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KAPA Hyper Prep/HyperPlus Library Preparation on the Hamilton STAR

Nathan Quon

NGS Automation Engineer

PCR qPCR ATCGGCA TTTGGCC CGCATCG GACTACG NGS

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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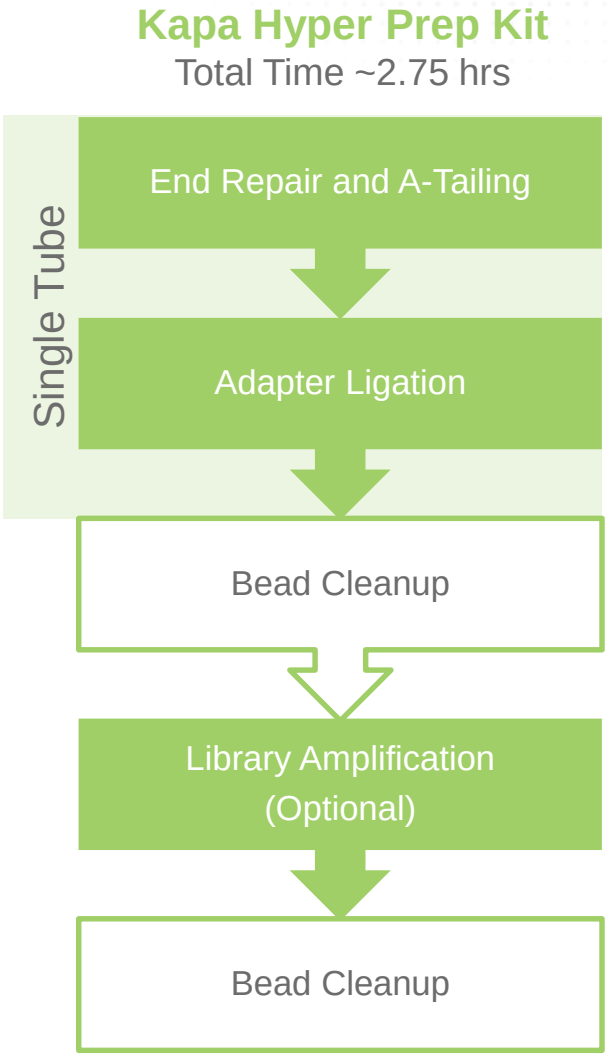
Hyper Prep

- 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



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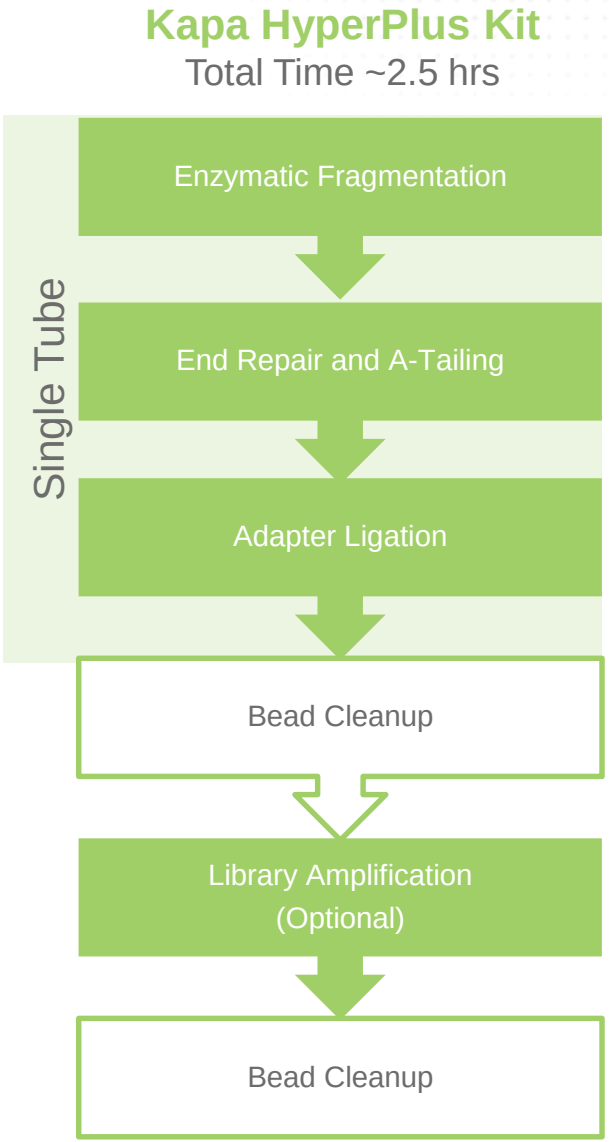
HyperPlus

- 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



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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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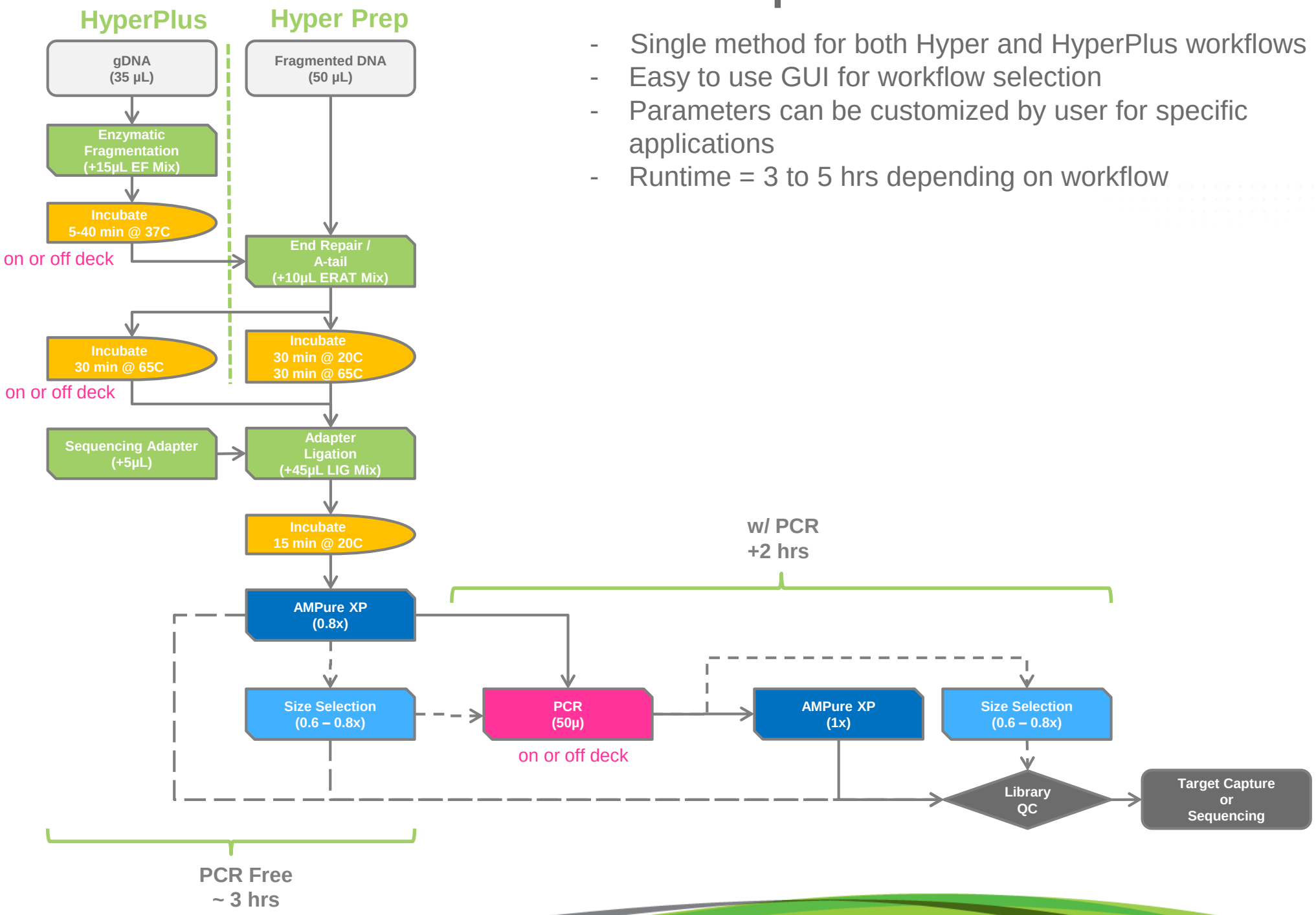
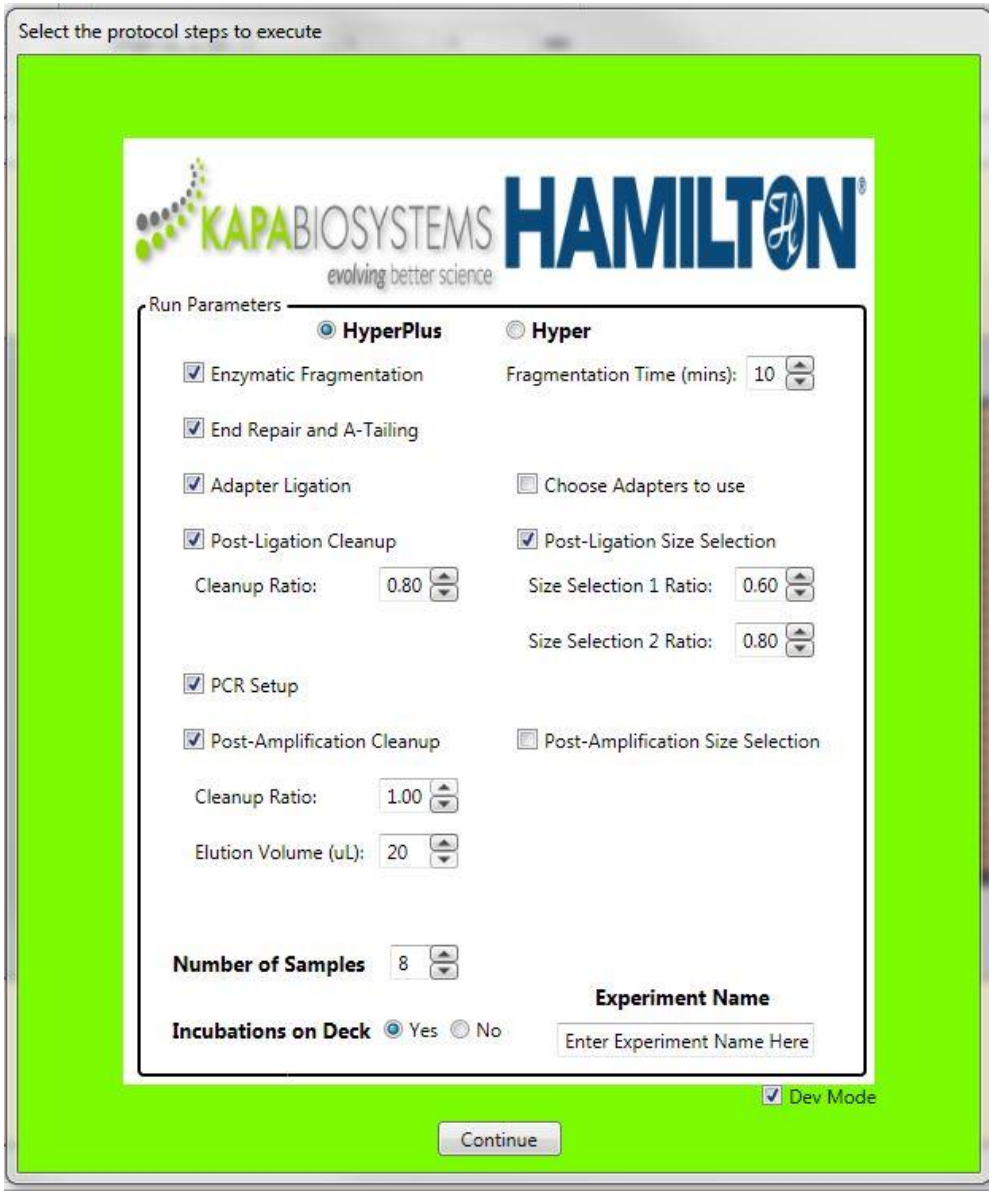
Automated Workflow Options

- 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



- 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

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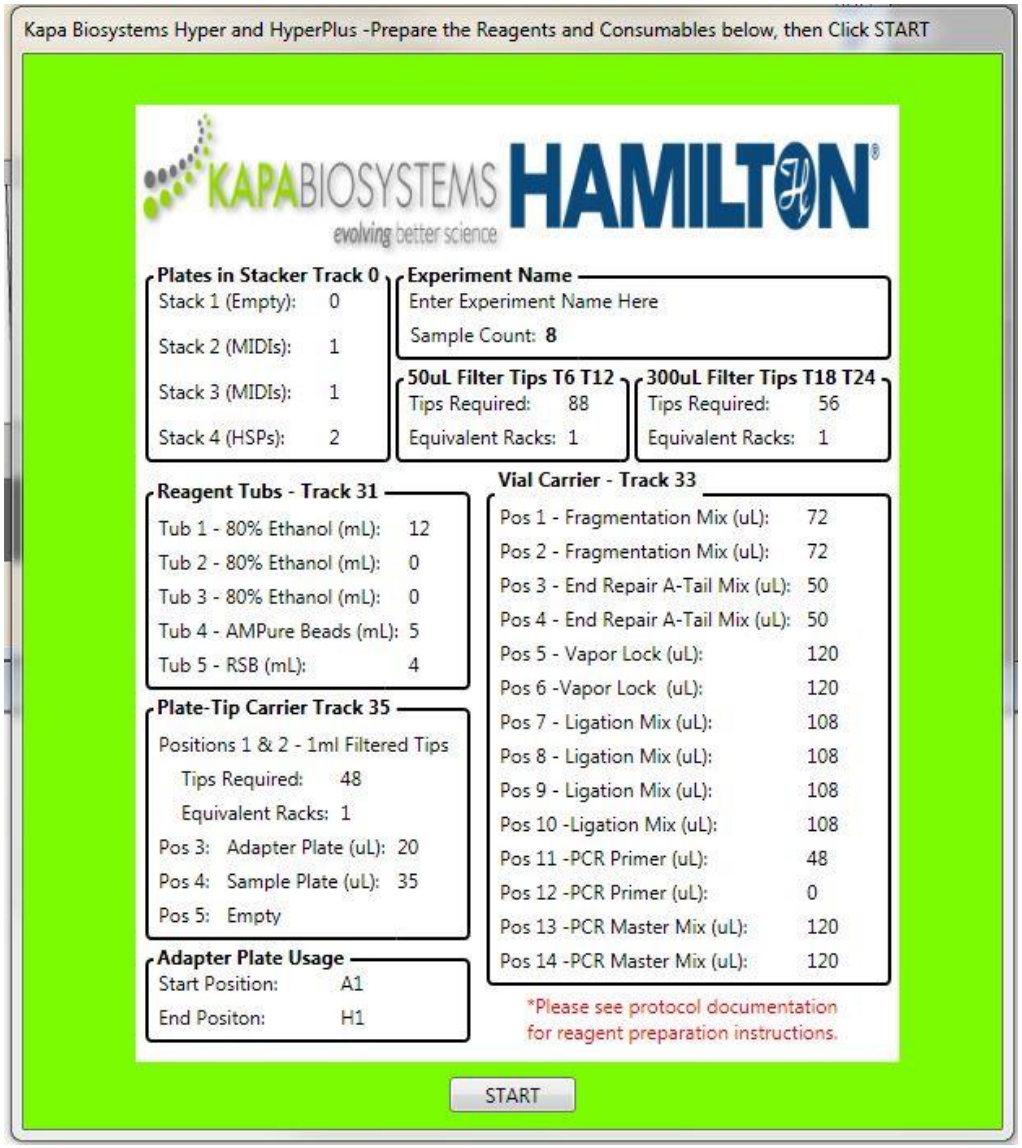
Reagent and Consumable Calculator



- 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

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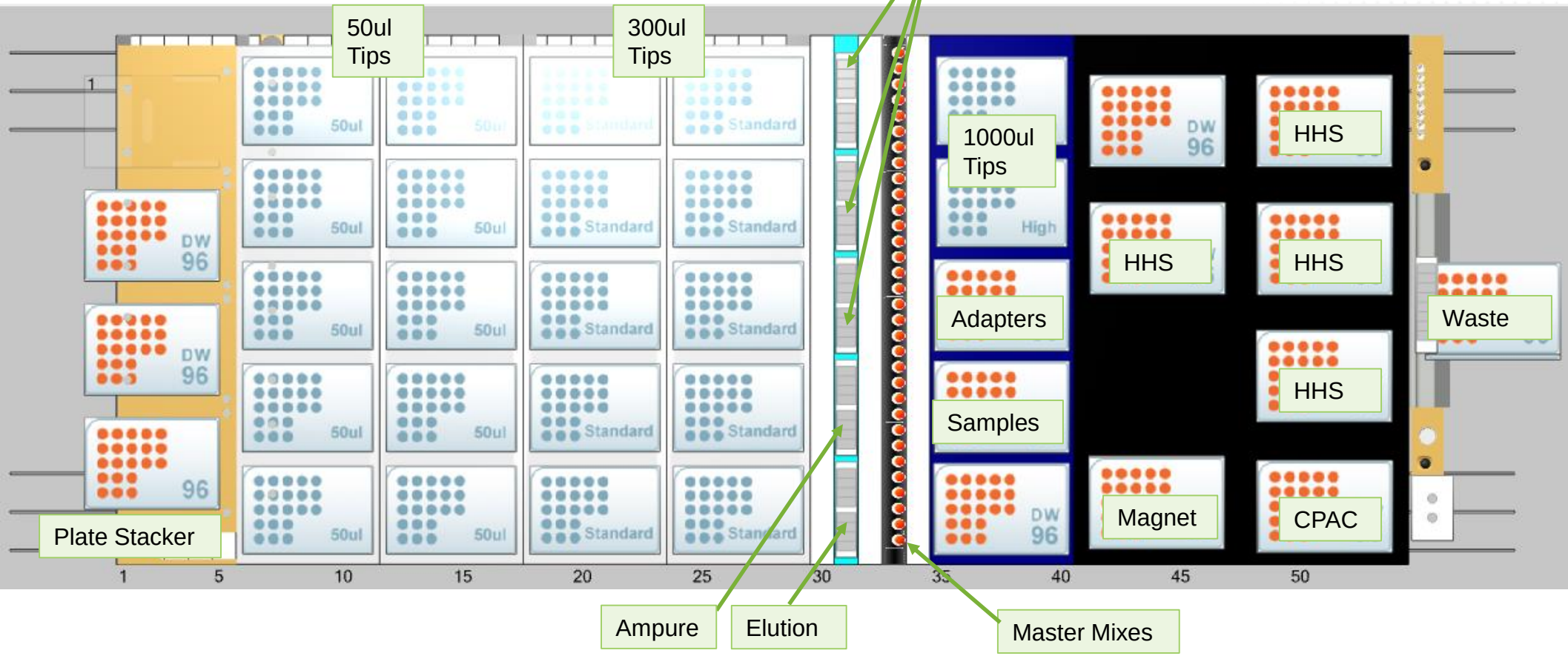
Deck Layout



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

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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	https://www.thermofisher.com/order/catalog/product/AM10027

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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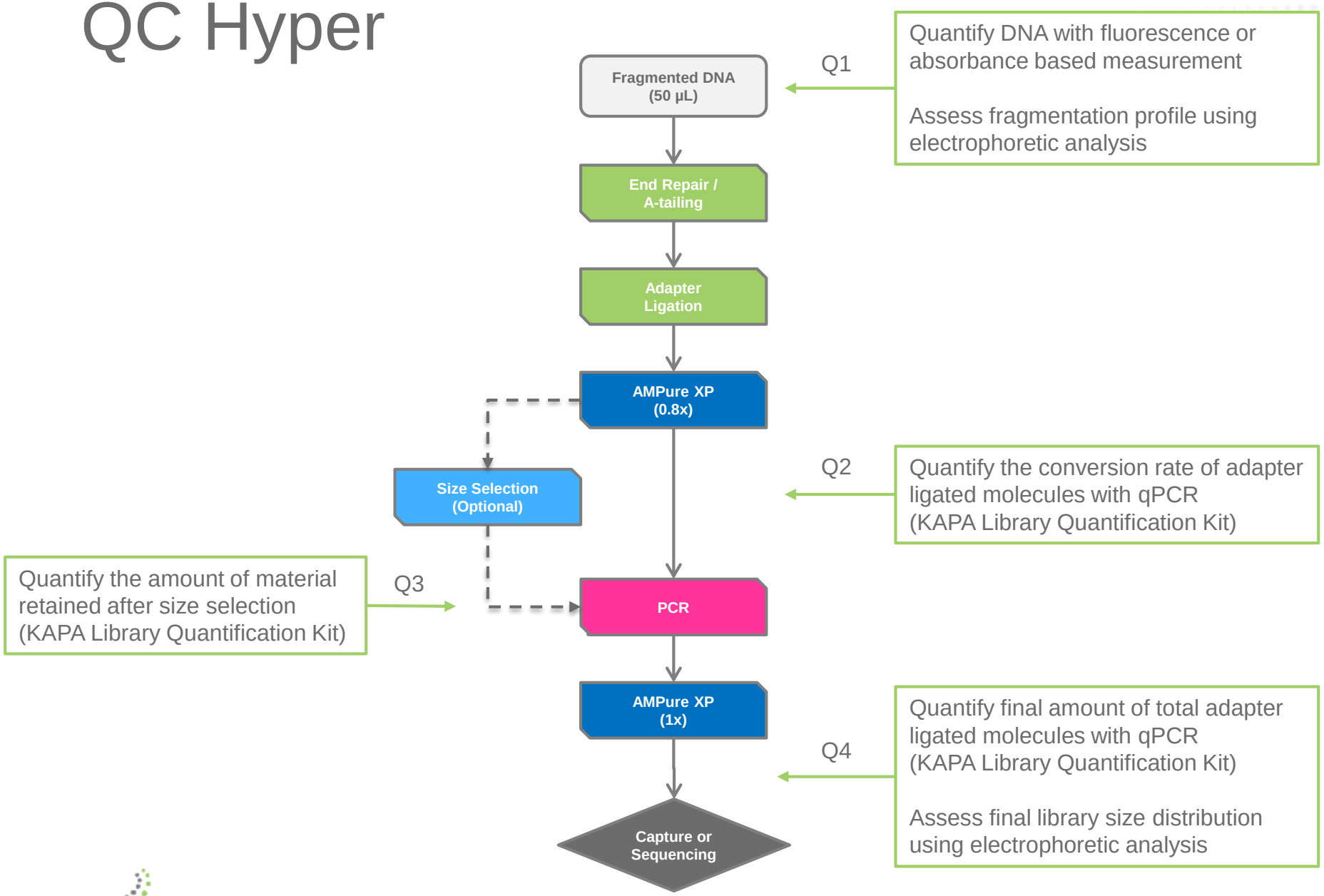
Validation Objectives

- 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



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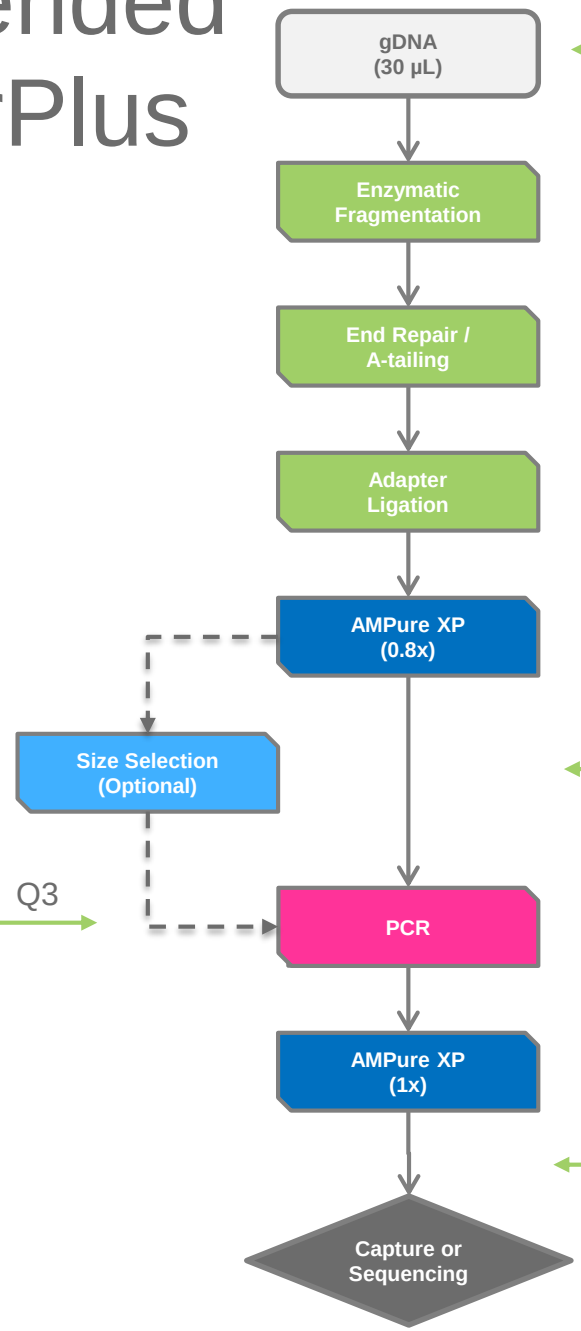
Recommended QC HyperPlus



Q1

Quantify DNA with fluorescence or absorbance based measurement

Q2

Quantify the conversion rate of adapter ligated molecules with qPCR (KAPA Library Quantification Kit)

Q3

Quantify the amount of material retained after size selection (KAPA Library Quantification Kit)

Q4

Quantify final amount of total adapter ligated molecules with qPCR (KAPA Library Quantification Kit)

Assess final library size distribution using electrophoretic analysis

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Performance Metrics

- % input DNA converted to adapter-ligated library
  - = (Q2 / Q1) * 100
  - Hyper Prep should be in the range of 15 – 40% for inputs ≥100 ng
  - HyperPlus should be in the range of 50 – 100% for inputs ≥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 ≥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 in ng / Q3 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