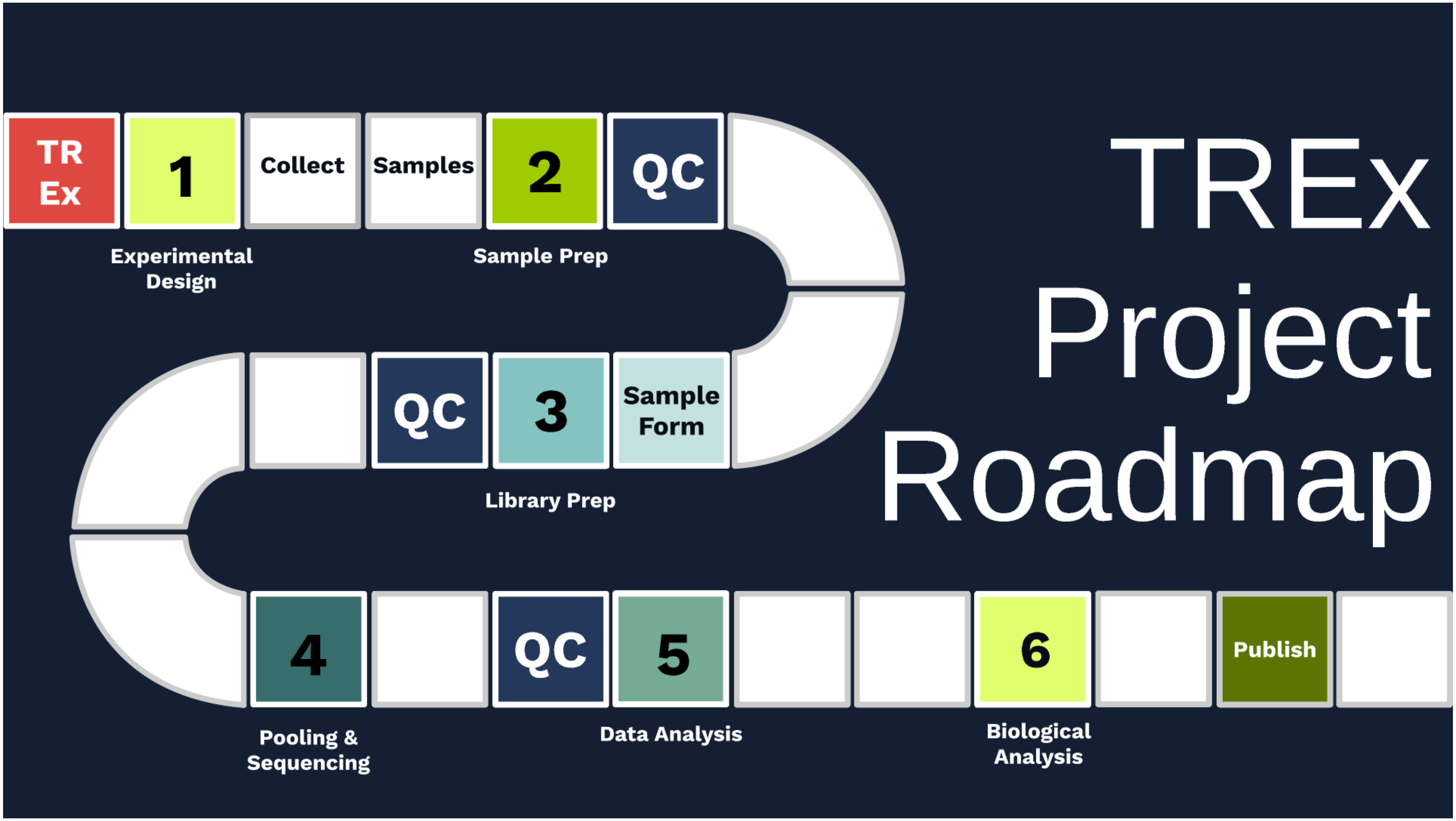
TREx Project Roadmap



TREx

Tech Talk

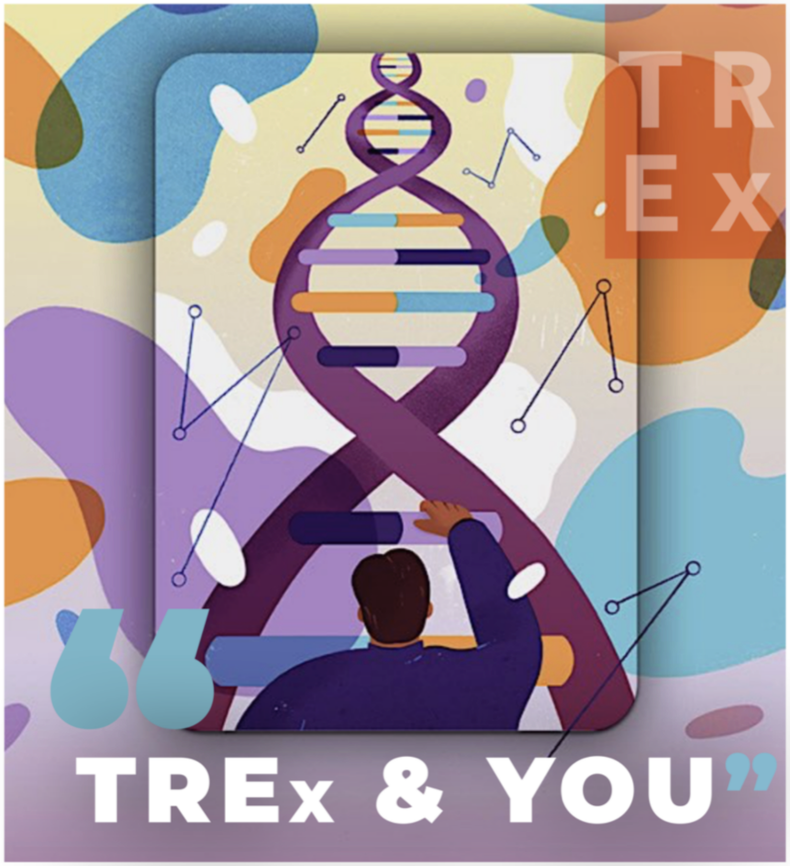
Transcriptional Regulation and Gene Expression Facility

Our Team

Workshops

New Pricing!

Sign up for Workshops!



GENOMICS PROJECT CONSULTATIONS

END-TO-END PROFILING SOLUTIONS

COMPREHENSIVE QUALITY ASSESSMENT

Our Team

Director:
Jen Grenier

Lab Manager:
Chrissy Butler

Data Analyst:
Faraz Ahmed

Project Manager:
Ann Tate

Upcoming Workshops

Sign up

1 **RNA Extraction- October 24th**

2 **From Library Prep to Analysis- 4 weeks, Wednesdays from October 30th-November 20th**

3 **Data Analysis Tools for Biological Discovery- December**

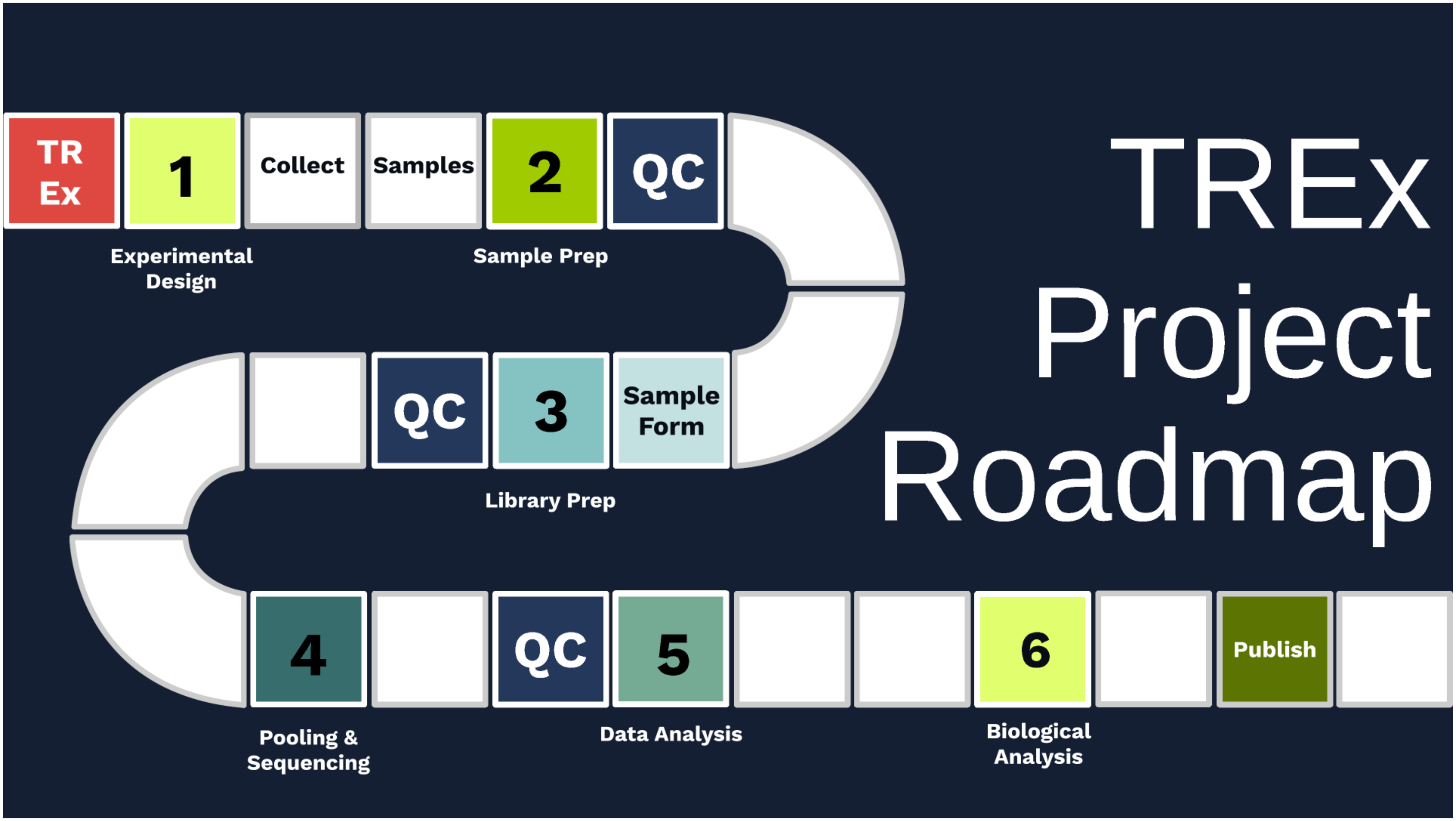
New Pricing

RNA Seq

OPTIONS	CORNELL PRICING SAMPLES 1-24	CORNELL PRICING SAMPLES 25+	ACADEMIC PRICING ALL SAMPLES
Full Package	$315	$265	$470
Add rRNA Subtraction	$50	$50	$100
Add 20M Additional Reads	$160	$160	$255

Small RNA

OPTIONS	CORNELL PRICING SAMPLES 1-24	CORNELL PRICING SAMPLES 25+	ACADEMIC PRICING ALL SAMPLES
Full Package	$265	$215	$375
Add 10M Additional Reads	$110	$110	$160



TREx Project Roadmap

Experimental Design

- You:
  - Bringing Biological Question
- TREx:
  - Bringing Technological Expertise

Project Planning

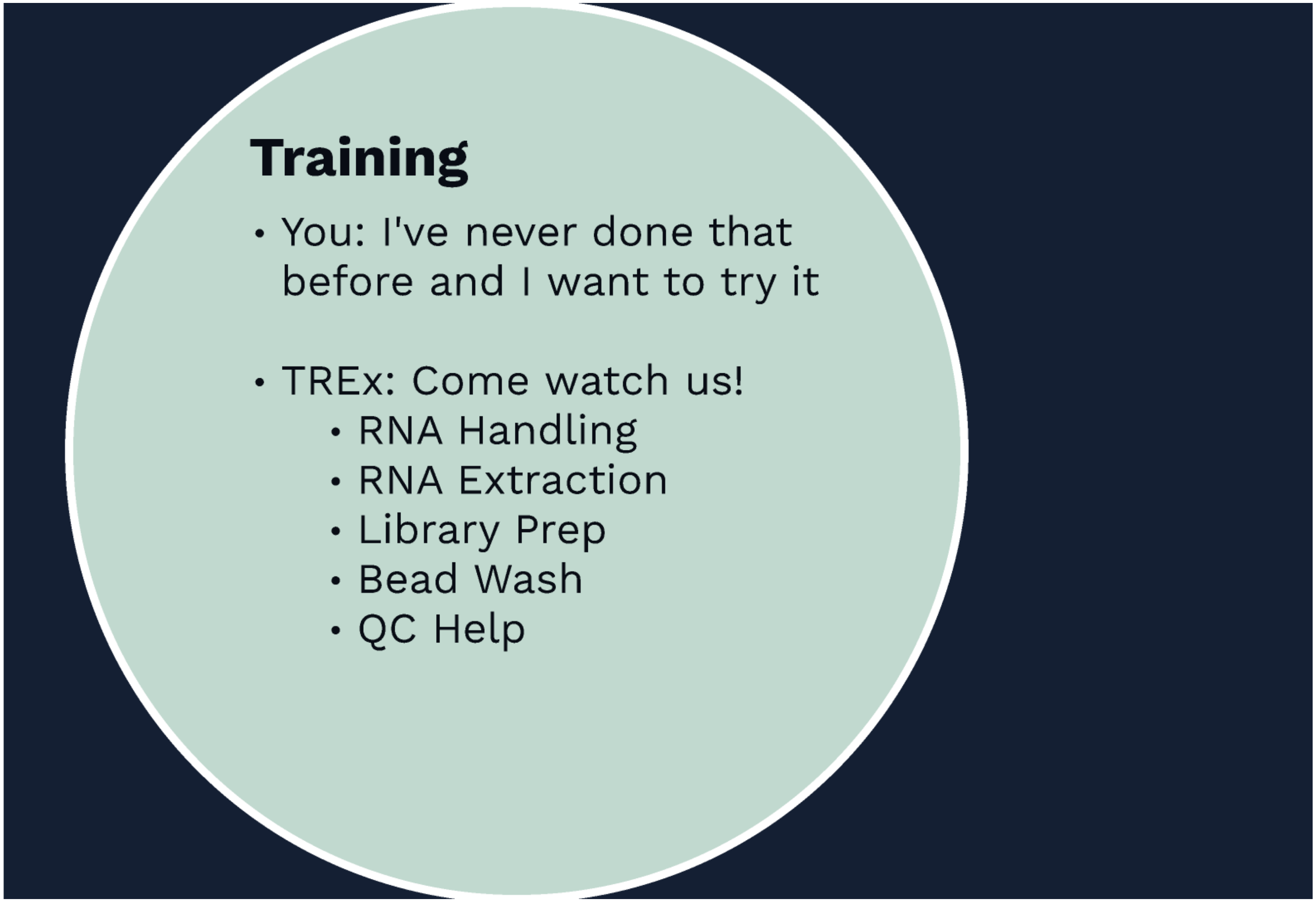
Training

New Tech

Project Planning

- You: Biological Question

- TREx: How to use the technology to answer that question
  - How many Replicates do you need?
  - How are you going to extract your samples?
  - How much RNA will you have?
  - What will the quality of that RNA be?
  - What is your budget?
  - What type of library do you need?



Training

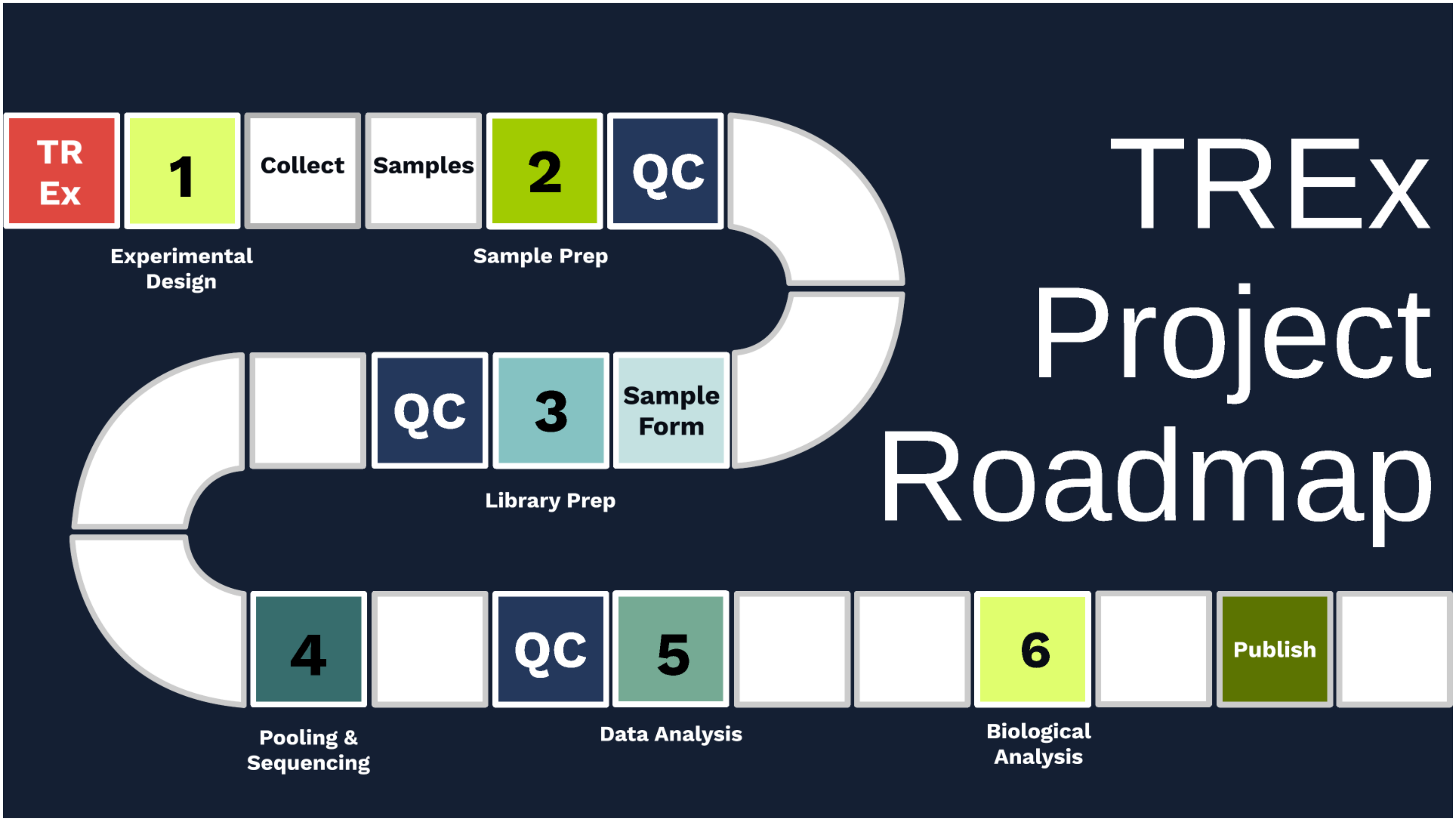
- You: I've never done that before and I want to try it
- TREx: Come watch us!
    - RNA Handling
    - RNA Extraction
    - Library Prep
    - Bead Wash
    - QC Help

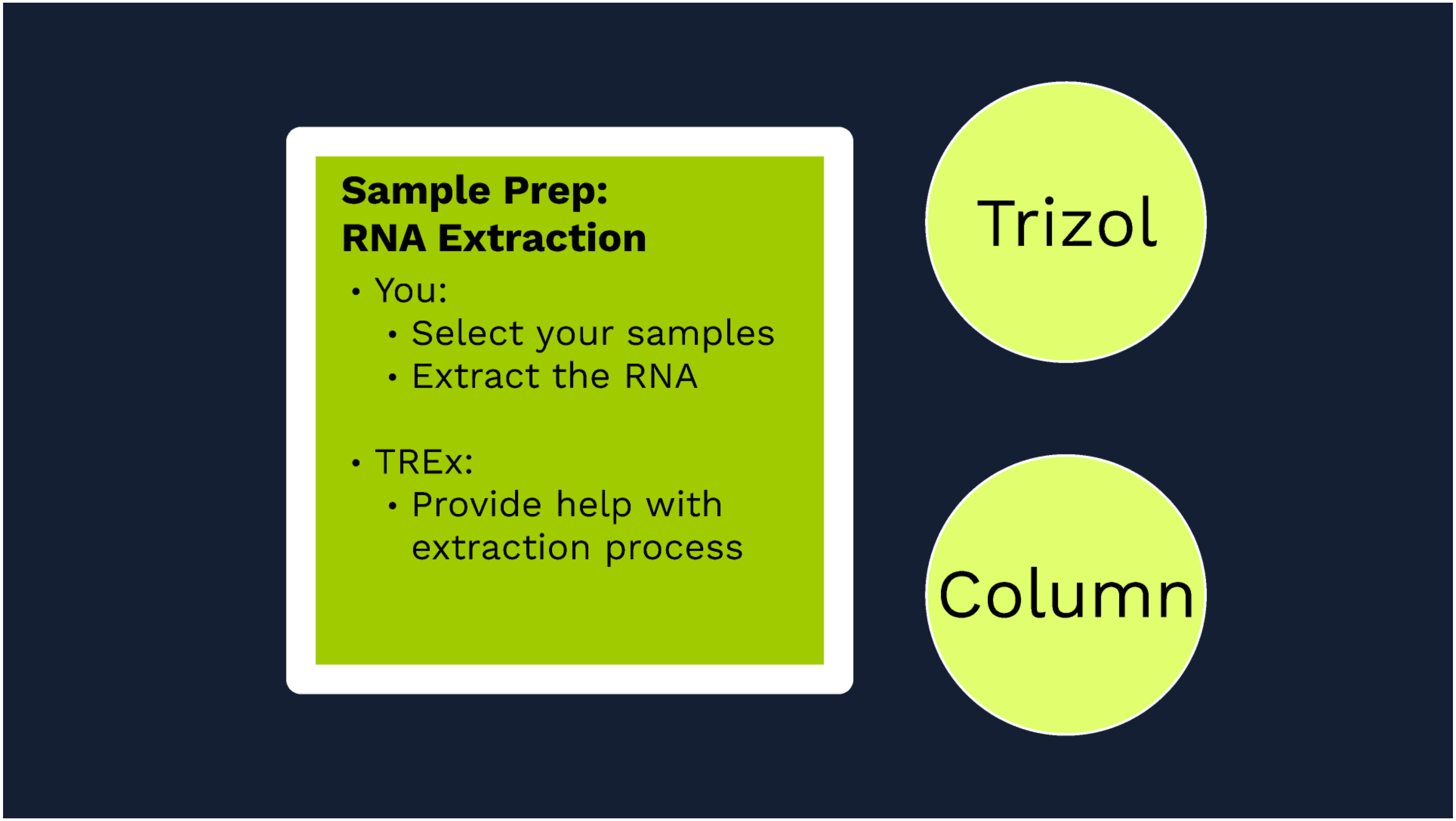
Emerging Technologies

- You: Biological Question

- TREx: Try this new technology, it may help answer your question in a better way
  - ATAC-Seq
  - Pro-Seq
  - Single Cell Sequencing

TREx Project Roadmap





Sample Prep:
RNA Extraction

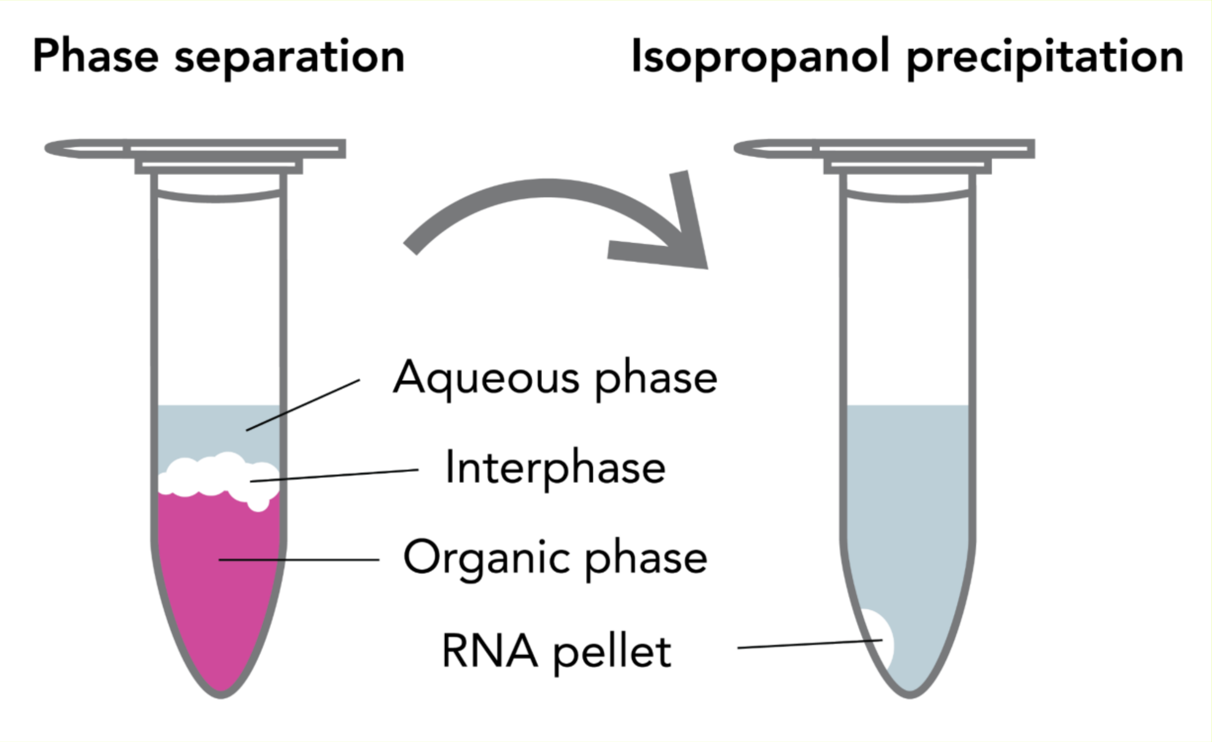
- You:
  - Select your samples
  - Extract the RNA

- TREx:
  - Provide help with extraction process

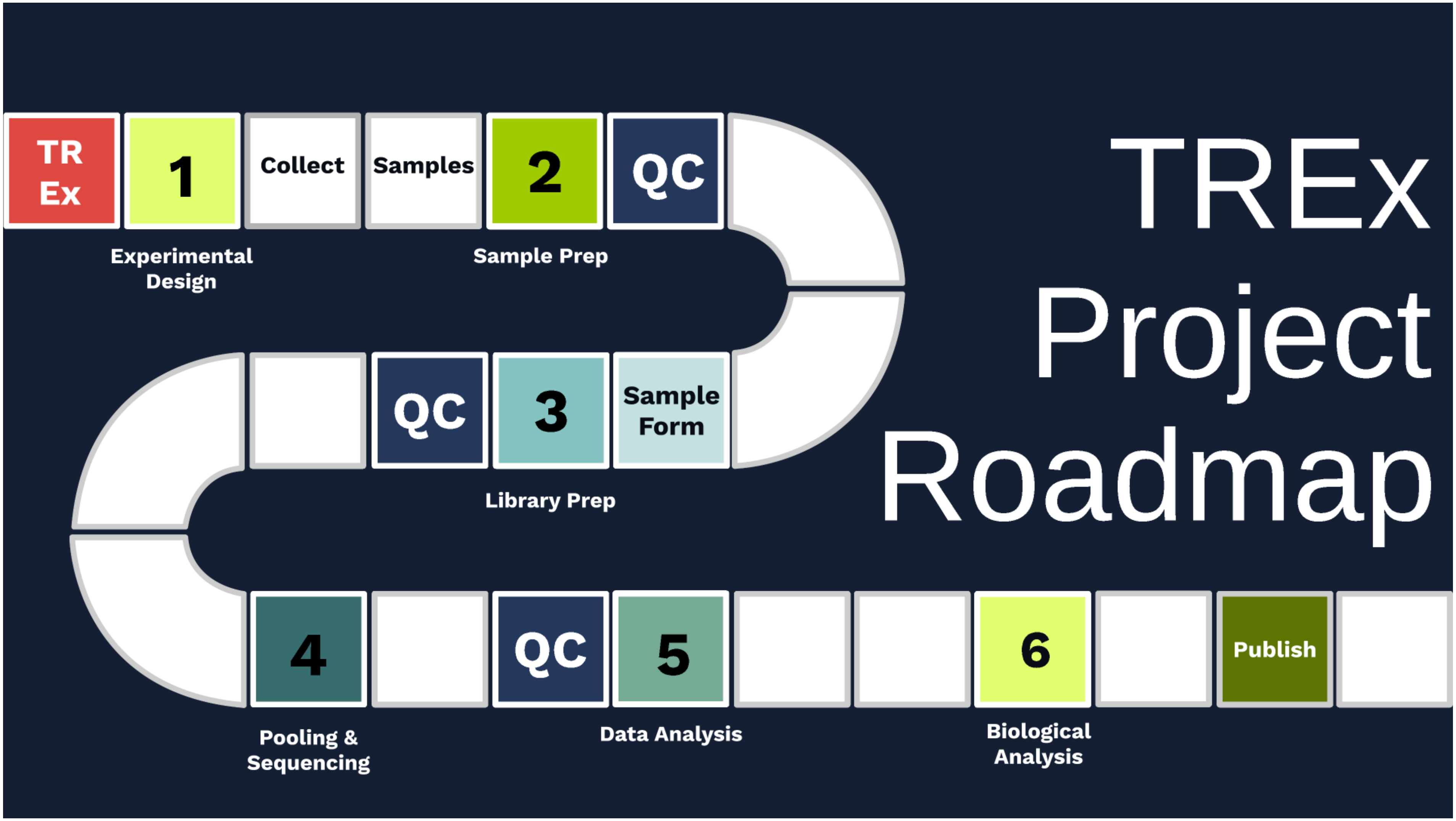
Trizol

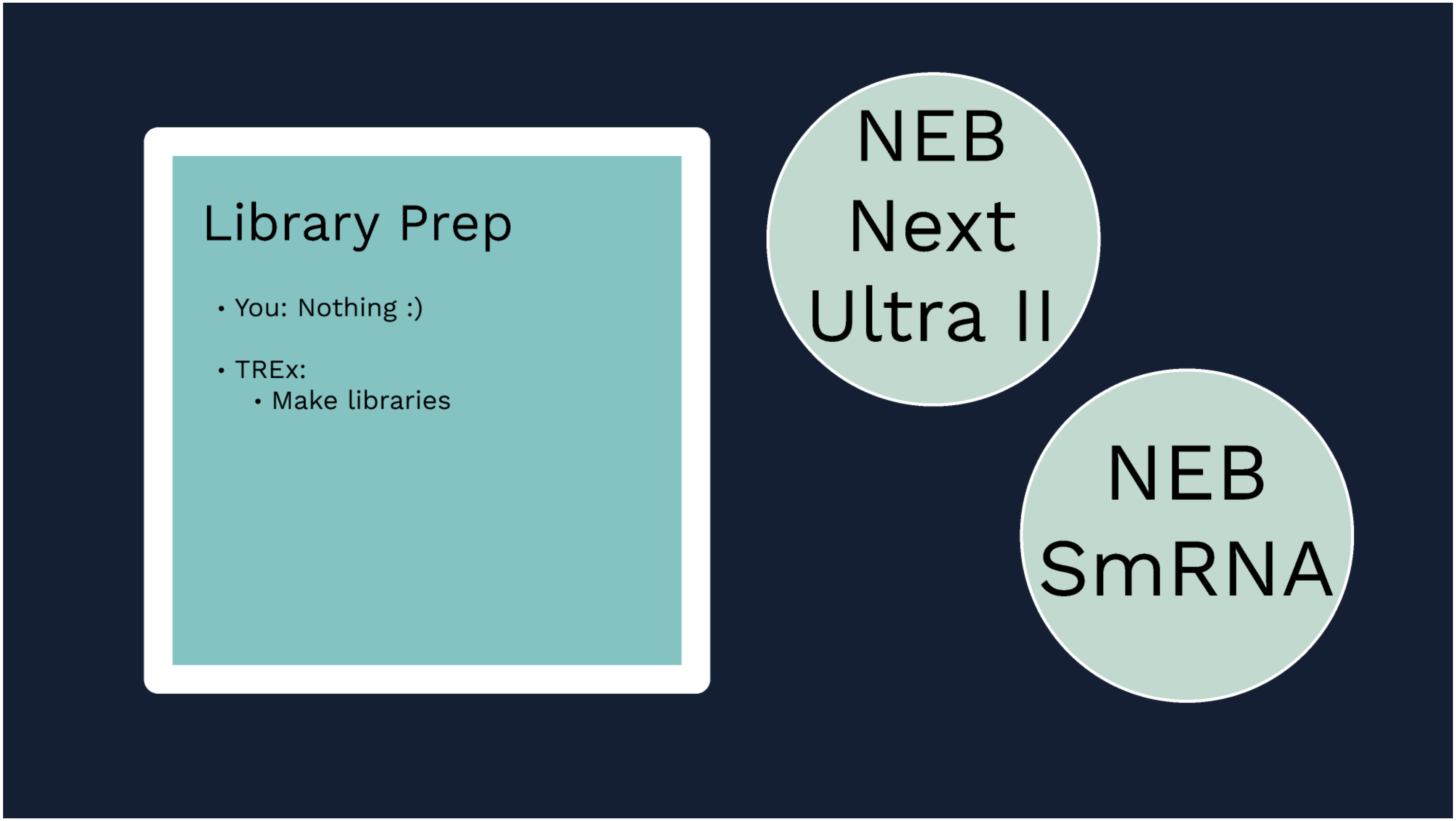
Column

Trizol Prep


Phase separation
Isopropanol precipitation
Aqueous phase
Interphase
Organic phase
RNA pellet

TREx Project Roadmap





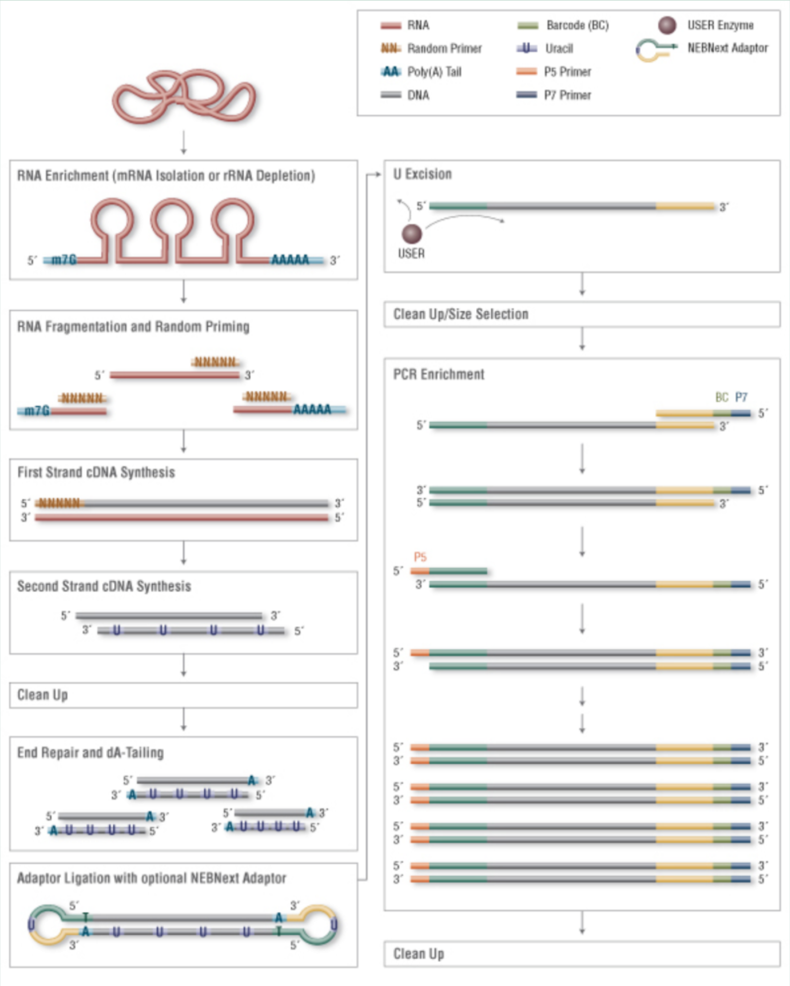
Library Prep

- You: Nothing :)
- TREx:
    - Make libraries

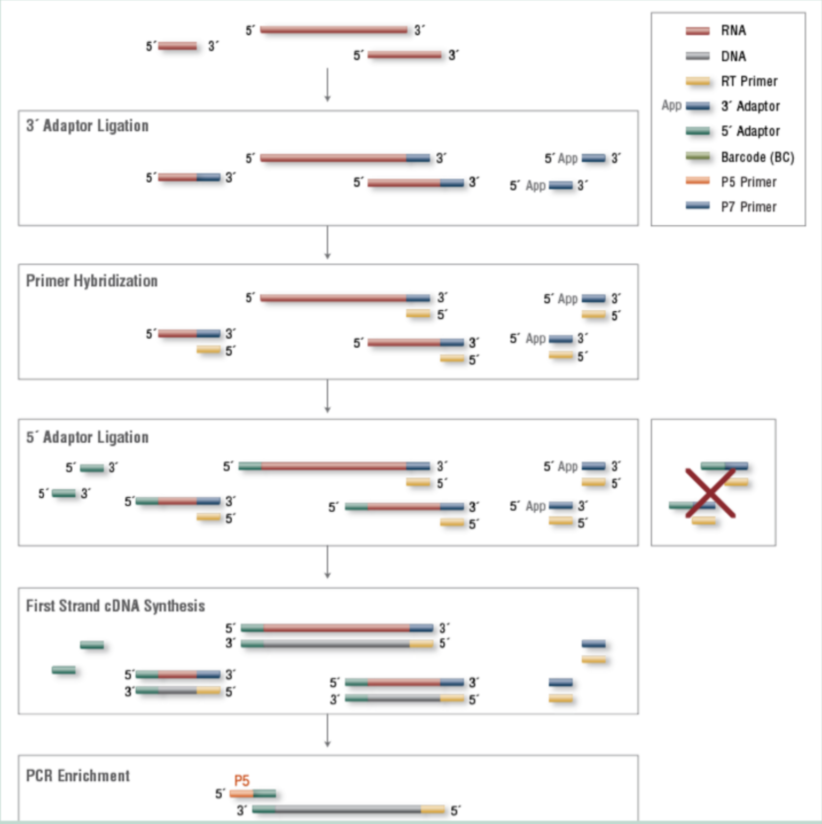
NEB Next Ultra II

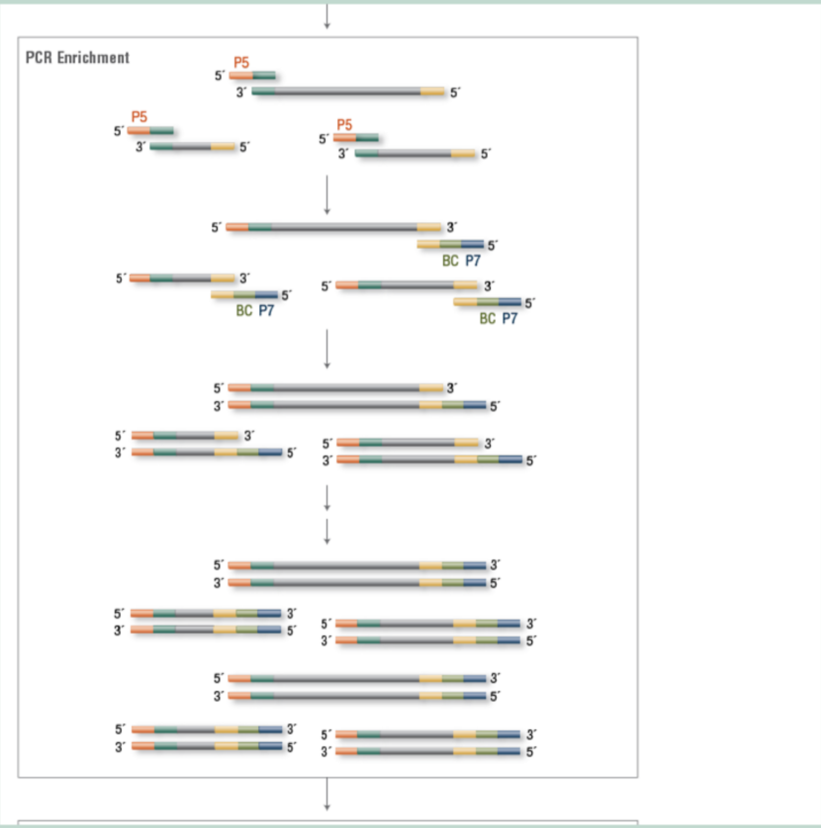
NEB SmRNA

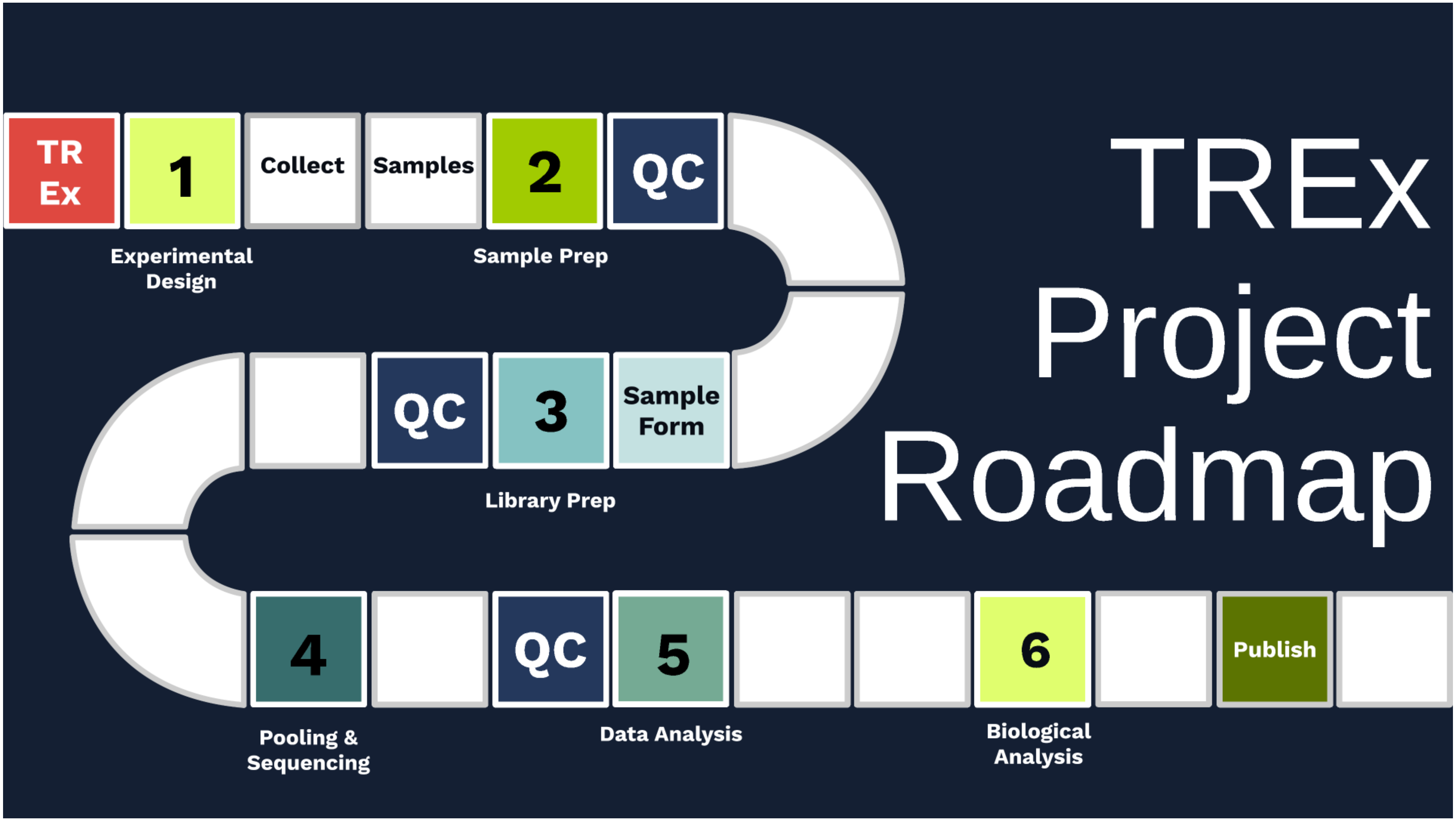
NEB Next Ultra II RNA



NEB SmRNA


3´ Adaptor Ligation
Primer Hybridization
5´ Adaptor Ligation
First Strand cDNA Synthesis
PCR Enrichment
RNA
DNA
RT Primer
App 3´ Adaptor
5´ Adaptor
Barcode (BC)
P5 Primer
P7 Primer
5´ App 3´
P5


PCR Enrichment
P5
BC P7



TREx Project Roadmap

TR Ex

1

Collect

Samples

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QC

Experimental Design

Sample Prep

Sample Form

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Library Prep

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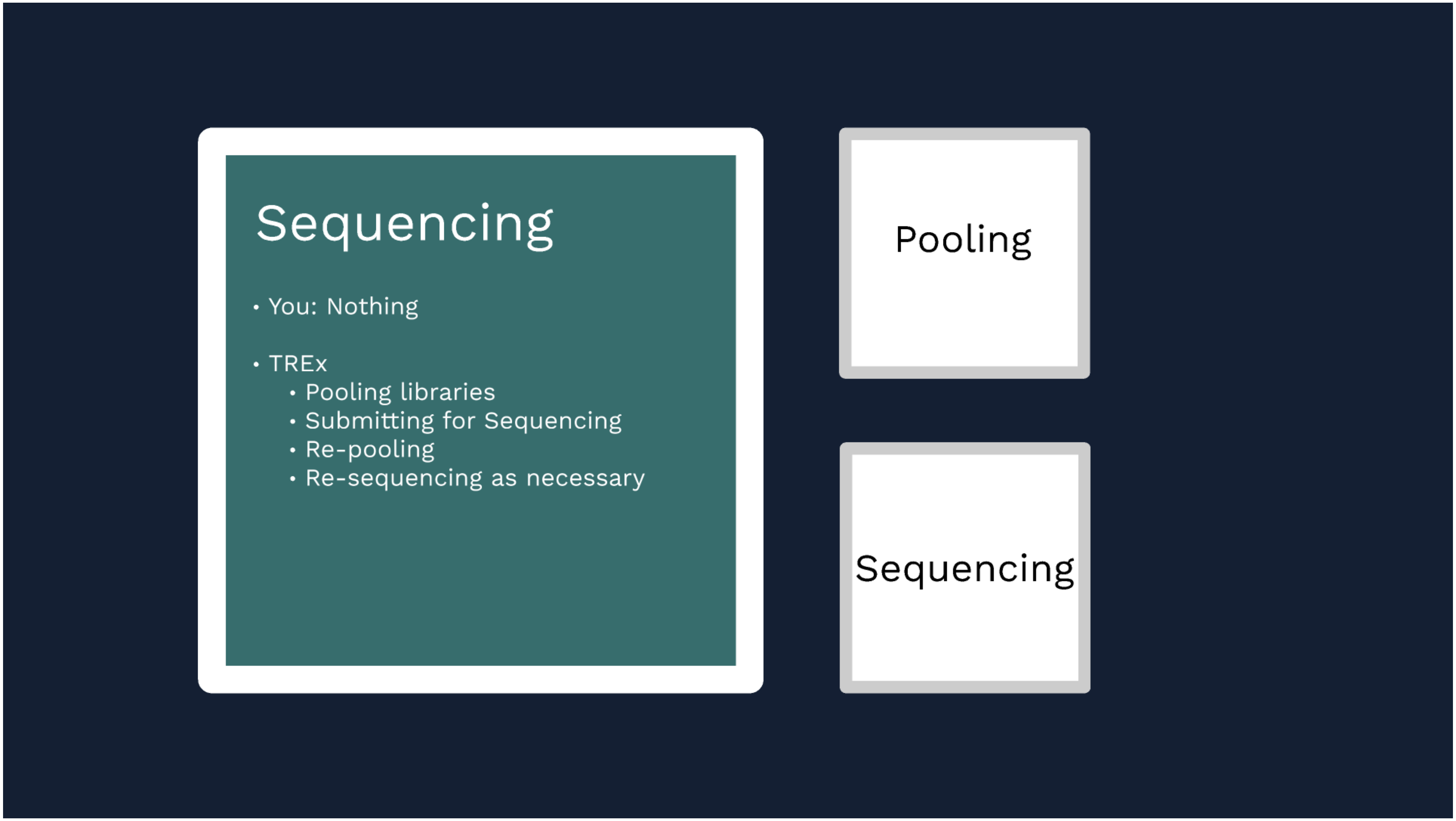
6

Publish

Pooling & Sequencing

Data Analysis

Biological Analysis



Sequencing

- You: Nothing
- TREx
  - Pooling libraries
  - Submitting for Sequencing
  - Re-pooling
  - Re-sequencing as necessary

Pooling

Sequencing

Sequencing

- Standard Read length 75bp
- Standard depth
  - RNAseq - 20Million raw reads
  - Small RNAseq- 10million raw reads

- Special Projects
  - Transcriptome assembly
    - longer paired end reads
    - deeper sequencing
  - Pathogen ID
    - deeper sequencing

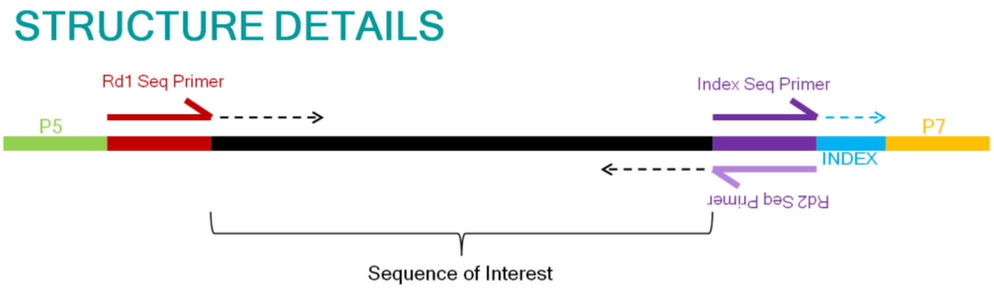
Nextseq 500

- BRC Specifications:
  - 333-400 Million Reads/Flowcell
    - 16-RNAseq libraries
    - 32-smRNA libraries

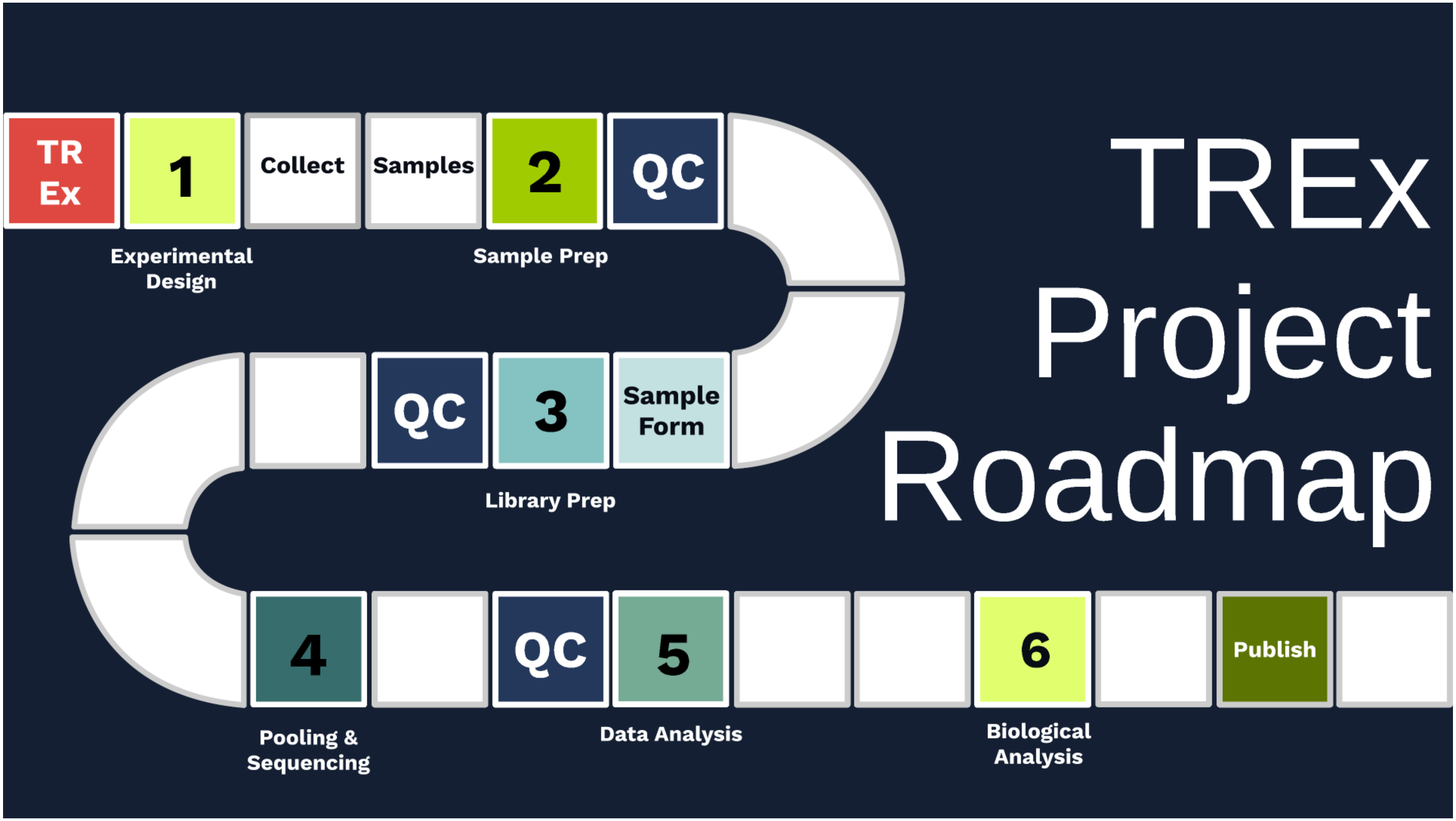
- TREx Benefit:
  - We will fill the flowcell
    - Select barcodes
    - Combine Projects
    - Guaranteed Balancing

Pooling Libraries

- Barcode/Index: Unique identifier
- Pool: Combine different libraries into one tube



TREx Project Roadmap



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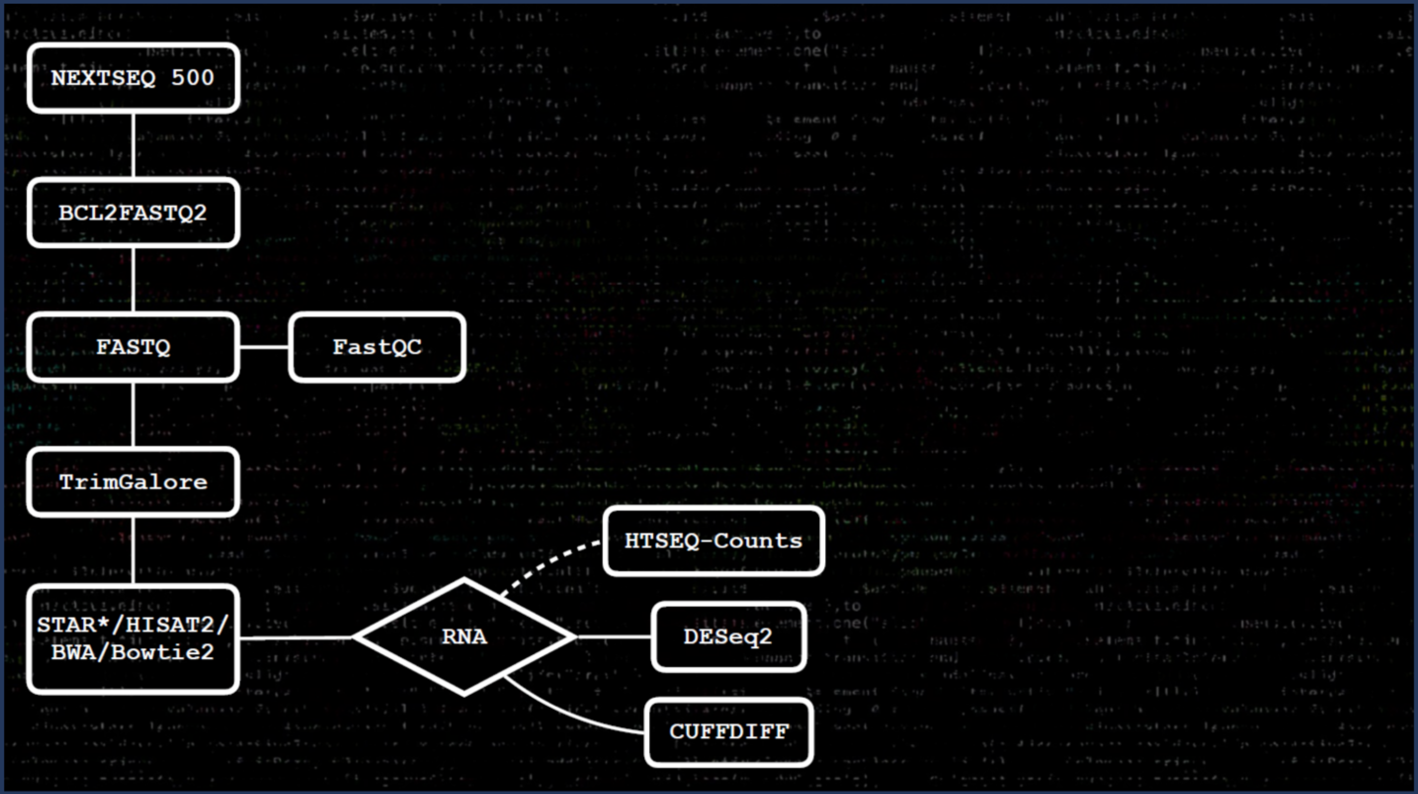
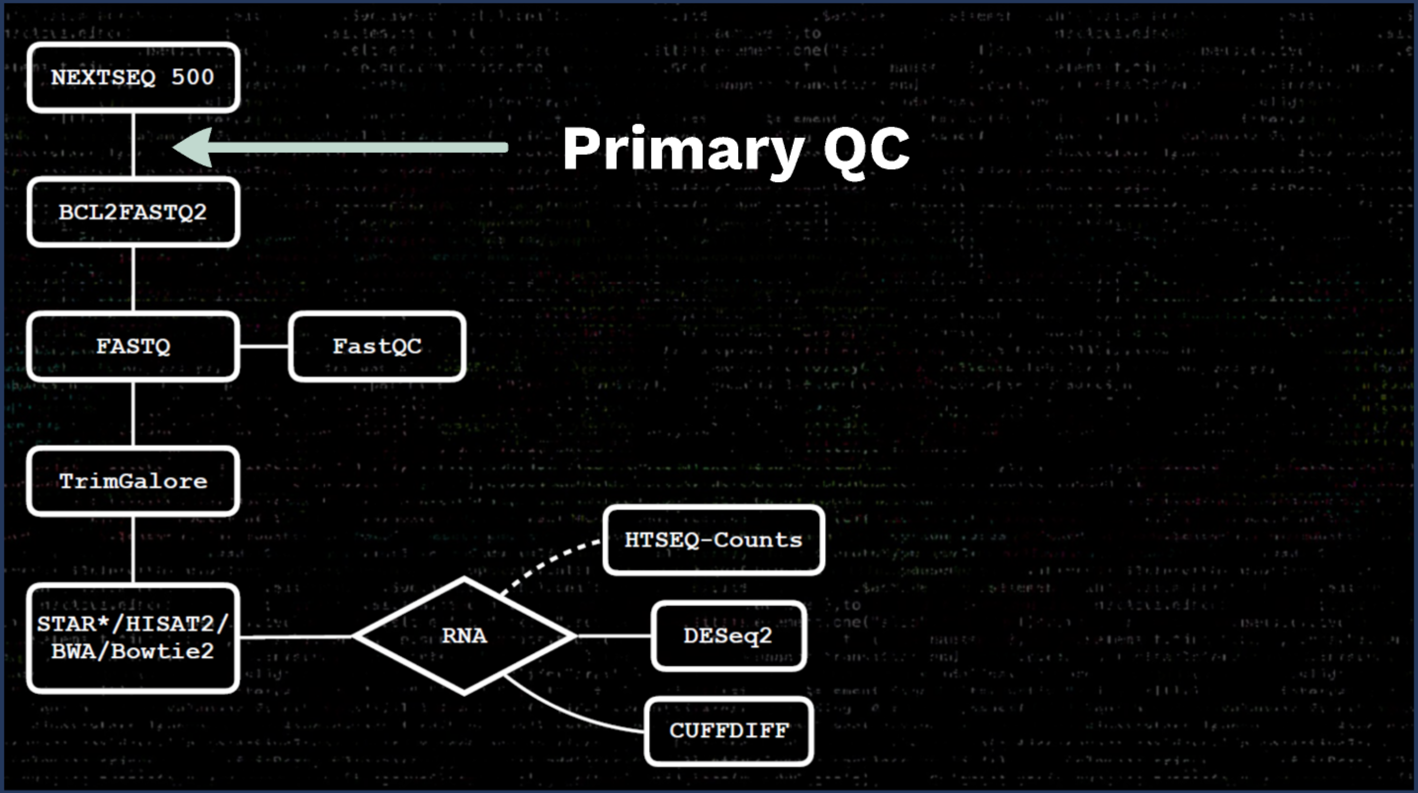

NEXTSEQ 500
BCL2FASTQ2
FASTQ
FastQC
TrimGalore
HTSEQ-Counts
STAR*/HISAT2/
BWA/Bowtie2
RNA
DESeq2
CUFFDIFF

Data QC


NEXTSEQ 500
Primary QC
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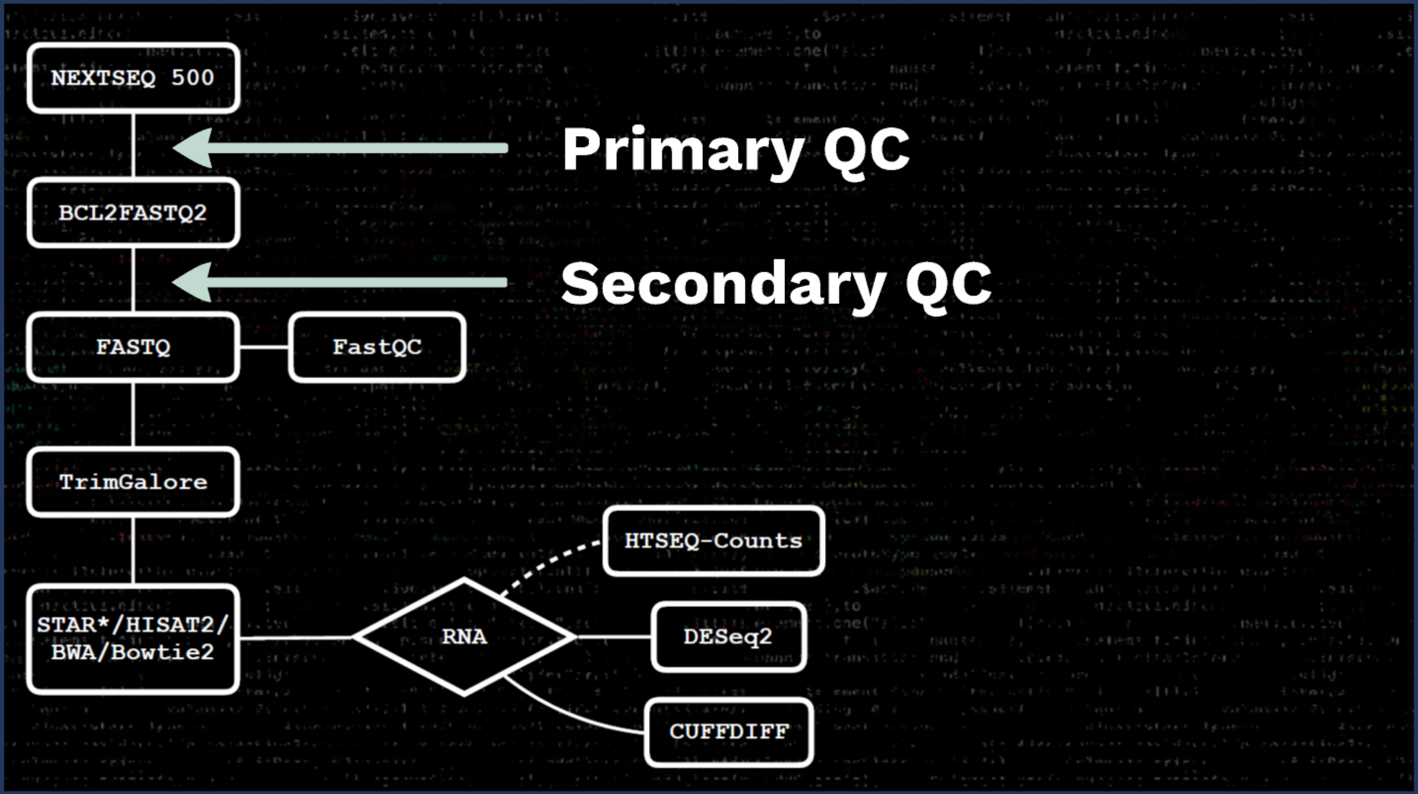
Data QC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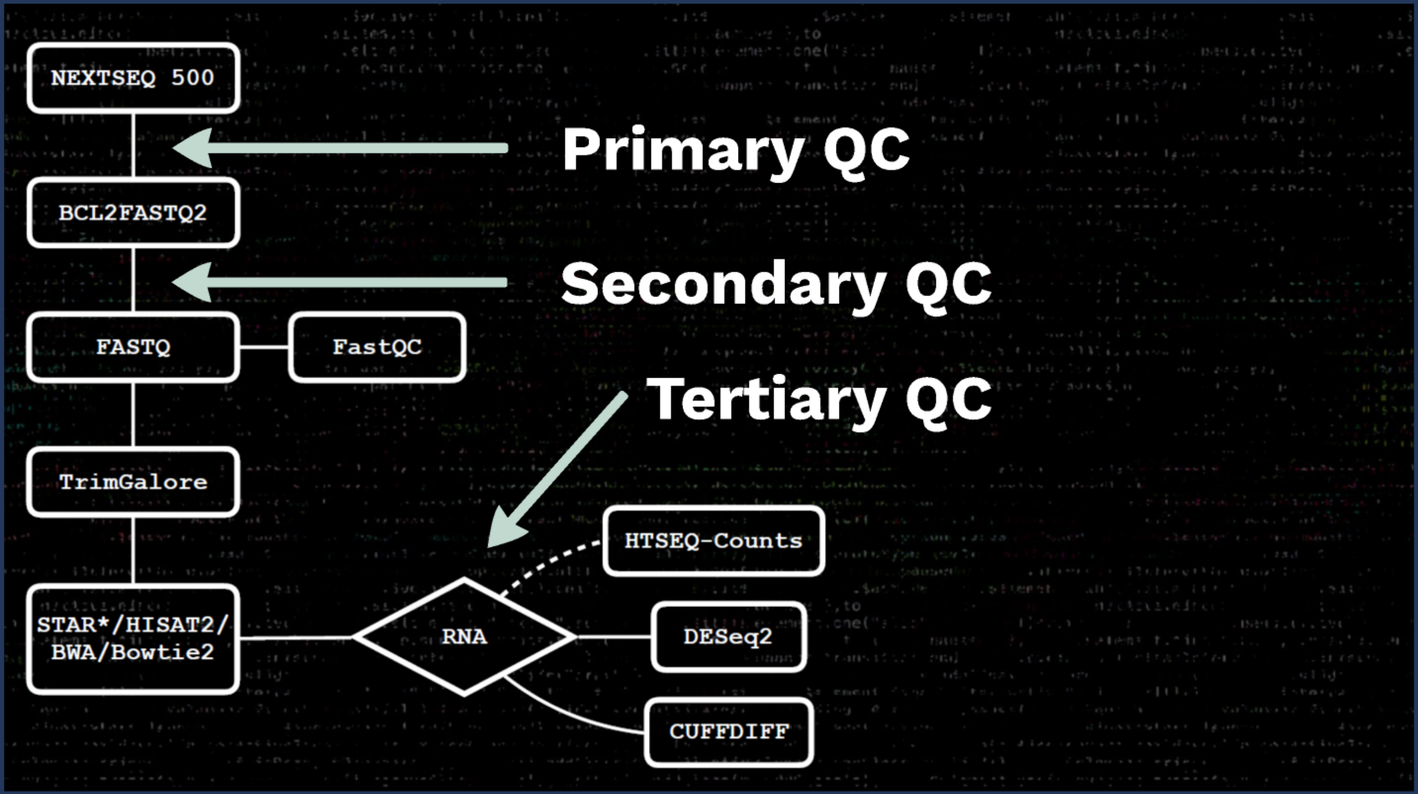
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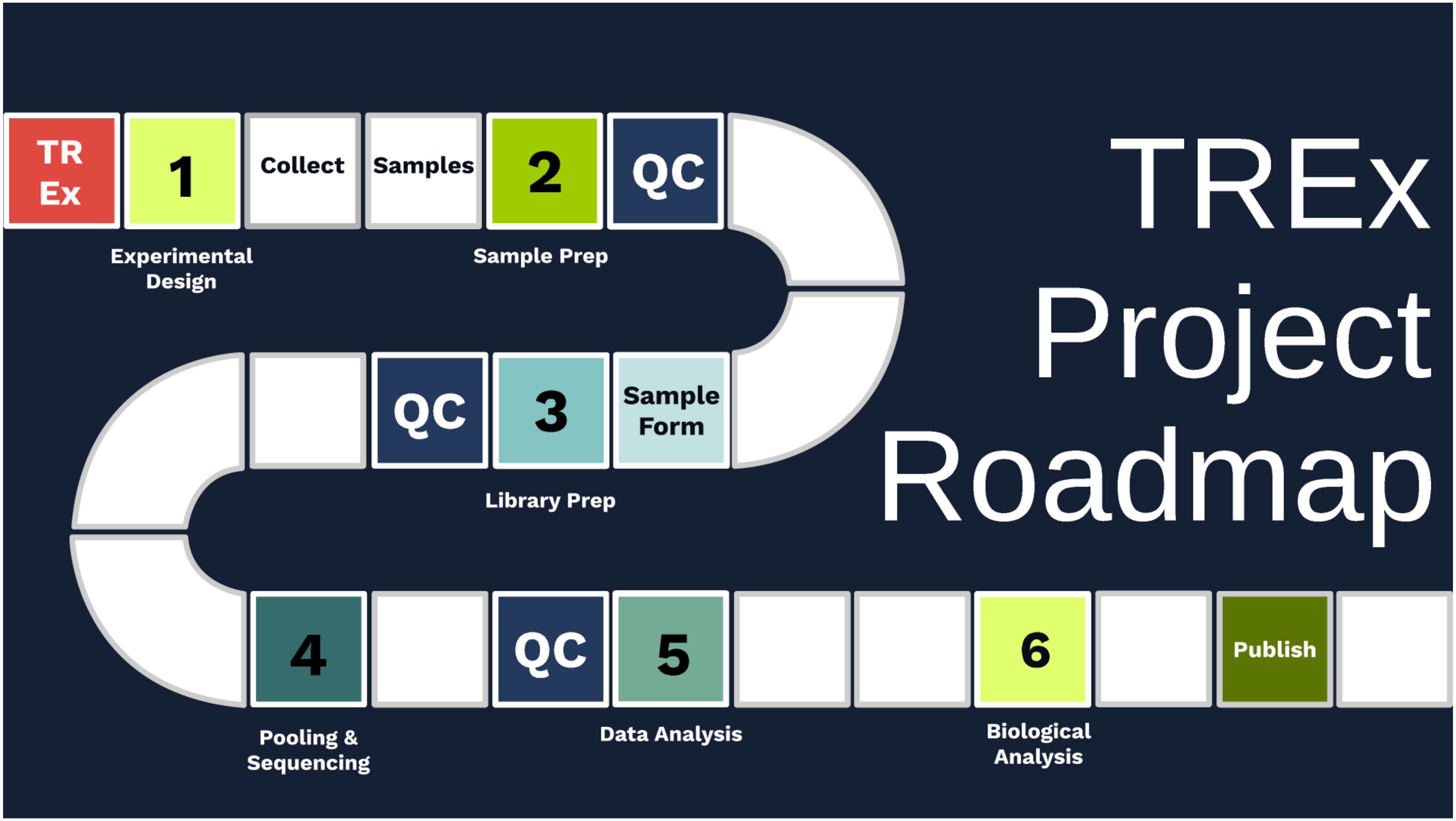

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Data Analysis

Biological Analysis

Preliminary Data Analysis:

You:
- Review desired outcomes
- Make space for data

TREx:
- Review QC data
- Generate reports
- Hand off data

Data

Data

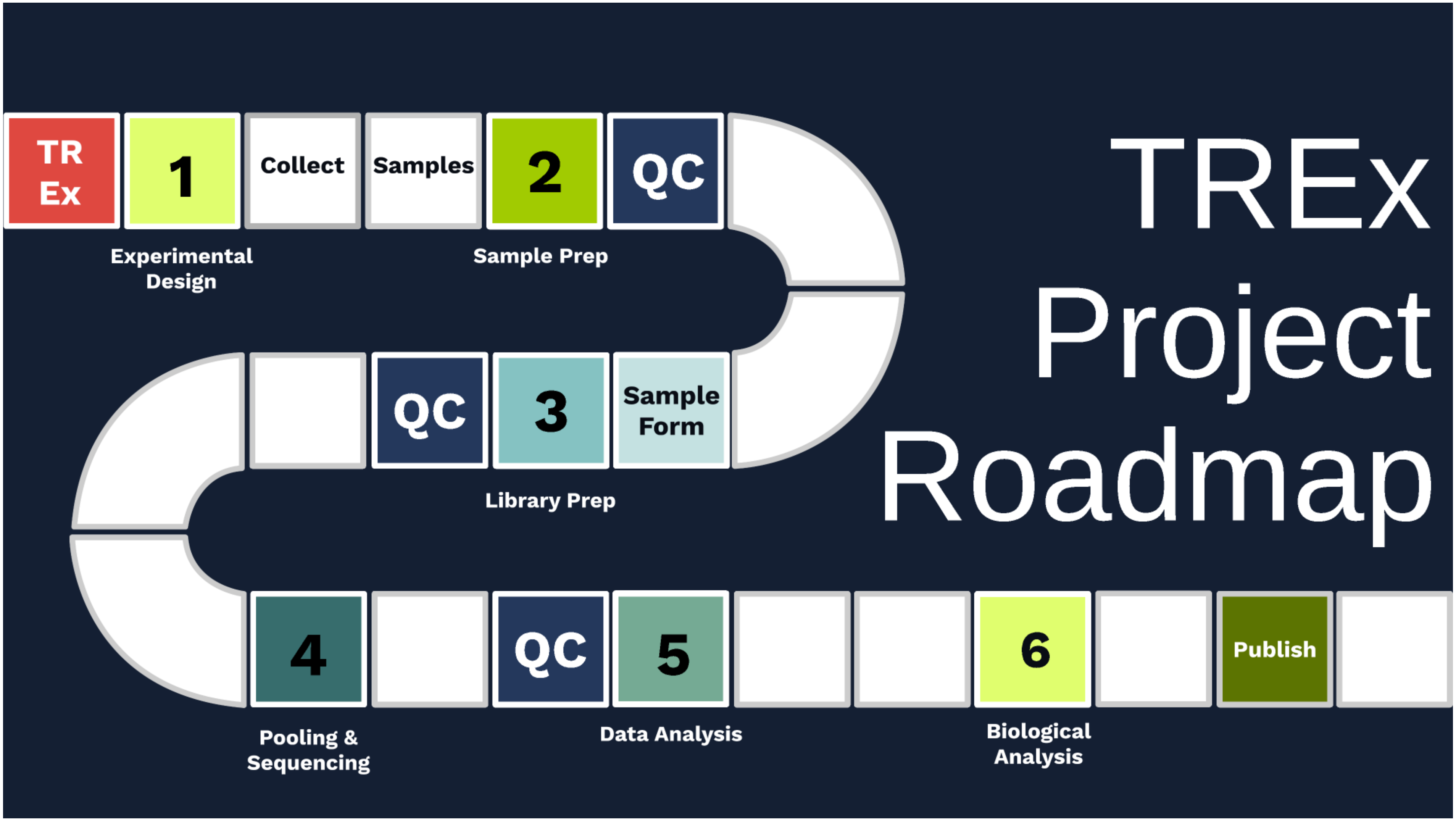
RNA Seq

- Excel report with differentially expressed genes
- HTML reports
  - Mapping Summary
  - Analysis QC Plots
  - Clustering
- FASTQ files on request

Small RNA

- Excel report: Analysis of Annotated miRNAs
  - mirdeep2 quantifications
  - read count summary
  - Log2 fold change calculation
- FASTQ files on request

TREx Project Roadmap



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Biological Analysis

RNA QC

- You:
  - Complete QC

- TREx:
  - Help with QC interpretation
  - Advise on how QC affects technology

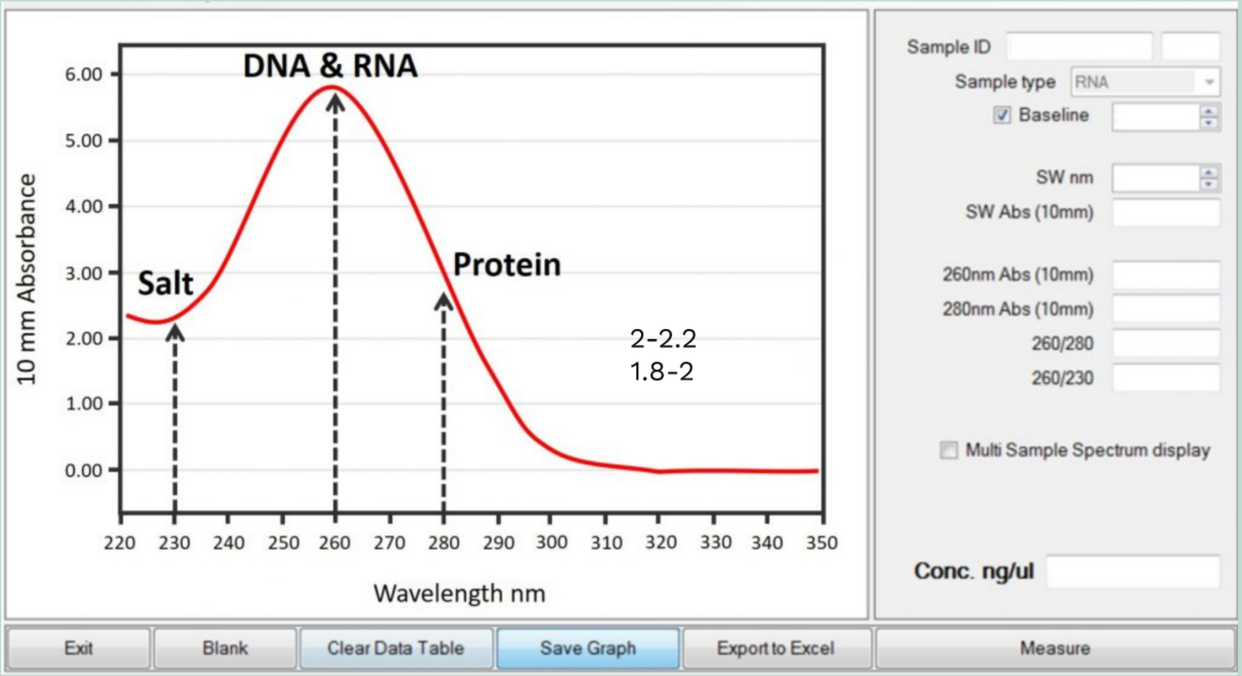
Nanodrop

Fragment Analyzer

Qubit

Nanodrop:

- Chemical Purity
- Concentration: >20ng/uL



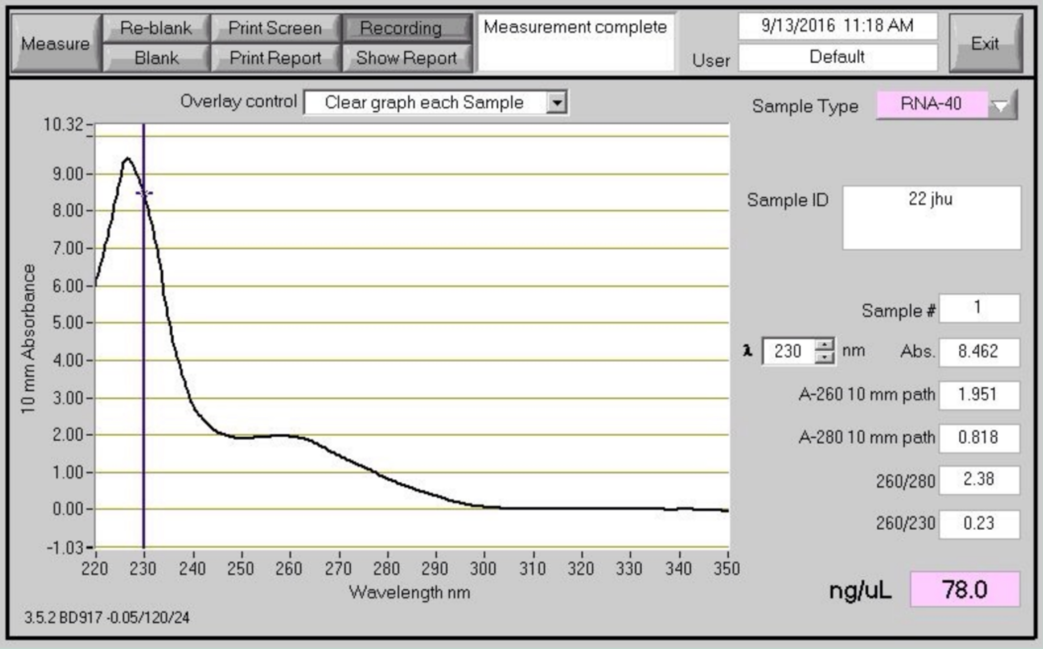
Sample type: RNA

☑ Baseline

2-2.2
1.8-2

Bad 260/230: Salty RNA

- Cause
  - Touching the interface
  - Not drying the pellet enough
  - Column clean-up
- Solution
  - Repeat final clean-up step
  - Be careful to avoid the interface
  - Ethanol Wash
    - Move the pellet
    - Dry the pellet
    - Repeat 2-3x



Measurement complete

9/13/2016 11:18 AM

User: Default

Overlay control: Clear graph each Sample

Sample Type: RNA-40

Sample ID: 22 jhu

Sample #: 1

λ 230 nm Abs. 8.462

A-260 10 mm path: 1.951

A-280 10 mm path: 0.818

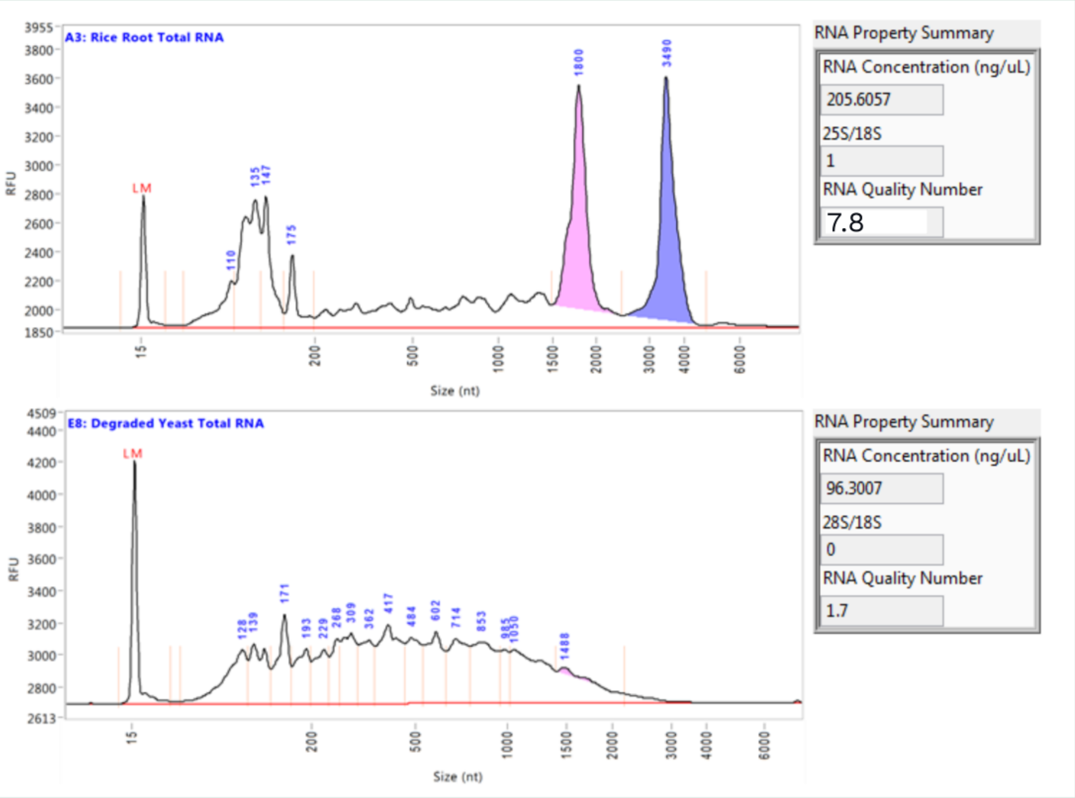
260/280: 2.38

260/230: 0.23

ng/uL: 78.0

3.5.2 BD917 -0.05/120/24

Fragment Analyzer: RNA Quality



RNA Property Summary

RNA Concentration (ng/uL): 205.6057

25S/18S: 1

RNA Quality Number: 7.8

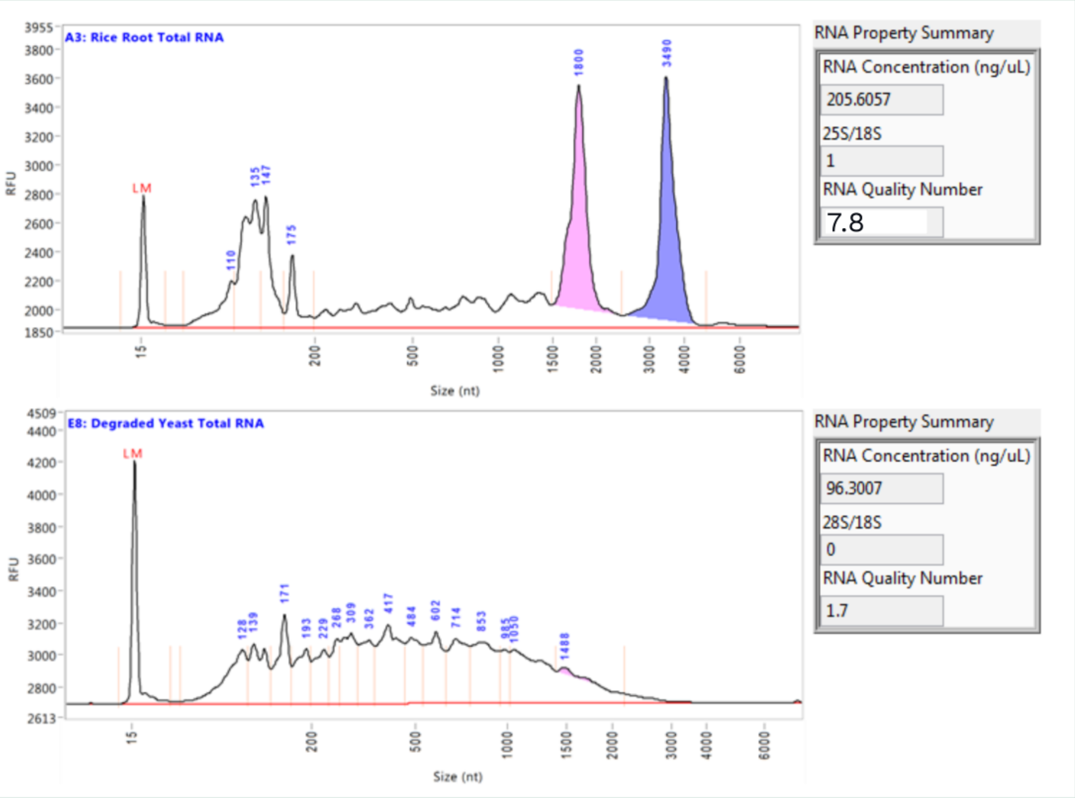
RNA Property Summary

RNA Concentration (ng/uL): 96.3007

28S/18S: 0

RNA Quality Number: 1.7

Fragment Analyzer: RNA Quality



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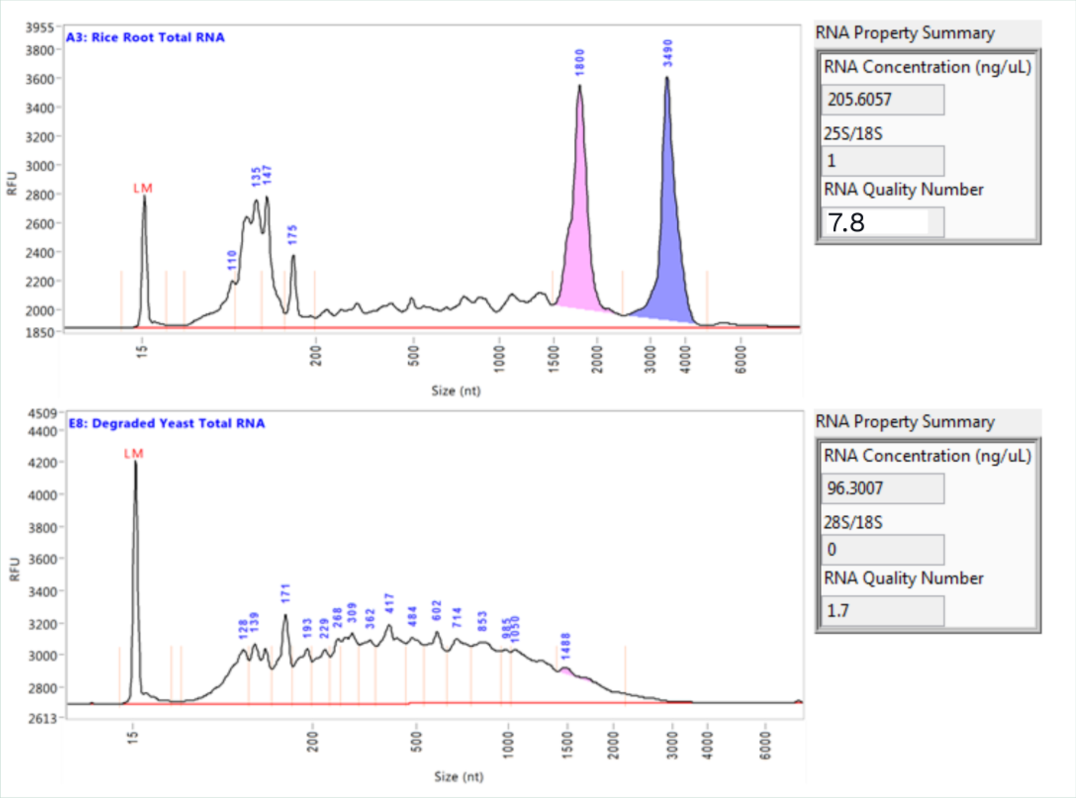
→ High Quality
Intact
RQN>7

Fragment Analyzer: RNA Quality



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Intact
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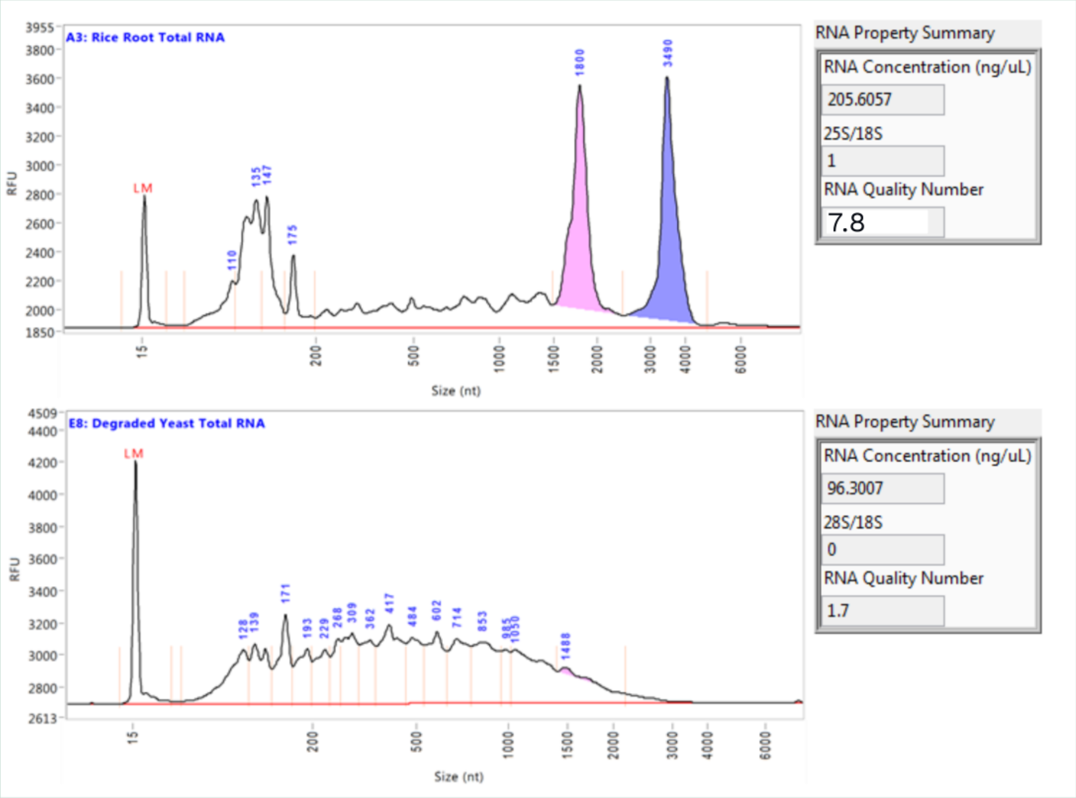
Poly A
Selection

Fragment Analyzer: RNA Quality



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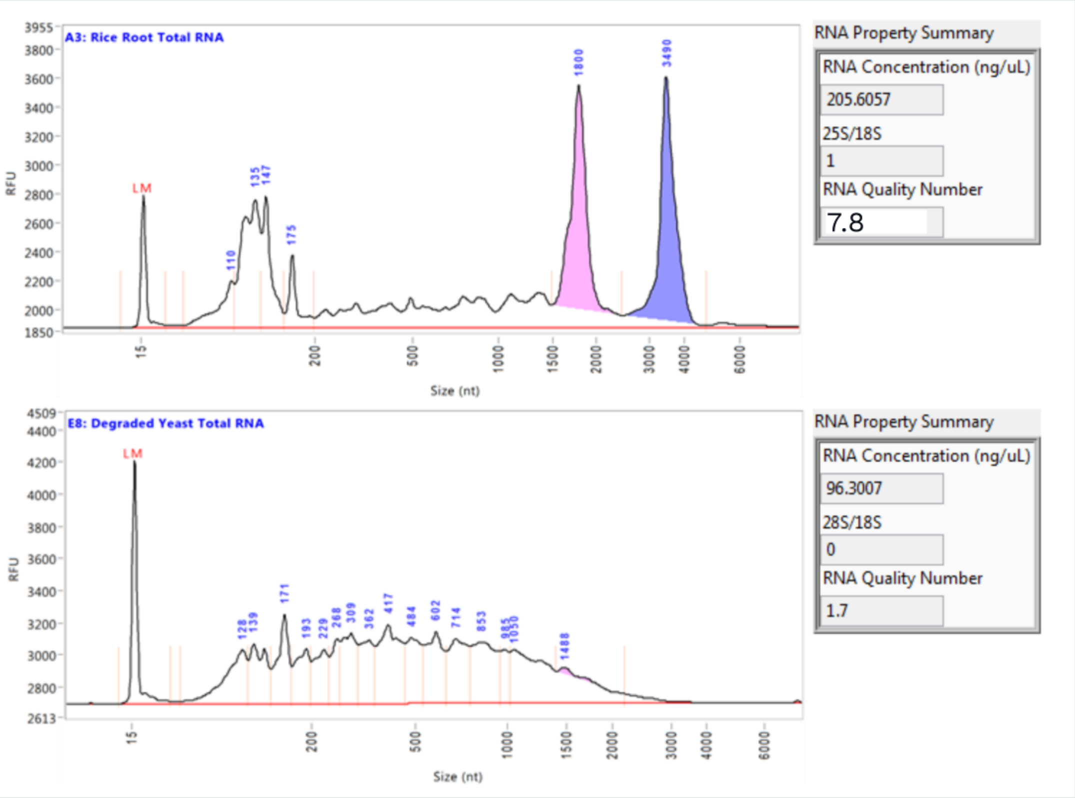
RQN>7

Poly A
Selection

Degraded
RQN<7

Fragment Analyzer: RNA Quality



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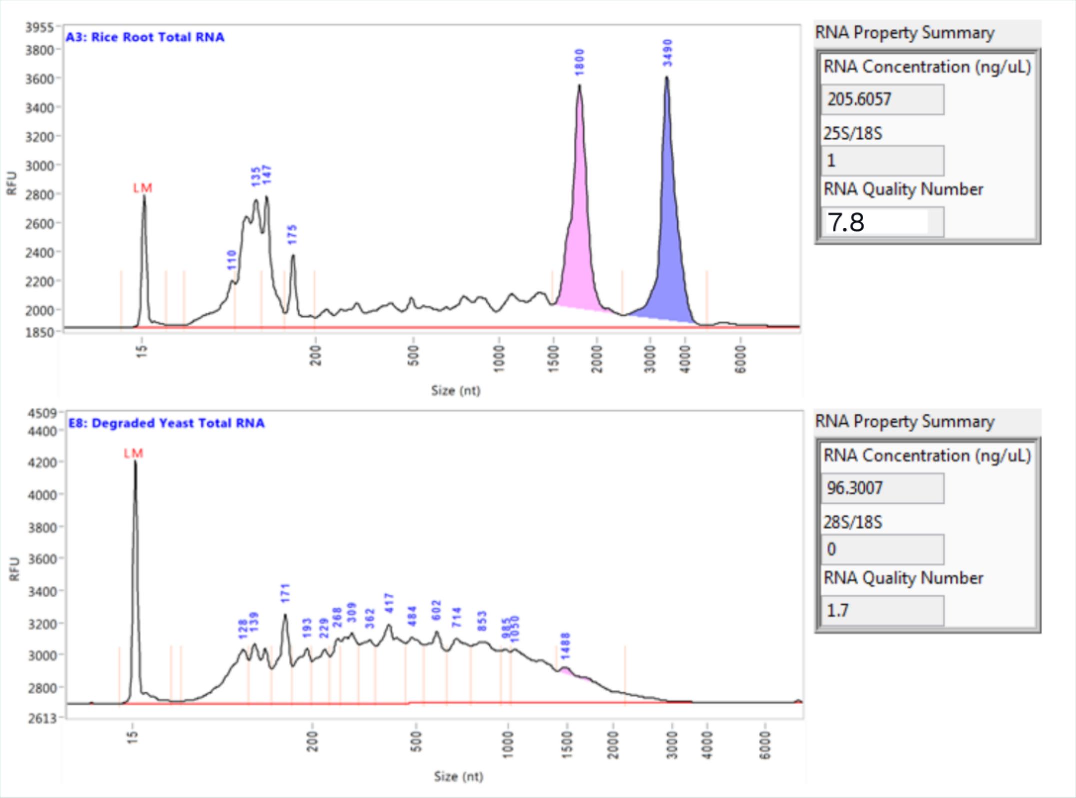
Discuss

Fragment Analyzer: RNA Quality



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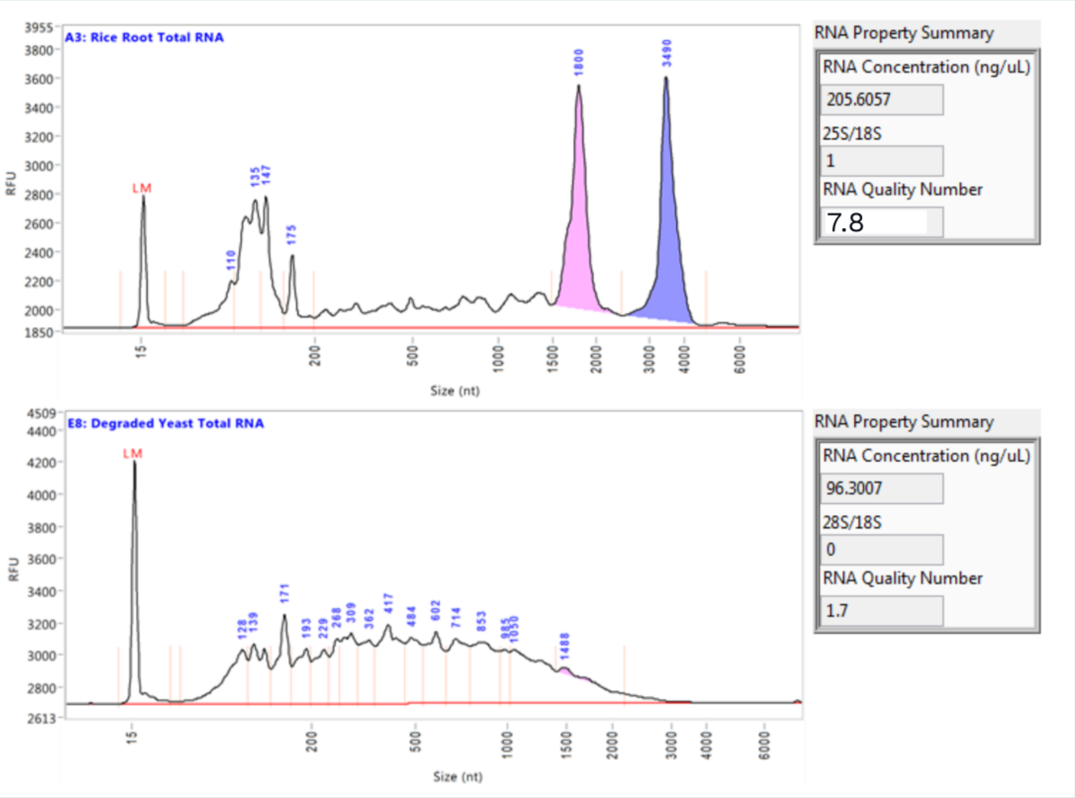
RQN<7

Ribosomal
Depletion

Discuss

Fragment Analyzer: RNA Quality



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→ High Quality
Intact
RQN>7

→ Poly A
Selection

→ Degraded
RQN<7

→ Discuss

Ribosomal
Depletion

Re-Extract

Qubit:

Concentration for <20ng/uL

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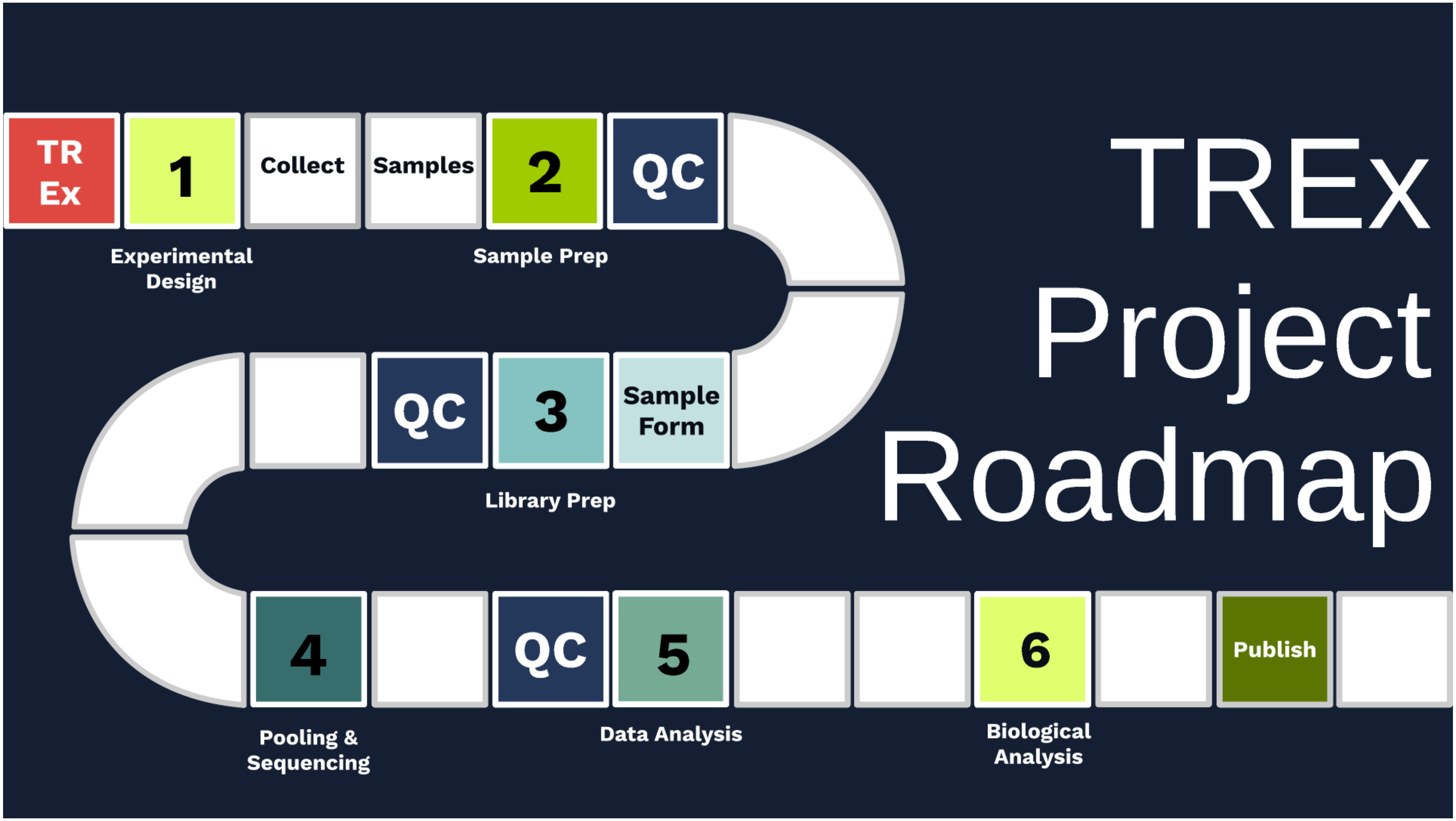


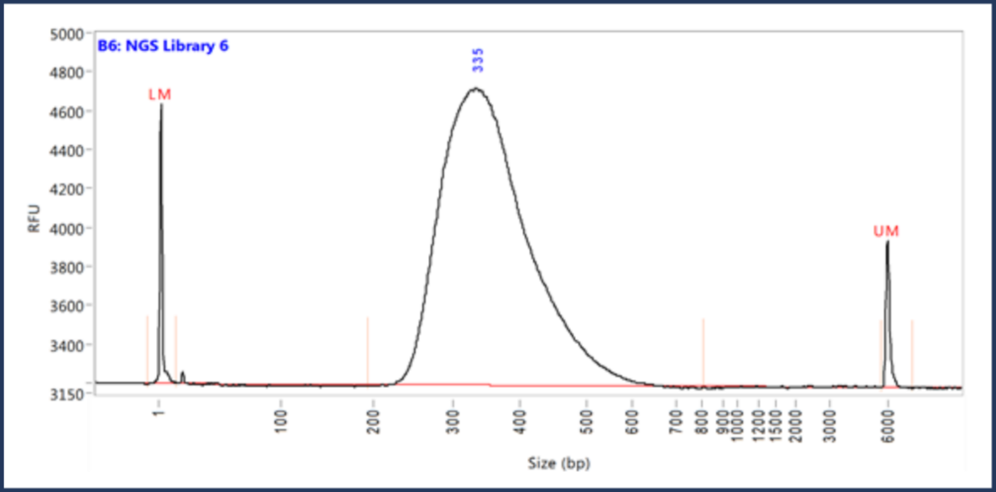
Library QC

- Qubit: Concentration

- Fragment Analyzer:
  - Size Distribution

Library QC

- Qubit: Concentration
- Fragment Analyzer:
  - Size Distribution



Library QC

- Qubit: Concentration
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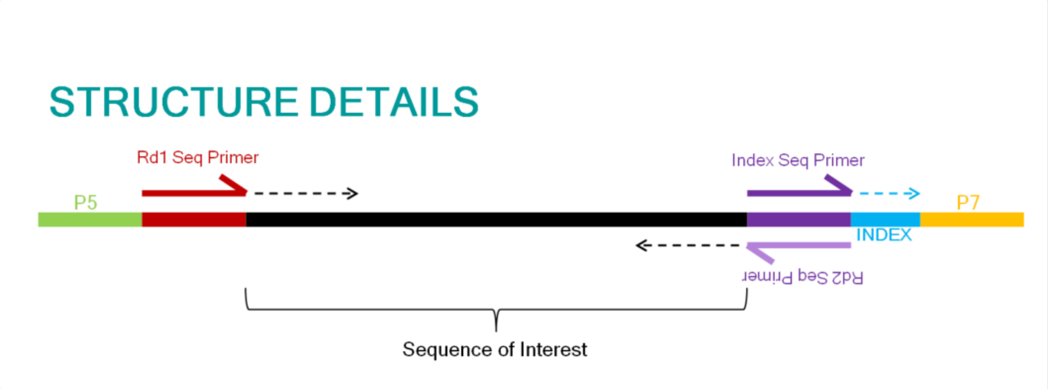

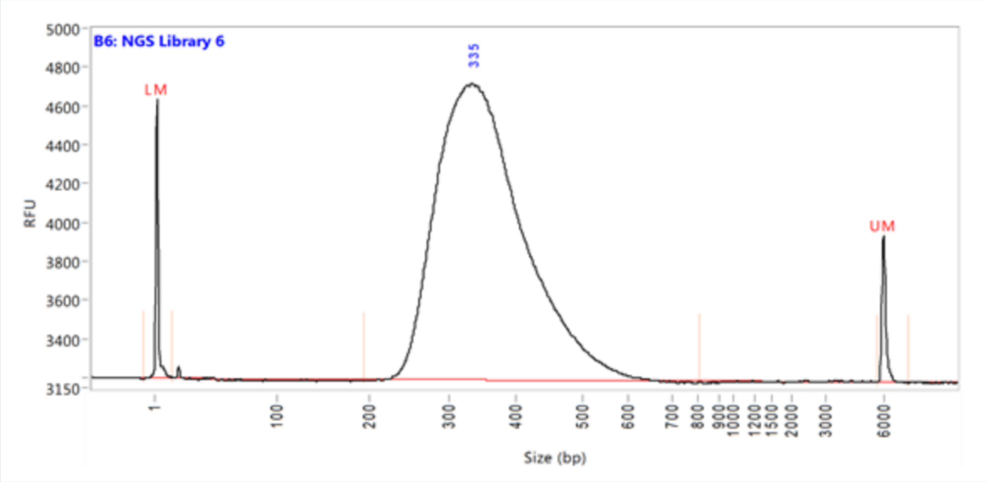

Library QC

- Qubit: Concentration

- Fragment Analyzer:
  - Size Distribution
  - Primer: <10%
  - Adapter Dimer <10%

Library QC

- Qubit: Concentration

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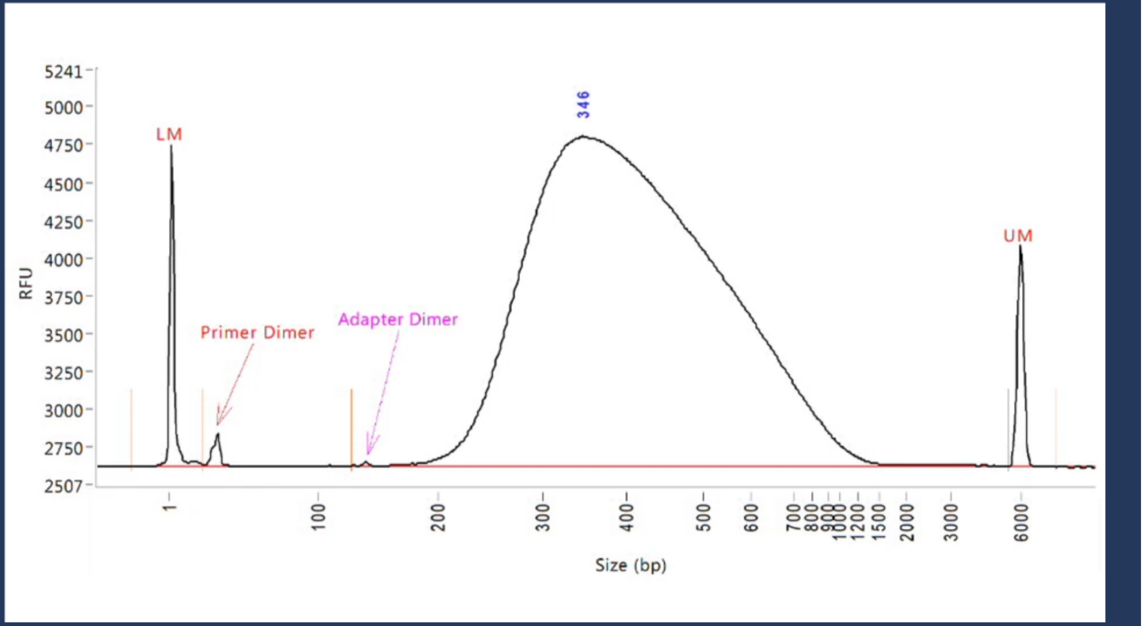

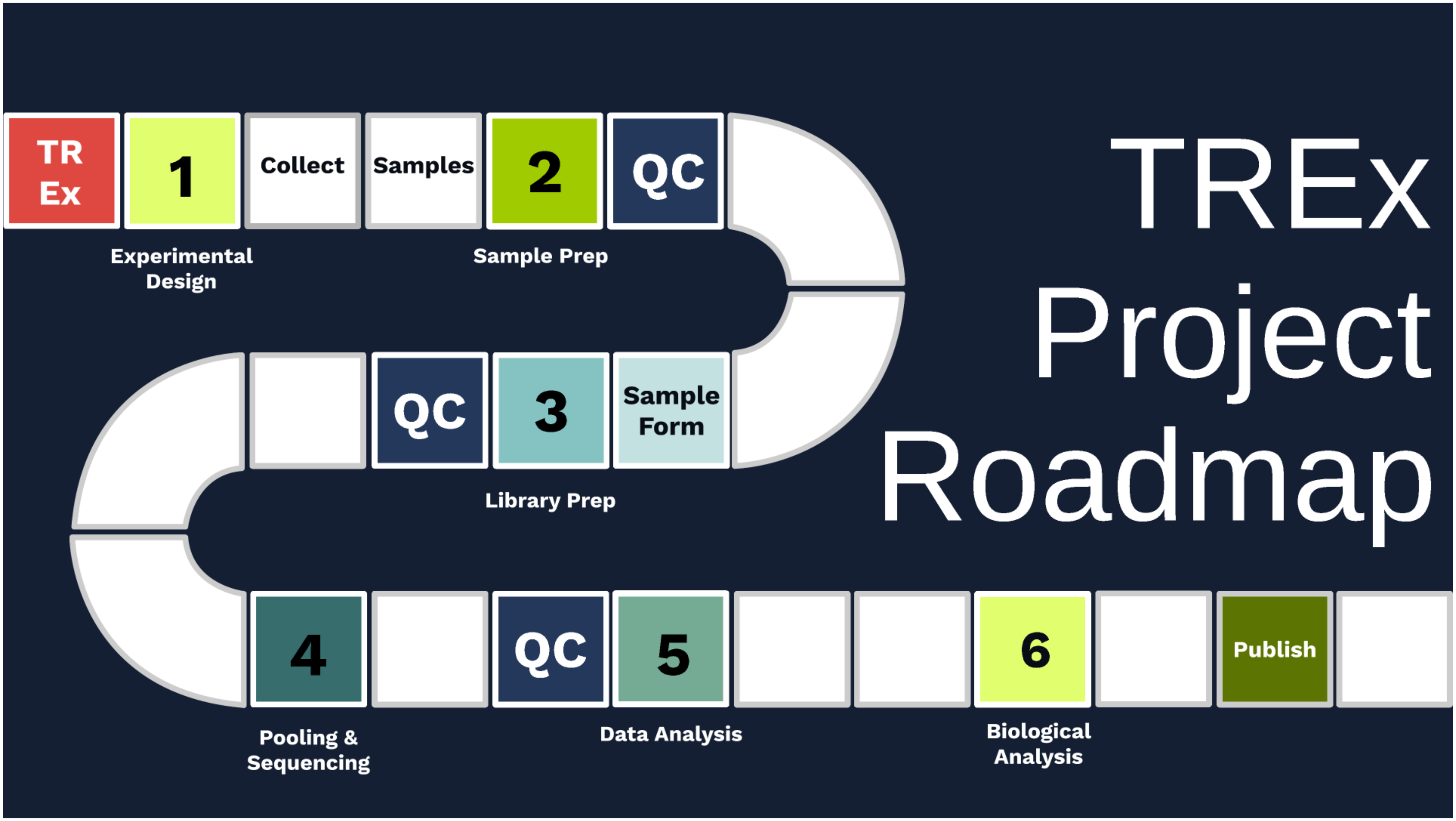
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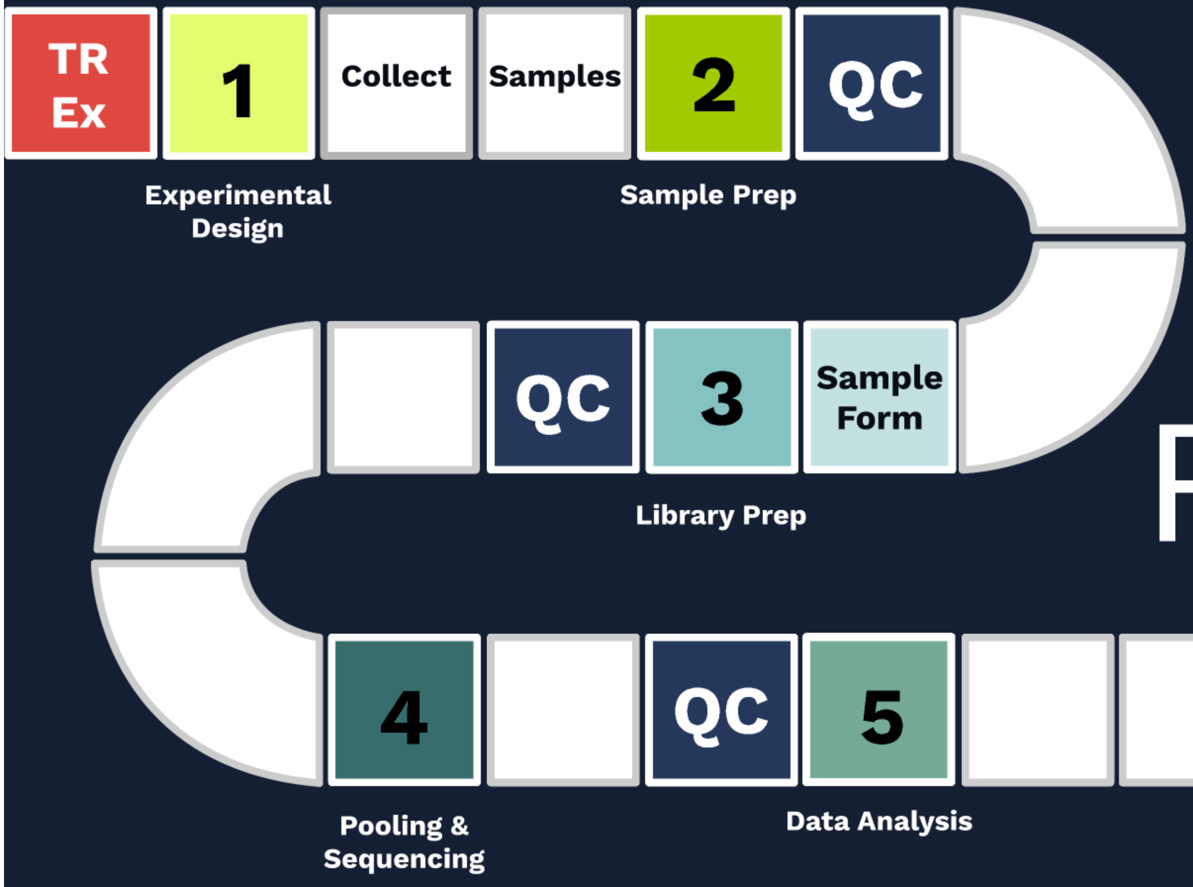
Sample Submission Form:

- You:
  - Fill out form
  - Submit form via e-mail
  - Drop off samples
    - VRT 4th floor -80
    - Biotech 333

- TREx:
  - Double check everything looks good
  - Start Library Prep

TREx Project Roadmap



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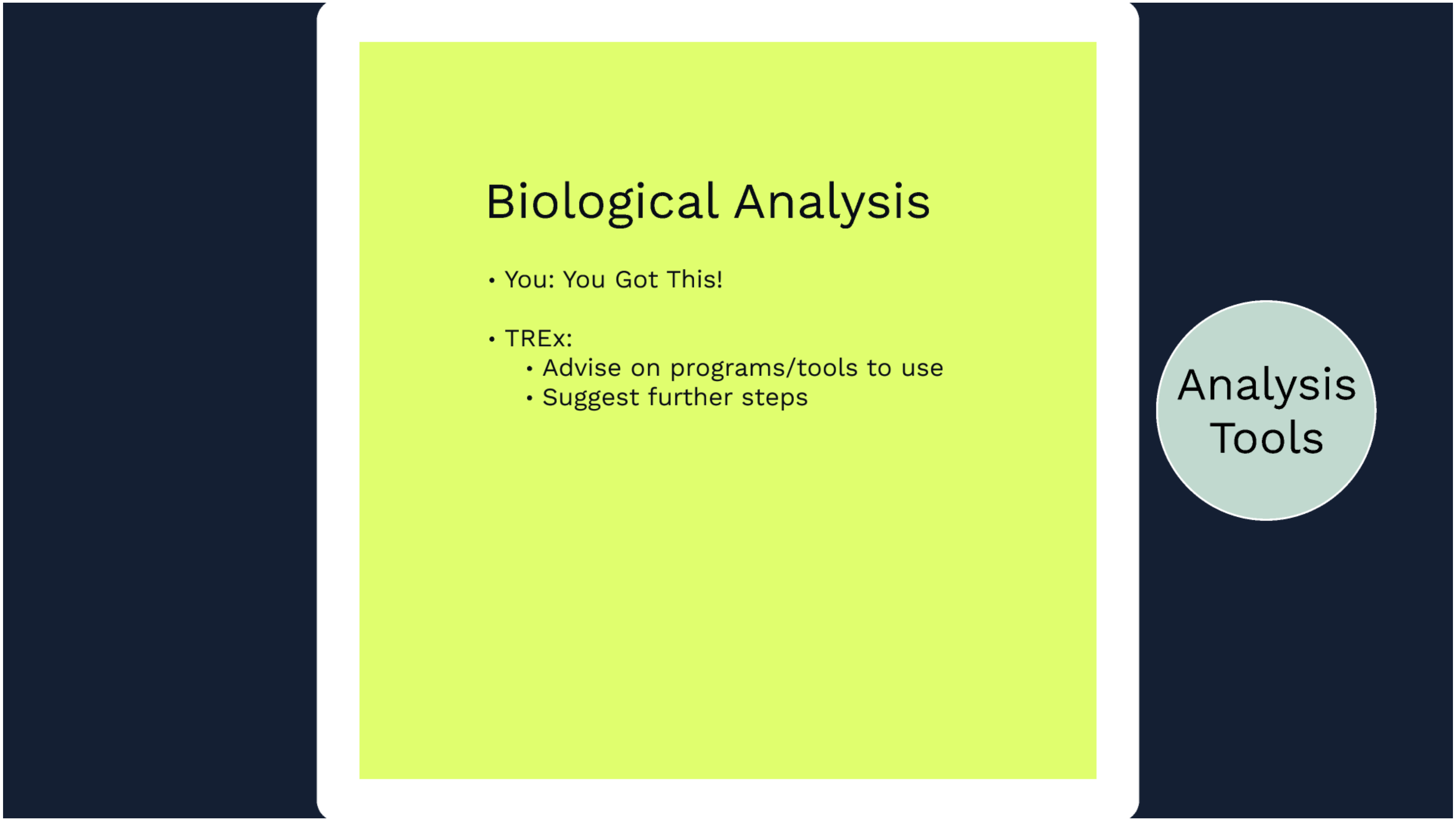

Biological Analysis

- You: You Got This!
- TREx:
  - Advise on programs/tools to use
  - Suggest further steps

Analysis Tools

Biological Analysis Tools

- **R (RStudio)** www.rstudio.com windows-style environment for R, Shiny apps
- **iDEP** bioinformatics.sdstate.edu/idep web interface for PCA, clustering, DEgenes (R tools)
- **Heatmapper** www2.heatmapper.ca/expression heatmap with clustering
- **Panther** pantherdb.org GO term, pathway enrichment in DE genes
- **DAVID** david.ncifcrf.gov GO term, pathway enrichment in DE genes
- **Reactome** reactome.org pathway enrichment in DE genes, expression overlay
- **GSEA/MSig*** software.broadinstitute.org/gsea gene set enrichment across full log2FC
- **Ingenuity†** www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis/

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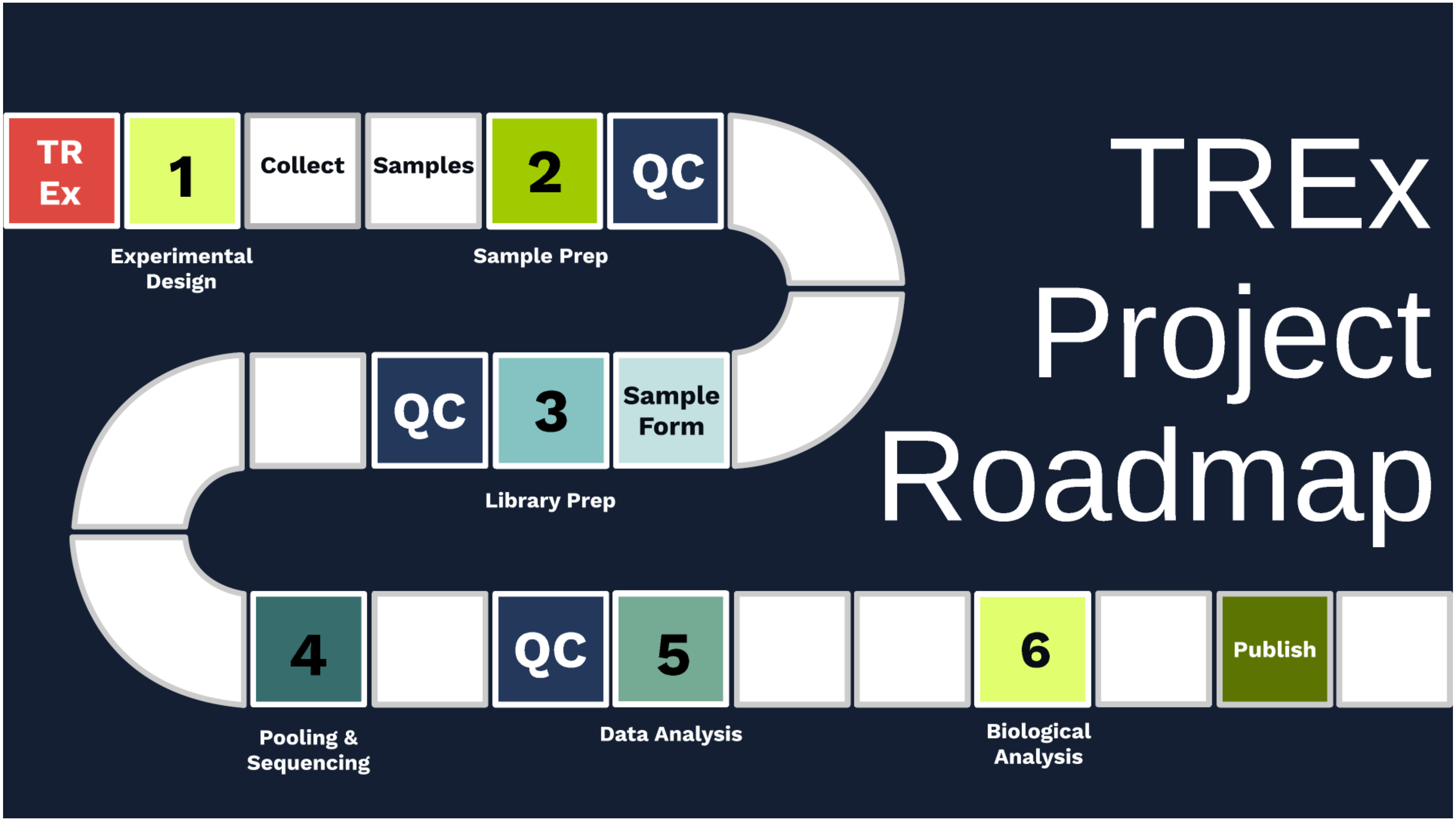

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