

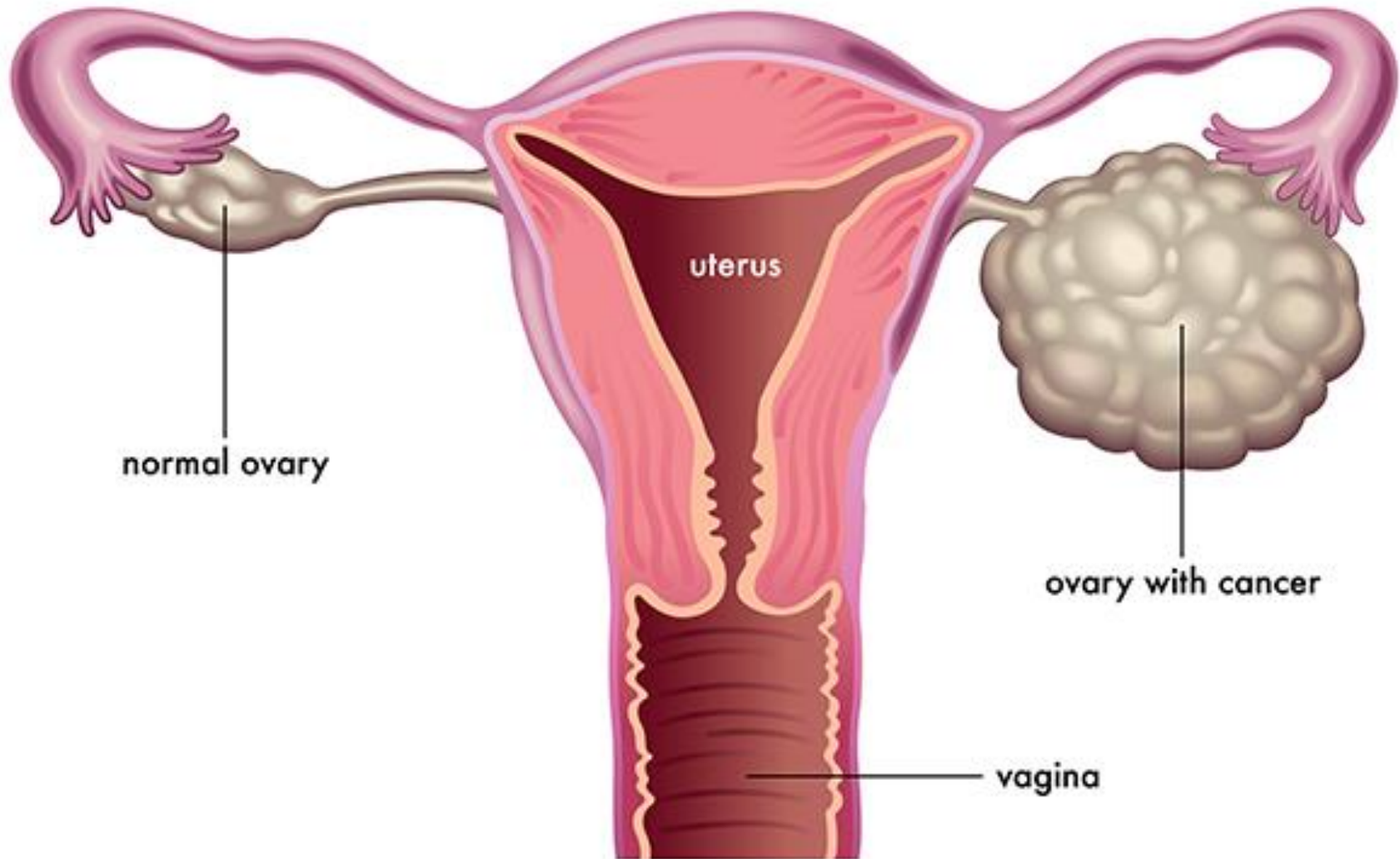
Ovarian Cancer and *RAD51D*

Jonathan Lombardino

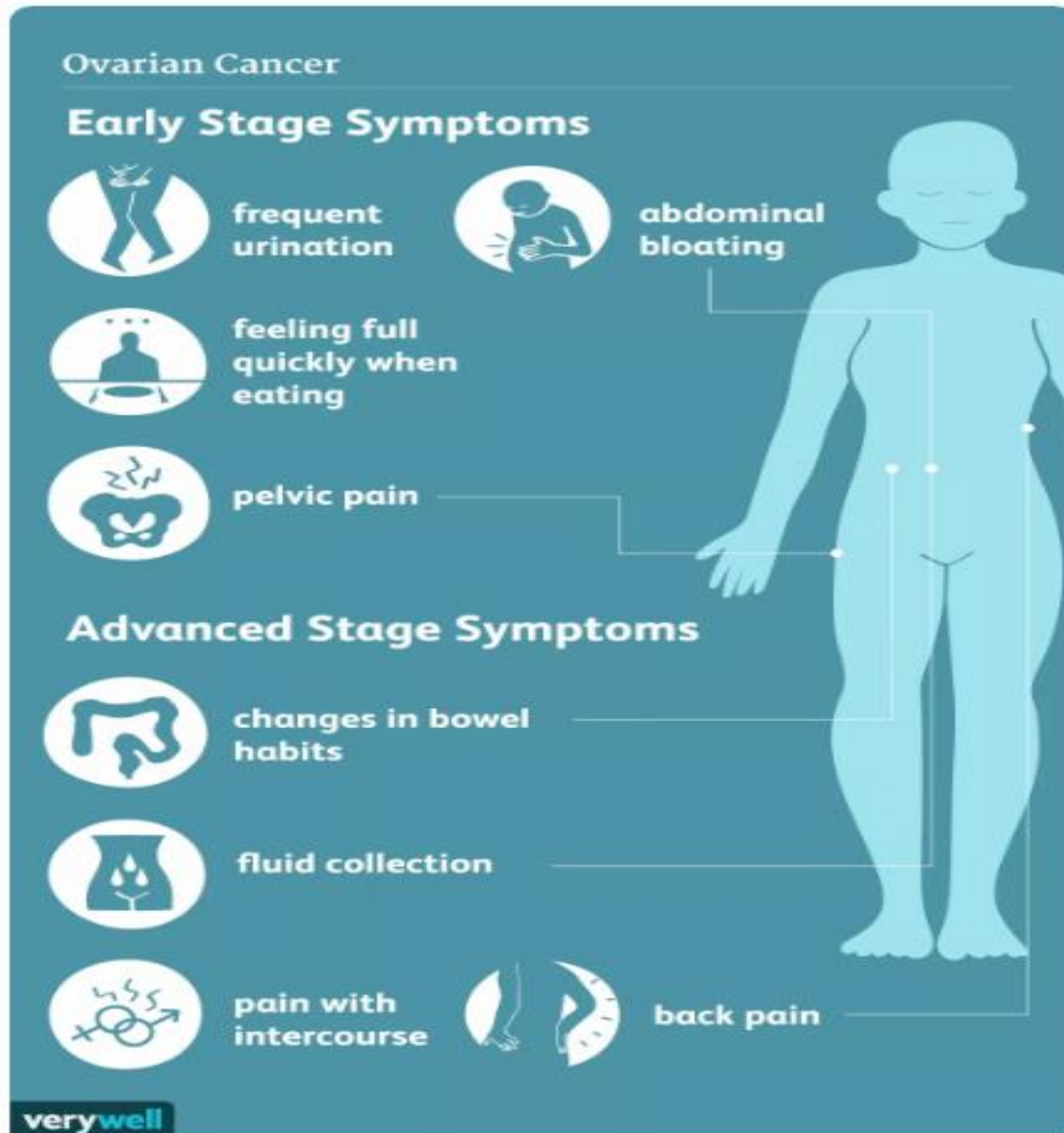


WISCONSIN
UNIVERSITY OF WISCONSIN-MADISON

What is ovarian cancer?



What are symptoms of ovarian cancer?



RAD51D is associated with ovarian cancer

2KZ3|A

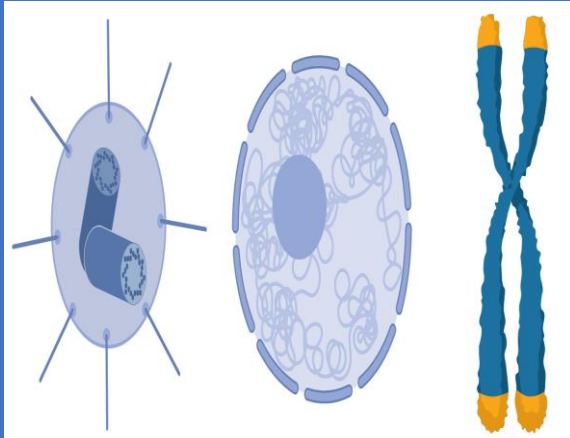
RAD51/AAA

328

ssDNA binding domain

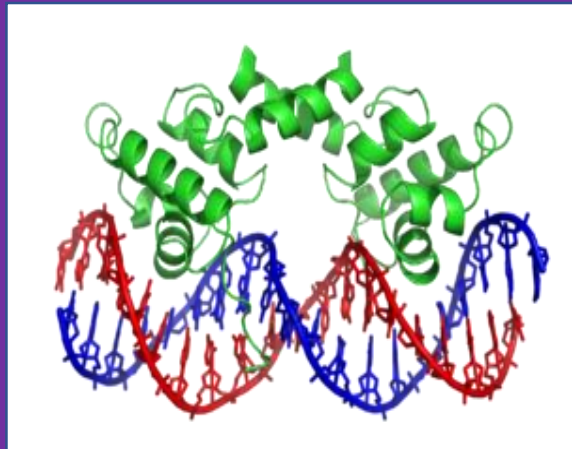
RAD51 family domain

Cellular Component



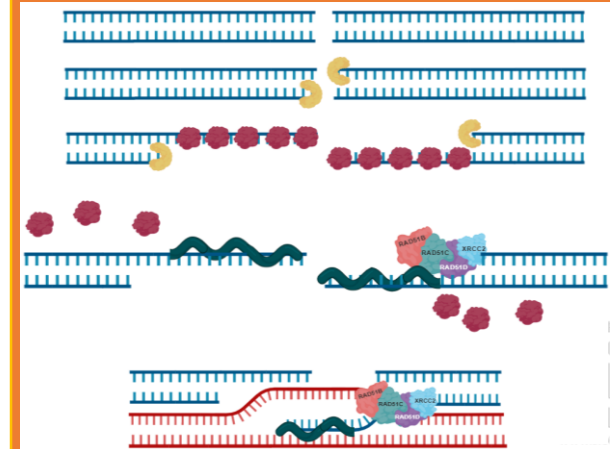
Centrosome, Nucleus,
Telomeres

Molecular Function



DNA Binding

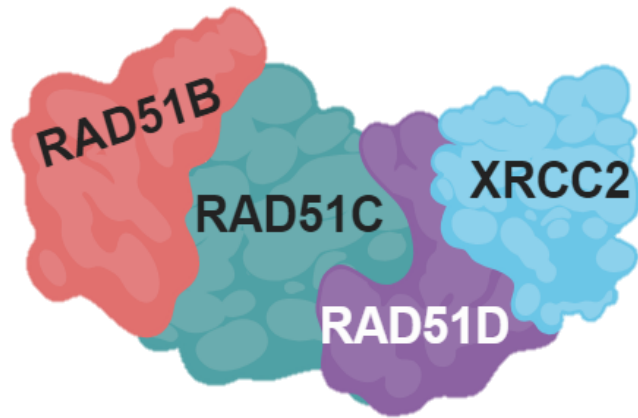
Biological Processes



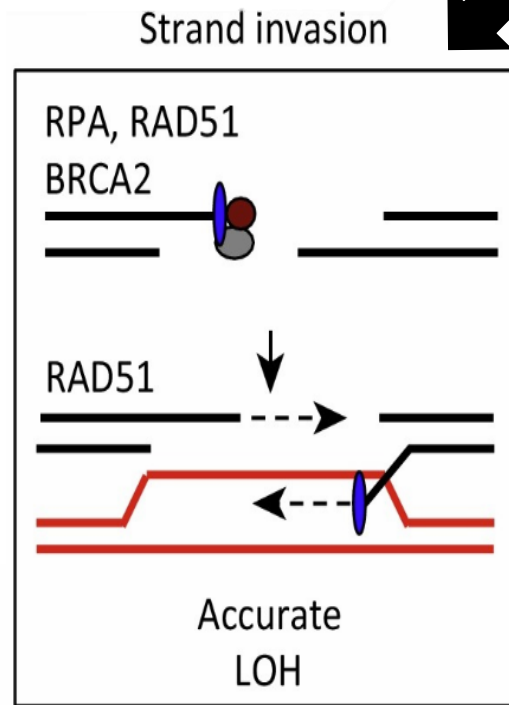
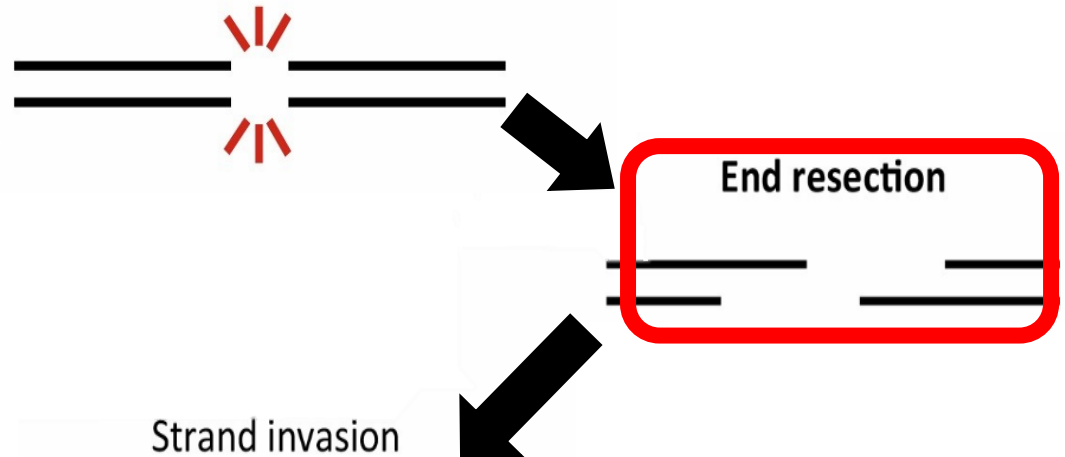
Homologous Recombination

RAD51D knockouts affect DNA repair

DNA double-strand break (DSB)



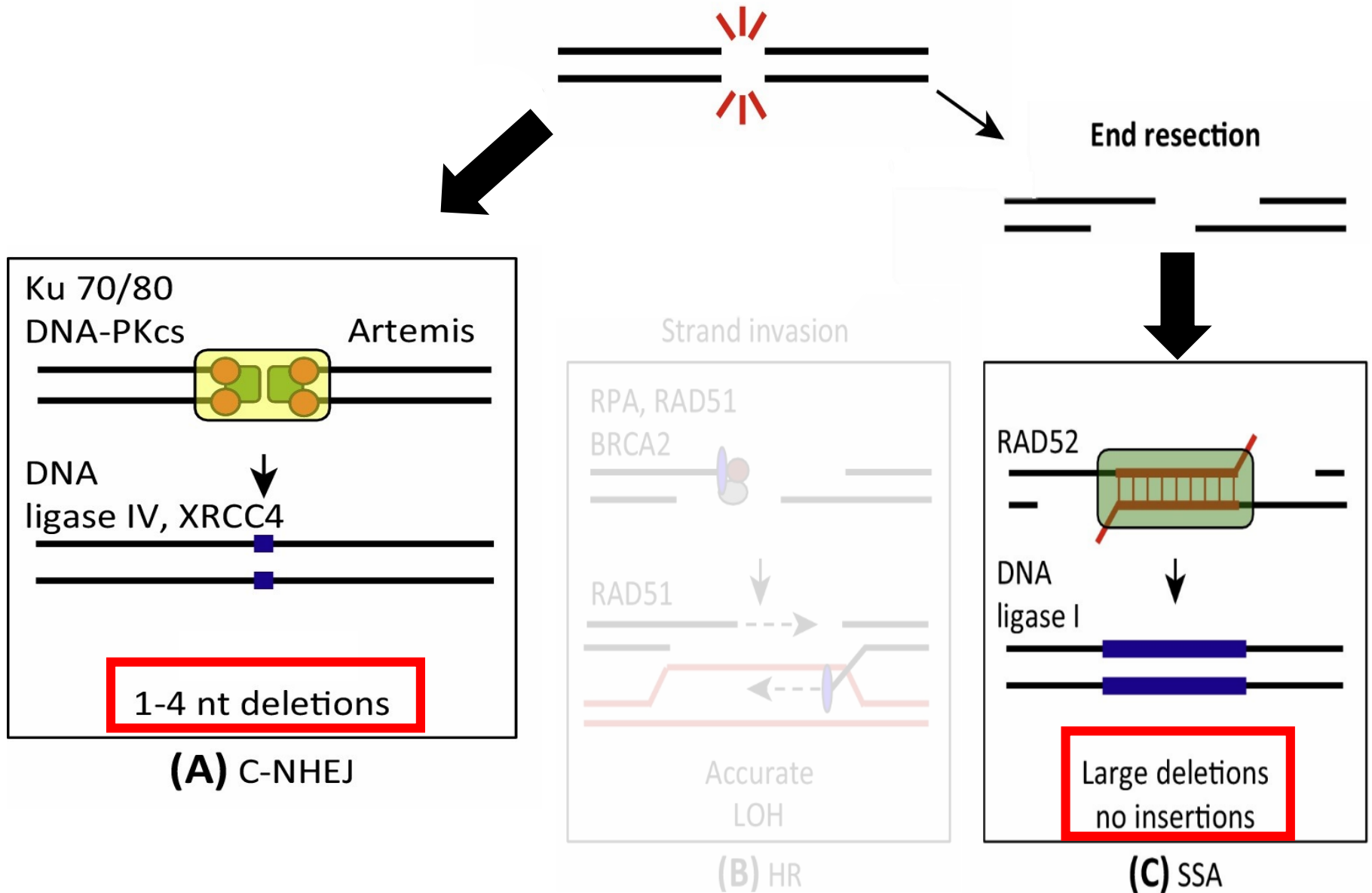
RAD51D is needed for accurate DNA repair



(B) HR

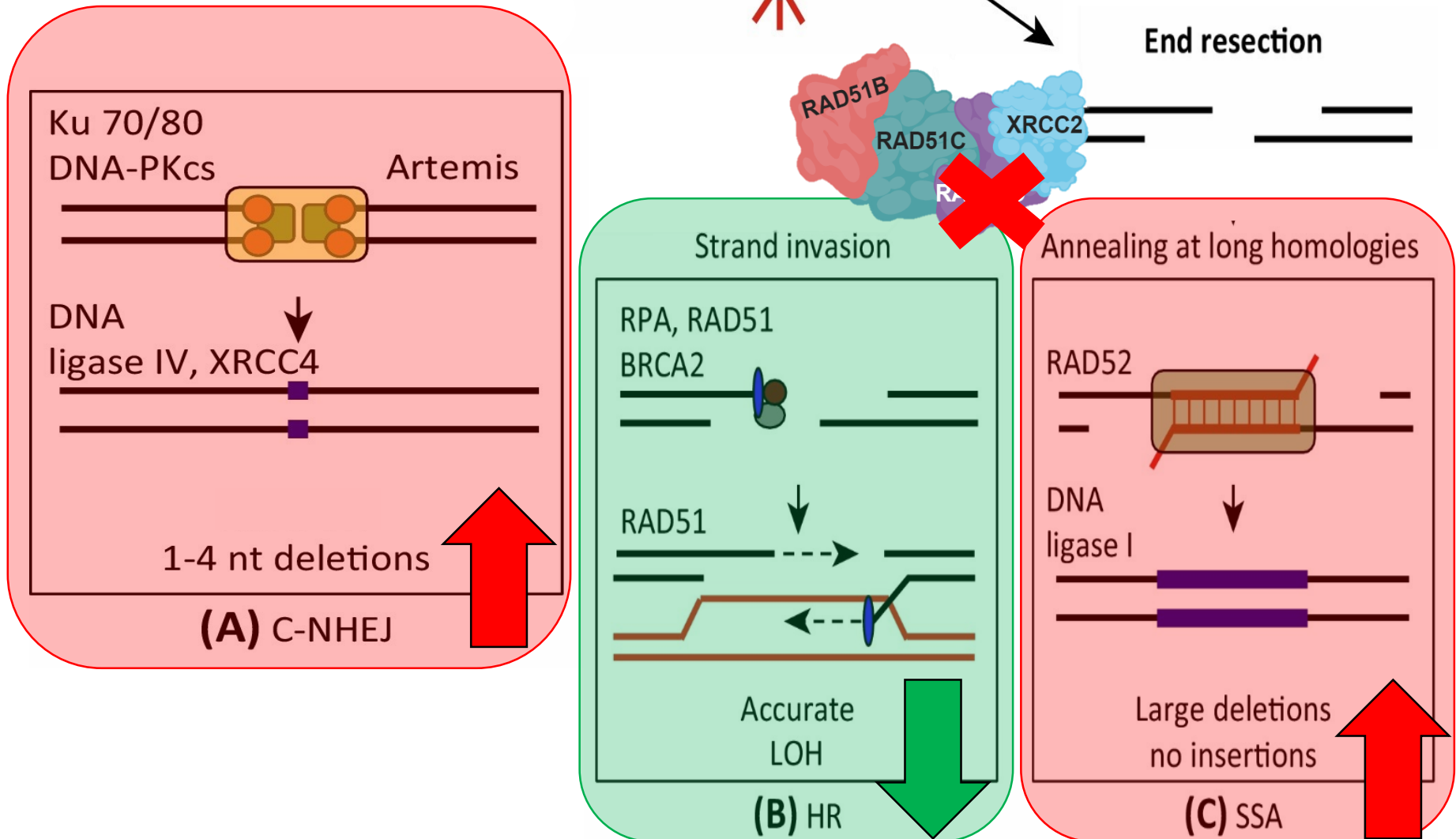
RAD51D knockouts affect DNA repair

DNA double-strand break (DSB)



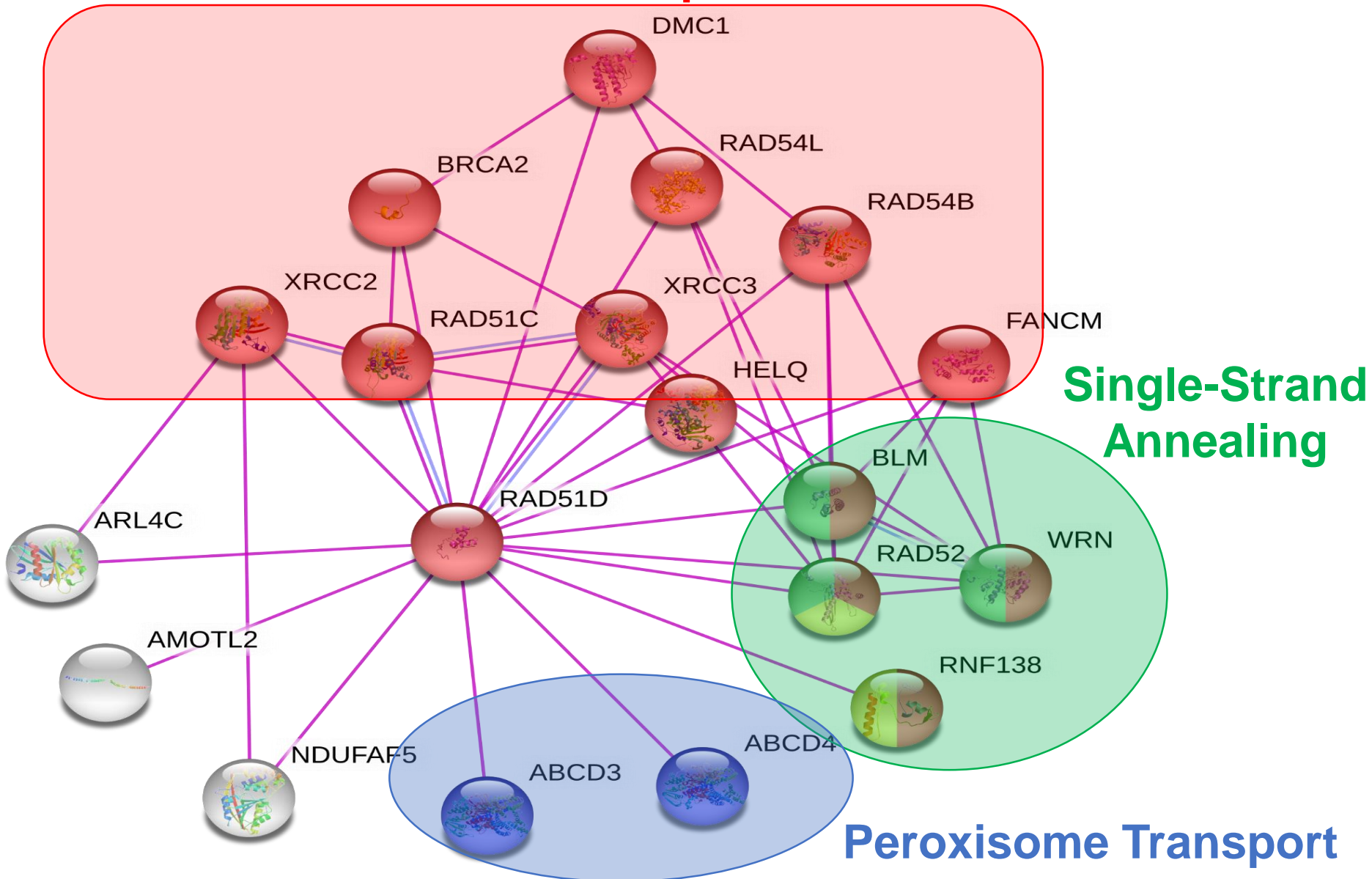
RAD51D knockouts affect DNA repair

DNA double-strand break (DSB)

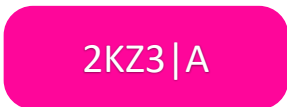


What proteins interact with *RAD51D*?

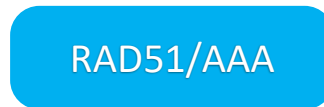
DNA Repair



The RAD51 domain is highly conserved

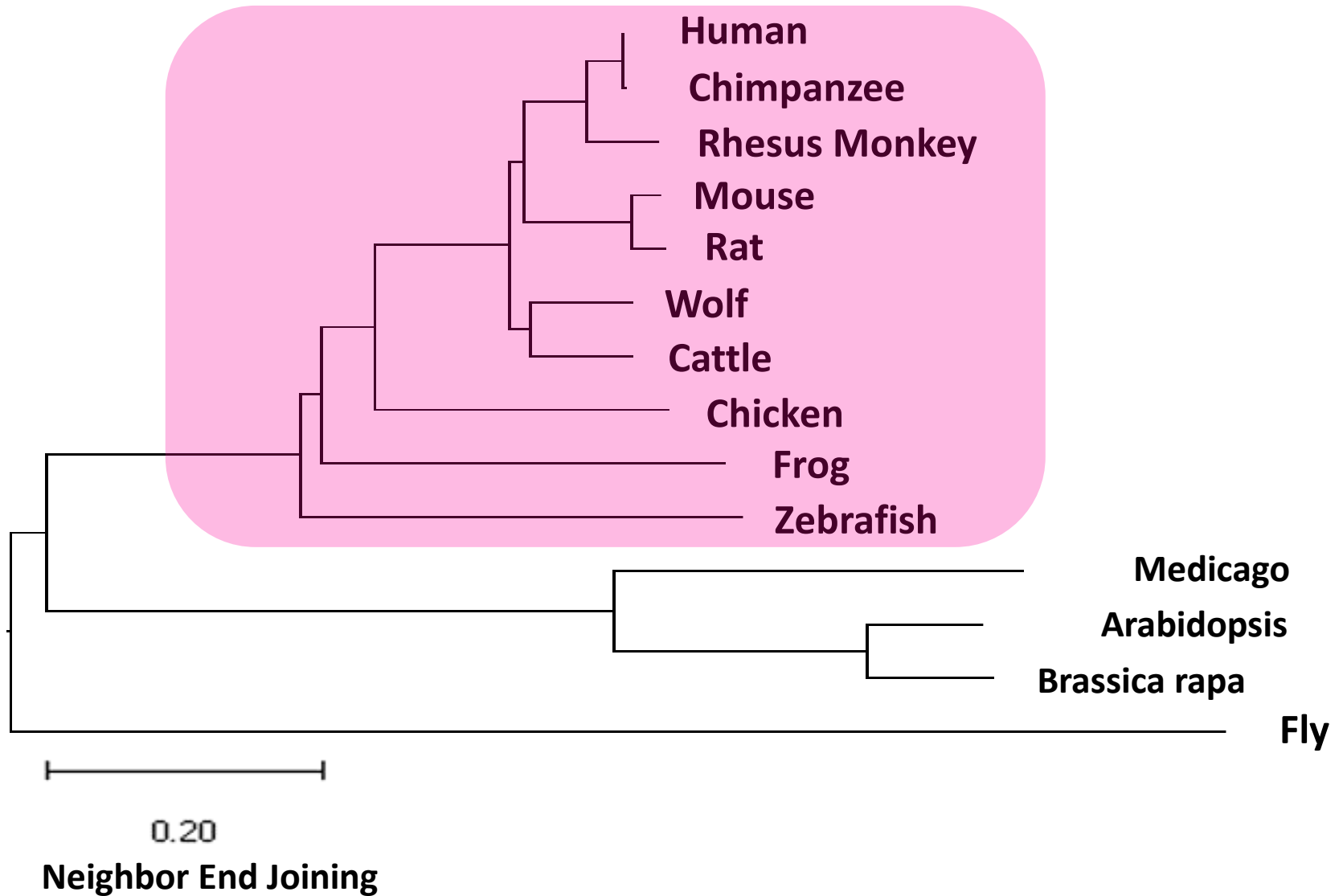


ssDNA binding domain

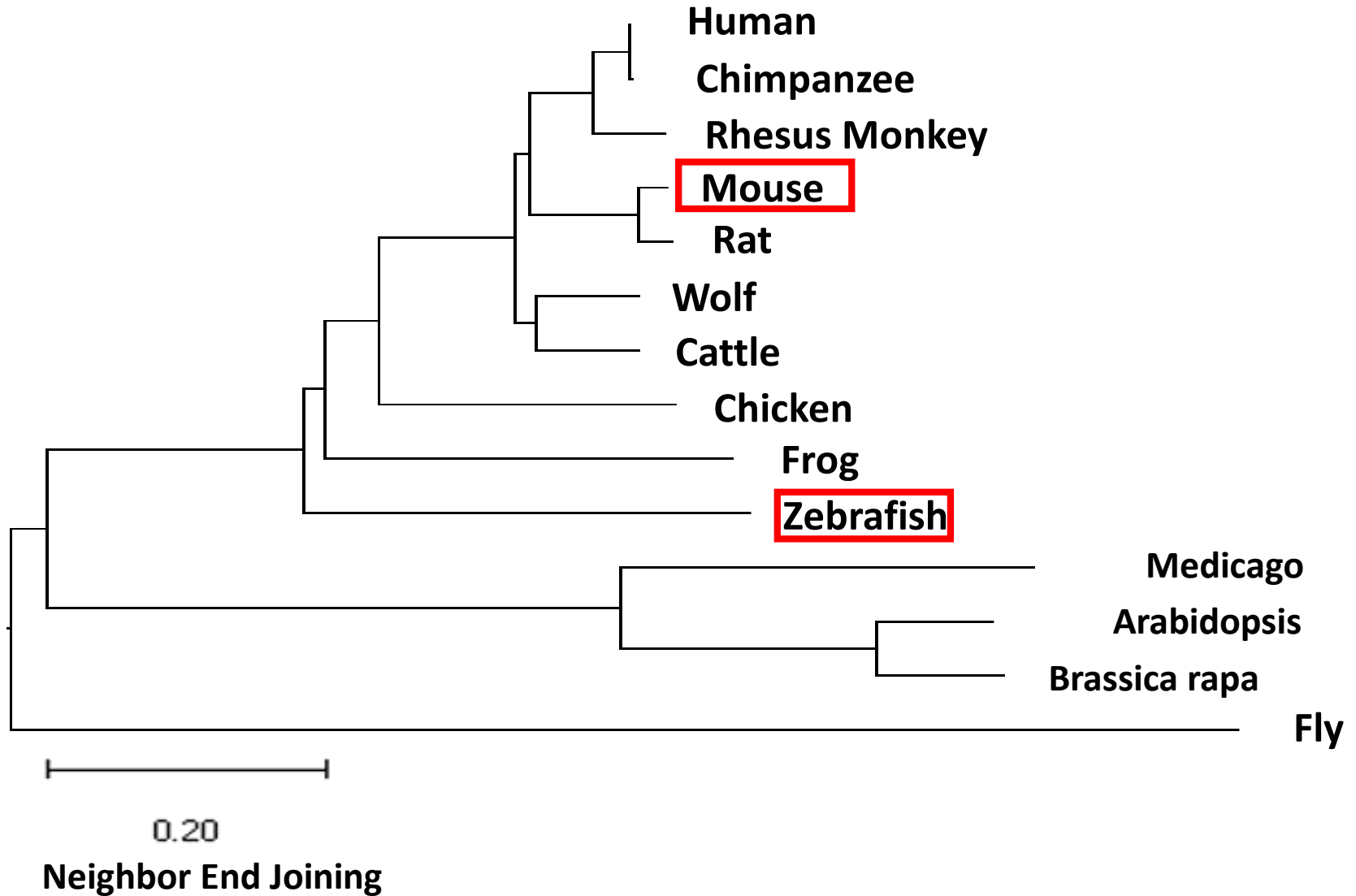


RAD51 C terminus Domain

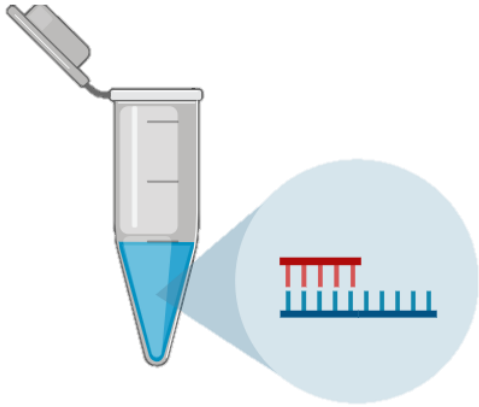
RAD51D's phylogeny is informative



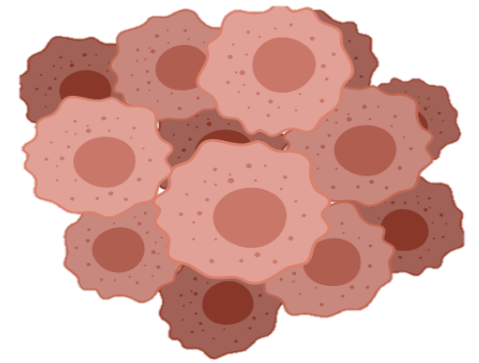
RAD51D's phylogeny is informative



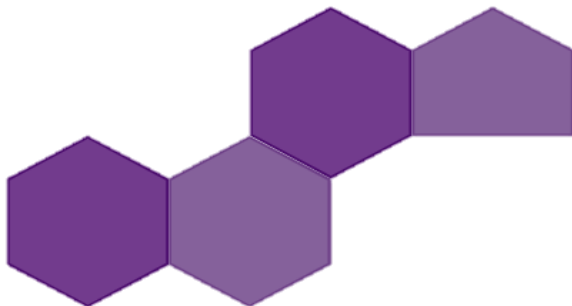
Model organism 1: Mus musculus



Genetic engineering



Xenografting tools

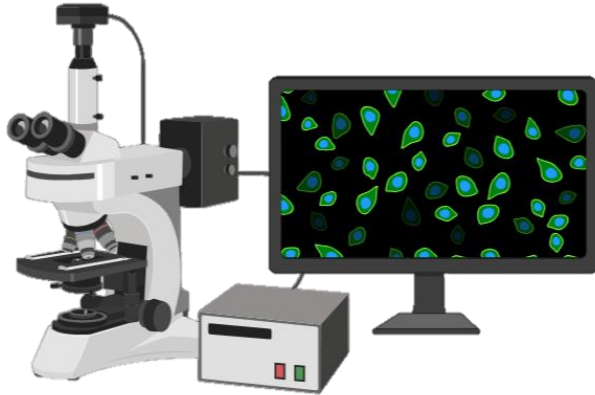


Similar Physiology

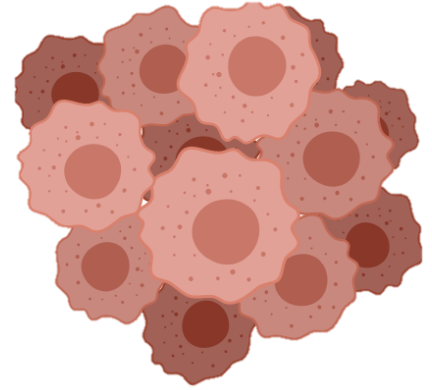


Similar ovarian microenvironment

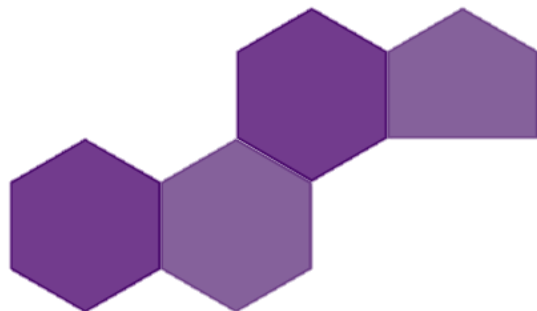
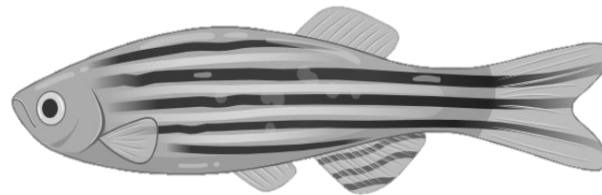
Model organism 2: Danio rerio



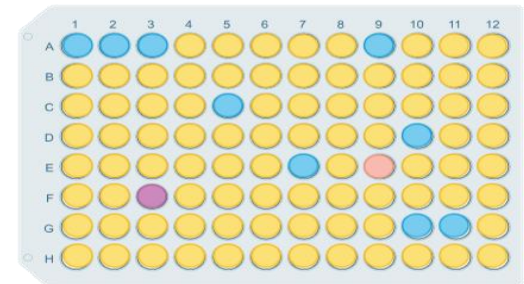
Fluorescent microscopy



Xenografting tools

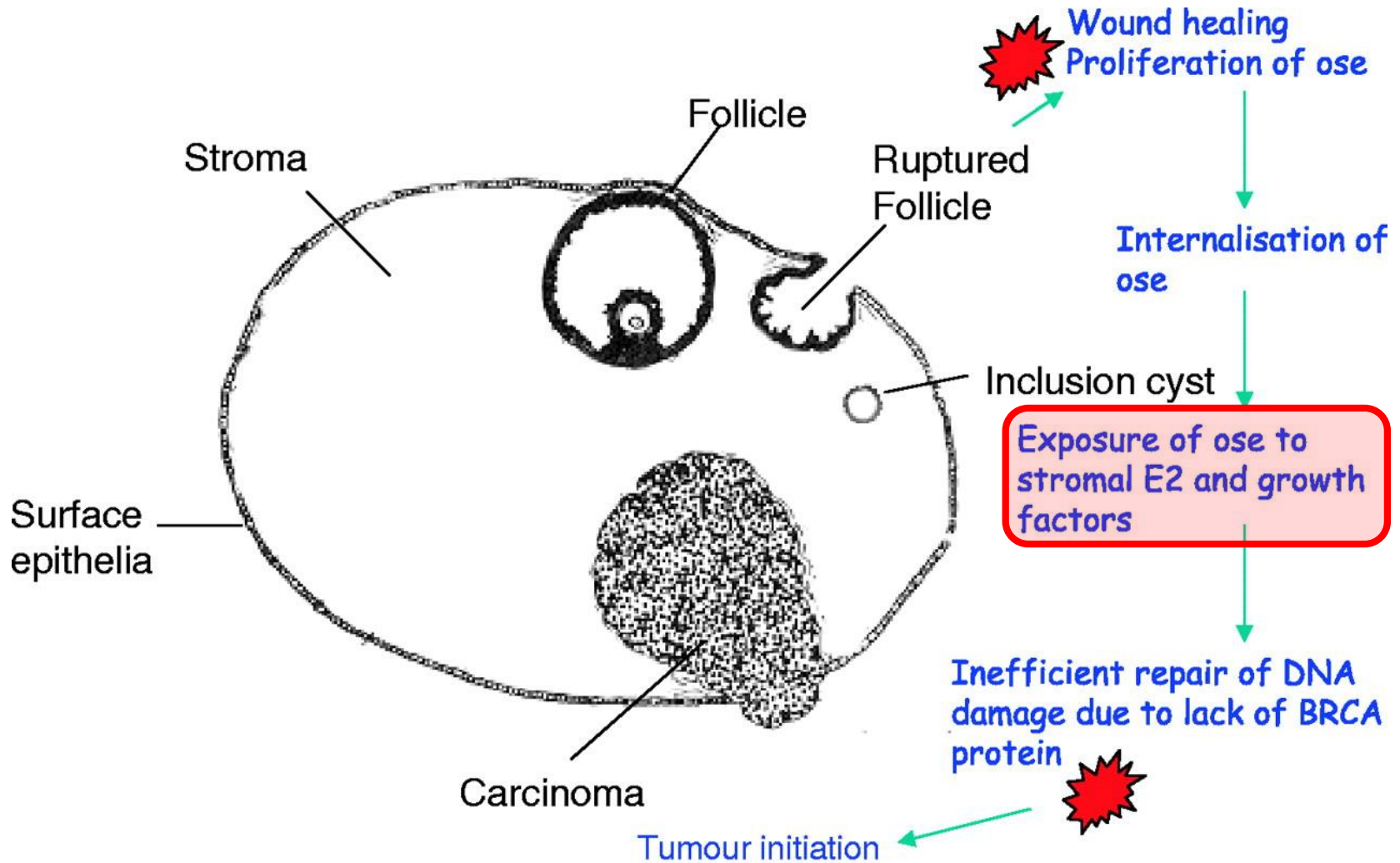


Steroid Hormone Receptors



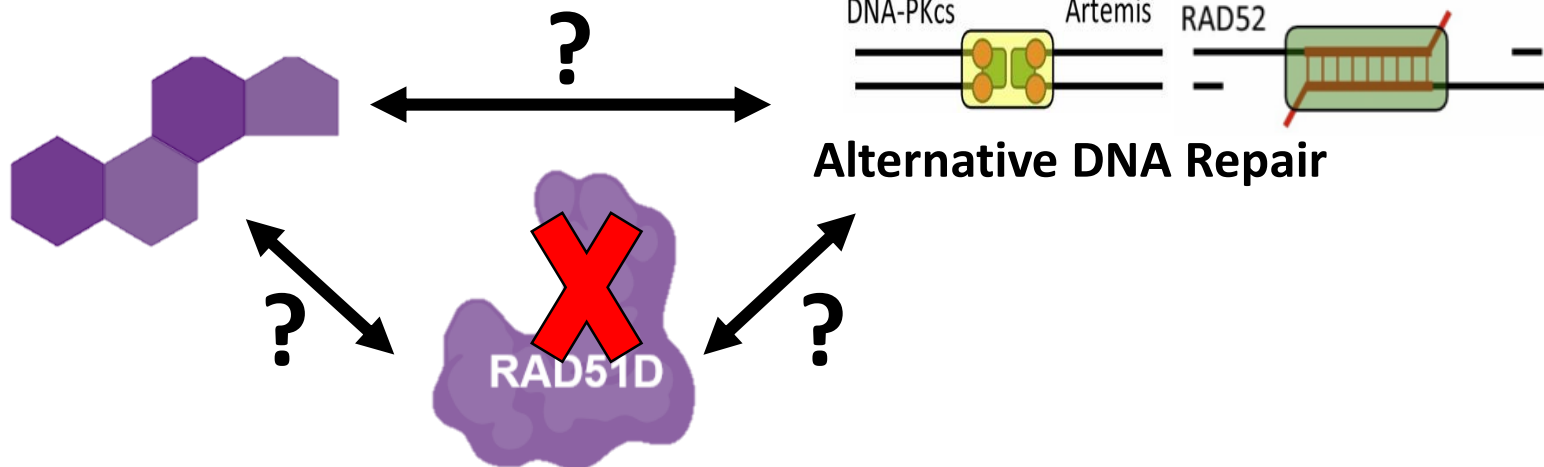
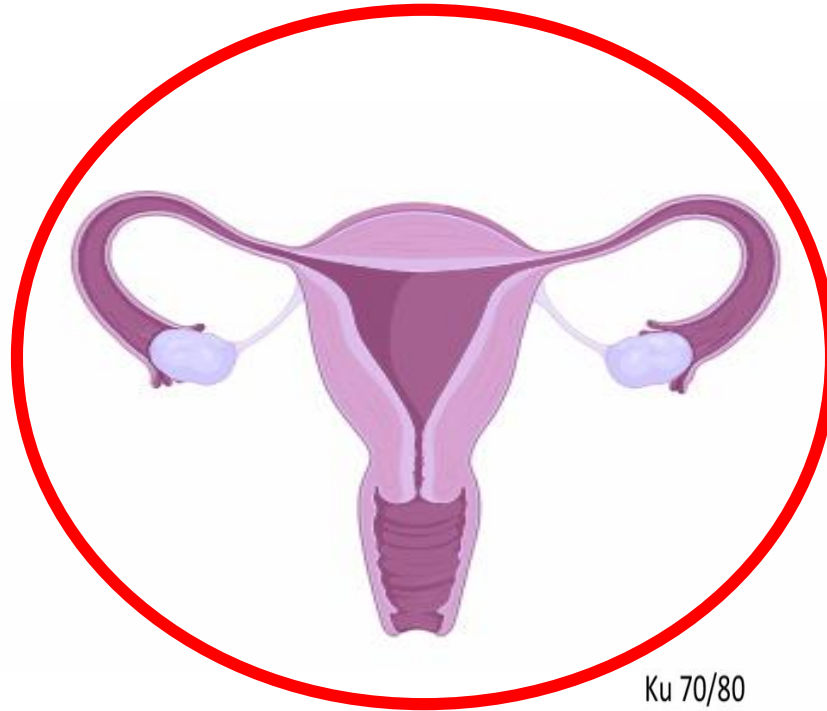
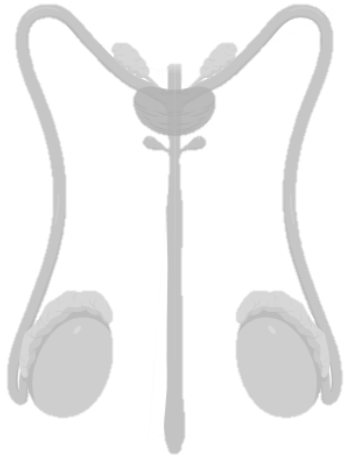
**Cheap and Fast
Genetic Screens**

Estrogen signaling damages DNA



 **Stages at which genetic damage can occur and accumulate**

RAD51D is a tissue-specific risk allele



What is the Primary Goal?



To better understand how the *RAD51D* protein regulates DNA repair in the ovarian microenvironment

Aim 1:

Characterize conserved domains and motifs in *RAD51D* that are crucial for genomic integrity in the ovaries.

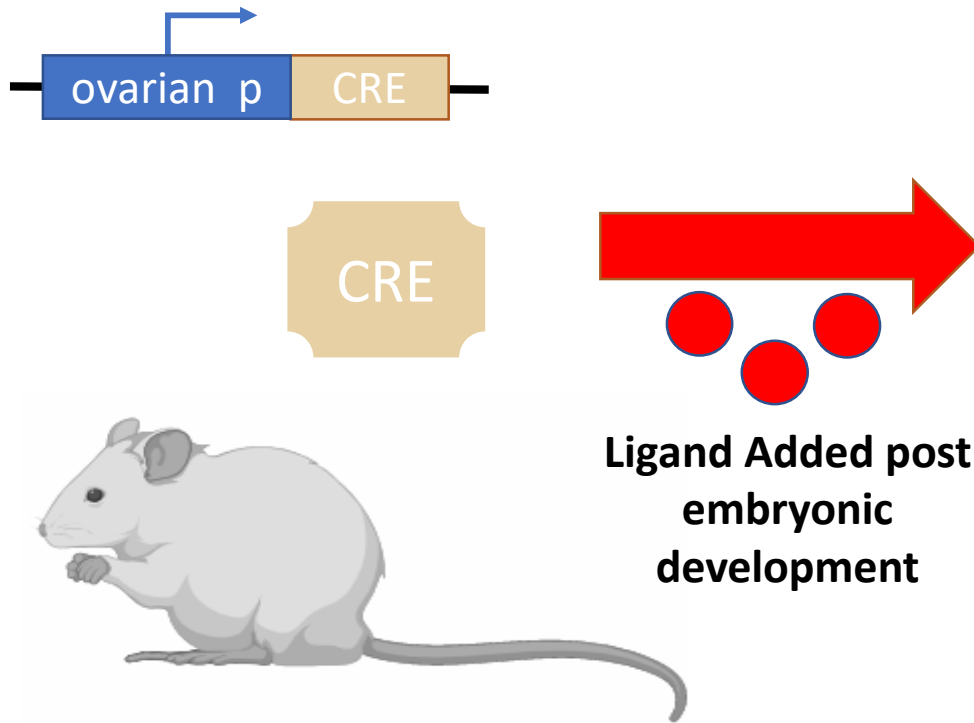
Aim 2:

Identify hormones that alter ovarian genomic integrity in both WT and mutant *RAD51D*

Aim 3:

Identify how changes in *RAD51D*'s structure alters DNA repair regulation

Aim 1: Characterize regions of RAD51D essential for ovarian genetic integrity

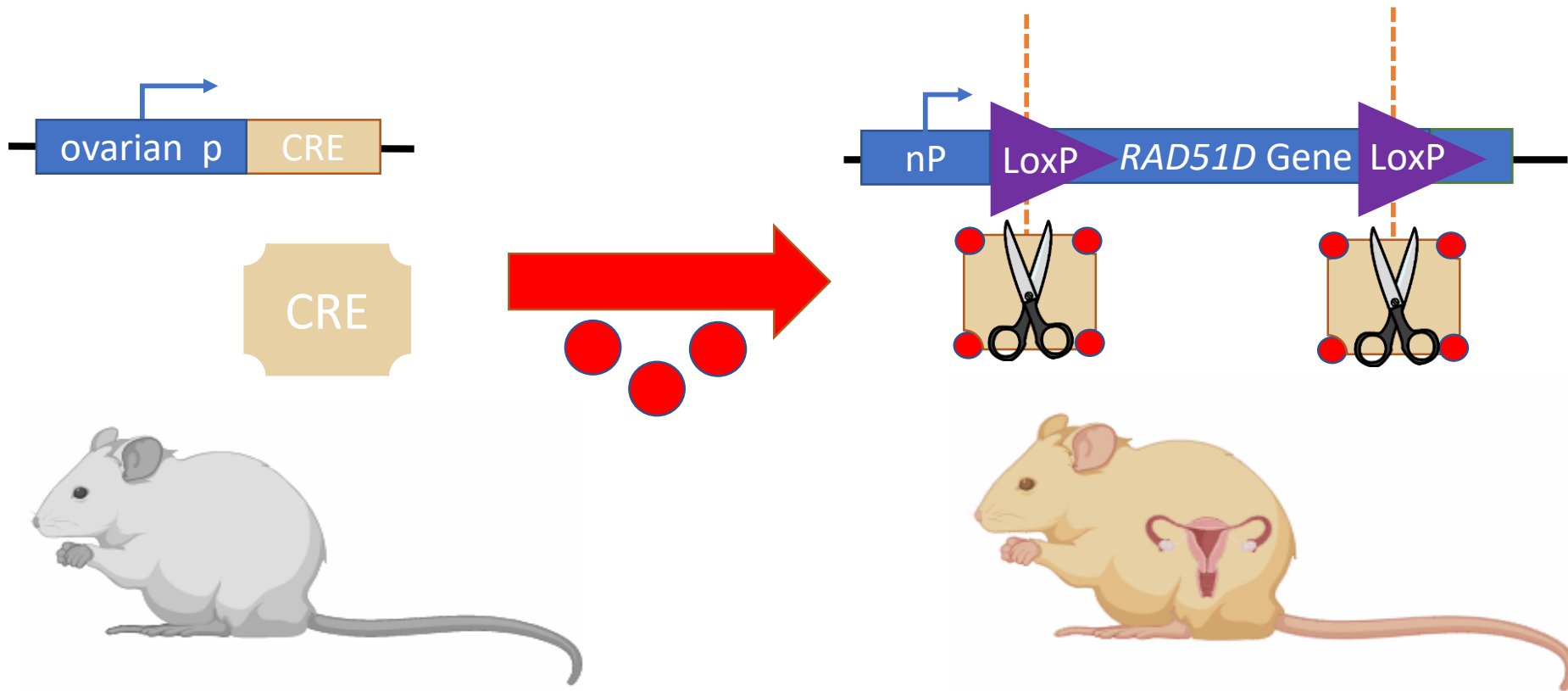


Clustal W

Cre - Loxp

Assess DNA Repair

Aim 1: Characterize regions of RAD51D essential for ovarian genetic integrity



Clustal W

Cre - Loxp

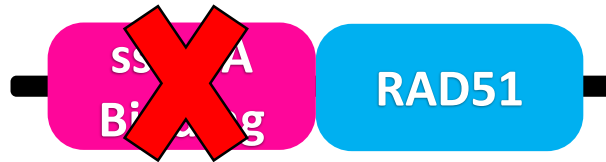
Assess DNA Repair

Aim 1: Characterize regions of RAD51D essential for ovarian genetic integrity

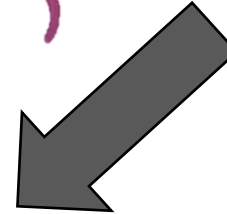
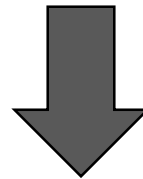
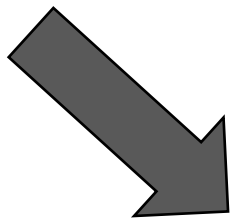
Wild Type
Normal Binding



Mutant N:
No ssDNA Binding



Mutant C:
C terminus truncation



Sequence tumors using
Next-Gen sequencing

Clustal W

Cre - Loxp

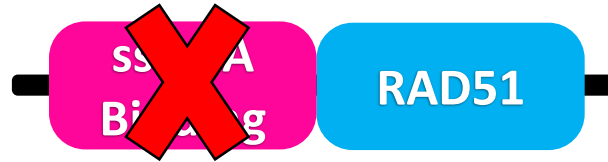
Assess DNA Repair

Aim 1: Characterize regions of RAD51D essential for ovarian genetic integrity

Wild Type
Normal Binding



Mutant N:
No ssDNA Binding



Mutant C:
C terminus truncation



Align results to reference genome to identify deletions

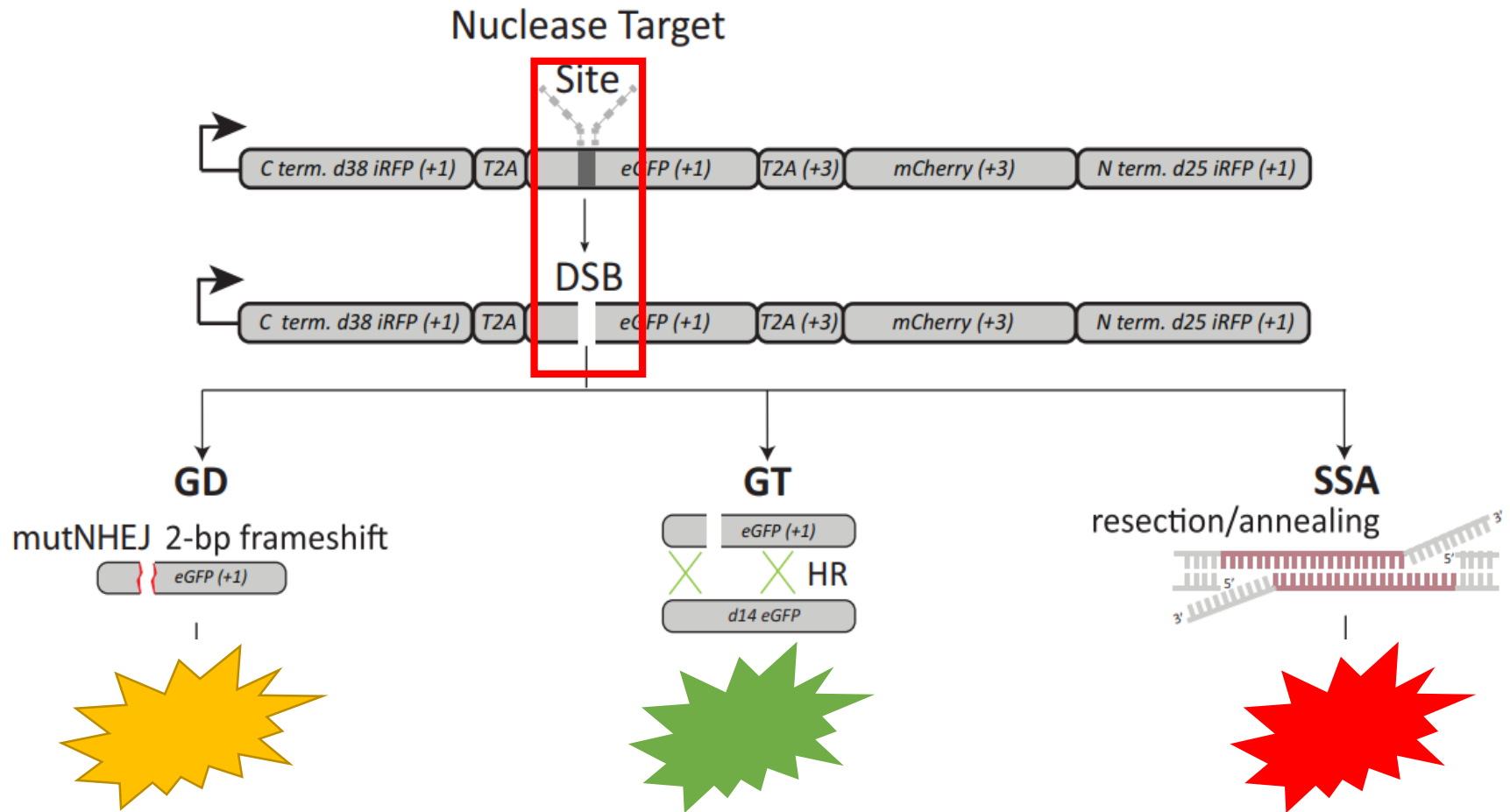
Hypothesis: Disrupting conserved regions will result in increased chromosomal deletions

Clustal W

Cre - Loxp

Assess DNA Repair

Aim 2: Identify hormones that affect ovarian genetic integrity

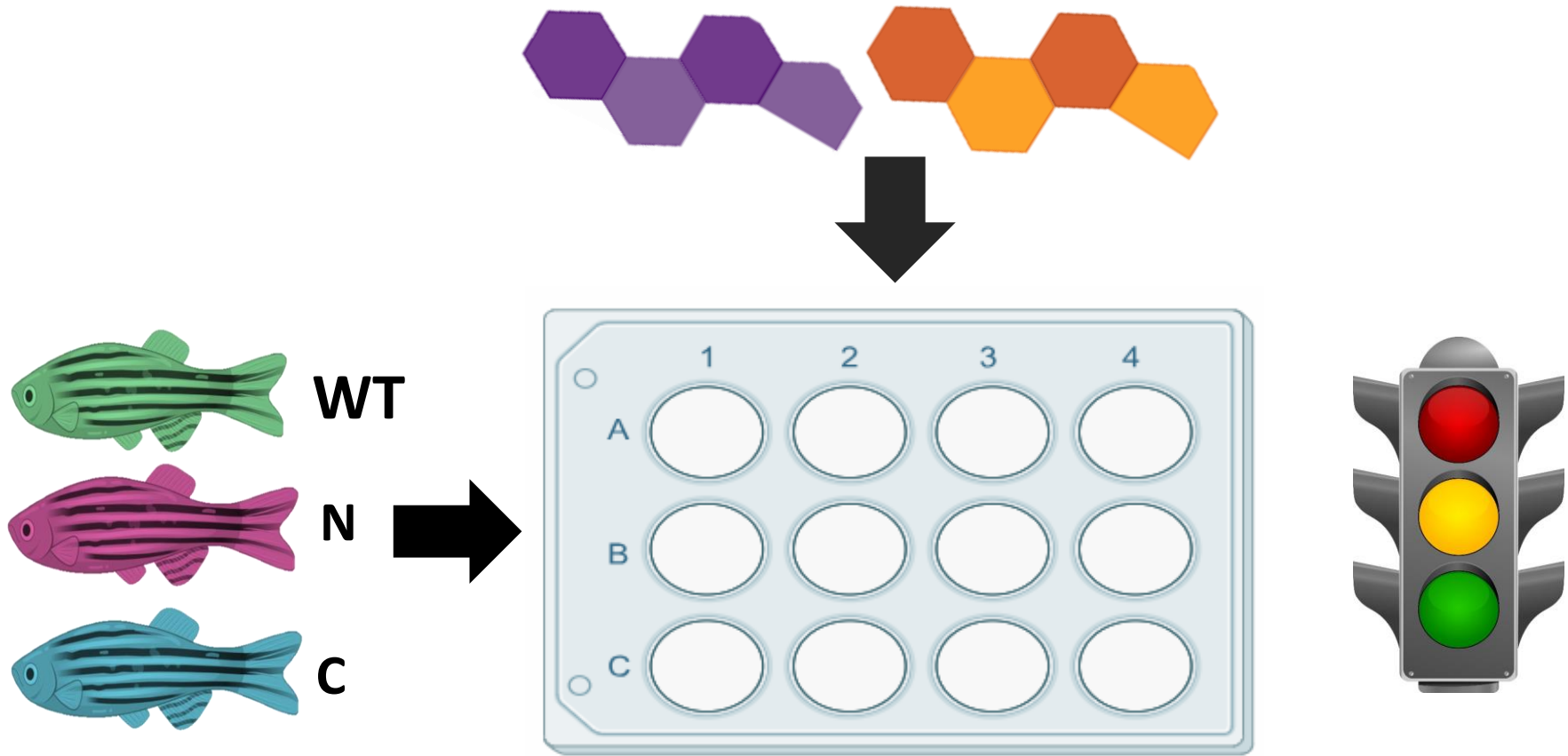


Clone Reporter

Chemical
Genomic Screen

Assess DNA Repair

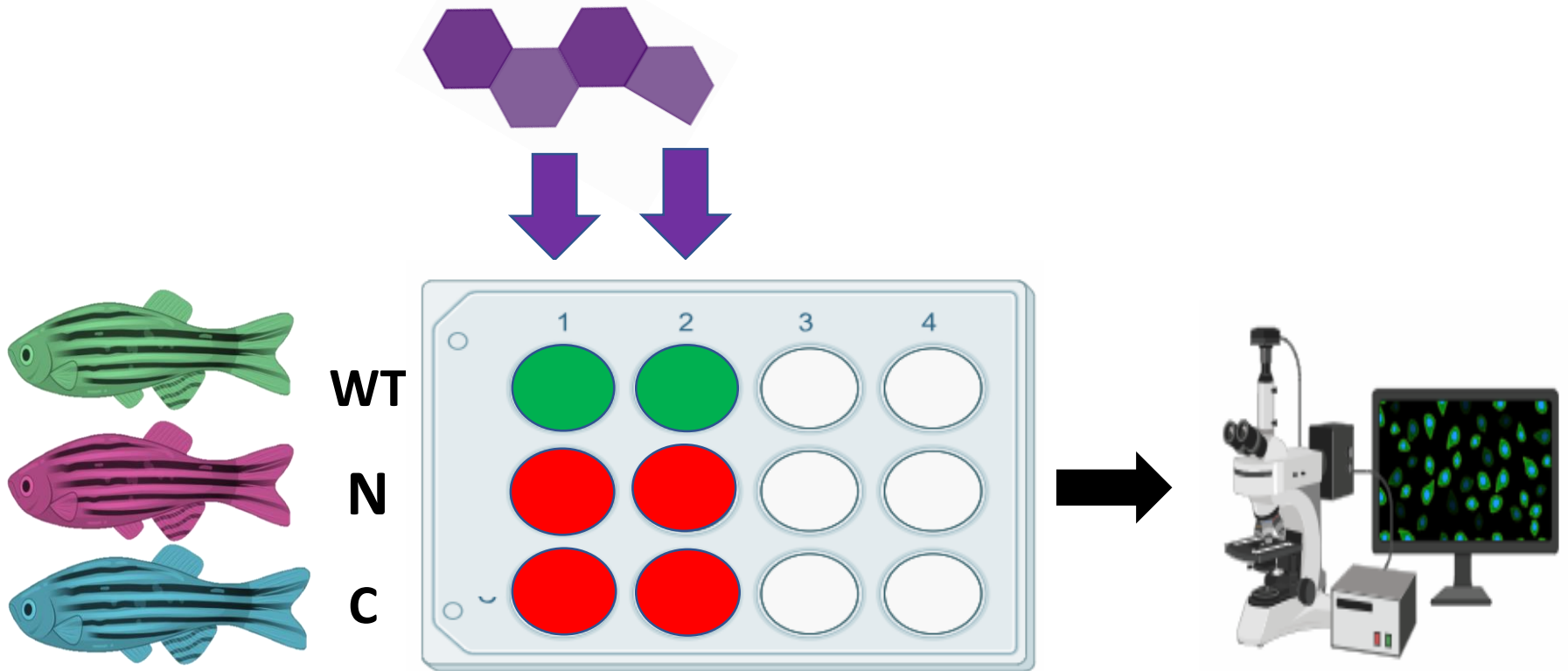
Aim 2: Identify hormones that affect ovarian genetic integrity



Chemical Genomic Screen

Assess DNA Repair

Aim 2: Identify hormones that affect ovarian genetic integrity

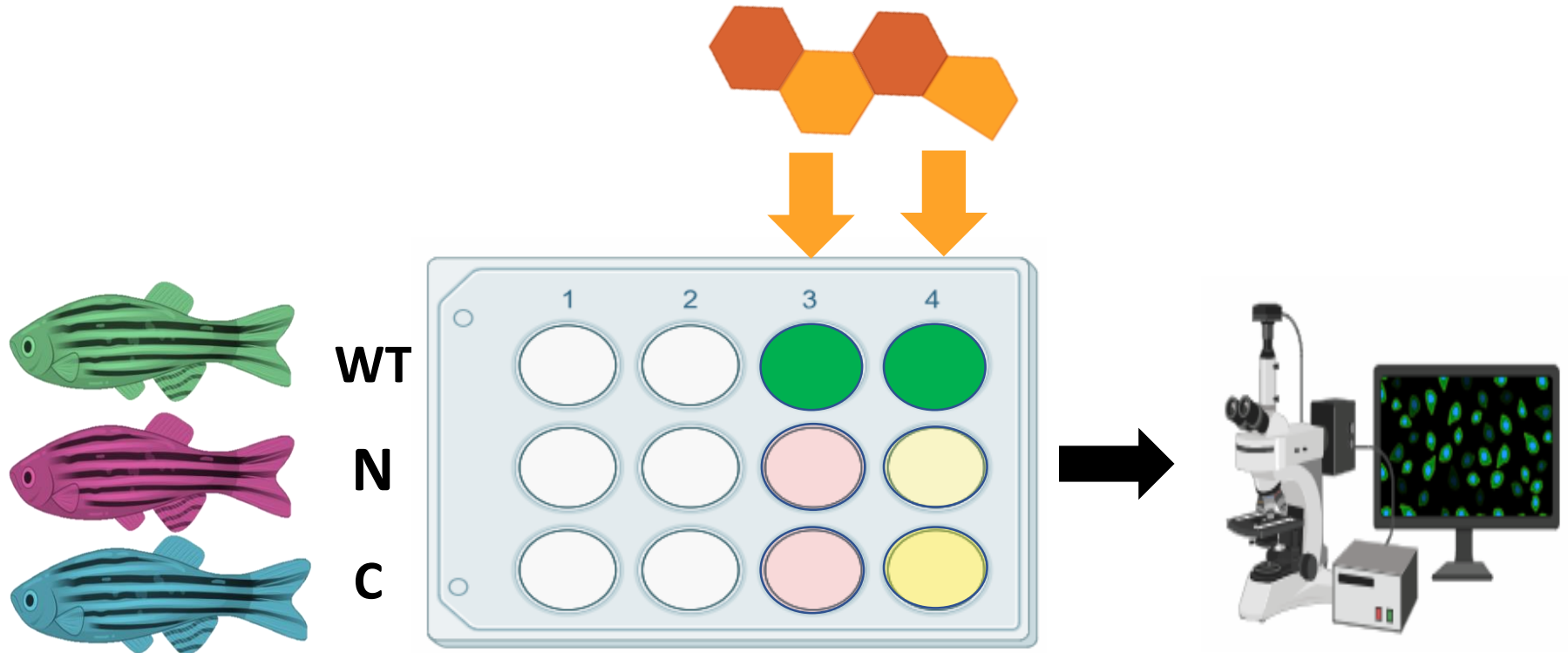


Hypothesis: Estrogen and growth factors will lead to an increase use of large deletion prone pathways in the *RAD51D* mutants

Chemical Genomic Screen

Assess DNA Repair

Aim 2: Identify hormones that affect ovarian genetic integrity

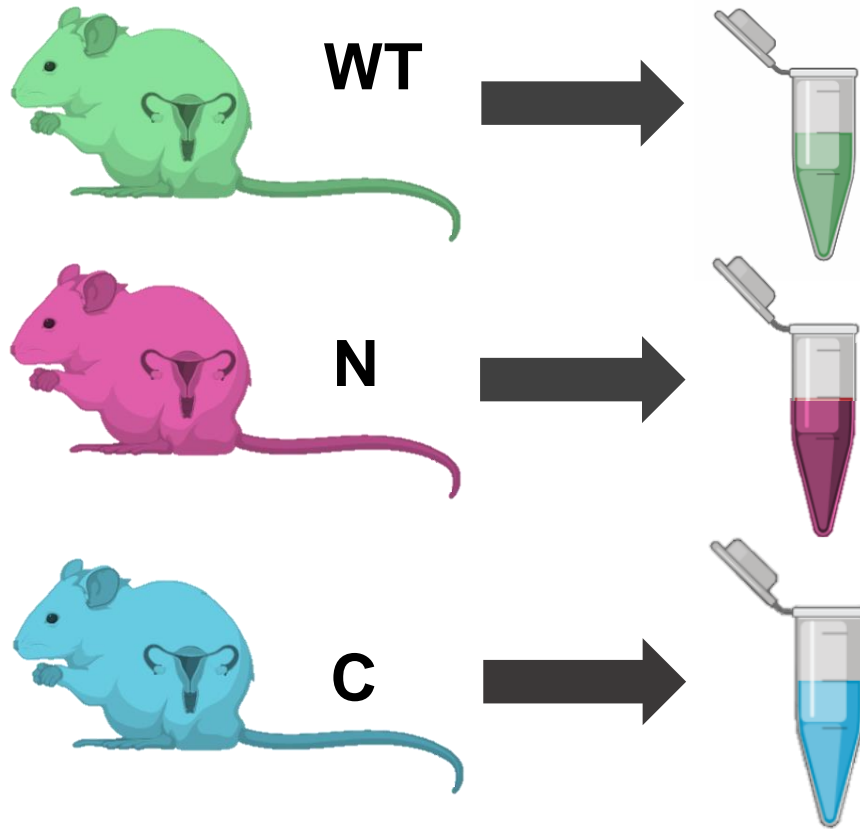


Hypothesis: Some ovarian hormones, such as progesterone, will not significantly affect DNA repair.

Chemical Genomic Screen

Assess DNA Repair

Aim 3: Identify changes in protein-protein binding interactions in *RAD51D* mutants



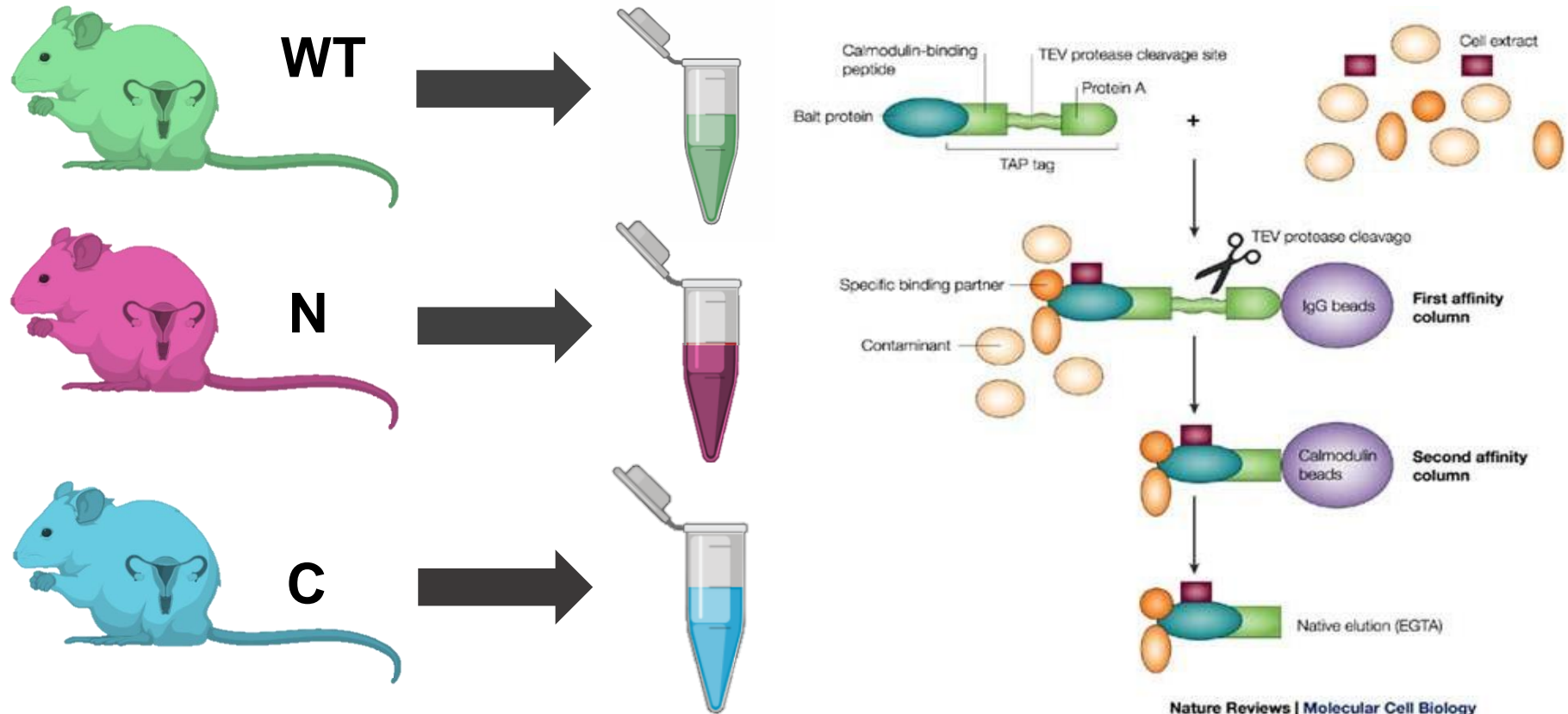
Hypothesis: Disrupting the N and C terminus of RAD51D will alter its ability to bind targets that regulate DNA repair

Isolate Proteins

Tandem-Affinity

Mass-Spec

Aim 3: Identify changes in protein-protein binding interactions in *RAD51D* mutants



Hypothesis: Disrupting the N and C terminus of *RAD51D* will alter its ability to bind targets that regulate DNA repair

Isolate Proteins

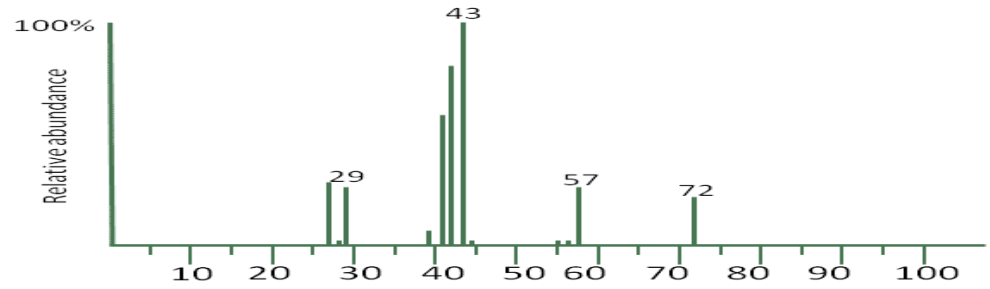
Tandem-Affinity

Mass-Spec

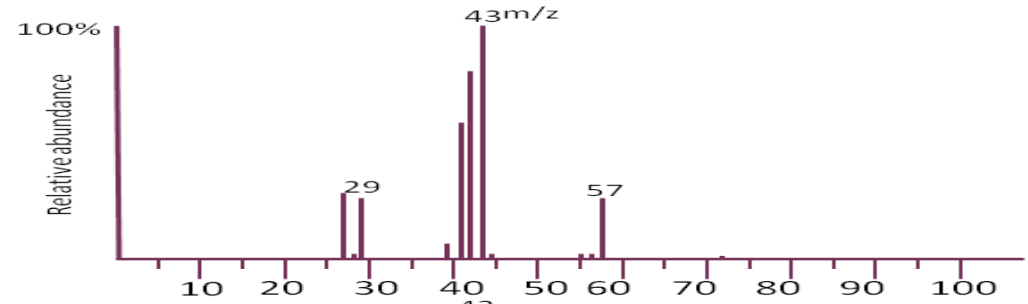
Aim 3: Identify changes in protein-protein binding interactions in *RAD51D* mutants



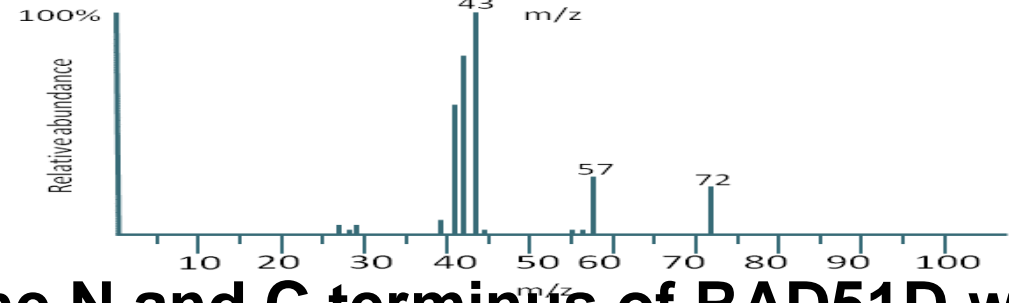
WT



N



C



Hypothesis: Disrupting the N and C terminus of RAD51D will alter its ability to bind targets that regulate DNA repair

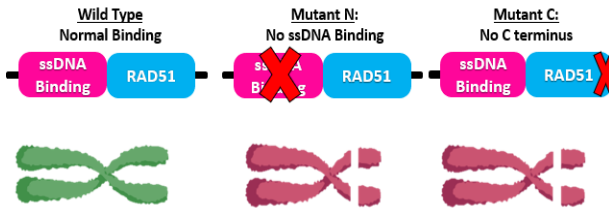
Isolate Proteins

Tandem-Affinity

Mass-Spec

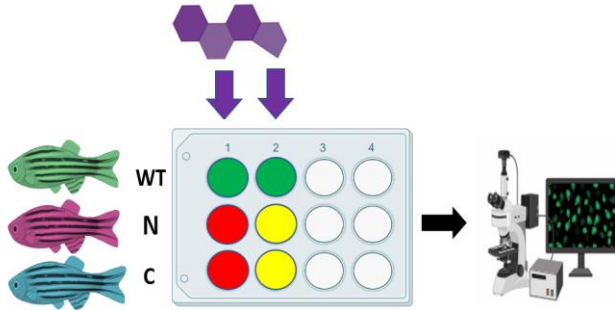
Summary

Aim 1: Characterize conserved domains essential for ovarian integrity

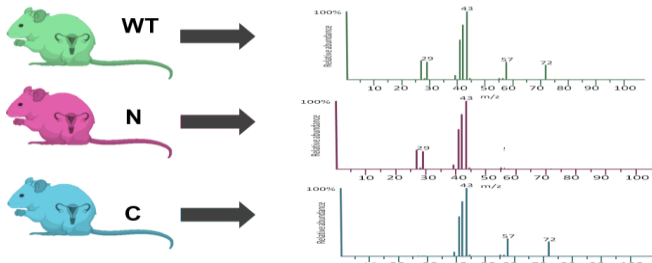


Align results to reference genome to identify deletions

Aim 2: Identify small molecules that affect ovarian genetic integrity



Aim 3: Identify changes in protein-protein binding interactions in *RAD51D* mutants



Identify conserved amino acids that are important for ovarian genetic integrity

Identify how different hormones alter DNA repair in *RAD51D* mutants

Identify how the C/N termini regulate DNA repair cross talk through protein-protein interactions in the ovaries

Future Directions

Wild Type
Normal Binding

Mutant N:
No ssDNA Binding

Mutant C:
No C terminus

ssDNA
Binding

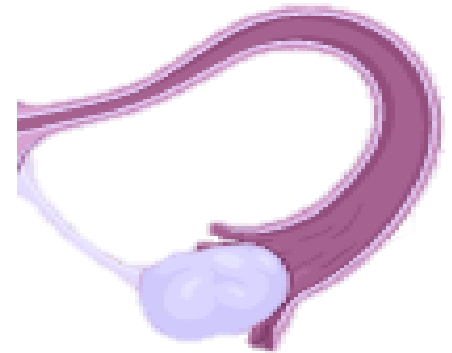
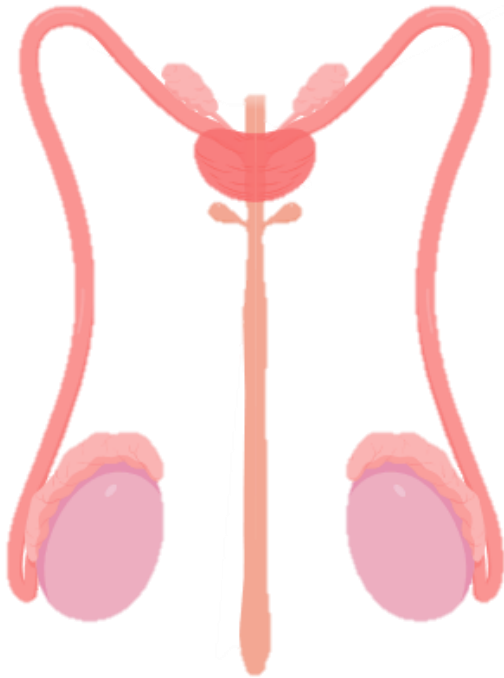
RAD51

~~ssDNA
Binding~~

RAD51

ssDNA
Binding

~~RAD51~~



Investigate the effect of mutant N/C in other reproductive tissues to determine if there are other *RAD51D* tissue specific phenotypes



Questions?

References

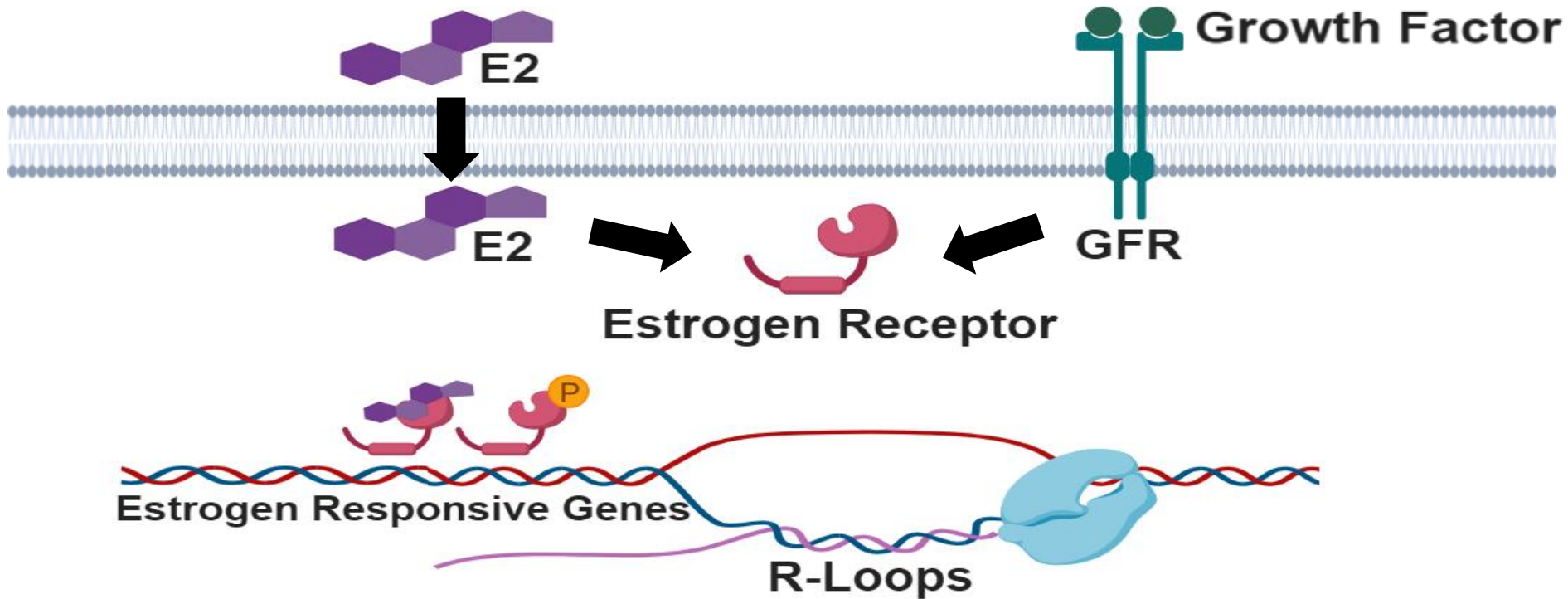
1. American Cancer Society. Key Statistics for Ovarian Cancer (2018, April 11). Retrieved from <https://www.cancer.org/cancer/ovarian-cancer/about/key-statistics.html>
2. Lisa Fayed. Very Well Health. Symptoms of Ovarian Cancer. Retrieved from <https://www.verywellhealth.com/ovarian-cancer-causes-risk-factors-2509671>
3. Reh, W. A., Nairn, R. S., Lowery, M. P., & Vasquez, K. M. (2016). The homologous recombination protein RAD51D protects the genome from large deletions. *Nucleic acids research*, 45(4), 1835–1847. doi:10.1093/nar/gkw1204
4. Heidi, S., Alan., (2005) *BRCA1* and *BRCA2* as ovarian cancer susceptibility genes. *Carcinogenesis*, 26(10), 1651-1656. <https://doi.org/10.1093/carcin/bgi136>
5. Kuhar, R., Gwiazda, K. S., Humbert, O., Mandt, T., Pangallo, J., Brault, M., ... Certo, M. T. (2013). Novel fluorescent genome editing reporters for monitoring DNA repair pathway utilization at endonuclease-induced breaks. *Nucleic acids research*, 42(1), e4. doi:10.1093/nar/gkt872

References - Images

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16. Double Strand Break: <https://innovativegenomics.org/resources/educational-materials/glossary/double-strand-break/>
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- 22: https://www.researchgate.net/publication/257754595_Novel_fluorescent_genome_editing_reporters_for_monitoring_DNA_repair_pathway_utilization_at_endonuclease-induced_breaks
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24. Biorender: <https://biorender.com/>
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28. Biorender: <https://biorender.com/> , Mass Spec: <https://bitesizebio.com/6016/how-does-mass-spec-work/>
30. Biorender: <https://biorender.com/>

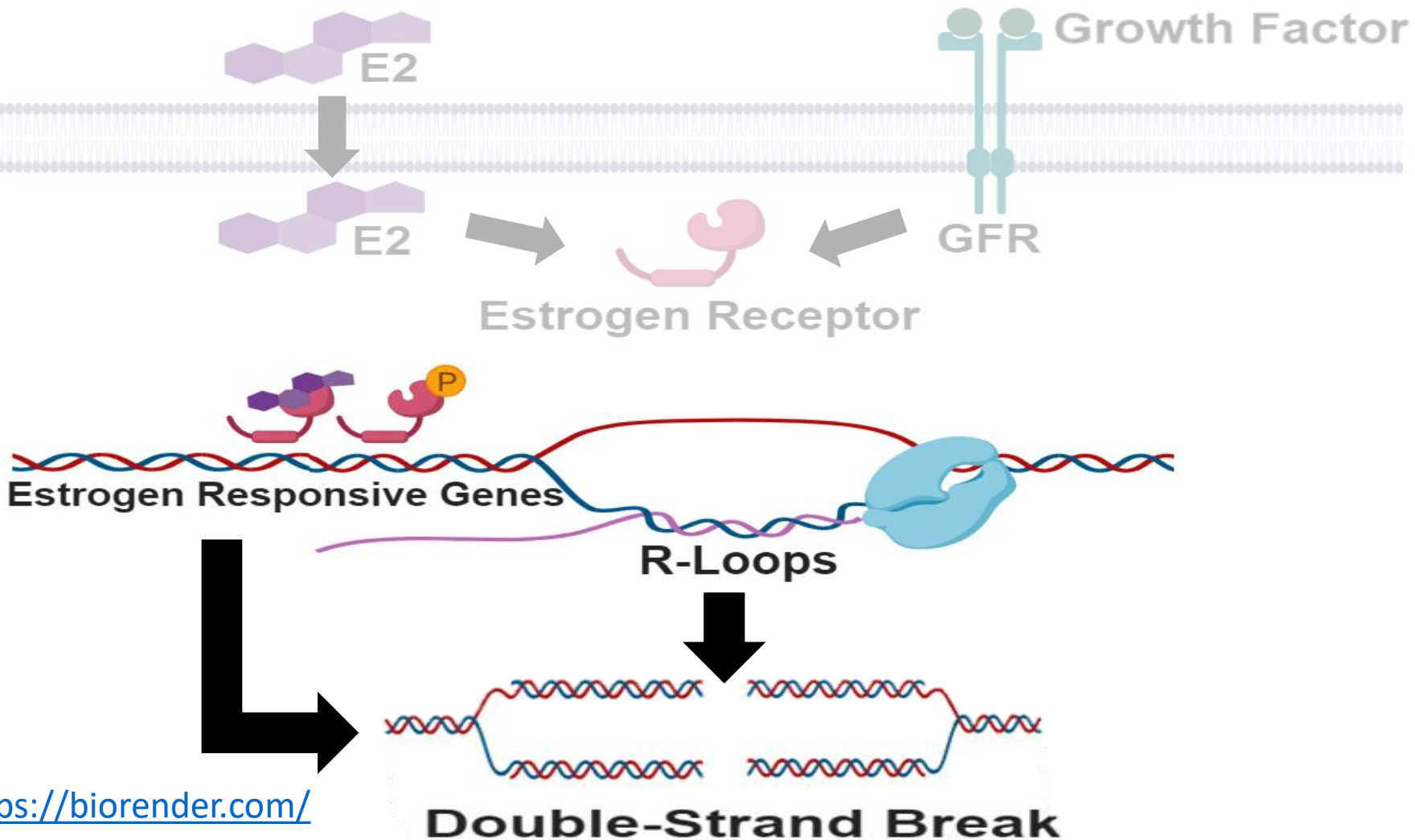
Supplemental Slides

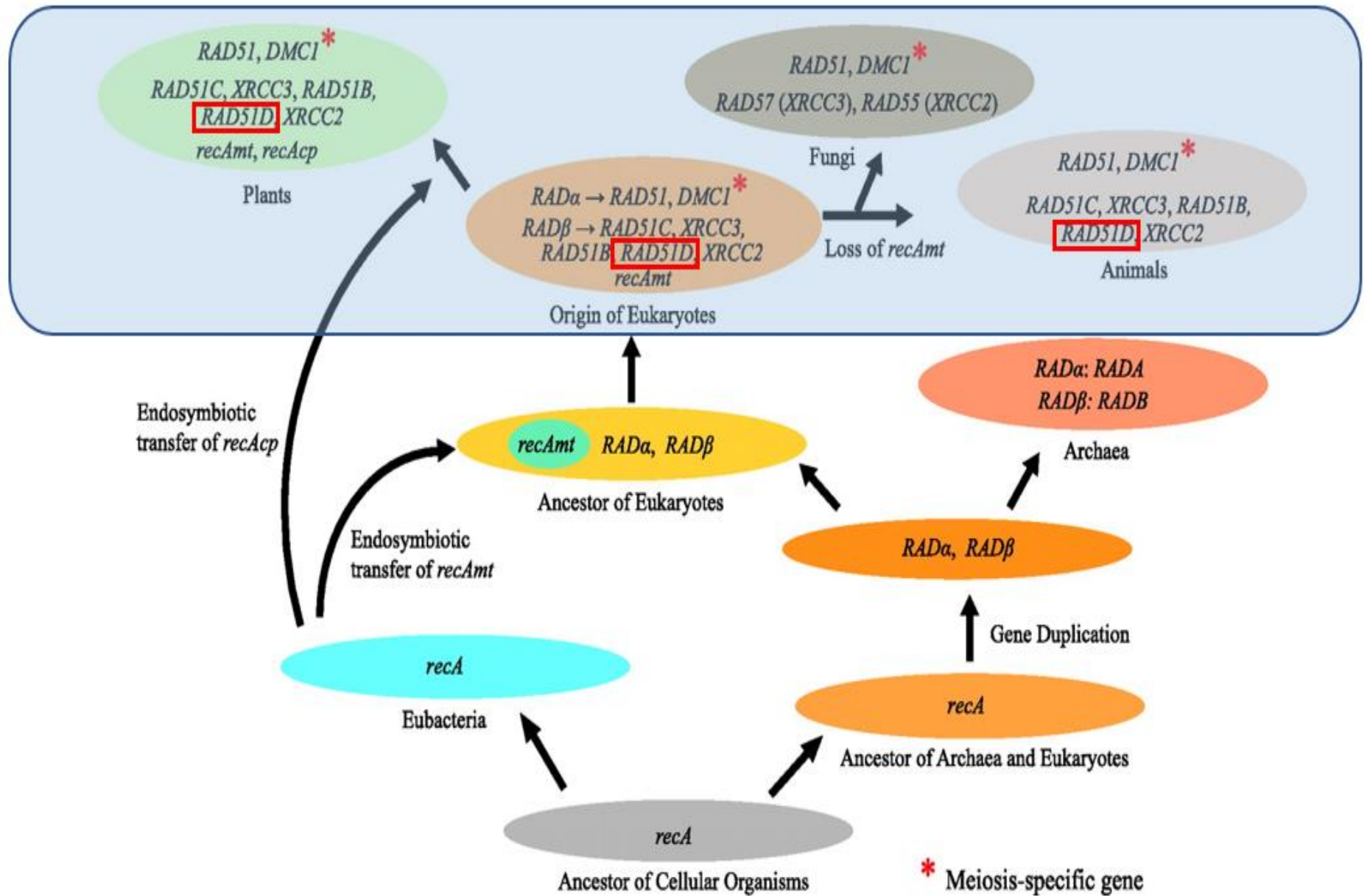
Estrogen signaling induces DNA damage



<https://biorender.com/>

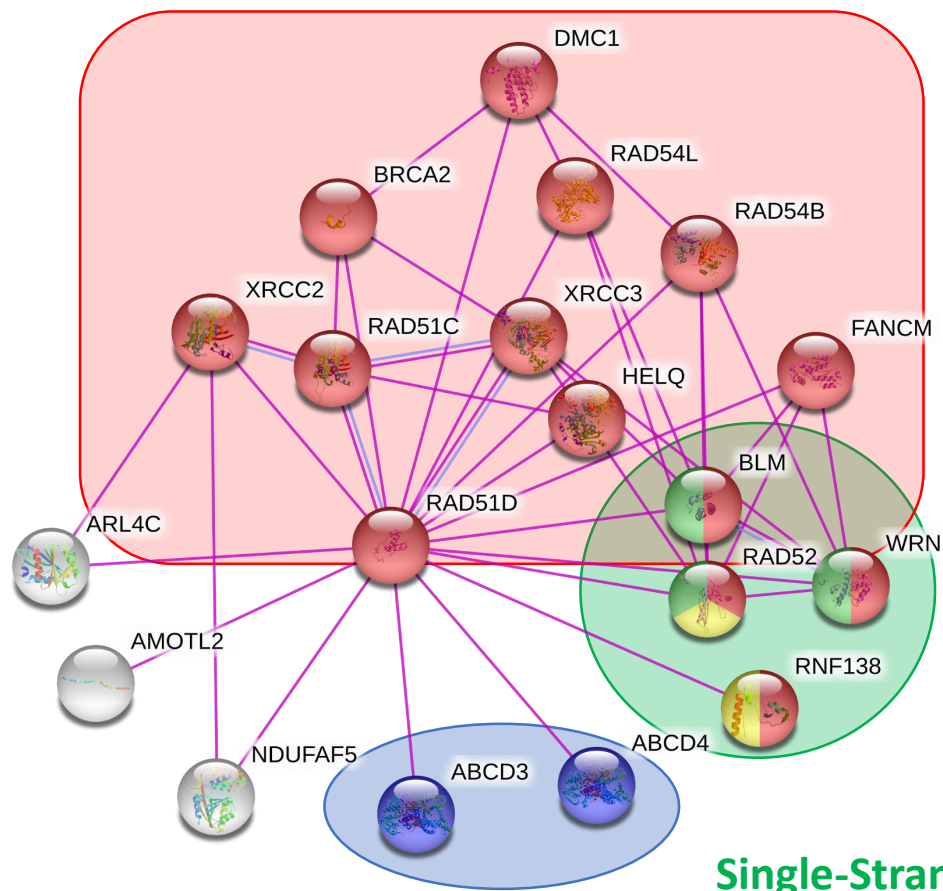
Estrogen signaling induces DNA damage





What proteins interact with *RAD51D*?

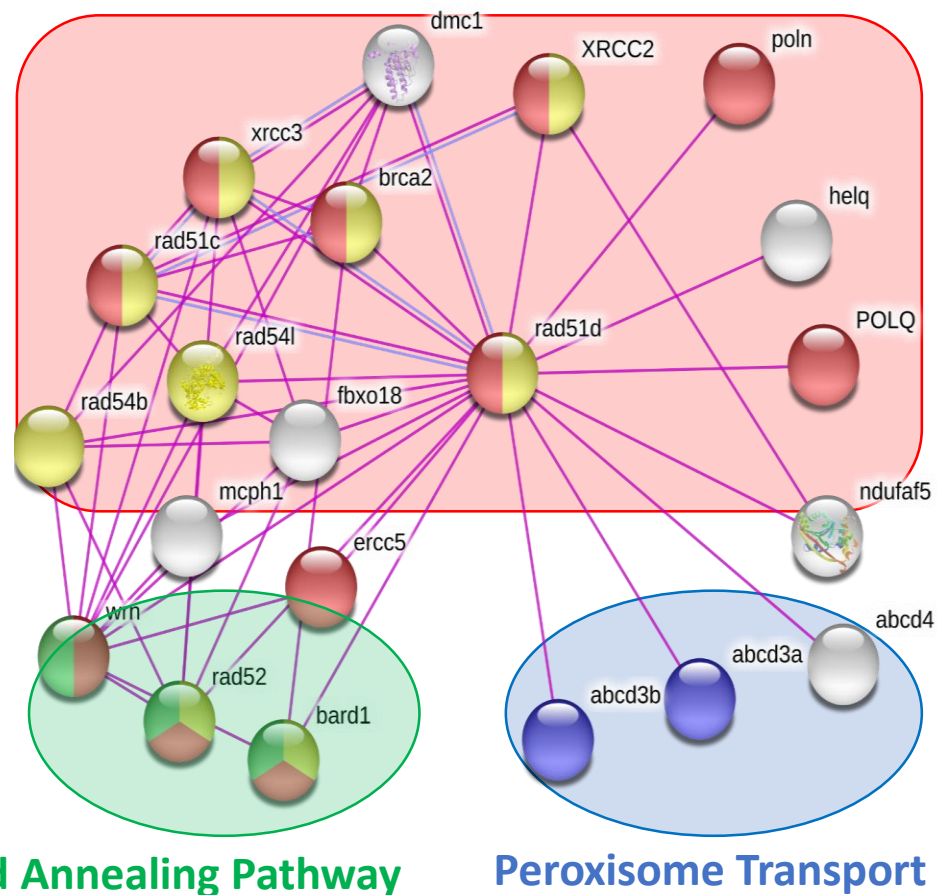
DNA Repair



Peroxisome Transport

Human

DNA Repair



Peroxisome Transport

Zebrafish