Utah Agricultural Experiment Station
UtahStateUniversity

USTAR Applied Nutrition Research

USDA

NIFA

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

Sumira **Phatak**
Aaron **Thomas**
Rakesh **Kaundal**
Rousselene **Jones**
Korry **Hintze**
Abby **Benninghoff**

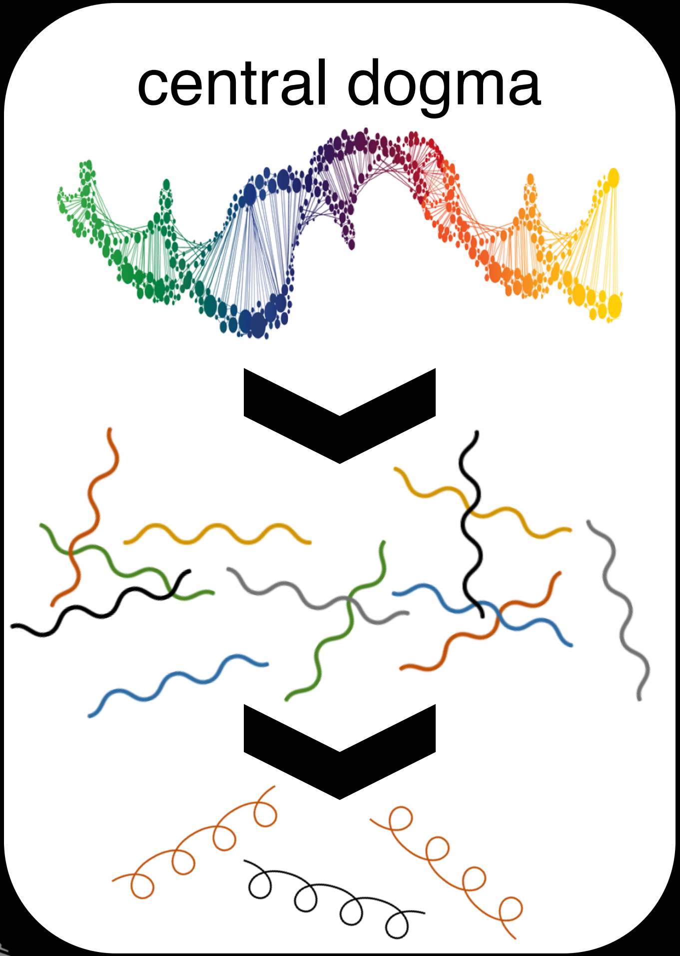
Colorectal cancer (CRC)

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

American Cancer Society. Colorectal Cancer Facts & Figures 2017-2019.
Pericleous et al. 2013. J Gastrointest Oncol.

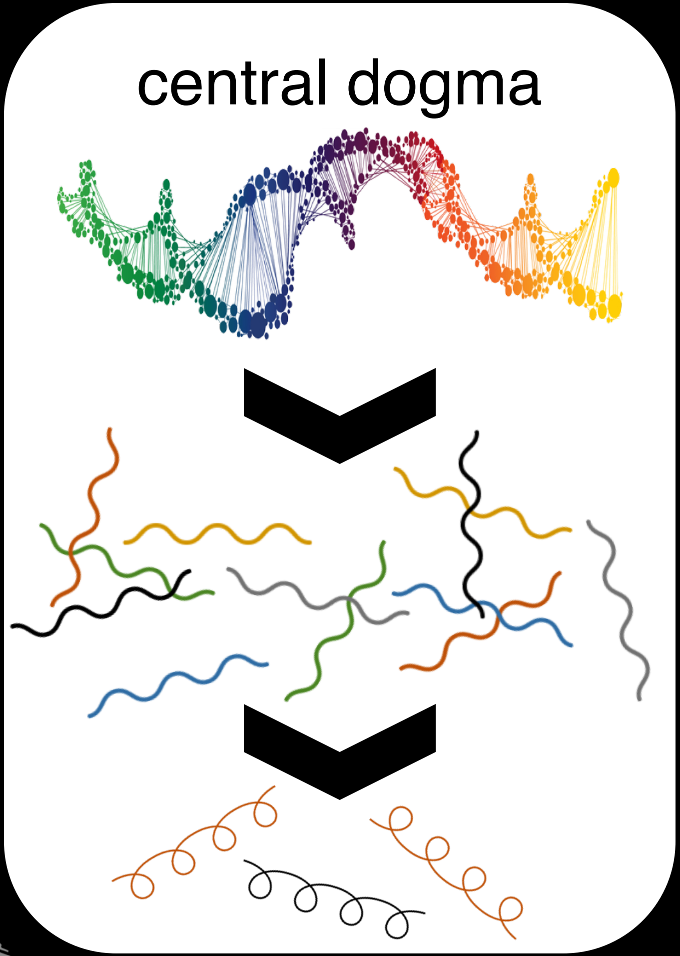
UtahStateUniversity
ANIMAL, DAIRY & VETERINARY SCIENCES

Epigenetic gene expression signatures

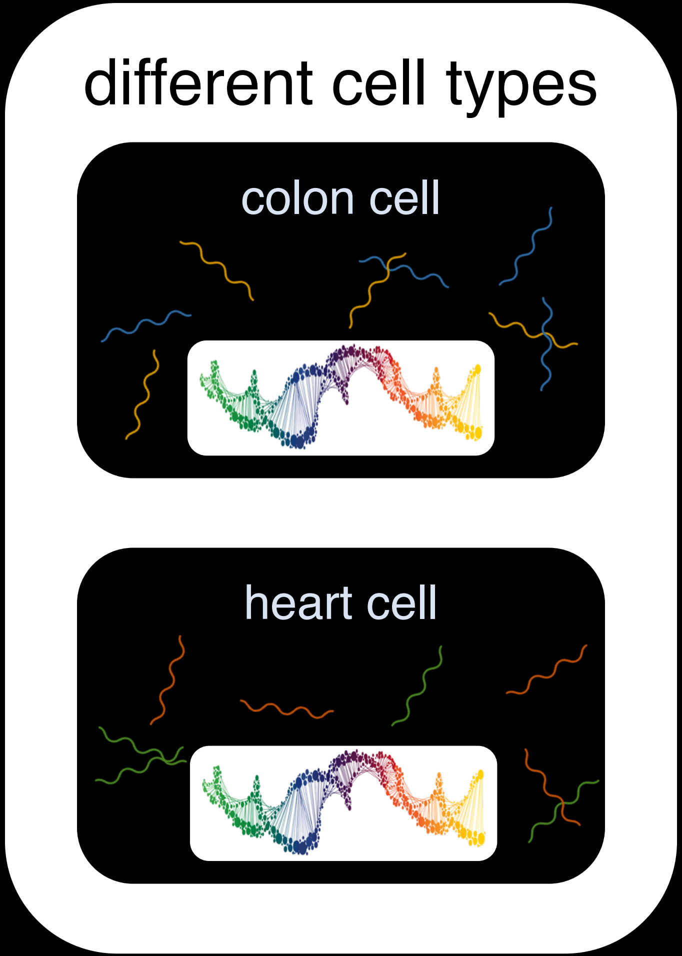


Rapin et al. 2014. Blood.
Yang et al. 2017. Oncol Lett.

Epigenetic gene expression signatures



central dogma



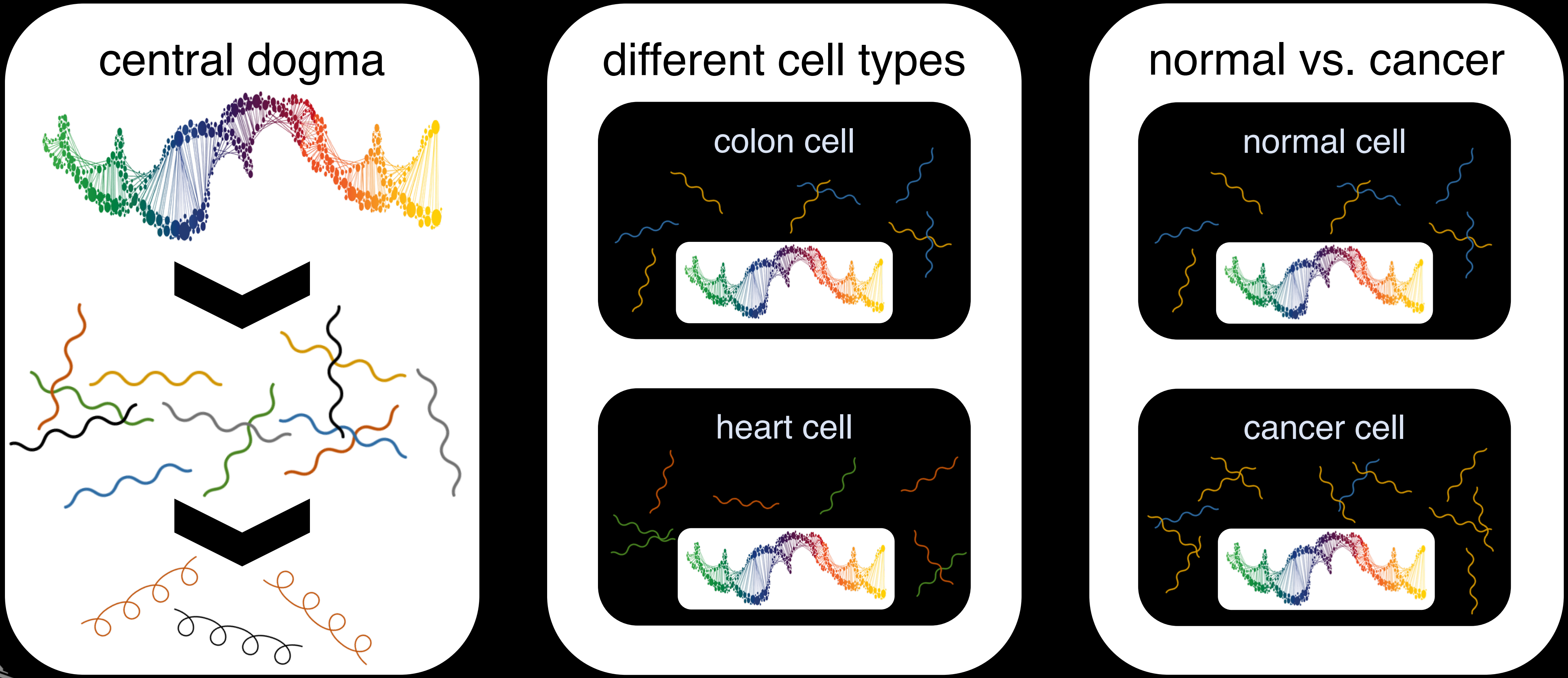
different cell types

colon cell

heart cell

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

Epigenetic gene expression signatures



central dogma

different cell types

colon cell

heart cell

normal vs. cancer

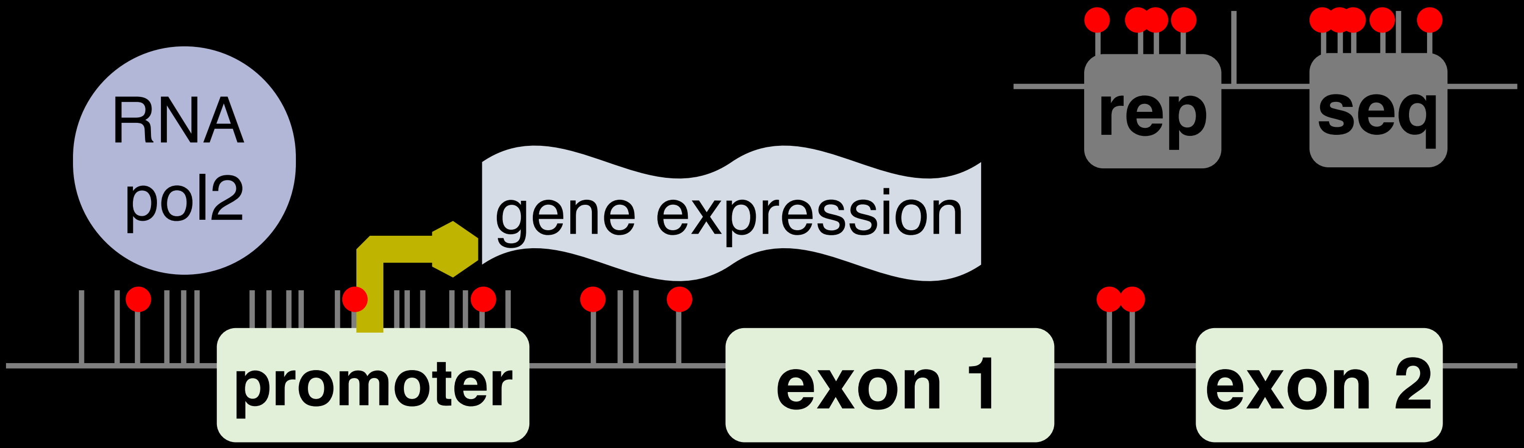
normal cell

cancer cell

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

UtahStateUniversity
ANIMAL, DAIRY & VETERINARY SCIENCES

Heritable DNA methylation



normal cell

TSG hypomethylation

active transcription

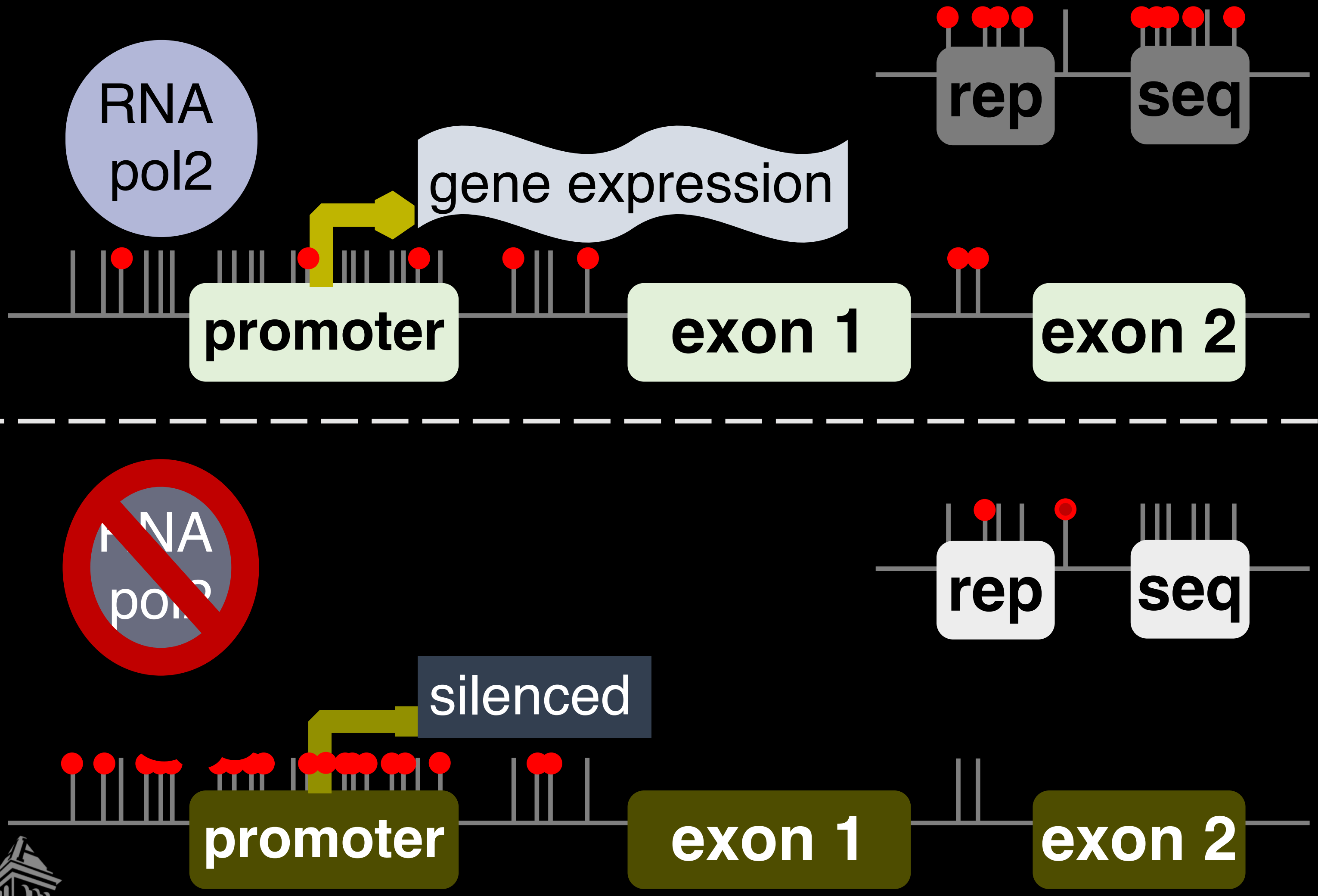
global hypermethylation

RepSeq inhibition

Seisenberger et al. 2013. Philos Trans R Soc Lond B Biol Sci.

UtahStateUniversity
ANIMAL, DAIRY & VETERINARY SCIENCES

Heritable DNA methylation



normal cell

- TSG hypomethylation
- active transcription
- global hypermethylation
- RepSeq inhibition

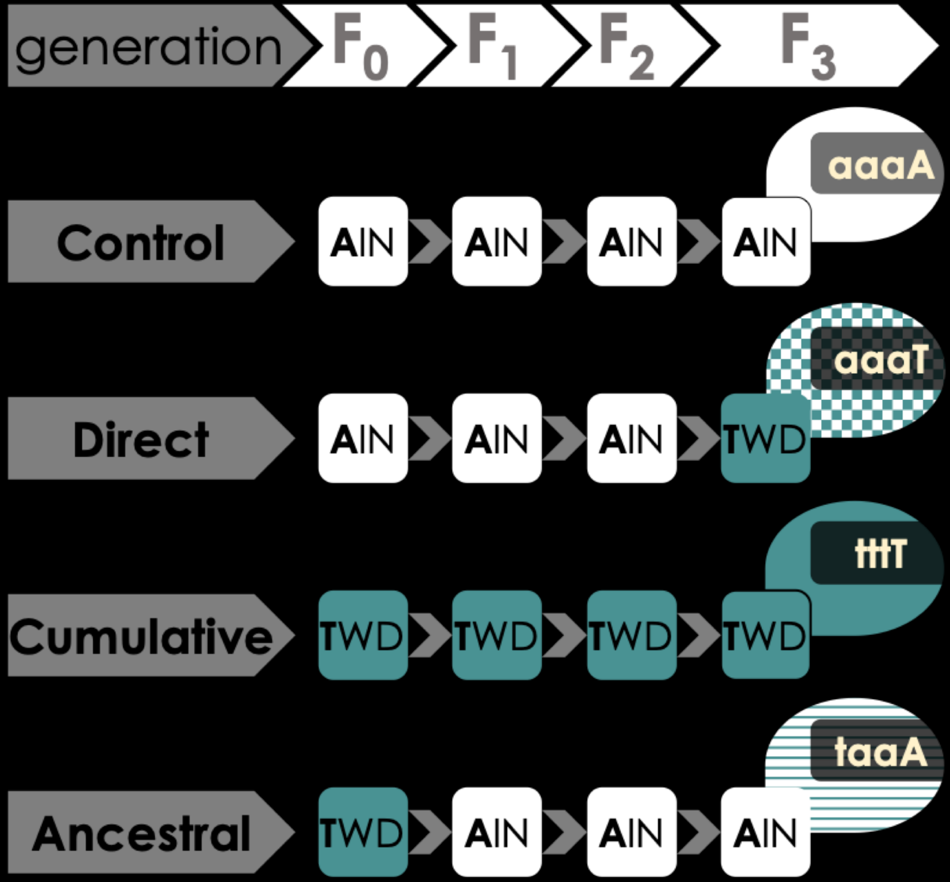
tumor cell

- TSG hypermethylation
- silenced transcription
- global hypomethylation
- genome instability

Utah State University
ANIMAL, DAIRY & VETERINARY SCIENCES

Seisenberger et al. 2013. Philos Trans R Soc Lond B Biol Sci.

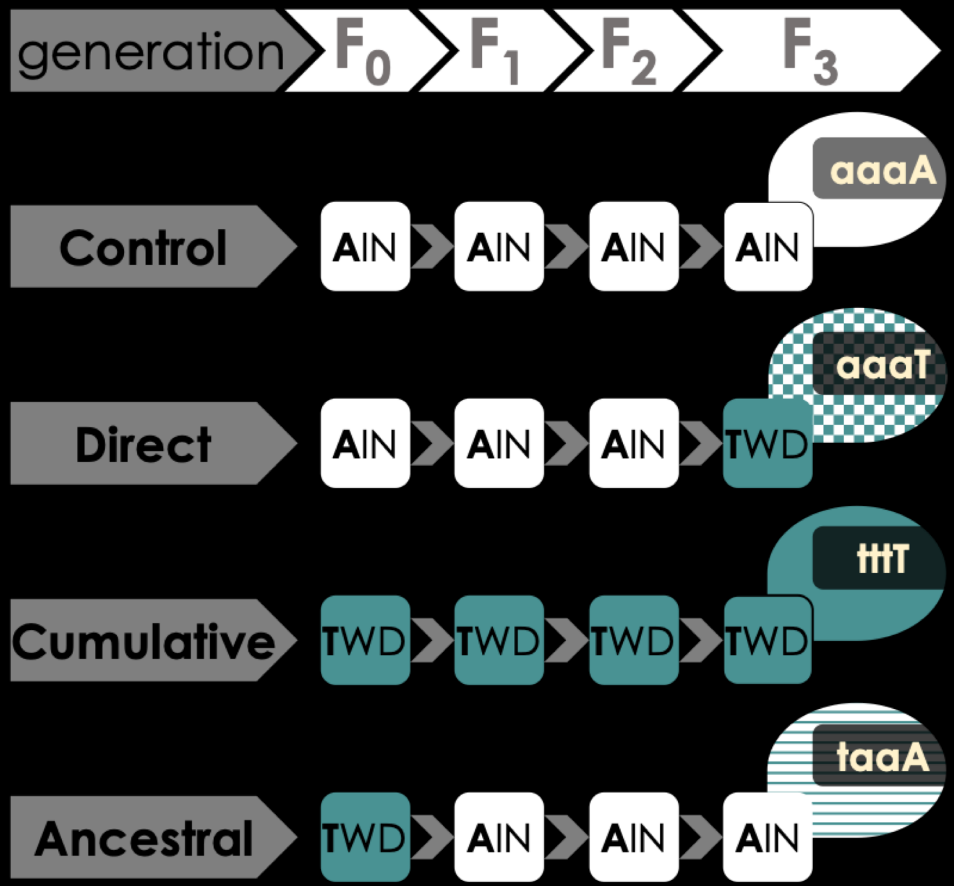
Study design



generation	F_0	F_1	F_2	F_3	
Control	AIN	AIN	AIN	AIN	aaaA
Direct	AIN	AIN	AIN	TWD	aaaT
Cumulative	TWD	TWD	TWD	TWD	tttT
Ancestral	TWD	AIN	AIN	AIN	taaA

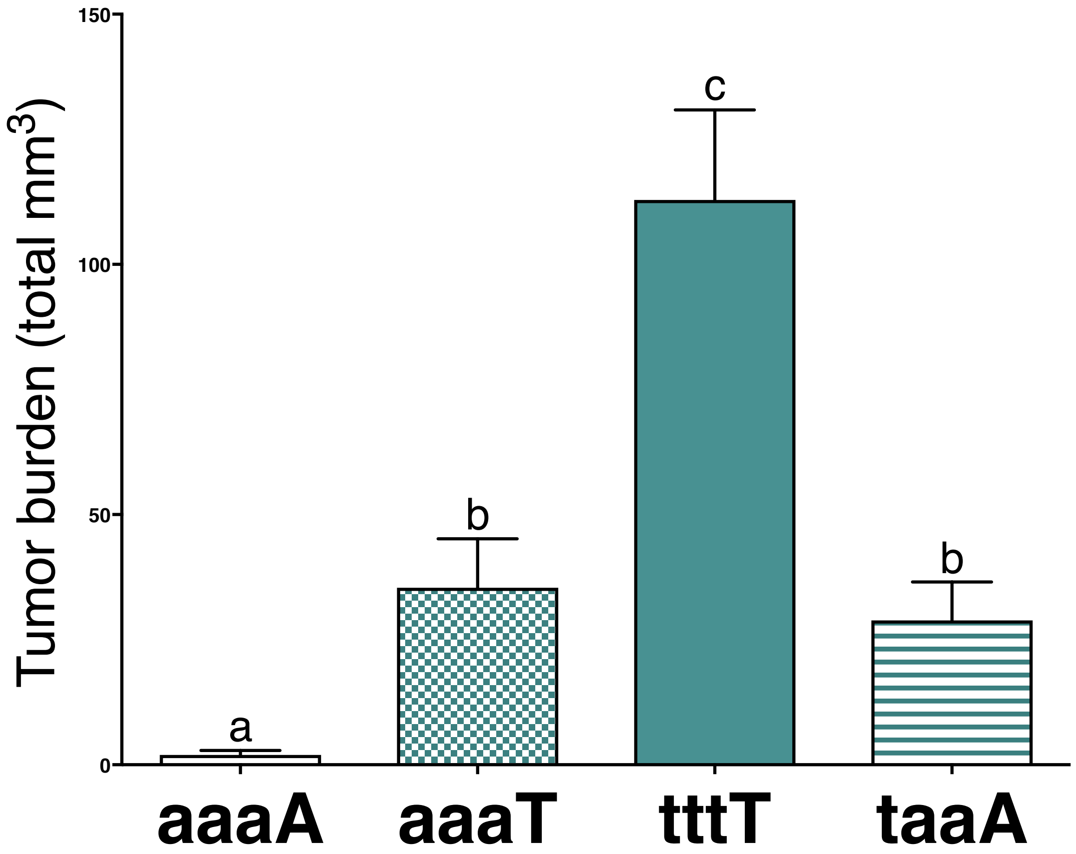
UtahStateUniversity
ANIMAL, DAIRY & VETERINARY SCIENCES

Study design



generation	F0	F1	F2	F3	
Control	AIN	AIN	AIN	AIN	aaaA
Direct	AIN	AIN	AIN	TWD	aaaT
Cumulative	TWD	TWD	TWD	TWD	tttT
Ancestral	TWD	AIN	AIN	AIN	taaA

CRC outcome



Knowledge gap

Objective:

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

Knowledge gap

Objective:

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

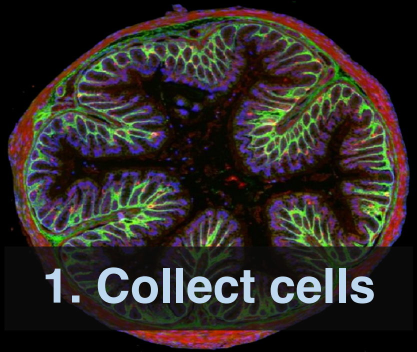
→

Hypothesis:

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

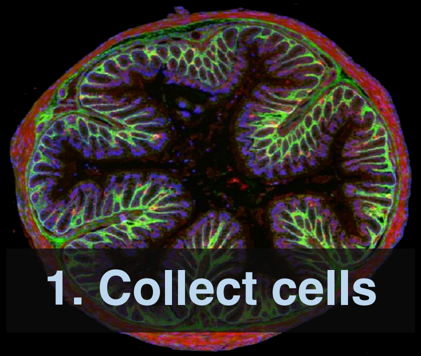
UtahStateUniversity
ANIMAL, DAIRY & VETERINARY SCIENCES

Methods

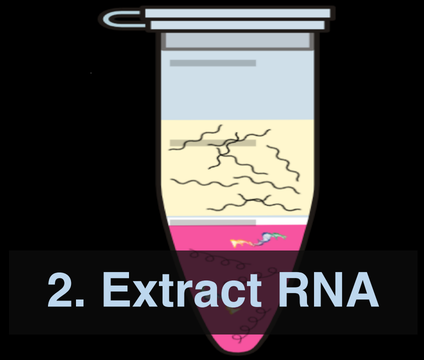

1. Collect cells

UtahStateUniversity
ANIMAL, DAIRY & VETERINARY SCIENCES

Methods

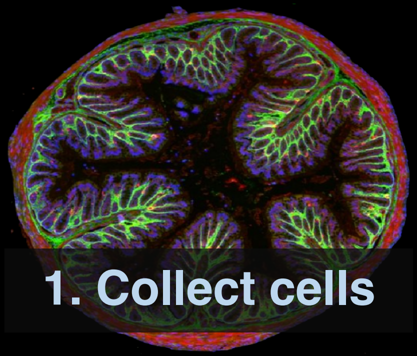


1. Collect cells

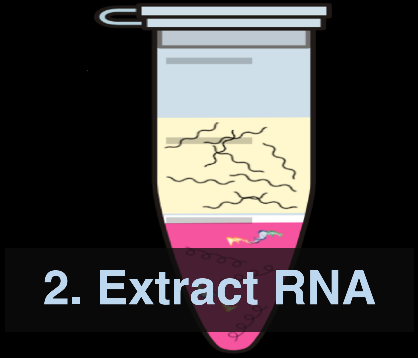


2. Extract RNA

Methods



1. Collect cells

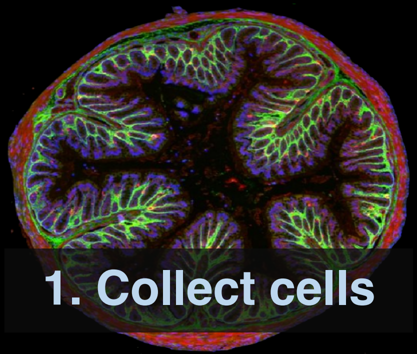


2. Extract RNA

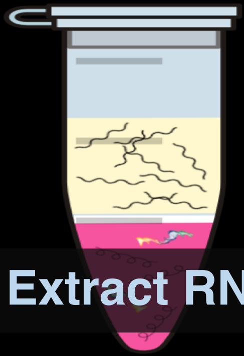
3. Sorting

UtahStateUniversity
ANIMAL, DAIRY & VETERINARY SCIENCES

Methods



1. Collect cells

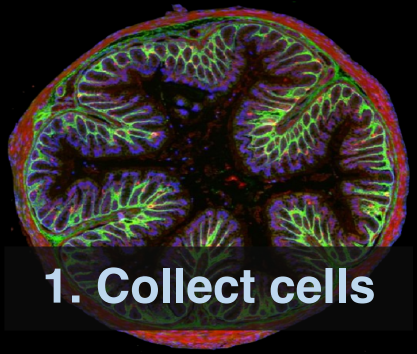


2. Extract RNA

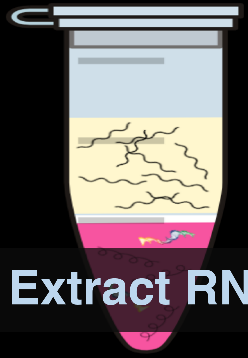
3. Sorting

4. Sequencing

Methods



1. Collect cells



2. Extract RNA

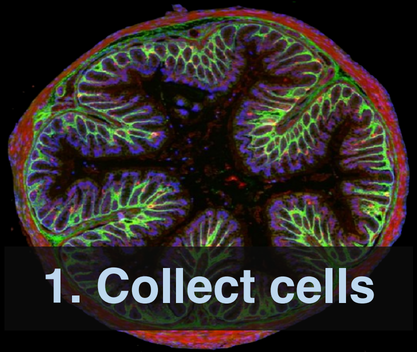
3. Sorting

4. Sequencing

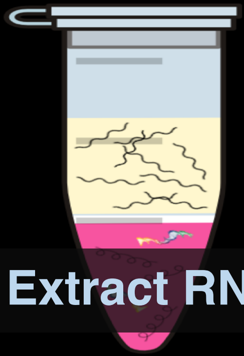
5. Quality control

UtahStateUniversity
ANIMAL, DAIRY & VETERINARY SCIENCES

Methods



1. Collect cells



2. Extract RNA

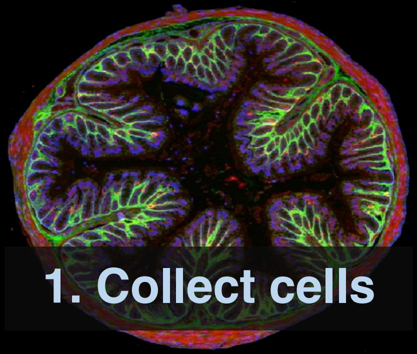
3. Sorting

4. Sequencing

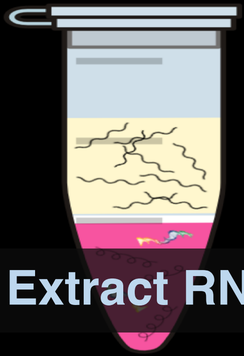
5. Quality control

6. Trimming

Methods



1. Collect cells



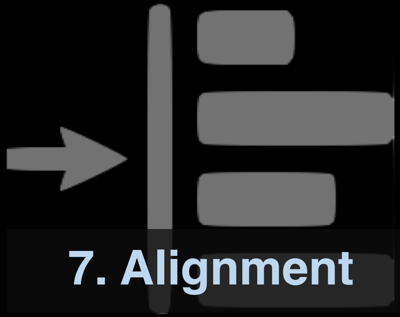
2. Extract RNA

3. Sorting

4. Sequencing

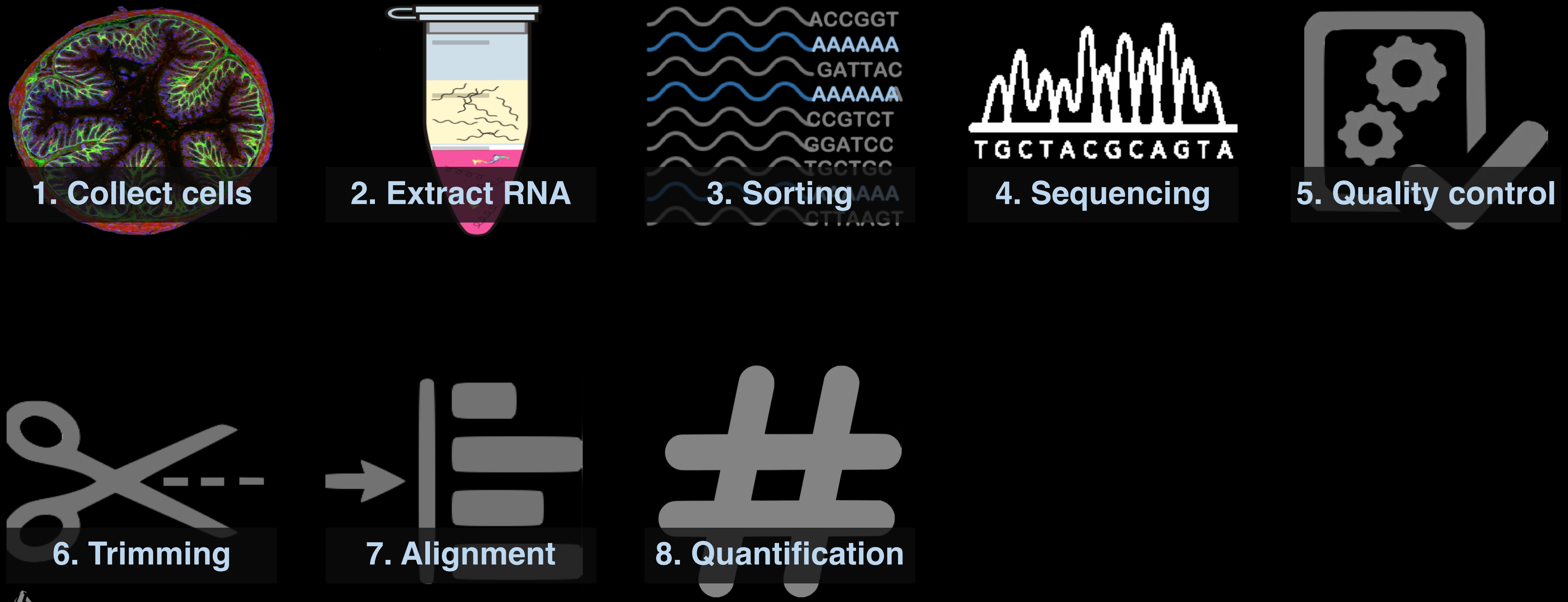
5. Quality control

6. Trimming



7. Alignment

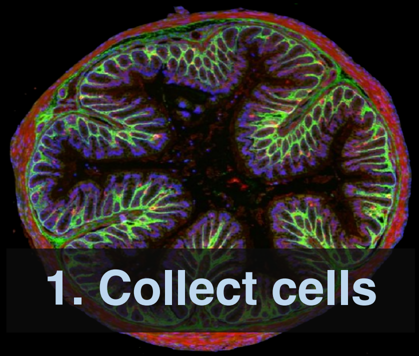
Methods



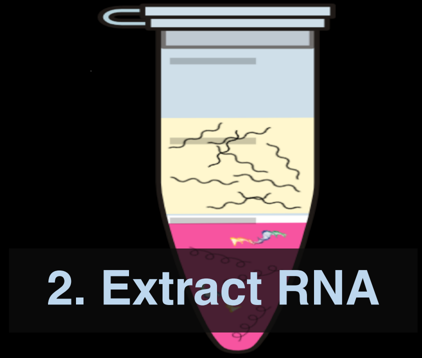
1. Collect cells
2. Extract RNA
3. Sorting
4. Sequencing
5. Quality control
6. Trimming
7. Alignment
8. Quantification

UtahStateUniversity
ANIMAL, DAIRY & VETERINARY SCIENCES

Methods



1. Collect cells



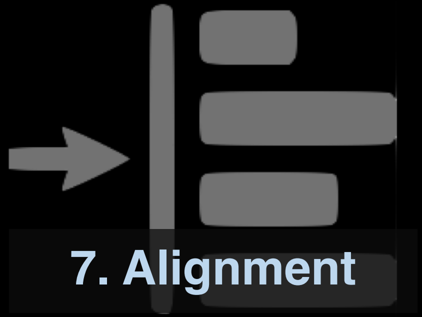
2. Extract RNA

3. Sorting

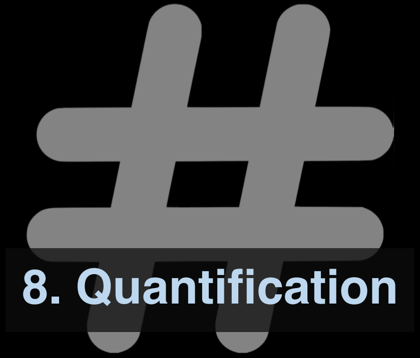
4. Sequencing

5. Quality control

6. Trimming



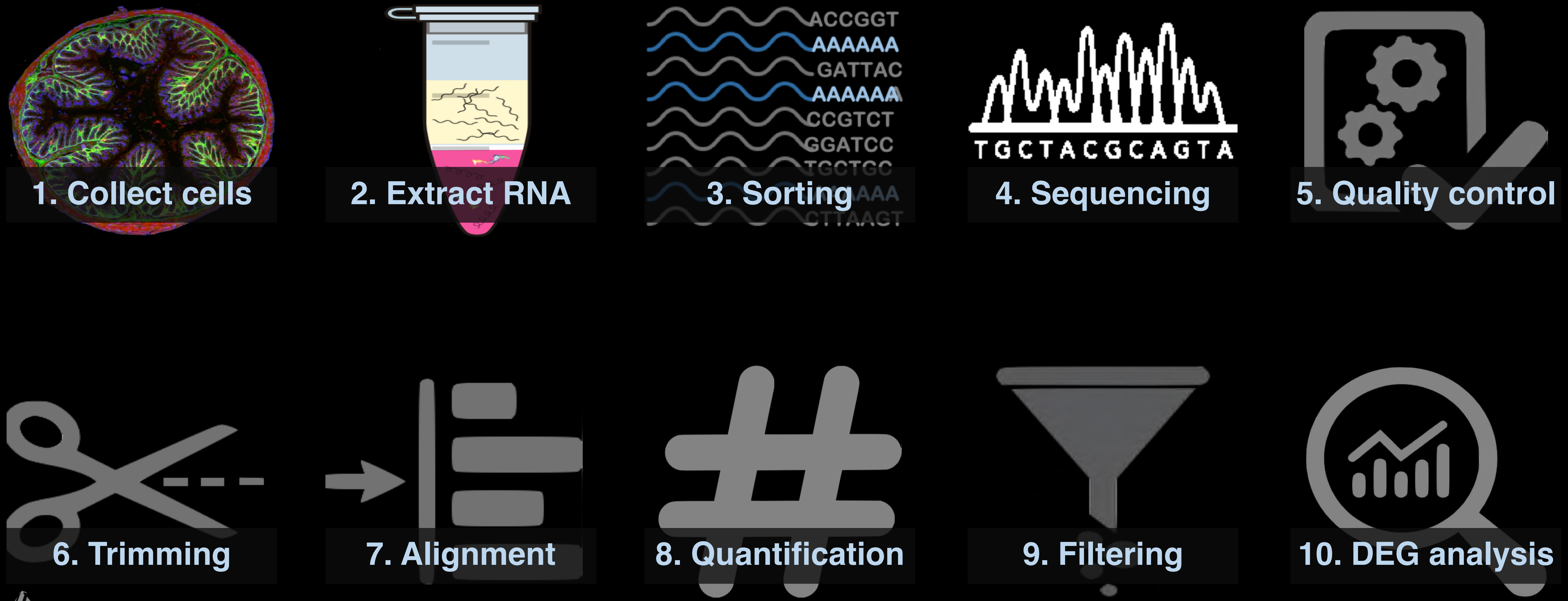
7. Alignment



8. Quantification

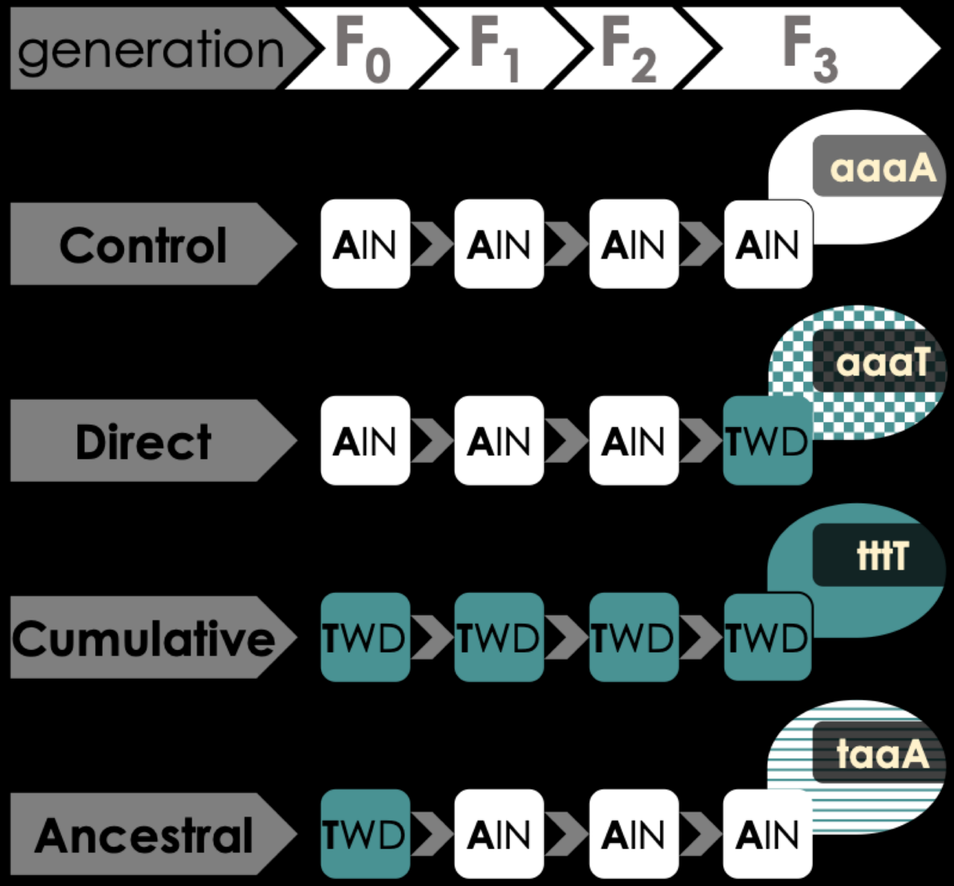
9. Filtering

Methods



1. Collect cells
2. Extract RNA
3. Sorting
4. Sequencing
5. Quality control
6. Trimming
7. Alignment
8. Quantification
9. Filtering
10. DEG analysis

Study design

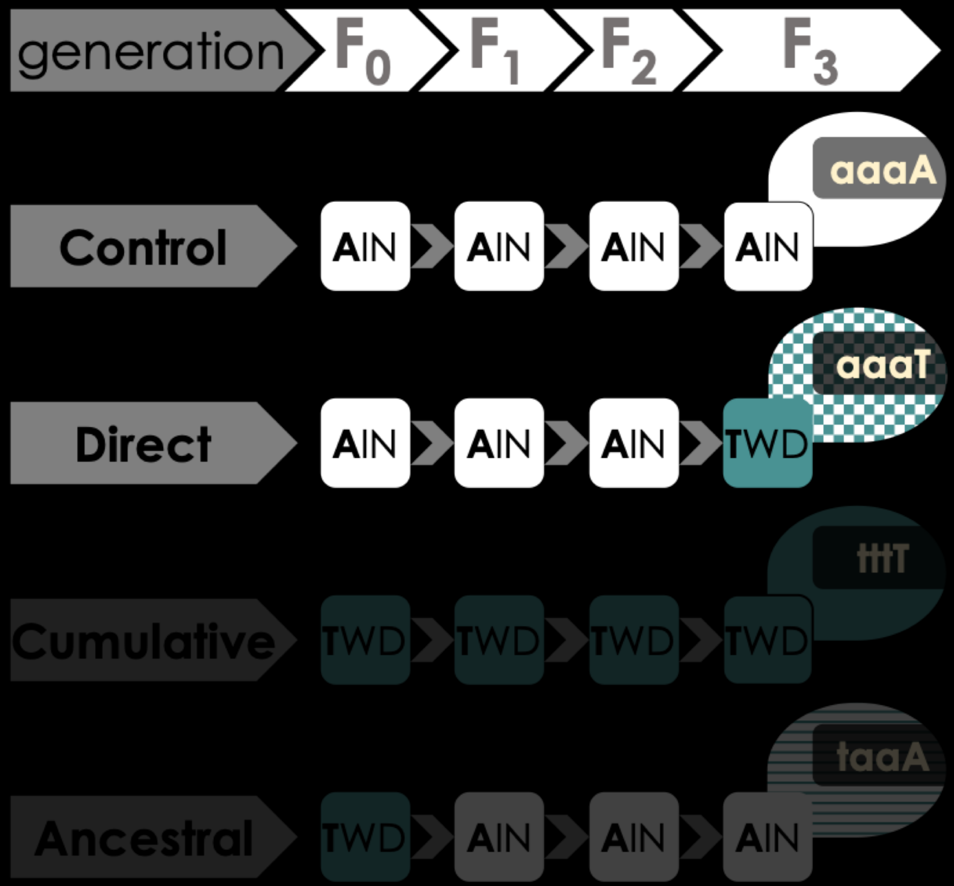


generation	F_0	F_1	F_2	F_3	
Control	AIN	AIN	AIN	AIN	aaaA
Direct	AIN	AIN	AIN	TWD	aaaT
Cumulative	TWD	TWD	TWD	TWD	tttT
Ancestral	TWD	AIN	AIN	AIN	taaA

Preliminary results

- Cancer vs. controls
  - ~700-4500 DEGs

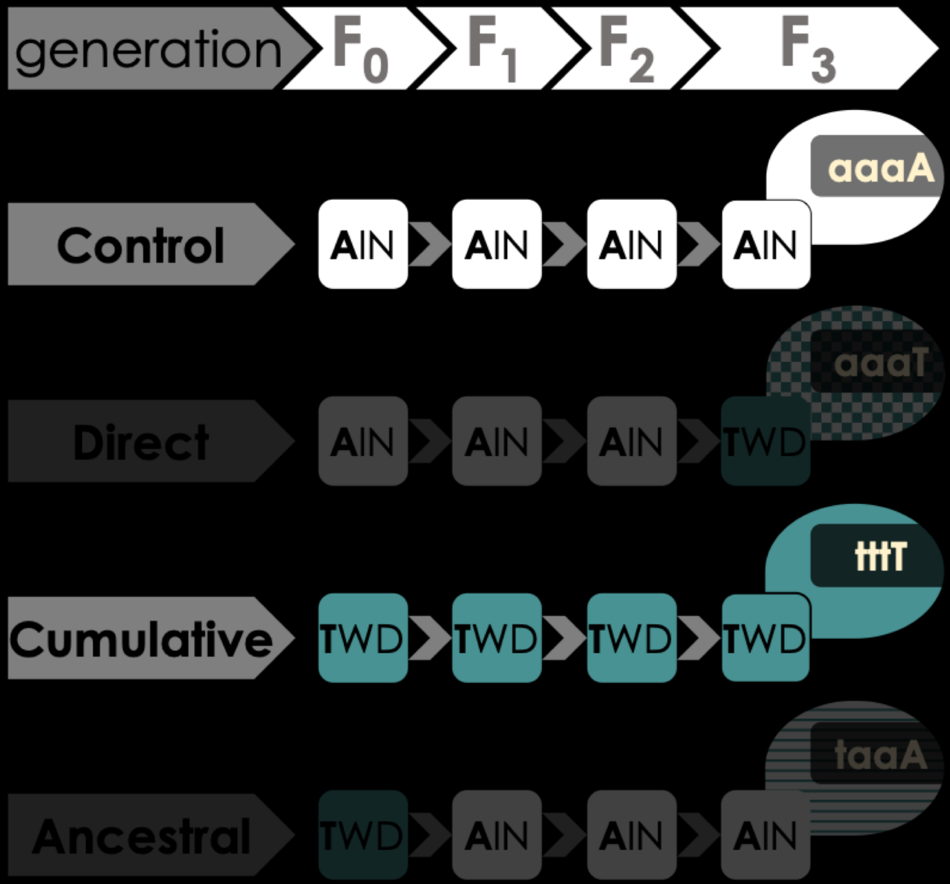
Study design



Preliminary results

- Cancer vs. controls
  - ~700-4500 DEGs
- Cancer cohorts
  - aaaA vs aaaT
    36 DEGs

Study design



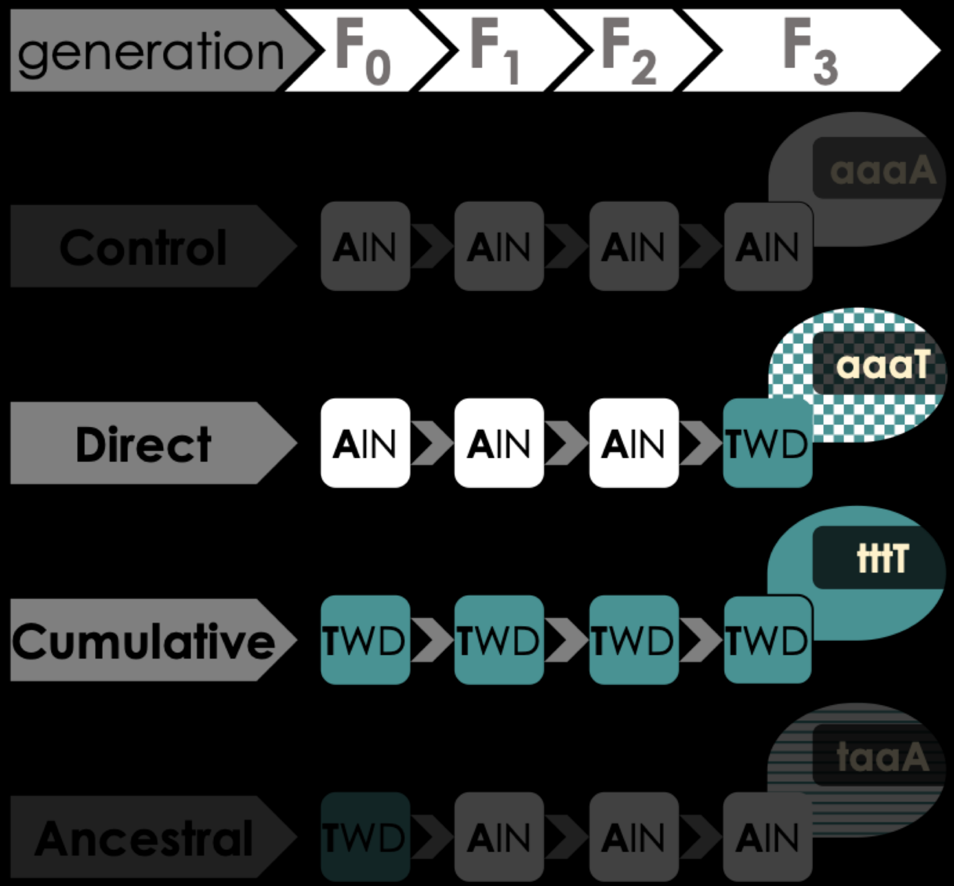
generation	F_0	F_1	F_2	F_3	
Control	AIN	AIN	AIN	AIN	aaaA
Direct	AIN	AIN	AIN	TWD	aaaT
Cumulative	TWD	TWD	TWD	TWD	tttT
Ancestral	TWD	AIN	AIN	AIN	taaA

UtahStateUniversity
ANIMAL, DAIRY & VETERINARY SCIENCES

Preliminary results

- Cancer vs. controls
  - ~700-4500 DEGs
- Cancer cohorts
  - aaaA vs aaaT
    36 DEGs
  - aaaA to tttT
    119 DEGs

Study design



Preliminary results

- Cancer vs. controls
  - ~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

- Multigenerational exposure to the Western dietary pattern may alter gene expression and health outcome in offspring.

Fehlker et al. 2014. BMC Cancer.
Kosmidis et al. 2018. J Cancer.

Sun et al. 2018. PeerJ.
Wong et al. 2007. Gut.

Summary & conclusions

- Multigenerational exposure to the Western dietary pattern may alter gene expression and health outcome in offspring.
  - defense/immune response: down regulation promotes CRC metastasis
  - response to interferon: down regulation promotes CRC development

Fehlker et al. 2014. BMC Cancer.
Kosmidis et al. 2018. J Cancer.

Sun et al. 2018. PeerJ.
Wong et al. 2007. Gut.

UtahStateUniversity
ANIMAL, DAIRY & VETERINARY SCIENCES

Summary & conclusions

- Multigenerational exposure to the Western dietary pattern may alter gene expression and health outcome in offspring.
  - defense/immune response: down regulation promotes CRC metastasis
  - response to interferon: down regulation promotes CRC development
- Metanalysis of clinical CRC gene expression signature reveals DEGs related to immune function.

Fehlker et al. 2014. BMC Cancer.
Kosmidis et al. 2018. J Cancer.

Sun et al. 2018. PeerJ.
Wong et al. 2007. Gut.

UtahStateUniversity
ANIMAL, DAIRY & VETERINARY SCIENCES

Summary & conclusions

- Multigenerational exposure to the Western dietary pattern may alter gene expression and health outcome in offspring.
  - defense/immune response: down regulation promotes CRC metastasis
  - response to interferon: down regulation promotes CRC development
- Metanalysis of clinical CRC gene expression signature reveals DEGs related to immune function.
- Many DEGs in human CRC are associated with aberrant DNA methylation.

Fehlker et al. 2014. BMC Cancer.
Kosmidis et al. 2018. J Cancer.

Sun et al. 2018. PeerJ.
Wong et al. 2007. Gut.

UtahStateUniversity
ANIMAL, DAIRY & VETERINARY SCIENCES

Summary & conclusions

- Multigenerational exposure to the Western dietary pattern may alter gene expression and health outcome in offspring.
  - defense/immune response: down regulation promotes CRC metastasis
  - response to interferon: down regulation promotes CRC development
- 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.

Fehlker et al. 2014. BMC Cancer.
Kosmidis et al. 2018. J Cancer.

Sun et al. 2018. PeerJ.
Wong et al. 2007. Gut.

UtahStateUniversity
ANIMAL, DAIRY & VETERINARY SCIENCES

Acknowledgements

Benninghoff Laboratory

USTAR Applied Nutrition Research

USDA NIFA/AFRI Grant 2014-67017-21755

Utah Agricultural Experiment Station Project UTA-01178