

# Impact of the Total Western Diet for rodents on colon mucosal gene expression in a multi-generational murine model of colitis-associated colorectal cancer

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Rousselene **Jones**

Korry **Hintze**

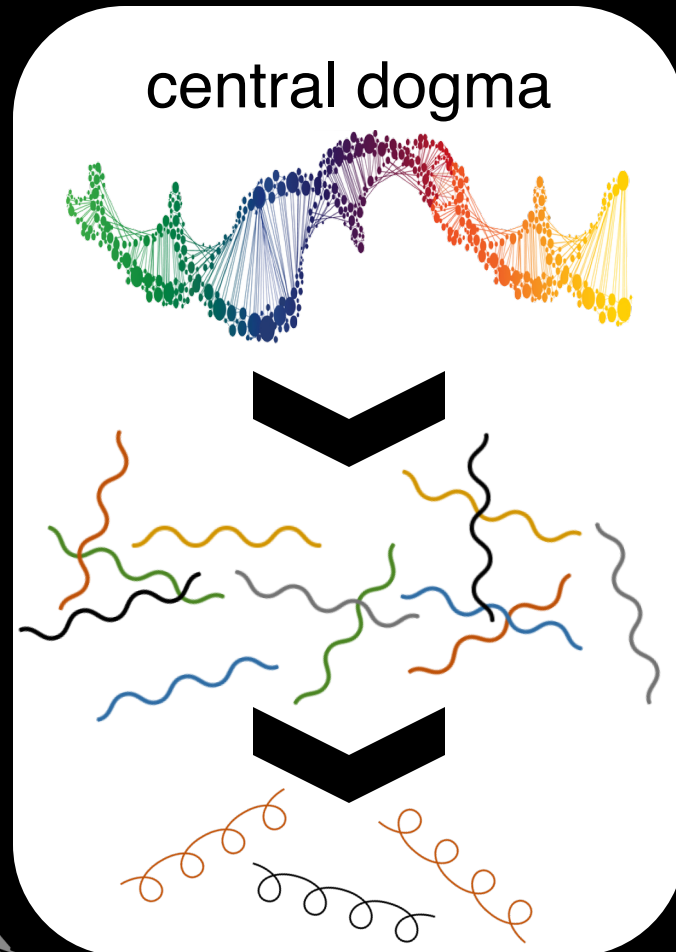
Abby **Benninghoff**

# Colorectal cancer (CRC)

- CRC is the 2<sup>nd</sup> leading cause of cancer-related death in the US.
- Majority of CRC incidence is attributed to diet.



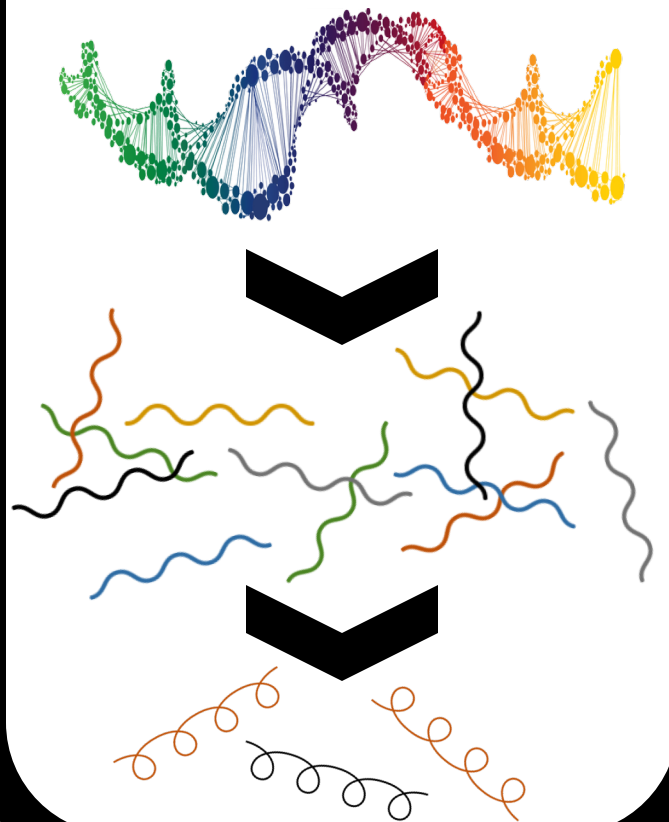
# Epigenetic gene expression signatures



Rapin et al. 2014. Blood.  
Yang et al. 2017. Oncol Lett.

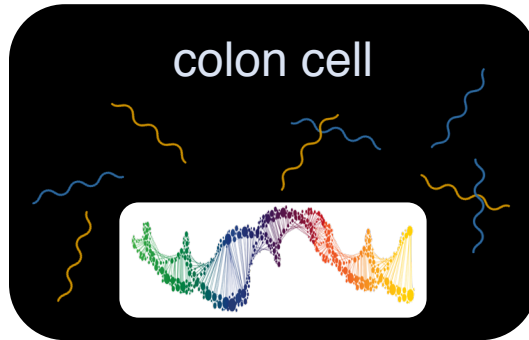
# Epigenetic gene expression signatures

central dogma

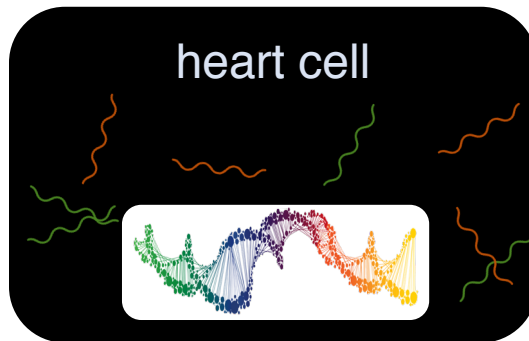


different cell types

colon cell



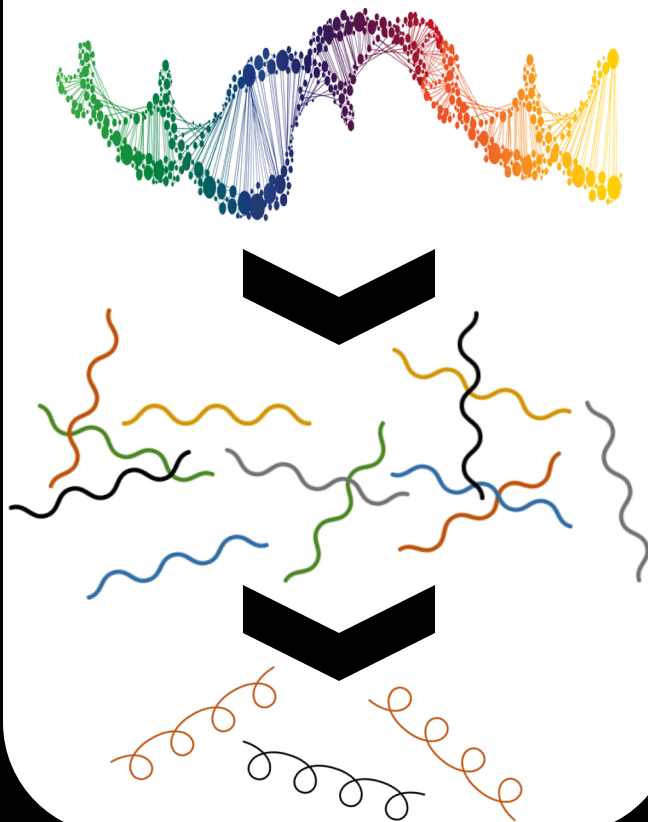
heart cell





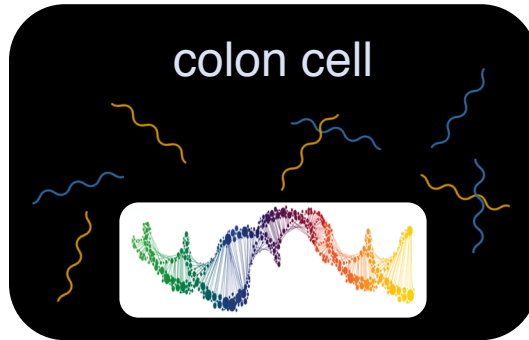
# Epigenetic gene expression signatures

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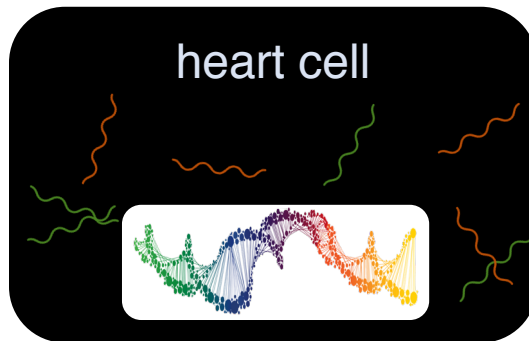


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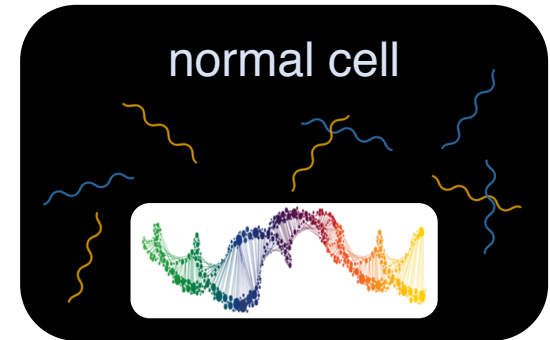


heart cell

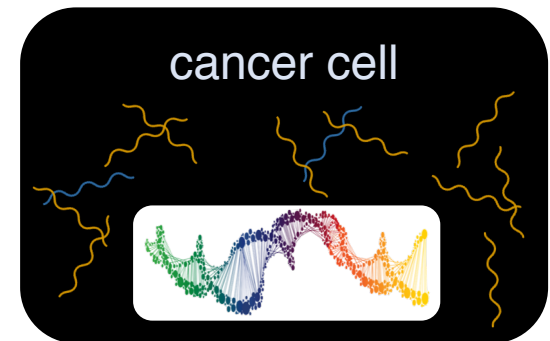


normal vs. cancer

normal cell



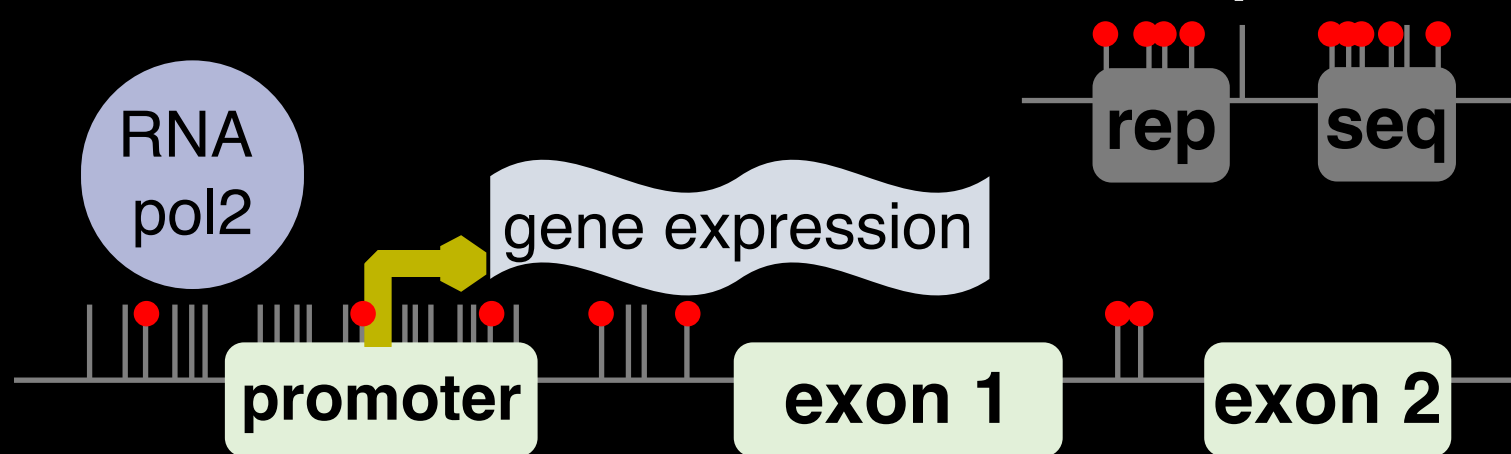
cancer cell





## RepSeq inhibition

# Heritable DNA methylation



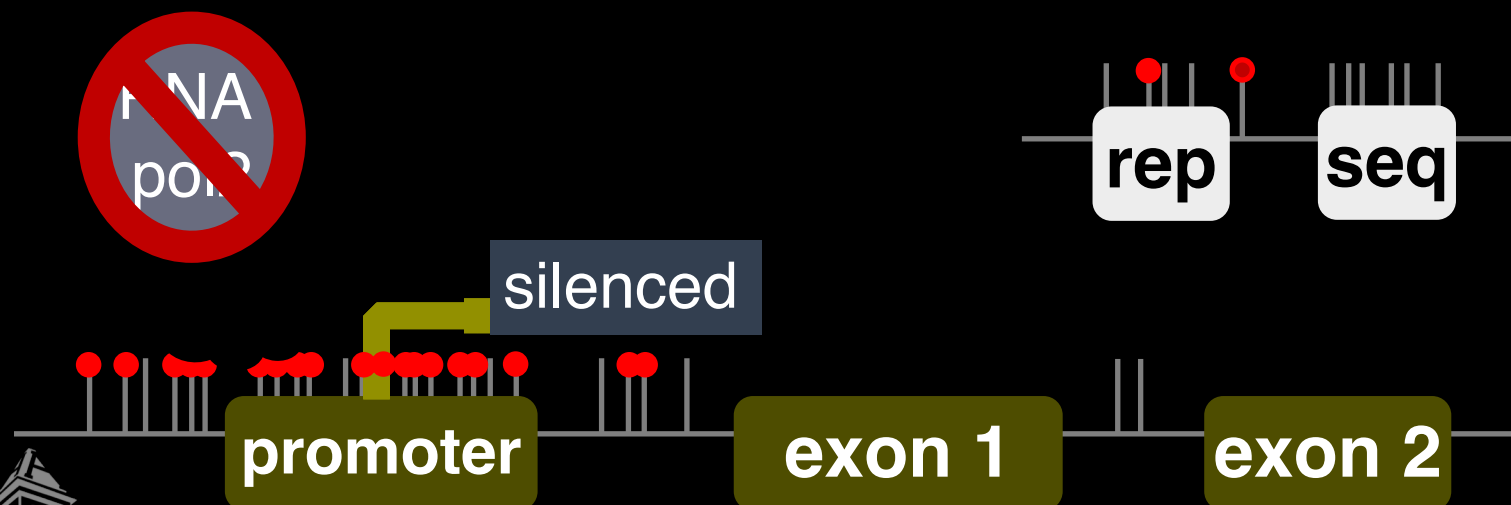
**normal cell**

TSG hypomethylation

active transcription

global hypermethylation

RepSeq inhibition



**tumor cell**

TSG hypermethylation

silenced transcription

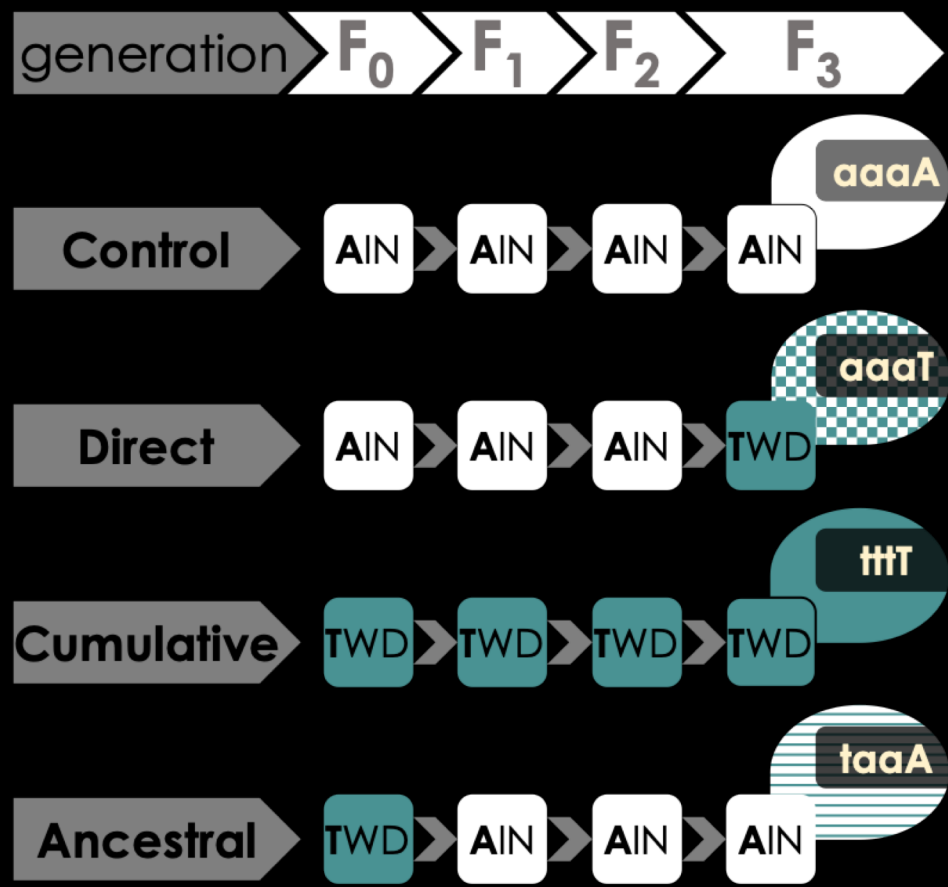
global hypomethylation

genome instability



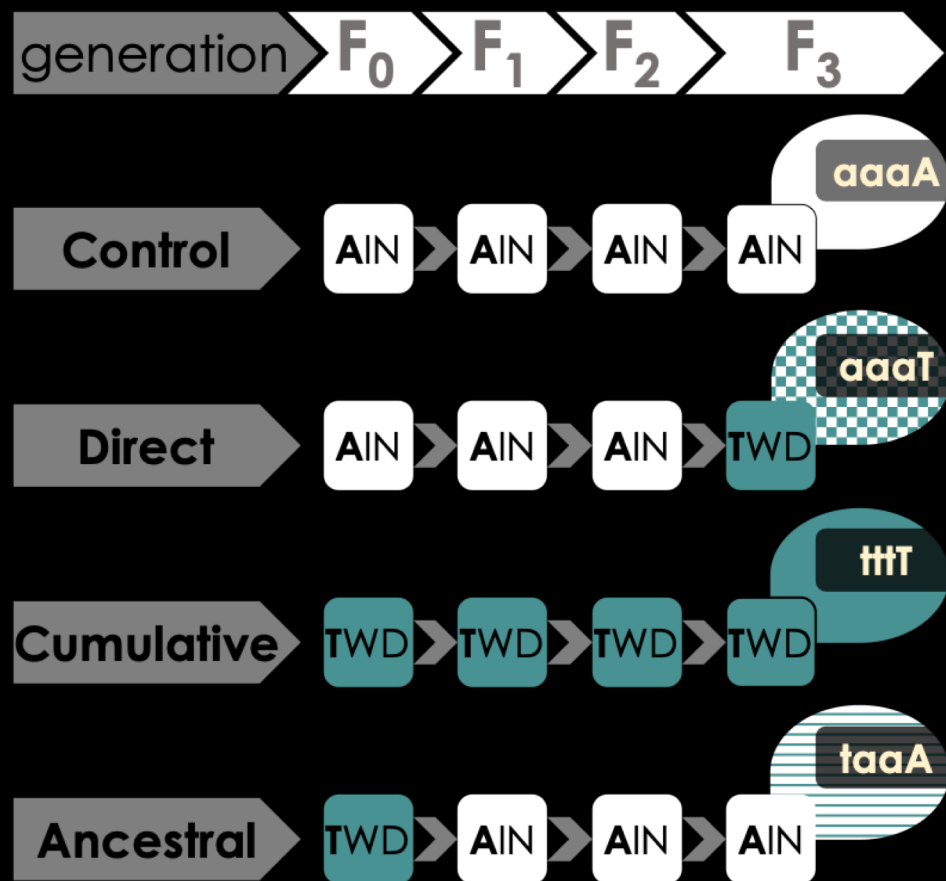


# Study design

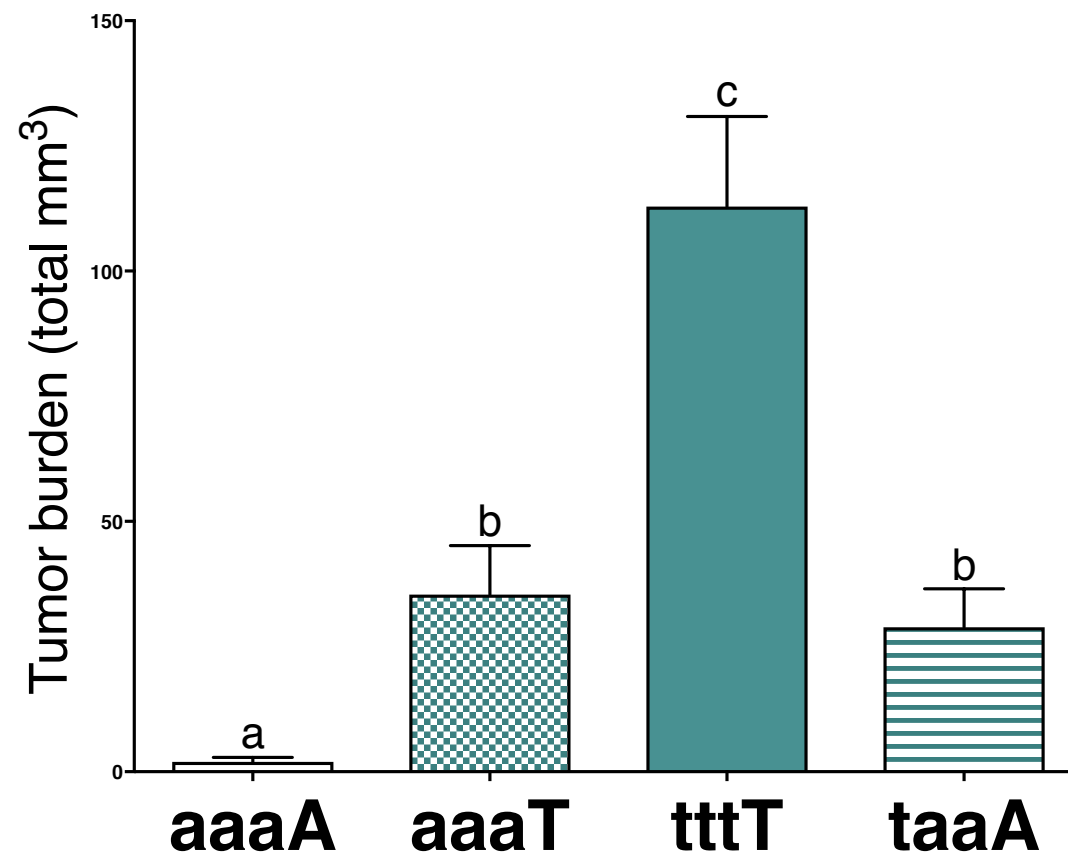




# Study design



# CRC outcome





# Knowledge gap

## Objective:

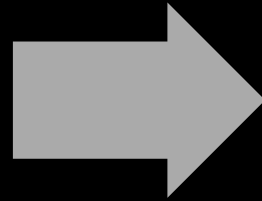
evaluate differentially expressed genes (DEGs) of colonic mucosal cells from 3<sup>rd</sup> generation offspring.



# Knowledge gap

## Objective:

evaluate differentially expressed genes (DEGs) of colonic mucosal cells from 3<sup>rd</sup> generation offspring.

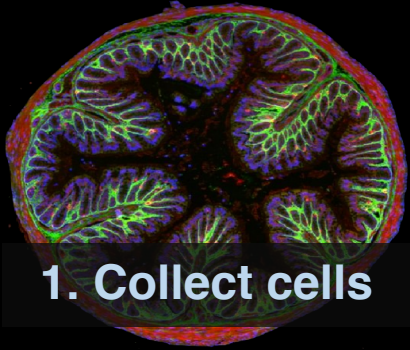


## Hypothesis:

Total Western Diet (TWD) exposure will upregulate or downregulate genes that play a role in CRC.



# Methods

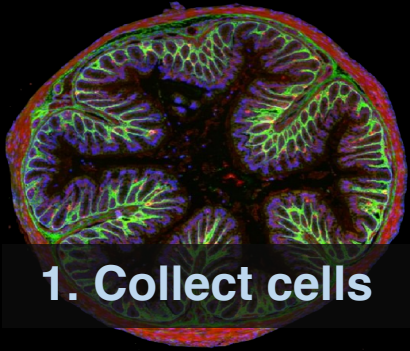


**1. Collect cells**

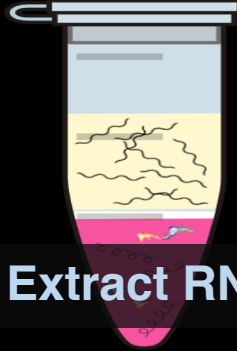




# Methods



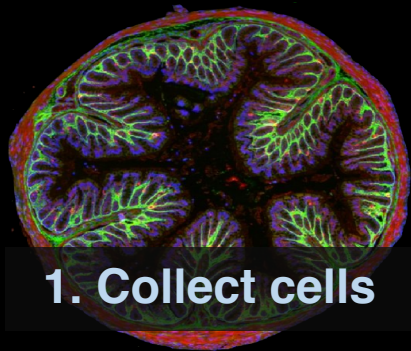
**1. Collect cells**



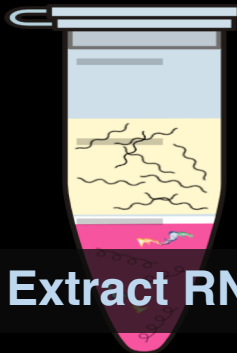
**2. Extract RNA**



# Methods



**1. Collect cells**



**2. Extract RNA**

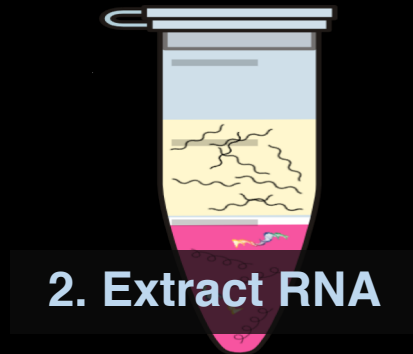
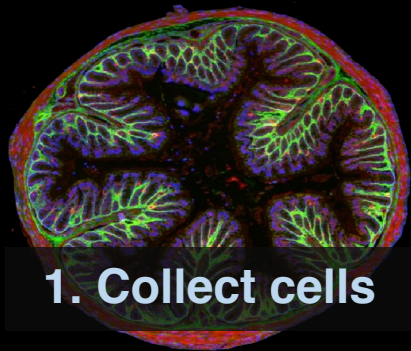


**3. Sorting**



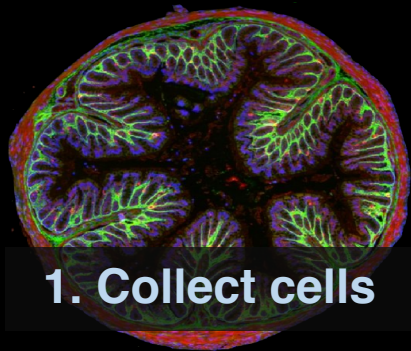


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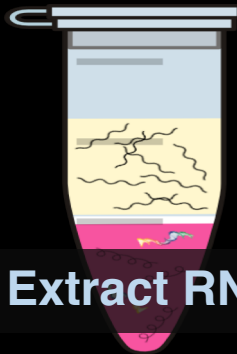




# Methods



1. Collect cells



2. Extract RNA



3. Sorting



4. Sequencing



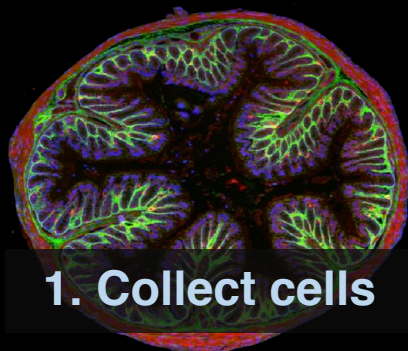
5. Quality control



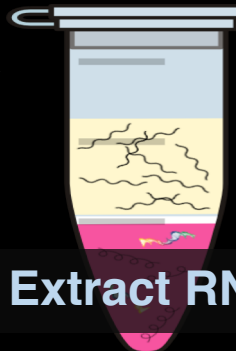




# Methods



1. Collect cells



2. Extract RNA



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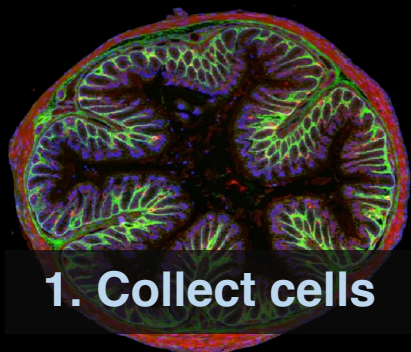


6. Trimming

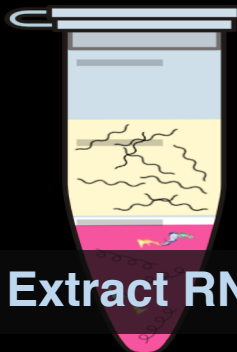




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3. Sorting



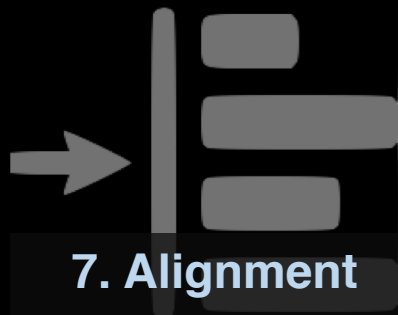
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5. Quality control



6. Trimming

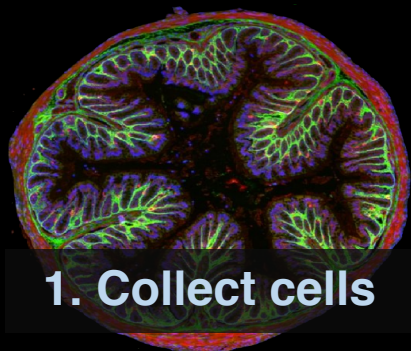


7. Alignment

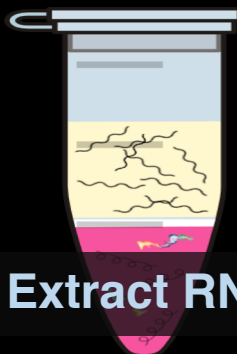




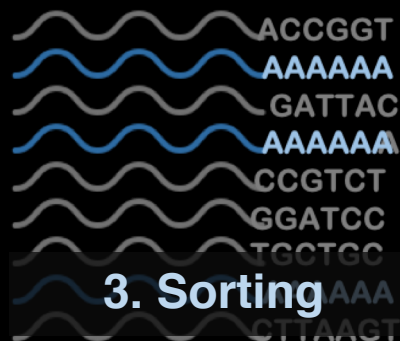
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2. Extract RNA



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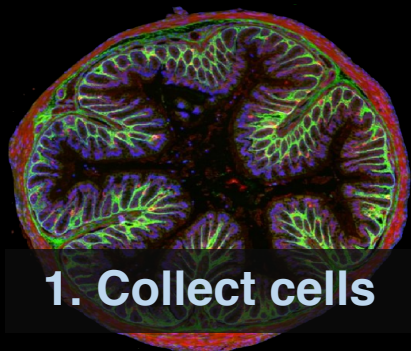
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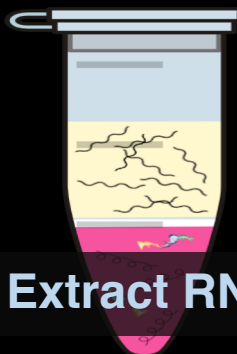
8. Quantification



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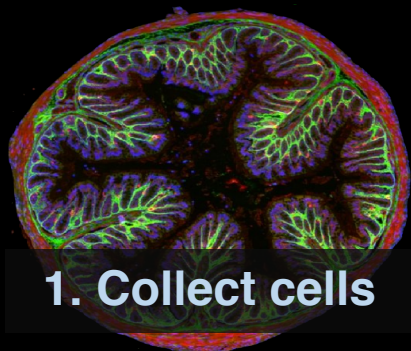
9. Filtering



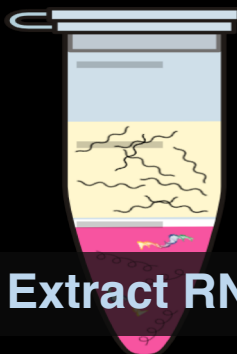




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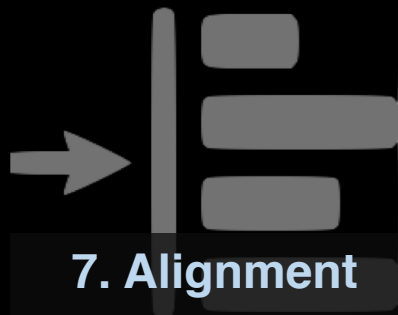
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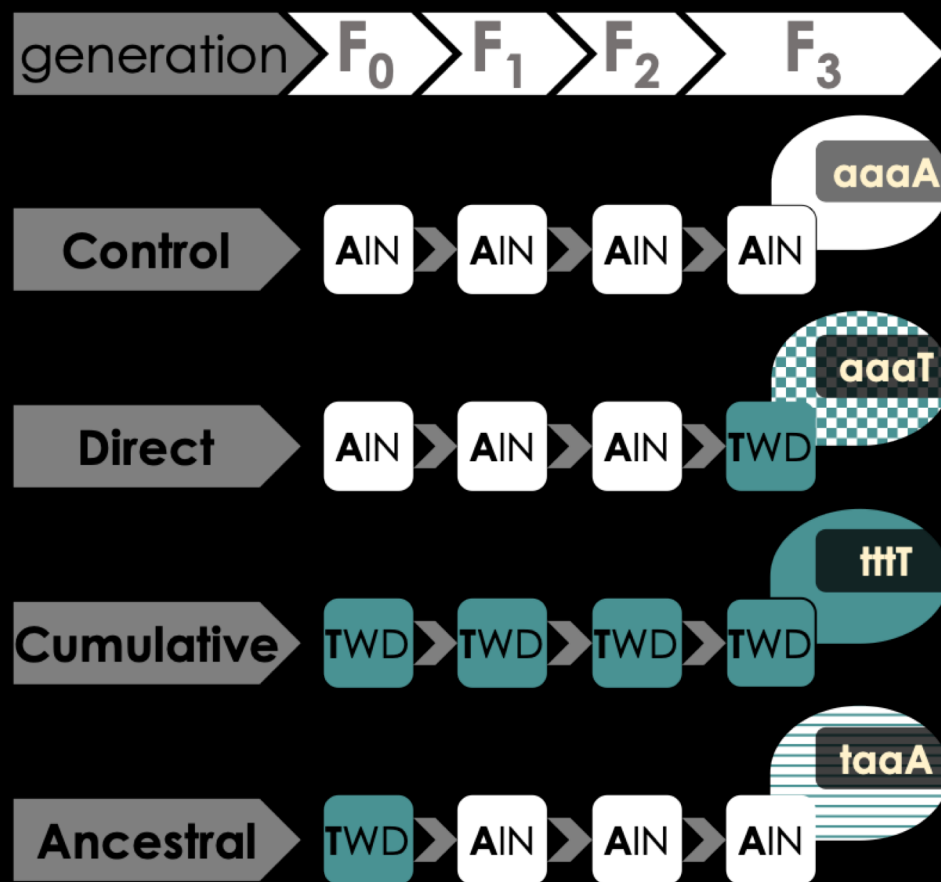


9. Filtering



10. DEG analysis

# Study design

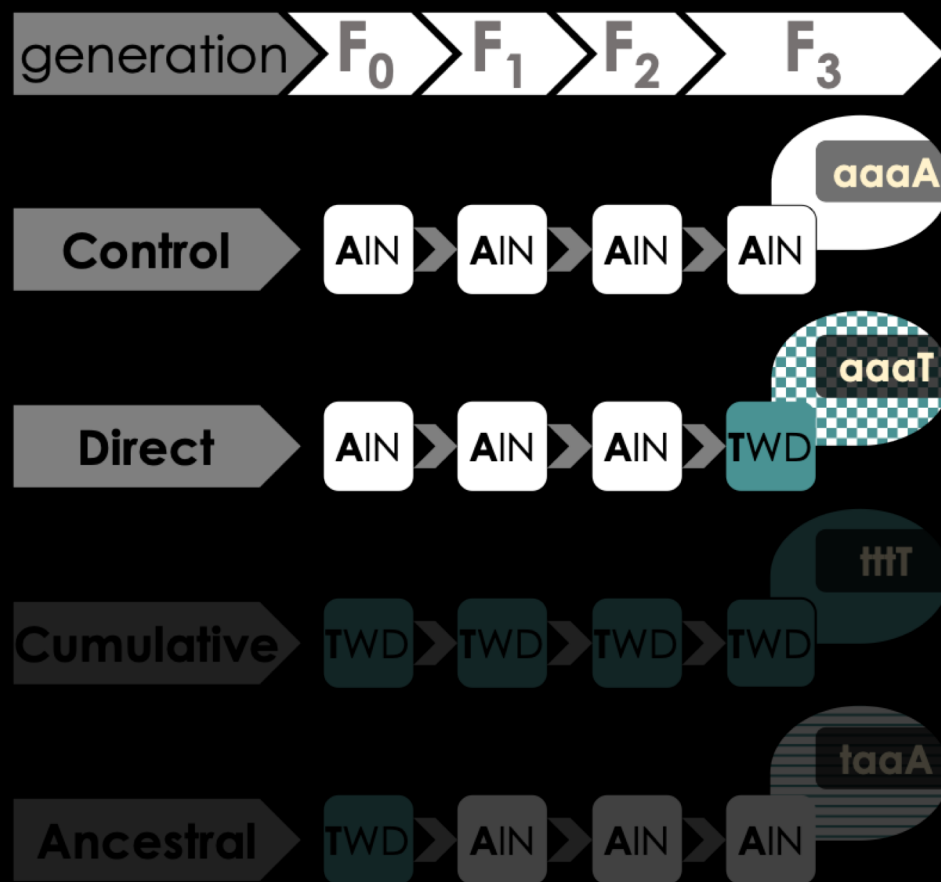


# Preliminary results

- Cancer vs. controls
  - ~700-4500 DEGs



# Study design

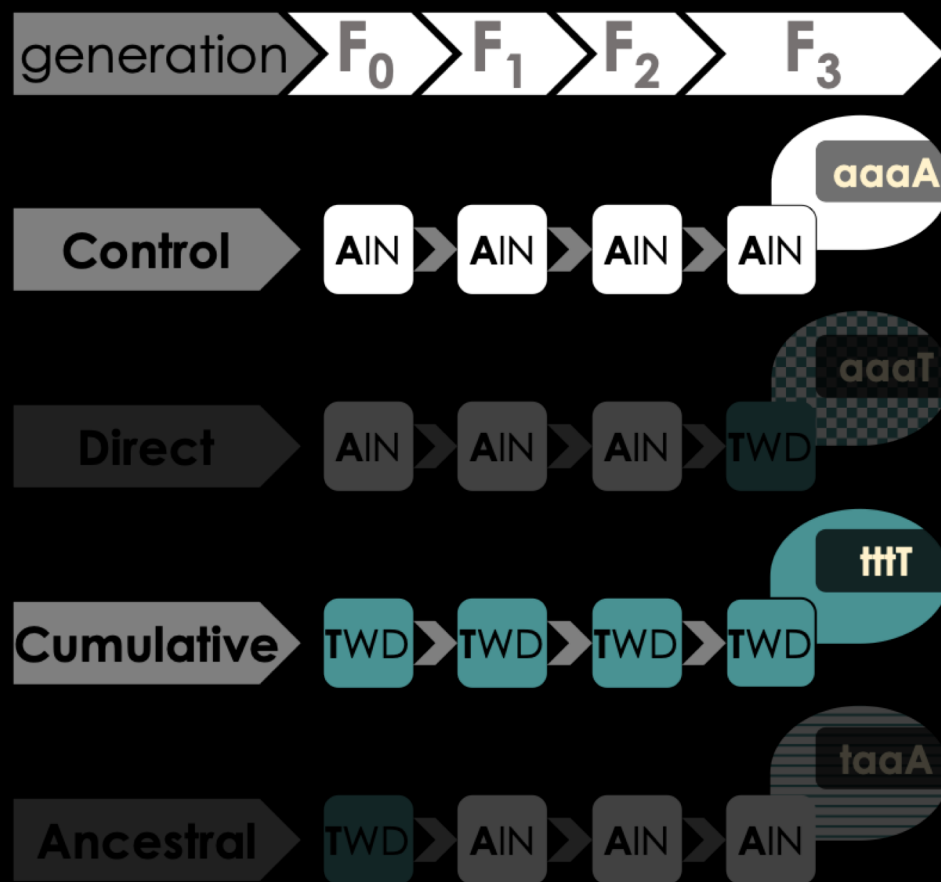


# Preliminary results

- Cancer vs. controls
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- Cancer cohorts
  - aaaA vs aaaT
  - 36 DEGs



# Study design

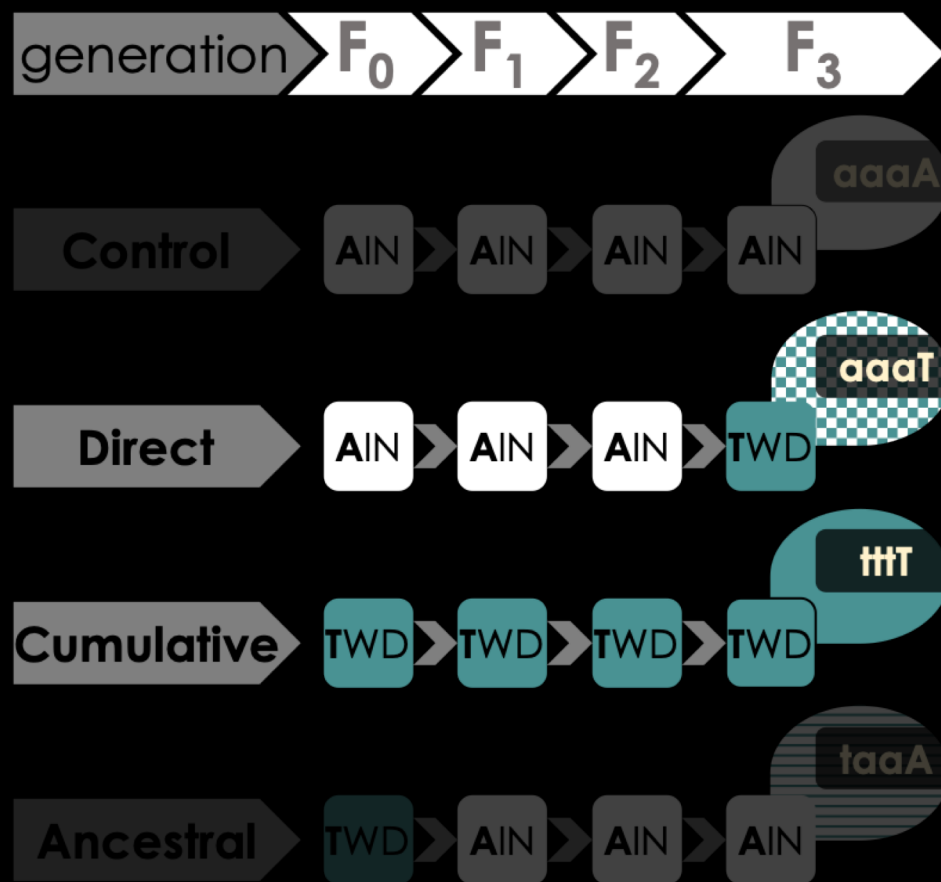


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119 DEGs



# Study design



# Preliminary results

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  - ~700-4500 DEGs
- Cancer cohorts
  - aaaA vs aaaT  
36 DEGs
  - aaaA to tttT  
119 DEGs
- Sham cohorts
  - aaaT vs. tttT  
101 DEGs
    - defense response
    - immune response
    - response to interferon





# Summary & conclusions

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- Metanalysis of clinical CRC gene expression signature reveals DEGs related to immune function.
- Many DEGs in human CRC are associated with aberrant DNA methylation.
- Ongoing analysis will include methylation status.





# Acknowledgements

Benninghoff Laboratory  
USTAR Applied Nutrition Research  
USDA NIFA/AFRI Grant 2014-67017-21755  
Utah Agricultural Experiment Station Project UTA-01178