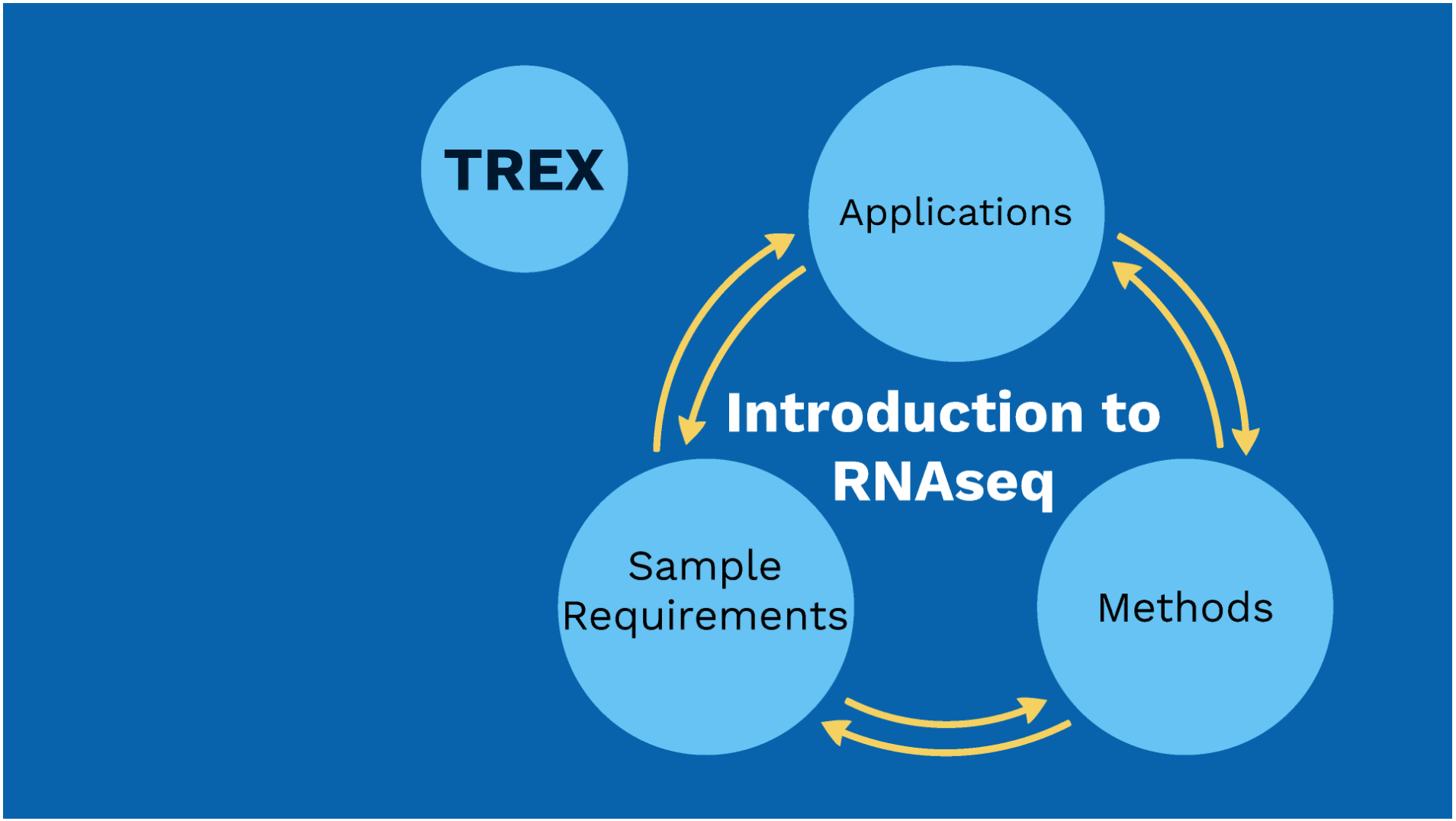
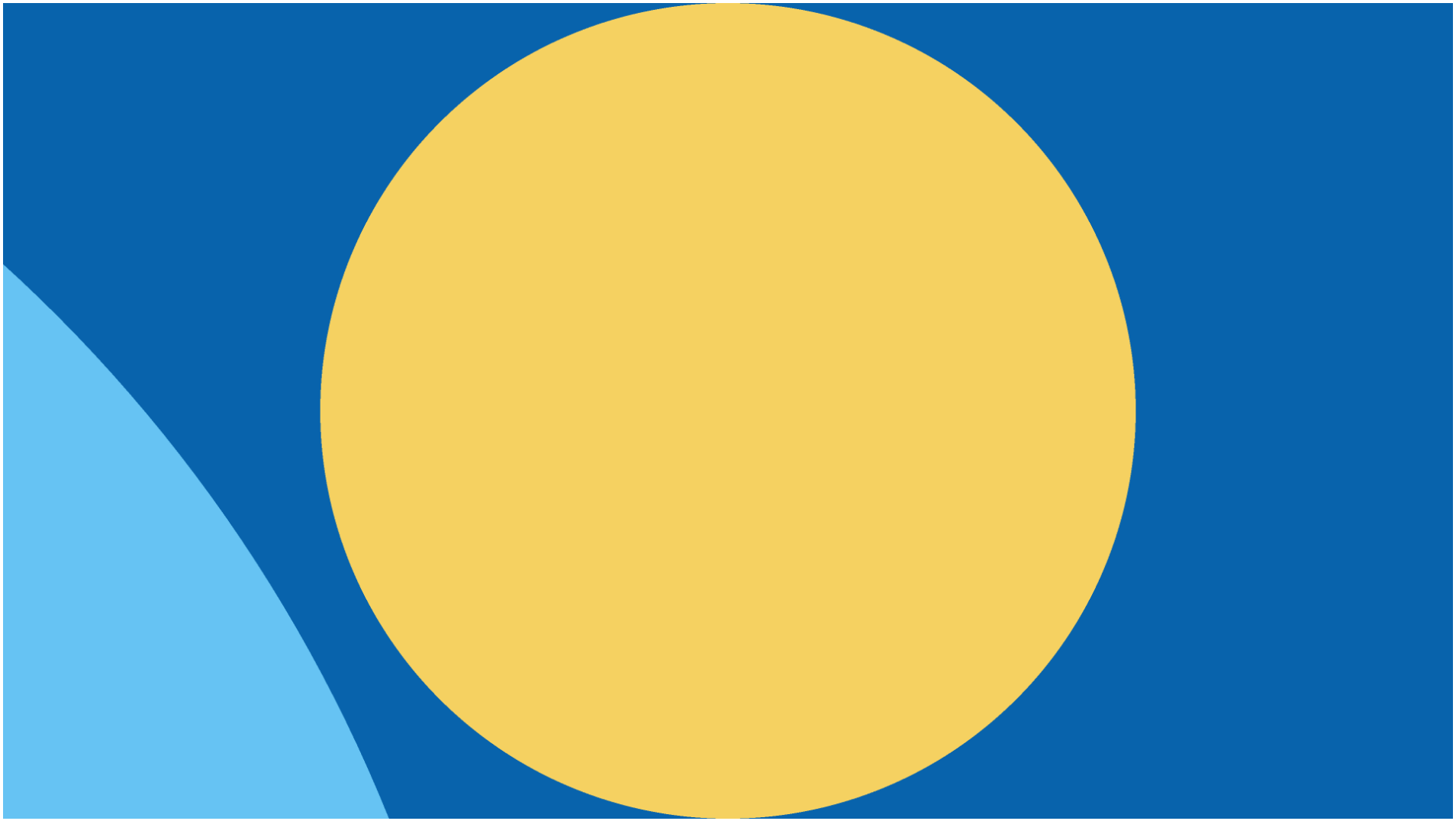
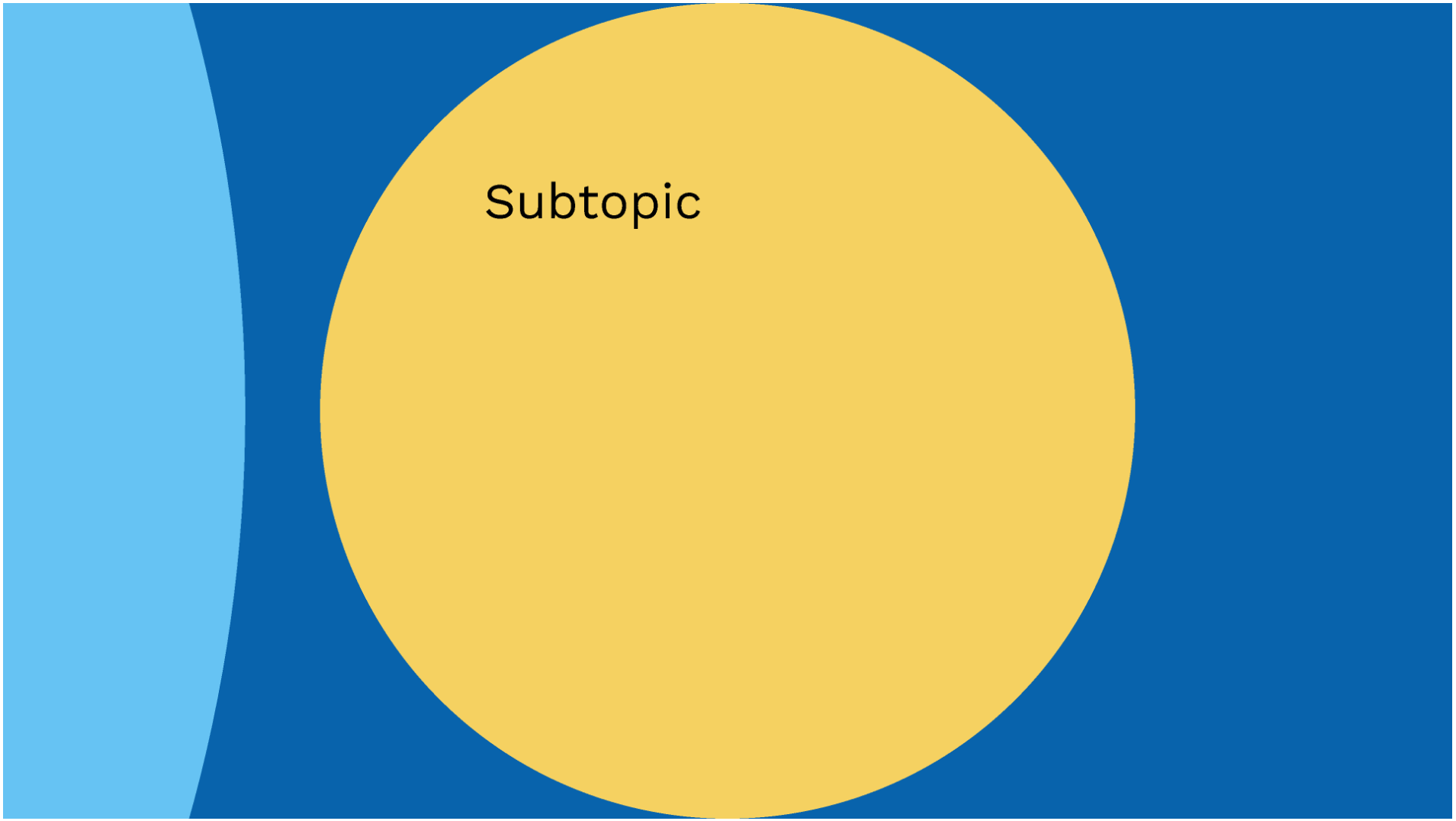
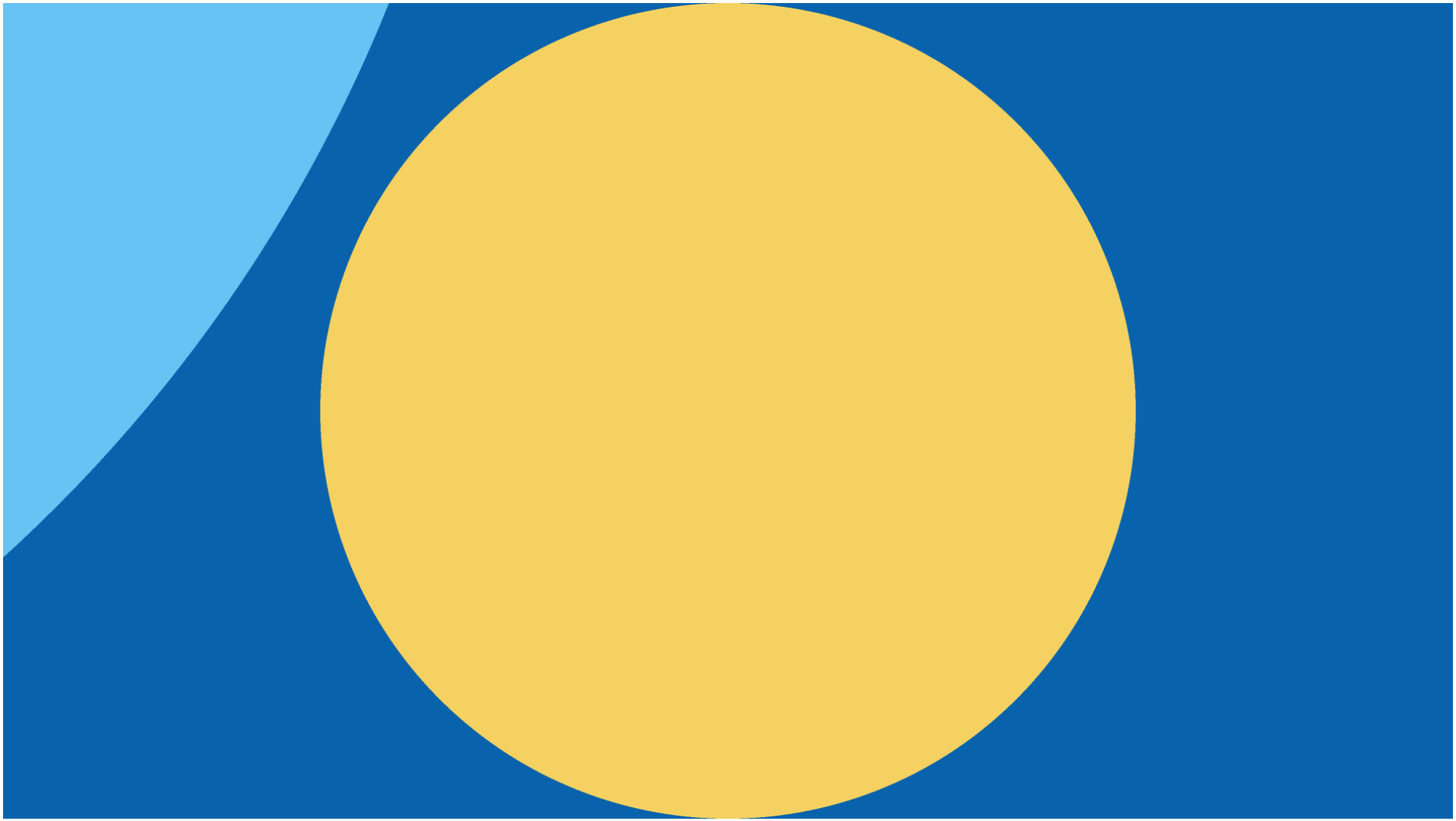
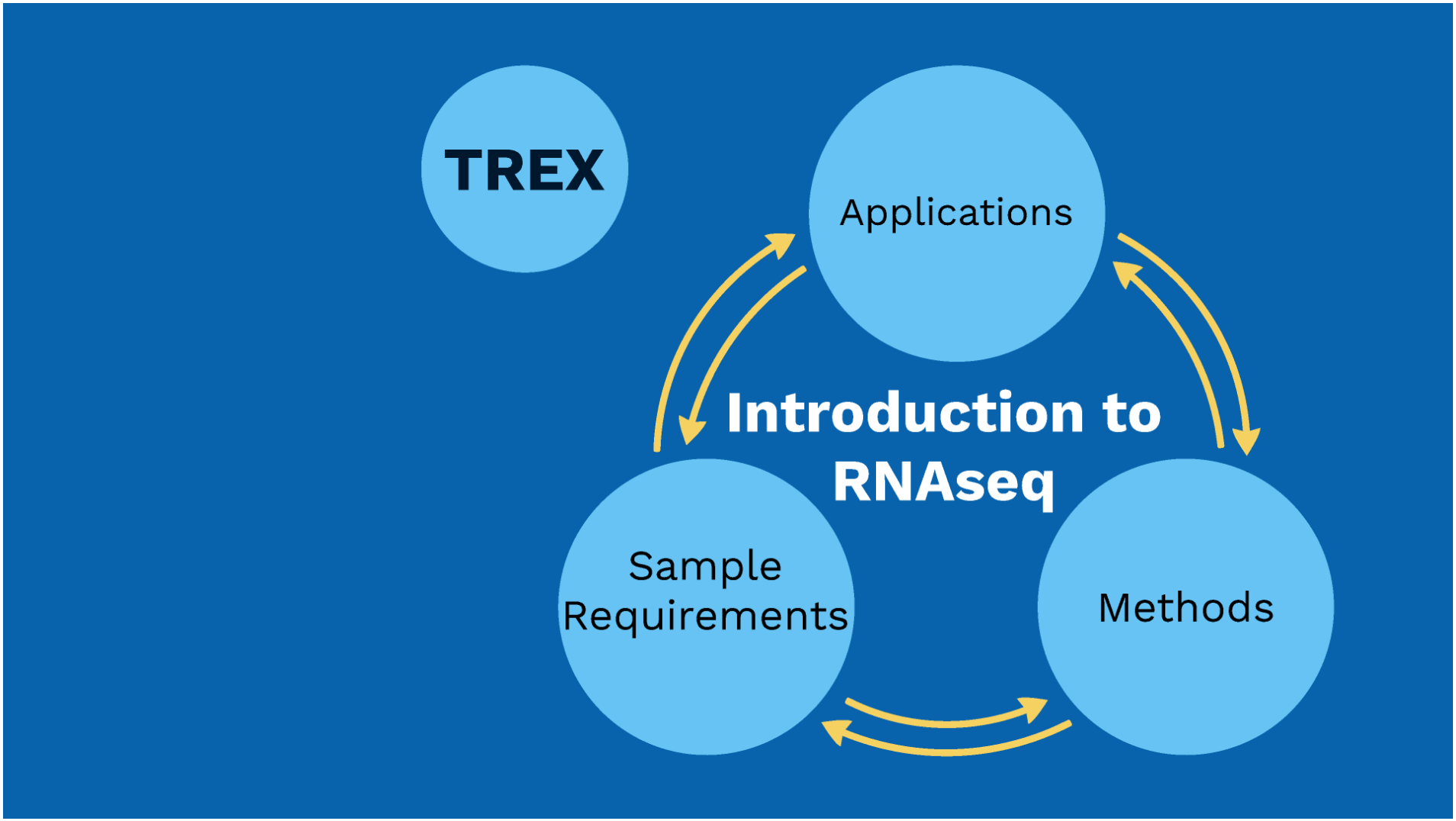

TREX
Applications
Introduction to RNAseq
Sample Requirements
Methods

Having high quality RNA is the MOST important thing you can do to insure the success of your experiment!
RNA Handling
RNA Extraction
RNA QC




Subtopic




TREX
Applications
Introduction to RNAseq
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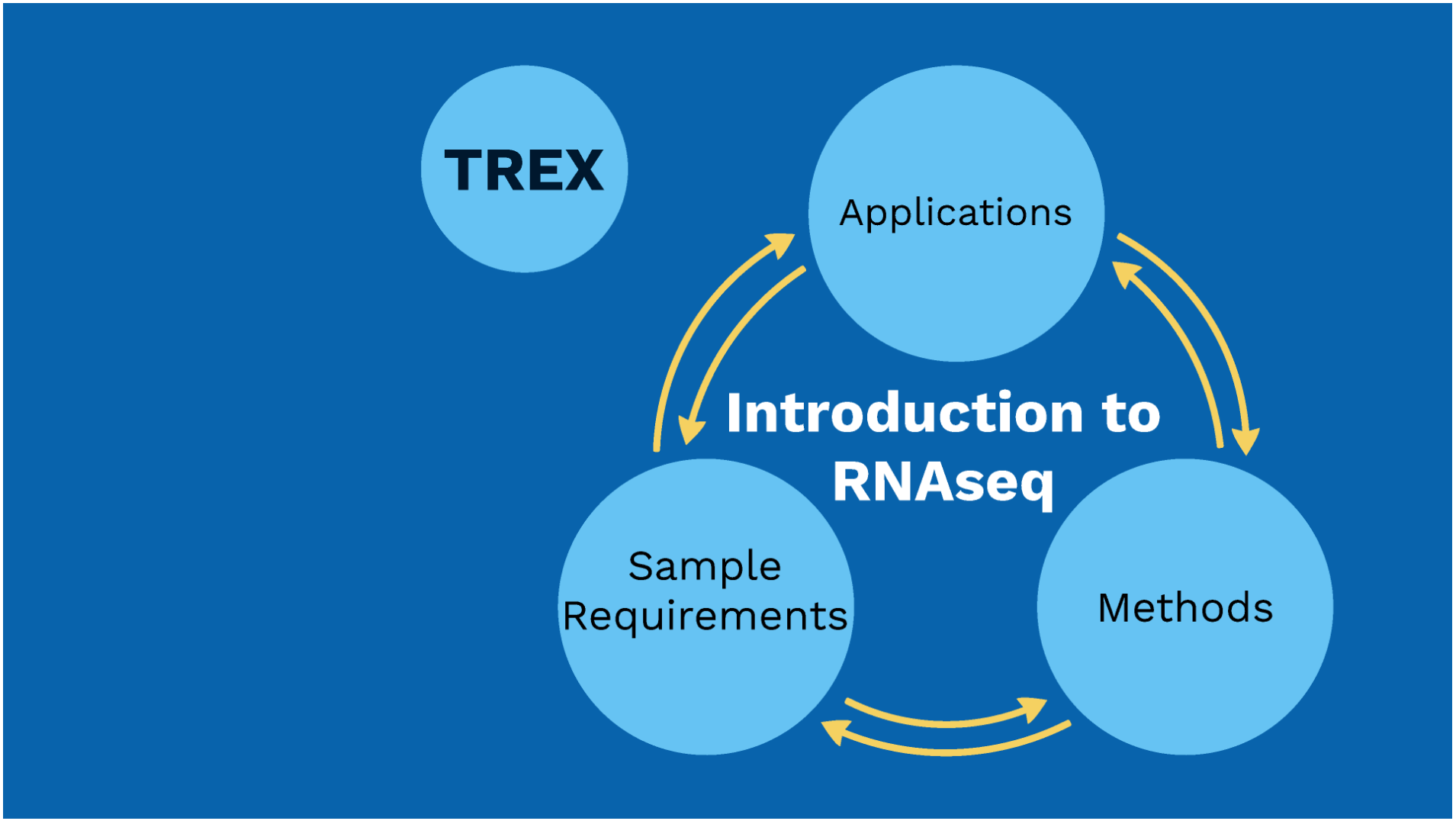
Why do RNASeq ?

- Gene Expression Profiling
  - Reference (Annotated)
  - De Novo (discovery)
- Variant Analysis or Discovery
- Pathogen ID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- What are my research goals?
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Lexogen 3' RNASeq
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NEBNext Ultra II
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Why Choose Lexogen 3'RNA Seq?

High Throughput: BRC Service requires >32 samples

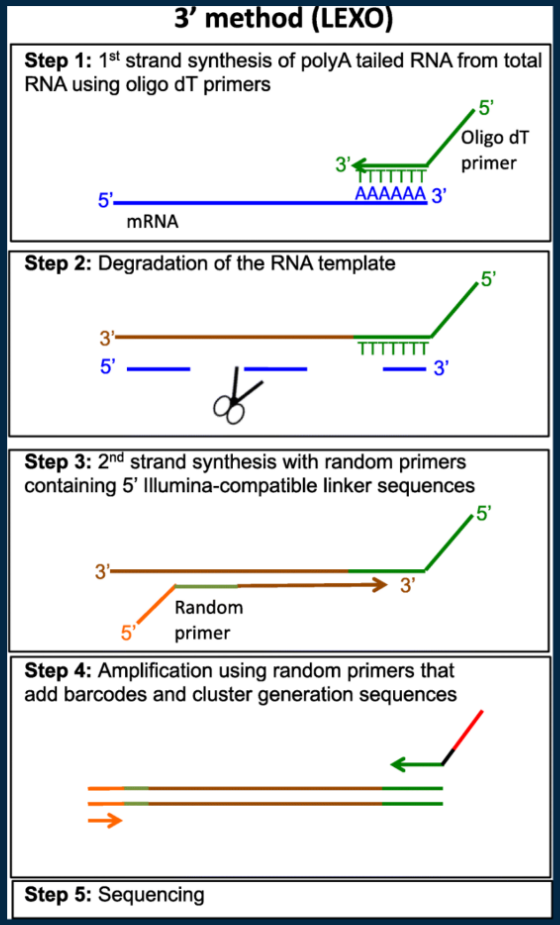
Experimental design tolerant of dropouts

The information you are interested in is at the 3' end of the RNA strand

Tolerant of:

- Input concentration diversity
- RNA quality diversity

Prep Chemistry


3’ method (LEXO)
Step 1: 1st strand synthesis of polyA tailed RNA from total RNA using oligo dT primers
5’
Oligo dT primer
3’
AAAAAA 3’
5’
mRNA
Step 2: Degradation of the RNA template
5’
3’
5’
3’
Step 3: 2nd strand synthesis with random primers containing 5’ Illumina-compatible linker sequences
5’
3’
3’
5’
Random primer
Step 4: Amplification using random primers that add barcodes and cluster generation sequences
Step 5: Sequencing

Why Choose Truseq RNA

Flexible:

- Input type:
  - Total RNA
  - Ribosomal Depleted RNA
  - Poly A Selected RNA
- Input Concentration: 1000-100ng
- RNA Integrity: Intact OR Degraded
- Sample Number: <384

Directional Or Non-Directional

Highly Supported

Chemistry

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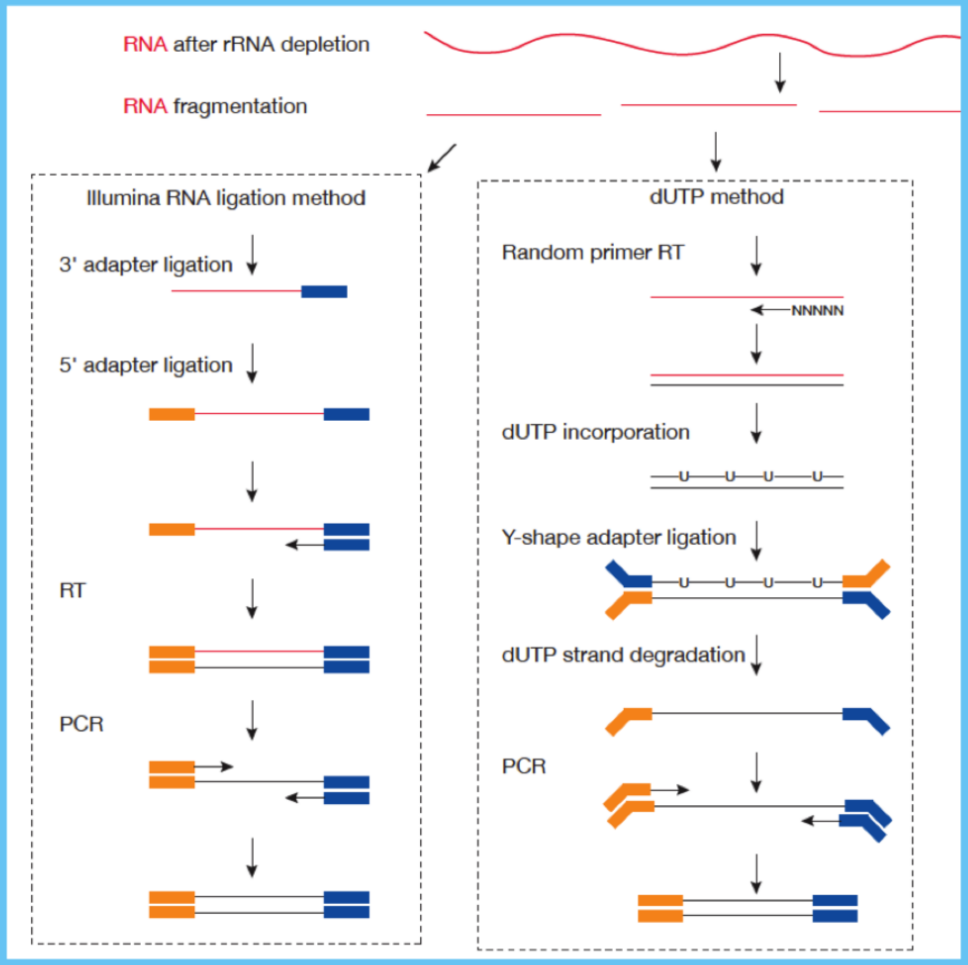
RNA after rRNA depletion
RNA fragmentation
Illumina RNA ligation method
3' adapter ligation
5' adapter ligation
RT
PCR
dUTP method
Random primer RT
NNNNN
dUTP incorporation
U U U U
Y-shape adapter ligation
dUTP strand degradation
PCR

Why choose NEB Next Ultra II

Flexible:

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  - Ribosomal Depleted RNA
  - Poly A Selected RNA
- Input Concentration: 1000-100ng
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- Sample Number: <384

Fully Supported

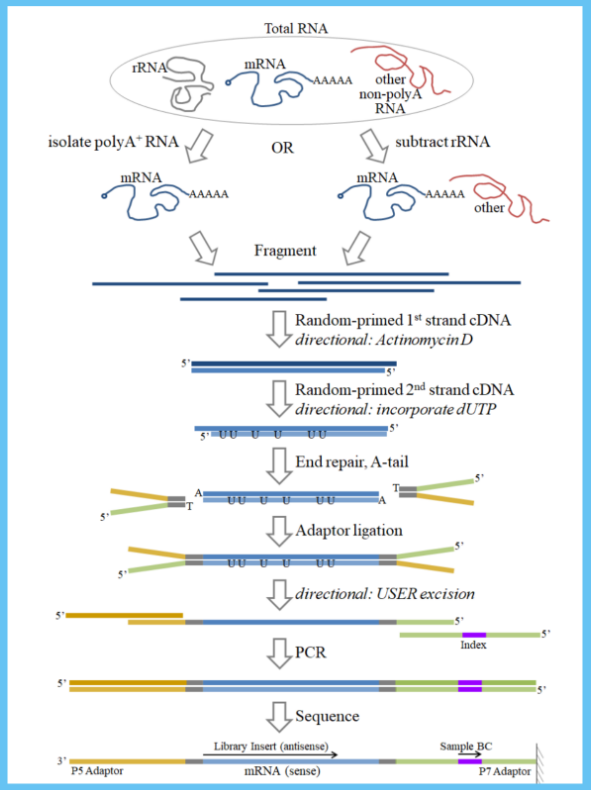
Directional Or Non-Directional

Modular

Prep Chemistry

Decision Tree

NEB Next Ultra II

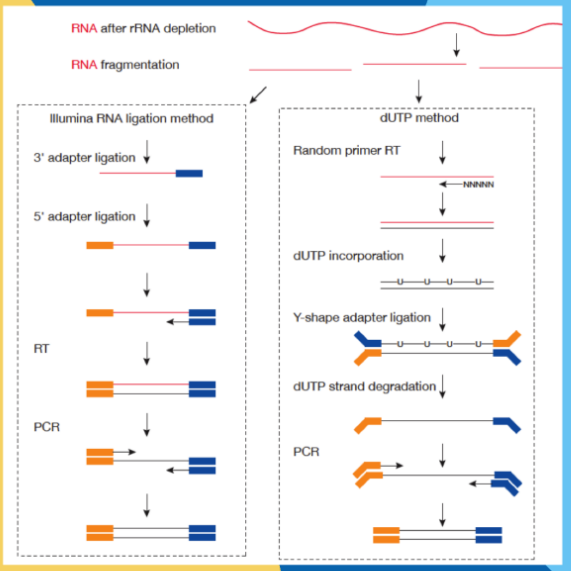


Poly A

Ribosomal Depletion

Directional vs. Non-Directional

Illumina Truseq



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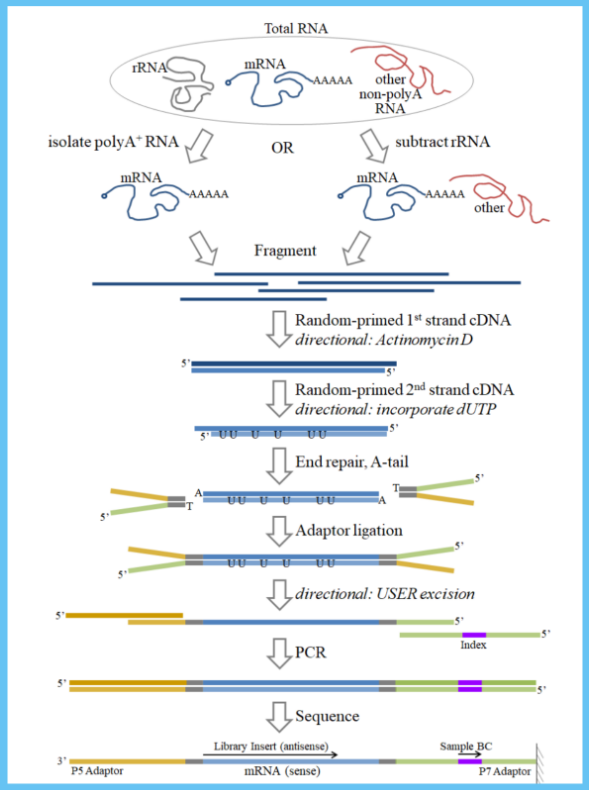

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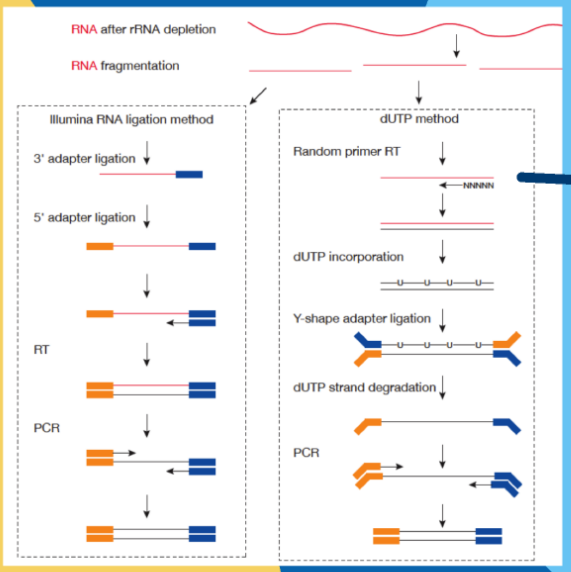

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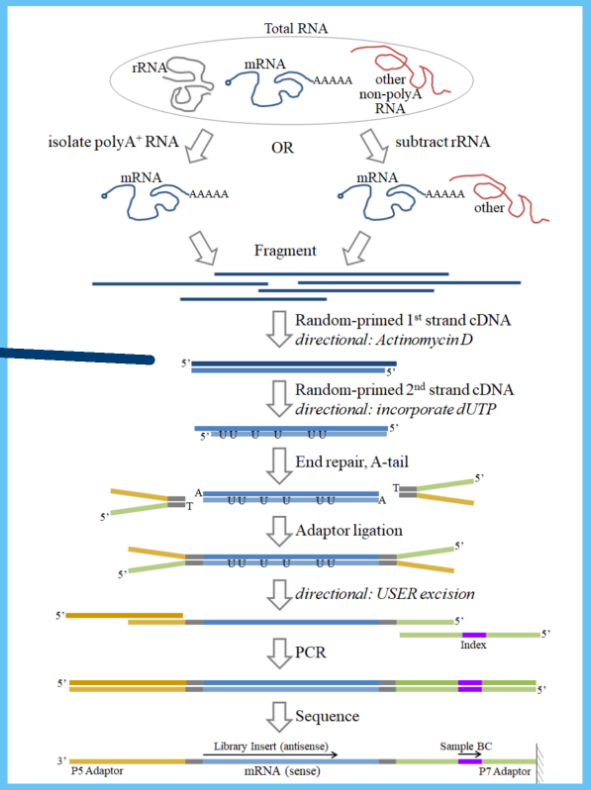

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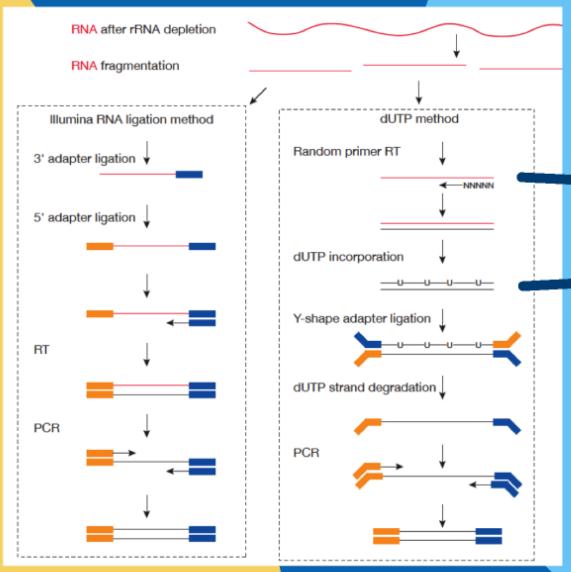

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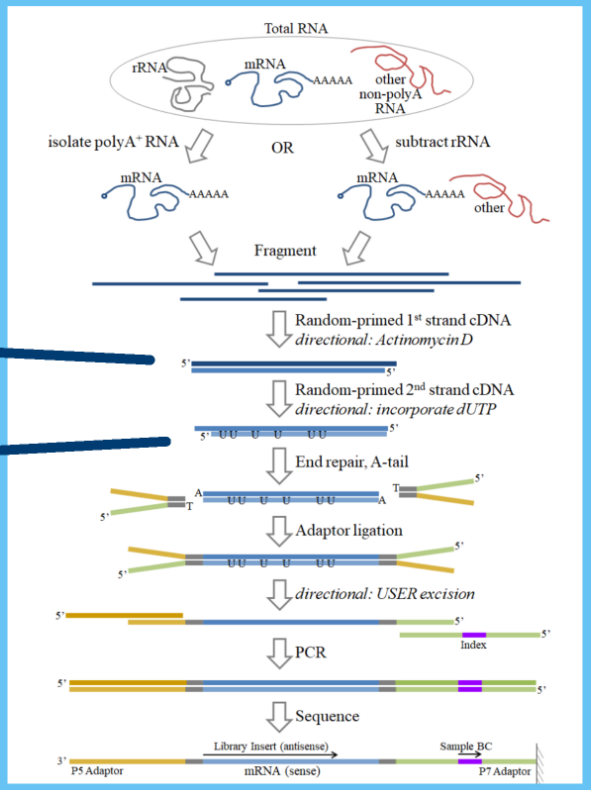

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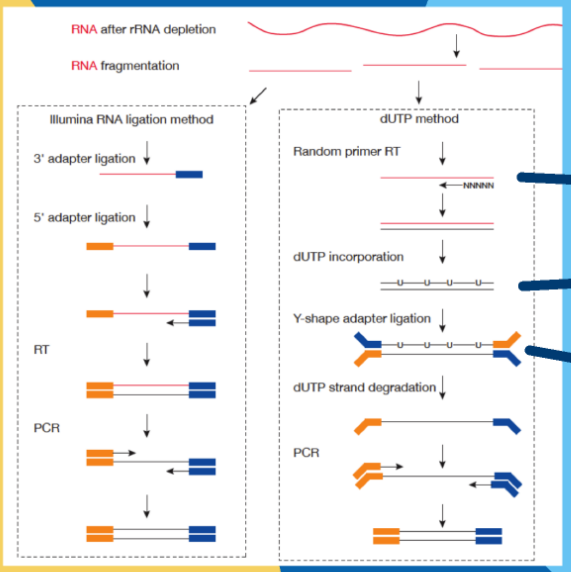

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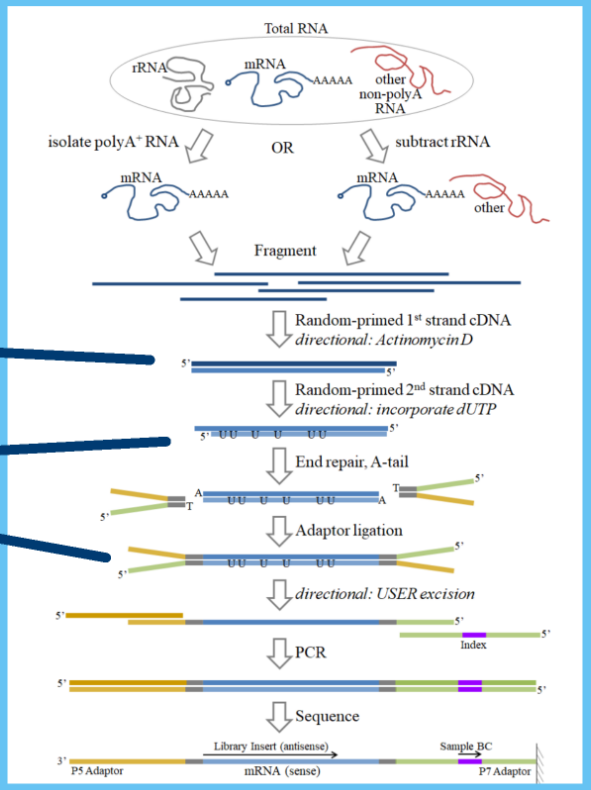

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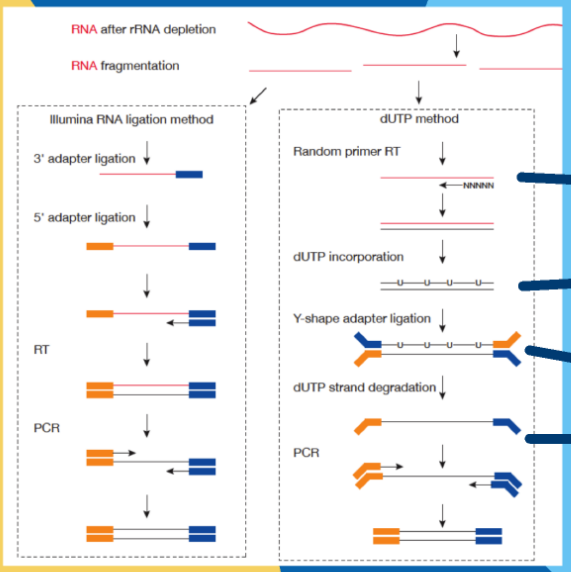

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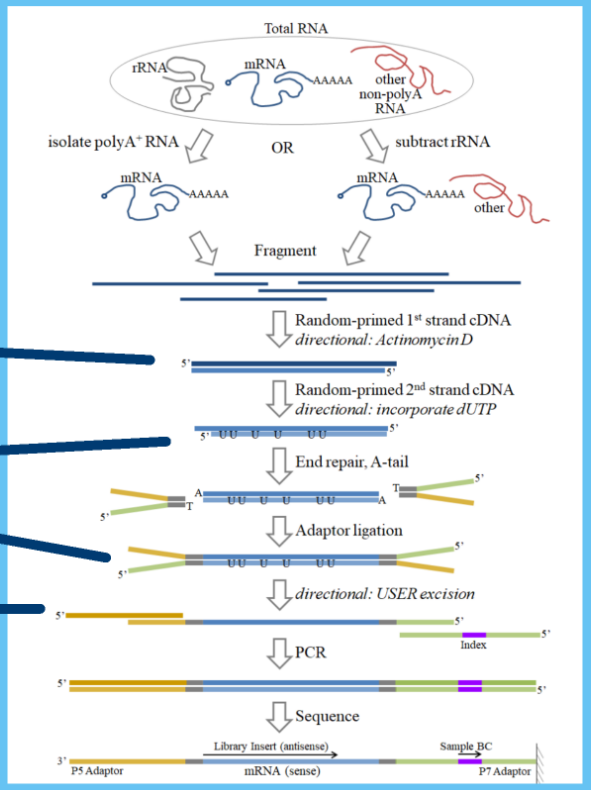

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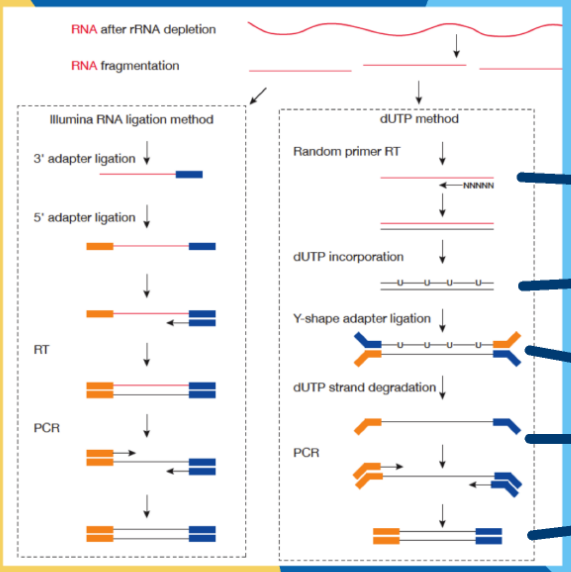

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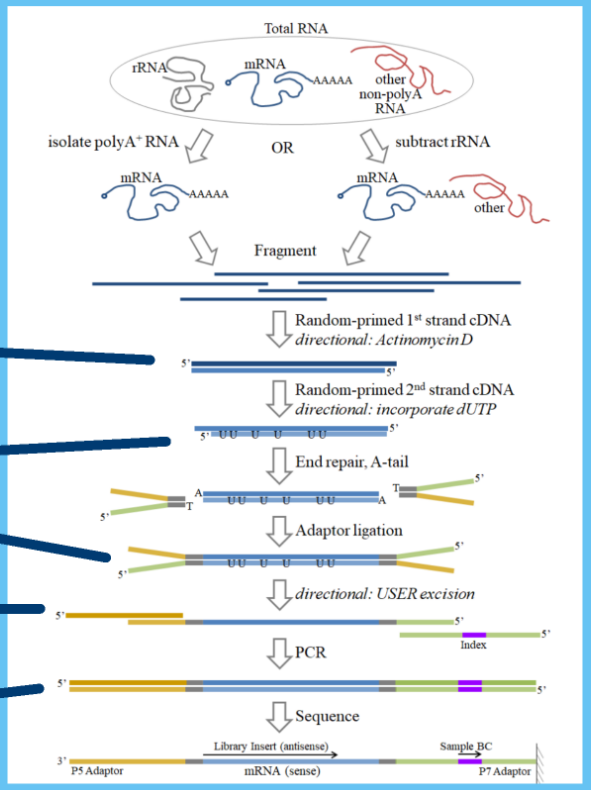

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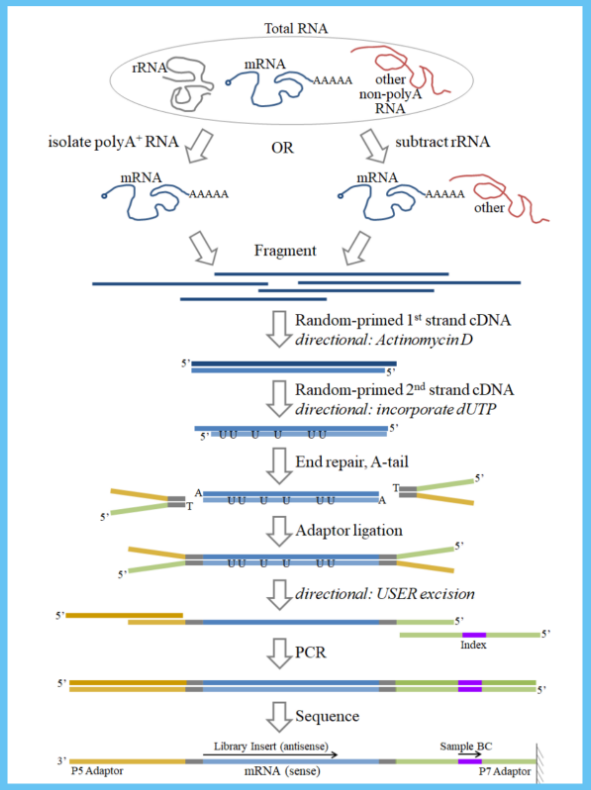

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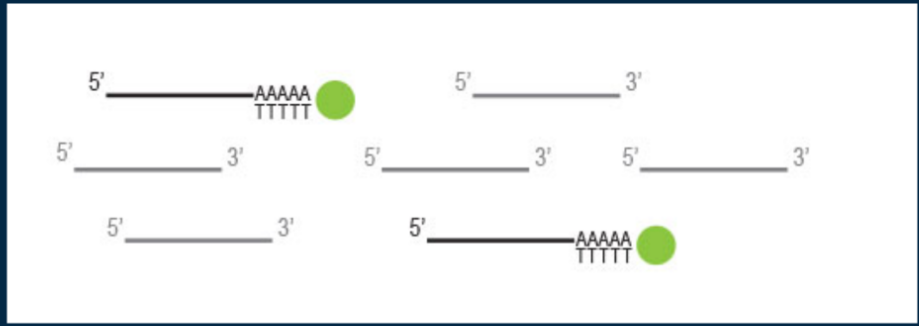

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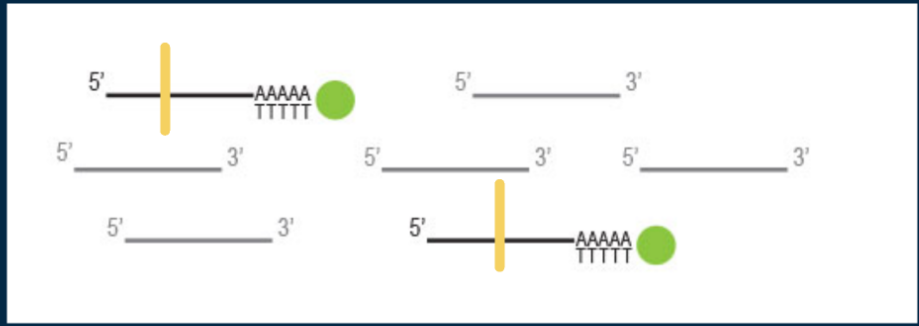

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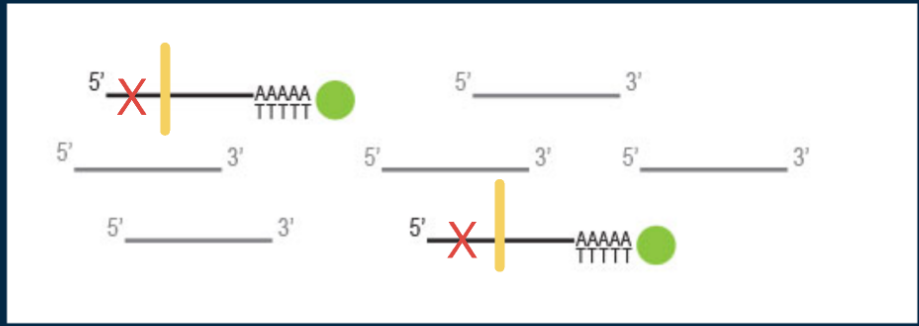

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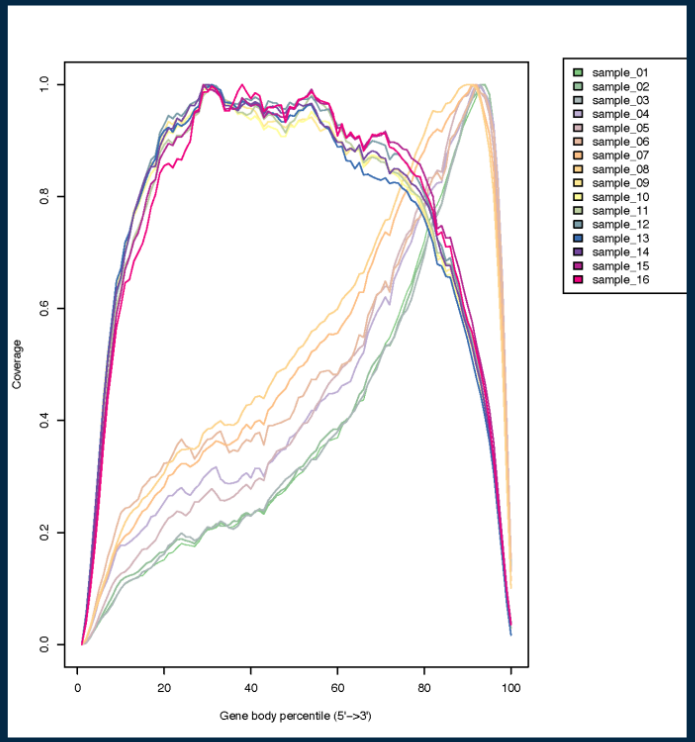
3' Bias



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  - Bacteria don't have a Poly A tail
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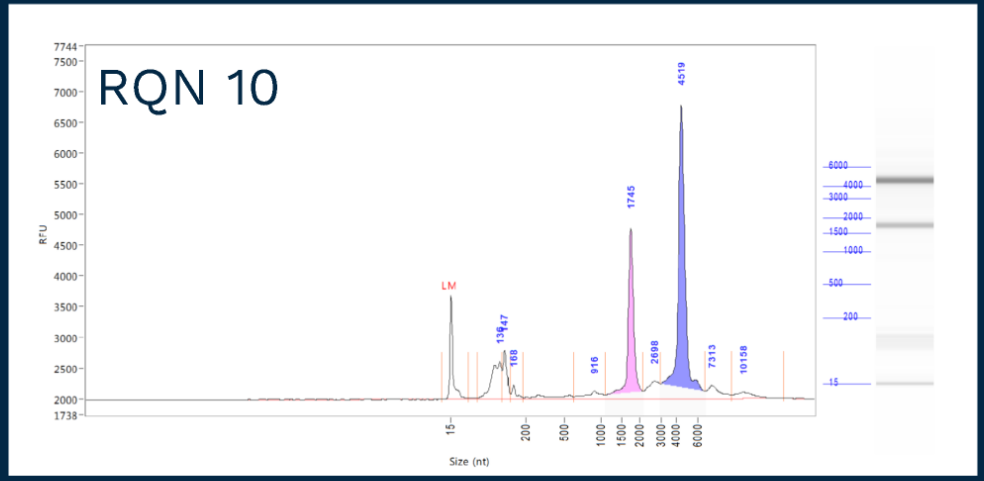

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  - If you are looking for RNA's that don't have a Poly A tail, ex: LNC RNA
- If your RNA is intact:
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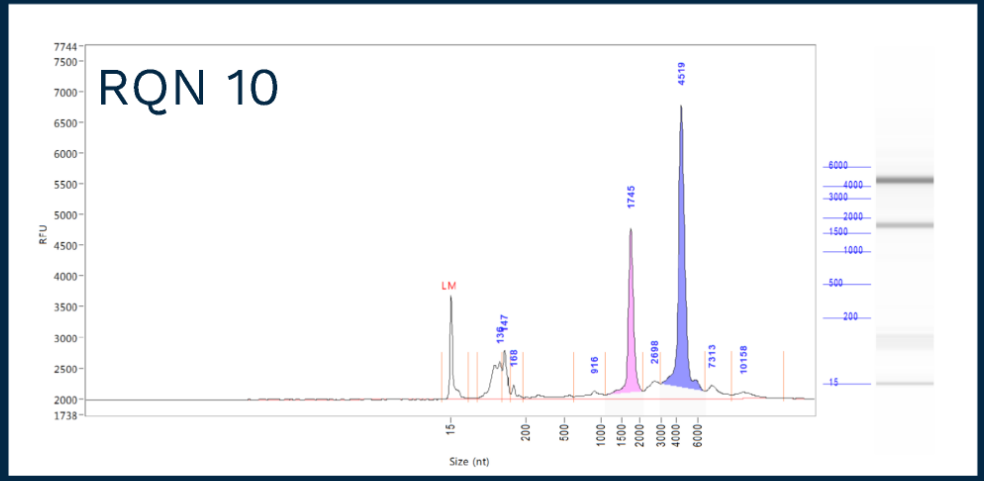
- If you don't care about 3' bias



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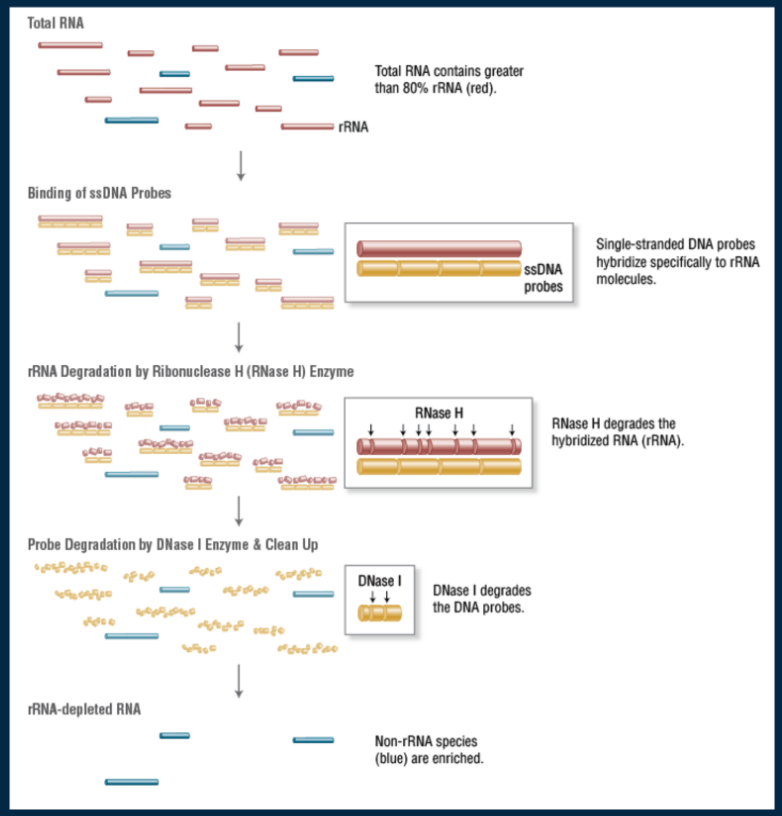
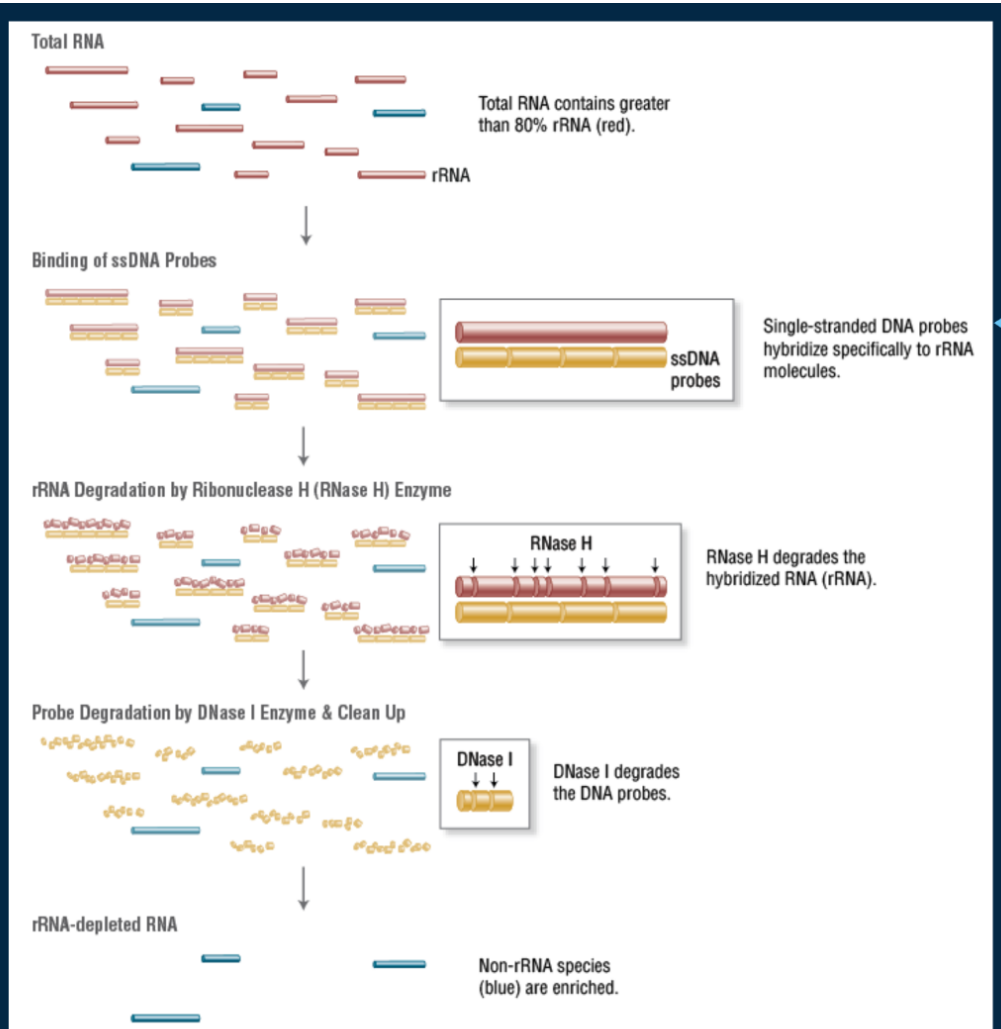
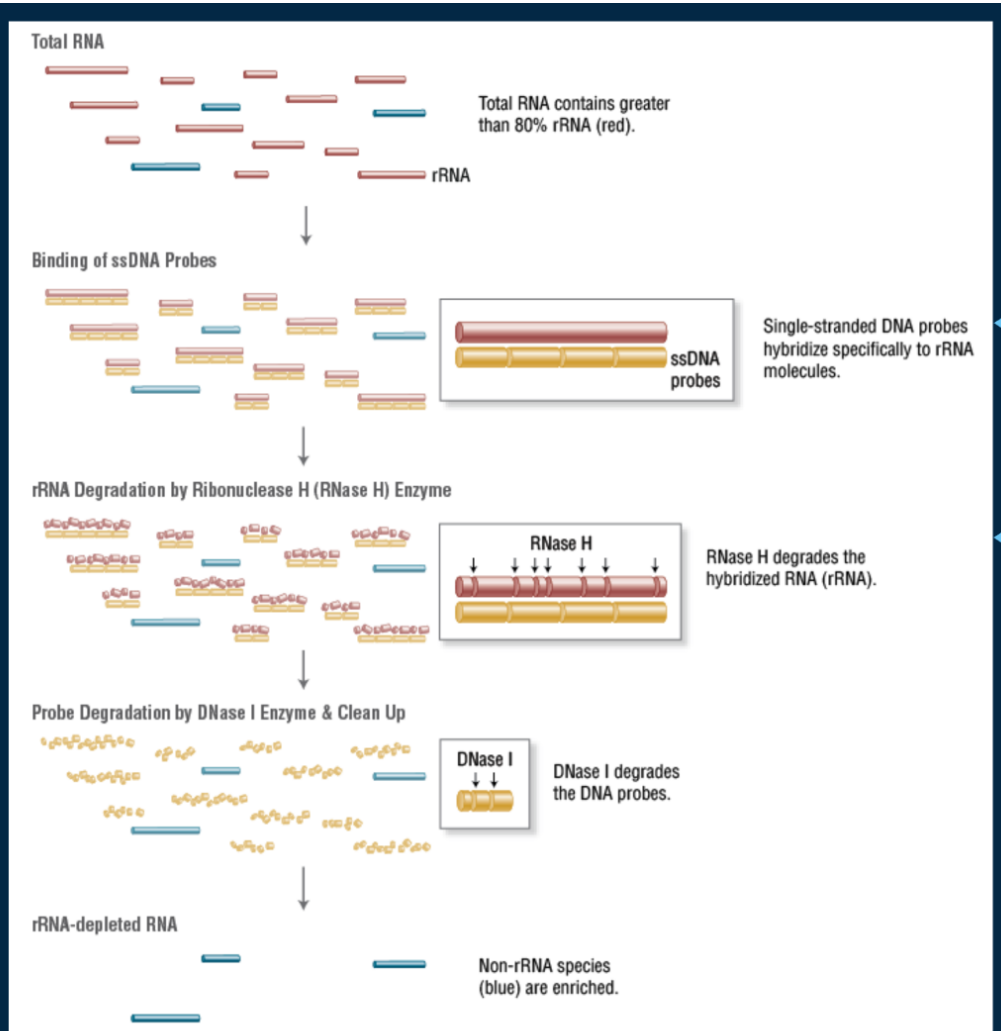


Total RNA
Total RNA contains greater than 80% rRNA (red).
rRNA
Binding of ssDNA Probes
ssDNA probes
Single-stranded DNA probes hybridize specifically to rRNA molecules.
rRNA Degradation by Ribonuclease H (RNase H) Enzyme
RNase H
RNase H degrades the hybridized RNA (rRNA).
Probe Degradation by DNase I Enzyme & Clean Up
DNase I
DNase I degrades the DNA probes.
rRNA-depleted RNA
Non-rRNA species (blue) are enriched.

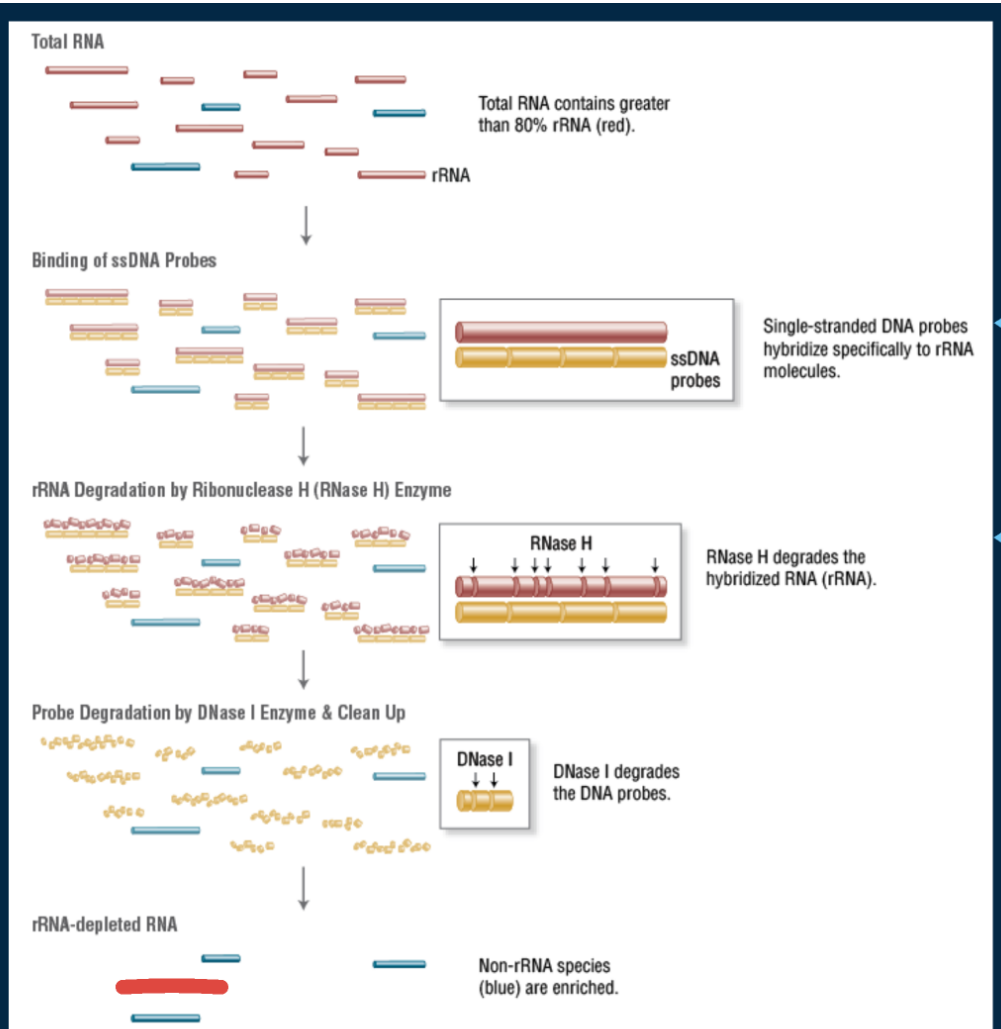


ssDNA probes are organism specific



ssDNA probes are organism specific

ONLY the hybridized RNA is degraded



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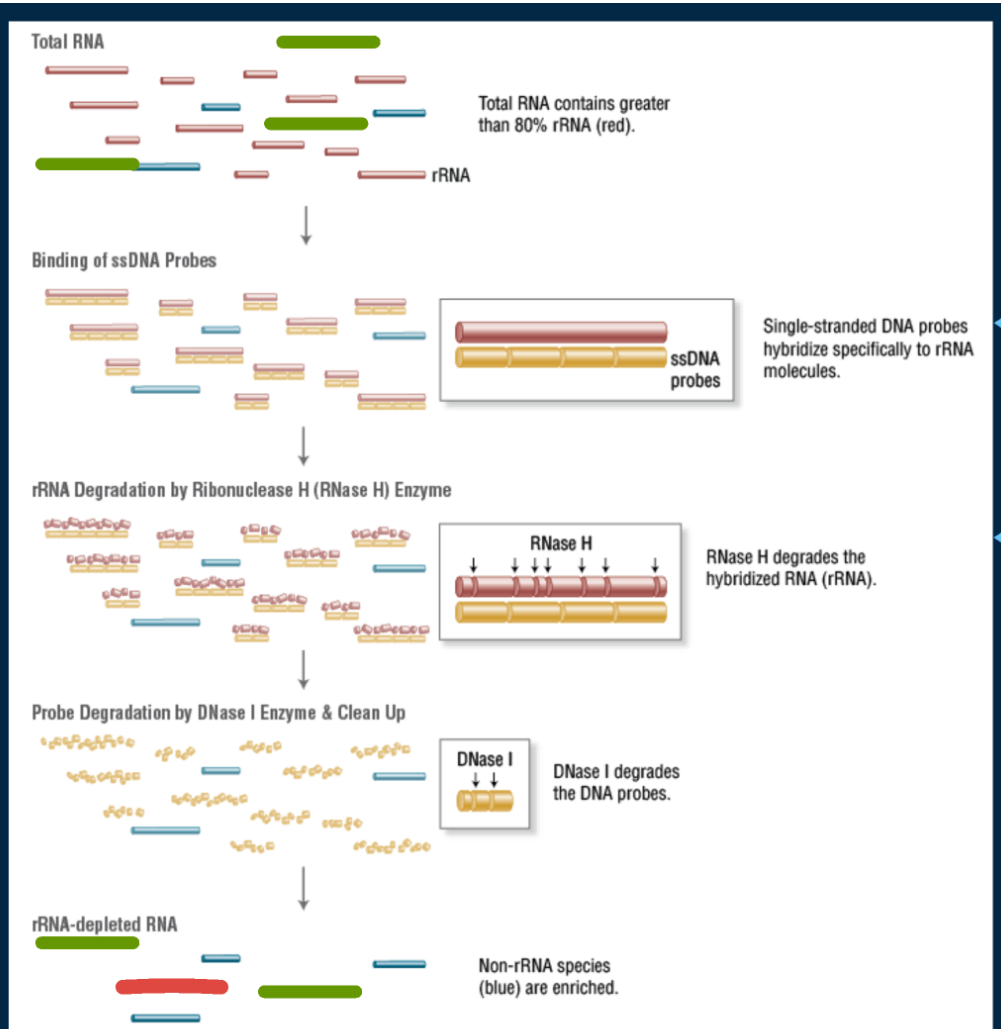

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Housekeeping RNAs

Why Choose Ribosomal Depletion?

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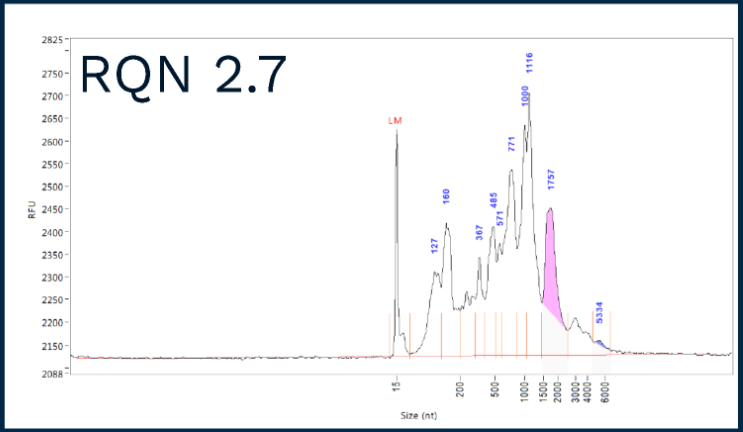
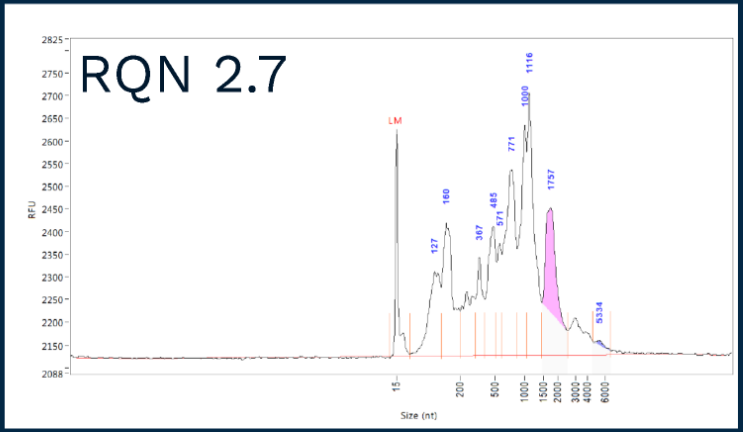


Why Choose Ribosomal Depletion?

If your RNA is degraded: RQN <7

If your organism is not compatable with Poly A



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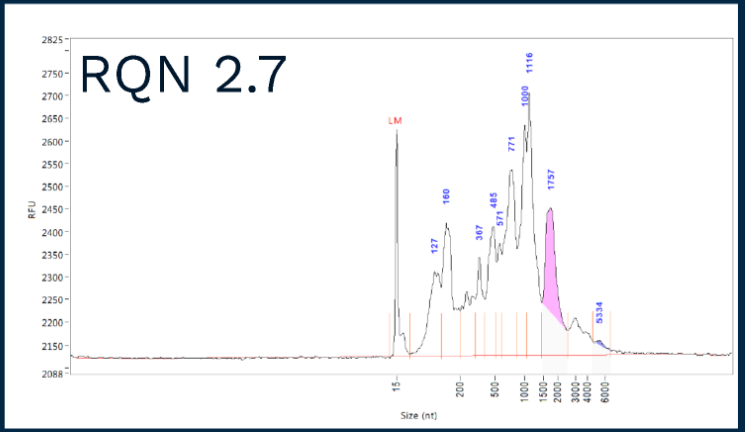
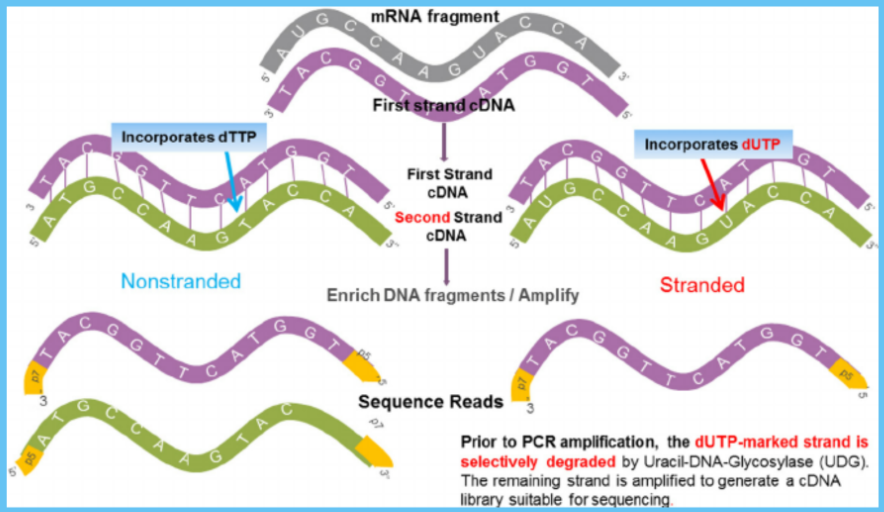
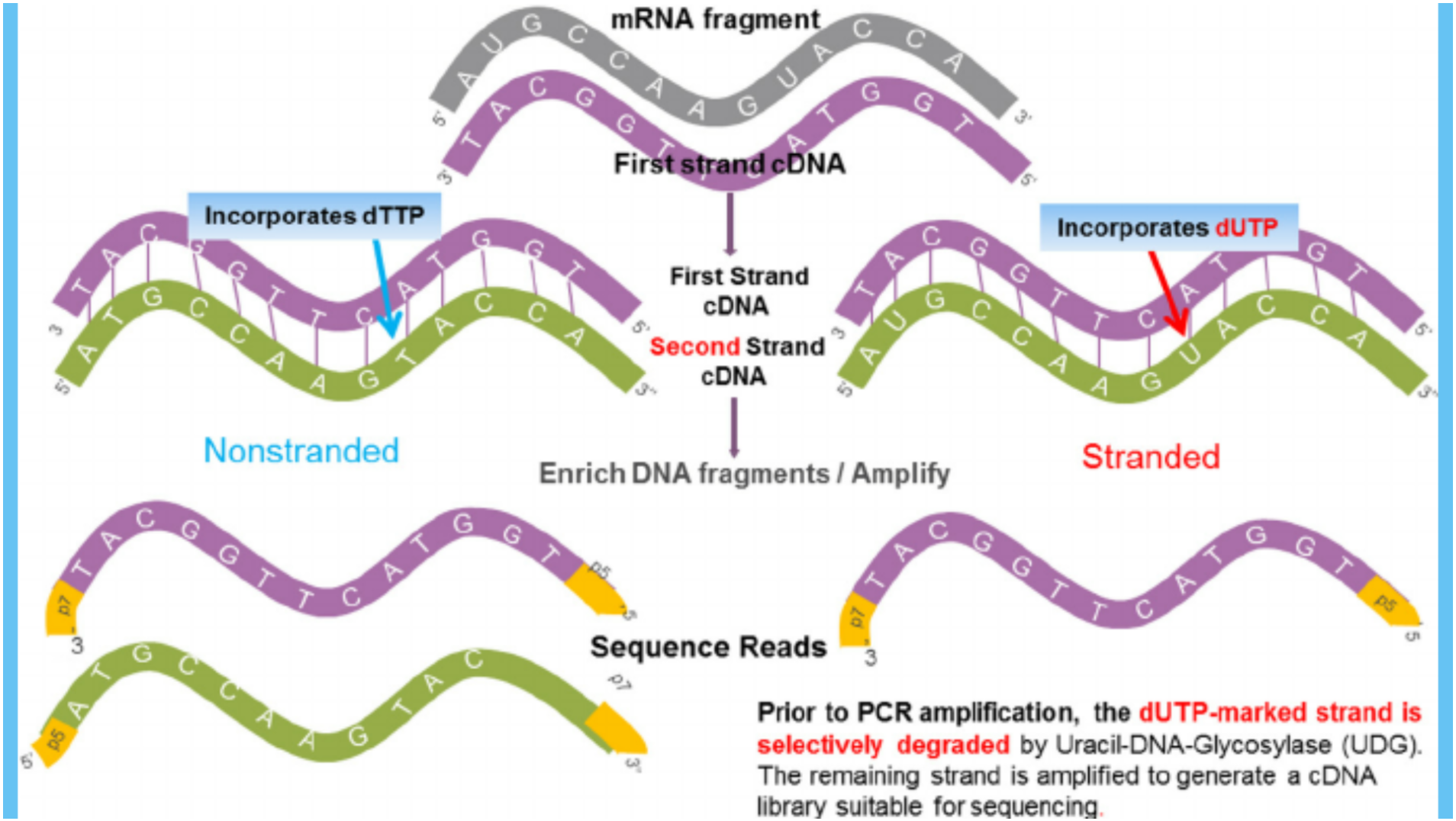
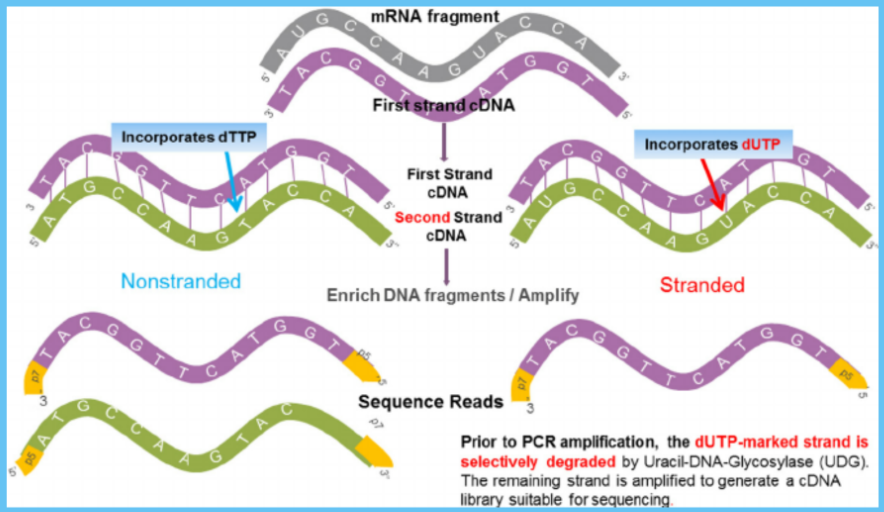


What is the difference between Directional and Nondirectional?




mRNA fragment
First strand cDNA
Incorporates dTTP
Incorporates dUTP
First Strand cDNA
Second Strand cDNA
Nonstranded
Stranded
Enrich DNA fragments / Amplify
Sequence Reads
Prior to PCR amplification, the dUTP-marked strand is selectively degraded by Uracil-DNA-Glycosylase (UDG). The remaining strand is amplified to generate a cDNA library suitable for sequencing.

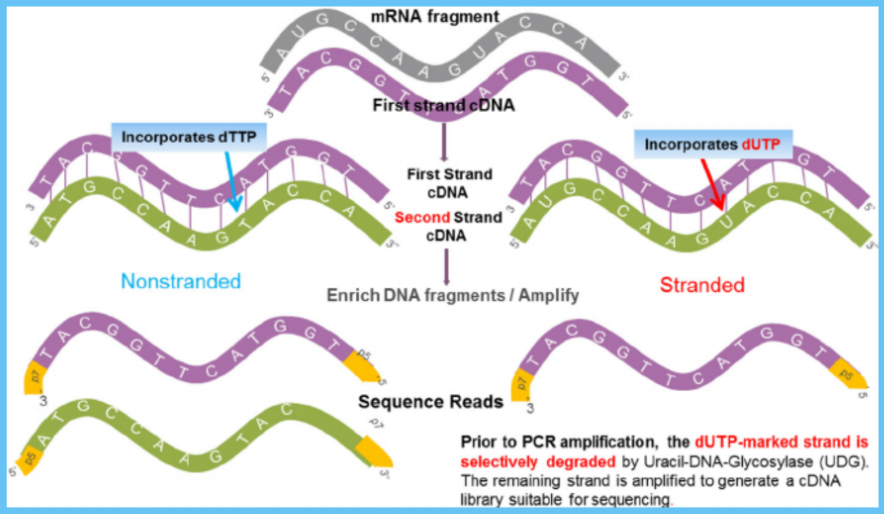
What is the difference between Directional and Nondirectional?



What is the difference between Directional and Nondirectional?

Why choose Directional?

- More information
  - Which strand your RNA is being transcribed from
  - More accurate count of genes in differential expression analysis



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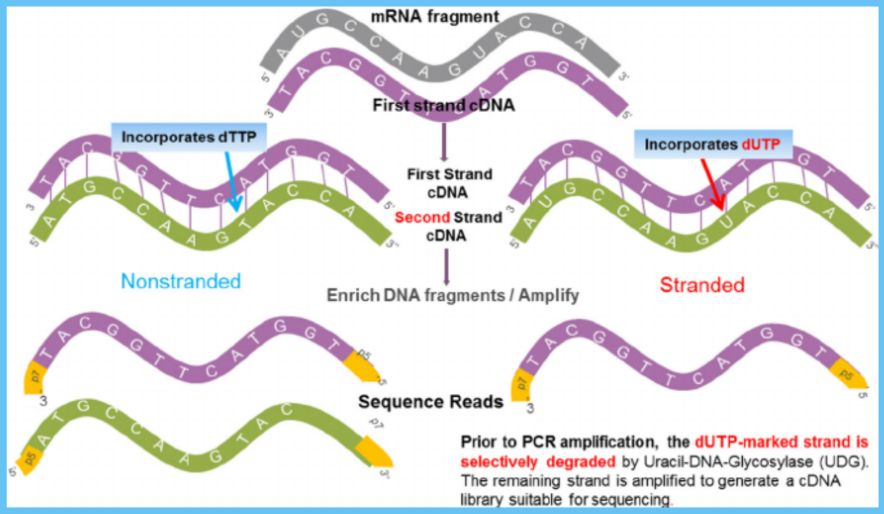
Why choose Nondirectional?

- If you have <10ng of total RNA



What is the difference between Directional and Nondirectional?

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  - Which strand your RNA is being transcribed from
  - More accurate count of genes in differential expression analysis

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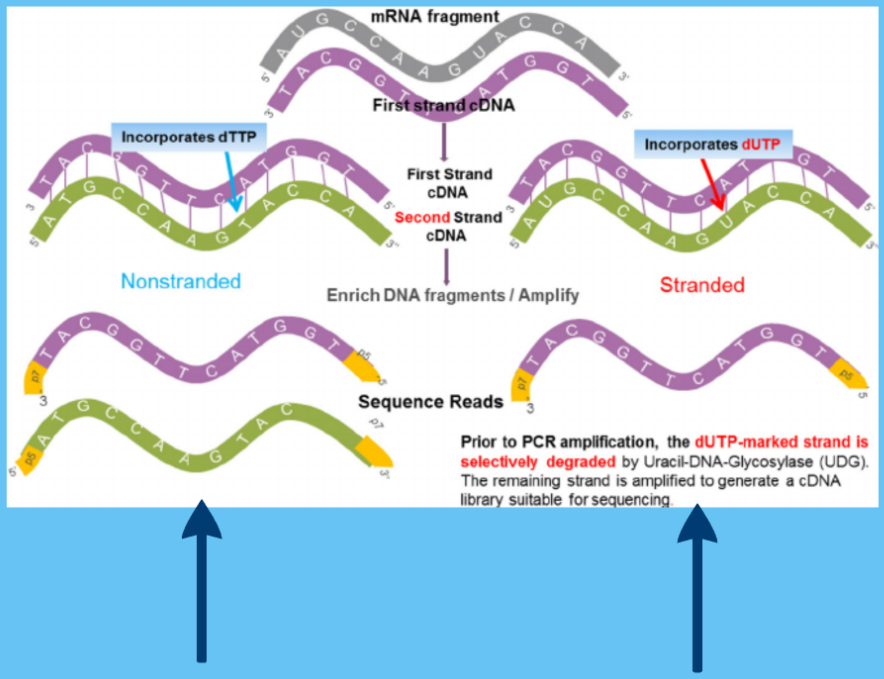

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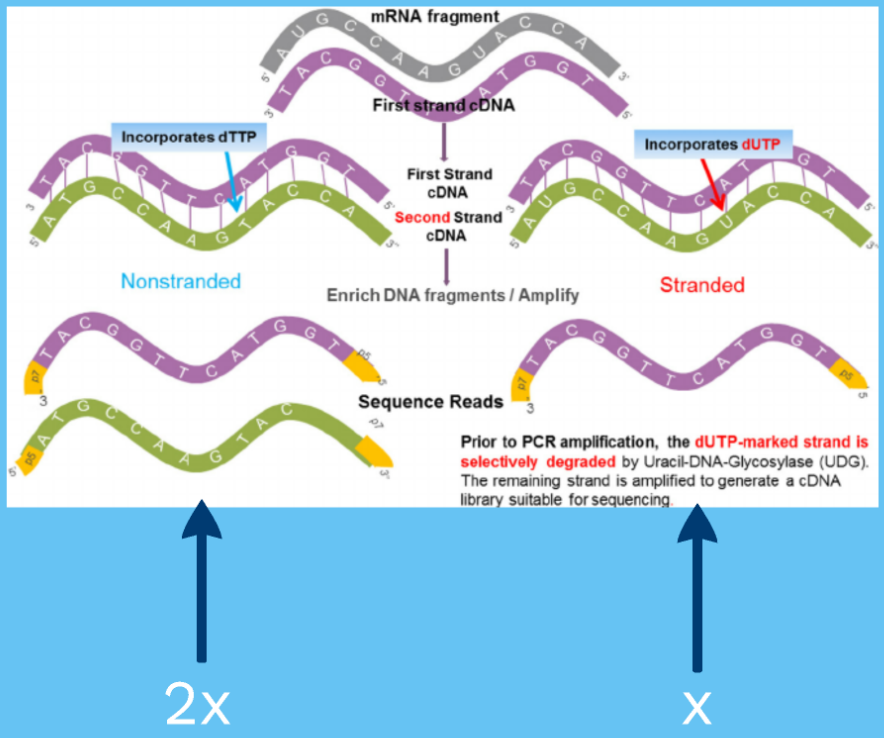
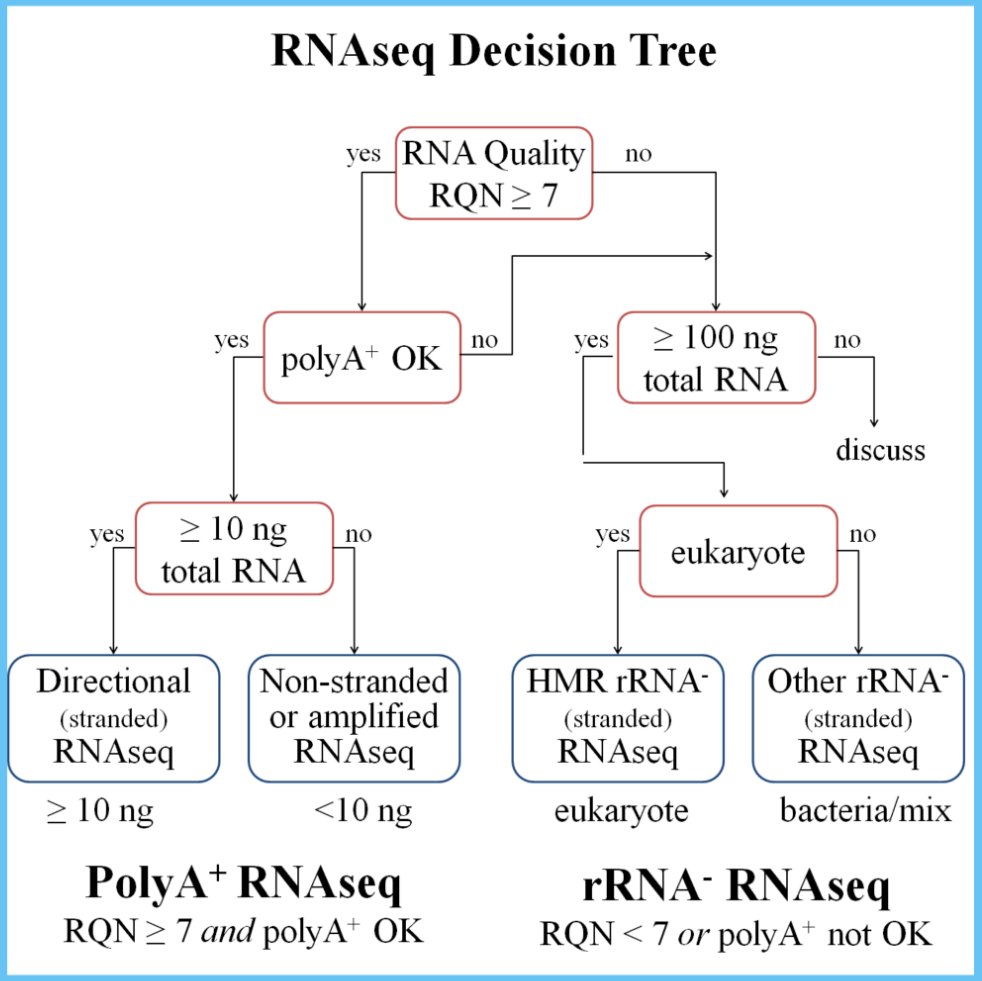
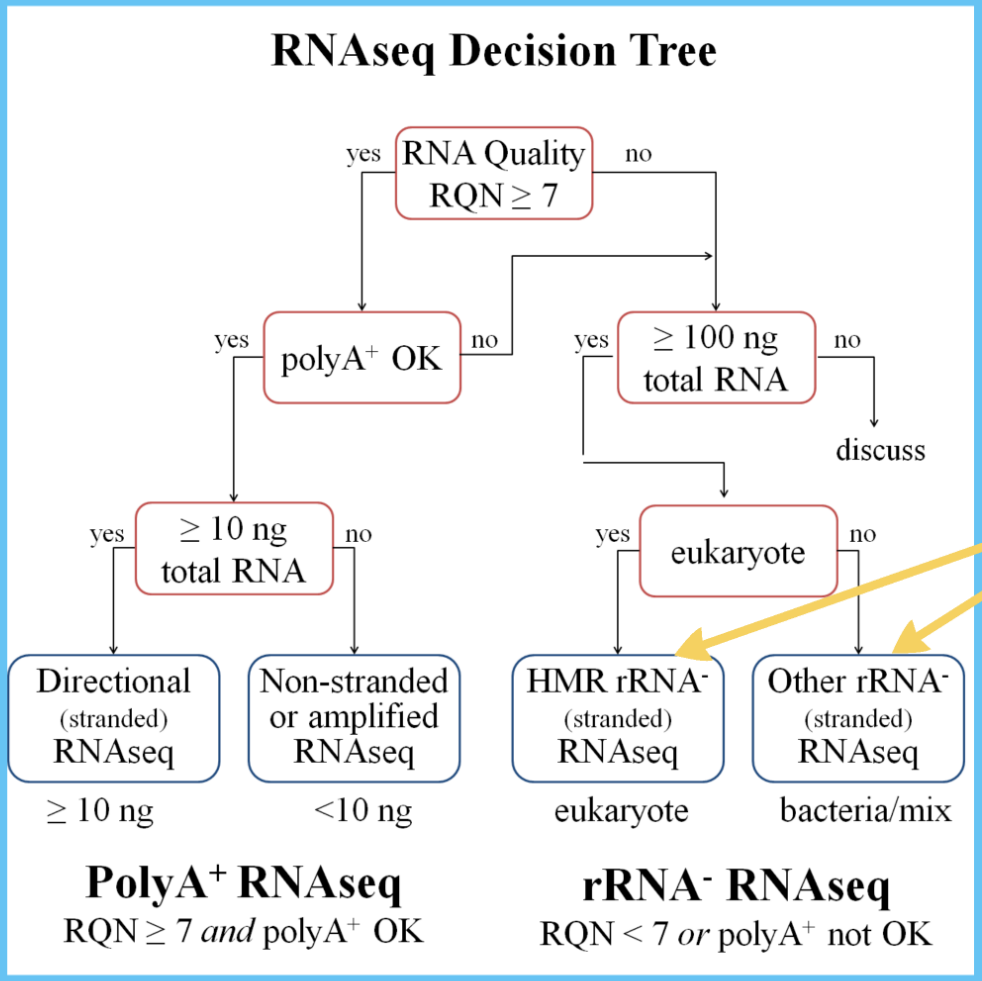
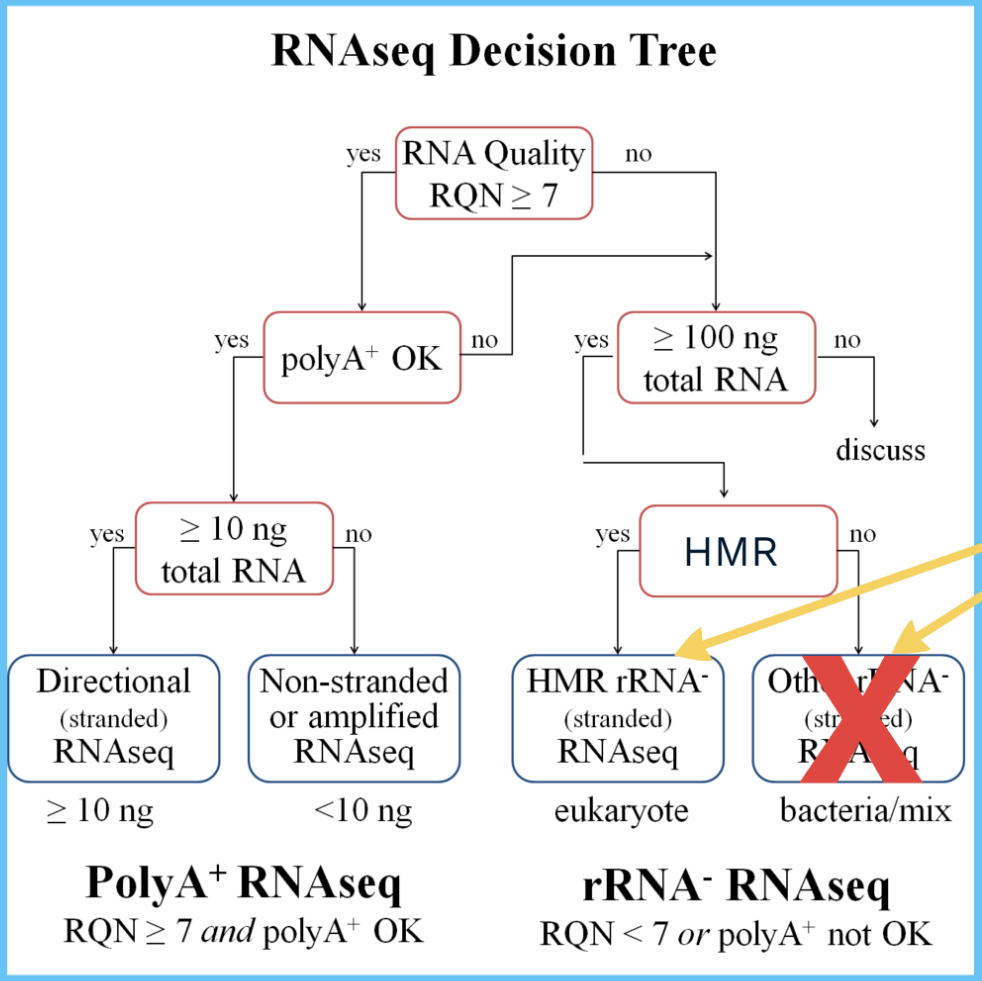



RNAseq Decision Tree
RNA Quality
RQN ≥ 7
yes
no
polyA+ OK
yes
no
≥ 100 ng
total RNA
yes
no
discuss
≥ 10 ng
total RNA
yes
no
eukaryote
yes
no
Directional
(stranded)
RNAseq
Non-stranded
or amplified
RNAseq
HMR rRNA-
(stranded)
RNAseq
Other rRNA-
(stranded)
RNAseq
≥ 10 ng
<10 ng
eukaryote
bacteria/mix
PolyA+ RNAseq
RQN ≥ 7 and polyA+ OK
rRNA- RNAseq
RQN < 7 or polyA+ not OK



Ribo Zero from illumina has been discontinued!



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Why choose Small RNA?

Analyzing microRNAs, siRNAs, piRNAs
- Selecting for 20-30nt small RNAs
- Minimun input of 100ng of cellular Total RNA

What is small RNA

Prep Chemistry

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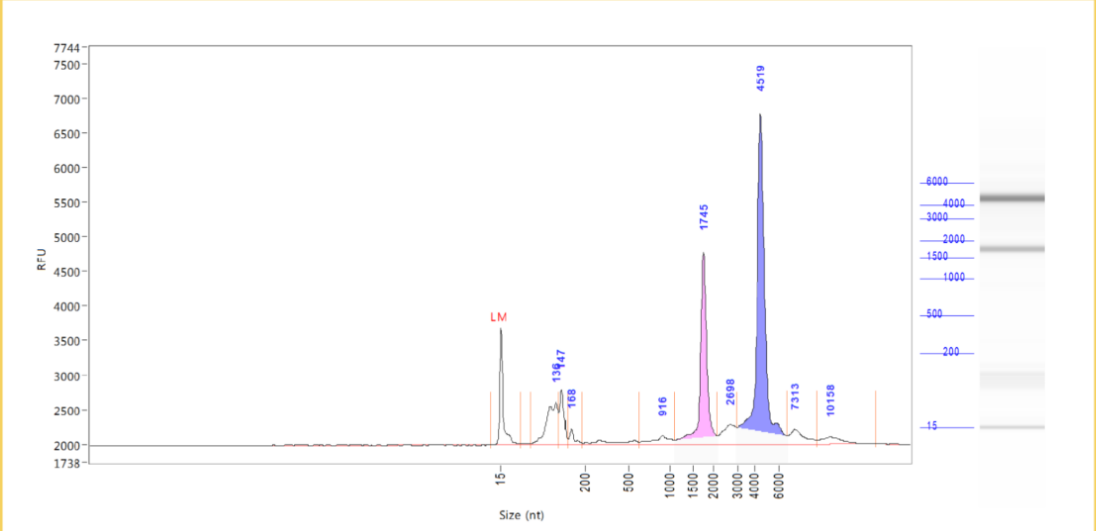
* For RNA Extraction: make sure you use a method that keeps small RNA's *

What is small RNA

Prep Chemistry

What is small RNA

- Type of ncRNA
- Small, 25-250NT's
- Involved in regulating translation of target RNA's



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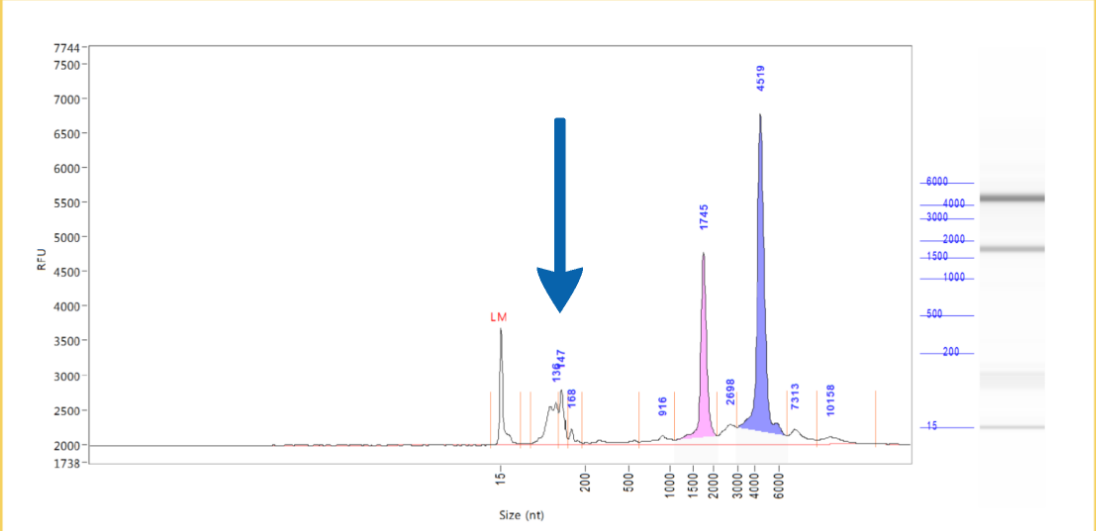
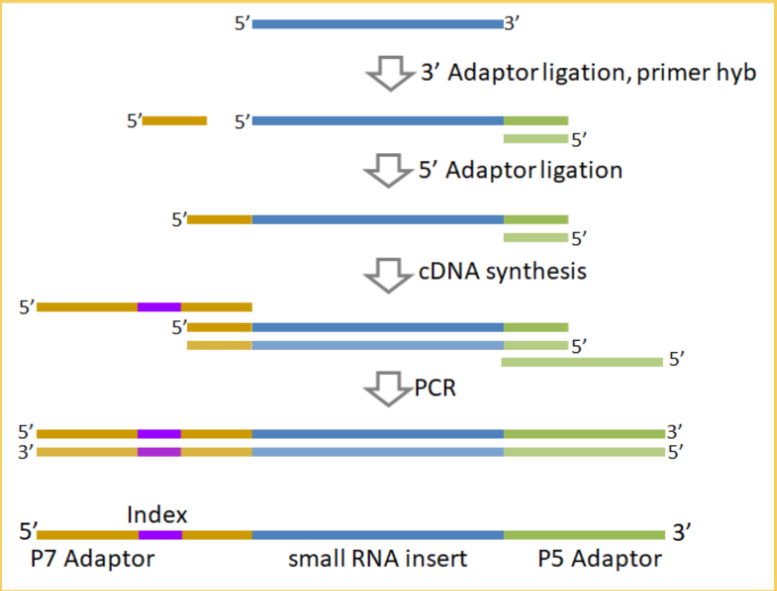


Small RNA Seq



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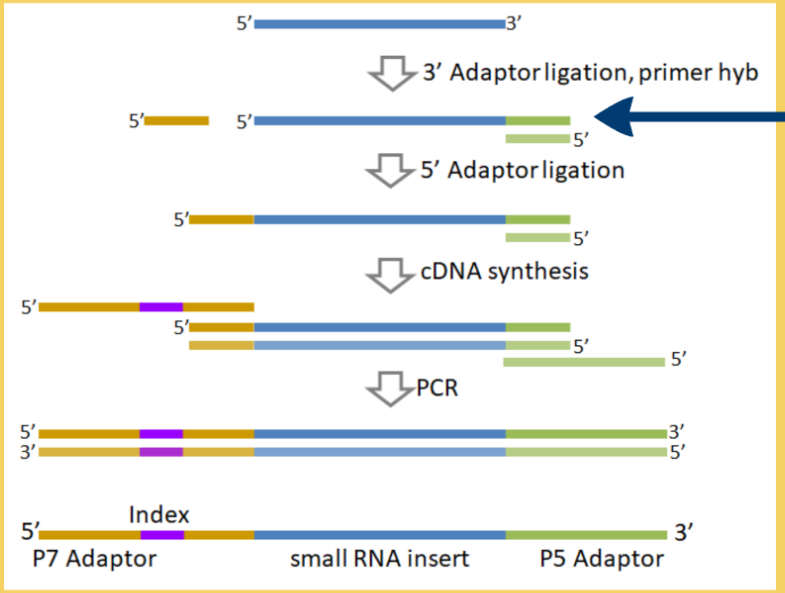
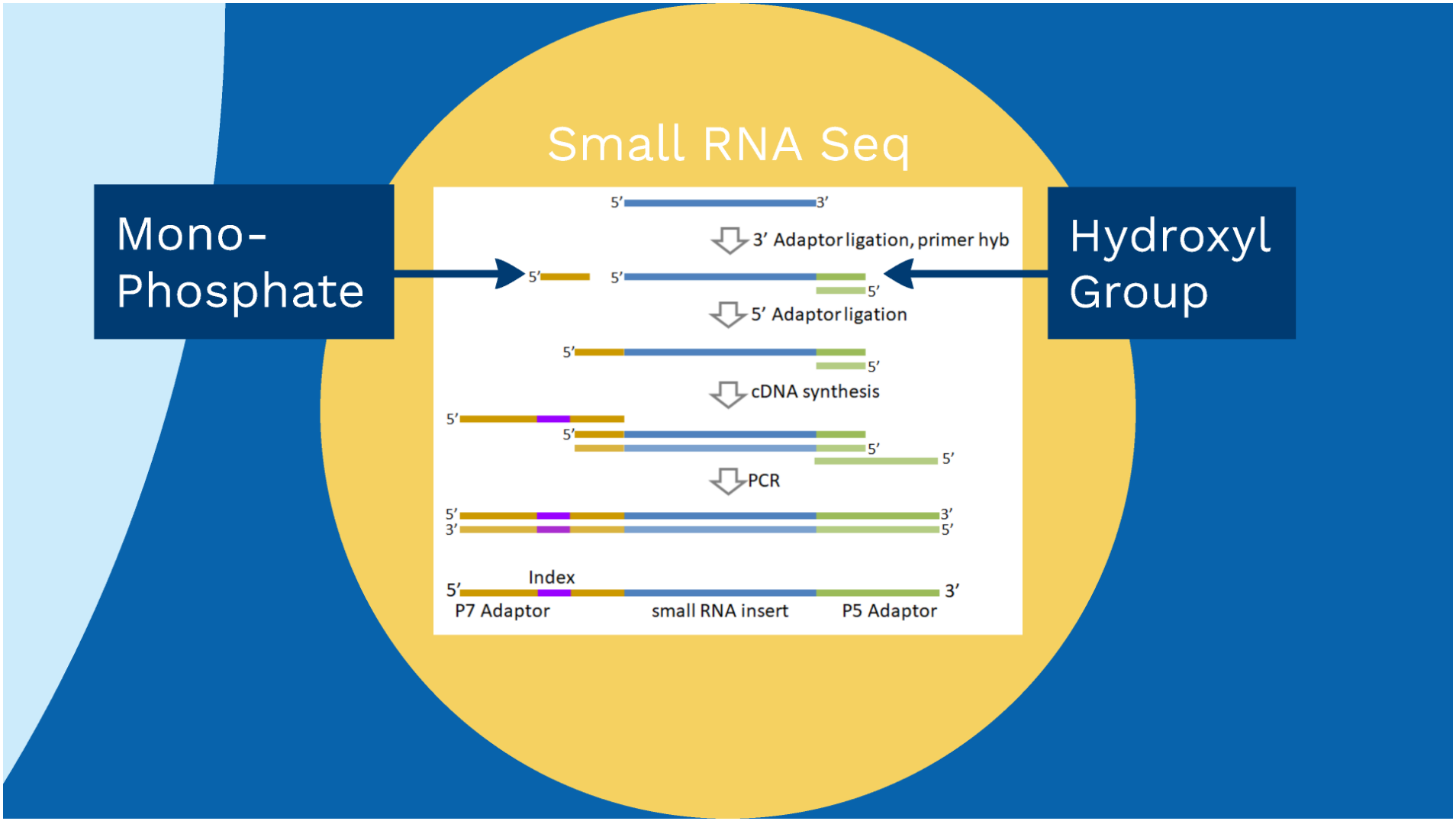
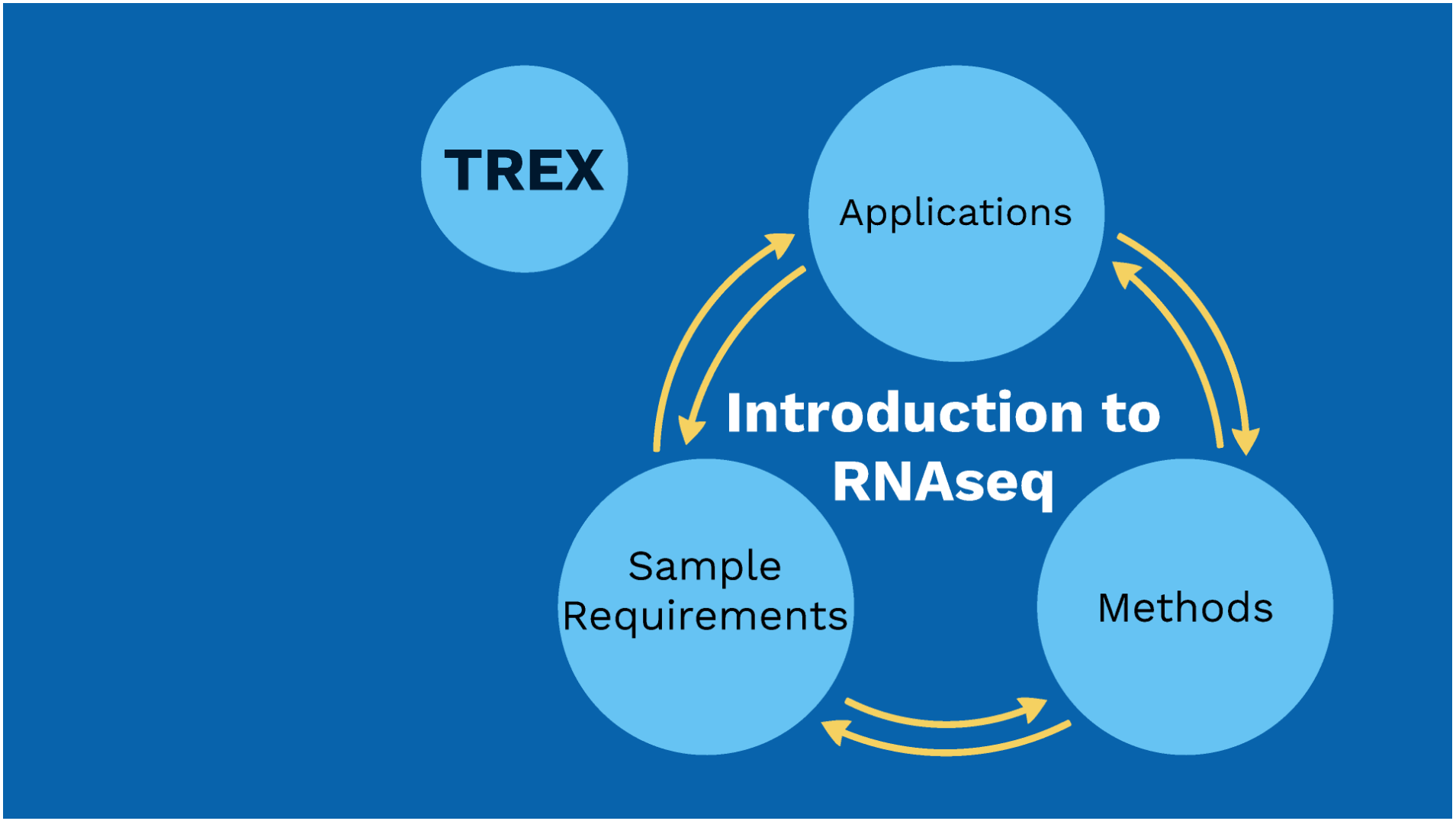
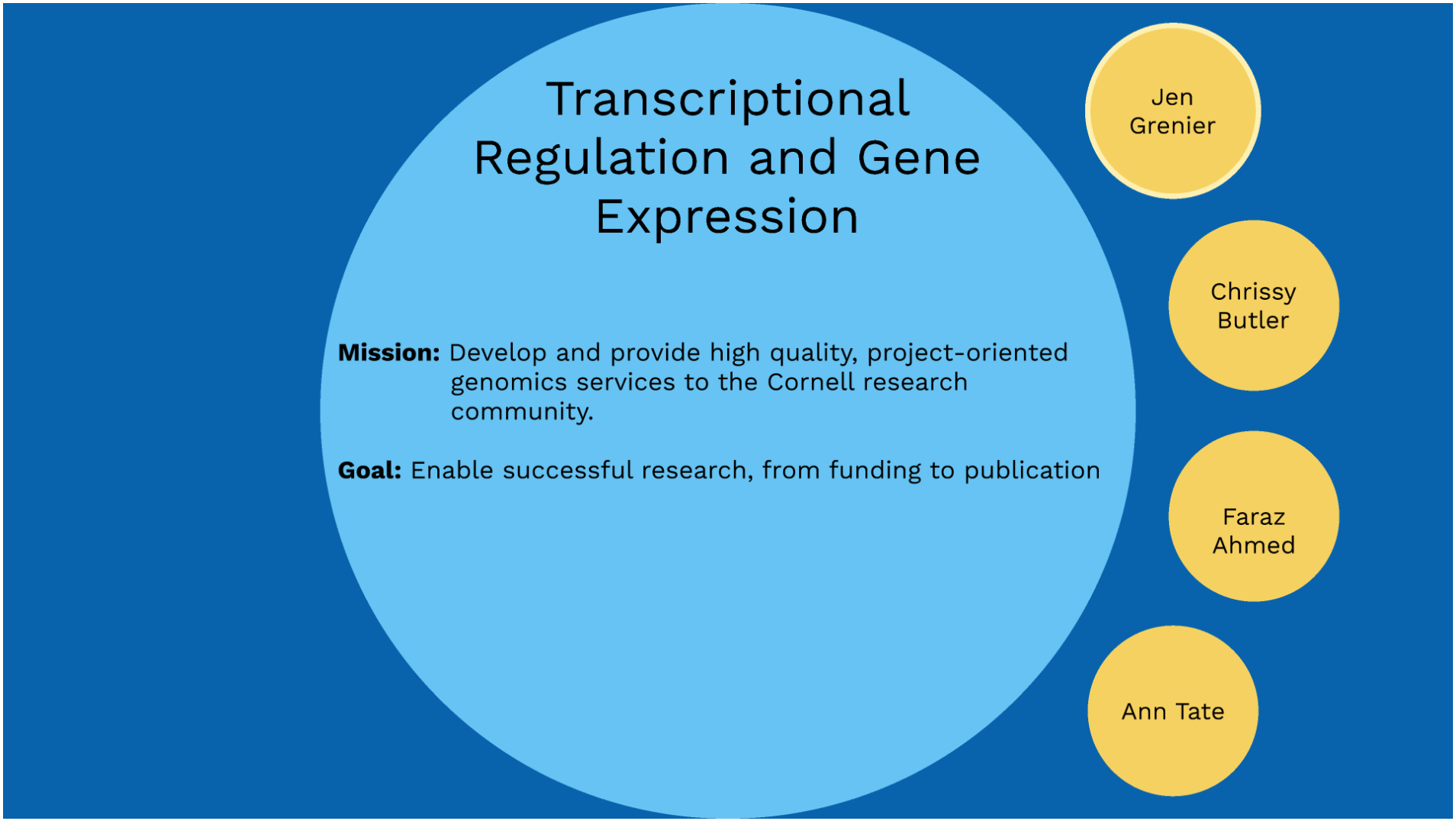
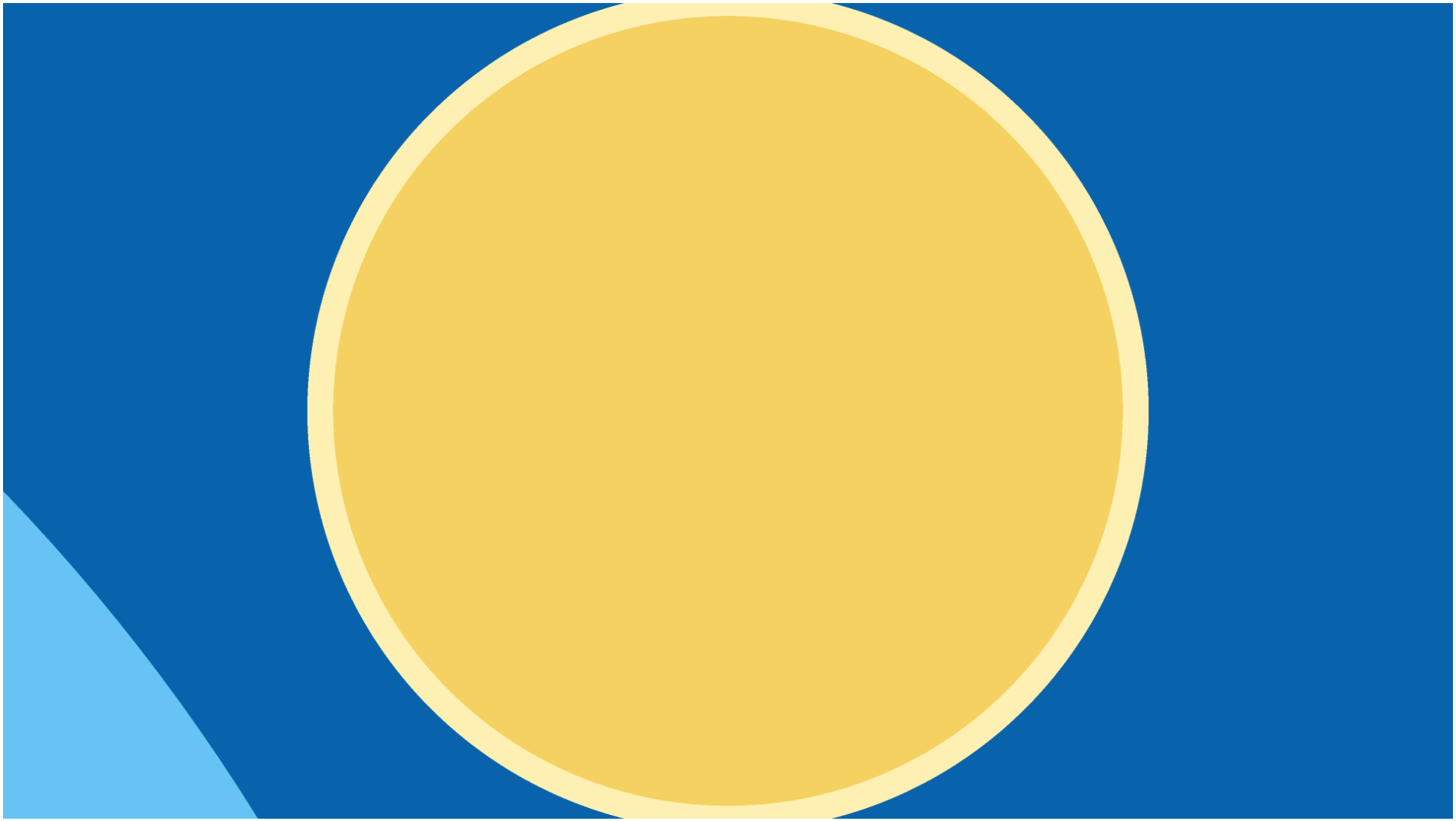


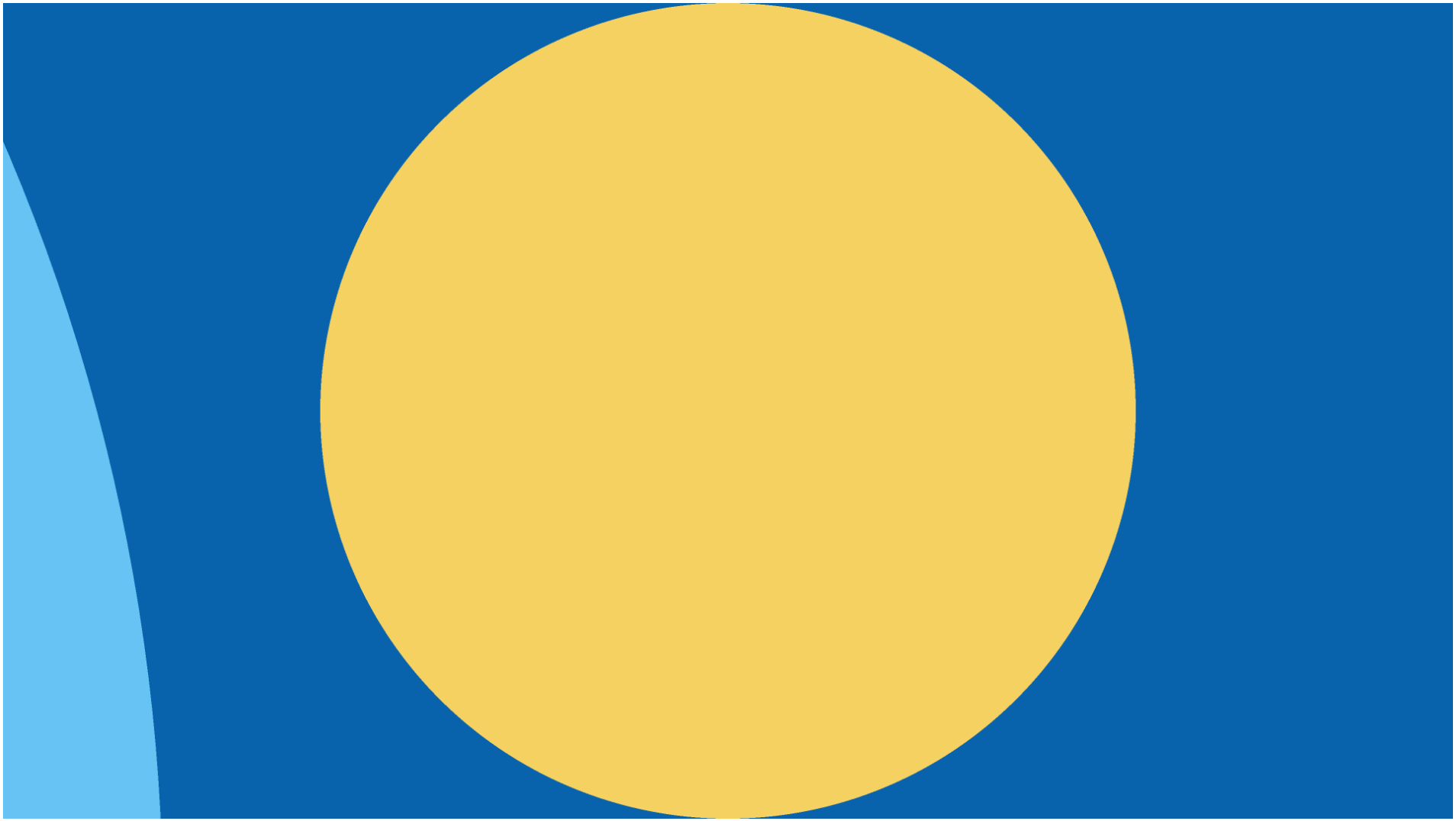
Hydroxyl Group

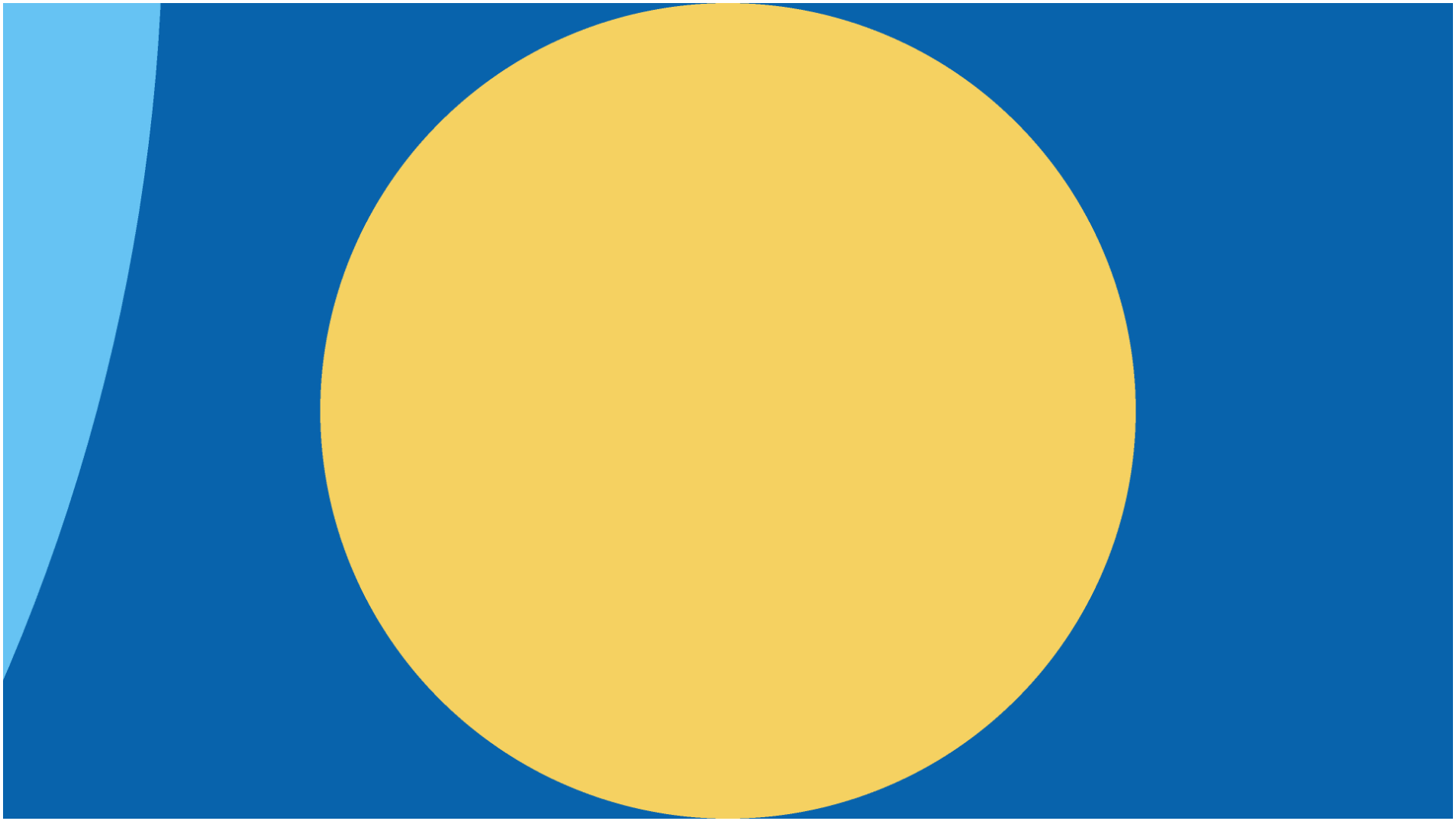

Small RNA Seq
Mono-Phosphate
Hydroxyl Group
5′
3′
3′ Adaptor ligation, primer hyb
5′
5′
5′
5′ Adaptor ligation
5′
5′
cDNA synthesis
5′
5′
5′
5′
PCR
5′
3′
3′
5′
Index
5′
3′
P7 Adaptor
small RNA insert
P5 Adaptor

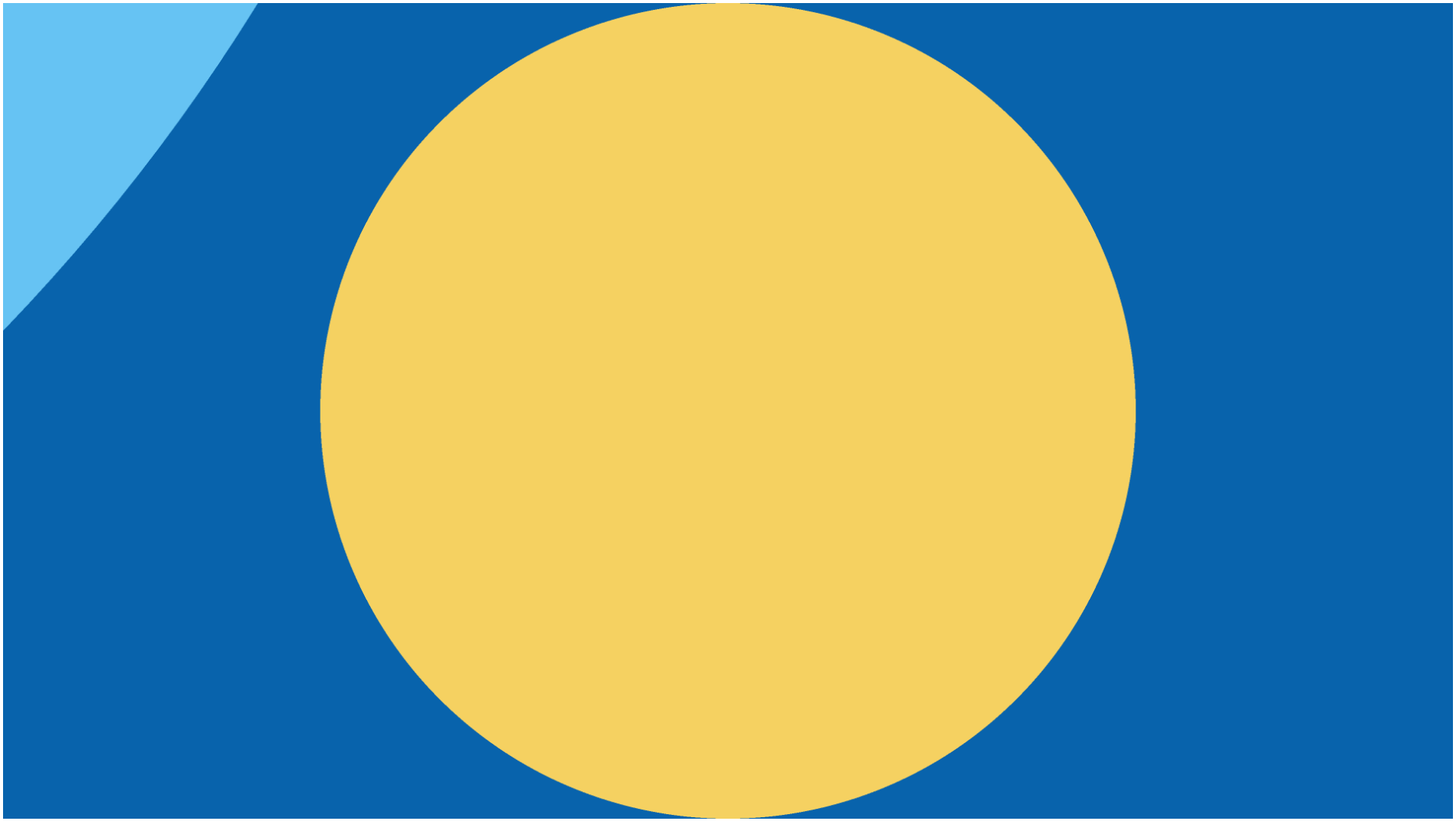

TREX
Applications
Introduction to RNAseq
Sample Requirements
Methods

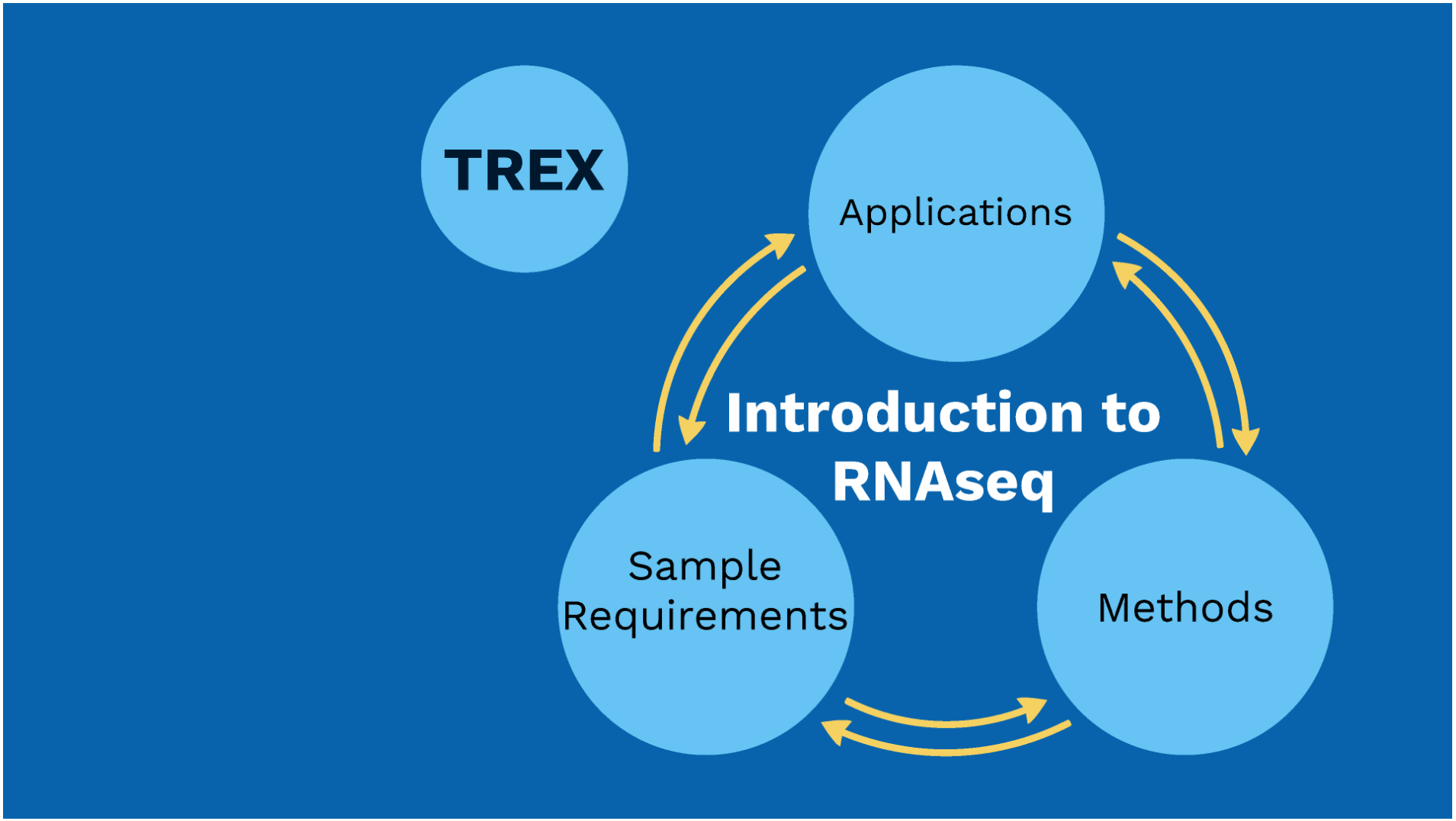

Transcriptional Regulation and Gene Expression
Mission: Develop and provide high quality, project-oriented genomics services to the Cornell research community.
Goal: Enable successful research, from funding to publication
Jen Grenier
Chrissy Butler
Faraz Ahmed
Ann Tate







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