

POLL 1



RRBS-SEQ
ICE
OS-SEQ
MAINE-SEQ
RIBO-SEQ
CHIP-SEQ

CLASH-SEQ
TIF-SEQ/PEAT
IN-SEQ
TAB-SEQ

5-C
SMM-SEQ
OXBS-SEQ
RIP-SEQ

4-C
TC-SEQ
NET-SEQ
UMI
CAP-SEQ
FAIRE-SEQ
DUPLEX-SEQ

PAR-CLIP-SEQ

BS-SEQ
MEDIP-SEQ
GRO-SEQ

MEIP-SEQ
DIGITAL
CHIA-PET
ATAC-SEQ
CIP-TAP

ICLIP

MBDCAP-SEQ

PARE-SEQ/GMUT

HITS-CLIP

HI-C/3-C

TRAP-SEQ
RC-SEQ
FRAG-SEQ

WORKSHOP 16: LIBRARY PREP FOR NGS

DAY 3

Marco Morselli

Pellegrini Lab

QCBio Collaboratory Fellow

April 16th, 2020

TUESDAY (day 1)

- **INTRO to SEQUENCING**
- **LIBRARY PREP 101**

YESTERDAY (day 2)

- **Specialty DNA Library Preps**
- **RNA Library Preps**
 - RNAseq
 - small RNA-seq
 - Low-input RNAseq

TODAY : ONLINE LAB Experiments (day 3)

(Use the TapeStation for QC; Library Preparation; submit libraries for sequencing)

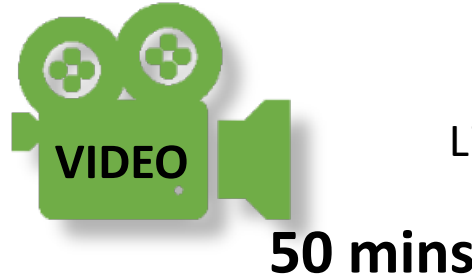
RNA: Quality check of extracted Eukaryotic RNA using the TapeStation

PART 1



LIBRARY pt1 : Library Preparation

PART 2 - NEW



Library Prep and AMPure XP beads

LIBRARY pt2 : Visualization of Purified DNA using the TapeStation

PART 3



LIBRARY pt3 : Library Submission

PART 4

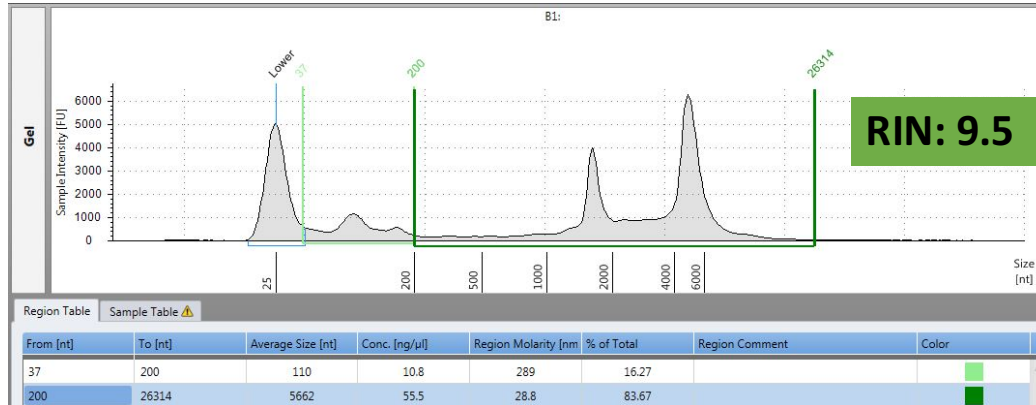
Qubit and Molarity Calculation



PART 1

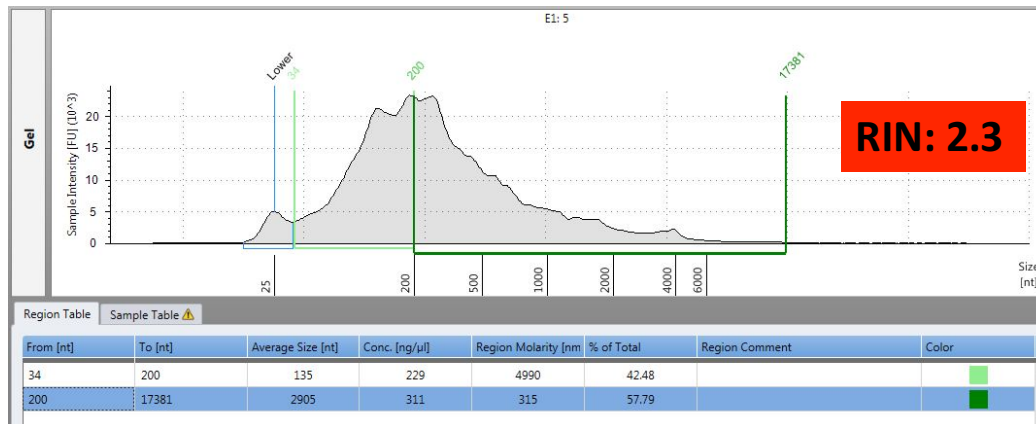
LAB

RNA: Quality check of extracted Eukaryotic RNA using the TapeStation



GOALS:

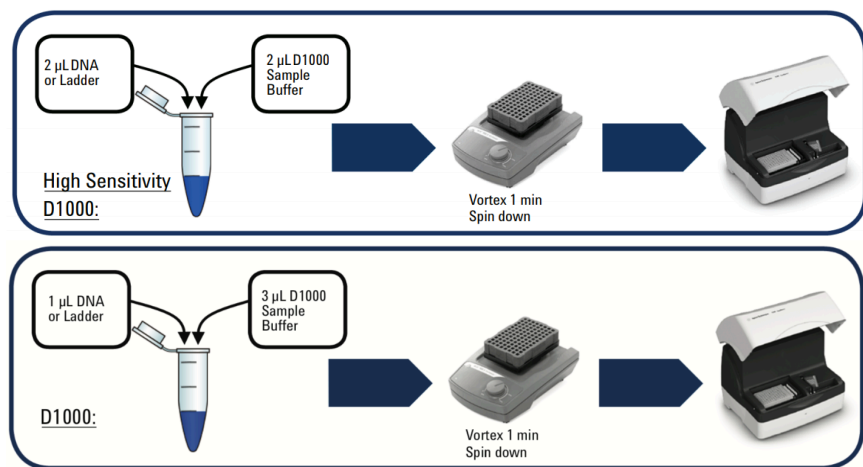
1. Learn how to use the TapeStation (RNA)
2. Learn to estimate the integrity of RNA
3. Decide the RNAseq strategy



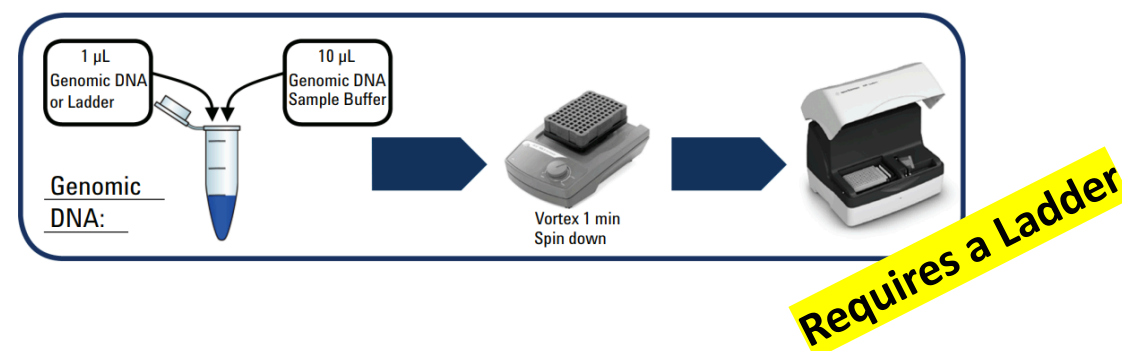
Agilent 2200 TapeStation System



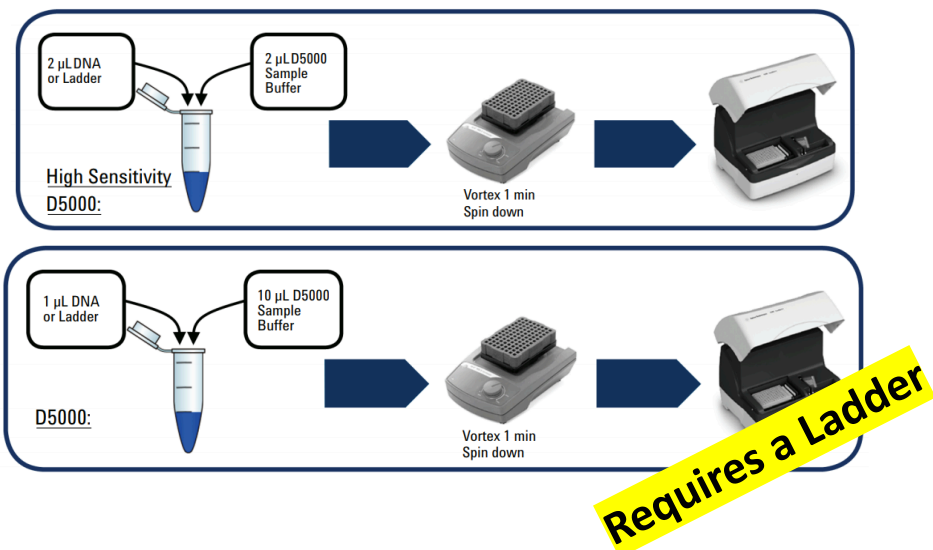
D1000: DNA up to 1 Kb



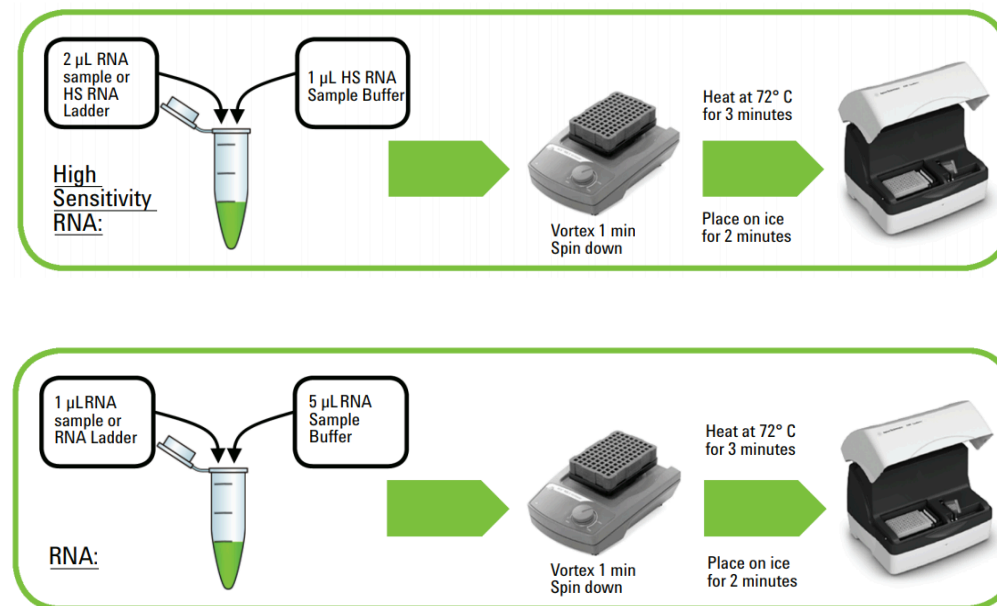
Genomic DNA: DNA up to 60 Kb



D5000: DNA up to 5 Kb



RNA



WHAT DO YOU NEED TO RUN THE TapeStation?

Short DNA (up to 1 Kb) - D1000 (Regular Sensitivity)	
D1000 ScreenTape For the analysis of DNA from 35 to 1000 bp. Includes 7 ScreenTape	5067-5582
D1000 Reagents For the analysis of DNA from 35 to 1000 bp. Includes ladder and sample buffer [OPTIONAL – The Ladder is not Required for the run, you can buy reagents only ↓]	5067-5583
D1000 Sample Buffer Additional buffer for the D1000 ScreenTape assay	5067-5602

Short DNA (up to 1 Kb) - D1000 (High Sensitivity)	
High Sensitivity D1000 ScreenTape for the analysis of DNA from 35 to 1000 bp. Includes 7 ScreenTape	5067-5584
D1000 Reagents For the analysis of DNA from 35 to 1000 bp. Includes ladder and sample buffer [OPTIONAL – The Ladder is not Required for the run, you can buy reagents only ↓]	5067-5585
High Sensitivity D1000 Sample Buffer Additional buffer for the High Sensitivity D1000 ScreenTape assay	5067-5603

Medium DNA (up to 5Kb) - D5000 (Regular Sensitivity)	
D5000 ScreenTape For the analysis of DNA from 100 to 5000 bp. Includes 7 ScreenTape devices	5067-5588
D5000 Reagents For the analysis of DNA from 100 to 5000 bp. Includes ladder and sample buffer [The Ladder is required for each run]	5067-5589
D5000 Ladder Additional ladder for the D5000 ScreenTape assay	5067-5590
D5000 Reagents For the analysis of DNA from 100 to 5000 bp. Includes ladder and sample buffer.	5067-5589

Medium DNA (up to 5Kb) - D5000 (High Sensitivity)	
High Sensitivity D5000 ScreenTape For the analysis of DNA from 100 to 5000 bp. Includes 7 ScreenTape devices	5067-5592
High Sensitivity D5000 Reagents For the analysis of DNA from 100 to 5000 bp. Includes ladder and sample buffer [The Ladder is required for each run]	5067-5593
High Sensitivity D5000 Ladder Additional ladder for the D5000 ScreenTape assay. [you often run out of ladder before the Tapes and Buffer]	5067-5594
High Sensitivity D5000 Reagents For the analysis of DNA from 100 to 5000 bp. Includes ladder and sample buffer.	5067-5593

Genomic DNA (up to 60Kb) - Regular Sensitivity	
Genomic DNA ScreenTape For the analysis of genomic DNA from 200 to > 60,000 bp. Includes 7 ScreenTape	5067-5365
Genomic DNA Reagents For the analysis of genomic DNA from 200 to > 60,000 bp. Includes ladder and sample buffer [The Ladder is required for each run]	5067-5366

RNA - Regular Sensitivity	
RNA ScreenTape For the analysis of total RNA from 25 to 500ng/μL. Includes 7 ScreenTape	5067-5576
RNA ScreenTape Sample Buffer For use with the RNA ScreenTape assay	5067-5577
RNA ScreenTape Ladder RNA ladder for use with the RNA ScreenTape assay [OPTIONAL – The Ladder is not Required for the run]	5067-5578

RNA - High Sensitivity	
High Sensitivity RNA ScreenTape For the analysis of total RNA from 500 to 10,000 pg/μL. Includes 7 ScreenTape	5067-5579
High Sensitivity RNA ScreenTape Sample Buffer For use with the High Sensitivity RNA ScreenTape assay	5067-5580
High Sensitivity RNA ScreenTape Ladder RNA ladder for use with the High Sensitivity RNA ScreenTape assay (dilute to 20μL with RNase free water) [OPTIONAL – The Ladder is not Required for the run]	5067-5581

Consumables – common to all applications

Loading Tips (1pK of 384 tips)	5067-5153
Mx3000P Strip Tubes	401428
Mx3000P Optical Strip Caps	401425
96-well Sample Plates	5067-5150
96-well Plate Foil Seal	5067- 5154

WHAT DO YOU NEED TO RUN THE TapeStation?



Tips

**Tubes
and caps**

Sample Buffer

**Ladder
(optional)**

ScreenTape



**KEEP
CALM
IT'S
BREAK
TIME**

**CHECK FOR
QUESTIONS**

QUESTIONS
#12

LAB

GOALS:

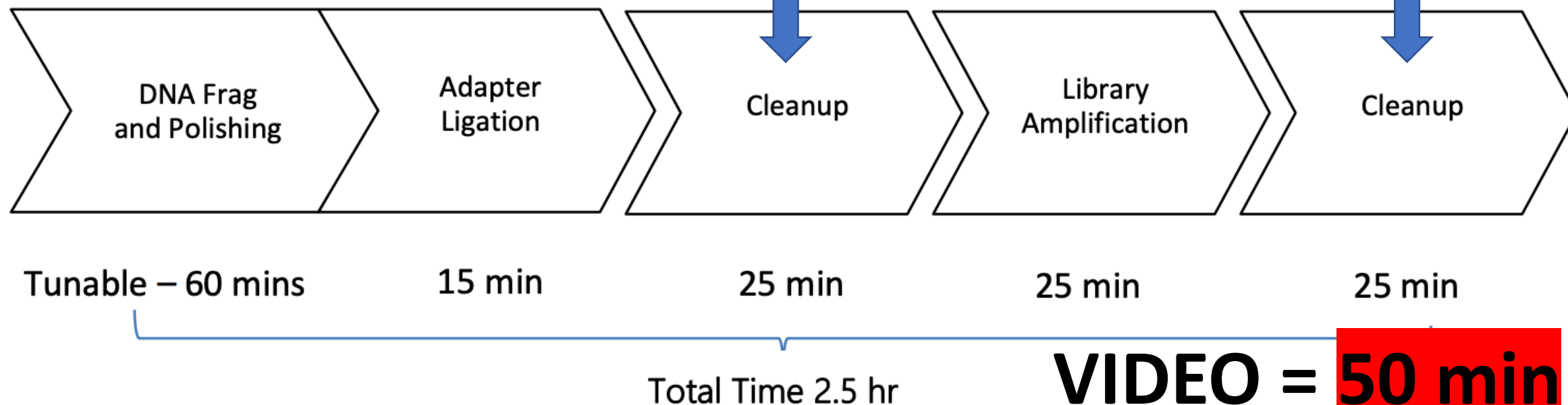
1. Prepare a Library
2. Use AMPure beads for Purification and Adapter-Dimer Removal

— **Quanta**bio —
sparQ DNA Frag & Library Prep Kit



PURIFICATIONS

Workflow Overview



Purification Beads

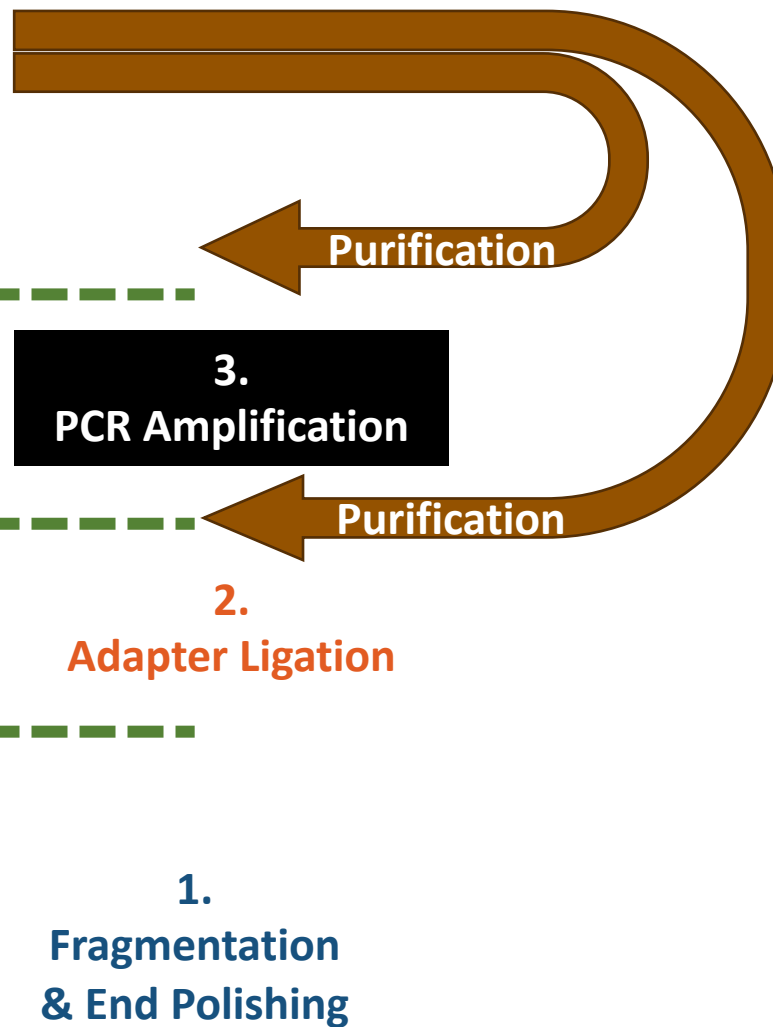
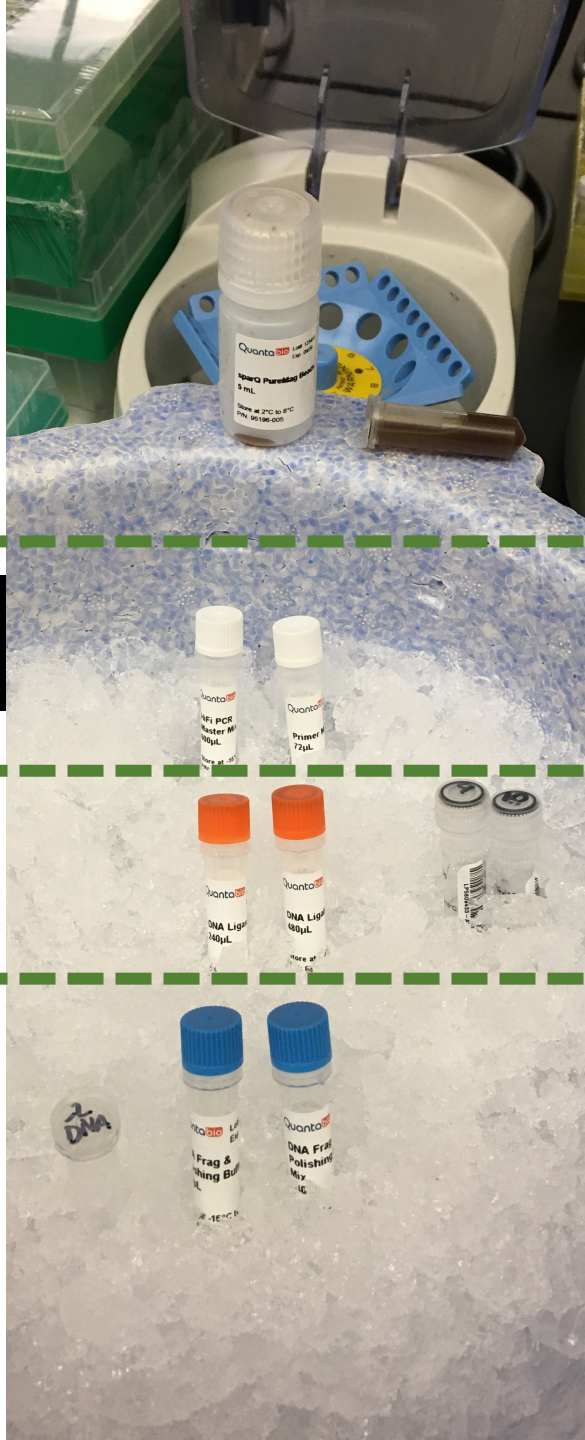
PCR Primer Mix +
HiFi PCR Master Mix

DNA Ligation Buffer
& DNA Ligase

+ DNA Adapters (15 μ M)

λ phage gDNA

DNA Frag &
Polishing Buffer +
Enzyme Mix



STANDARD PROTOCOL

500 ng of λ
phage gDNA

1.8x beads

1.8x beads

4X DNA
Adapters

500 ng of λ
phage gDNA



**KEEP
CALM
IT'S
BREAK
TIME**

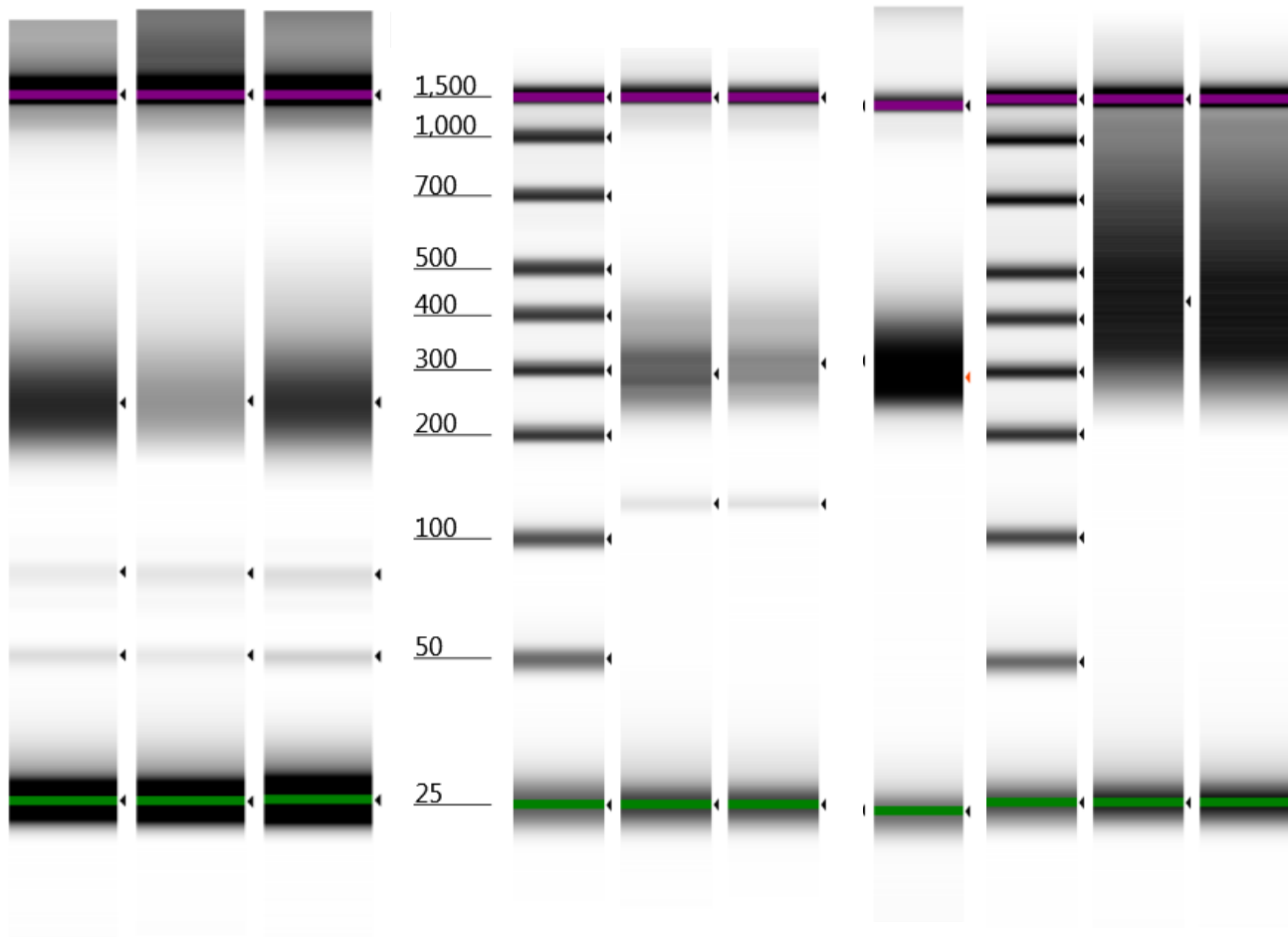
**CHECK FOR
QUESTIONS**

QUESTIONS
#13

PART 3



LIBRARY pt2 : Visualization of Purified DNA using the TapeStation



GOALS:

1. Learn how to use the TapeStation (DNA)
2. Assess the quality of a final library and its average size



**KEEP
CALM
IT'S
BREAK
TIME**

**CHECK FOR
QUESTIONS**

QUESTIONS
#14

PART 4



LIBRARY pt3 : Library Submission

GOALS:

Prepare your libraries for sequencing:

1. Calculate the concentration (**Qubit**)
2. Calculate the molarity (http://molbiol.ru/eng/scripts/01_07.html) or use the formula:



illumina®

Pooling Calculator

$$\frac{(\text{concentration in ng/}\mu\text{l})}{(660 \text{ g/mol} \times \text{average library size in bp})} \times 10^6 = \text{concentration in nM}$$

c. Calculate the molarity: http://molbiol.ru/eng/scripts/01_07.html

dsDNA **Size of the nucl. acid [kb]:** 0.3kb

Mw for "olig" :

"weight" --> "mole":

1

molecules

"mole" --> "weight":

1

molecules

2. Concentration

Calculation of the molar concentration:

21 in 1

107.86

3. Molarity

→ Formula:

$$\frac{\text{concentration} \left(\frac{\text{ng}}{\mu\text{l}} \right) * 10^6}{660 \left(\frac{\text{g}}{\text{mol}} \right) * \text{average size (bp)}} = \text{concentration in nM}$$

Tube	μl used	Qubit Tube	Stock Conc.	Average Size	Molarity
Standard 1	10 μl	too low	too low		
Standard 2	10 μl	5.0 μg/ml	100 ng/μl		

DNA 1 (A)	2 μl	0.419 μg/ml	41.9 ng/μl	426 bp	149 nM
DNA 2 (B)*	2 μl	0.433 μg/ml	43.3 ng/μl	379 bp	?????

POLL 2



→ Formula:

$$\frac{\text{concentration} \left(\frac{\text{ng}}{\mu\text{l}} \right) * 10^6}{660 \left(\frac{\text{g}}{\text{mol}} \right) * \text{average size (bp)}} = \text{concentration in nM}$$

*It requires more purification to get rid of Adapter/Primer Dimers

Tube	μl used	Qubit Tube	Stock Conc.	Average Size	Molarity
Standard 1	10 μl	too low	too low		
Standard 2	10 μl	5.0 μg/ml	100 ng/μl		

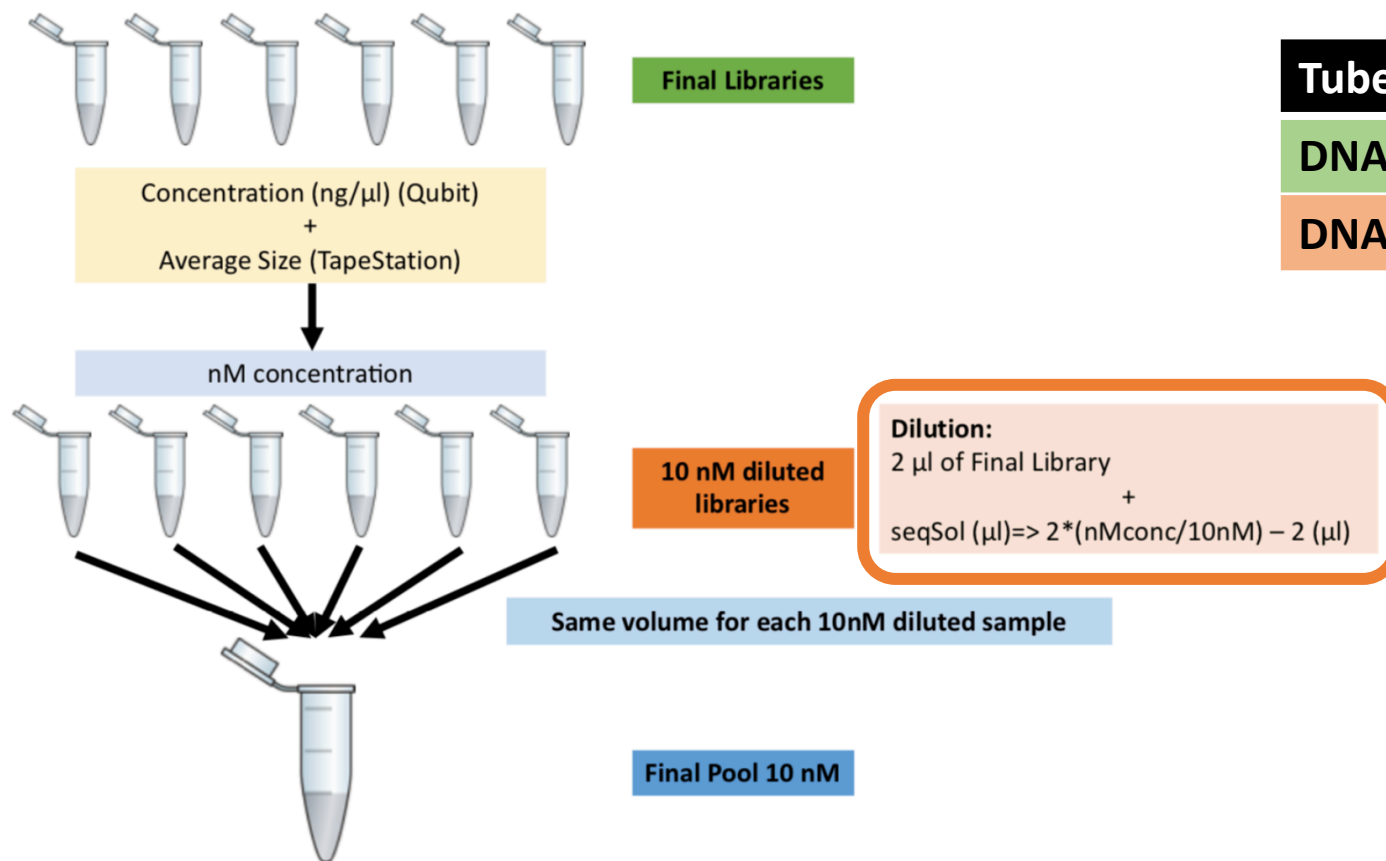
DNA 1 (A)	2 μl	0.419 μg/ml	41.9 ng/μl	426 bp	149 nM
DNA 2 (B)*	2 μl	0.433 μg/ml	43.3 ng/μl	379 bp	173 nM

→ Formula:

$$\frac{\text{concentration} \left(\frac{\text{ng}}{\mu\text{l}} \right) * 10^6}{660 \left(\frac{\text{g}}{\text{mol}} \right) * \text{average size (bp)}} = \text{concentration in nM}$$

*It requires more purification to get rid of Adapter/Primer Dimers

2. Dilute your sample to 10 nM using Sequencing Solution (EB + 0.1% Tween-20)
3. Submit your diluted final library (libraries) to your favorite Sequencing Core



Tube	Stock Conc.	Average Size	Molarity
DNA 1 (A)	41.9 ng/μl	426 bp	149 nM
DNA 2 (B)*	43.3 ng/μl	379 bp	???

e.g. DNA 1:

$$2 * (149 \text{ nM} / 10 \text{ nM}) - 2 (\mu l) = 27.8 \mu l$$

2 μl of DNA 1 Final Library
+
27.8 μl of Sequencing Solution

e.g. DNA 2:

$$2 * (??? \text{ nM} / 10 \text{ nM}) - 2 (\mu l) = ??? \mu l$$

2 μl of DNA 1 Final Library
+
??? μl of Sequencing Solution

POLL 3

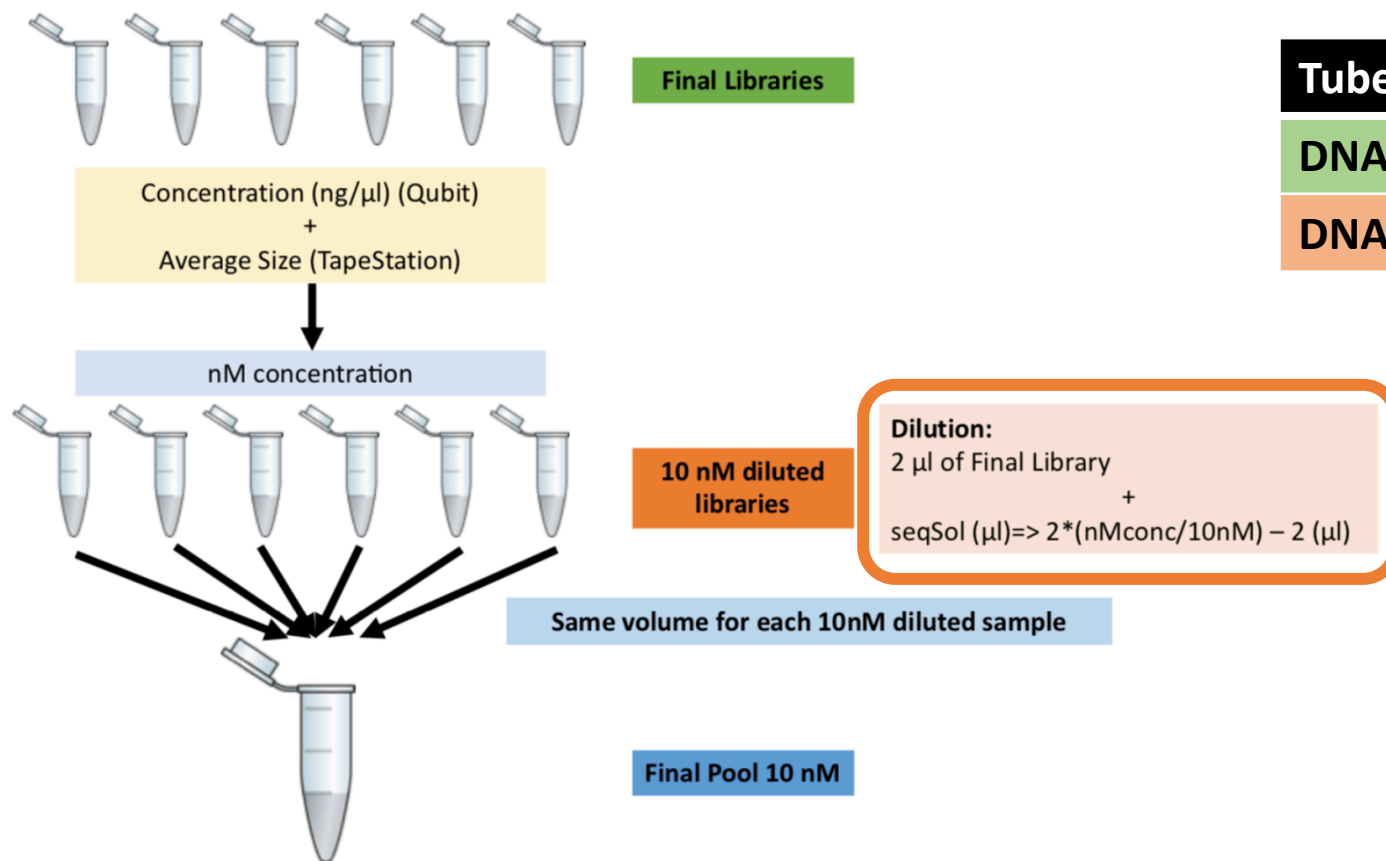


Or: <https://support.illumina.com/help/pooling-calculator/pooling-calculator.htm>

illumina®

Pooling Calculator

2. Dilute your sample to 10 nM using Sequencing Solution (EB + 0.1% Tween-20)
3. Submit your diluted final library (libraries) to your favorite Sequencing Core



Tube	Stock Conc.	Average Size	Molarity
DNA 1 (A)	41.9 ng/μl	426 bp	149 nM
DNA 2 (B)*	43.3 ng/μl	379 bp	???

e.g. DNA 1:

$$2 \times (149 \text{ nM} / 10 \text{ nM}) - 2 (\mu\text{l}) = 27.8 \mu\text{l}$$

2 μl of DNA 1 Final Library
+
27.8 μl of Sequencing Solution

e.g. DNA 2:

$$2 \times (173 \text{ nM} / 10 \text{ nM}) - 2 (\mu\text{l}) = 32.6 \mu\text{l}$$

2 μl of DNA 1 Final Library
+
32.6 μl of Sequencing Solution

Or: <https://support.illumina.com/help/pooling-calculator/pooling-calculator.htm>

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Pooling Calculator



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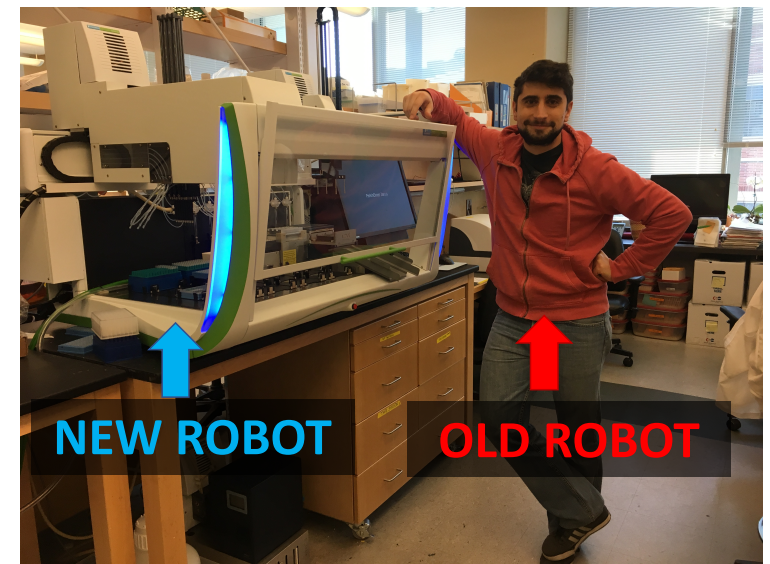
**CHECK FOR
QUESTIONS**

QUESTIONS
#15

RESOURCES OTHER THAN WORKSHOPS THAT WE OFFER:

- 1) **LIBRARY PREPARATION: FREE***** and under the form of collaboration (contact Matteo Pellegrini matteop@mcd.b.ucla.edu)
- 2) **“OFFICE HOURS”**: consult me to design experiment for NGS and solve problems (mmorselli@ucla.edu)
- 3) **Library Prep Calendar**:
https://calendar.google.com/calendar/embed?src=sdhmv1tsnqtdna4dpr2bkigt4%40group.calendar.google.com&ctz=America/Los_Angeles
- 4) **LIQUID HANDLER: Training**

***reagents and consumables needs to be provided by the user



**GOOGLE
FORMs for
ATTENDANCE
and mini-QUIZ
(LIBRARY PREP)**

TIME FOR ONLINE QUESTIONS

QUESTIONS #16

CHECK FOR
QUESTIONS

Pellegrini's Lab Tape Station
Joined the COVID-19 FIGHT!

04/07/2020