

Scaling Up NGS Sample Preparation:

Automation and Lab Preparation



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AGILENT AUTOMATED NGS SAMPLE PREPARATION

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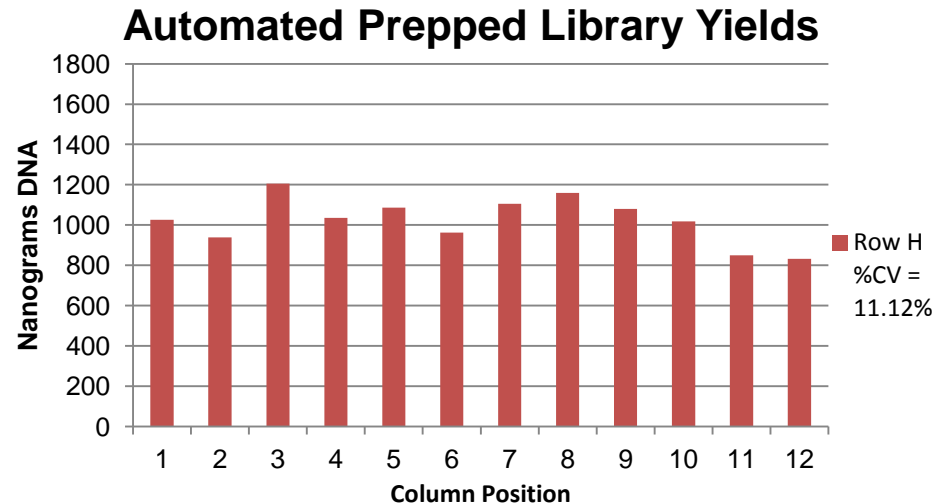
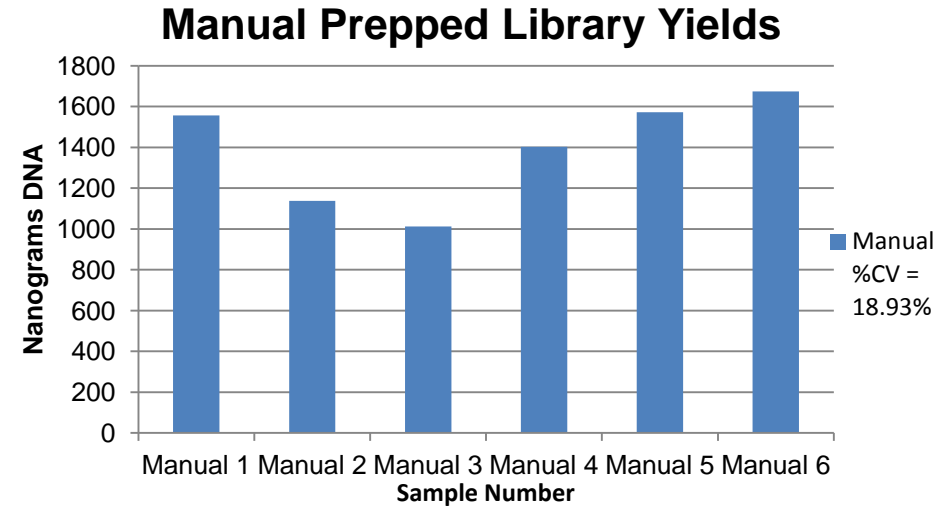


Benefits of Automation

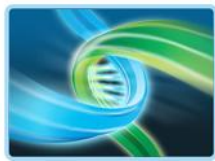
- **Scale Up Sample Throughput**
 - Increase throughput up to 10 times
- **Reduced Hands on time**
- **Increased reproducibility**
 - Reduce variation in library prep yield

Automation Increases Reproducibility

- Increased reproducibility
- CV for automated preparation is lower than CV for manual preparation



Automation Increases Laboratory Throughput



	MANUAL METHOD		AUTOMATED METHOD	
	Hands-On Time	Maximum Number of Samples Processed/Day ¹	Hands-On Time	Maximum Number of Samples Processed/Day ²
Library Prep	375	20	25 ²	96
Pre-Capture PCR	90	20	13	96
Hybridization Preparation	60	20	7	96
Capture	210	20	20	96
Post-Capture PCR	90	20	40	96
Total Minutes	825	20 - 40 per week	105	96 - 192 per week
Total Hours	13.75		1.75	

¹Assumes individual tube preps with an experienced user staggering sample processing

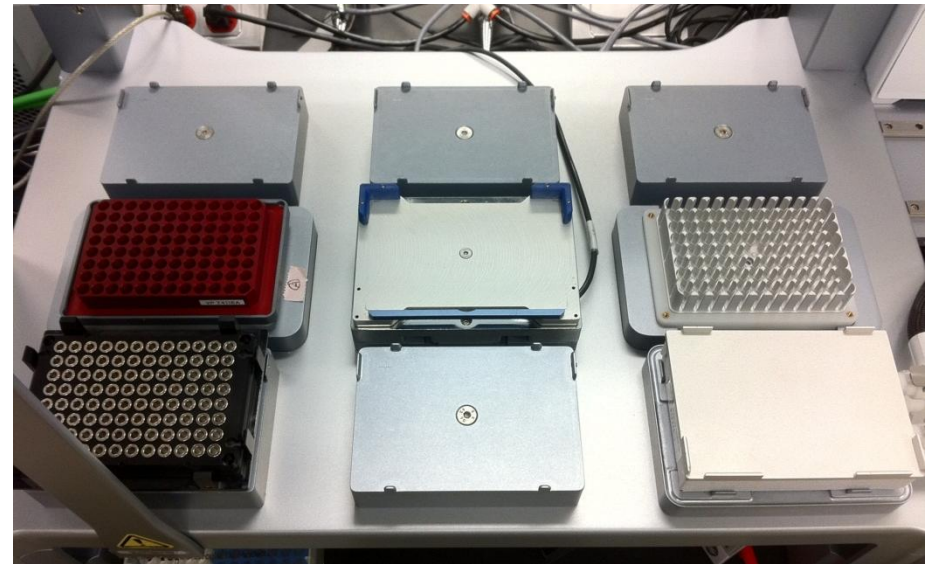
²Library prep master mixes done once

Agilent NGS Automation Instruments



Agilent NGS Bravo Option A

- Capable of automating almost any NGS reagents
- Higher throughput and greater reproducibility than manual preparation
- Deck features:
 - Temperature control
 - Shaking
 - Magnetic bead station



Agilent NGS Option B

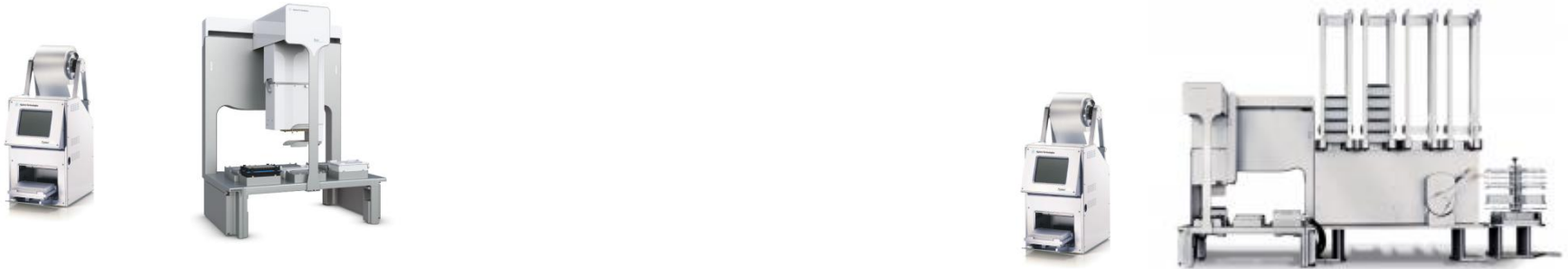
- Additional automation and labware storage offer greater walkaway time
- Some NGS protocols include >55 pipetting steps
- BenchCel stores pipette tips to reduce number of manual interventions



Workstation Options for Increasing Walkaway Time

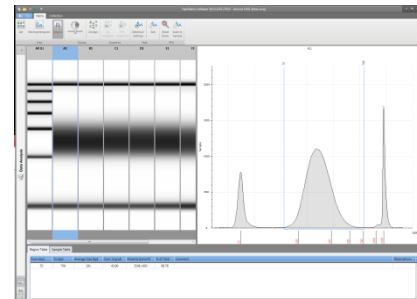
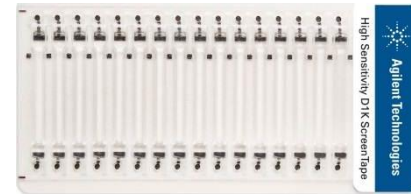
- Preconfigured workstations are designed for increasing walkaway time
- Bravo liquid handler is capable of automating common library preparation and sequence capture methods

Increasing Walkaway Time



Agilent TapeStation 2200 Simplifies Sample QC

- DNA sample QC is required at 3 steps in targeted enrichment workflow
- TapeStation processes 96 samples in 1.5 hours
- Significant time savings over BioAnalyzer



Agilent PlateLoc Thermal Microplate Sealer

- Ideal for sealing plates for heated overnight incubations
- Prevents evaporation of precious samples
- Works as a walk-up instrument so it can be used to seal other plates in lab for PCR or other applications



Example VWorks Form for NGS Protocols

Agilent Technologies
SureSelect

1) Select Protocol to Run
AMPureXP_XT_Illumina_v1.0.pro

2) Select additional Parameters
AMPureXP_XT_Illumina_v1.0.pro Only
a. Select DNA Product to Cleanup
Covaris Shearing Cleanup
b. Select Plate type containing DNA for cleanup
96 ABI PCR half skirt in black carrier

3) Select Number of Columns of Samples*
1 Columns selected
*needs to be 1, 2, 3, 4, 6, or 12 columns

4) Press button below to display Workstation Setup
Display Initial WorkStation State

5) Once Labware is loaded according to Workstation Setup, Click "Run Selected Protocol" to start run.
Run Selected Protocol Pause

Full Screen on/off
Elapsed Time: 00:00:00

	MiniHub Cassette 1	MiniHub Cassette 2	MiniHub Cassette 3	MiniHub Cassette 4
Shelf 5	Empty Nunc DeepWell Plate			
Shelf 4				
Shelf 3		Empty Eppendorf Twin.tec Plate		
Shelf 2		Nuclease-free Water Reservoir	AmpureXP Beads in Nunc DeepWell	
Shelf 1		70% Ethanol Reservoir		

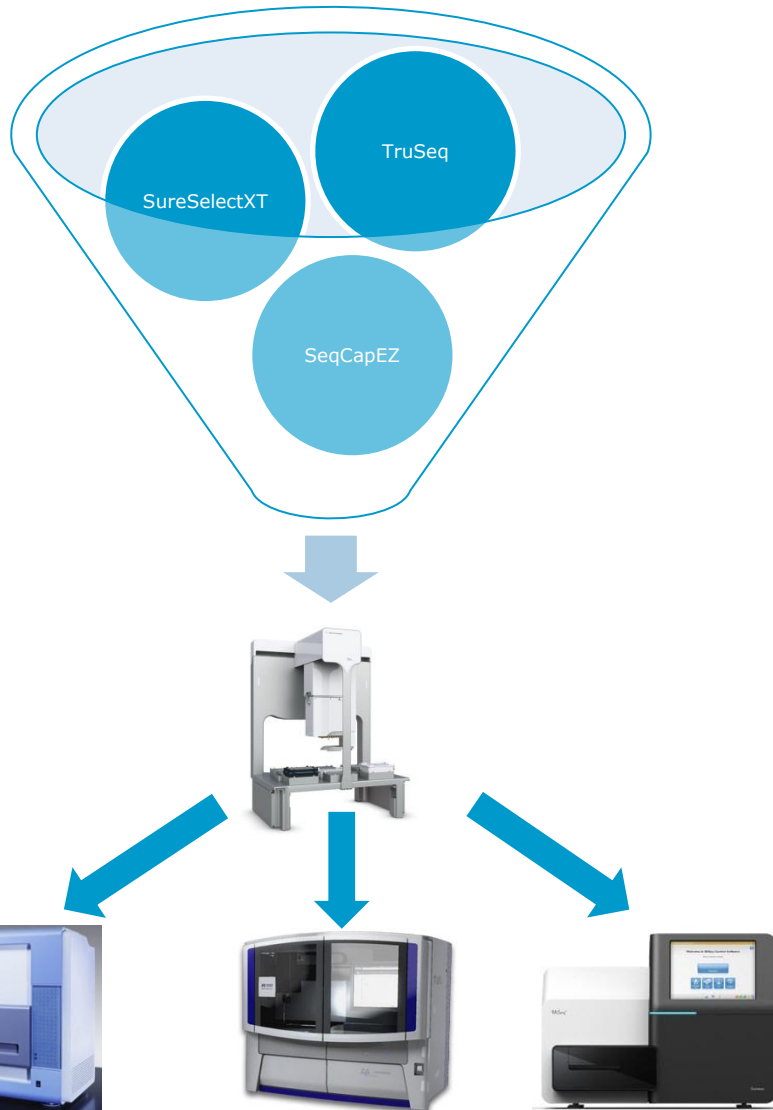
Bravo Deck

<Position 1> Waste Reservoir (Axygen 96DW)	<Position 2>	<Position 3>
<Pos 4: Peltier> DNA Plate for Cleanup	<Pos 5: Shaker>	<Pos 6: Peltier>
<Pos 7: Magnetic>	<Position 8> Empty Tip Box	<Pos 9: Chilled>

BenchCel Stacker 1	BenchCel Stacker 2	BenchCel Stacker 3	BenchCel Stacker 4
1 Tip Box	Empty	Empty	Empty

- VWorks software forms available to streamline processing
- Diagrams show location of accessories, labware, and reagents for Bravo deck and Minihub

Agilent NGS Workstation is an Open Platform



- Automation protocols are available for both Illumina and SOLiD sequencers
- Optimized protocols and user manual available for SureSelectXT and HaloPlex for Illumina
- Vworks automation files available for:
 - Illumina TruSeq RNA and DNA
 - NimbleGen SeqCapEZ
 - KAPA

Automated Agilent NGS Protocols

- Optimized Vworks protocols are available for many Agilent NGS reagents
- Step-by-step user manuals
- Genomics and automation technical support
 - SureSelect^{XT}
 - HaloPlex

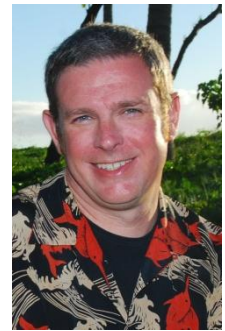


3rd Party NGS Reagents

- VWorks protocol available for fast scale-up
- VWorks protocols are open so users can modify protocols as necessary
 - Illumina TruSeq DNA and RNA
 - Roche NimbleGen SeqCap EZ
 - KAPA Library Prep Kit for Illumina

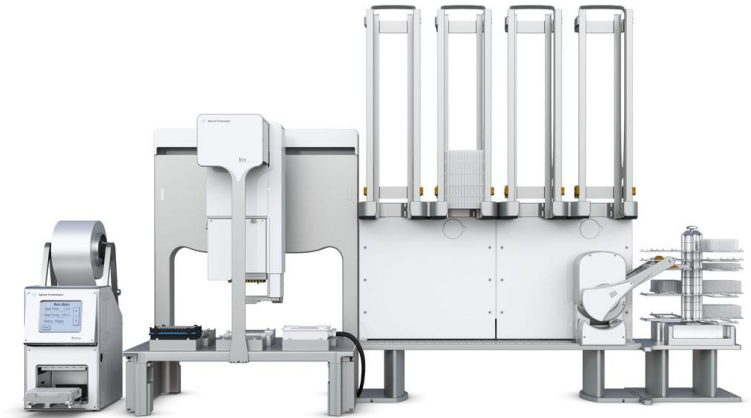
CONSIDERATIONS FOR AUTOMATING NGS SAMPLE PREPARATION

Steve Lappin
Application Scientist

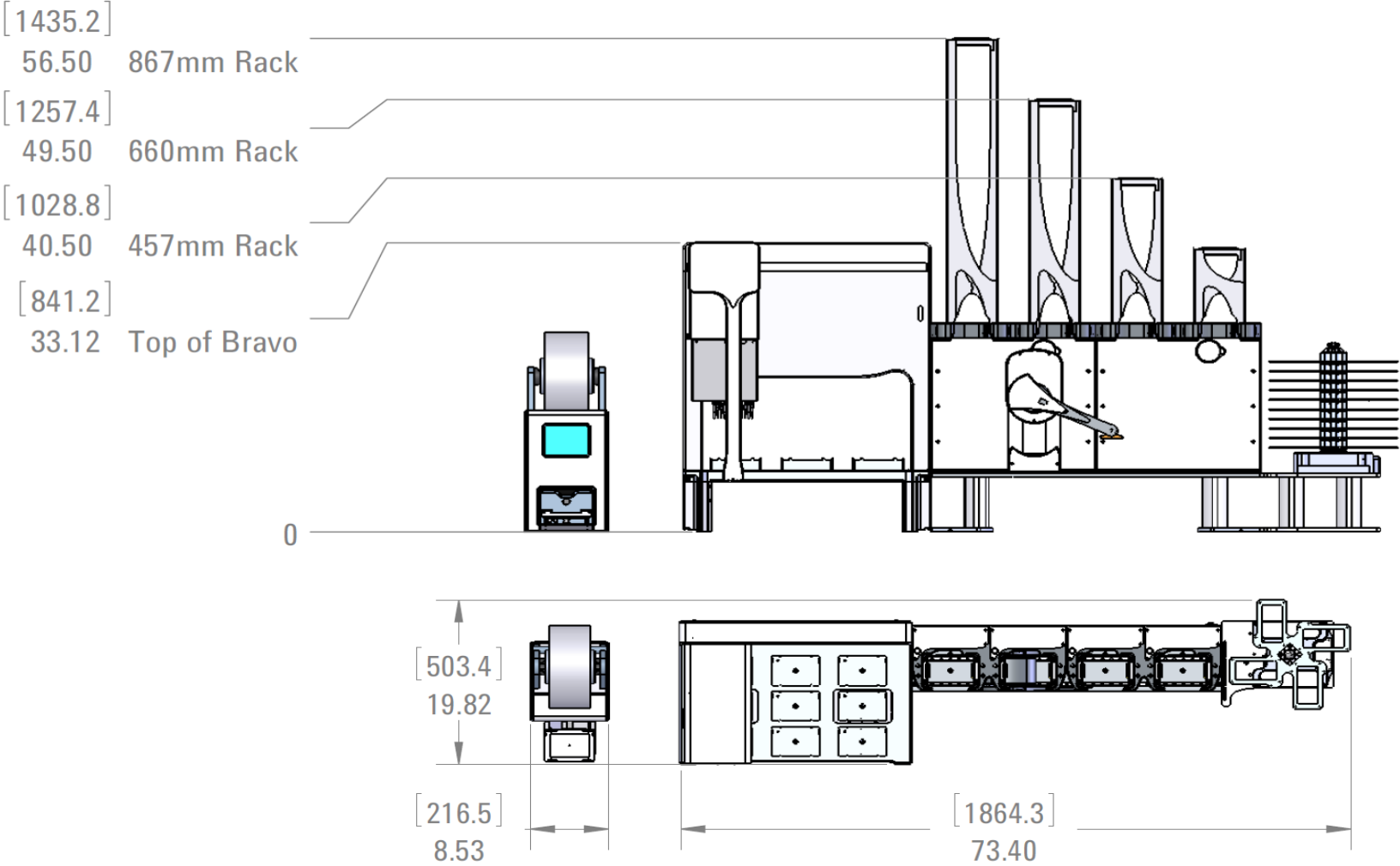


Lab Space Requirements

- Space Requirements for instruments and computer
 - Option A: 48" w, 34" h, 20"d
 - Option B: 105" w, 50" h, 20"d
- PCR instrument and plate sealer should be in close proximity to the robot
- Clean-dry 100 psi compressed air is required for BenchCel and PlateLoc
- House air or compressor options

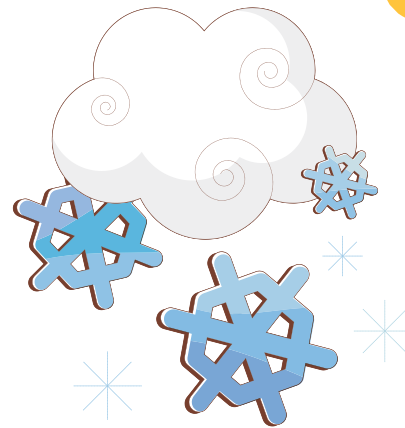
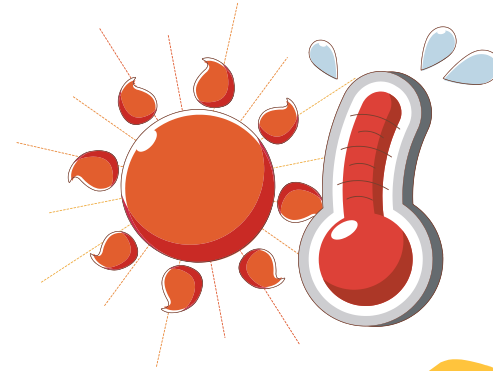


Option B Workstation Dimensions



Lab Environmental Considerations

- Instruments should not be placed directly under air vents (heating/cooling/dust)
- Third party vendors can provide containment solutions
- Ambient temp should be ~22-27C
 - If outside of normal range, additional temperature controlled steps can be introduced as needed
- Hybridization solutions are especially sensitive to cold



Multiple Systems Drive Throughput and Consistency

- Multiple systems enable users to increase throughput and build redundancy
- Multiple System Examples from current customers
 - Separate Pre/Post PCR Workstations
 - Pre-PCR library prep: NGS Option B
 - Post-PCR: NGS Option A
 - Labs in different locations: 1 lab director running similar projects in Singapore and USA
 - Massive throughput: >40 Bravos at the Broad institute
 - Ultra High throughput with maximum walkaway time: 6 NGS Option B workstations at 1 Sequencing Center

Labware Considerations

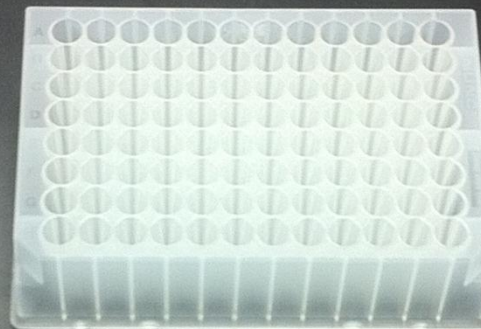
- Check protocols and order labware early, some protocols require labware from 4 or more vendors
- PCR plates determined by your thermalcycler
 - Full/half skirted with base
- Pipette Tips
 - Protocols with many pipetting steps require many pipette tips
 - Halo: 25 tips/sample
 - SureSelect: 55 tips/sample
 - Tips should be sterile, DNA/RNA free, we suggest filtered

Labware used:

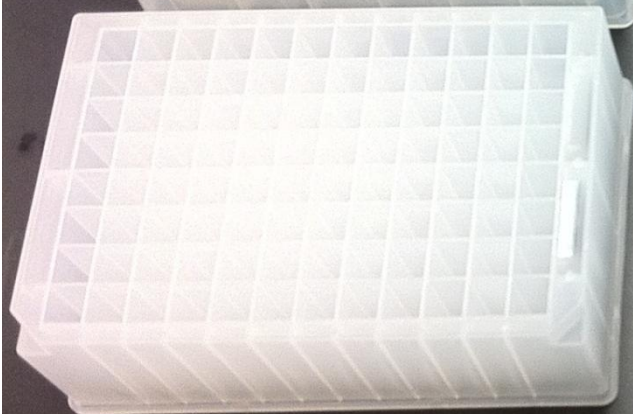
Agilent Filtered 250 μ L tips
(180 μ L capacity)



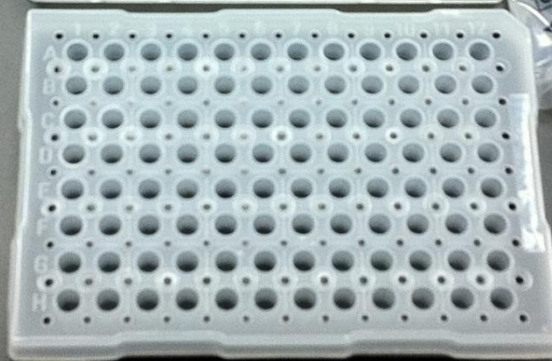
Thermo/Nunc 1.3mL
DeepWell Plate



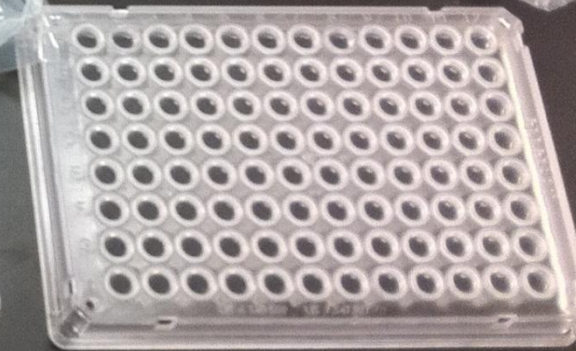
Thermo
Reservoir



E&K Scientific
2.2mL plate



ABI MicroAmp Plate
with Black Carrier

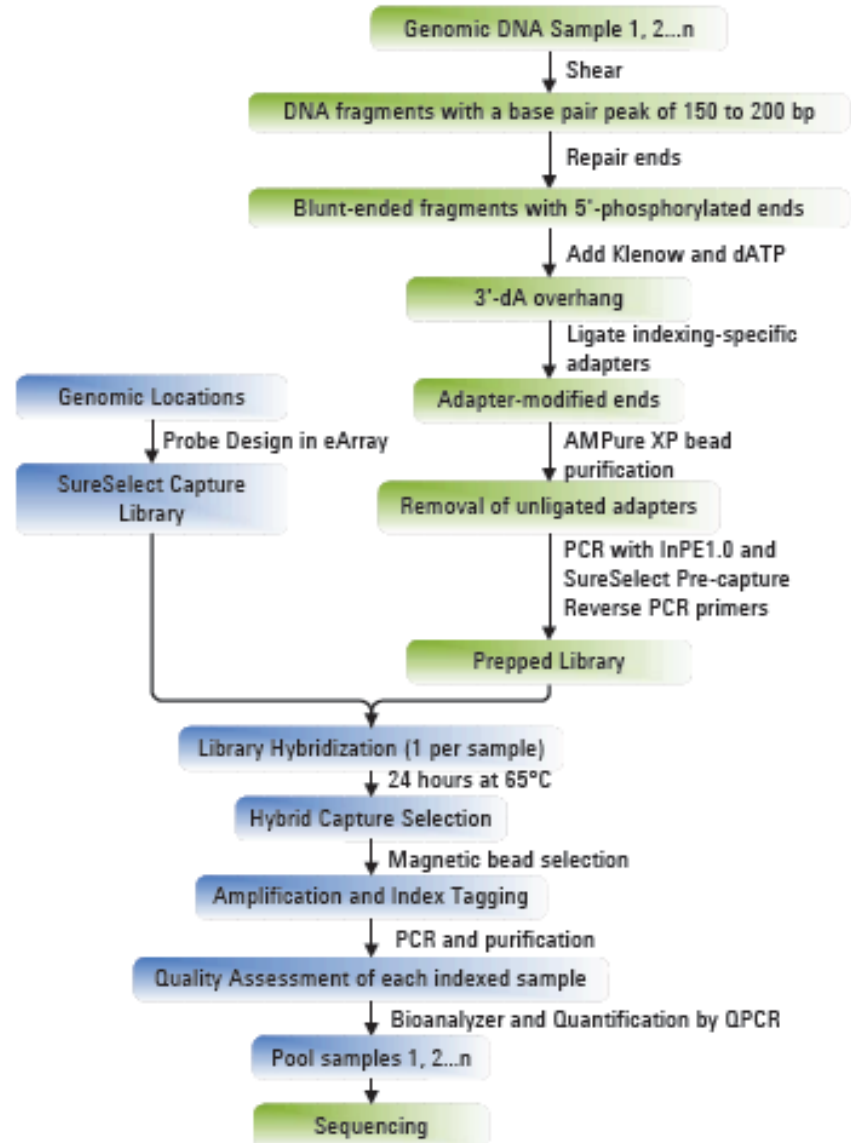


Eppendorf
Twin.tec plate

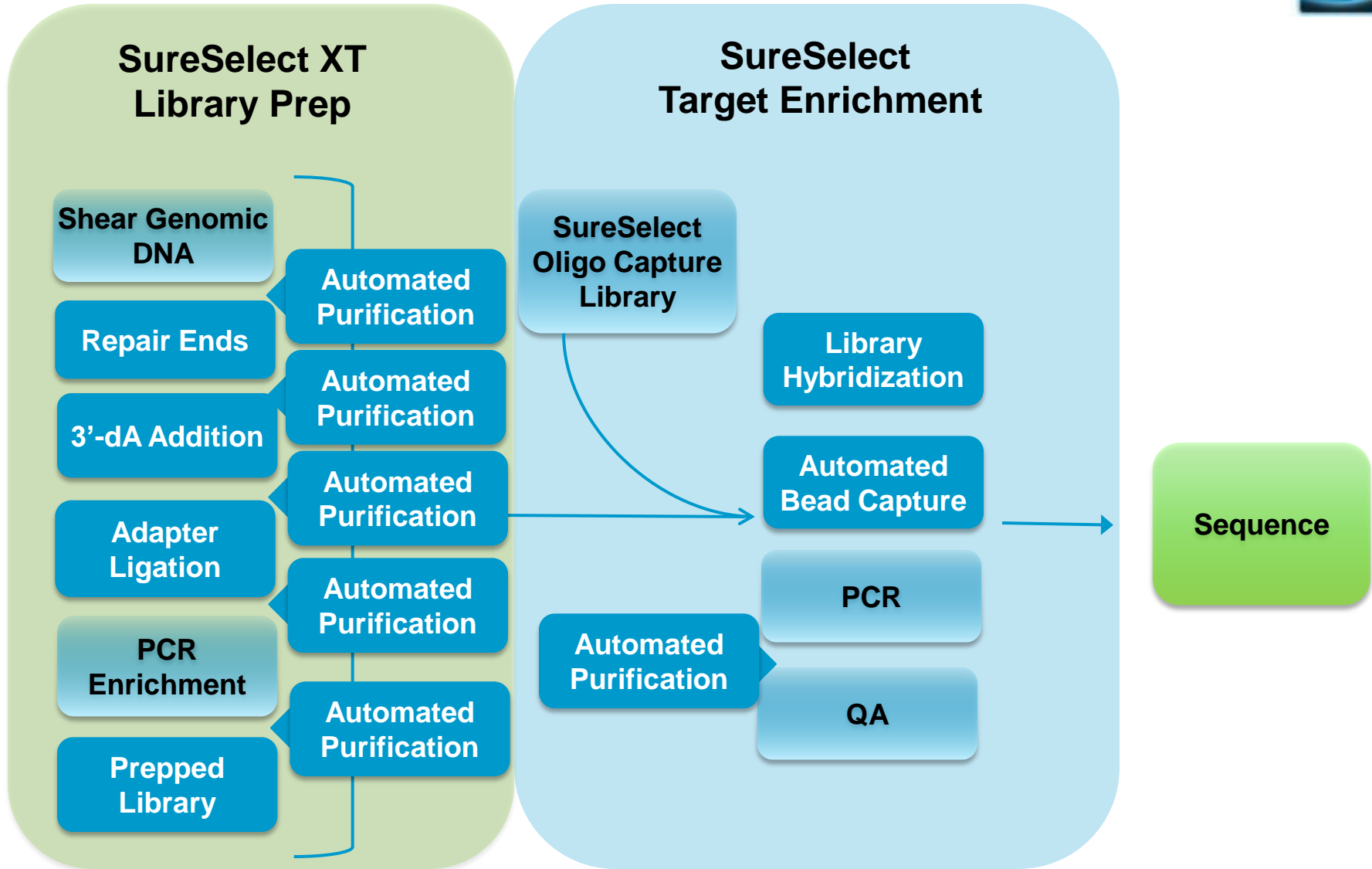
Overview of NGS Sample Preparation Workflow

Library Prep
highlighted in green

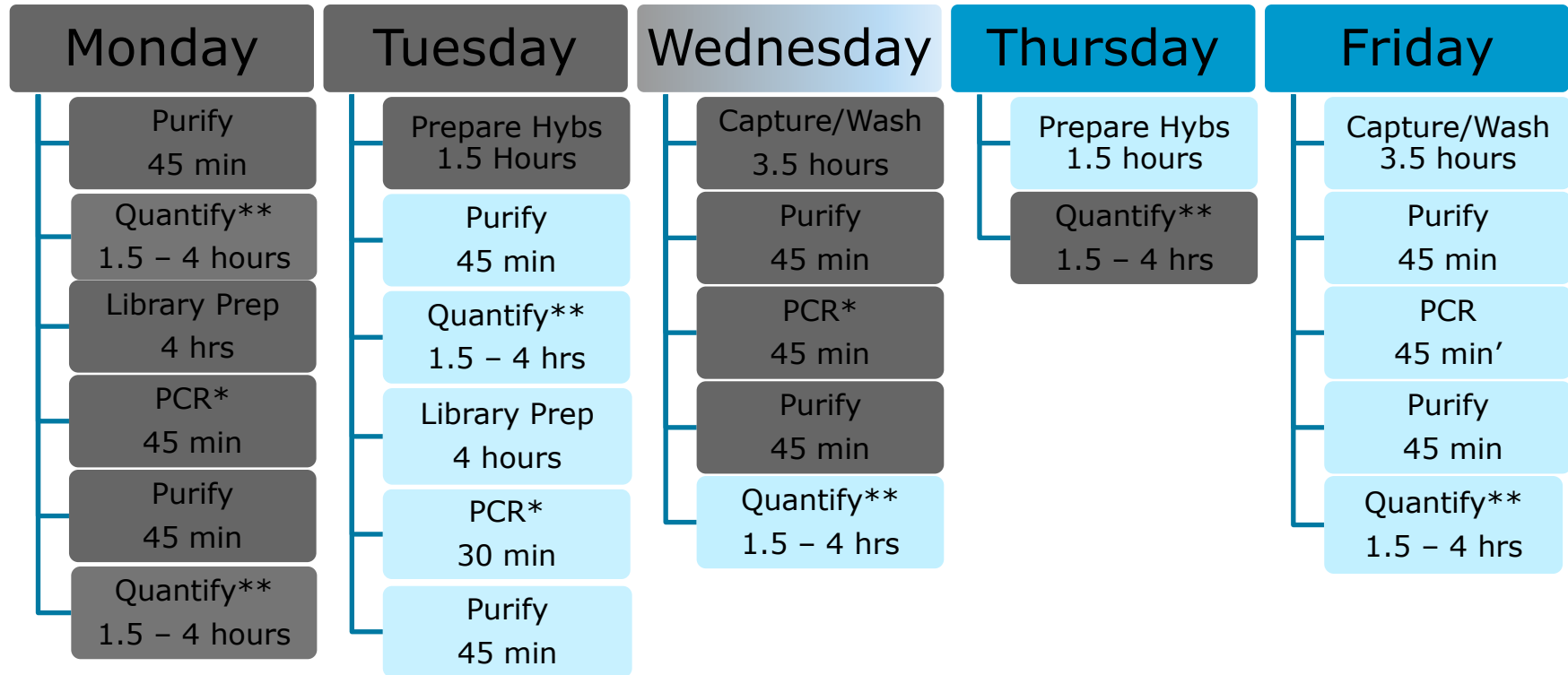
Target Enrichment
highlighted in blue



Automation of Next-Gen Sequencing Using SureSelect



Processing 192 Samples: An Example Workweek

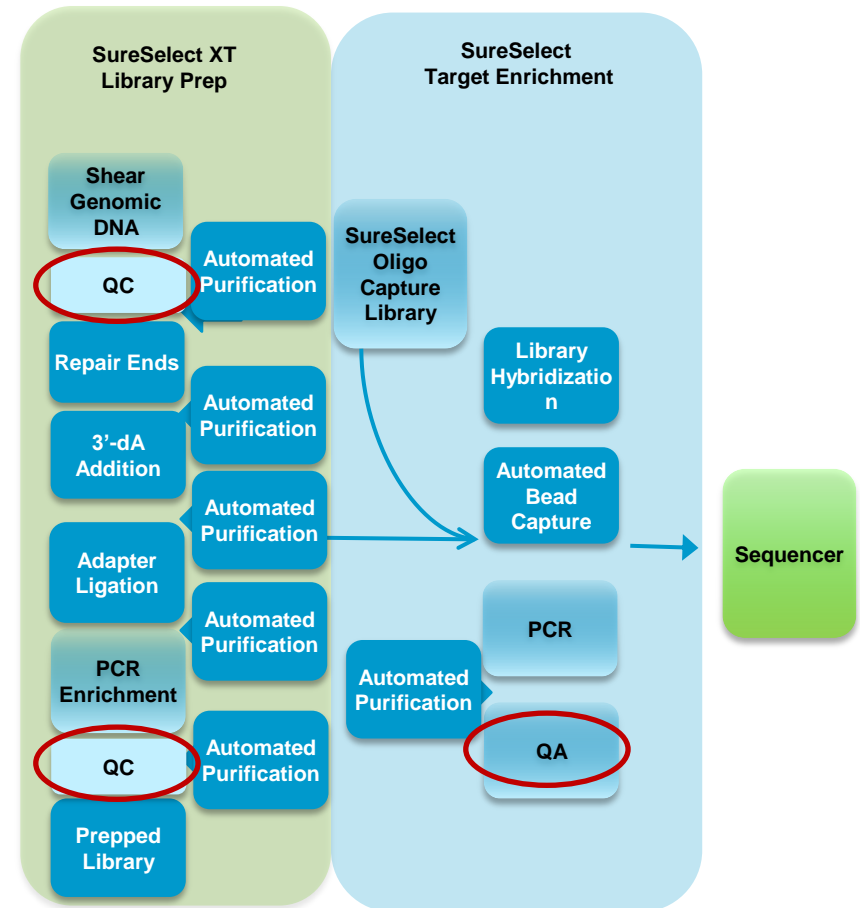


*Procedures take place off Bravo deck

**Quantification is off deck. Time for 96 samples: BioAnalyzer=4 hours, TapeStation=1.5 hours

Quality Control Scale Up

- With increased sample processing capacity, it is necessary to plan for scale up of sample QC
- Three QC steps are required in targeted resequencing protocols
- QC process for 96 samples can take 4 hours with BioAnalyzer
- TapeStation processes 96 samples in 90 minutes
- TapeStation offers time savings of 7.5 hours per plate of samples

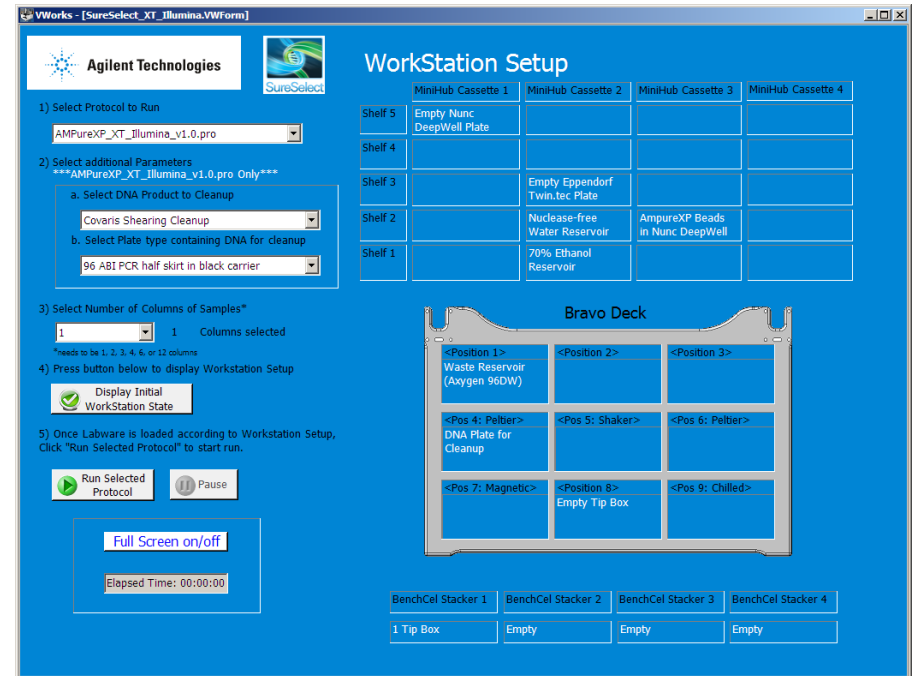


Minimize Reagent Waste

- Automation protocols require more dead volume than the same process done manually
- Many kits do not have enough dead volume to provide the advertised number of samples when automating
 - This adds to the cost per sample
- Even with “Automation Friendly” reagent kits, dead volumes can be an issue
 - Goal is to maximize reagent yield and minimize “dead volume” of residual reagents left in bottom of tubes
- For Agilent automation kits, to achieve the advertised number of samples, batch in minimum (or multiples) of 24 samples for highest reagent yield

VWorks Automation Software

- Tiered access for power user and end users
- Forms functionality to create GUI. Easily managing daily runs with less training needed
- Open system to completely control all aspects of automation protocols
- Modifications can often be made easily



Normalization and Pooling

- Before sequencing and hybridization, normalization and/or pooling is performed on NGS libraries
 - These processes can be error-prone and tedious
- Time savings is offered along with precision pipetting, utilizing data generated from your analysis device output
 - TapeStation, Pico, or BioA
- Protocols are included with NGS methods, but authoring modified protocols for specific applications is facilitated with a hit-picking wizard

The image displays three screenshots from the Hit Pick Replication Wizard software interface:

- Select Available Wells In Destination Plate:** A grid showing wells A-H and 1-12. Wells are marked as available (green) or unavailable (grey).
- Hit Pick Replication Format Wizard:** A configuration window with the following settings:
 - Dilution Series: Yes, No (applies in adjacent wells)
 - Number of dilutions: 3 (>= 0)
 - Dilution factor: 1.00 (>= 1.0)
 - Stock concentration: 5 (<= 0.000000)
 - Starting concentration: 5 (<= 0.000000)
 - Final volume: 50 (0.1-200 µL)An output table is shown below:

	Dilution1	Dilution2	Dilution3
Transfer volume(µL)	50	5	0.5
Concentration(nM)	5	0.5	0.05
- Destination Plate Format Preview:** A grid showing a color-coded layout for wells A-H and 1-12.
- Workflow Diagram:** A detailed process flow for the hit-picking routine, including steps like "Hit Pick Routine (Begin)", "Set head mode to One barrel", "Tip On in 1 selection(s) from TipsOn", "Dilute to final volume from Reservoir to Destination", "Mix 10 µL in selection determined by hit pick task from Destination", "Tip Off in 1 selection(s) from TipsOff", and "Hit Pick Routine (End)".

Normalization and Pooling

Hit Picking Wizard to automate the setup and data handling of these processes

Hit Pick Routine Wizard

Input File Specification

Operation type: **hit picking**

Select Input File

Specify an input file when the protocol runs

Specify an input file now

Auto skip headings

Skip heading lines: 1

C:\Documents and Settings\mbeban\My Documents\MyVWorks\Hit Pick Input File ... Start from transfer number: 1

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8
Sample ID	Assay	Source Plate	Barcode	Well	Response	% Inhibition	Column 8
AGT123456	TNFA	plt0001	00001	A10	1.11E+04	97.2	1.
AGT234561	TNFA	plt0001	00001	G6	2.34E+03	98	5.
AGT345612	TNFA	plt0001	00001	C2	5.21E+05	88.5	2.
AGT456123	TNFA	plt0001	00001	A1	1.20E+04	92.3	1.
AGT561234	TNFA	plt0001	00001	D4	9.89E+03	99.8	2.

Source plate barcode/ID

Source plate (column in csv): 4

Source Well Format and Corresponding Input File Column

Well ID (column in csv): 3

Well row and column

Row (column in csv):

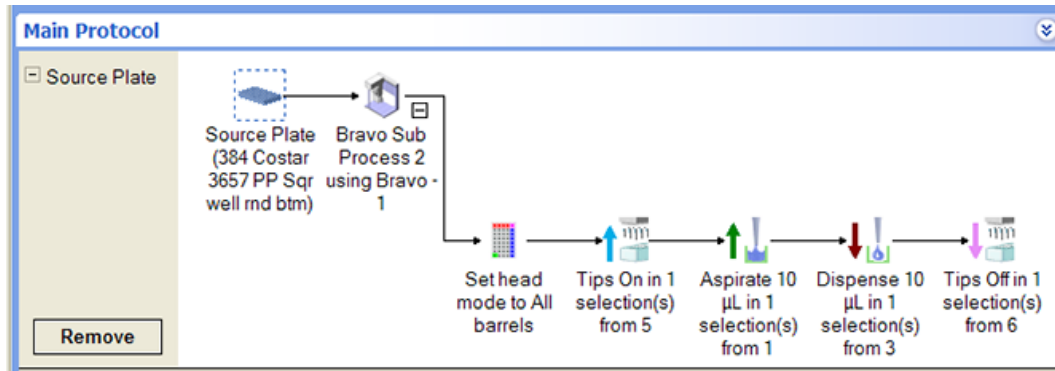
Column (column in csv):

Variable dilution factor (column in csv):

Variable transfer volume (column in csv): 7

Buttons: Cancel, << Back, Next >>, Finish

Setting up PCR plates with Bravo



Developing protocols such as PCR setup for other applications is very manageable for lab personnel using drag-and-drop tasks

Protocol examples are available from the Field Applications team

Setting up PCR plates with Bravo

Overview of VWorks Software User Interface

1 Available Tasks area

2 Protocol area

3 Task Parameters area

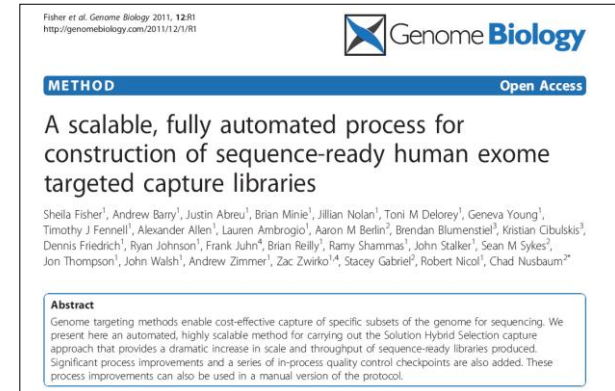
The screenshot displays the VWorks software interface for a Serial Dilution protocol. The interface is divided into three main sections:

- 1 Available Tasks area:** Located on the left, it contains a list of tasks such as Define Plate Set, Define Variables, Deld, Dismount, Downstack, Incubate, and JavaScript. A search filter is provided at the bottom of this panel.
- 2 Protocol area:** The central workspace shows the 'Startup Protocol' with two main steps: 'Source (384 Greiner 781101 PS clr fit btm)' and 'Destination (384 Greiner 781101 PS clr fit btm)'. Each step includes a 'Remove' button and a visual flow diagram showing the liquid handling process from the source to the destination via a stacker and liquid handling unit.
- 3 Task Parameters area:** Located on the right, it contains a 'Task Parameters' panel with sections for 'Plate identity' (Plate name: Source, Plate type: 384 Greiner 781101 PS) and 'Process control' (Simultaneous plates: 1, Use single instance of: , Automatically update: , Enable timed release: , Release time: 0:00:30).

At the bottom of the interface, there is a 'Main Log' window showing a list of events with timestamps and descriptions, such as 'Info File saved' and 'Info Opening diagnostics'. The status bar at the very bottom indicates 'Ready' and 'a is logged in'.

Citations for Agilent NGS Automation

- S. Fisher et al *Genome Biol.* 2011;12(1):R1. doi: 10.1186/gb-2011-12-1-r1.
 - 1200 sampls/week
- M. Garber *Mol Cell.* 2012 Sep 14;47(5):810-22.
 - ChIP Seq sample preparation
- Rohland N, Reich D. Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. *Genome Res.* 2012 May;22(5):939-46.



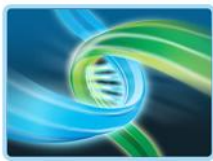
A High-Throughput Chromatin Immunoprecipitation Approach Reveals Principles of Dynamic Gene Regulation in Mammals

Method

Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture

Nadin Rohland¹ and David Reich

Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA; Broad Institute of Harvard and MIT, Cambridge, Massachusetts 02139, USA



**Benefits to automating NGS sample preparation:
Increase throughput, increase reproducibility**

Why Automate with Agilent?

- **Comprehensive Solution**
 - Automation and reagents developed and supported by one company
- **Automation Expertise**
 - Applications team focused on instrumentation
- **Flexibility**
 - Automation can be easily set up to perform a variety of tasks
 - Potential to build scalable high throughput systems
- **Satisfied Customers**
 - Over 100 Agilent NGS Systems in use worldwide
 - Key sequencing centers (Broad, BGI, Sanger) use Agilent Bravo
 - Three peer-reviewed publications