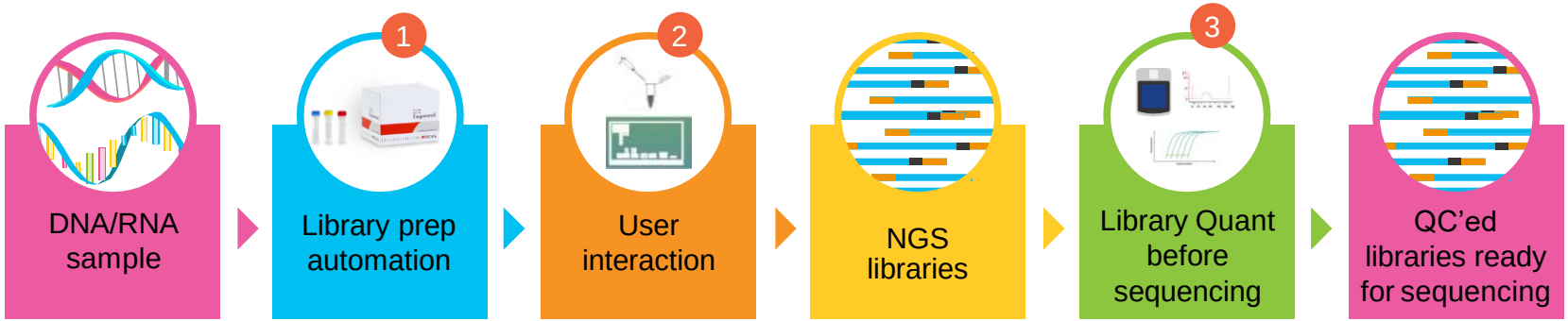
DreamPrep™ NGS.

TECAN.

Challenges in current NGS library preparation and quantification workflow



PROBLEM

1. Complicated workflow validating kits on existing automation platform
2. Increased variability due to manual interaction for automated prep
3. Time consuming and expensive library quant with sample loss

DreamPrep NGS featuring Tecan Genomics NGS library preps

DreamPrep NGS offers unprecedented speed and accuracy for automated library preparation and quantification with Tecan Genomics reagents.

- **Full walk-away solution allowing two library preps in a day**
  DNA-seq libraries quantified, normalized and ready to sequence in less than 4 hours.
- **NuQuant technology for integrated library QC in less than 6 min with No sample loss**
  Measure 96 samples simultaneously in the final plate without sample transfer.
- **User friendly interface & system performance monitoring**
  Intuitive touchscreen operation to reduce operator training. Introspect software is tracking your system productivity, incl. runtime, consumable usage and error rates.

TECAN IS YOUR SINGLE POINT OF CONTACT FOR NGS Automation, Tecan's reagents, consumables, application support, troubleshooting & service

DreamPrep NGS brings you to a superior level of automation

Single point of contact for automation & reagents

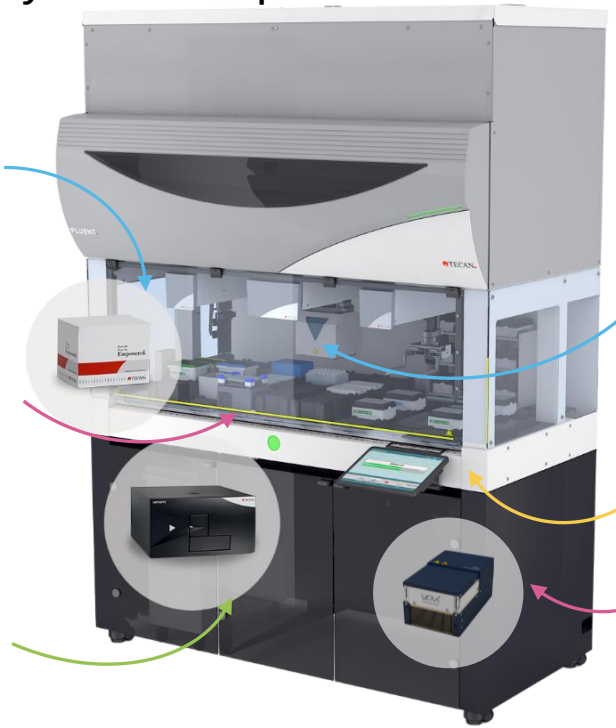
OPTIMIZED for Tecan NGS library preparation kits#

Optimal performance of reagents with samples

THERMAL DEVICES and shaker+

Library QC in less than 6 min without sample loss

INTEGRATED PLATE READER for full walk away QC and normalization



3 ARMS for increased speed and flexibility

Individual well and full plate manipulation for optimal speed

TOUCH SCREEN For intuitive operations

User friendly interface to reduce user training

THERMAL CYCLER INCLUDED for longer walk away times

Walk-away solution with minimal manual interaction

+ CPACs, Thermoshake and ODTC manufactured by Inheco Industrial Heating and Cooling GmbH. Martinsried, Germany

For research use only. Not for use in diagnostic procedures. The DreamPrep NGS method needs to be validated in your lab based on your workflow according to your desired use and laboratory protocols. Tecan makes no claims regarding the performance of the example methods.

Trust DreamPrep NGS with your assays

RELIABLE

with adaptive signal technology for reagent saving

FLEXIBLE PROCESSING

of up to 2x96 samples per run

FAST

with up to 2 normalized libraries in less then 4h

FUNCTIONAL

with reader & thermal cycler integrated

DreamPrep NGS worktable overview

POGO

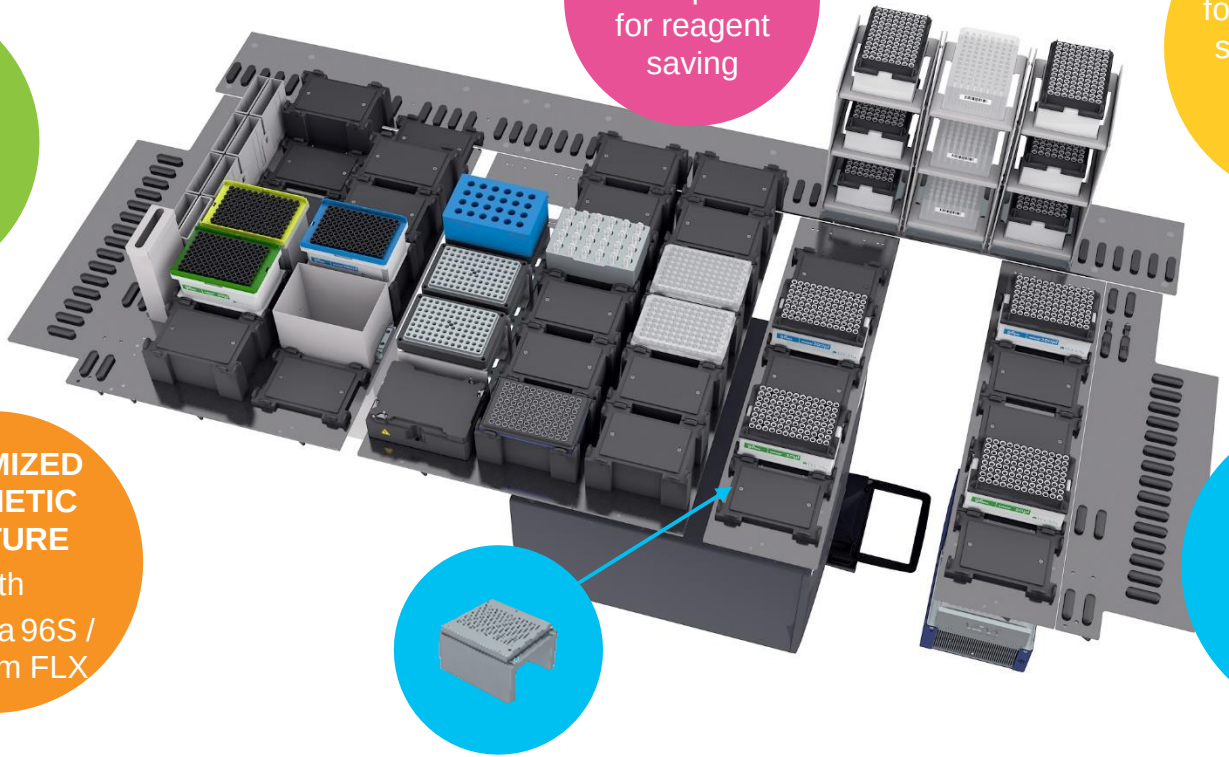
adapter for reagent saving

HOTELS

for consumables storage in high throughput workflows

COOLING HEATING

for safe reagent storage and on deck incubations



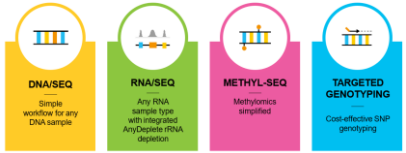
OPTIMIZED MAGNETIC CAPTURE

with

Alpaqua 96S / Magnum FLX

ADAPTER MCA96 DITI MCA384

DreamPrep NGS – Highlights



Open Platform 1

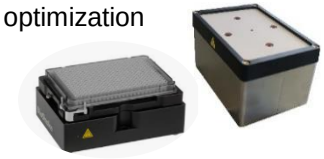
2 **8 independent channels**
Precision
Flexibility
Speed

3 **96/384 Head**
Speed
Reproducibility

Fluent GX & Magellan 9
Traceability
Monitoring

Fluent Gx **Assurance Software**
(User Mgmt., Method approval, sample tracking, Electronics records, Audit tool)

4 **4x active modules & accessories**
Samples and reagents safetv
Workflow optimization



User Interface 8
No errors
Protected scripts

5 **Independent gripper**
Workflow optimization
Speed
Integrations

Reader 7
Q&N integrated

6 **Thermalcycler**
Walk-away protocols

DreamPrep NGS – Modular scripting

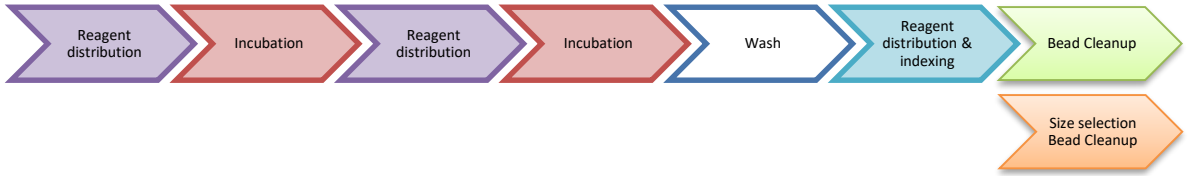
NGS modules development

NGS workflows are based on the repetition of similar steps multiple times throughout the same protocol and between protocols

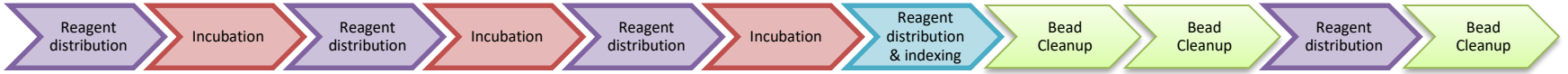
Time saving using the already developed modules

1. Quick and easy run optimization
2. New workflows development in weeks rather than months

Workflow 1



Workflow 2



DreamPrep NGS - Software package

Fluent GX Assurance software for regulated environments
Touchscreen-based everyday workflow interactions for fast onboarding of operator.
Advanced process security features (user management, sample tracking and more).

Tecan Connect™ app for remote monitoring of instrument status

Introspect™ to receive valuable insights on your instrument usage and availability

1. Understand your system utilization
2. Increase productivity
3. Attain faster production and lower costs
4. Understand the cost of consumable usage
5. Reap the benefits of automated solutions
6. Get notified wherever you are

Arms &
Active Modules

TECAN FCA – Flexible Channel Arm with 8 independent pipetting channels

Tecan FCA

- Automatic spacing from 9 - 38mm between labware formats
- Positional definition +/- 0.1mm in all axes for high density plate formats
- Teach-free 384-well access
- Patented* next generation liquid level detection
- Access to 6 plates or tip boxes in depth

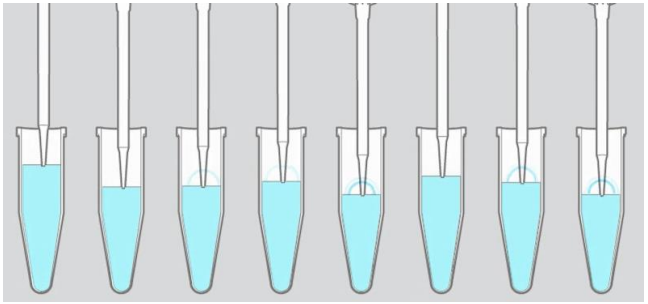
Feature	Description
Tip Ejection System	Designed to keep aerosols away from the deck area
In-line filter	• Protection against contamination • Safety stop in case of liquid infiltration into the channel • Pierce and aspirate • Plate seal piercing • Low maintenance • Just clean DiTi cones at end of day • High chemical compatibility (no sensitive o-ring on liquid path needing to be exchanged)
High definition pipetting	0.5µl to 1000µl 0.5µl CV<5% at ± 10% accuracy 200µl CV<0.2% at ± 0.5% accuracy 6 x 5µl CV<6% at ± 5% accuracy

Adaptive Signal Technology

ADVANCED LIQUID LEVEL DETECTION

- **Proprietary* safe & fast liquid detection**
- Double signal confirms each detection
- **Save sample and precious reagent**
- Calculation of liquid volume present
- Increased sensitivity
- Low dead volumes
- **Aspiration monitoring**
- **Liquid arrival check**

RELIABLE



Fluent offers a patented liquid level detection technology, independent for each channel and with increased sensitivity

* Link or refer to patents on www.tecan.com/intellectual_property/ patent_portfolios.

TECAN MCA – Multiple Channel Arm 384/96 for keeping reproducibility between samples

Tecan MCA
Access to 6 microplates in depth

Feature	Description
Automatic exchange 384 ⇔ 96 tip adapters within seconds	• saves space • reduces investment versus complete head exchange
96 tip EVA (Extended Volume Adapter)	• Pipette with 96 tips • 50, 100, 150, 200, 500 µl disposable tips
Row & column functions	• 384, 96, 24, 16,12 & 8… channels

TECAN RGA – Robotic Gripper Arm for safe resources transfer over the deck and to the lower cabinet

Tecan RGA

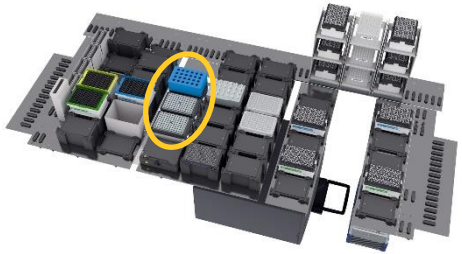
Move labware between worktable and periphery

Feature	Description
Fast, precise & quiet	• < 5 second average move • 360 degree continuous rotation
Long-Z Arm	• extends vertical reach for accessing devices anywhere below the deck
Eccentric gripper fingers	• Extend reach beyond pipetting work area for more device & storage access

INHECO CPLC - Keeping reagents and samples always at the right temperature with the cooling / heating modules

INHECO CPLCs

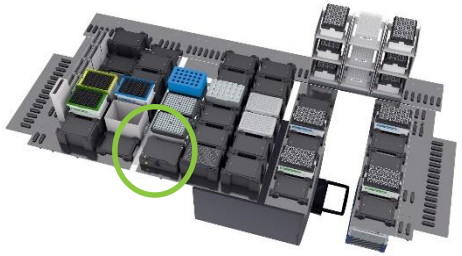
- 1 module for ALPAQUA POGO reagent tubes adapter
- 2 modules for PCR plates



CPLC Model	Parameters
Input voltage / max. current	12Vdc / 4.5 A
Temperature range	+4°C to +70°C [+39.2°F to +158°F]
Temperature range ambient	15°C to +32°C [+59°F to 89.6°F]
Temperature cycling	no
Max reachable temperature difference in the heating mode	up to 80K
Max. reachable temperature difference in the cooling mode	up to 30K
Outer dimensions for Length x width x height in mm	129 x 89 x 80
Weight incl. cord	approx. 1.0 kg

QInstruments BioShake 3000 - Mixing reagents in the most efficient way

QInstruments BioShake 3000
1 unit with the PCR plate adapter

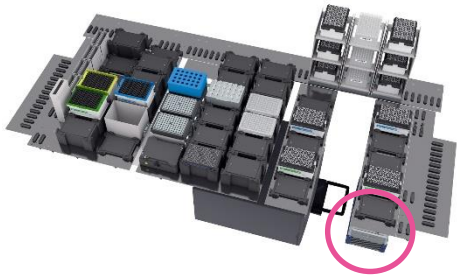


BioShake 3000	Parameters
Outer dimensions for Length x width x height in mm	142 x 99 x 48.2 (5.59 x 3.897 x 1.8976 in)
Shaking Frequency	200 – 3000 RPM
Amplitude (mm)	2
Input	24Vdc
Environmental operating range	+5°C to 45°C
Relative humidity	80% max.

INHECO ODTC - Increasing walk away time with the thermal cycler integration in the lower cabinet

INHECO ODTC

1 unit integrated in the lower cabinet

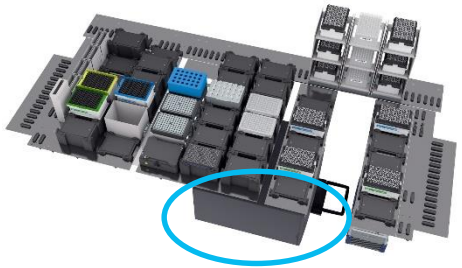


ODTC 96 Model	Parameters
Temperature range	4°C to 99°C [39°F to 210°F]
Temperature accuracy	±0.3K @ 55°C [131°F]
Temperature uniformity	±0.2K @ 55°C [131°F] ±0.2K @ 72°C [162°F] ±0.2K @ 95°C [203°F]
Adjustable heating rate	from 0.1 to 4.4K/s
Adjustable cooling rate	from 0.1 to 2.2K/s
Heating rate average	max. 4.4K/s
Cooling rate average	max. 2.2K/s
Heated lid temperature	adjustable between 30°C to 115°C

TECAN Infinite 200 PRO Reader – Integrating Norm & Quant steps for fully walk-away solutions

Tecan Infinite 200 PRO Reader

1 unit integrated in the lower cabinet

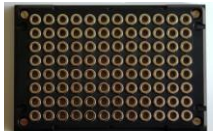


Infinite 200 PRO F NANO+ Model	Parameters
Input voltage / max. current	12Vdc / 4.5 A
Temperature range	+4°C to +70°C [+39.2°F to +158°F]
Temperature range ambient	15°C to +32°C [+59°F to 89.6°F]
Temperature cycling	no
Max reachable temperature difference in the heating mode	up to 80K
Max. reachable temperature difference in the cooling mode	up to 30K
Outer dimensions for Length x width x height in mm	129 x 89 x 80
Weight incl. cord	approx. 1.0 kg

Accessories

ALPAQUA Ring Magnet Plates – the right Magnet to each protocol

1. **One dedicated magnet** for each well means fewer edge effects and greater uniformity of results.
2. Immobilized beads are spread over a larger area, improving subsequent resuspension and elution.
3. Near universal microplate compatibility. Post and bar magnets tend to be customized for specific well geometries.
4. Integrated **spring cushion technology** give way when tips come in contact with a well bottom, thus compensating for physical tolerances between labware and pipettors that can compromise precision aspiration:
   - •Maximizes sample recovery & reagent usage
   - •Improves pipetting consistency



Magnet Plate 96S

Recommended reaction volume up to 1ml

Magnet Plate 96 MAGNUM FLX

up to 4.5x faster separation than 96S

Sample volume range 2ml - 10µl

ALPAQUA POGO – to save till the last drop of expensive reagents

1. Solid aluminum for efficient cooling
2. 24 tube capacity
3. Compatible with many screw top vials
4. Spring Cushion Technology to maximize usage of precious reagents
5. Helps avoid tip crashes and occlusions

Consumables

TECAN FCA Tips

1. Conductive & filtered
2. **Tecan Pure**: lot tested to ensure they are free from human DNA, PCR inhibitors, RNase and DNase
3. 2304 PCE. SBS
4. Does not come with SBS box (Boxes need to be purchased separately)

DITI LiHa 50µl

PN 30057813

DITI LiHa 200µl

PN 30057815

DITI LiHa 1000µl

PN 30057817

TECAN FCA Tip Boxes

1. **Tecan Standard** purity level: manufactured in a controlled environment with premium raw material
2. 10 PCE. SBS
3. Empty box

LiHa DITI SBS BOX Refill Small

PN 30058506

Empty box for 10µl, 50µl and 200µl LiHa DITI

LiHa DITI SBS BOX Refill Large

PN 30058507

Empty box for 1000µl LiHa DITI

TECAN MCA Tips

1. Sterile & filtered
2. Tecan Sterile: lot tested to ensure they are free of human DNA, RNase, DNase and endotoxins. Tested and certified to a specified sterility assurance level (SAL).
3. 3840 PCE. SBS
4. MCA96

DITI 50µl

PN 30038608

Maximum pipetting volume 45µl

DITI 150µl

PN 30038618

Maximum pipetting volume 150µl

DITI 200µl

PN 30038616

Maximum pipetting volume 200µl

Other non-Tecan needed consumables

Bio-Rad arched auto-sealing lids

Bio-Rad Cat. No. MSL2032

Bio-Rad hard shell PCR plates

Bio-Rad Cat. No. HSP9611

Replaceable by

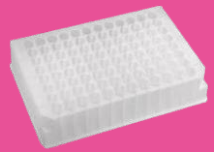
4titude/Brooks lids

4titude/Brooks Cat. No. 4ti-0291

Perkin Elmer hard shell PCR plates

Perkin Elmer Cat. No. 50-6008870

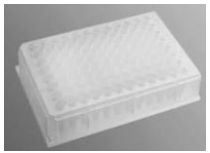
Other non-Tecan needed consumables



Nunc 96-Well DW 2ml Plate
ThermoFisher Cat. No. 95040452

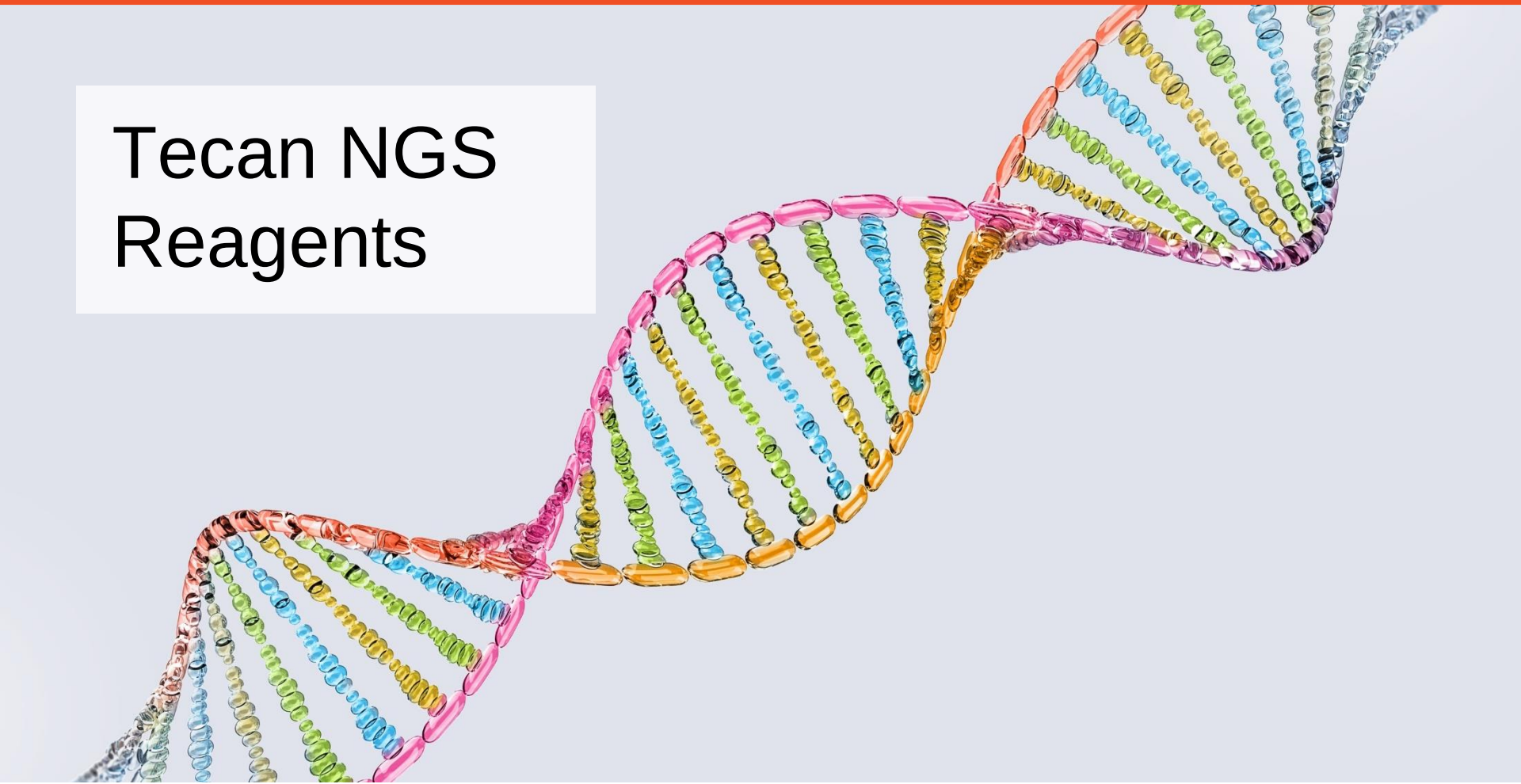
Nunc 96-Well DW 1ml Plate
ThermoFisher Cat. No. 260251

Sarstedt 2.0ml SC Micro Tube PCR-PT
Sarstedt Cat. No. 72.694.406



Axygen 96-Well DW 500µL Plate
P-96-450V-C-S:
- *Fisher scientific*
- *VWR*
- *Corning*

Tecan NGS Reagents



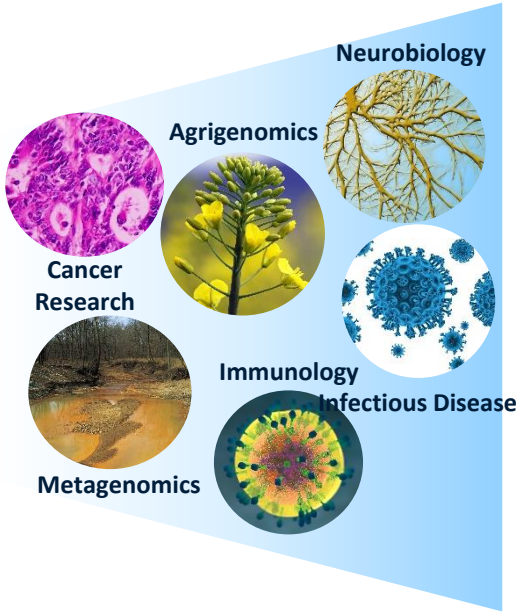
Tecan develops innovative technologies for Next Generation Sequencing (NGS) library preparation kits

Any sample type

- Purified Nucleic Acids
- LCM
- Liquid Biopsy
- Fresh and Frozen Tissues
- FFPE
- Cell-free Nucleic Acids
- Single and Sorted Cells
- Cell Lines

Innovative solutions

Highly informative data for your research



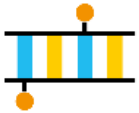
Tecan provides library preparation solutions for a broad range of sample types and applications

DNA/SEQ

Simple workflow for any DNA sample

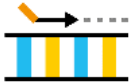
RNA/SEQ

Any RNA sample type with integrated AnyDeplete rRNA depletion



METHYL-SEQ

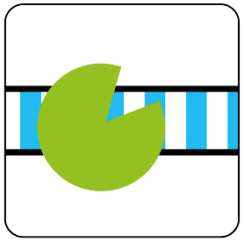
Methylomics simplified



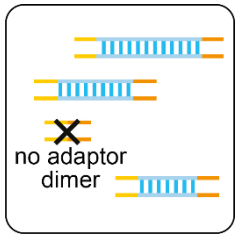
TARGETED GENOTYPING

Cost-effective SNP genotyping

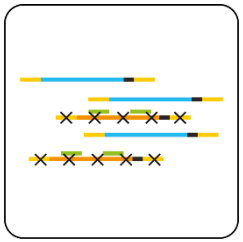
Combining core technologies in innovative products to deliver market leading solutions



Enzymatic Fragmentation

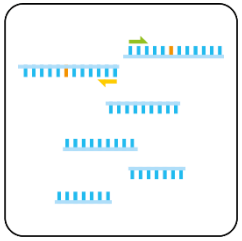


DimerFree®

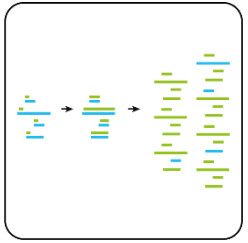


AnyDeplete®

NuQuant®

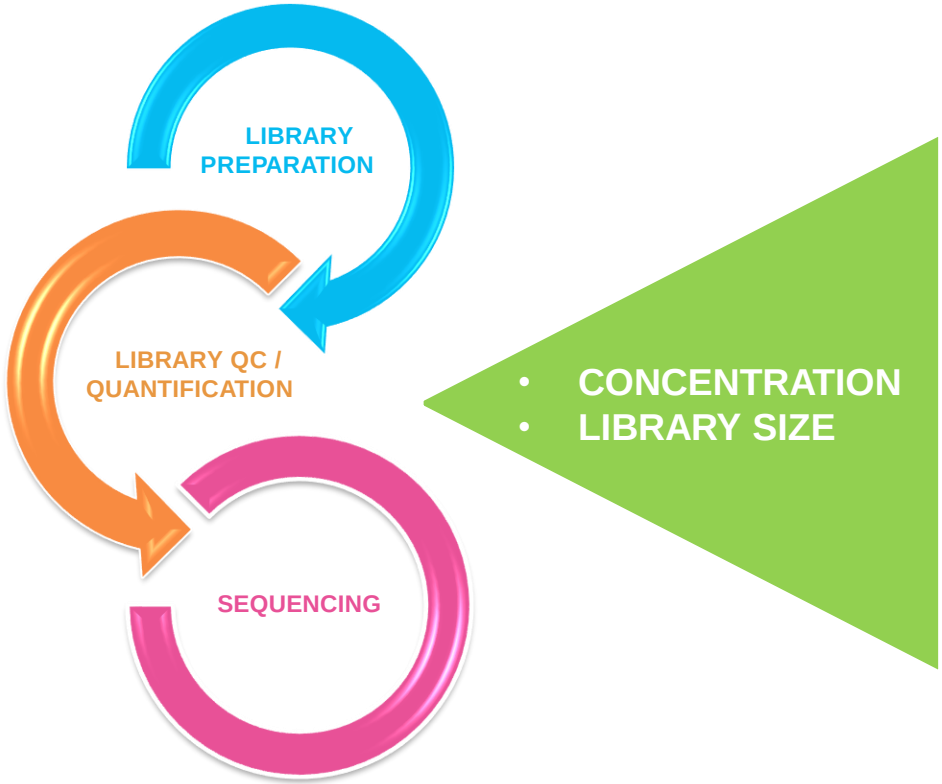


SPET: **S**ingle **P**rimer **E**nrichment **T**echnology



SPIA®: **S**ingle **P**rimer **I**sothermal **A**mplification

NGS workflow for sequencing



QUBIT

- Simple and fast
- Does not differentiate adaptor dimers
- Requires average library size in order to calculate molarity

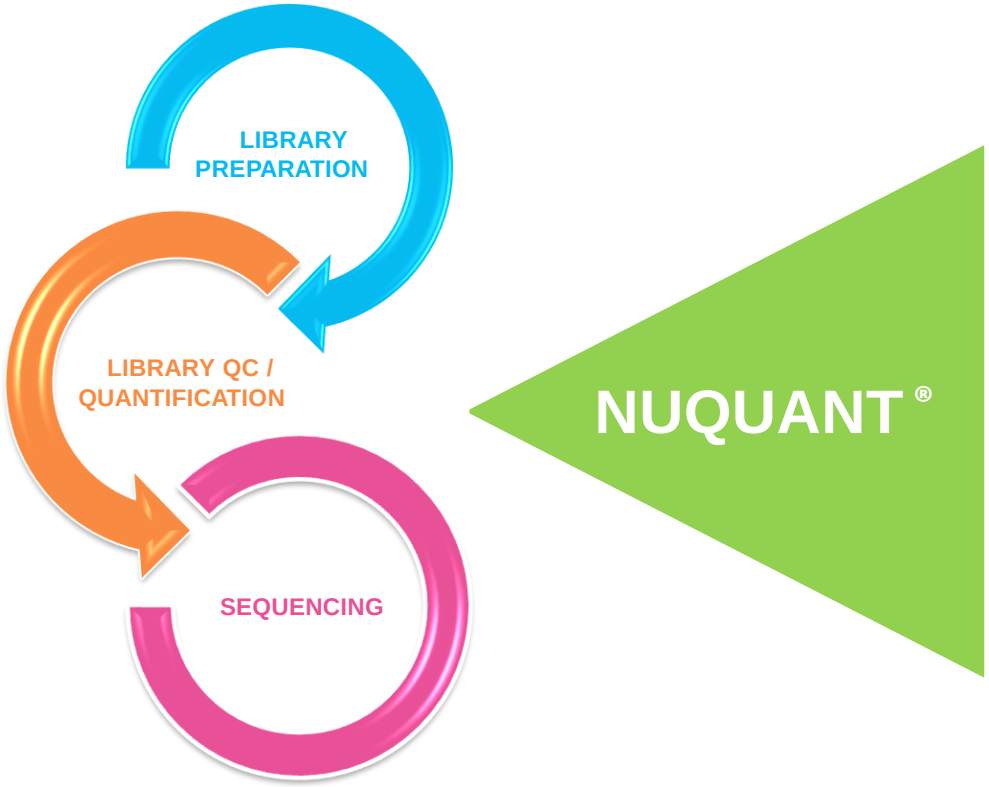
BIOANALYZER

- Gives average size
- Can calculate molarity but can be variable

QPCR

- Calculates mass with intercalating dye
- Error prone with dilution of sample and standards
- Requires average library size to calculate molarity

Re-imagining the NGS workflow for sequencing



- Integrated into the library preparation workflow
- Simple and fast fluorometric assay
- Direct calculation of library molarity
- Accurate multiplexing of libraries for sequencing

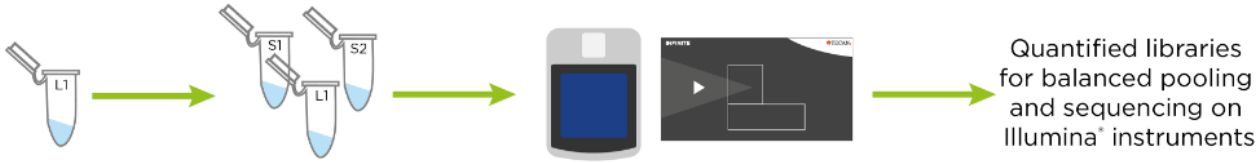
NuQuant – Library QC in under 6 minutes

Measures only sequenceable molecules unlike Qubit or Bioanalyzer

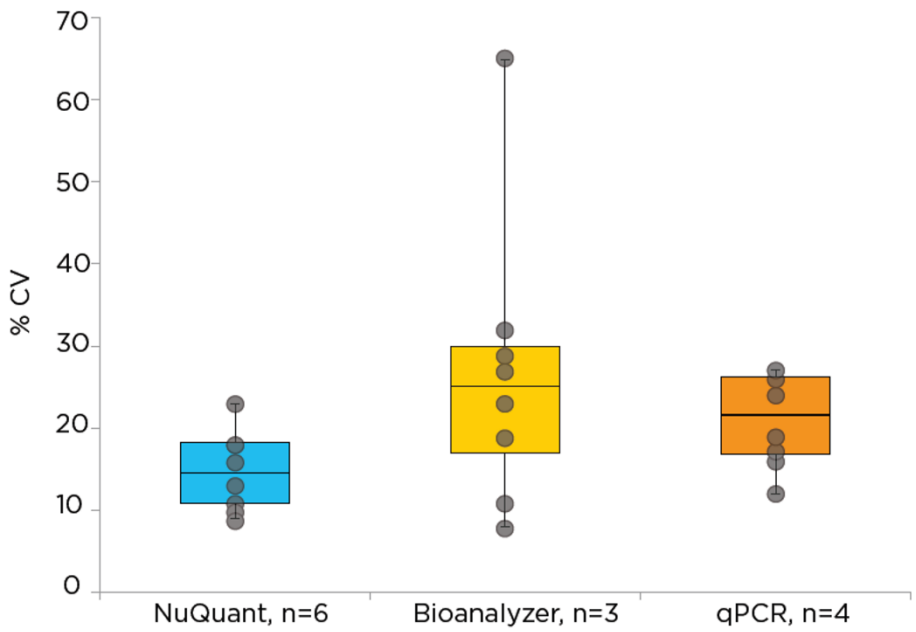
No serial dilution steps like qPCR, no additional reagents

Does not interfere with other quantitation methods and does not affect sequence clustering

Compatible with fluorometers like Qubit and standard plate readers



Accuracy of NuQuant library quantification



- Eight libraries quantified at external sites
- Molar concentrations of library compared to qPCR and Bioanalyzer

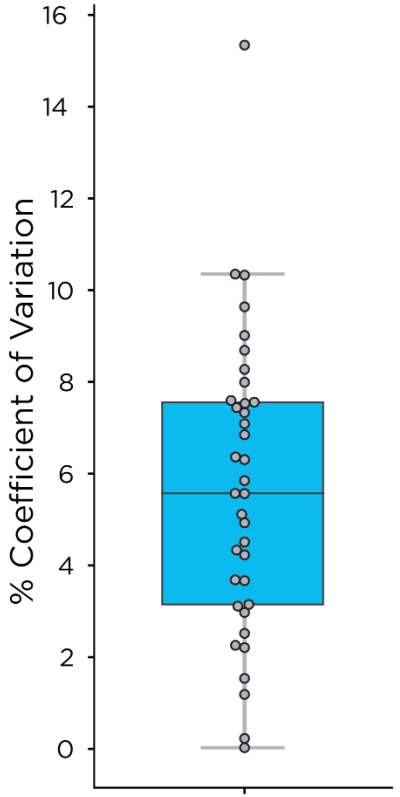
NuQuant showed the lowest variability and highest consistency between the different quantitation methods.

Consistent library quantification using NuQuant

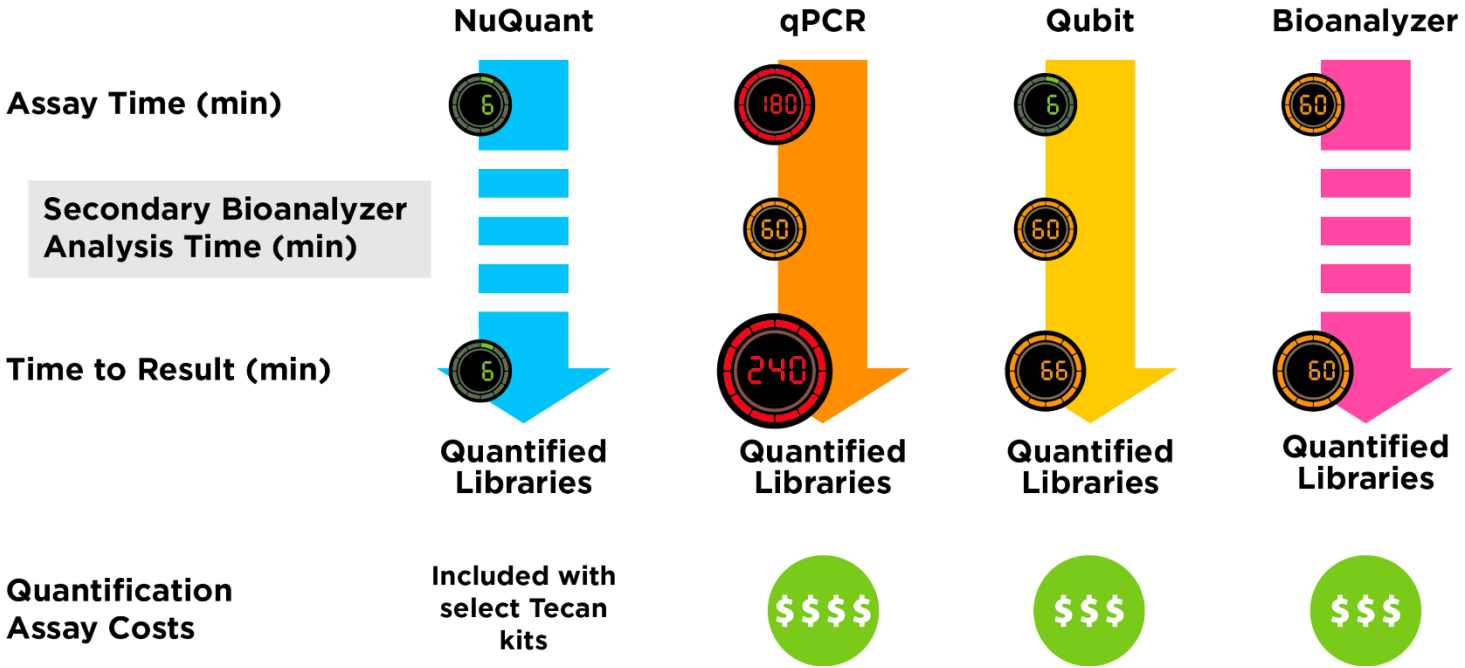
- 147 libraries were generated using Celero DNA-Seq and quantified using NuQuant*
- Quantified libraries were multiplexed into 37 pools and sequenced

NuQuant showed gives consistent library quantification across multiple samples and library pools.

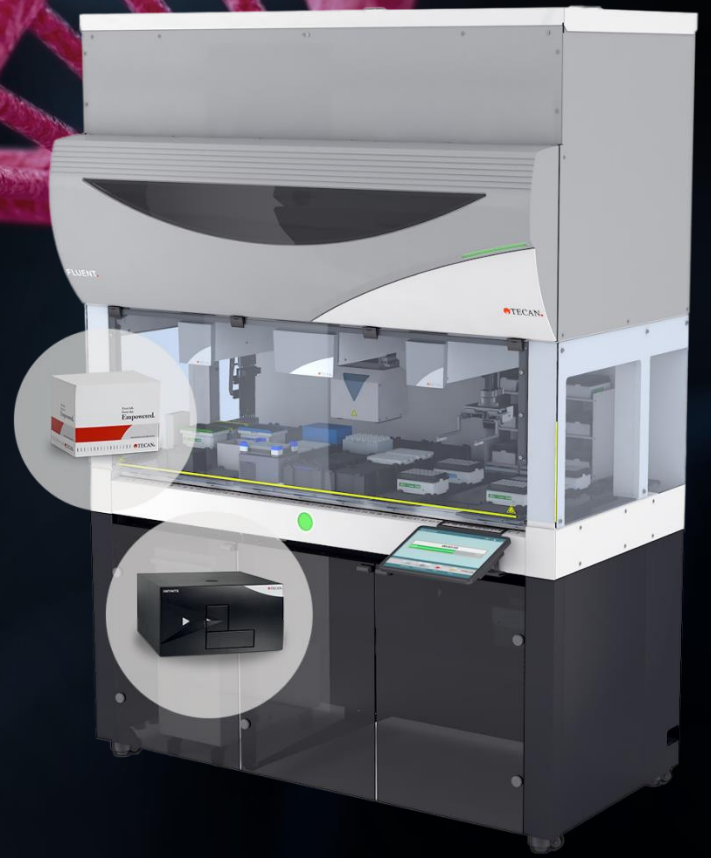
*Data on file at Tecan.



Reduce the time and cost associated with library quantification



Celero™ DNA-Seq on DreamPrep NGS



Celero DNA-Seq – Features

1. Celero is an **addition only workflow** with 3 master mixes and only 1 bead purification clean-up following library construction
2. **DimerFree™** technology eliminates adapter dimers
3. Multiplexing up to 384 samples; 96 UDI adaptors
4. **NuQuant** quantitation method for PCR workflow eliminates Bioanalyzer and qPCR for multiplex pooling
5. Robust **enzymatic fragmentation**
6. **Automation ready**

Celero DNA-Seq – Library prep and QC streamlined

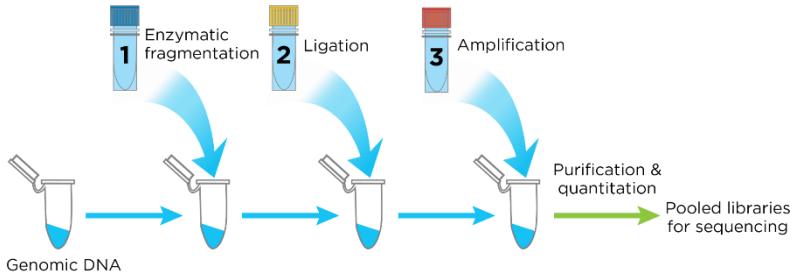
Simple:
Single tube, addition-only workflow with ONE post-amplification bead purification step

&

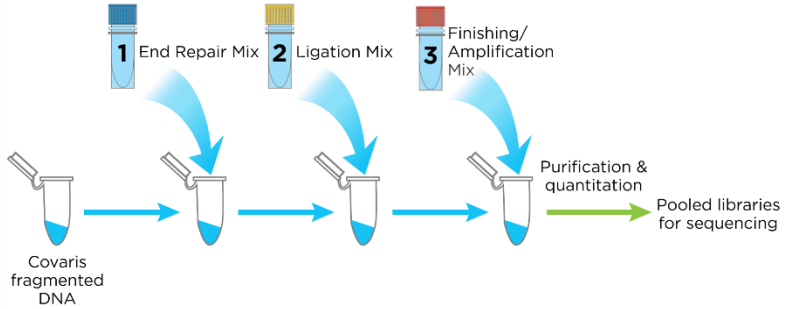
Flexible:

Consistent across a broad input range

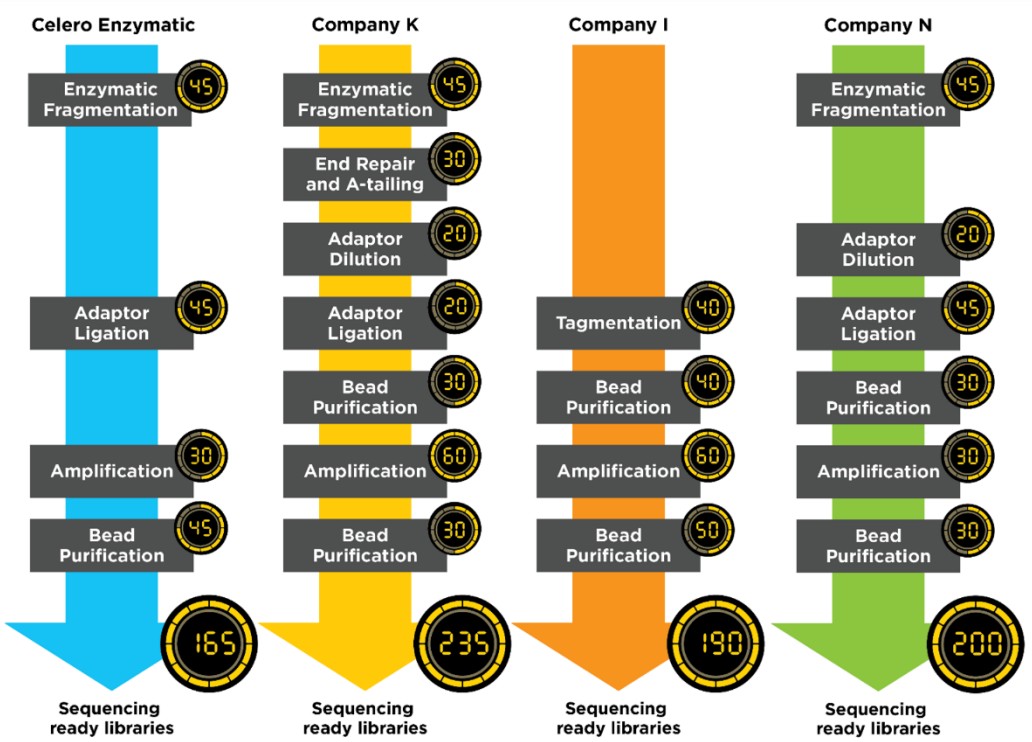
Enzymatic Fragmentation



Mechanical Fragmentation



Celero Enzymatic has a simpler and shorter workflow for quantified libraries



Celero DNA-Seq (Enzymatic) on DreamPrep NGS – Experimental results

STARTING MATERIALS

1) 10 or 75 ng of Human genomic DNA NA12878

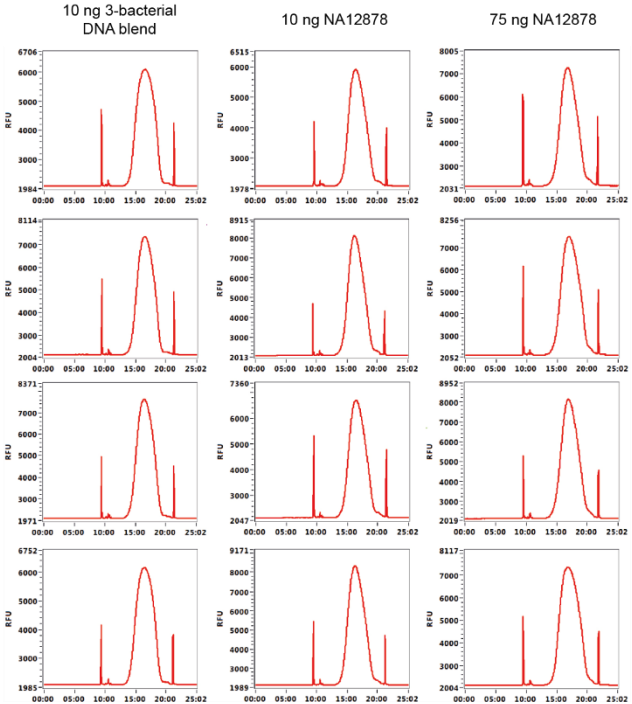
2) 10 ng of a blend of bacterial DNA (3-bacterial DNA blend) at equal mass

KIT

Celero PCR Workflow with Enzymatic Fragmentation (9363A-A01)

INSTRUMENTS

Fluent Liquid Handler with integrated **Infinite plate reader** and **Inheco On-Deck Thermal Cycler**



No library failures for all sample type and input

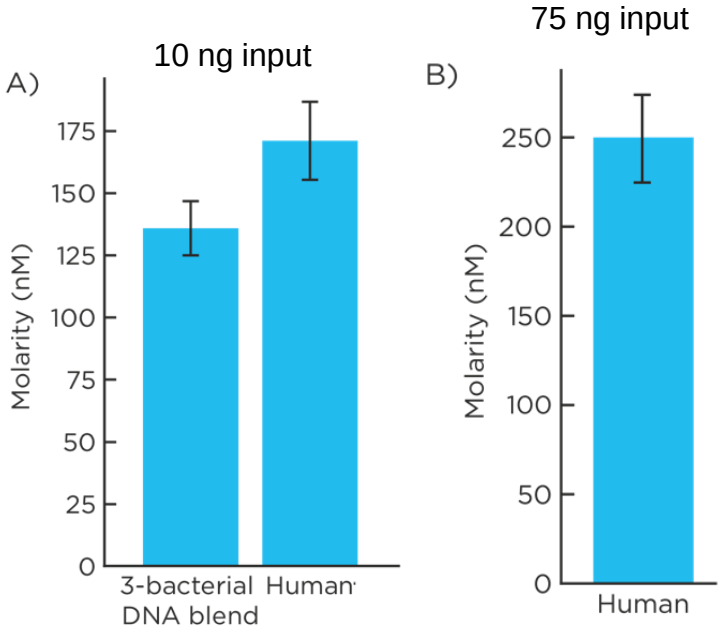
Library QC with NuQuant resulted in %CV of less than 13%

Reference: DreamPrep™ NGS – automated DNA library preparation and quantification on the Fluent® Automation Workstation.

Celero DNA-Seq (enzymatic) DreamPrep NGS Experimental results

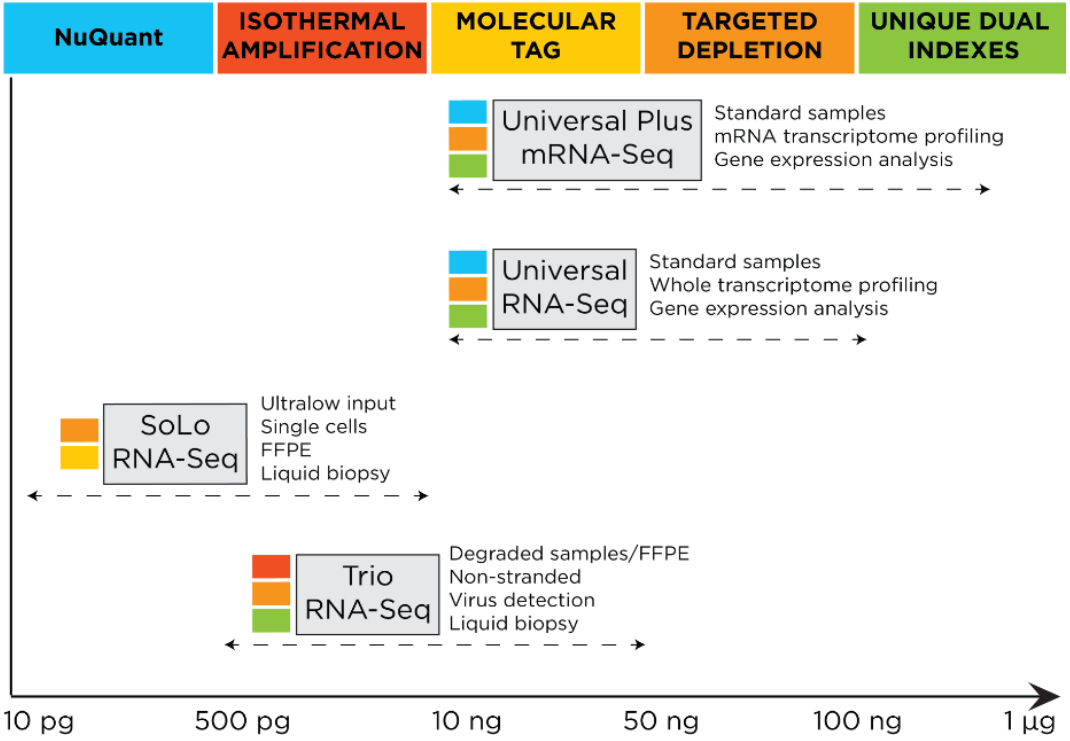
DNA LIBRARY YIELD MEASURED BY NUQUANT

SAMPLE	# OF REPLICATES	AVG INSERT SIZE	AVG% ALIGNED READS
3-bacterial DNA blend	12	294 bp (2.27 % CV)	98.8 (0.09 % CV)
Human Genomic DNA (NA12878)	12	280 bp (1.42 % CV)	96.5 (0.09 % CV)

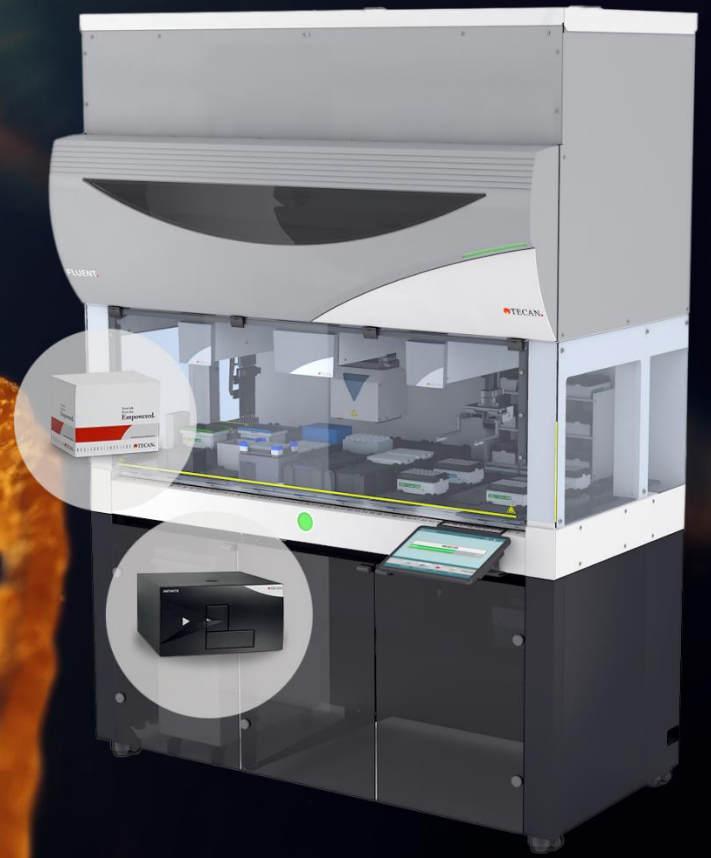


Reference: Application note "DreamPrep™ NGS – automated DNA library preparation and quantification on the Fluent® Automation Workstation"

Tecan’s RNA-Seq portfolio

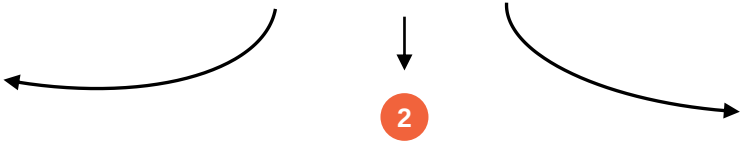


Universal Plus mRNA-Seq on DreamPrep NGS



Universal Plus mRNA-Seq with NuQuant

KEY BENEFITS:



1

An
“**ALL INCLUSIVE**”
kit from sample
to quantified libraries
ready for sequencing

2

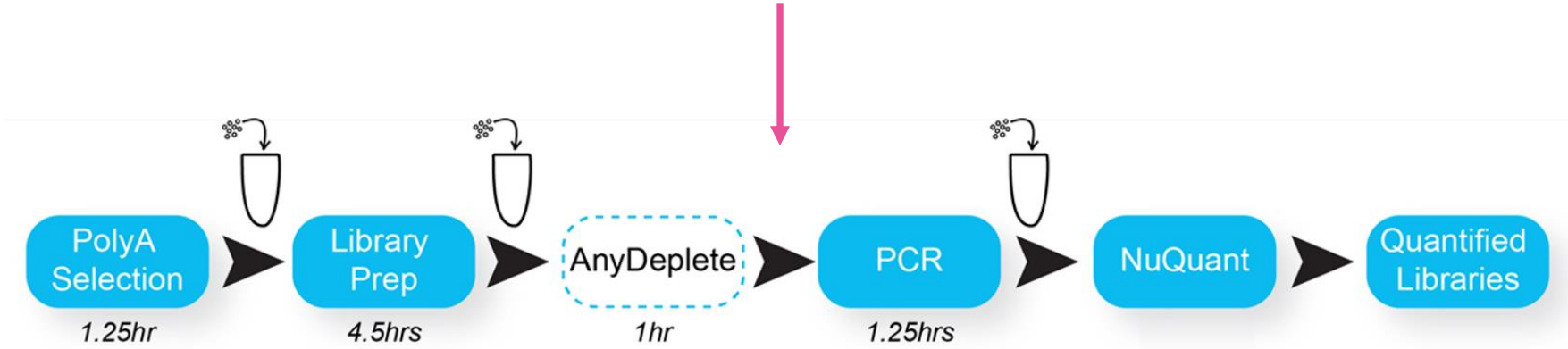
FASTEST
WORKFLOW
from sample
to quantified libraries

3

ONLY KIT
WITH INPUT
range from
10 ng to 1 µg

Universal Plus mRNA-Seq with NuQuant helps you to get quantified libraries faster with a short, simplified workflow

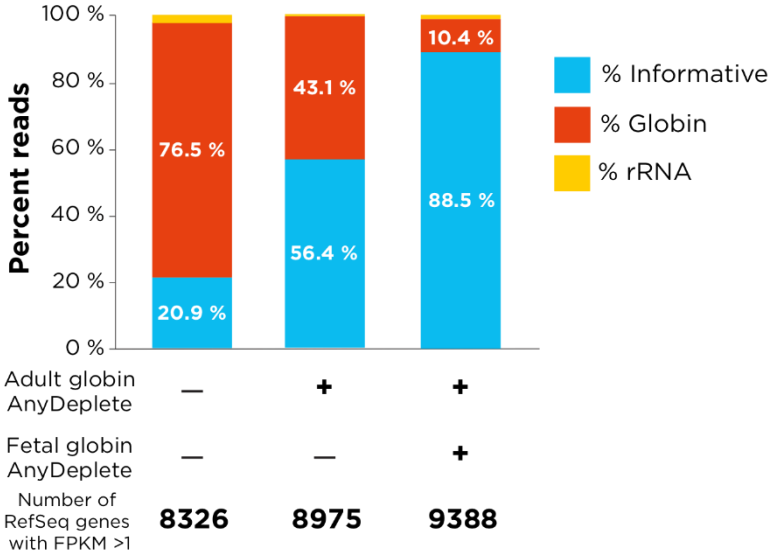
ONLY 7 HRS (8 WITH ANYDEPLETE)
TO COMPLETE LIBRARIES!



Customized transcript depletion maximizes sequencing results

STUDY RESULTS:

SAMPLE	NUMBER OF REFSEQ GENES WITH FPKM > 1
Without AnyDeplete	8326
Adult Globin AnyDeplete	8975
Feta + Adult Globin AnyDeplete	9388



Customizable transcript depletion with AnyDeplete®, increases informative data providing more biological information from each mRNA sequencing experiment.

Universal Plus mRNA-Seq automated on Fluent – Experimental results

STARTING MATERIALS

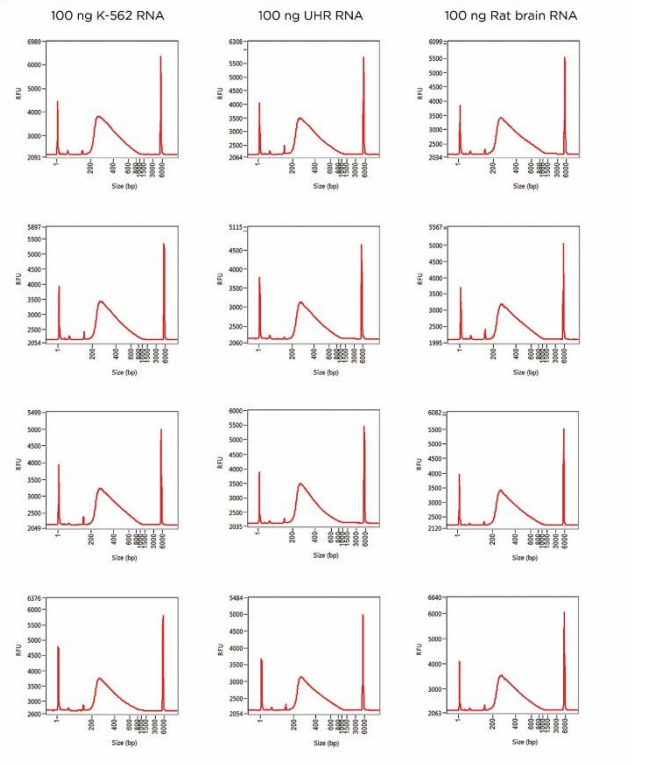
1) 100 ng Rat Brain RNA or Univeral Human Reference RNA
or K562 RNA

KIT

Universal Plus mRNA-Seq with NuQuant (0520-A01)

INSTRUMENTS

Fluent Liquid Handler with integrated **Infinite plate reader** and **Inheco On-Deck Thermal Cycler**



No library failures for all sample type and input

Library QC with NuQuant resulted in %CV of less than 10%

Average % alignment of 98%, 92% uniquely aligned

Reference: Application note "NGS DreamPrep- automated mRNA library preparation and quantification on the Fluent Automation workstation"

Allegro SNP Genotyping on DreamPrep NGS

Allegro Targeted Genotyping V2 benefits

FLEXIBLE SNP DESIGNS FOR ANY ORGANISM

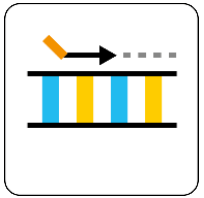
Up to hundreds of thousands of SNPs per assay

LOW COST PER DATA POINT

High multiplexing of > 3,000 samples

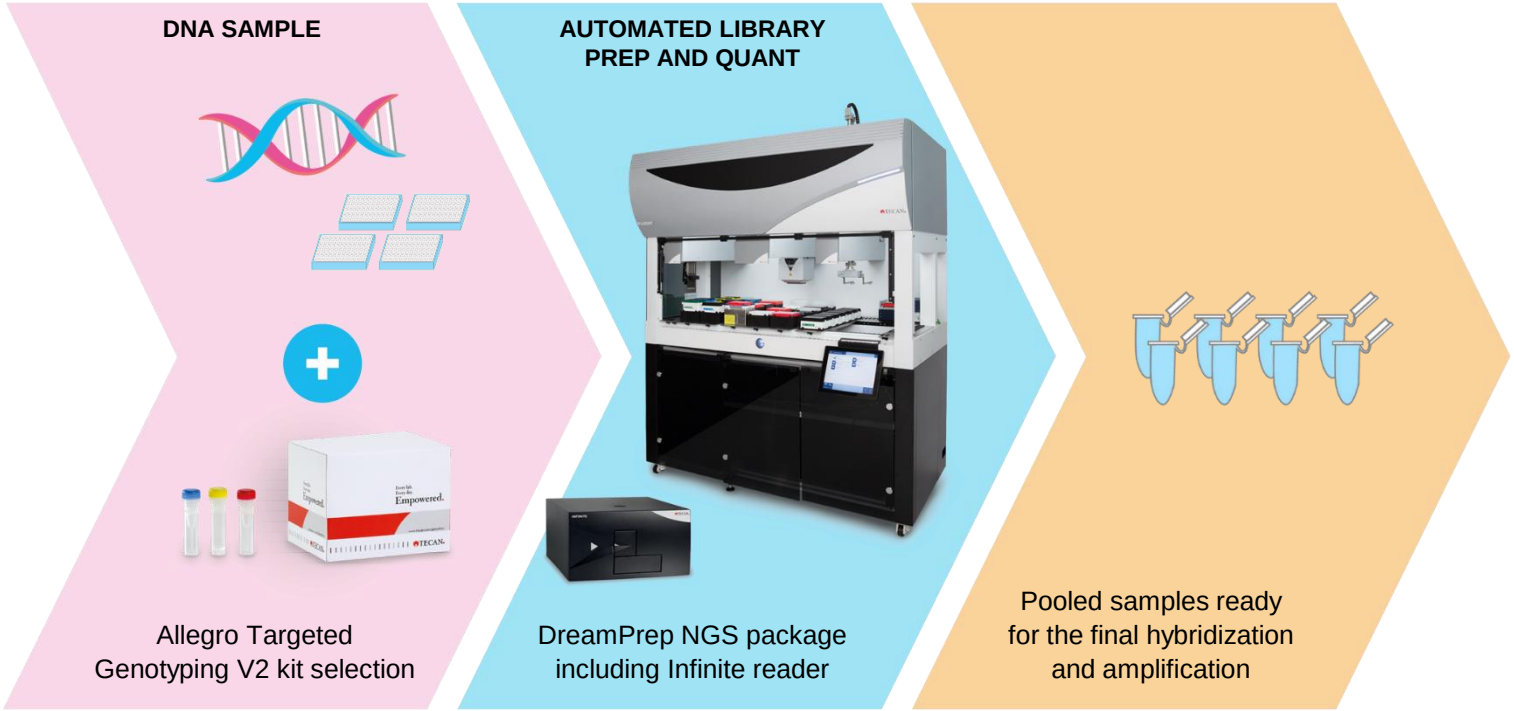
ROBUST, SINGLE-TUBE ASSAY WORKFLOW

Automatable



Allegro Targeted Genotyping V2 workflow on the DreamPrep™ NGS liquid handling automation platform currently under development.

Allegro Genotyping on DreamPrep NGS



Allegro Targeted Genotyping V2 workflow on the DreamPrep™ NGS liquid handling automation platform currently under development.

SNP Genotyping with Maize on DreamPrep NGS

MAIZE GENOTYPING: ALIGNMENT METRICS

	TOTAL READS	PERCENT ALIGNED	PERCENT ON-TARGET	PERCENT ON-TARGET WITH 0X COVERAGE	TOTAL PROBES
Sample Average (n=48)	560,000	90.77	84.40	15.9	50,000

MAIZE MAGIC LINES SEGREGATION ANALYSIS

	Minimum recall 90%	Minimum recall 80%	Minimum recall 50%
Total MAGIC-informative SNPs	37,409	51,651	68,108
W153R SNPs	12,934	18,260	24,857
Mo17 SNPs	15,263	21,728	29,450
HP301 SNPs	16,367	22,976	30,923
H99 SNPs	15,485	21,607	29,305
F7 SNPs	17,344	24,322	32,544

Allegro Targeted Genotyping V2 workflow on the DreamPrep™ NGS liquid handling automation platform currently under development.

Revelo™ RNA-Seq

HIGH-SENSITIVITY SOLUTION FOR
INFECTIOUS DISEASE RESEARCH

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Revelo RNA-Seq for high-sensitivity detection of viral sequences

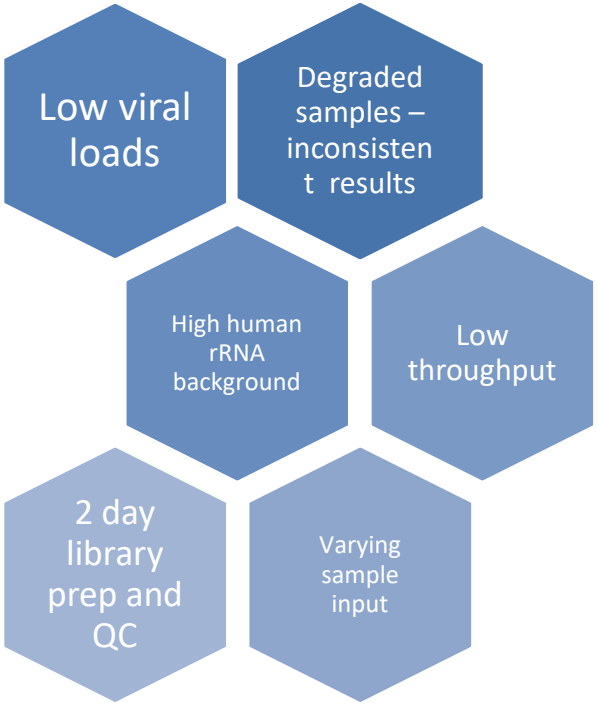
KEY AREAS OF RESEARCH

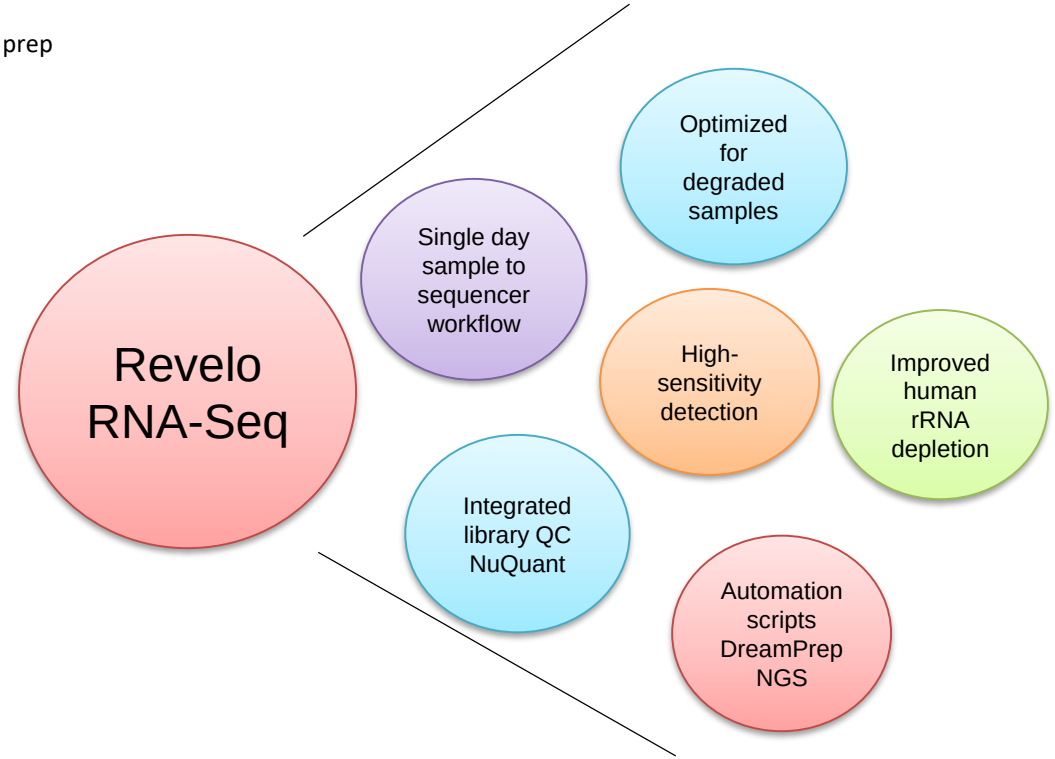
- Pathogen detection and characterization
- RNA virus sequencing (e.g. SARS-CoV-2, Flu (H1N1), HIV)
- Whole transcriptome studies for bacterial pathogens
- Impact of infectious disease on human transcript expression
- Meta-transcriptome studies in humans to understand the effects of an infection

- ✓ Solution for infectious disease – pathogen NGS applications
- ✓ Human samples
- ✓ Limiting samples such as nasal swabs
- ✓ Single day workflow with integrated library quantification
- ✓ Low-input solution

Revelo RNA-Seq resolves challenges in processing infectious disease sample types

Integrated with novel technologies for high-quality NGS library prep

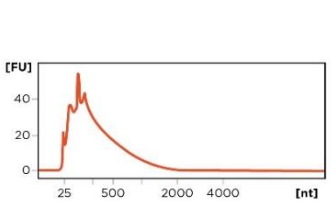


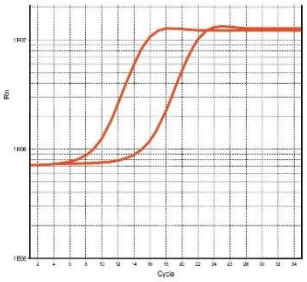


Real-world sample testing with Revelo RNA-Seq

This kit has been optimized to ensure high-quality libraries from degraded samples

Samples of varying quality and viral copies from nasal swabs



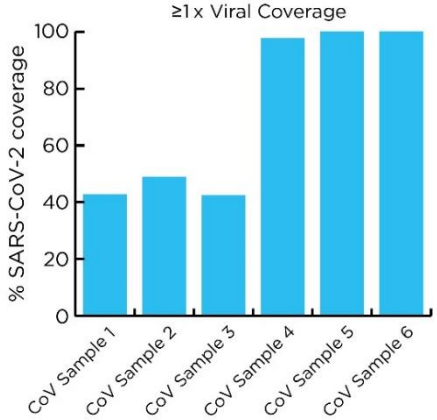


Input samples with RIN < 3.0 and viral copies ranging from ~500 – 7M

NGS library preparation and quantification

Revelo RNA-Seq

High detection sensitivity of SARS-CoV-2 using Revelo RNA-Seq, from as low as ~500 viral copies



TECAN.

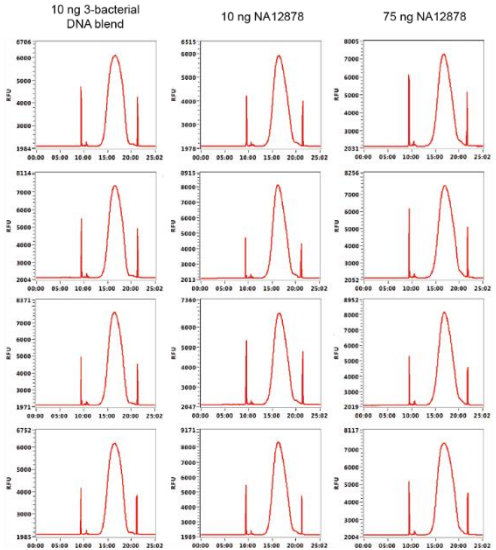
Revelo RNA-Seq – single day sample-to-sequencer fully automated workflow for large scale infectious disease sample testing

Single day, sample-to-sequencer workflow

Integrated sample QC with NuQuant

High detection sensitivity

Improved rRNA depletion



No library failures for all sample type and input

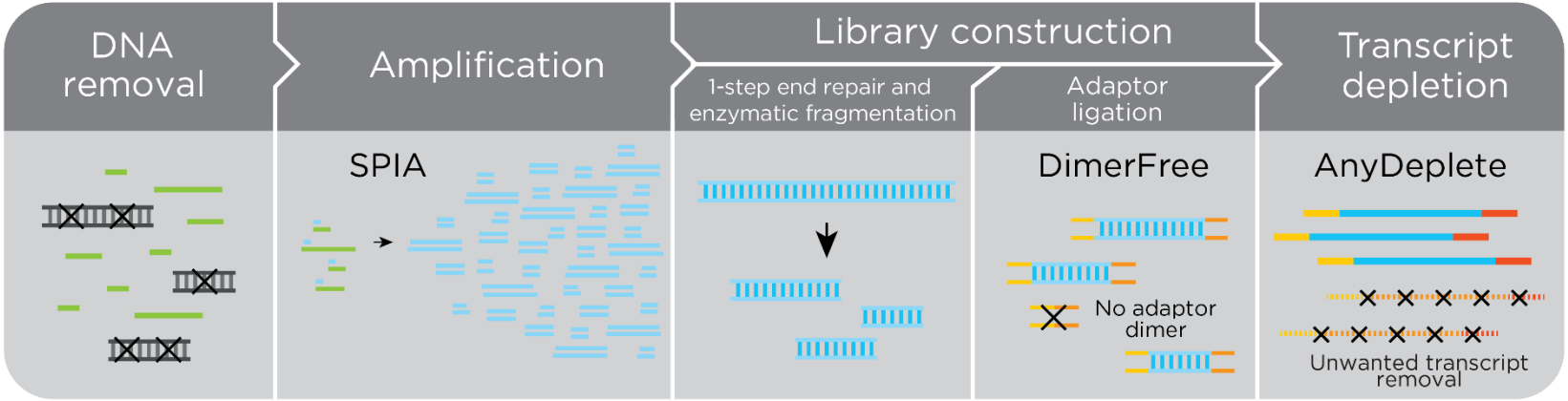
SARS-CoV-2 spike-in samples achieved consistent enrichment

Average ~20 fold viral sequences enrichment

Trio RNA-Seq

For Research Use Only. Not for use in diagnostic procedures.

Trio RNA-Seq: Robust library prep for low-input samples



Inputs of varying degraded quality

SPIA technology for robust global amplification of limiting material

Any sample type

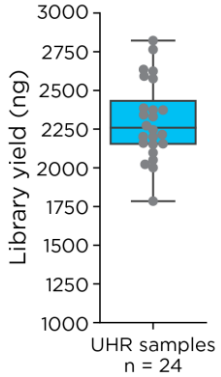
AnyDeplete for unwanted transcript depletion

Trio RNA-Seq on DreamPrep NGS – Experimental results

Starting materials: 500 pg UHR total RNA

Kit: Trio RNA-Seq (Part no. 0506)

Instruments: Fluent Liquid Handler integrated with integrated Infinite plate reader and Inheco On-Deck Thermal Cycler



	Average percent rRNA (SD)	Average percent aligned (SD)	Average percent exon (SD)
500 pg UHR (n = 8)	6.5 (2.0)	96.5 (0.1)	41.1 (0.8)

- **No library failures for all samples**
- **Consistent library yields across replicates with a 10.9% CV.**
- **Significant depletion of unwanted human rRNA**

DreamPrep NGS available in two options

1

STAND-ALONE OFFER

AUTOMATION INCLUDES FLUENT GX 780 INTEGRATED WITH INFINITE READER AND INHECO ON-DECK THERMAL CYCLER

- Configuration supports library prep and quant
- Consumables not included

TECAN GENOMICS REAGENTS AVAILABLE

- Celero DNA-Seq kit
- Universal Plus mRNA-Seq kit

2

REAGENT RENTAL OFFER

- Same automation configuration as standalone
- Annual reagent contract

Summary

- DreamPrep NGS is the only solution that automates library preparation, quantification, normalization and pooling in a single workflow
- Minimal to no manual interaction for library prep and QC
- Library QC done in less than 6 min with no sample loss
- NuQuant quantitation method eliminates Bioanalyzer and qPCR for multiplex pooling
- Robust pooling with NuQuant validated by sequencing results
- Additional NGS library preparation kits will be available on DreamPrep NGS

Disclaimer:

For research only. Not for use in diagnostic procedures. Example methods have not been validated. Please make a copy of this method in order to customize it to accommodate your workflow according to your desired intended use and laboratory protocols.
Tecan makes no claims regarding the performance of example methods.

Tecan – Who we are

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Italy +39 02 92 44 790 **Japan** +81 44 556 73 11 **Netherlands** +31 18 34 48 17 4 **Nordic** +46 8 750 39 40 **Singapore** +65 644 41 886 **Spain** +34 93 595 25 31
Switzerland +41 44 922 89 22 **UK** +44 118 9300 300 **USA** +1 919 361 5200 **Other countries** +41 44 922 81 11

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