

# KAPA Hyper Prep/HyperPlus Library Preparation on the Hamilton STAR

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# Outline

- Kapa Hyper Prep and HyperPlus Intro
  - Key Benefits
  - Recommended DNA Inputs
- Kapa Hyper Prep / HyperPlus using the Hamilton STAR
  - Protocol Overview
  - System Requirements (Hardware, Labware and Consumables)
- Kapa Hyper Prep / HyperPlus Method
  - Evaluation/Verification
  - Support: KAPA vs. Hamilton

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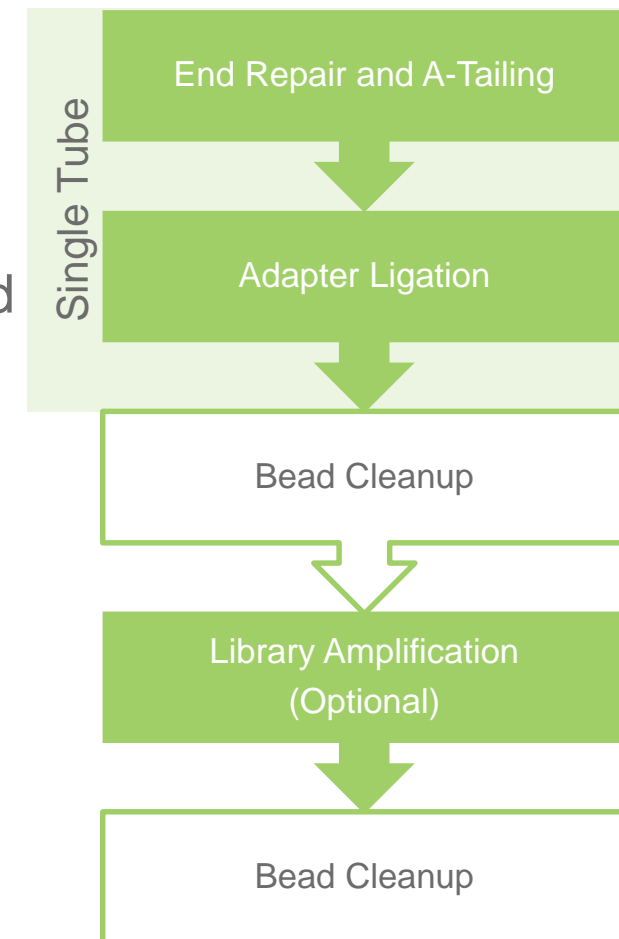
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# Hyper Prep

- A novel, single-tube library construction method
- Library construction from fragmented DNA in less than 3 hours
- Robust performance with inputs from 1 ng – 1 µg
- Bead-based size selection steps can be incorporated to achieve desired final library fragment-size distribution
- Contains KAPA HiFi and an optimized Library Amplification Primer Mix
- Library amplification may be omitted for PCR-free workflows

## Kapa Hyper Prep Kit

Total Time ~2.75 hrs

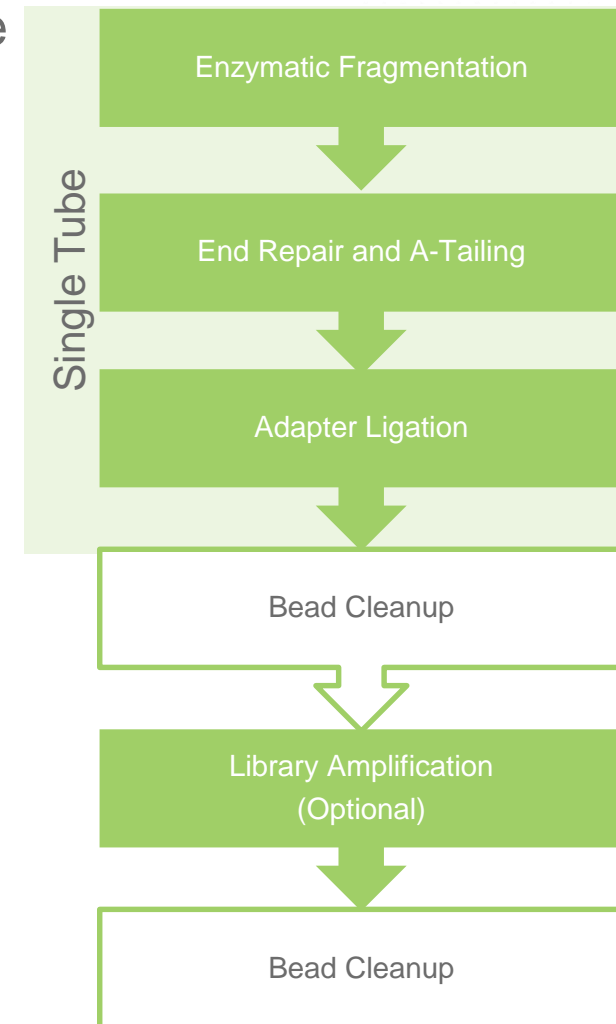


# HyperPlus

- Streamlined workflow that includes enzymatic fragmentation and library preparation in a single tube
- Library construction from gDNA in less than 3 hours
- Supports a wide range of DNA types and input amounts (1 ng – 1 µg)
- Robust performance with challenging sample types (such as FFPE)
- Enzymatic Fragmentation = Automation-friendly workflow
- Adjust library insert sizes from 150 - 800 bp by varying fragmentation time

## Kapa HyperPlus Kit

Total Time ~2.5 hrs



# Recommended DNA Inputs

Application	Sample Type	Recommended Input
WGS	Complex gDNA (high quality)	50 ng – 1 µg
Target Capture (WES, custom panels)	Complex gDNA (high quality)	10 ng – 1 µg
WGS, target capture	FFPE DNA	≥50 ng (quality dependent)
WGS	Microbial DNA	1 ng – 1 µg
WGS (PCR-free)	Complex gDNA (high quality)	≥50 ng (no SS)* ≥500 ng (w/SS)*
Targeted Sequencing	Long amplicons	≥1 ng
RNA-Seq	Full-length / unfragmented cDNA	≥1 ng

\*SS = size selection; results in the loss of 60 – 95% of DNA,  
irrespective of whether a bead or gel-based technique is used.

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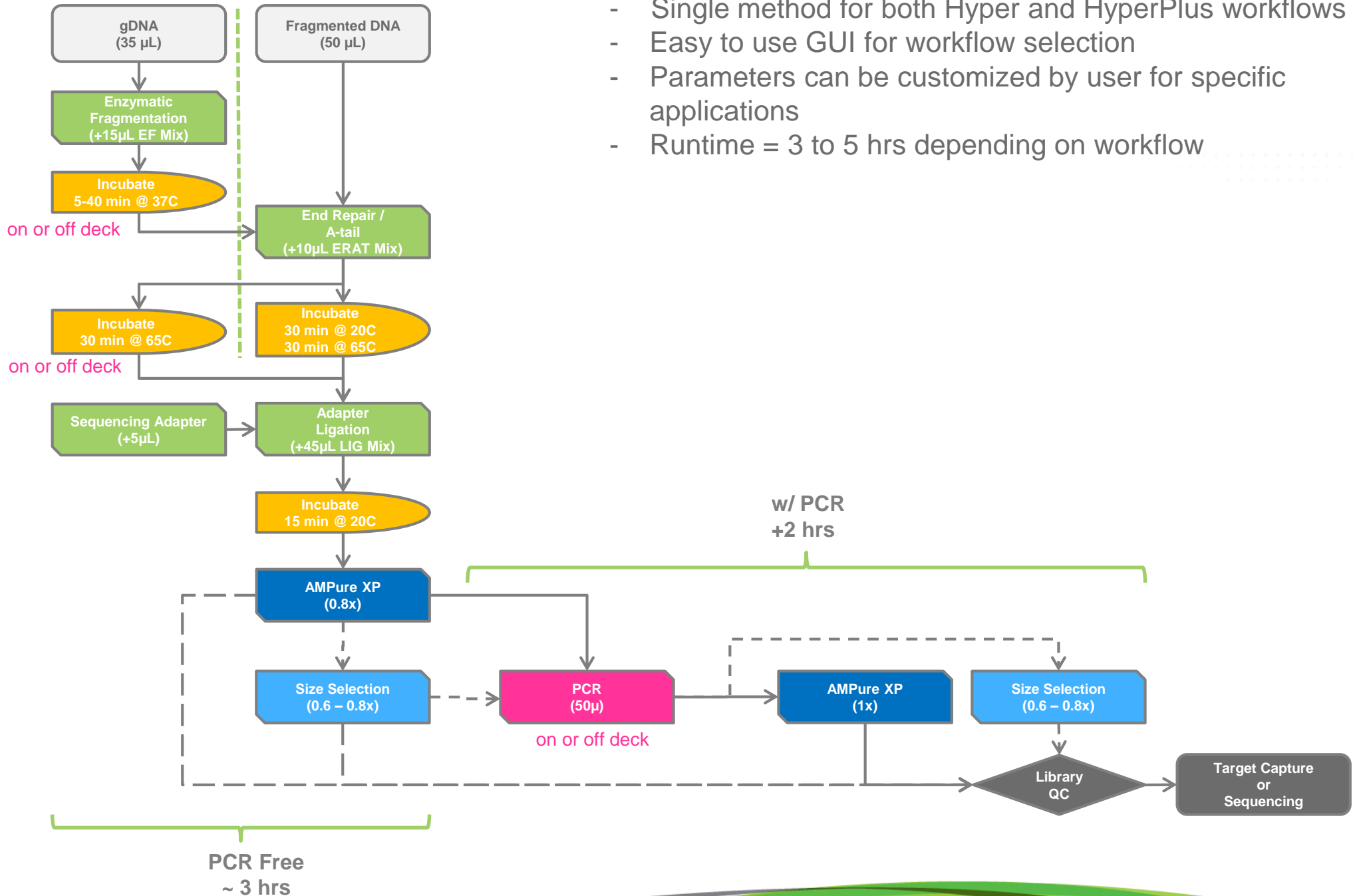
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# Automated Workflow Options

## HyperPlus

## Hyper Prep

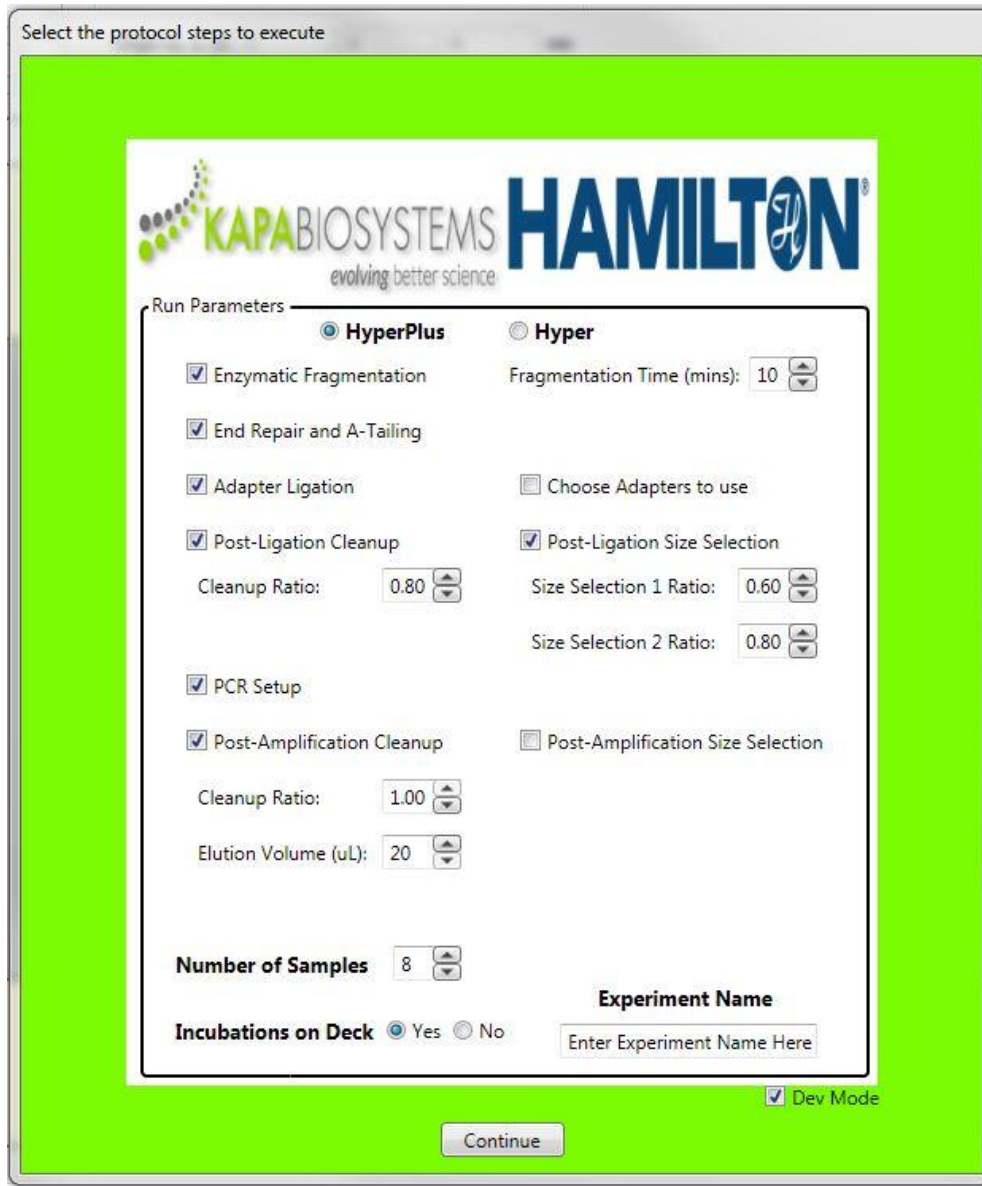
- Single method for both Hyper and HyperPlus workflows
- Easy to use GUI for workflow selection
- Parameters can be customized by user for specific applications
- Runtime = 3 to 5 hrs depending on workflow





# User Interface

Select the protocol steps to execute



The screenshot shows a software window titled "Select the protocol steps to execute". Inside, there's a header with the KAPA BIOSYSTEMS logo and "HAMILTON" in large blue letters, with the tagline "evolving better science". Below this is a "Run Parameters" section with two tabs: "HyperPlus" (selected) and "Hyper". Under "HyperPlus", there are several checked checkboxes: "Enzymatic Fragmentation", "End Repair and A-Tailing", "Adapter Ligation", "Post-Ligation Cleanup", "PCR Setup", and "Post-Amplification Cleanup". To the right of these, there are input fields for "Fragmentation Time (mins): 10", "Choose Adapters to use" (unchecked), "Post-Ligation Size Selection" (checked), "Size Selection 1 Ratio: 0.60", "Size Selection 2 Ratio: 0.80", "Post-Amplification Size Selection" (unchecked), "Cleanup Ratio: 0.80", and "Elution Volume (uL): 20". At the bottom left, there's a "Number of Samples" field set to 8, and "Incubations on Deck" with "Yes" selected. A text field for "Experiment Name" is labeled "Enter Experiment Name Here". A "Continue" button is at the bottom center. A "Dev Mode" checkbox is checked at the bottom right.

KAPABIOSYSTEMS HAMILTON<sup>®</sup>  
evolving better science

Run Parameters

☒ HyperPlus ☐ Hyper

☒ Enzymatic Fragmentation Fragmentation Time (mins): 10

☒ End Repair and A-Tailing

☒ Adapter Ligation ☐ Choose Adapters to use

☒ Post-Ligation Cleanup ☒ Post-Ligation Size Selection

Cleanup Ratio: 0.80 Size Selection 1 Ratio: 0.60

Size Selection 2 Ratio: 0.80

☒ PCR Setup

☒ Post-Amplification Cleanup ☐ Post-Amplification Size Selection

Cleanup Ratio: 1.00

Elution Volume (uL): 20

Number of Samples: 8

Experiment Name

Incubations on Deck ☒ Yes ☐ No Enter Experiment Name Here


☒ Dev Mode

Continue

- Method execution can be defined by the user through simple interface
- Parameters can be adjusted by the user for different applications from one program
- Program can be started from any main step in the process for easy error-recovery and process optimization

# Reagent and Consumable Calculator

Kapa Biosystems Hyper and HyperPlus - Prepare the Reagents and Consumables below, then Click START



evolving better science

<b>Plates in Stacker Track 0</b>		<b>Experiment Name</b> Enter Experiment Name Here Sample Count: 8	
Stack 1 (Empty):	0		
Stack 2 (MIDIs):	1		
Stack 3 (MIDIs):	1		
Stack 4 (HSPs):	2		
<b>50uL Filter Tips T6 T12</b>		<b>300uL Filter Tips T18 T24</b>	
Tips Required: 88		Tips Required: 56	
Equivalent Racks: 1		Equivalent Racks: 1	

<b>Reagent Tubs - Track 31</b>		<b>Vial Carrier - Track 33</b>	
Tub 1 - 80% Ethanol (mL):	12	Pos 1 - Fragmentation Mix (uL):	72
Tub 2 - 80% Ethanol (mL):	0	Pos 2 - Fragmentation Mix (uL):	72
Tub 3 - 80% Ethanol (mL):	0	Pos 3 - End Repair A-Tail Mix (uL):	50
Tub 4 - AMPure Beads (mL):	5	Pos 4 - End Repair A-Tail Mix (uL):	50
Tub 5 - RSB (mL):	4	Pos 5 - Vapor Lock (uL):	120
		Pos 6 - Vapor Lock (uL):	120
		Pos 7 - Ligation Mix (uL):	108
		Pos 8 - Ligation Mix (uL):	108
		Pos 9 - Ligation Mix (uL):	108
		Pos 10 - Ligation Mix (uL):	108
		Pos 11 - PCR Primer (uL):	48
		Pos 12 - PCR Primer (uL):	0
		Pos 13 - PCR Master Mix (uL):	120
		Pos 14 - PCR Master Mix (uL):	120

<b>Plate-Tip Carrier Track 35</b>	
Positions 1 & 2 - 1ml Filtered Tips	
Tips Required:	48
Equivalent Racks:	1
Pos 3: Adapter Plate (uL):	20
Pos 4: Sample Plate (uL):	35
Pos 5: Empty	

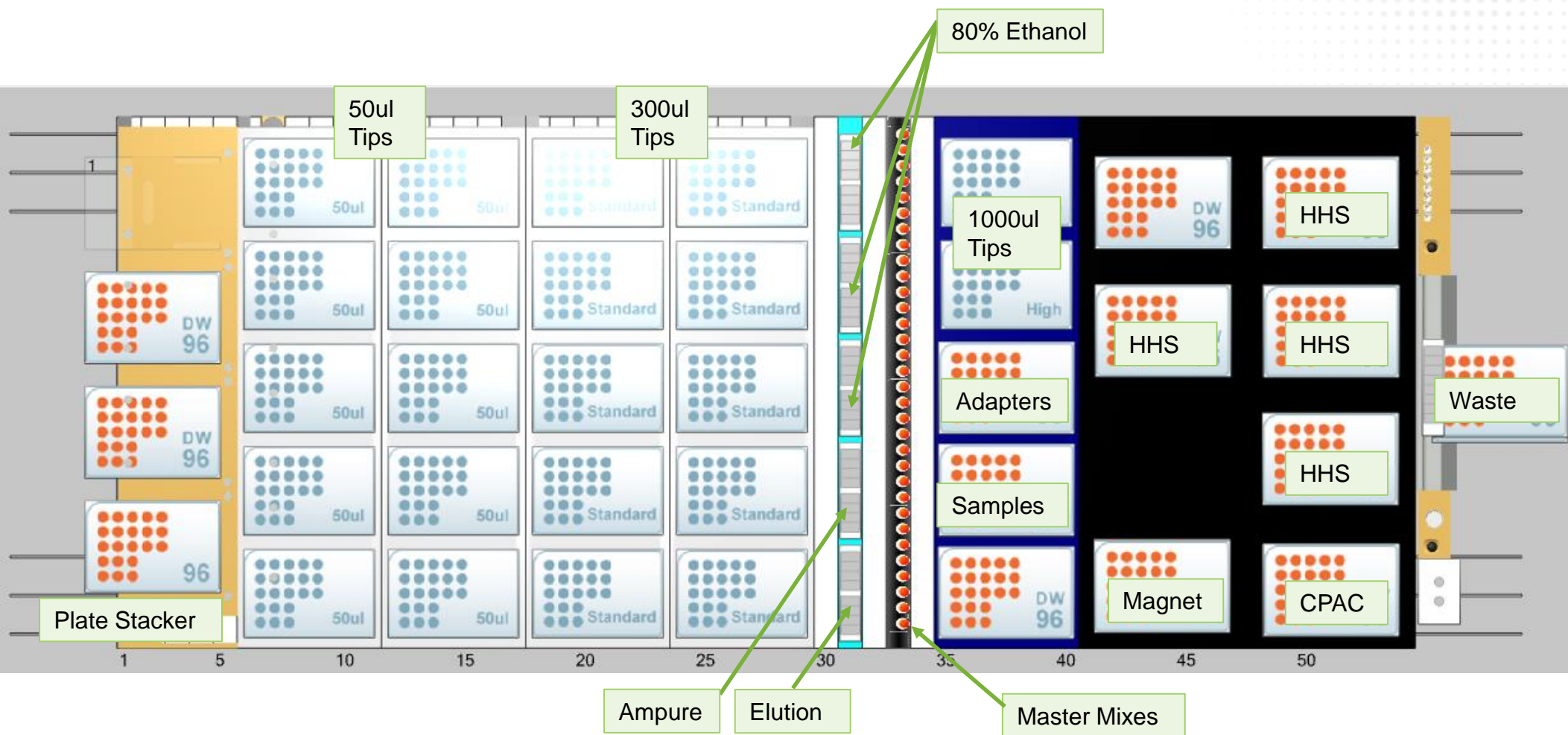
<b>Adapter Plate Usage</b>	
Start Position:	A1
End Position:	H1

\*Please see protocol documentation for reagent preparation instructions.

START

- Based on run parameters specified in the user interface, labware, tip, and reagent requirements will be calculated and provided to the user
- Experiment name, sample count and adapter usage may be confirmed

# Deck Layout



Method can be tailored to alternate deck layouts provided that all hardware and minimum number of positions are available

# Hardware Requirements

Hardware	Qty	Comments
1000uL channels	8	Independent channels with liquid level detection, capable of pipetting 0.5 to 1000uL
CO-RE gripper or iSwap	1	Gripper will be used for plate movements
Autoload Barcode Reader	Optional	Loading and scanning ensures that proper tips and samples are being loaded
Hamilton Heater Shaker Positions	3	Mixing by shaking and Enzymatic incubations from 22°C to 65°C
CPAC Position	1	All reagent additions to sample plate will occur at 4°C
Plate Stack carrier	1	Stacker holds extra MIDI and BioRad HSPs for cleanups
Liquid Waste chute	1	Liquid waste from cleanups will be disposed into attached carboy

# Labware Requirements

Labware	Qty	Comments
96-well PCR plate adapter for HHS	2	Enables more surface contact with wells during incubations
Ambion Magnetic Stand 96 or equivalent SuperMagnet	1	<a href="https://www.thermofisher.com/order/catalog/product/AM10027">https://www.thermofisher.com/order/catalog/product/AM10027</a>



# Consumable Requirements

Labware	Qty	Comments
CORE 50uL filtered tips	1 - 10 racks	Quantity required depends on number of samples
CORE 300uL filtered tips	1 - 10 racks	Quantity required depends on number of samples
CORE 1000uL filtered tips	1 - 2 racks	Quantity required depends on number of samples
Hard-shell PCR Plates (Bio-Rad HSP9601, etc.)	2 – 4	Sample plate (1) and Adapter plate (1), and up to 2 additional plates for cleanup/size selection elution
96-well 0.8mL MIDI plates	2 - 4	Quantity depends on cleanups/size selections selected
50 mL tubs for Cleanup Reagents	3 - 5	Ampure beads (1), Ethanol (1-3), and Elution buffer (1)
1.5mL tubes for Master Mixes	Up to 14	Quantity depends on run parameters selected

# Reagent Requirements

- KAPA Hyper or HyperPlus Library Preparation Kit
- gDNA or cDNA
- Adapters (TruSeq style adapters from an Illumina kit or from another source, e.g. IDT).
- PCR-grade water
- Freshly prepared 80% ethanol
- AMPure XP reagent
- Sterile elution buffer (10 mM Tris-HCl, pH 8.0 at 25 °C)
- Reagents for electrophoretic assessment of library size
- Reagents for library quantification (e.g. KAPA Library Quantification Kit)
- Qiagen Vapor Lock

# Lab Requirements

- Single-channel pipettes and filtered tips
- Sterile microtubes
- Vortex mixer and microcentrifuge
- PCR cycler (for library amplification and incubations)
- PerkinElmer LabChip GX, Agilent Bioanalyzer or TapeStation, or other electrophoretic system (to assess library size)
- qPCR cycler (for qPCR-based library quantification)
- Qubit or other fluorometer (for library quantification using PicoGreen assay)



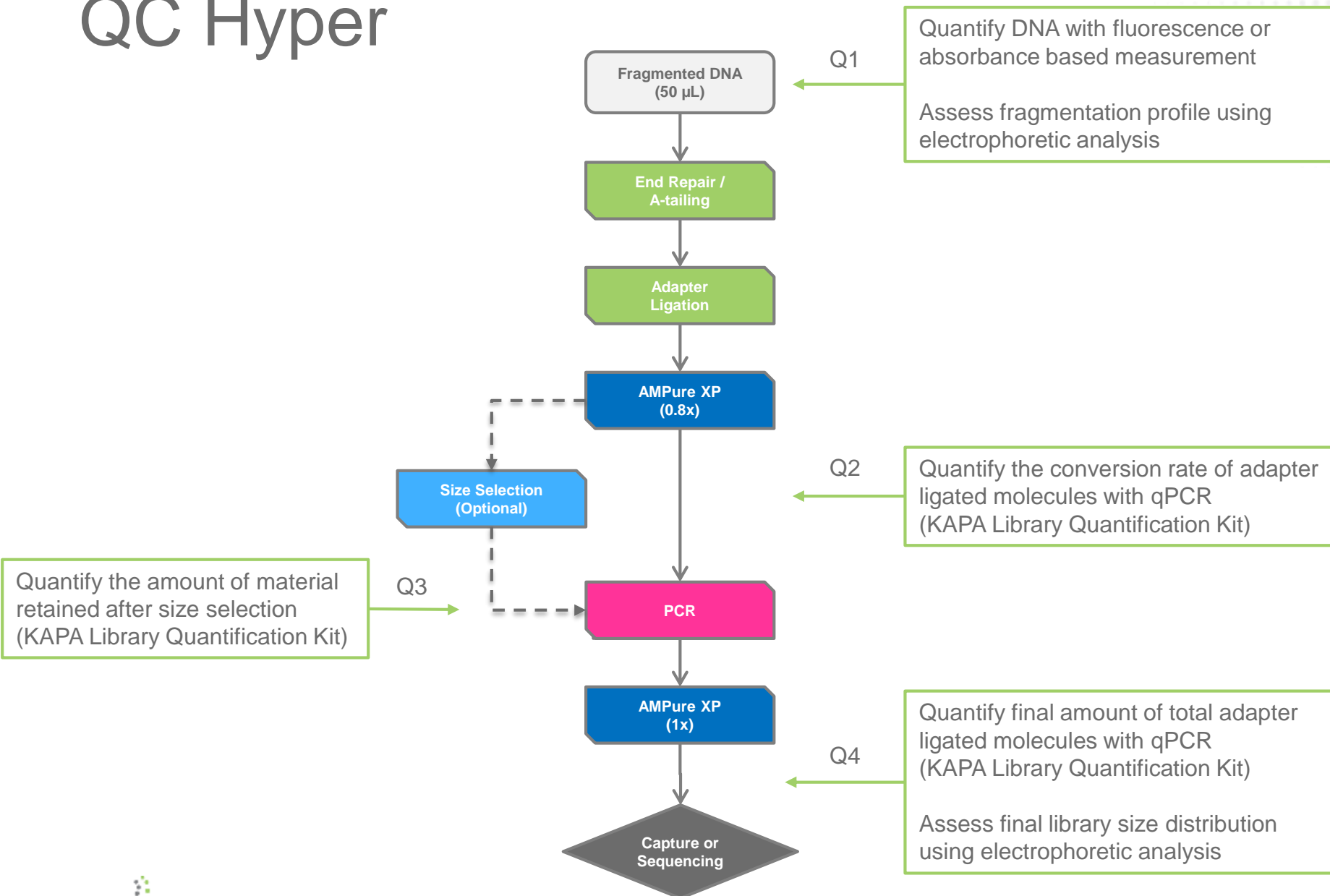
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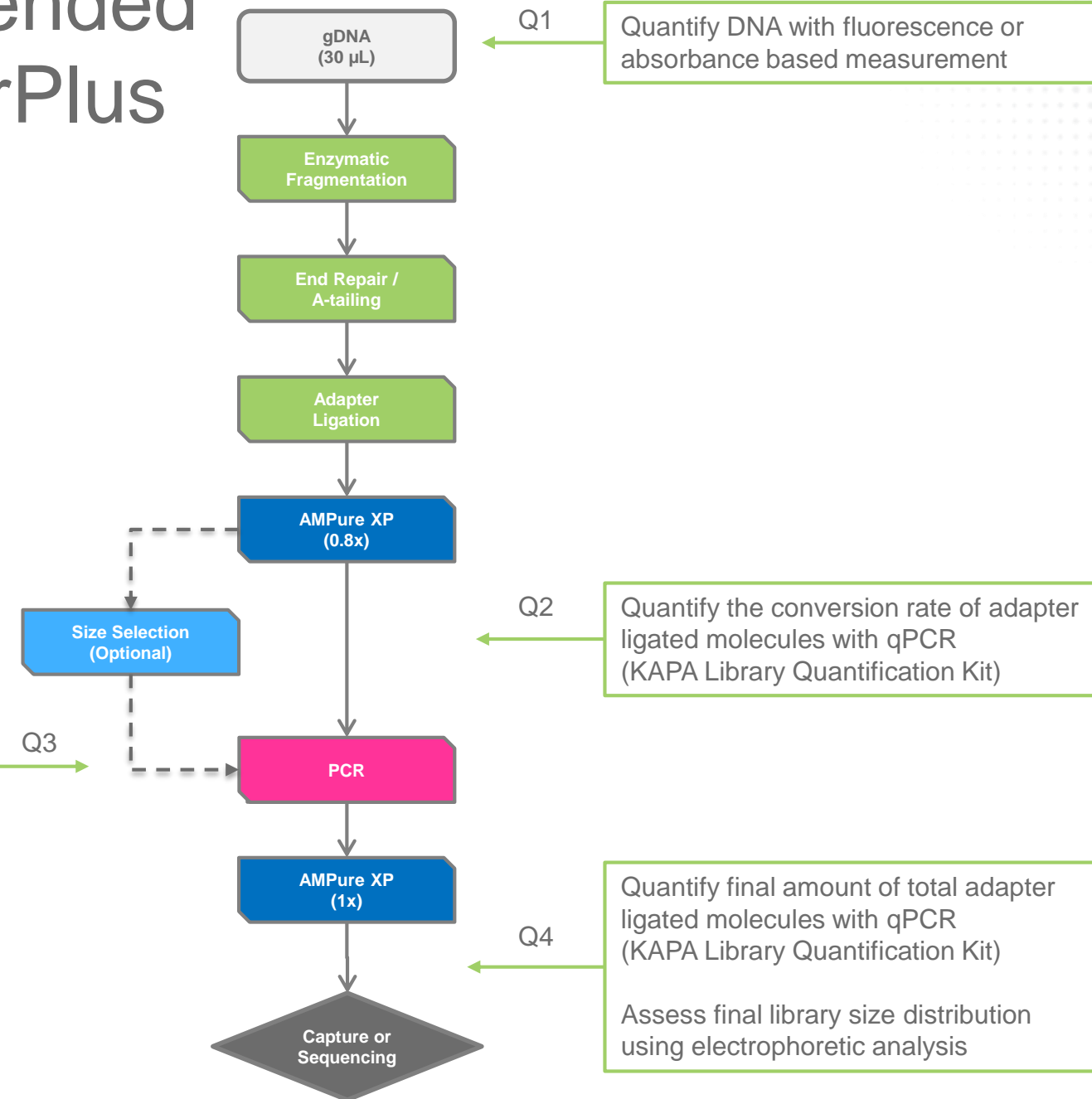
# Validation Objectives

- During the validation process only, it is recommended that QC samples are taken at the following stages of the process (for troubleshooting and/or process optimization see next 2 slides for more detail):
  - At the end of post-ligation cleanup (i.e. before library amplification)
  - If size selection is done: before and after size selection
  - At the end of post-amplification cleanup
- qPCR quantification of the above samples (during method validation) allows for the following questions to be answered:
  - Is the process up to the end of ligation as efficient as expected?
    - What % of input DNA was converted to adapter-ligated molecules?
  - Is library amplification as efficient as expected?
    - Theoretical yield can be calculated from amount of template
  - How much material is lost during size selection?
    - How does this influence the number of library amplification cycles need to ensure a sufficient yield of the final library?
    - Can size selection be avoided (particularly if input is limited)?

# Recommended QC Hyper



# Recommended QC HyperPlus



# Performance Metrics

- % input DNA converted to adapter-ligated library
  - $= (Q2 / Q1) * 100$
  - Hyper Prep should be in the range of 15 – 40% for inputs  $\geq 100$  ng
  - HyperPlus should be in the range of 50 – 100% for inputs  $\geq 100$  ng
- % loss of adapter-ligated library as a result of size selection
  - $= (1 - (Q3 / Q2)) * 100$
  - Usually in the range of 80 – 95%
- Use Q2 (or Q3 with SS) to predict the optimal number of pre-amplification cycles
  - Amplification efficiency is  $\geq 80\%$  for good-quality DNA; 20 – 50% for FFPE samples
  - Use lowest number of cycles to obtain sufficient material for capture or sequencing, QC, and/or archiving
- $(Q4 \text{ in ng} / Q3 \text{ in ng}) * 100\%$  gives actual amplification efficiency; should be in expected range for specific sample type

# Distribution / Support

- Distribution, Installation, and Support of the software application is provided by Hamilton Robotics including:
  - Method (Instructions for robot e.g. where to go and what to do)
  - Labware definitions (exact dimensions of each consumable)
- Support of all product chemistry is provided by Kapa Biosystems including:
  - Technical support
  - Protocol optimization
  - Field Training
- Current method is designed to accommodate the NGS STAR Illumina workstation deck layout and hardware configuration (Alternative deck layouts will require further customization)
- Hardware support including initial setup, calibration, and preventative maintenance are the responsibility of Hamilton Robotics & customer

# Hamilton Method Demo