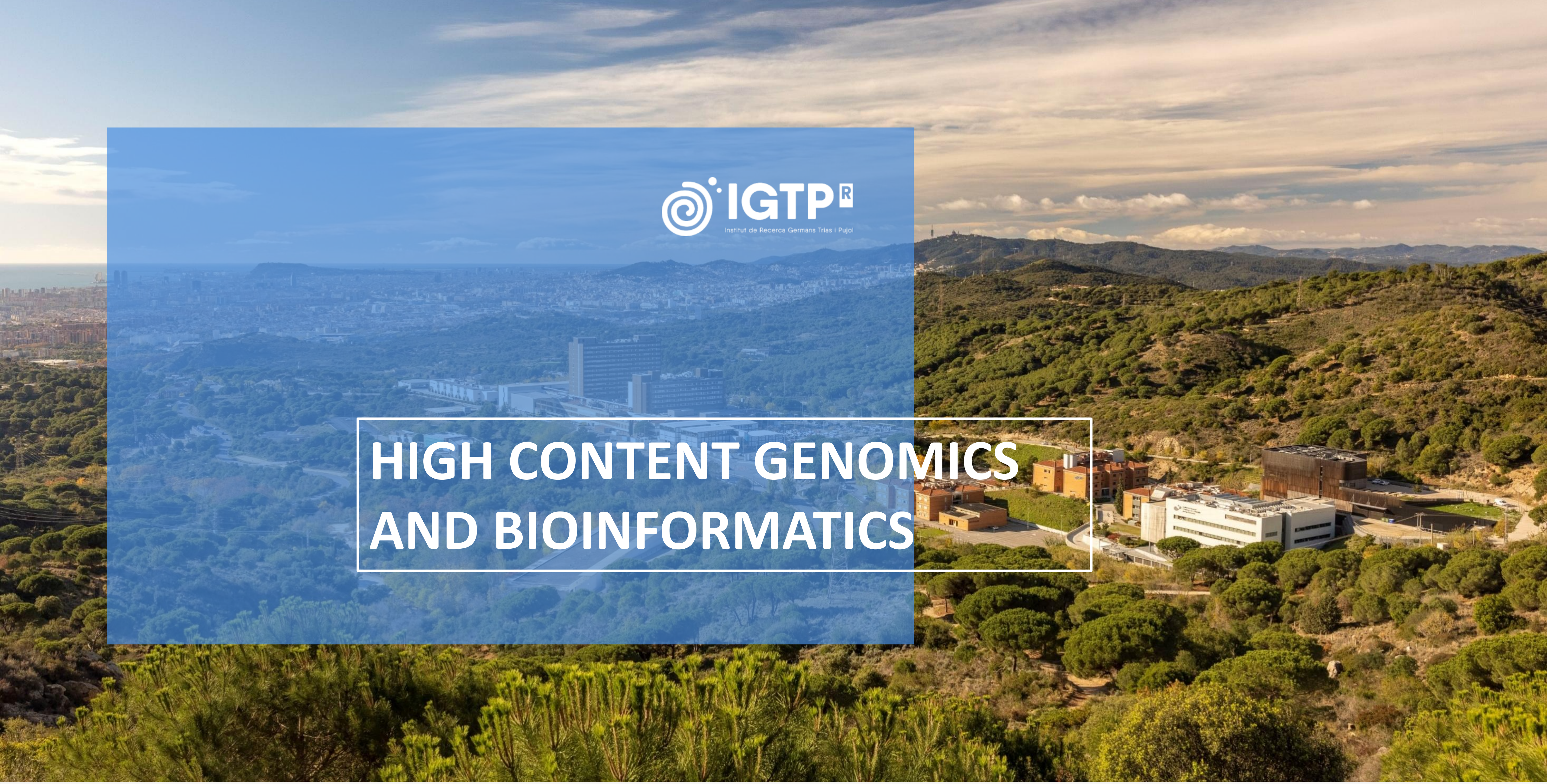
IGTP Core Facility Seminars

El 3r dilluns de cada mes a les 15 h
Modalitat en línia i presencial (Sala Polivalent IGTP)



IGTP
Institut de Recerca Germans Trias i Pujol

HIGH CONTENT GENOMICS AND BIOINFORMATICS

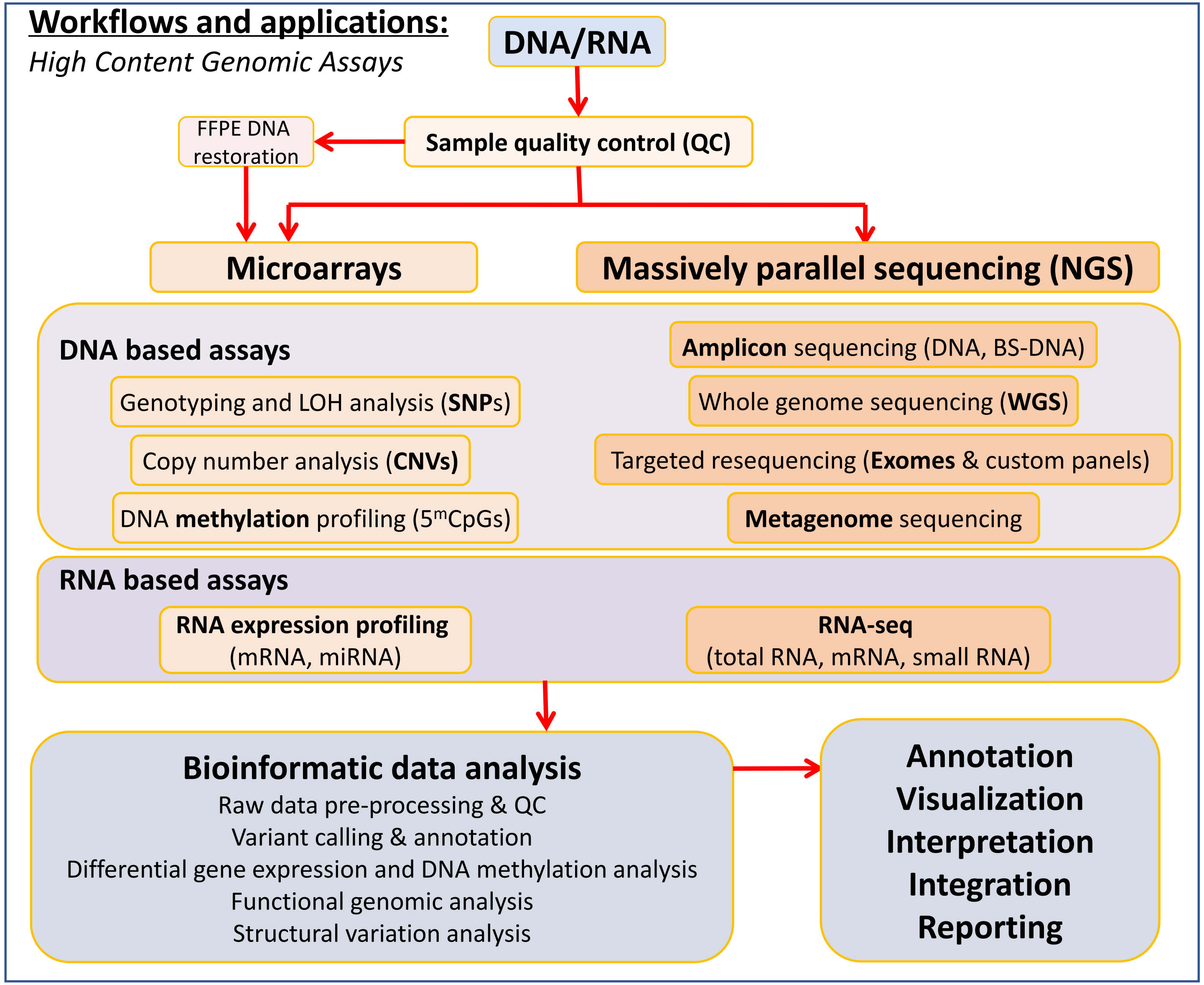
Generalitat de Catalunya
Germans Trias i Pujol Hospital
UAB Universitat Autònoma de Barcelona
ICO Institut Català d'Oncologia
Ajuntament de Badalona
IDIAP Jordi Gol
IrsiCaixa Institut de Recerca de la Sida
Institut Guttmann Hospital de Neurorehabilitació
Consorci Sanitari del Maresme
Banc de Sang i Teixits
Institut Català de la Salut
Josep Carreras Leukaemia Research Institute

Sequencing library preparation automation solutions

- 04/11/2024 15:00
- Sala Polivalent, Edifici Mar, IGTP / online

- **Lauro Sumoy**
- IGTP High Content Genomics and Bioinformatics Facility

HCGB Services



Workflows and applications:

High Content Genomic Assays

DNA/RNA

Sample quality control (QC)

FFPE DNA restoration

Microarrays

Massively parallel sequencing (NGS)

DNA based assays

Genotyping and LOH analysis (**SNP**s)

Copy number analysis (**CNVs**)

DNA **methylation** profiling (5^{m}CpGs)

Amplicon sequencing (DNA, BS-DNA)

Whole genome sequencing (**WGS**)

Targeted resequencing (**Exomes** & custom panels)

Metagenome sequencing

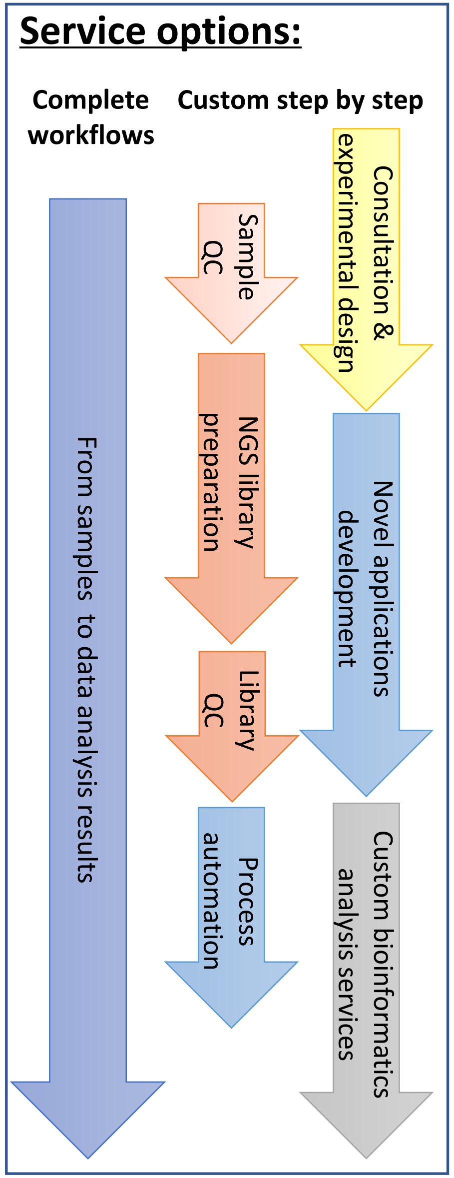
RNA based assays

RNA expression profiling
(mRNA, miRNA)

RNA-seq
(total RNA, mRNA, small RNA)

Bioinformatic data analysis
Raw data pre-processing & QC
Variant calling & annotation
Differential gene expression and DNA methylation analysis
Functional genomic analysis
Structural variation analysis

Annotation
Visualization
Interpretation
Integration
Reporting



Service options:

Complete workflows

Custom step by step

From samples to data analysis results

Sample QC

NGS library preparation

Library QC

Process automation

Consultation & experimental design

Novel applications development

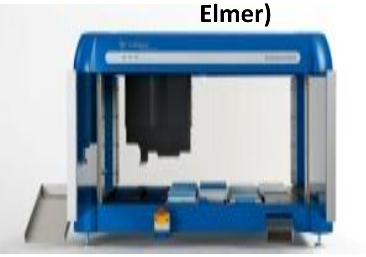
Custom bioinformatics analysis services

HCGB

Technologies and equipment:

NGS

Library prep and QC automation on **Sciclone NGS** **robot (Perkin Elmer)**



Microarrays

Infinium assay automation on **Tecan Evo-150** **robot (Illumina)**

Illumina SBS

Amplicon sequencing,
Sequencing panels,
16S rRNA,
RNA-seq,
WGS,
Exomes,
BS-seq,
metagenomes,
bacterial genomes,
viromes,
SARS-COV2

Illumina Infinium

genotyping / copy number / LOH & DNA methylation assays

Oligonucleotide beadarrays

0.2-5 M features per chip

Outsourcing

- Amplicon sequencing, small genome resequencing, panels , 16S rRNA
- RNA-seq, WGS, Exomes, BS-seq, Metagenomes, ChIP-seq

MiSeq

NextSeq 1000/2000

50-400 M reads per run, 50-300 nt, single or paired end reads (IGTP)

NextSeq500

NovaSeq

300 M-24 B reads per run, 75-250nt,
Single or paired end reads (UPF, CRG, CNAG, VHIO, MACROGEN, BGI...)

Scanning of beadarrays performed at (IJC)

iScan

HCGB automation capabilities

Liquid handling for NGS library prep, cleanup, size selection & qPCR setup

- ✓ With bead library prep (96-plex)

- ✓ AMPure cleanups / Magnetic bead based double size selection (96 plex)

- ✓ qPCR setup (384 well format)

Automated DNA fragment separation and collection

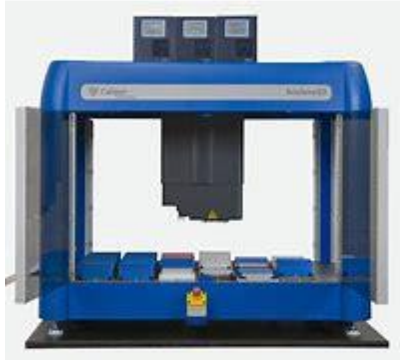
- ✓ Gel size selection with Pippin Prep (4 pools / run)

Automated array hybridization setup, washing and staining

- ✓ Illumina Infinium assays (up to 12 beadarrays, up to 192 samples/run)

HCGB automation equipment

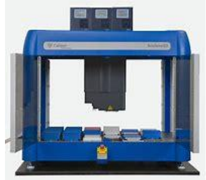
Sciclone NGS G3 (Caliper/PerkinElmer/Revvity):
Automated NGS library prep, SPRI cleanup & qPCR setup
- 96-tip – 96 to 384 well plate liquid handling robot



Pippin Prep (Sage Science):
Automated agarose cassette gel size selection

Freedom Evo 150 (Tecan -Illumina software driven-)
Automated Illumina Infinium beadarray assay - 8-tip - liquid handling robot

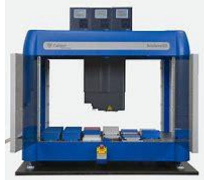
Applications on Sciclone NGS



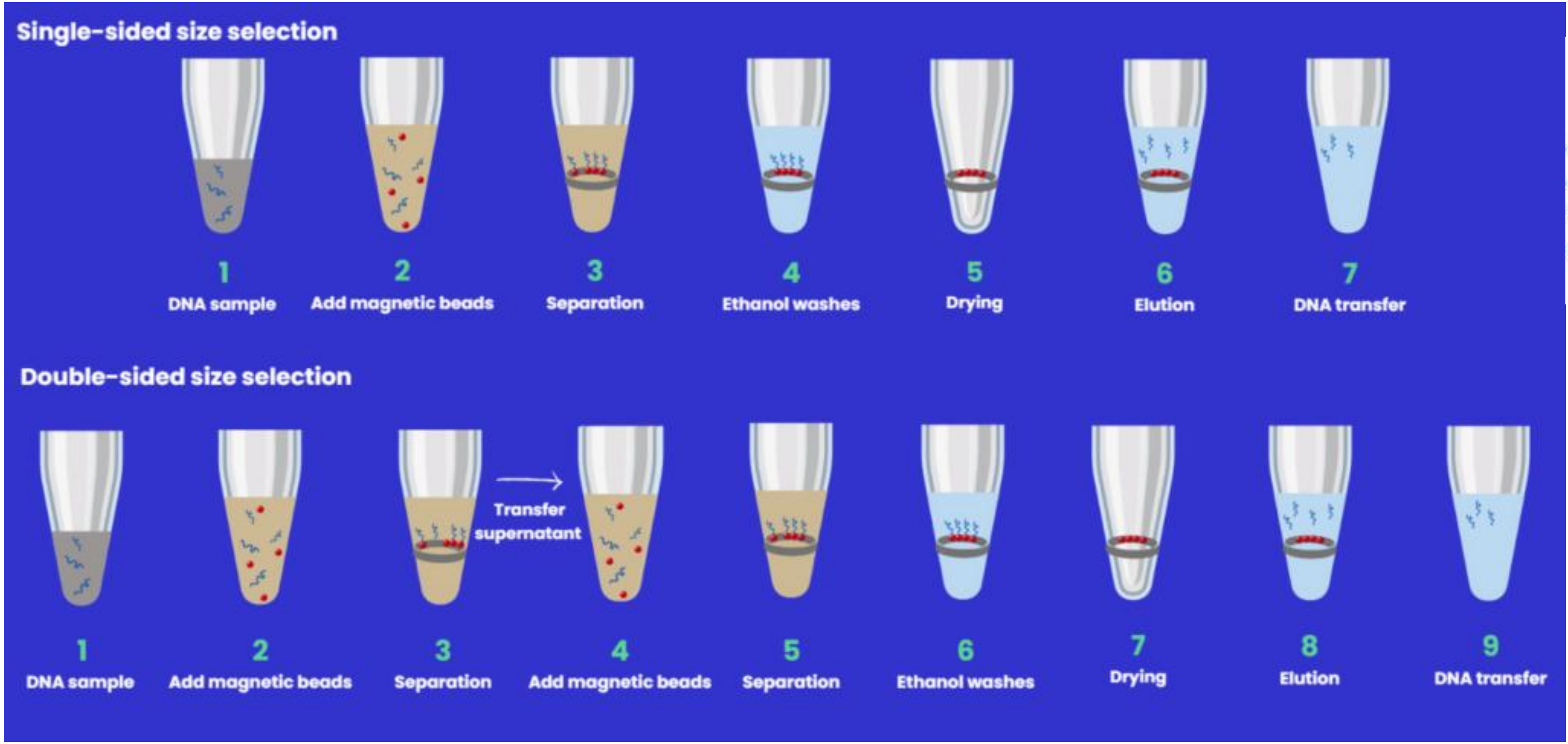
Automated NGS library preparation

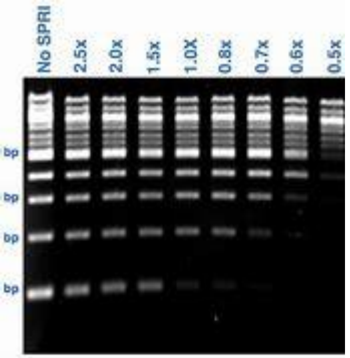
- ✓ RNA:
  - ✓ Illumina TruSeq RNA
  - ✓ Illumina TruSeq stranded mRNA (with beads protocol)
  - ✓ Illumina TruSeq stranded total RNA (with beads protocol)
  - ✓ Illumina Total RNA ribozero plus (tagmentation on beads)
- ✓ Exome:
  - ✓ Illumina Rapid Exome capture
- ✓ DNA
  - ✓ Illumina DNA Nextera Flex (for cDNA: SARS-COV2) - DNA prep
  - ✓ Illumina Nextera XT (clean up)
- ✓ smallRNA
  - ✓ Revvity (Bioo / PerkinElmer) NextFlex Small RNA v4 (with double size selection)

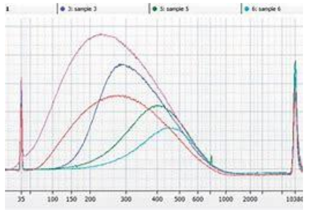
Applications on Sciclone NGS



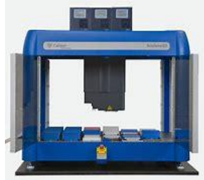
Size selection by solid phase reversible immobilization (SPRI)





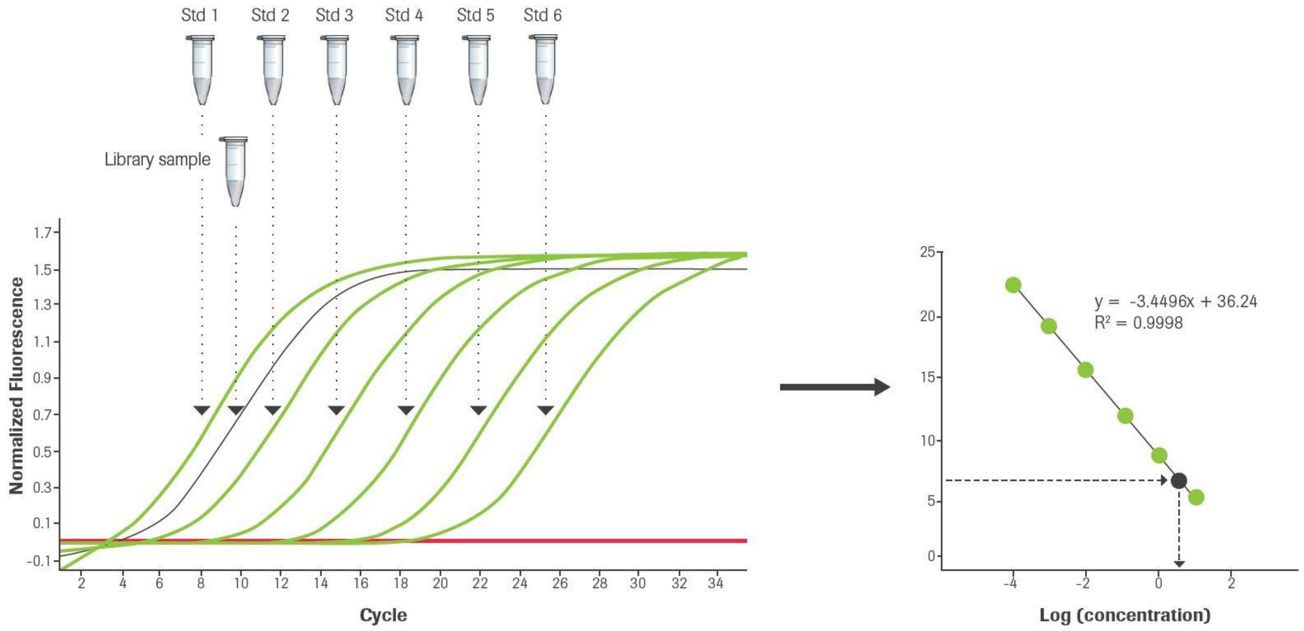


Applications on Sciclone NGS



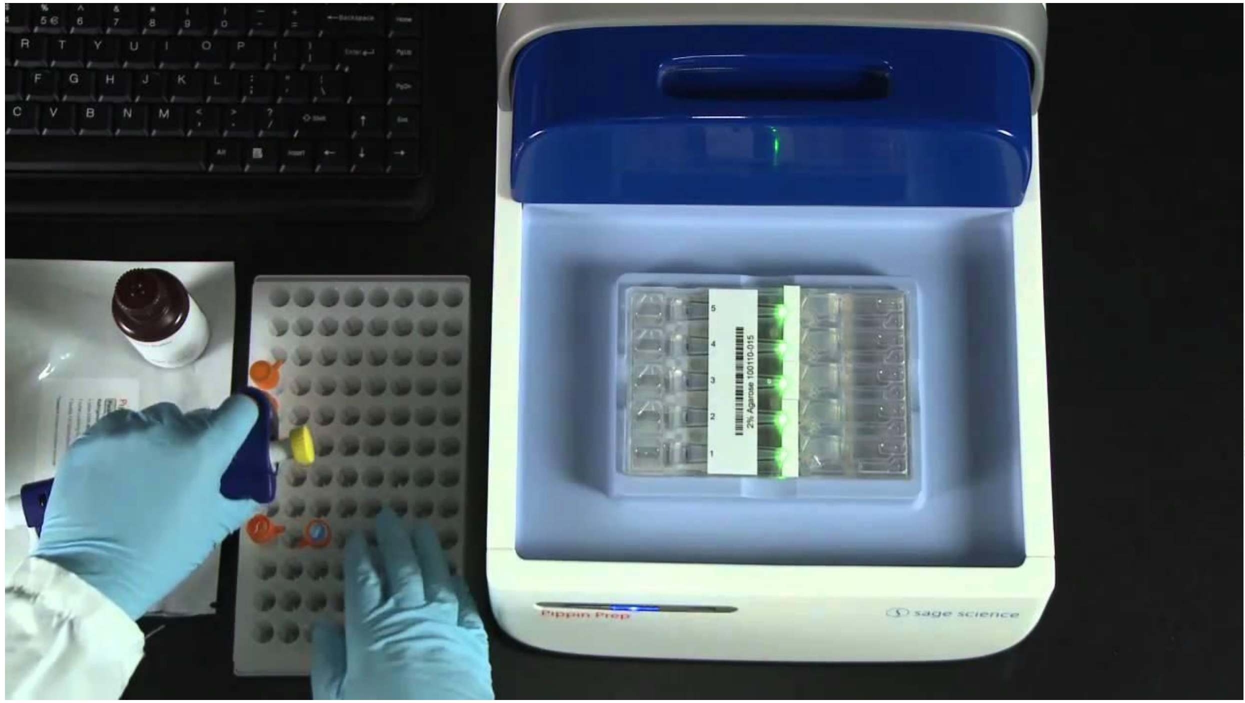
Automated qPCR setup

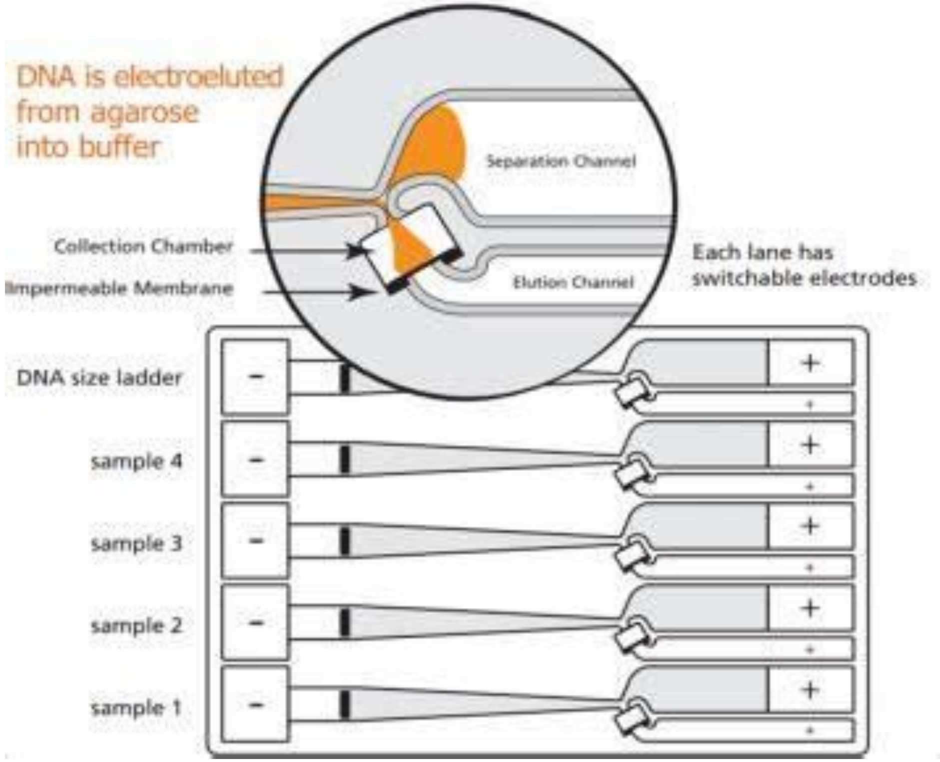
- ✓ Generic qPCR setup (SYBR green / Taqman)
- ✓ Taqman Advanced miRNA qPCR
- ✓ Ion Torrent NGS library Taqman qPCR quantification
- ✓ Illumina library prep KAPA qPCR quantification



Applications on Pippin Prep

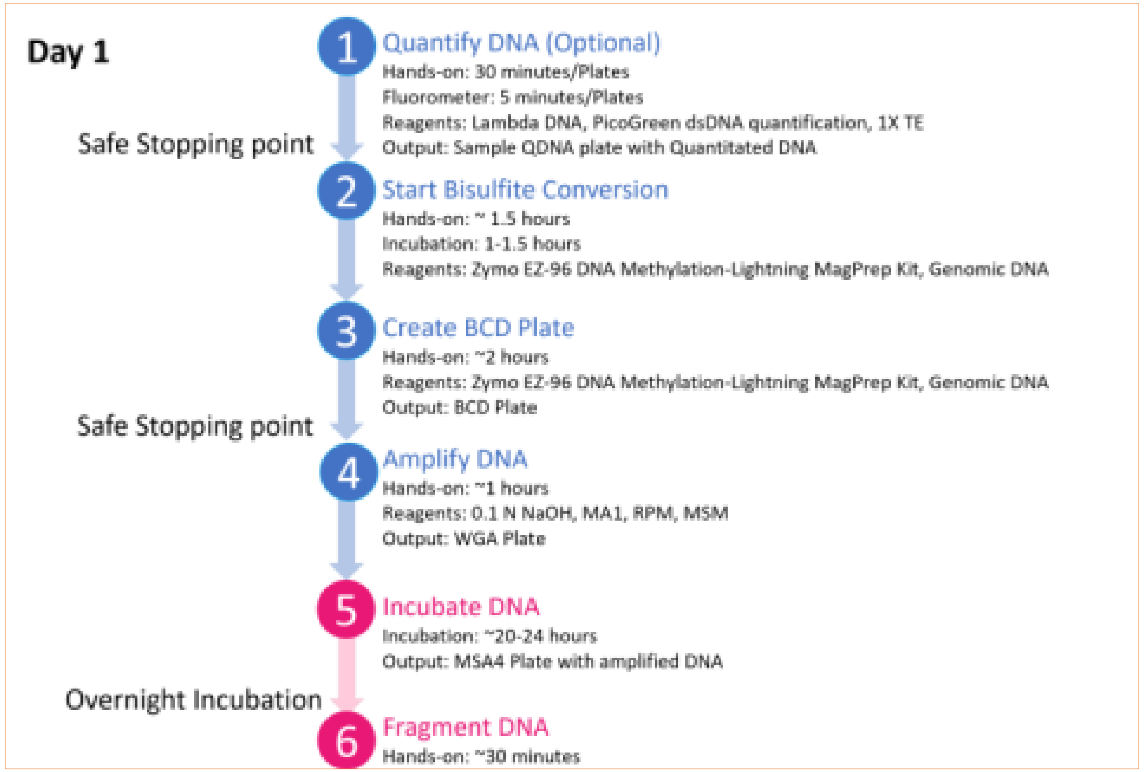
Automated gel size selection





Applications on Tecan

Infinium DNA methylation EPIC2 assay



Day 1

1 Quantify DNA (Optional)
Hands-on: 30 minutes/Plates
Fluorometer: 5 minutes/Plates
Reagents: Lambda DNA, PicoGreen dsDNA quantification, 1X TE
Output: Sample QDNA plate with Quantitated DNA

Safe Stopping point

2 Start Bisulfite Conversion
Hands-on: ~ 1.5 hours
Incubation: 1-1.5 hours
Reagents: Zymo EZ-96 DNA Methylation-Lightning MagPrep Kit, Genomic DNA

3 Create BCD Plate
Hands-on: ~2 hours
Reagents: Zymo EZ-96 DNA Methylation-Lightning MagPrep Kit, Genomic DNA
Output: BCD Plate

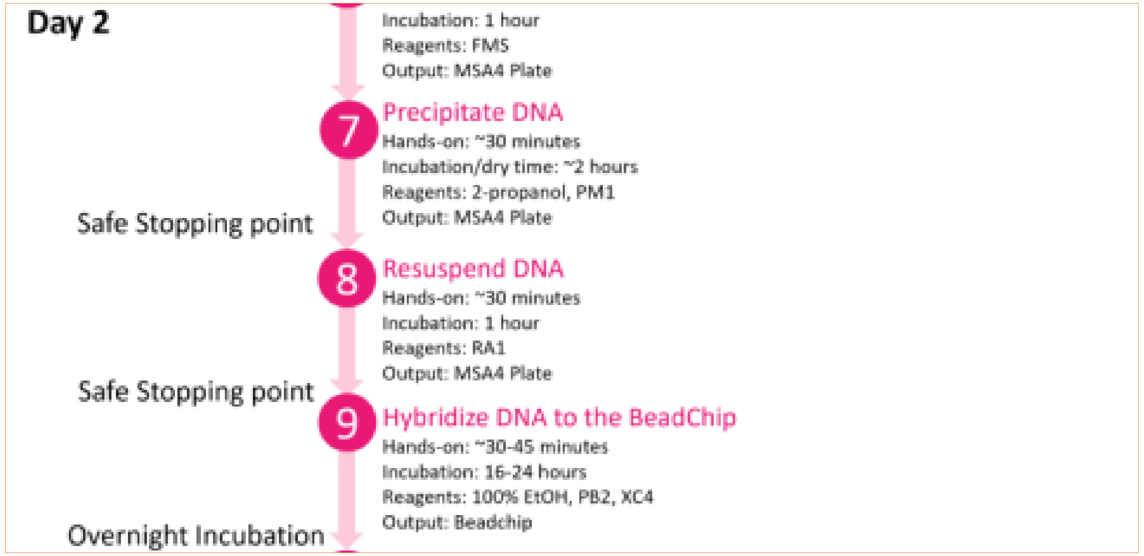
Safe Stopping point

4 Amplify DNA
Hands-on: ~1 hours
Reagents: 0.1 N NaOH, MA1, RPM, MSM
Output: WGA Plate

5 Incubate DNA
Incubation: ~20-24 hours
Output: MSA4 Plate with amplified DNA

Overnight Incubation

6 Fragment DNA
Hands-on: ~30 minutes



Day 2

Incubation: 1 hour
Reagents: FMS
Output: MSA4 Plate

7 Precipitate DNA
Hands-on: ~30 minutes
Incubation/dry time: ~2 hours
Reagents: 2-propanol, PM1
Output: MSA4 Plate

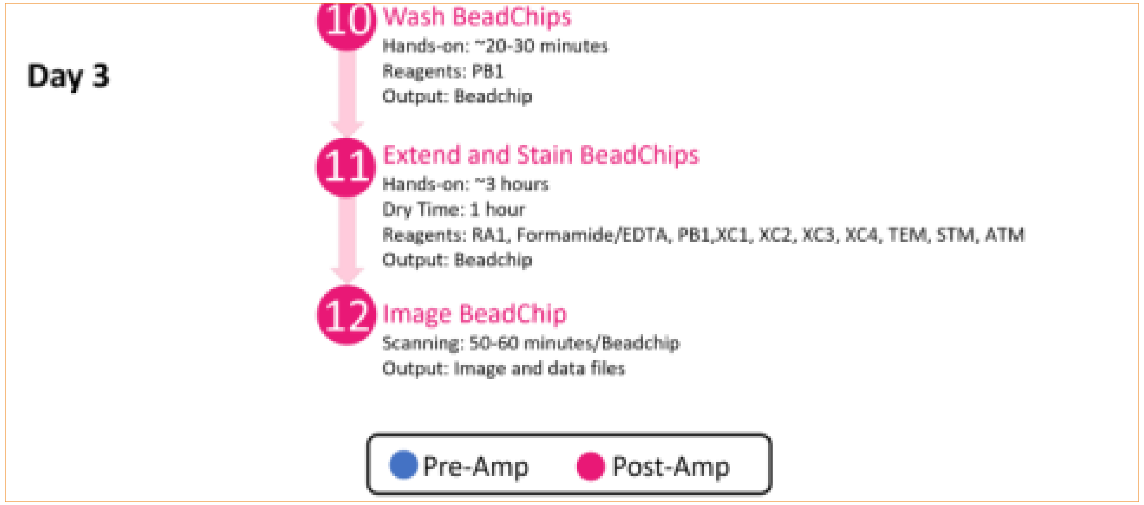
Safe Stopping point

8 Resuspend DNA
Hands-on: ~30 minutes
Incubation: 1 hour
Reagents: RA1
Output: MSA4 Plate

Safe Stopping point

9 Hybridize DNA to the BeadChip
Hands-on: ~30-45 minutes
Incubation: 16-24 hours
Reagents: 100% EtOH, PB2, XC4
Output: Beadchip

Overnight Incubation



Day 3

10 Wash BeadChips
Hands-on: ~20-30 minutes
Reagents: PB1
Output: Beadchip

11 Extend and Stain BeadChips
Hands-on: ~3 hours
Dry Time: 1 hour
Reagents: RA1, Formamide/EDTA, PB1,XC1, XC2, XC3, XC4, TEM, STM, ATM
Output: Beadchip

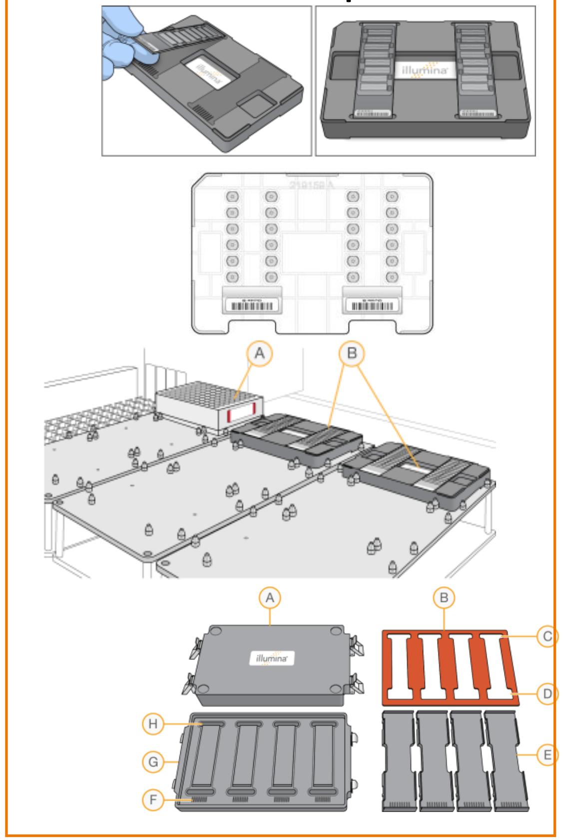
12 Image BeadChip
Scanning: 50-60 minutes/Beadchip
Output: Image and data files

● Pre-Amp ● Post-Amp

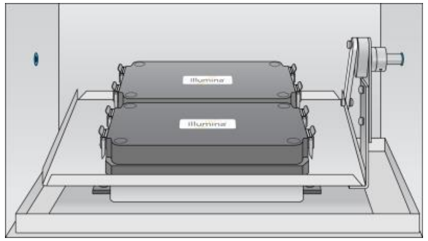
Applications on Tecan

Automated Illumina infinium assay beadarray processing

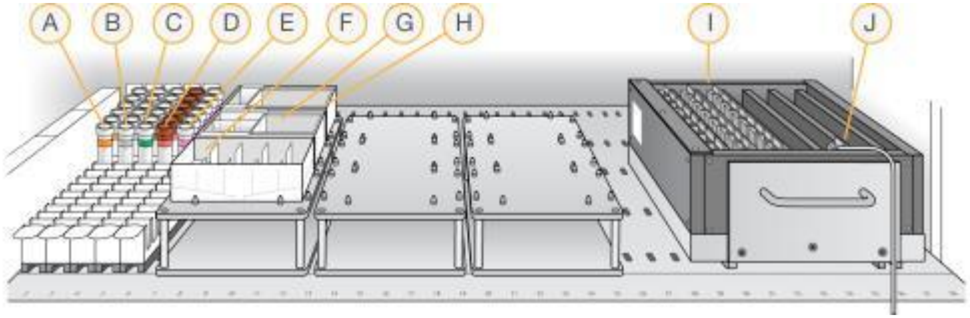
Day 2: Hybridization setup

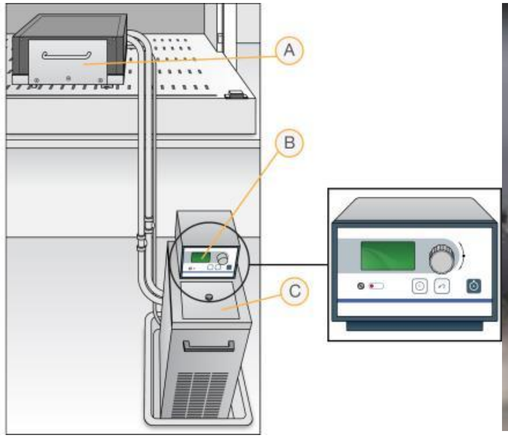


Hybridization oven



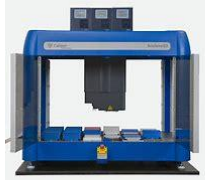
Day 3: High stringency washing and staining





iScan Beadarray scanner

Applications on Sciclone NGS

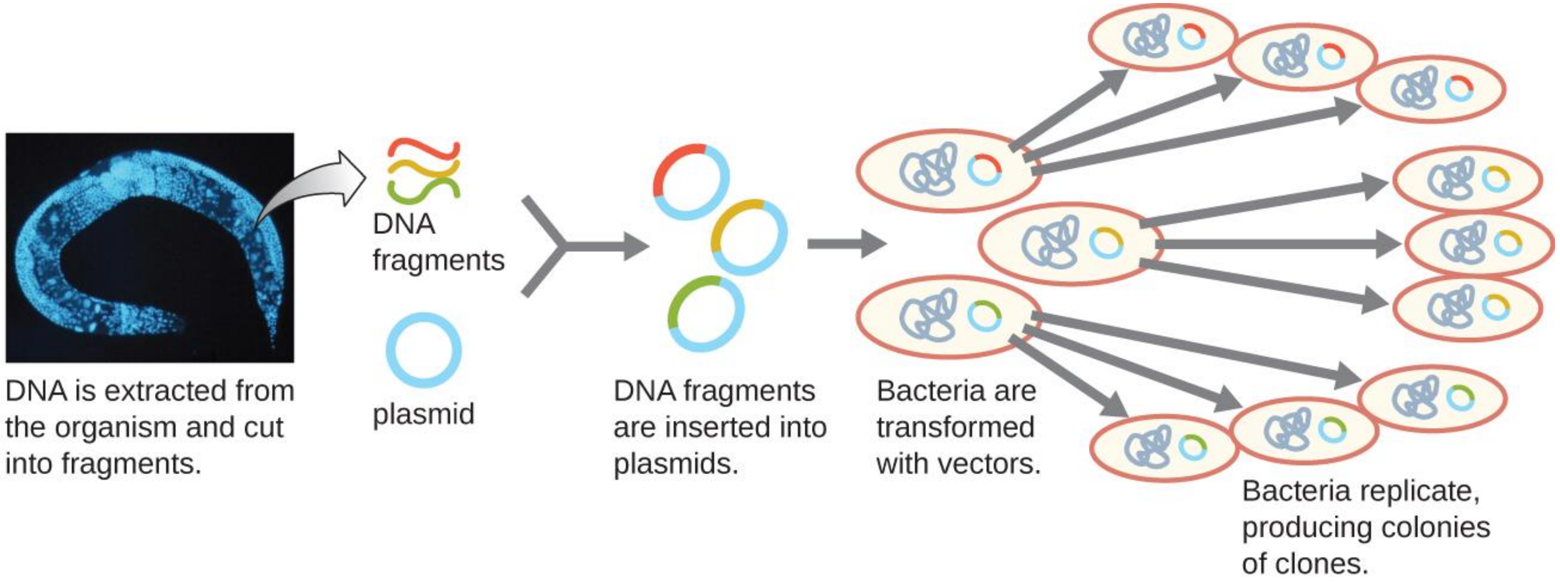


Automated NGS library preparation

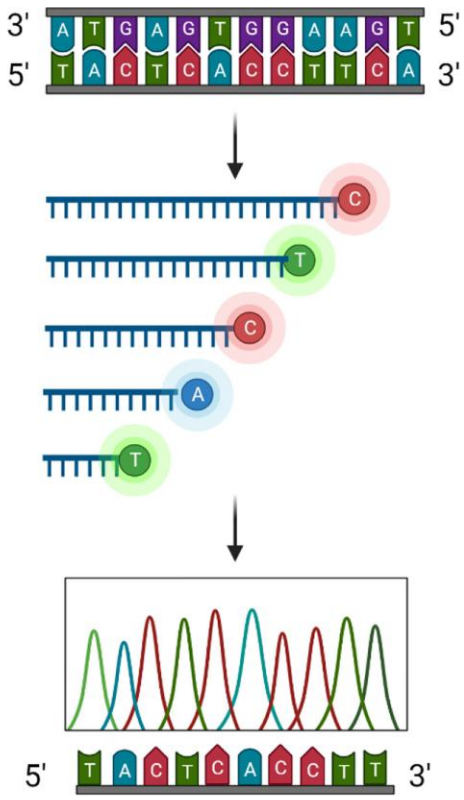
- ✓ RNA:
  - ✓ Illumina TruSeq RNA
  - ✓ Illumina TruSeq stranded mRNA (with beads protocol)
  - ✓ Illumina TruSeq stranded total RNA (with beads protocol)
  - ✓ Illumina Total RNA ribozero plus (with ligated adapters)
- ✓ Exome:
  - ✓ Illumina Rapid Exome capture
- ✓ DNA
  - ✓ Illumina DNA prep (tagmentation on beads, for cDNA: SARS-COV2)
  - ✓ Illumina Nextera XT (clean up)
- ✓ Small RNA
  - ✓ Revvity (Bioo/PerkinElmer) NextFlex Small RNA v4 (with double size selection)

DNA library as a concept

Classical DNA library preparation (plasmid/phage based clonal amplification)



Sanger sequencing (1 clone per capillary, 96-384-plex)



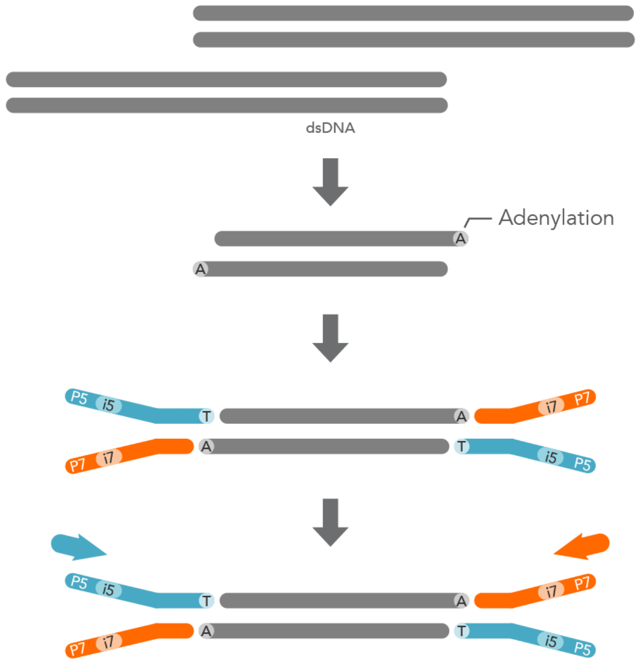
NGS library preparation
(PCR amplified)

Fragmentation

End repair and A-tailing

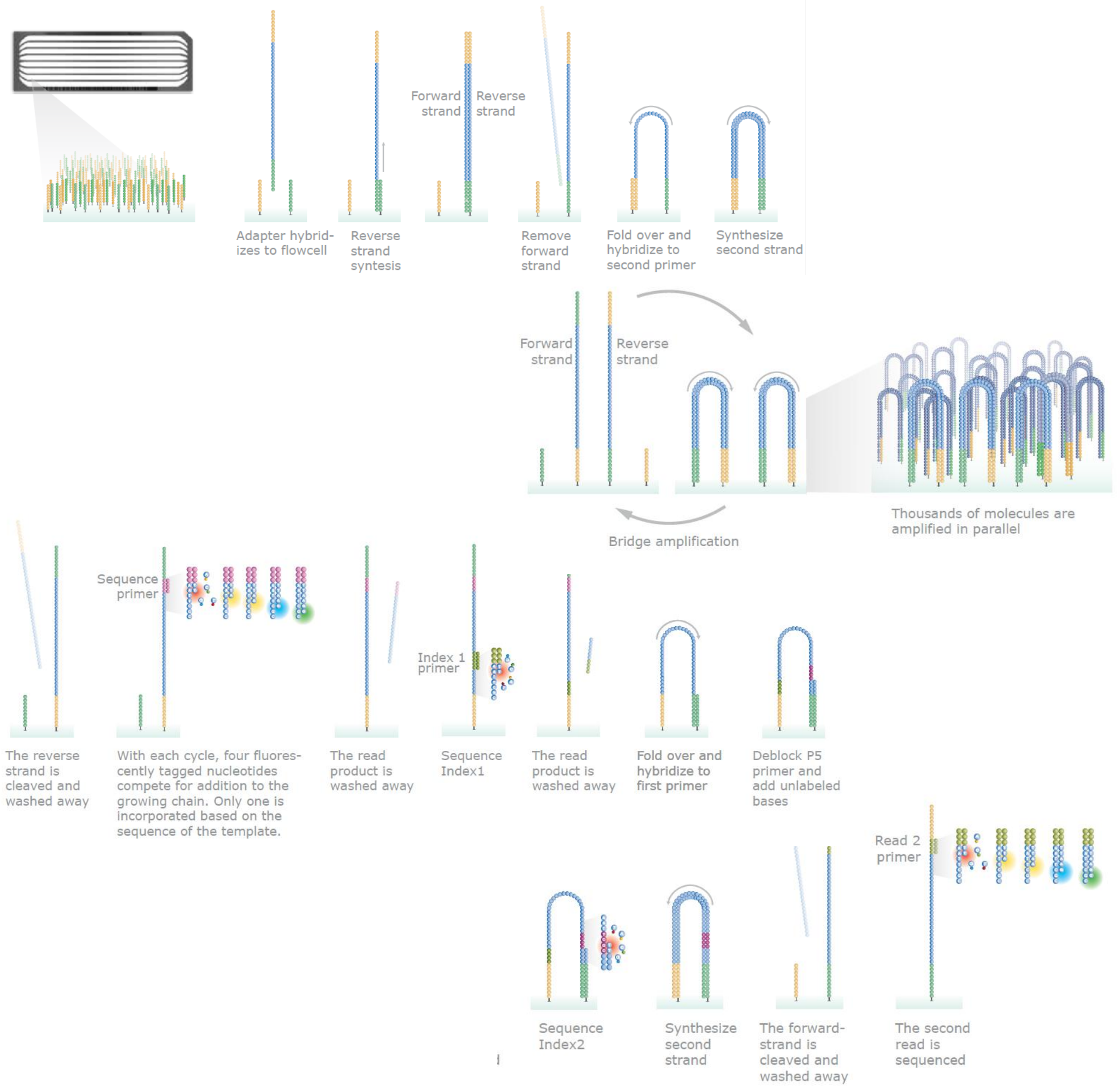
Ligation

PCR amplification



Next Generation Sequencing - NGS
(massively parallel sequencing, >>10E6 clones at a time)

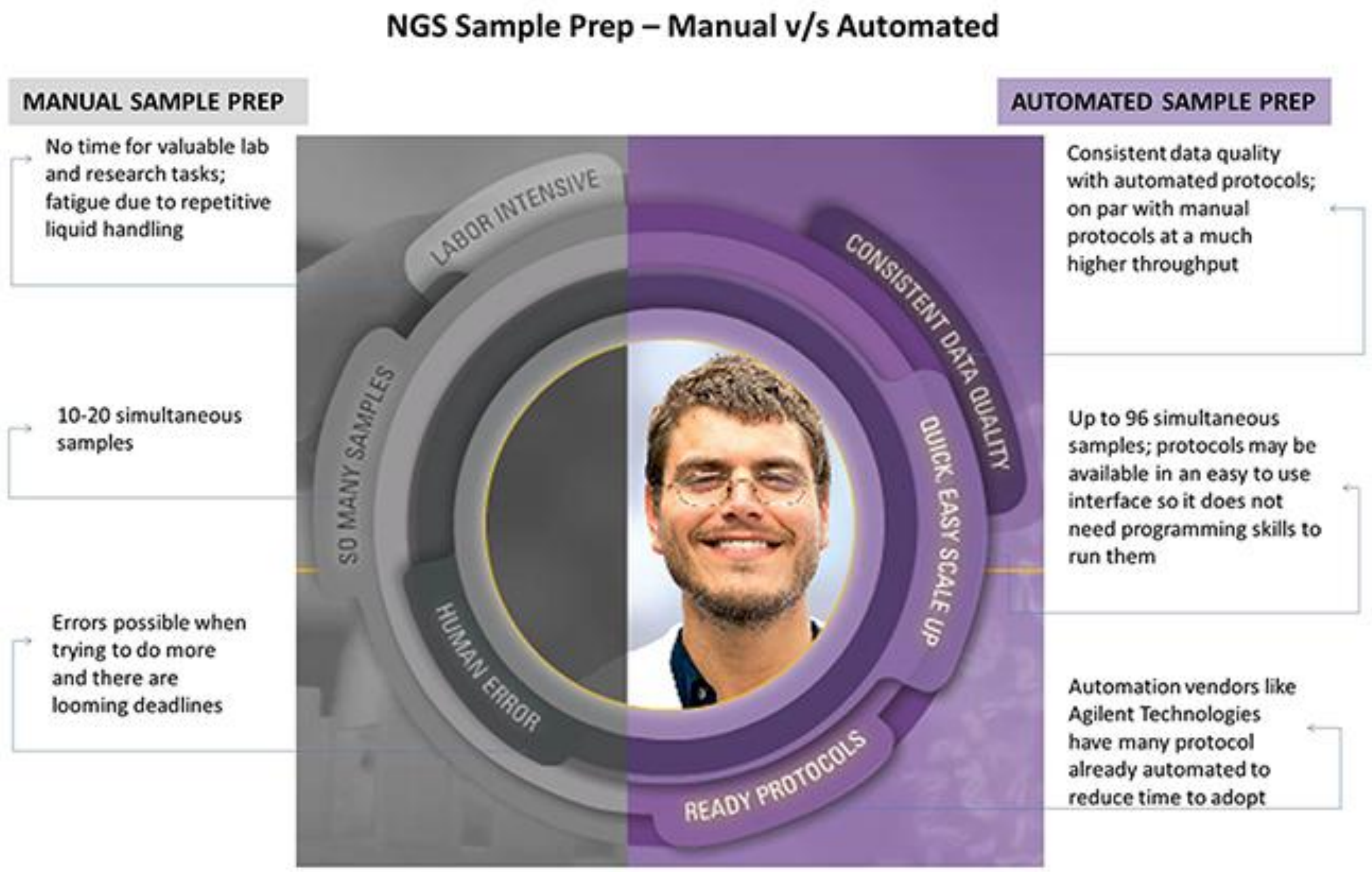
Sequencing by Synthesis



Library preparation is a complex multistep process

bulk RNA-seq	RNA extraction	input sample QC and normalization	cDNA synthesis	cDNA amplification	bead clean-up	cDNA QC and normalization	library preparation	[optional] fragmentation (physical or enzymatic)	end repair and A-tailing	ligation	library amplification	bead clean-up	library QC, normalization and pooling
single cell RNA-seq	single cell isolation	RNA extraction	cDNA synthesis	cDNA amplification	bead clean-up	QC and normalization	library preparation	[optional] fragmentation (physical or enzymatic)	end repair and A-tailing	ligation	library amplification	bead clean-up	library QC, normalization and pooling
bulk DNA-seq	gDNA extraction					QC and normalization	library preparation	[optional] fragmentation (physical or enzymatic)	end repair and A-tailing	ligation	library amplification	bead clean-up	library QC, normalization and pooling
single cell DNA-seq	single cell isolation	gDNA extraction		whole genome amplification	bead clean-up	QC and normalization	library preparation	[optional] fragmentation (physical or enzymatic)	end repair and A-tailing	ligation	library amplification	bead clean-up	library QC, normalization and pooling
amplicon sequencing	gDNA extraction					input sample QC and normalization	library preparation	[optional] fragmentation (physical or enzymatic)	end repair and A-tailing	ligation	library amplification	bead clean-up	library QC, normalization and pooling
whole exome sequencing	gDNA extraction	input sample QC and normalization	library preparation	library QC and pooling	probe hybridization and capture	PCR amplification	library preparation	[optional] fragmentation (physical or enzymatic)	end repair and A-tailing	ligation	library amplification	bead clean-up	library QC, normalization and pooling

Why automate library prep?



NGS Sample Prep – Manual v/s Automated

MANUAL SAMPLE PREP

No time for valuable lab and research tasks; fatigue due to repetitive liquid handling

10-20 simultaneous samples

Errors possible when trying to do more and there are looming deadlines

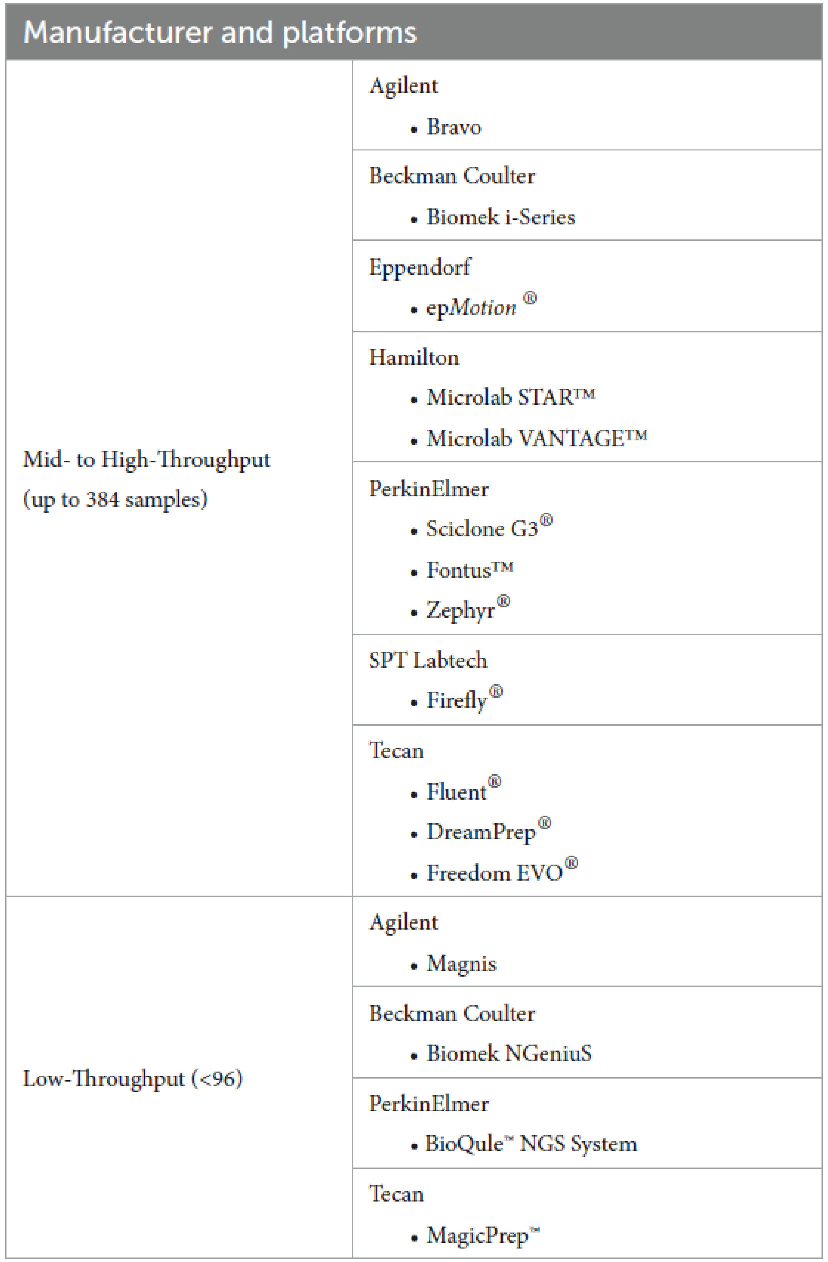
AUTOMATED SAMPLE PREP

Consistent data quality with automated protocols; on par with manual protocols at a much higher throughput

Up to 96 simultaneous samples; protocols may be available in an easy to use interface so it does not need programming skills to run them

Automation vendors like Agilent Technologies have many protocol already automated to reduce time to adopt

Automation provides consistent data quality with increased throughput while reducing hands-on time. Labware management can also provide walk away time.



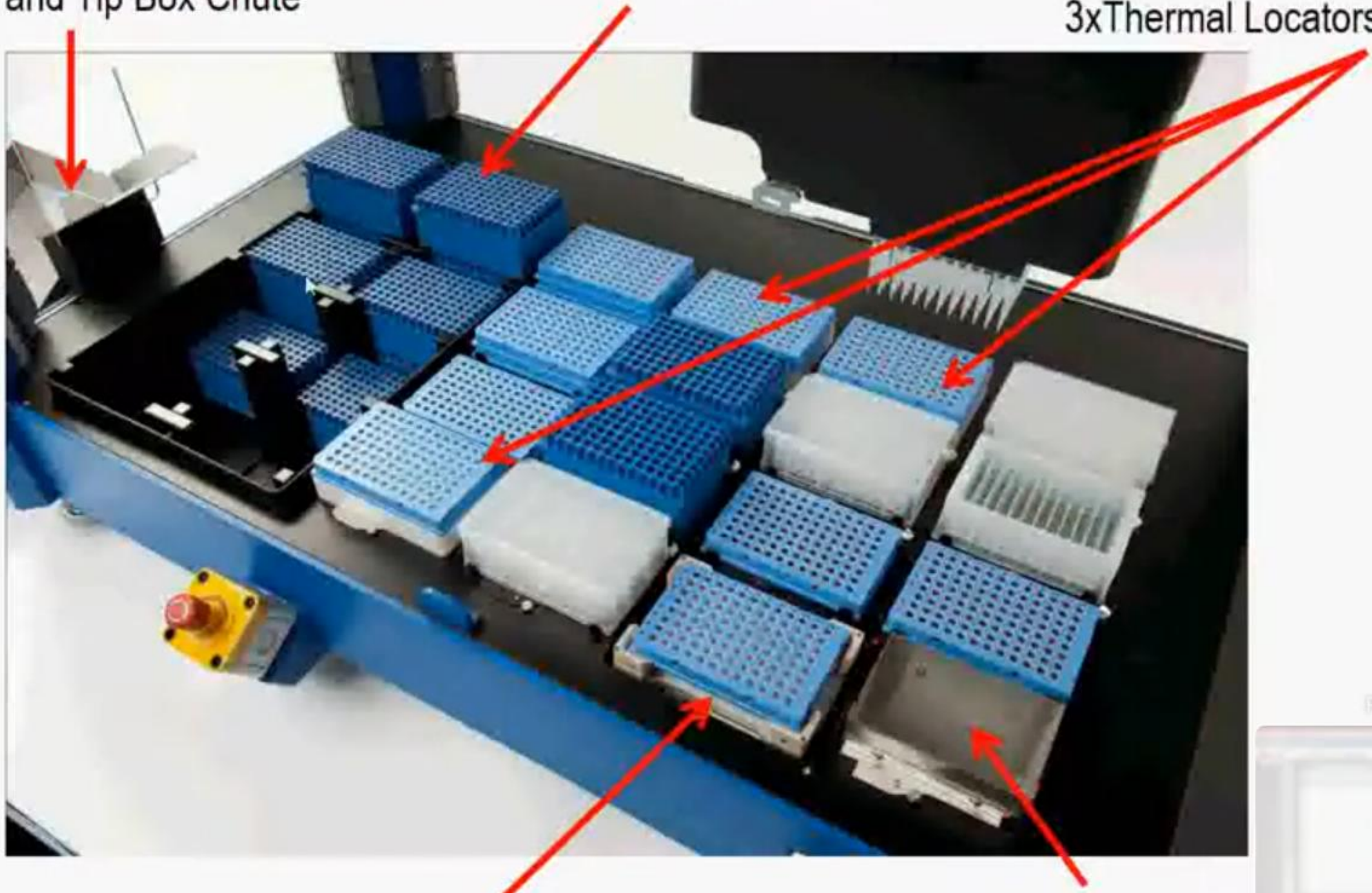
Manufacturer and platforms	
Mid- to High-Throughput (up to 384 samples)	Agilent • Bravo
	Beckman Coulter • Biomek i-Series
	Eppendorf • ep*Motion* ®
	Hamilton • Microlab STAR™ • Microlab VANTAGE™
	PerkinElmer • Sciclone G3® • Fontus™ • Zephyr®
	SPT Labtech • Firefly®
	Tecan • Fluent® • DreamPrep® • Freedom EVO®
Low-Throughput (<96)	Agilent • Magnis
	Beckman Coulter • Biomek NGeniuS
	PerkinElmer • BioQule™ NGS System
	Tecan • MagicPrep™

Sciclone NGSx Workstation

- Design of instrument
  - User friendliness
  - Overcoming fear of automation
  - Reduction in user intervention = reduced variability
  - Ease of use

Sciclone NGSx Workstation

PerkinElmer
For the Better



Used Plate and Tip Box Chute

Recessed Deck for Tip Reserves

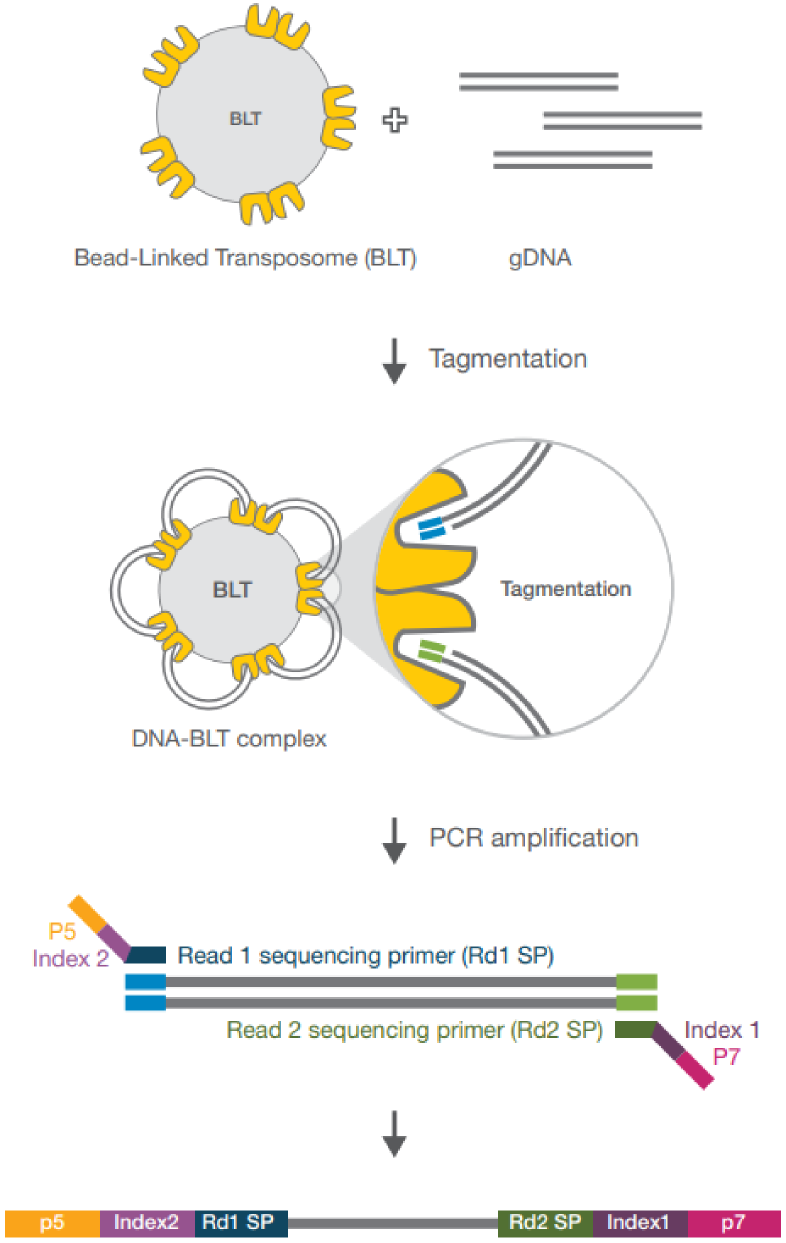
3xThermal Locators 4-110°

Thermal Shaker 4-70°

Used Tip Waste Chute

Walkaway Without Complexity

Figure 1 Illumina DNA Prep Workflow



Sequencing-ready fragment

gDNA

Blood Lysis
Hands-on: 20 minutes
Total: 40 minutes
Reagents: BLB, PK1, IPB, EtOH

Safe Stopping Point

Saliva Lysis
Hands-on: 20 minutes
Total: 40 minutes
Reagents: IPB, EtOH

Safe Stopping Point

1 Tagment Genomic DNA
Hands-on: 20 minutes
Total: 40 minutes
Reagents: BLT, TB1

2 Post Tagmentation Clean Up
Hands-on: 20 minutes
Total: 45 minutes
Reagents: TSB, TWB

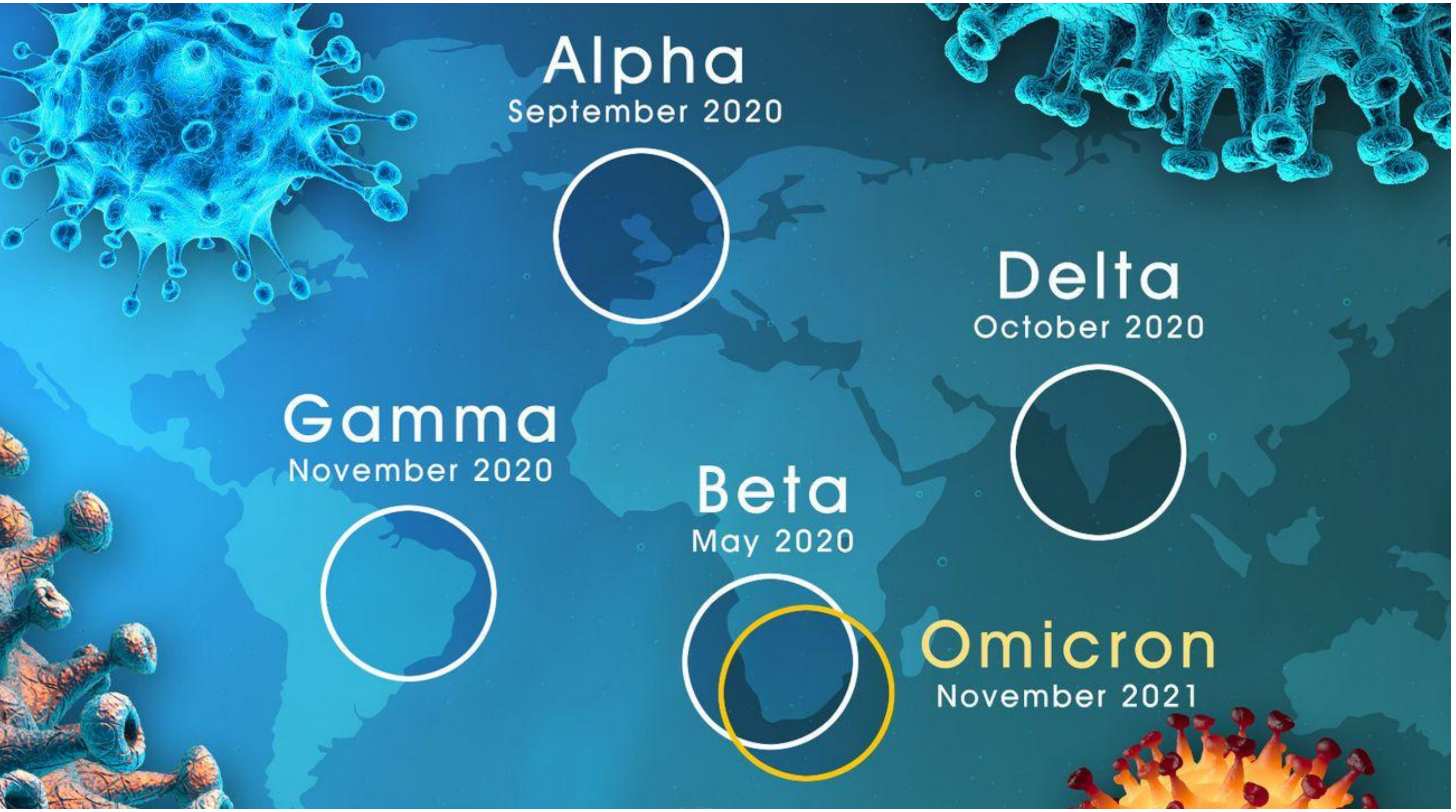
3 Amplify Tagmented DNA
Hands-on: 15 minutes
Total: 45 minutes
Reagents: EPM, Index Adapters

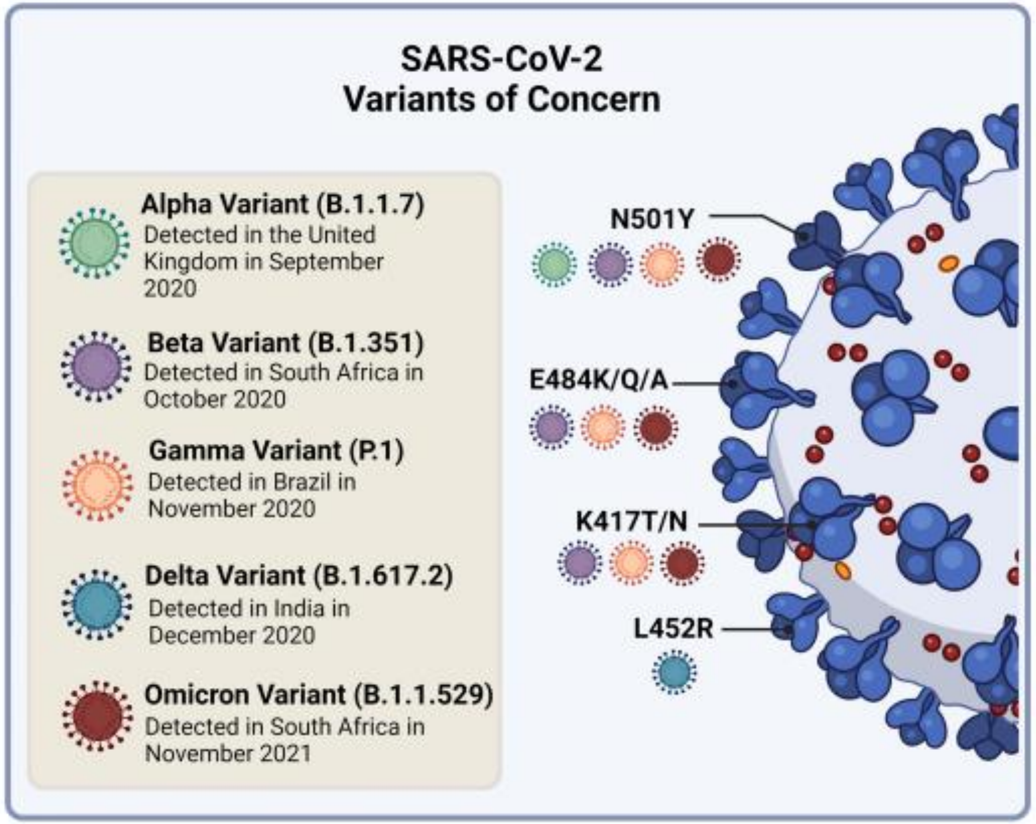
Safe Stopping Point

4 Clean Up Libraries
Hands-on: 10 minutes
Total: 50 minutes
Reagents: RSB, IPB, EtOH

Safe Stopping Point

5 Pool Libraries





SARS-CoV-2
Variants of Concern

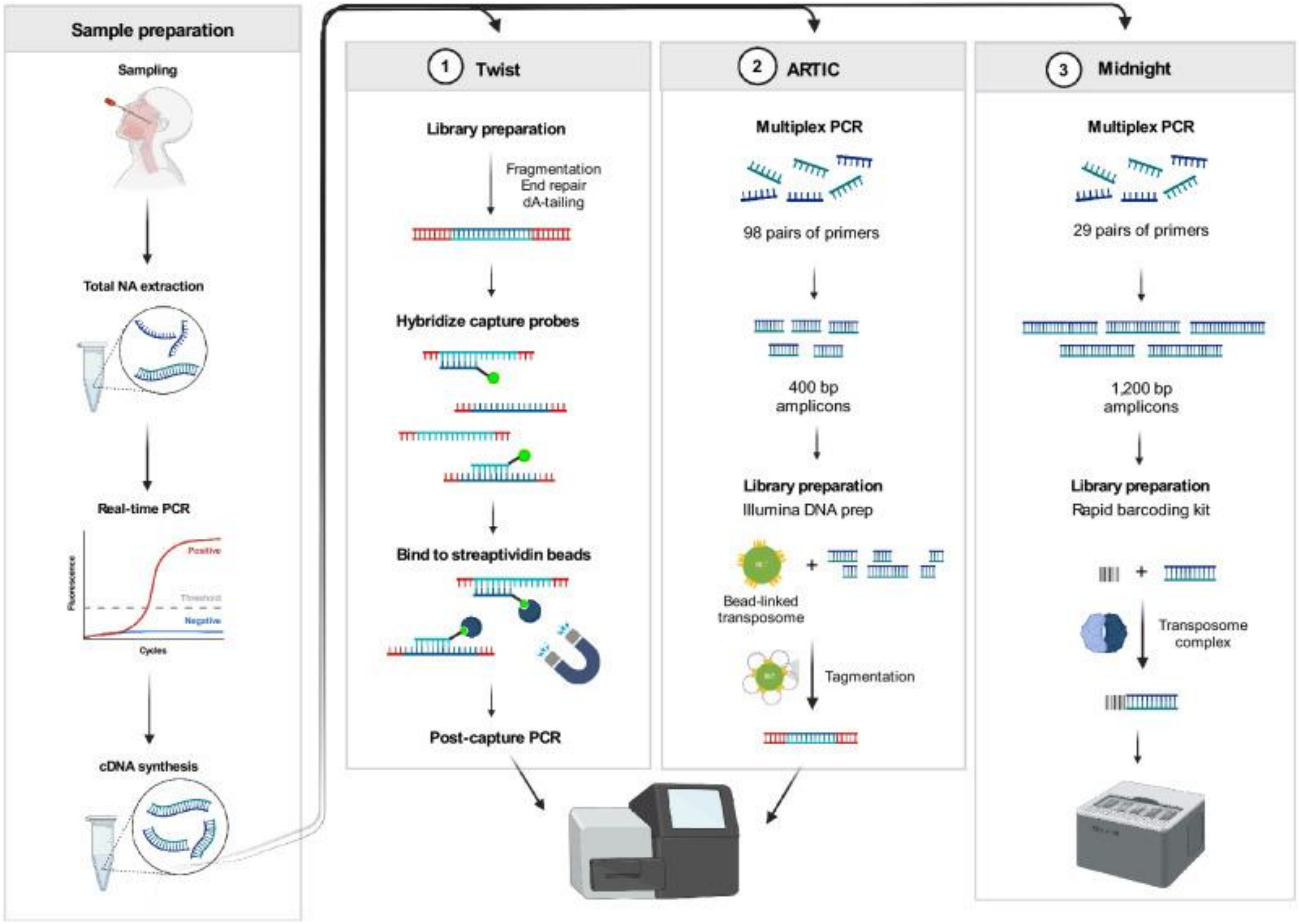
Alpha Variant (B.1.1.7)
Detected in the United Kingdom in September 2020

Beta Variant (B.1.351)
Detected in South Africa in October 2020

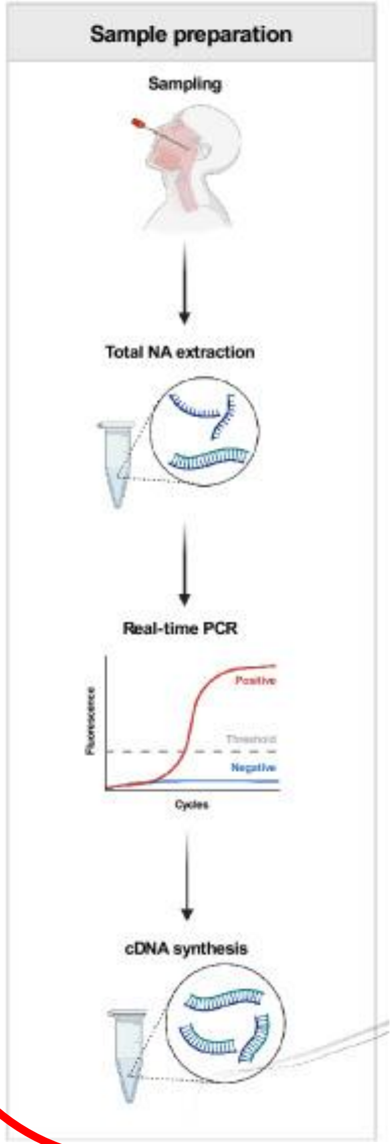
Gamma Variant (P.1)
Detected in Brazil in November 2020

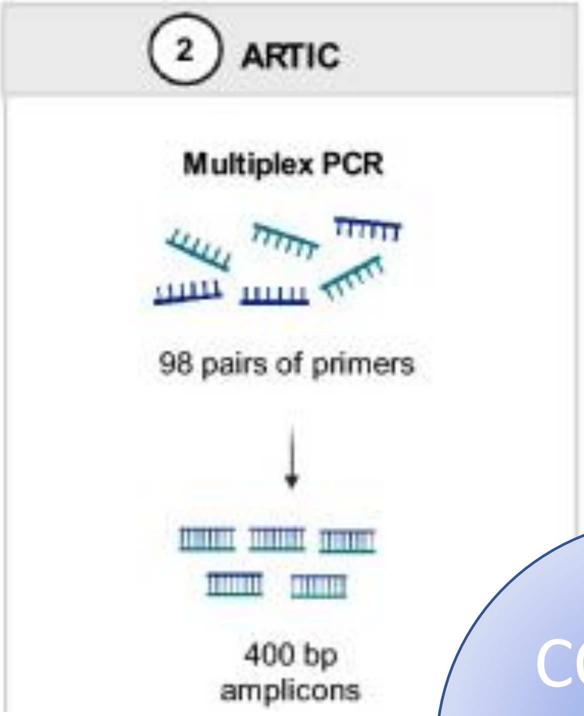
Delta Variant (B.1.617.2)
Detected in India in December 2020

Omicron Variant (B.1.1.529)
Detected in South Africa in November 2021


Sample preparation
Sampling
Total NA extraction
Real-time PCR
Fluorescence
Positive
Threshold
Negative
Cycles
cDNA synthesis
1 Twist
Library preparation
Fragmentation
End repair
dA-tailing
Hybridize capture probes
Bind to streaptividin beads
Post-capture PCR
2 ARTIC
Multiplex PCR
98 pairs of primers
400 bp
amplicons
Library preparation
Illumina DNA prep
+
Bead-linked
transposome
Tagmentation
3 Midnight
Multiplex PCR
29 pairs of primers
1,200 bp
amplicons
Library preparation
Rapid barcoding kit
+
Transposome
complex

Microbiologia HUGTiP
Metropolitana Nord





HCGB

1 Tagment **SARS-COV2 cDNA amplicons**

2 Post Tagmentation Clean Up

3 Amplify Tagmented DNA

4 Clean Up Libraries

COVID19
HUB
IGTP

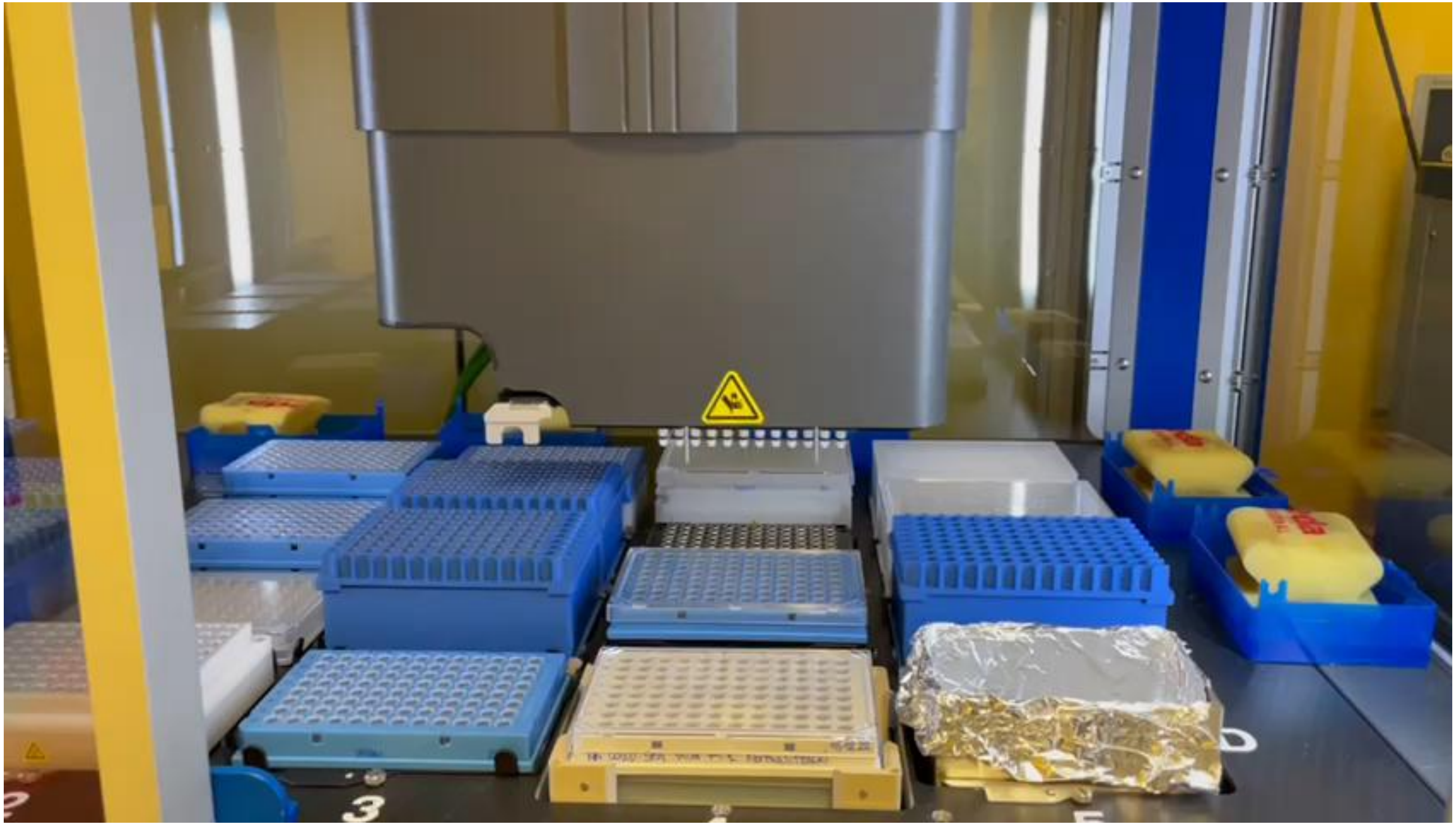
5 Pool Libraries **Microbiologia HUGTiP**
Metropolitana Nord

MiSeq

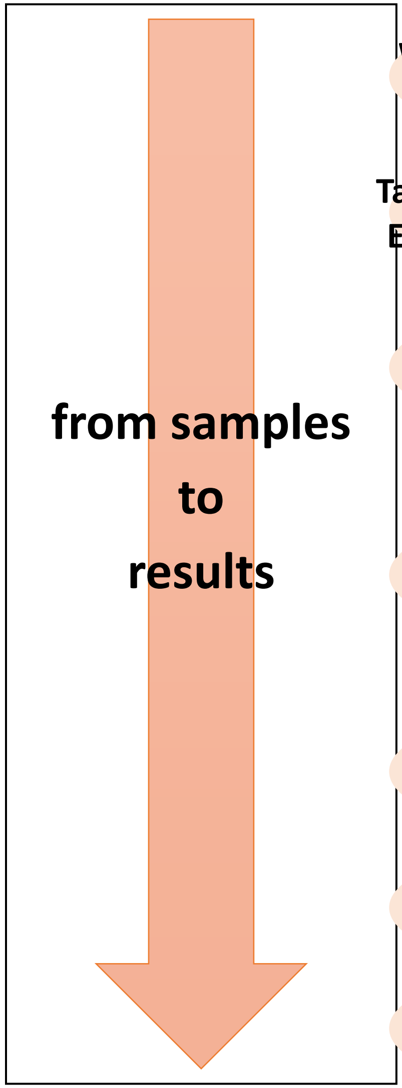
50M PE reads
Illumina SBS 2x 300bp

Genomica
Traslacional

	0	1	2	3	4	5	
A	1 tip box, full **SPARE TIP BOX**	1 tip box, full **SPARE TIP BOX**	3 PCR plates, all EMPTY (stacked) **top: TSB, middle: SPRI dil1, bottom: Elution**	1 PCR plate, EMPTY (on 96 THERMOBLOCK POSITION) **BLT/TB1**	1 V-StorPlate plate, with reagents ******WITH LID****** (on 96 THERMOBLOCK POSITION) **ENZYME MIXES**	1 clean seahorse plate (for LIQUID WASTE) **LIQUID WASTE PLATE**	**A**
B	1 tip box, full **SPARE TIP BOX**	1 tip box, full **SPARE TIP BOX**	2 PCR plates, with reagents (stacked) **top: SPB, bottom: RSB**	1 tip box, full **REFILL TIP BOX**	MAGNET POSITION, NO SPACER	1 deep well, 12 column septed reservoir, with reagent ******WITH LID****** **80% ETOH**	**B**
C	1 tip box, full **SPARE TIP BOX**	1 tip box, full **SPARE TIP BOX**	1 V-StorPlate plate, with wash buffer ******WITH LID****** **TWB**	1 tip box, full **WORKING TIP BOX**	1 PCR plate, with primers ******WITH LID****** ON MAGNET POSITION, WITH SPACER **INDEXED PRIMERS**	1 tip box, empty **SHELF FOR LID**	**C**
D	1 tip box, full **SPARE TIP BOX**	1 tip box, full **SPARE TIP BOX**	- 96 THERMOBLOCK POSITION	1 PCR plate, with water **WATER**	1 PCR plate, with samples ******WITH LID****** (on 96 THERMOBLOCK / SHAKER POSITION) **SAMPLES**	TIP WASTE	**D**
	0	1	2	3	4	5	


3

HCGB: COMPLETE WORKFLOWS VALIDATED PROTOCOLS IN PLACE



from samples
to
results

WGS: whole genome sequencing

Targeted resequencing: Exomes & customized

Metagenome sequencing

Expression profiling (mRNA, miRNA)

Genotyping (SNP analysis)

Ploidy analysis by aCGH (CNVs)

DNA methylation analysis

GENOMIC DNA SEQUENCING	IonExpress DNA	TruSeq DNA	KAPA DNA	Nextera XT		
DNA SEQUENCE CAPTURE	Sure Select XT			Illumina Rapid Exome		
AMPLICON SEQUENCING	IonExpress DNA	Ion Torrent fusion amplicon sequencing		Nextera XT	DNAflex	Indexing
RNA SEQUENCING	TruSeq RNA	TruSeq stranded mRNA	TruSeq stranded Total RNA	Clontech Smarter RNA	NEBNext RNA	Ribozero Plus with ligated adapters
SMALL RNA SEQUENCING	TruSeq small RNA	Nextflex small RNA	NEB next small RNA	NextFlex v4		
GENOTYPING / COPY NUMBER ANALYSIS / LOH ANALYSIS	Infinium SNP arrays: Core Exome, Omniexpress, Multi Ethnic Global, Global Screening Array, Immunoarray, Exome, Cyto-SNP, Omni-2.5, Omni-5					
METHYLATION PROFILING	Infinium methylation arrays: Human Methylation EPIC					

HCGB: COMPLETE WORKFLOWS VALIDATED PROTOCOLS IN PLACE AUTOMATED

from samples to results

- WGS: whole genome sequencing
- Targeted resequencing: Exomes & customized
- Metagenome sequencing
- Expression profiling (mRNA, miRNA)
- Genotyping (SNP analysis)
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GENOMIC DNA SEQUENCING	IonExpress DNA	TruSeq DNA	KAPA DNA	Nextera XT		
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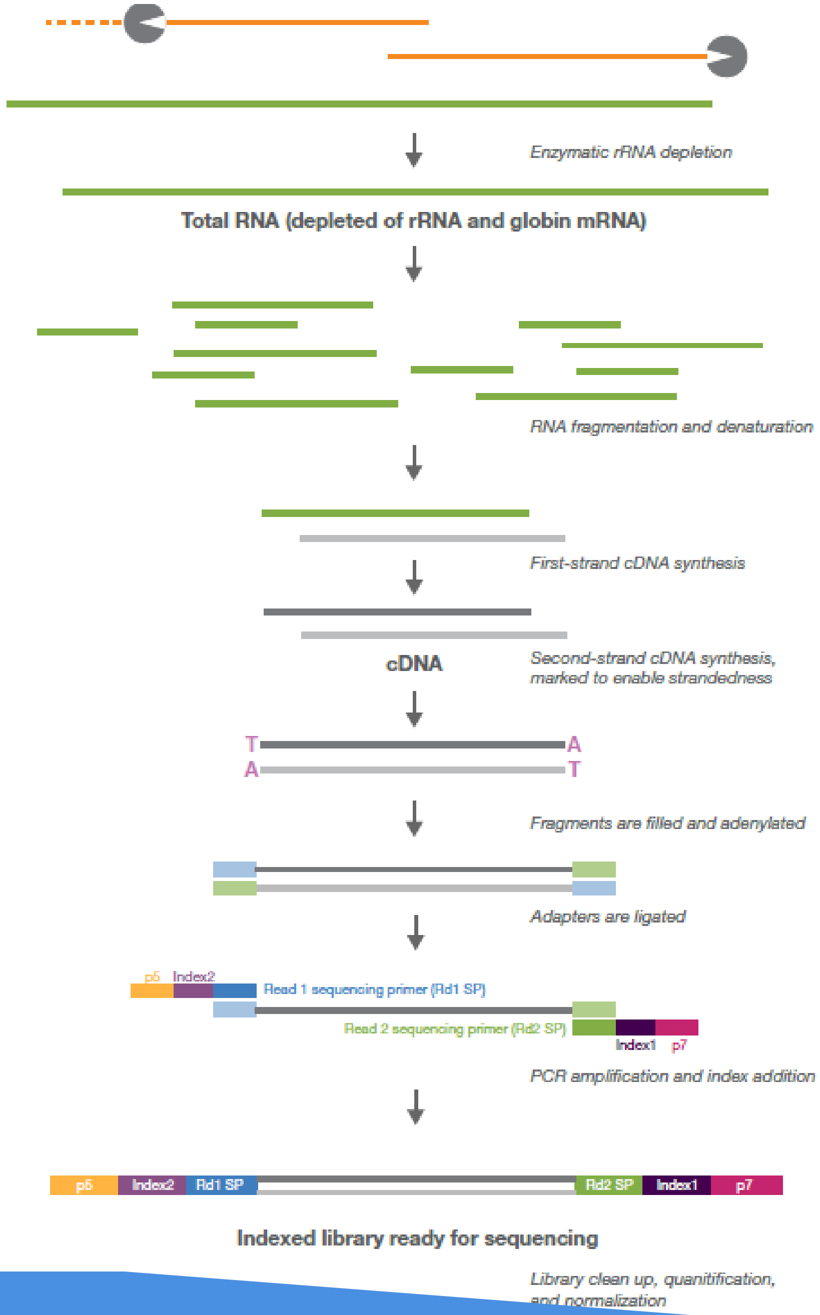
GENOTYPING / COPY NUMBER ANALYSIS / LOH ANALYSIS	Infinium SNP arrays: Core Exome, Omniexpress, Multi Ethnic Global, Global Screening Array, Immunoarray, Exome, Cyto-SNP, Omni-2.5, Omni-5					
METHYLATION PROFILING	Infinium methylation arrays: Human Methylation EPIC2					

Illumina Stranded Total RNA Prep with Ribo-Zero Plus



Enzymatic rRNA depletion

Total RNA (depleted of rRNA and globin mRNA)

RNA fragmentation and denaturation

First-strand cDNA synthesis

cDNA

Second-strand cDNA synthesis, marked to enable strandedness

Fragments are filled and adenylated

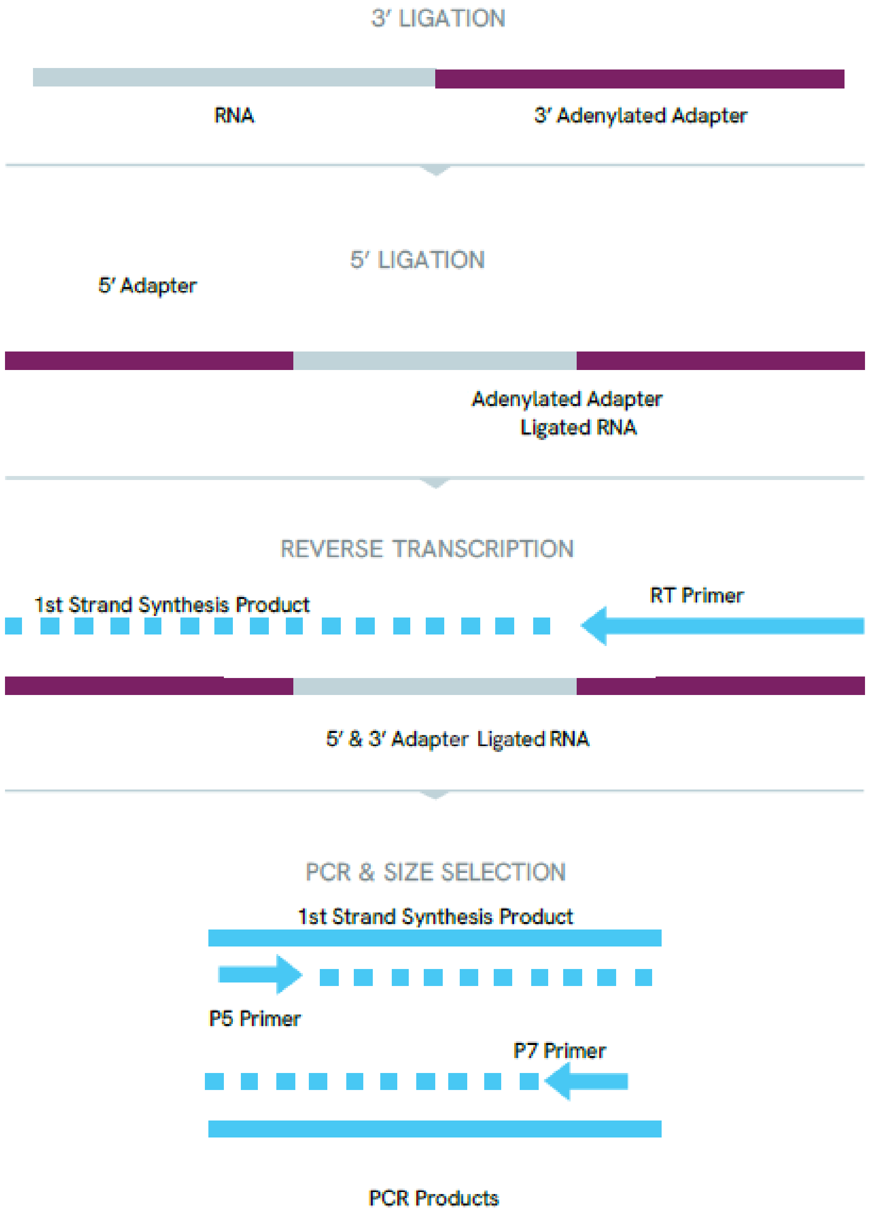
Adapters are ligated

PCR amplification and index addition

Indexed library ready for sequencing

Library clean up, quanitification, and normalization

NEXTFLEX Small RNA Sample Preparation Flow Chart



3′ LIGATION

RNA

3′ Adenylated Adapter

5′ LIGATION

5′ Adapter

Adenylated Adapter
Ligated RNA

REVERSE TRANSCRIPTION

1st Strand Synthesis Product

RT Primer

5′ & 3′ Adapter Ligated RNA

PCR & SIZE SELECTION

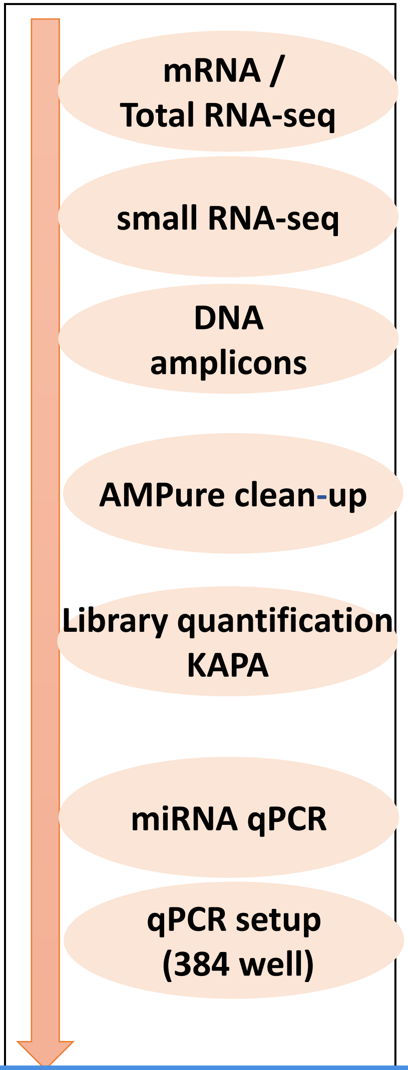
1st Strand Synthesis Product

P5 Primer

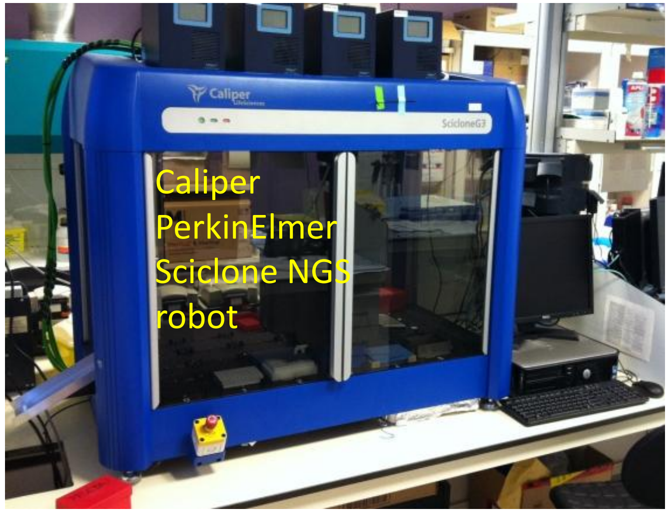
P7 Primer

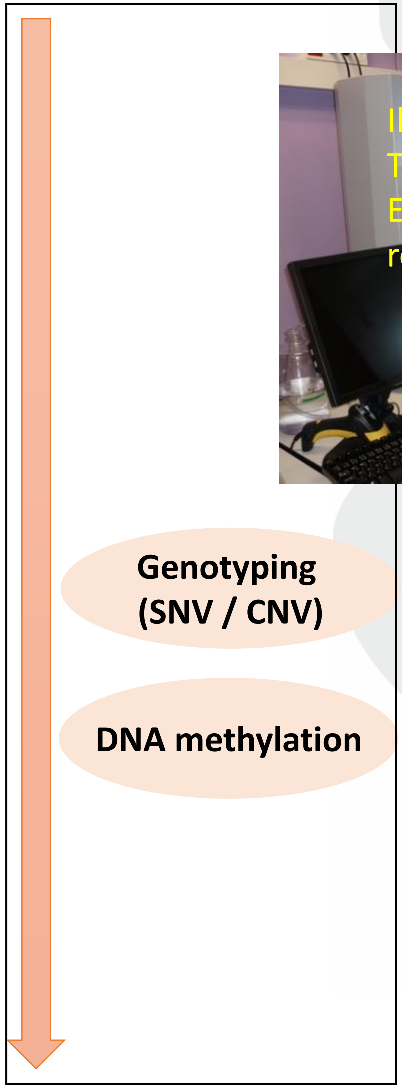
PCR Products

HCGB: COMPLETE WORKFLOWS VALIDATED PROTOCOLS IN PLACE AUTOMATED

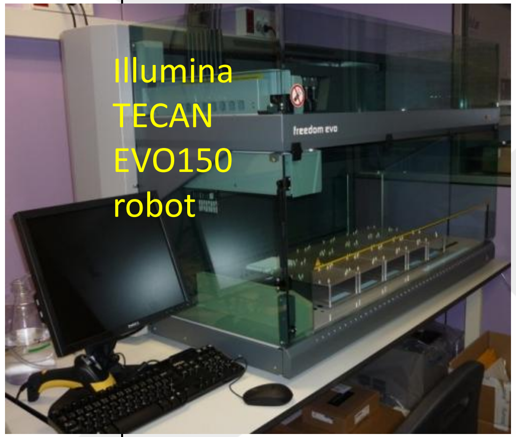


- mRNA / Total RNA-seq
- small RNA-seq
- DNA amplicons
- AMPure clean-up
- Library quantification KAPA
- miRNA qPCR
- qPCR setup (384 well)





- Genotyping (SNV / CNV)
- DNA methylation



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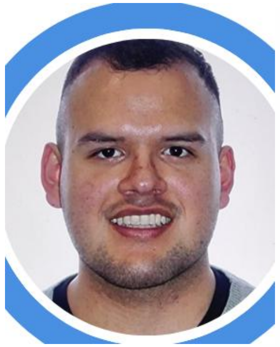

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Thank you!

Do you have any questions?

IGTP

Institut de Recerca Germans Trias i Pujol