a transgene and what are the benefits and disadvantages of each?
9. What is the role of a disarmed Ti plasmid in the Agrobacterium mediated transformation protocol?
10. What is a hemizygote? Explain the predicted pattern of inheritance of a transgene in an otherwise homozygous inbred diploid plant – starting with a biolistically transformed cell in a petri dish.
11. Explain the basis of Roundup Ready herbicide resistance, including source of the gene and general architecture of the construct. If a Roundup Ready variety has a construct using the CaMV promoter, is the gene likely to be expressed in all tissues and throughput the plant life cycle or only expressed when the herbicide is applied?
12. Tell the Golden Rice story in your own words – from rationale to the genes involved.
13. Tell the Applause Rose story in your own words – from rationale to the genes involved.
14. Summarize the importance of transgenic crops to US agriculture.
15. What are concerns regarding transgenic crops?
16. How are transgenic plants defined and detected?
17. Compare and contrast naturally occurring and induced mutations in terms of causal agents and mechanisms
18. What does TILLING stand for and what is it used for?
19. Compare and contrast transgenics and cisgenics.
20. Explain the principle of RNAi.
21. How is RNAi used in the case of the Arctic Apple?
22. Define the CRISPR and Cas9 acronyms.
23. What is concept of genome editing and how does the CRISPR technology differ from “classical transgenic technology”, e.g. agrobacterium-mediated Roundup Ready herbicide resistance?
24. If you get your dream job – as a geneticist working on your favorite plant – what is the appeal of CRISPR-Cas9 editing vs. mutagenesis by radiation or chemicals?
25. Based on [The MIT video](https://www.youtube.com/watch?v=2pp17E4E-O8) and [The Nature video](https://www.youtube.com/watch?v=4YKFw2KZA5o), answer the following questions.
	1. Briefly explain where the system is naturally occurring and what it does in that situation.
	2. What is meant by “gene editing”?
	3. What are the two principal components of the CRISPR-Cas9 system and what are their roles?
	4. How does the CRISP-Cas9 complex identify a specific target in the genome?
	5. What type of break does Cas9 make in the host double helix, and why is this break important?
	6. What is meant by “knocking out” a gene?
	7. Is gene “knock out” all that CRISPR-Cas 9 can do?
	8. Is CRISP-Cas9 really cheap and easy? Why or why not?
26. Explain how evidence of CRISPR gene editing can be “erased” and why this could be a goal.
27. Based on [*Gene drives – The TED talk*](https://www.youtube.com/watch?v=OI_OhvOumT0) , and the CRISPR-Cas9 resources presented in the lecture slides and online resources :
	1. What is a gene drive?
	2. Describe the pros and cons of implementing a transient edit in your favorite plant vs. implementing a gene drive.
28. Based on the Ueta et al. paper answer the following questions:
	1. What is the importance of parthenocarpy in tomato?
	2. What gene did the authors target for gene editing, and why?
	3. According to the authors, was their targeted mutation strategy successful?
	4. Were there any off-target mutations?
	5. Were there any pleiotropic effects of the edited gene?

**Supplementary Resources: Not required.**

**Background**

The Scientist article on CRISPR crops

<https://www.the-scientist.com/bio-business/companies-use-crispr-to-improve-crops-65362>

Bioluminescent trees

<https://www.smithsonianmag.com/innovation/creating-a-new-kind-of-night-light-glow-in-the-dark-trees-9600277/>

The Bt gene

<https://entomology.ca.uky.edu/ef130>

Transgene constructs for plants

<https://www.intechopen.com/books/new-visions-in-plant-science/transgenic-plants-gene-constructs-vector-and-transformation-method>

Constitutive promoters

[*https://link.springer.com/article/10.1007%2Fs00425-015-2278-4*](https://link.springer.com/article/10.1007/s00425-015-2278-4)

Tissue specific promoters

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3887779/>

Inducible promoters

[*https://doi.org/10.1007/s00425-017-2765-x*](https://doi.org/10.1007/s00425-017-2765-x)

EPSPS transgene and fecundity in Arabidopsis

<https://www.frontiersin.org/articles/10.3389/fpls.2018.00233/full>

Intron-mediated enhancement

<https://www.frontiersin.org/articles/10.3389/fpls.2016.01977/full>

Terminator sequences and gene expression

<https://academic.oup.com/pcp/article/51/2/328/1942394>

Reporter genes

<https://onlinelibrary.wiley.com/doi/full/10.1055/s-2003-40722>

More on GFP

<http://pdb101.rcsb.org/motm/42>

Luciferase example

<https://plantmethods.biomedcentral.com/articles/10.1186/s13007-018-0351-2>

**Transgenic examples**

Transgenic banana

<https://link.springer.com/article/10.1007/s11248-011-9574-y>

More on Agrobacterium-mediated gene transformation

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC150518/>

Golden Rice – the original paper

<http://www.goldenrice.org/PDFs/Ye_et_al_Science_2000.pdf>

Suntory Applause (blue) rose

<https://www.suntory.com/sic/research/s_bluerose/story/>

ISAAA GM approval database

<https://www.isaaa.org/gmapprovaldatabase/gene/default.asp?GeneID=7>

**Mutation review**

<https://www.ncbi.nlm.nih.gov/books/NBK21114/#A8425>

Mutation hot spots

<https://www.ncbi.nlm.nih.gov/pubmed/12888108>

Mutation rates

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2910838/>

Ionizing radiation

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6028737/>

DNA replication errors

<https://www.nature.com/scitable/topicpage/dna-replication-and-causes-of-mutation-409>

DNA repair mechanisms

<https://www.nature.com/scitable/topicpage/DNA-Damage-amp-Repair-Mechanisms-for-Maintaining-344>

TILLING

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3189332/>

**Cisgenics and RNAi**

<https://www.ncbi.nlm.nih.gov/pubmed/20573046>

Gene silencing through RNAi

<https://www.youtube.com/watch?v=cK-OGB1_ELE>

Arctic Apple RNAi

https://www.science20.com/kevin\_m\_folta/the\_hard\_science\_behind\_the\_nonbrowning\_arctic\_apple-224898

Arctic apple Extension bulletin

<http://nyshs.org/wp-content/uploads/2016/10/Pages-8-10-from-NYFQ-Book-Fall-2013-4.pdf>

**CRISP CRISPR-Cas9: how it works**

<https://www.youtube.com/watch?v=2pp17E4E-O8>

CRISPR-Cas9: so many possibilities

<https://www.youtube.com/watch?v=4YKFw2KZA5o>

CRISPR and Parthenocarpic tomato

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5428692/>

TED talk on gene drive

<https://www.ted.com/talks/jennifer_kahn_gene_editing_can_now_change_an_entire_species_forever?language=en>

Gene drive

<https://www.pnas.org/content/114/32/8452>

CRISPR mushroom

<http://agsci.psu.edu/magazine/articles/2016/fall-winter/a-crispr-mushroom>
Prime editing <https://science.sciencemag.org/content/366/6464/406>