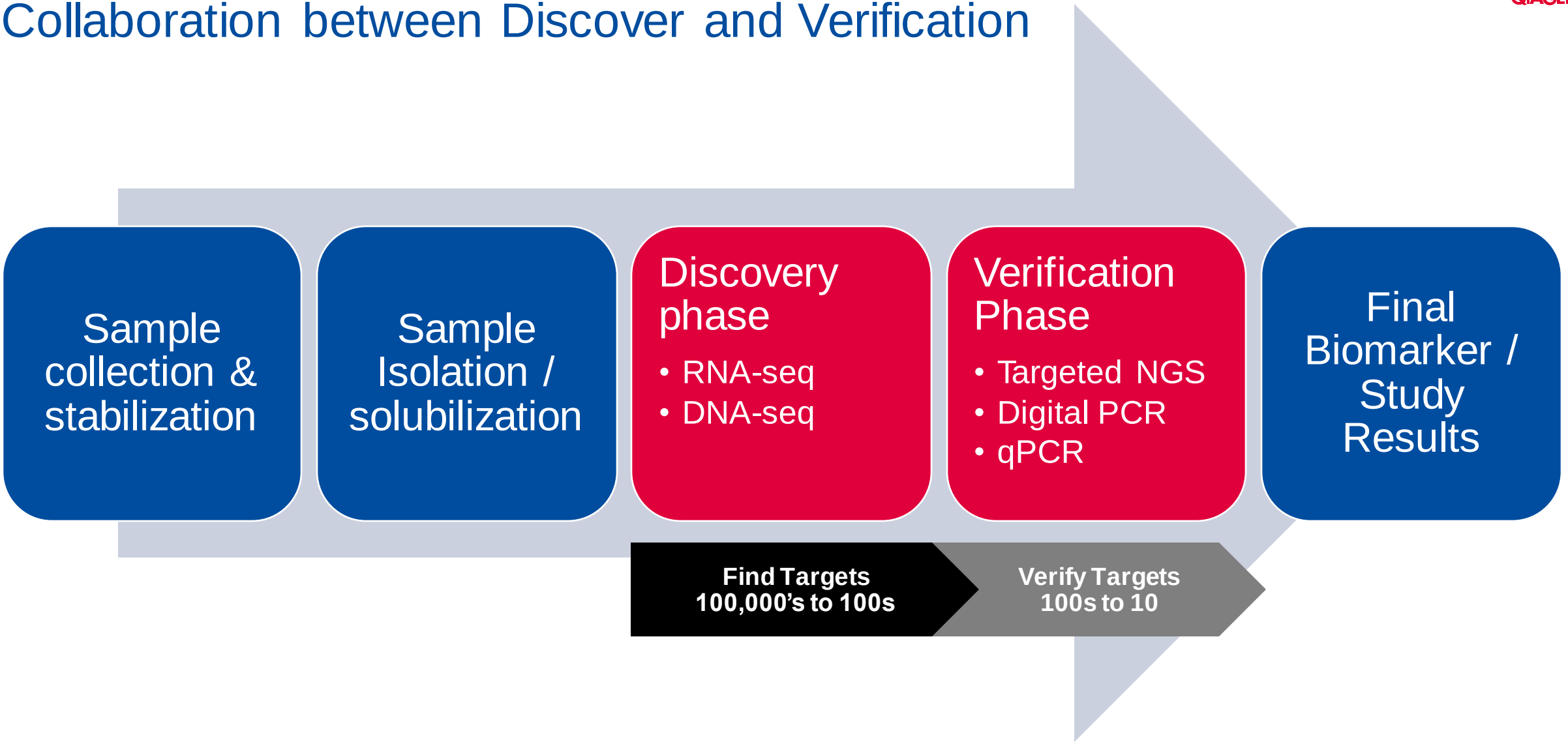
Simplify your NGS Workflows with QIAseq Lightspeed Collaboration

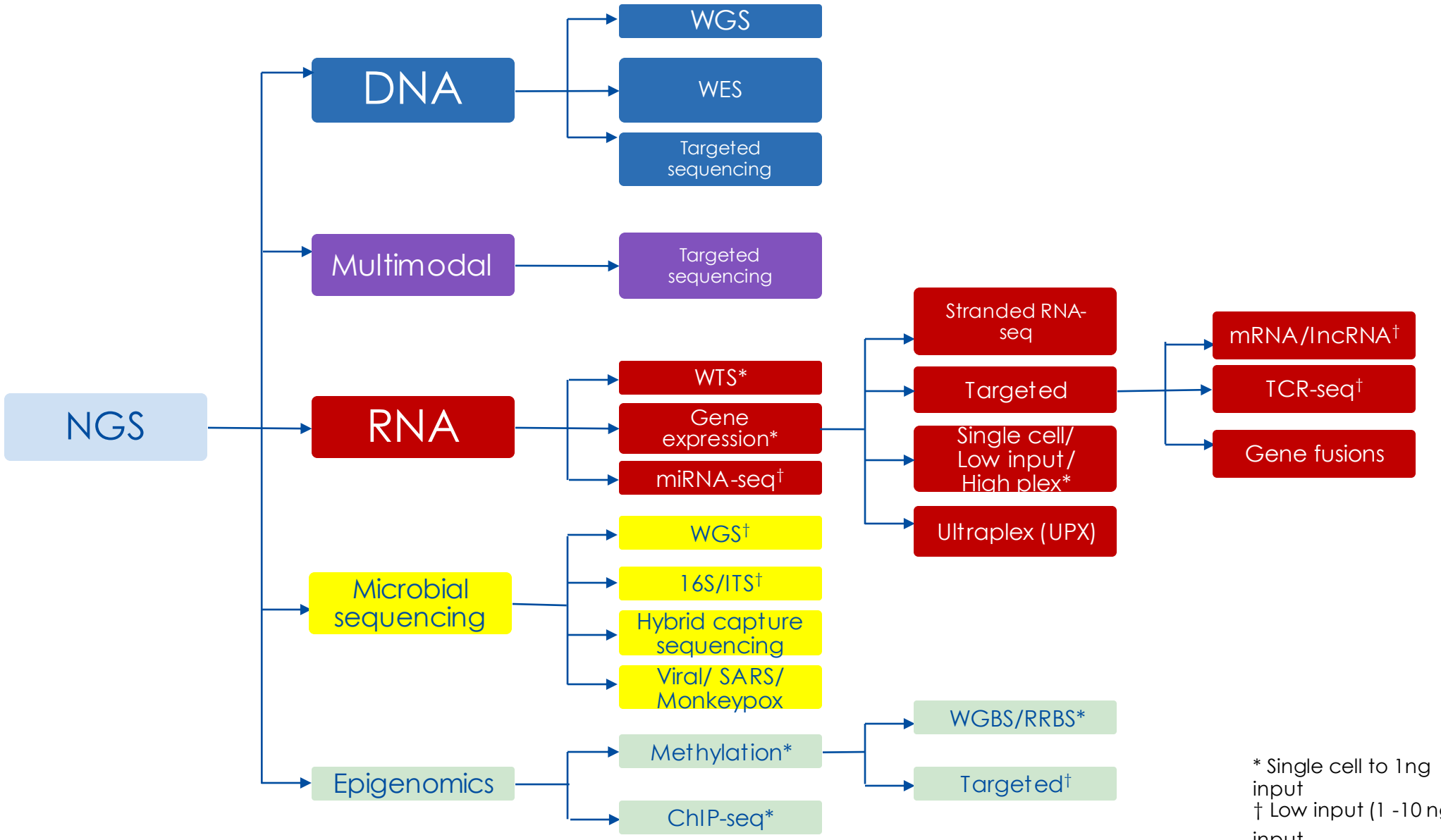
Samuel Rulli – Director Global Product Management NGS applications

October 2023

Typical Research Workflow- Collaboration between Discover and Verification

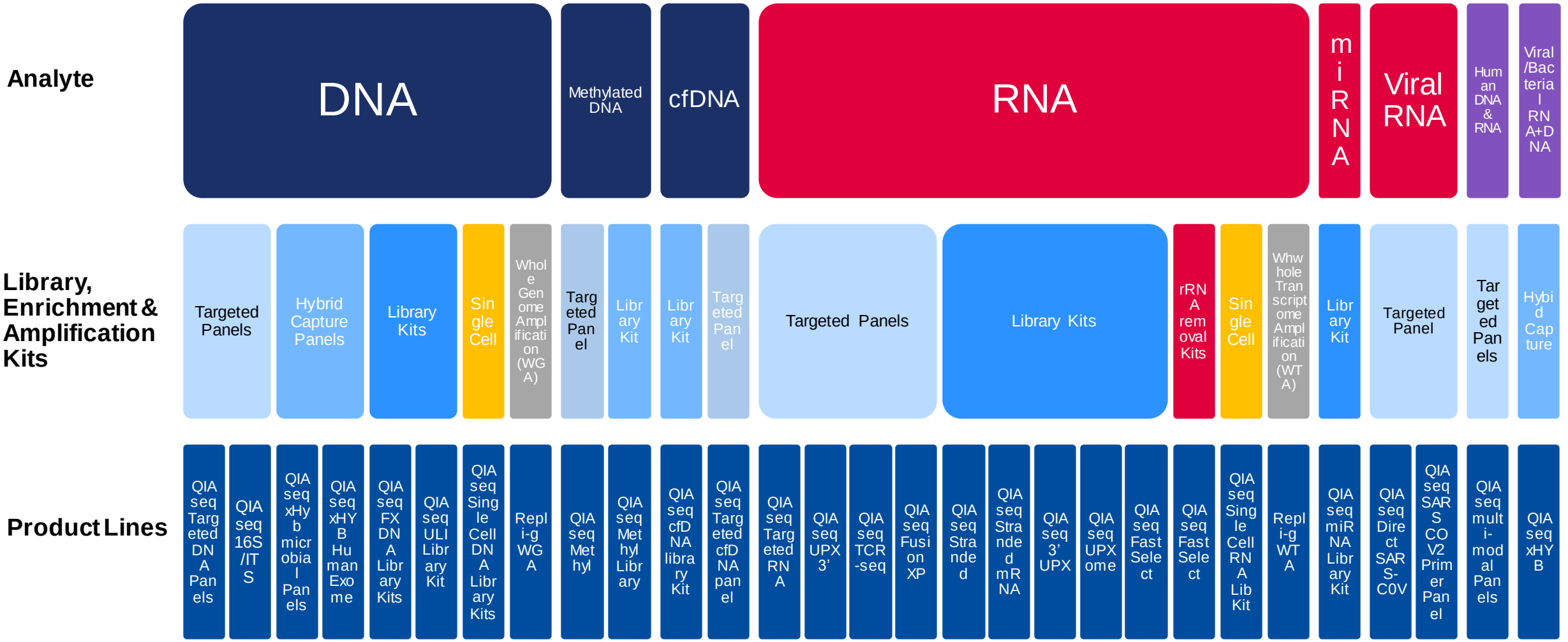


Introduction to the QIAseq portfolio



* Single cell to 1ng input
† Low input (1 -10 ng) input

QIAseq portfolio by Analyte



How can we speed up and simplify RNA-seq workflows?

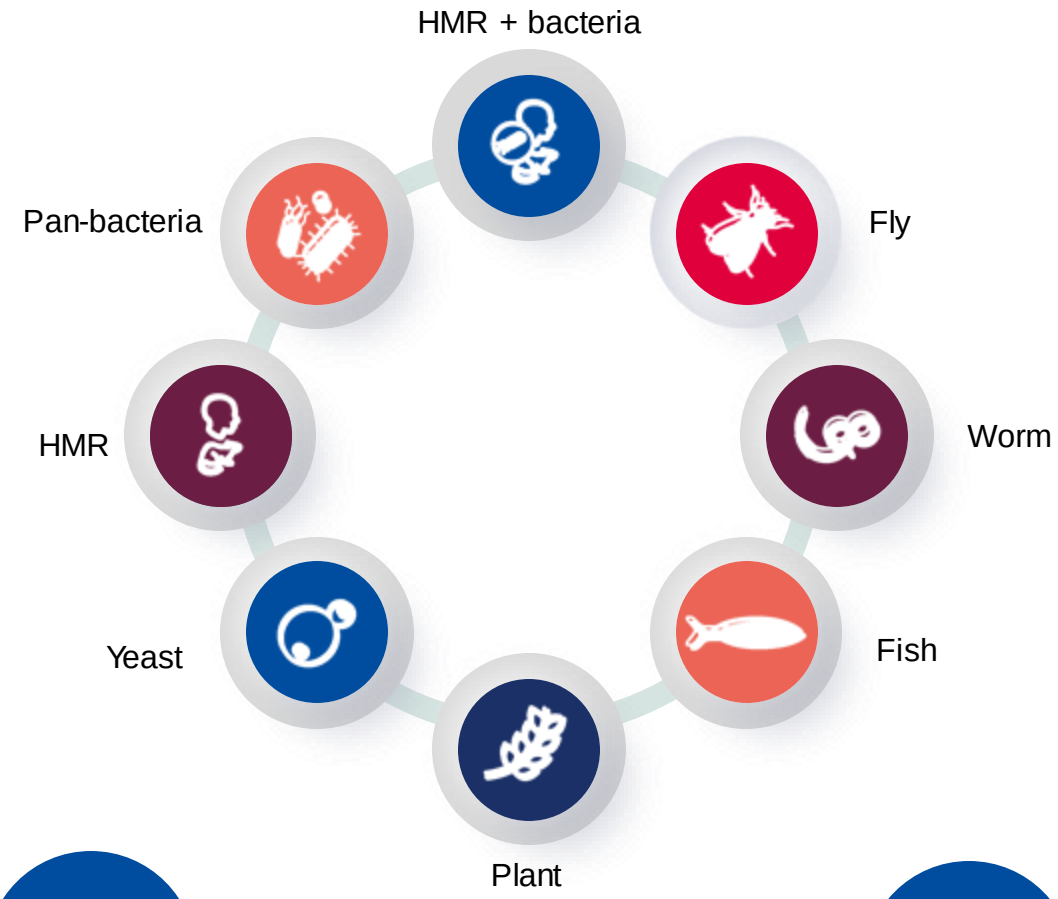
Speed up and Simplify your RNA-seq Workflows
QIAseq FastSelect RNA library kit and QIAseq Normalizer

Step	QIAseq FastSelect RNA Library Kit Workflow Time
rRNA Removal + RNA fragmentation	16 minutes
cDNA synthesis	90 minutes
QIAseq bead cleanup	45 minutes
Universal amplification and index assignment:	1000 ng input: 17 cycles – 36 minutes 100ng input: 20 cycles – 42 minutes 10 ng input: 24 cycles – 50 minutes 1 ng input: 27 cycles – 60 minutes
QIAseq bead cleanup	45 minutes
Library Normalization with QIAseq Normalizer	30 minutes – 45 minutes
Sequence Ready libraries @ 4nM concentration	

5 hours

RNA to library pooling

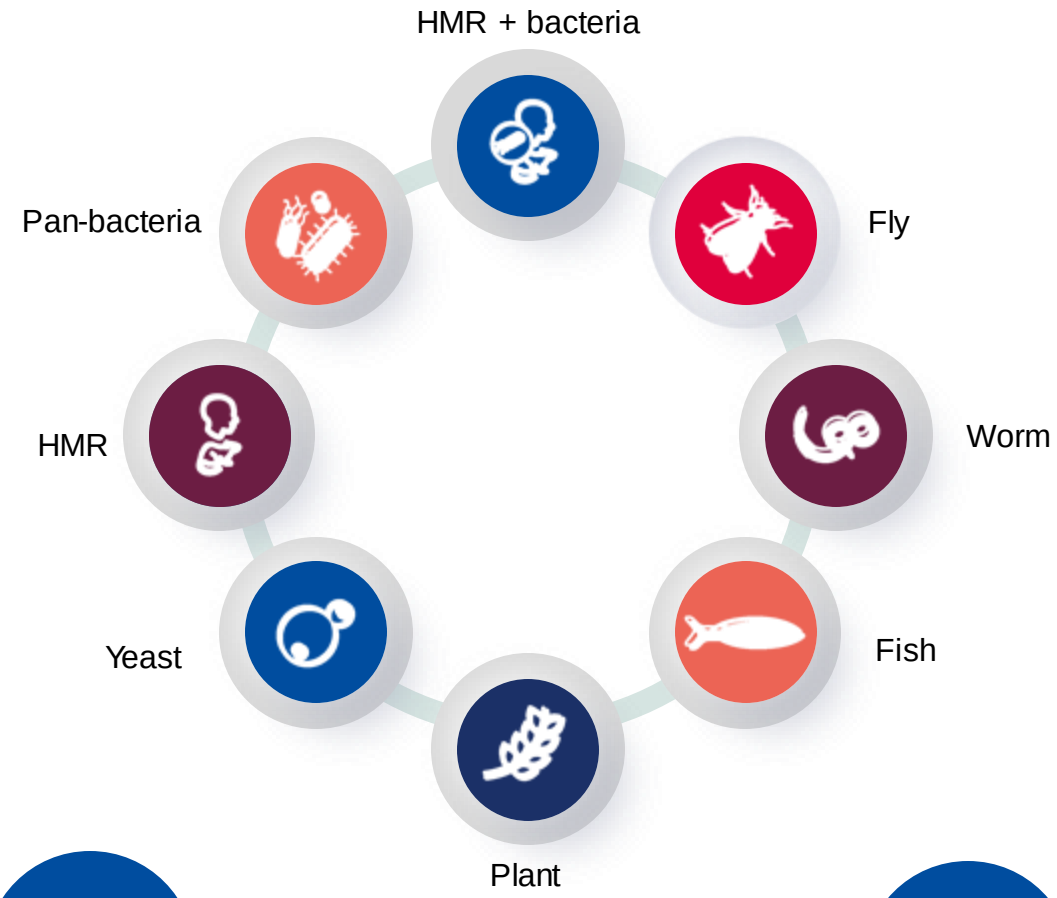
QIAseq FastSelect supports model organisms and more



Custom

Blood

QIAseq FastSelect supports model organisms and more



Custom

Blood

Add FastSelect to sample

Heat samples then cool sample

cDNA Synthesis

QIAseq FastSelect removed rRNA from FFPE samples

Experimental Setup

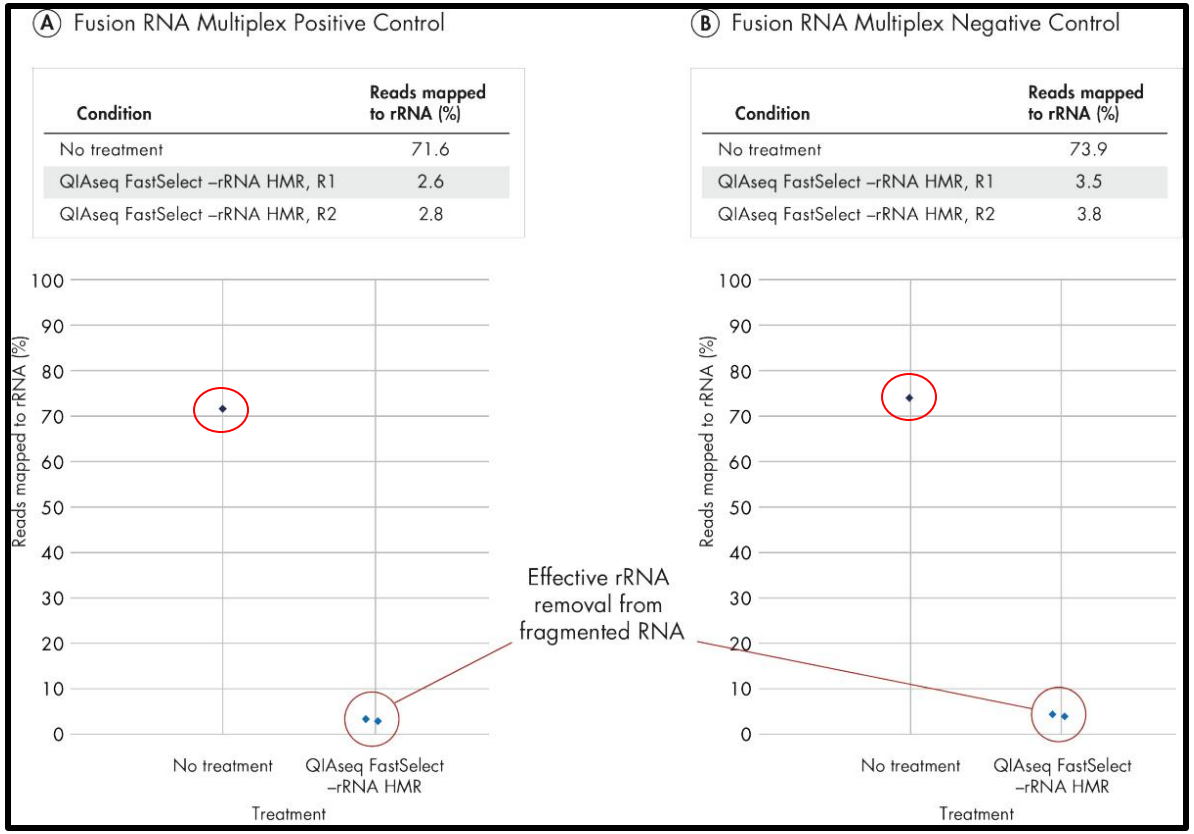
Sample: Horizon FFPE RNA reference standards

A FFPE Horizon 5-Fusion Multiplex Positive

B FFP E Horizon 5-Fusion Multiplex Negative Control

Amount of RNA used for library construction is dependent on the RNA library kit

Results: 95% of ribosomal RNA is removed



FastSelect has been used for what?

- **Oxford nanopore sequencing**

Fas
use

- Ox

viruses

MDPI

Protocol

Application of Oxford Nanopore Technology to Plant Virus Detection

Lia W. Liefting *, David W. Waite and Jeremy R. Thompson *

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* Correspondence: Lia.Liefting@mpi.govt.nz (L.W.L.); Jeremy.Thompson@mpi.govt.nz (J.R.T.)

Abstract: The adoption of Oxford Nanopore Technologies (ONT) sequencing as a tool in plant virology has been relatively slow despite its promise in more recent years to yield large quantities of long nucleotide sequences in real time without the need for prior amplification. The portability of the MinION and Flongle platforms combined with lowering costs and continued improvements in read accuracy make ONT an attractive method for both low- and high-scale virus diagnostics. Here, we provide a detailed step-by-step protocol using the ONT Flongle platform that we have developed for the routine application on a range of symptomatic post-entry quarantine and domestic surveillance plant samples. The aim of this methods paper is to highlight ONT's feasibility as a valuable component to the diagnostician's toolkit and to hopefully stimulate other laboratories towards the eventual goal of integrating high-throughput sequencing technologies as validated plant virus diagnostic methods in their own right.

FastSelect has been used for what?

- **Oxford nanopore sequencing**
- **Illumina sequencing with KAPA NEB, Illumina, Takara, Thermo-Fisher library Kits**
- **Removal of hemolytic miRNAs**
- **Removal of unwanted RNAs for hybrid capture workflows**
- **High quality RNA to FFPE RNA**
- **Single cell to bulk sequencing**

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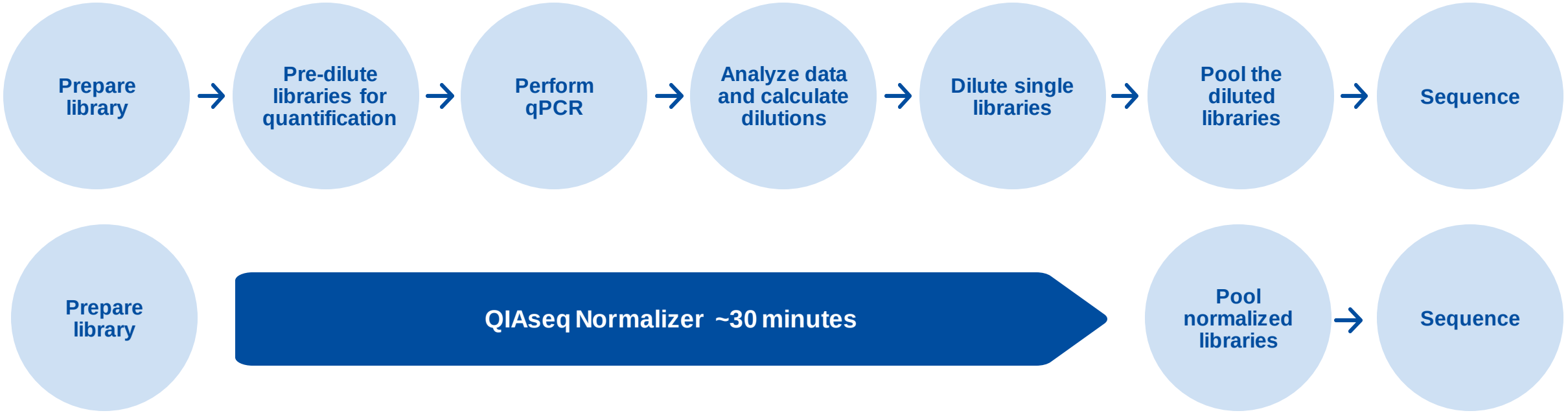
Sequence Ready libraries @ 4nM concentration	

5 hours

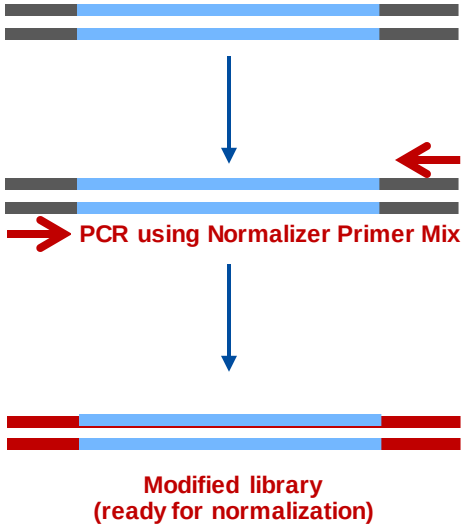
RNA to library pooling

QIAseq Normalizer Kits: A streamlined method to create ready-to-sequence normalized dsDNA libraries



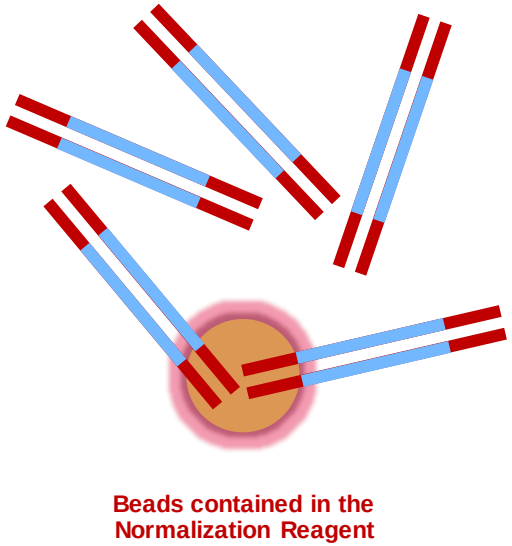
QIAseq Normalizer: Bind, wash, elute

Modify the libraries to be normalized



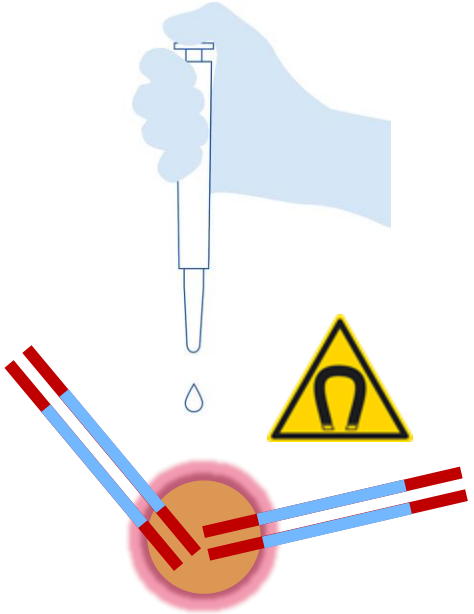
Modification is always required for normalization:

Bind modified libraries to beads



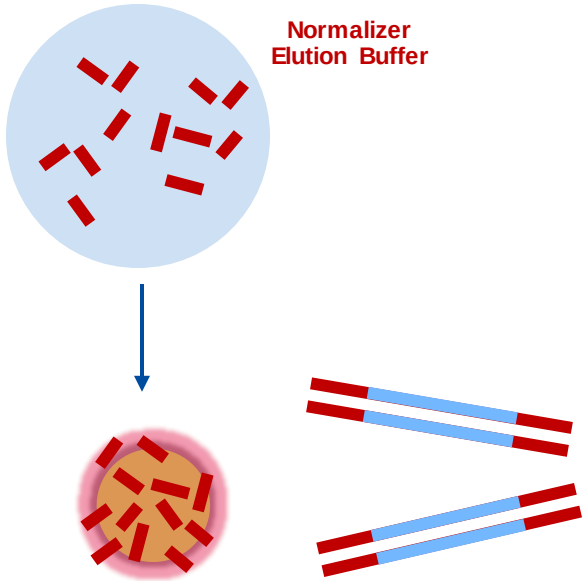
Limited bead capacity

Wash away unbound library molecules



Magnetic beads allow fast and easy washing

Elute the normalized libraries (~4 nmol/l)

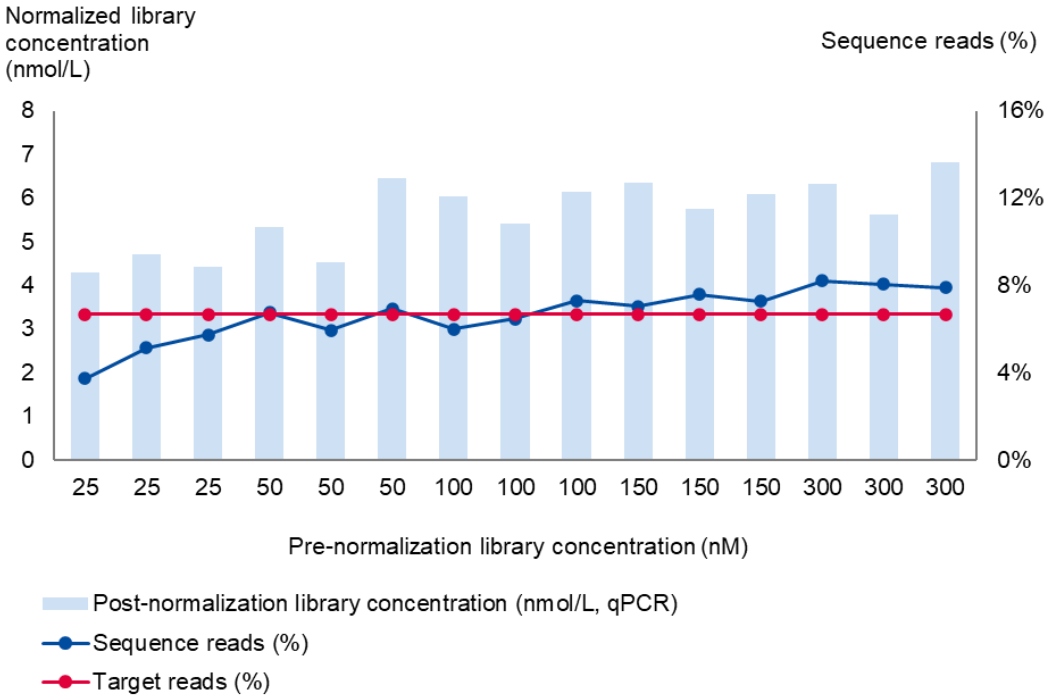


Specific elution of bound fragments by Normalizer Elution buffer

Eluted libraries are **dsDNA**

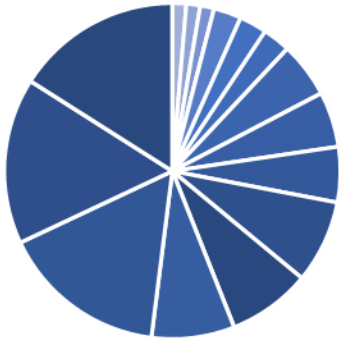
QIAseq Library Normalizer effectively normalizes QIAseq FX DNA libraries from a broad range of library concentrations

Normalization of QIAseq FX DNA libraries



Library representation

Before normalization



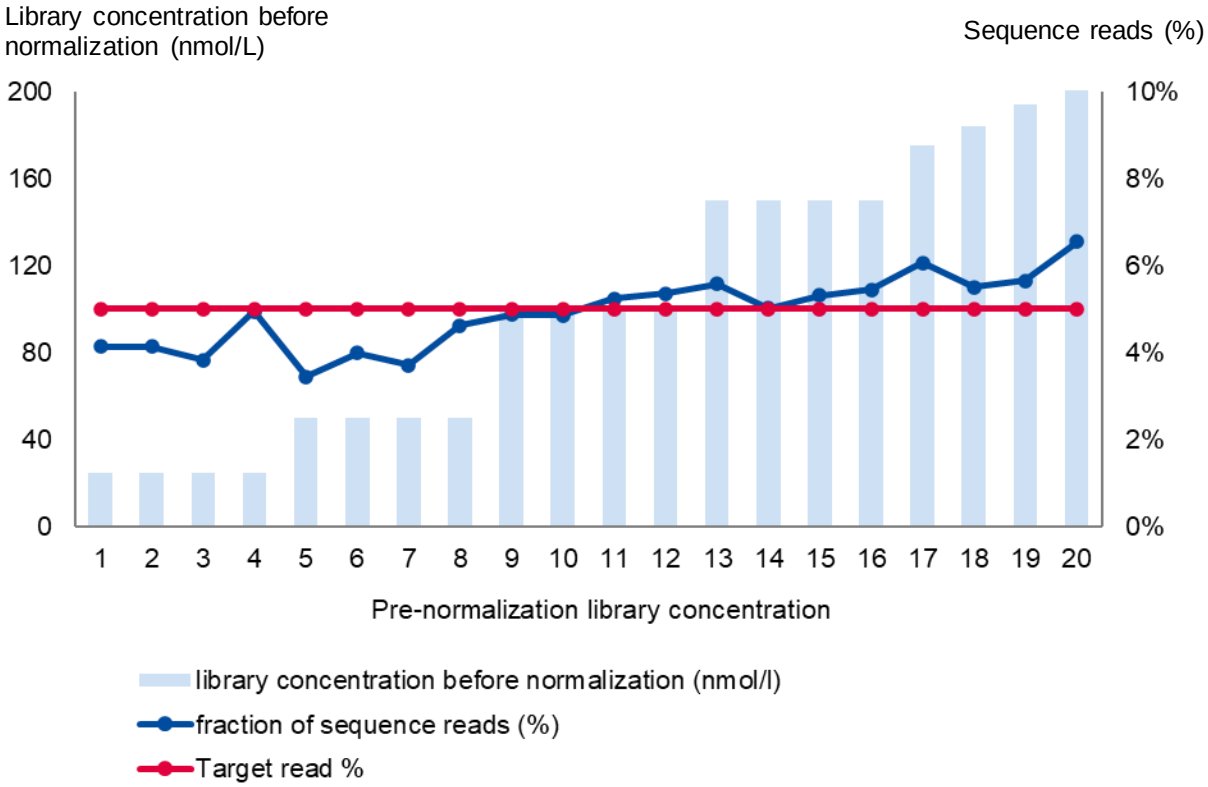
After normalization

	Before normalization (qPCR)	After normalization (sequencing)
CV%	80.7 %	18 %
GINI*	0.416	0.096

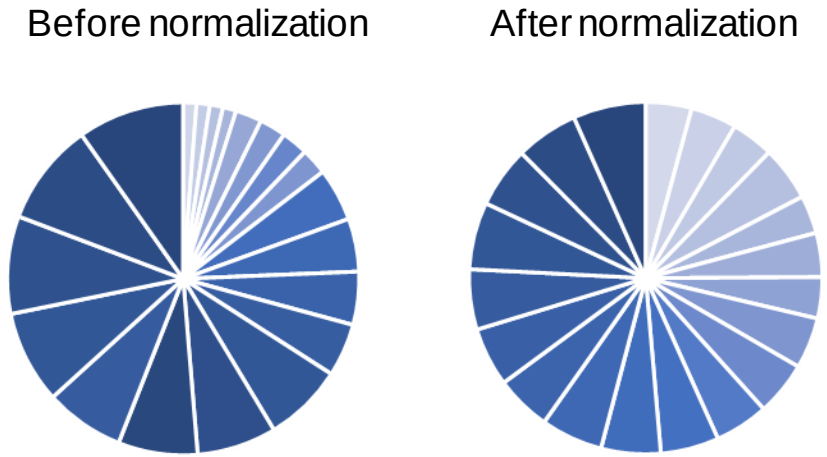
*GINI coefficient (0.0 –1.0); 0: All libraries are equally represented; 1: One library has all sequencing reads

QIAseq Library Normalizer for cfDNA libraries

QIAseq Ultralow Input with cfDNA as input



Library representation



	Before normalization (qPCR)	After normalization (qPCR)	After normalization (sequencing)
CV%	62 %	18.1 %	13.6 %
GINI	0.33	0.09	0.07

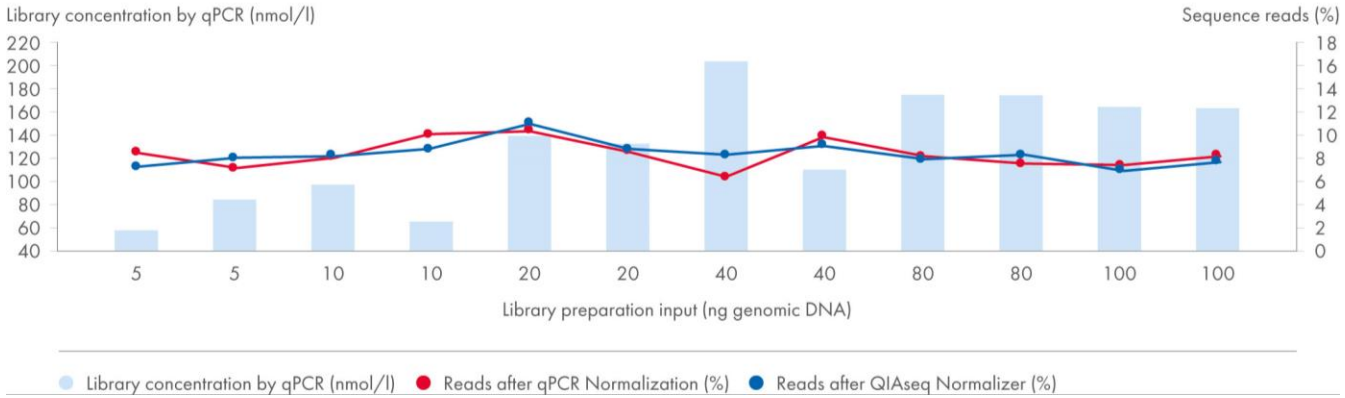
Effective normalization of cfDNA libraries with concentrations between 20 and 200 nmol/L

*GINI coefficient (0.0 –1.0); 0: All libraries are equally represented; 1: One library has all sequencing reads

QIAseq Universal Normalizer for targeted DNA libraries

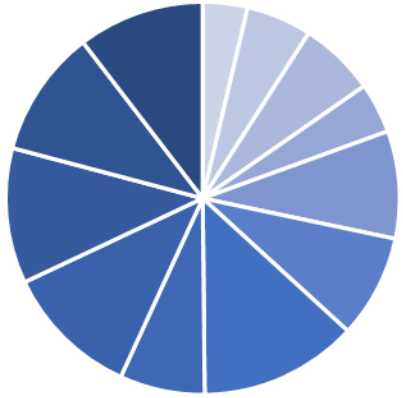
Universal workflow: Library preparation → Two-cycle normalizer PCR → Normalization

Normalization of targeted DNA panels (library input range: 5–100 ng)



Library representation

Before normalization



QIAseq Normalizer

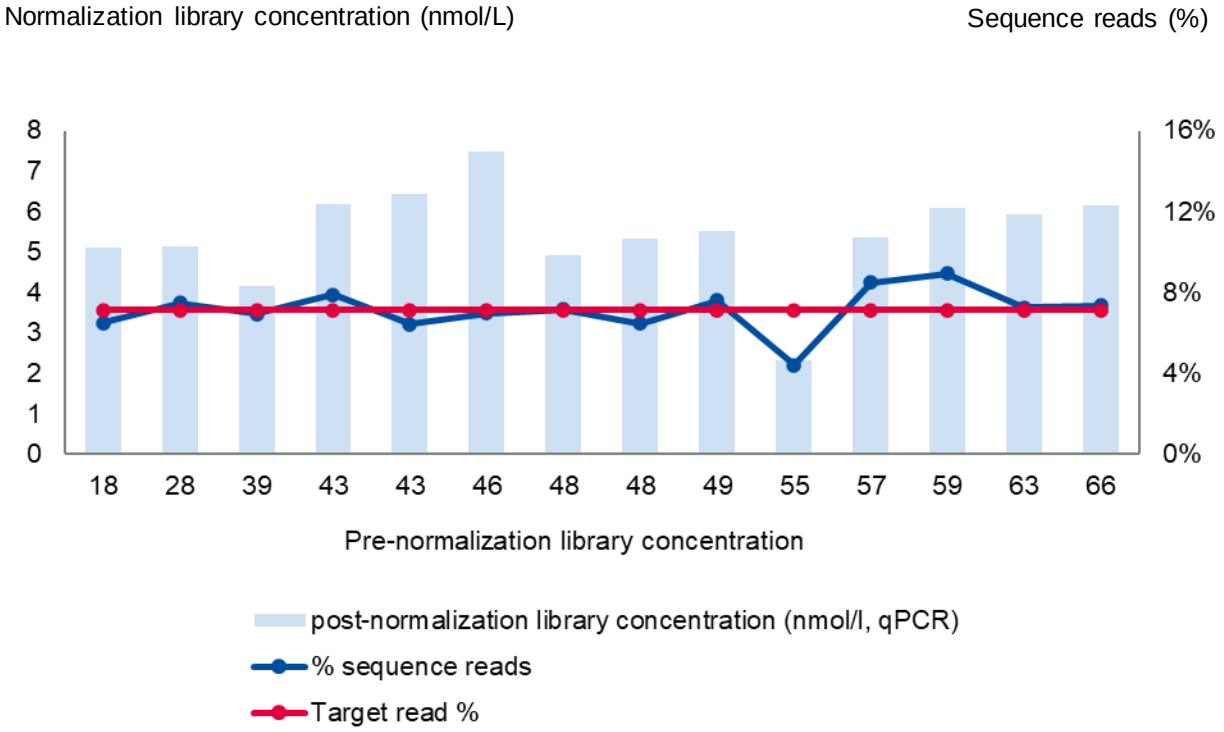
The QIAseq Normalizer matches the performance of the "qPCR+dilute" gold standard with minimized hands-on time

	Before normalization (qPCR of native library)	Sequence reads (qPCR/dilute/pool)	Sequence reads (QIAseq Normalizer)
CV%	36.4 %	14.8 %	12.4 %
GINI	0.2	0.08	0.06

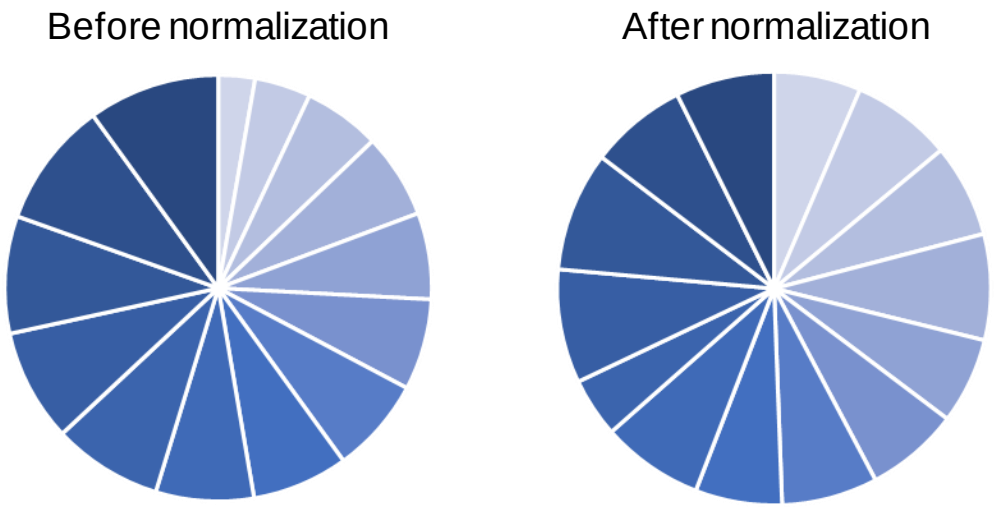
*GINI coefficient (0.0 –1.0); 0: All libraries are equally represented; 1: One library has all sequencing reads

QIAseq Library Normalizer for QIAseq Stranded RNA Libraries

Normalization of QIAseq Stranded RNA Libraries



Library representation



	Before normalization (qPCR)	After normalization (sequencing)
CV%	27.5%	15.0%
GINI	0.15	0.076

Integrated workflow: Library modification happens during library preparation/amplification

*GINI coefficient (0.0 –1.0); 0: All libraries are equally represented; 1: One library has all sequencing reads

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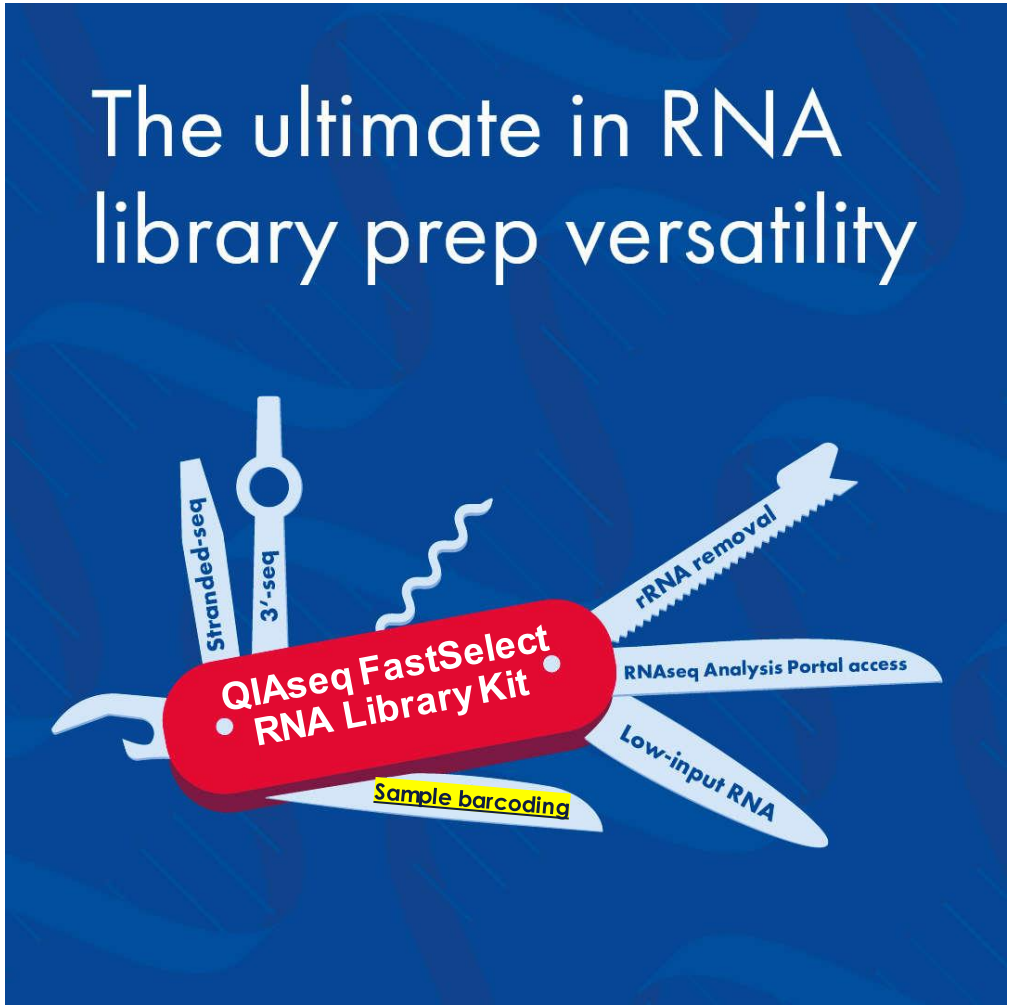
RNA to library pooling

Simplify your library preparation with 1 kit for most applications

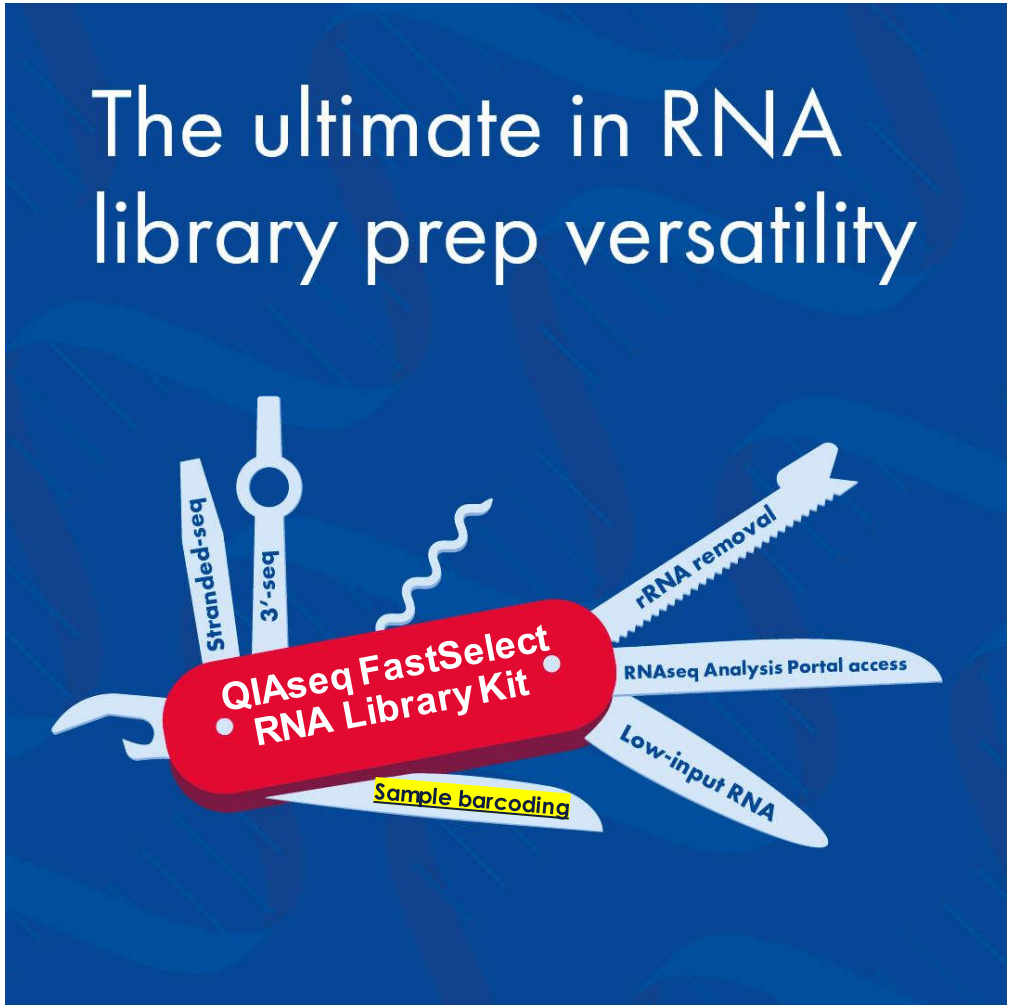
Reverse Transcription Reaction

Olgo-dt	**3’ RNAseq**
Random hexamer	**Full Transcriptome**
Oligo-dt + Random hexamer	**3’ RNAseq + Full Transcriptome**

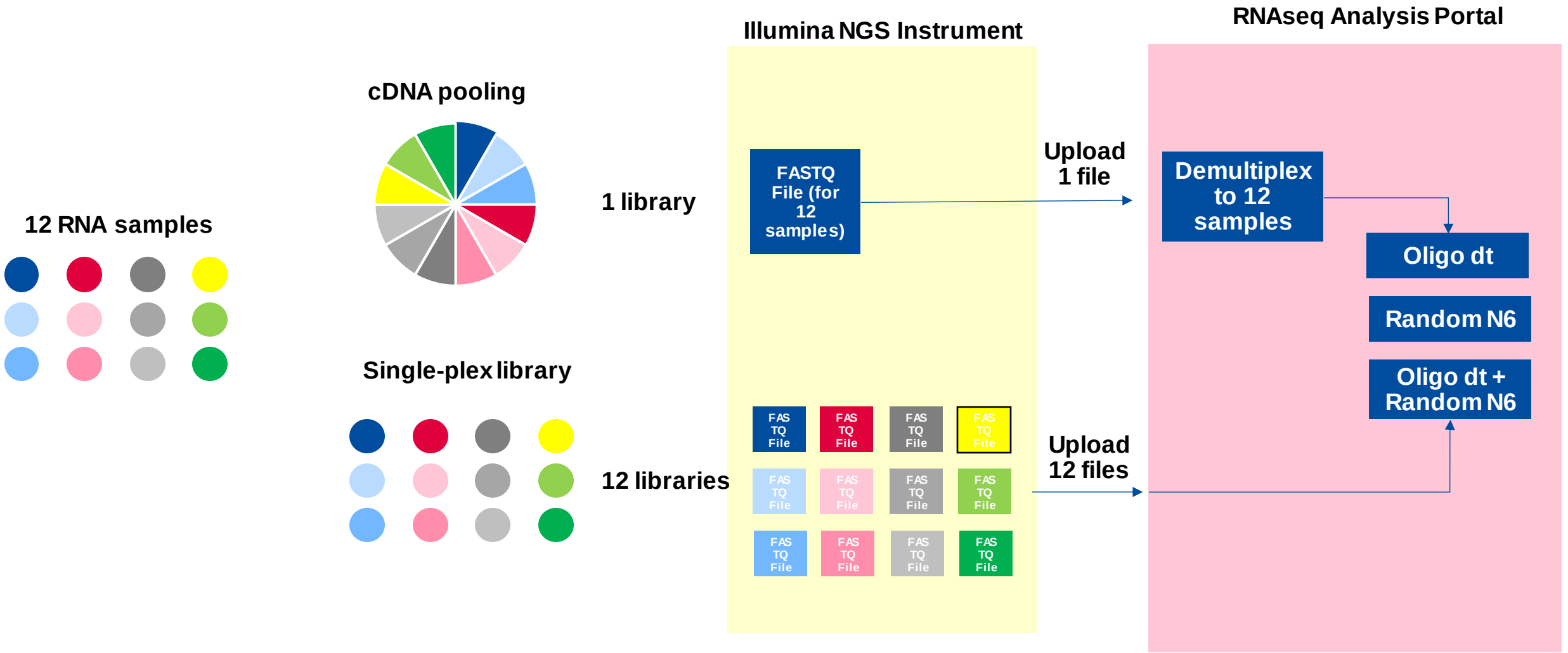


Simplify your library preparation with 1 kit for most applications

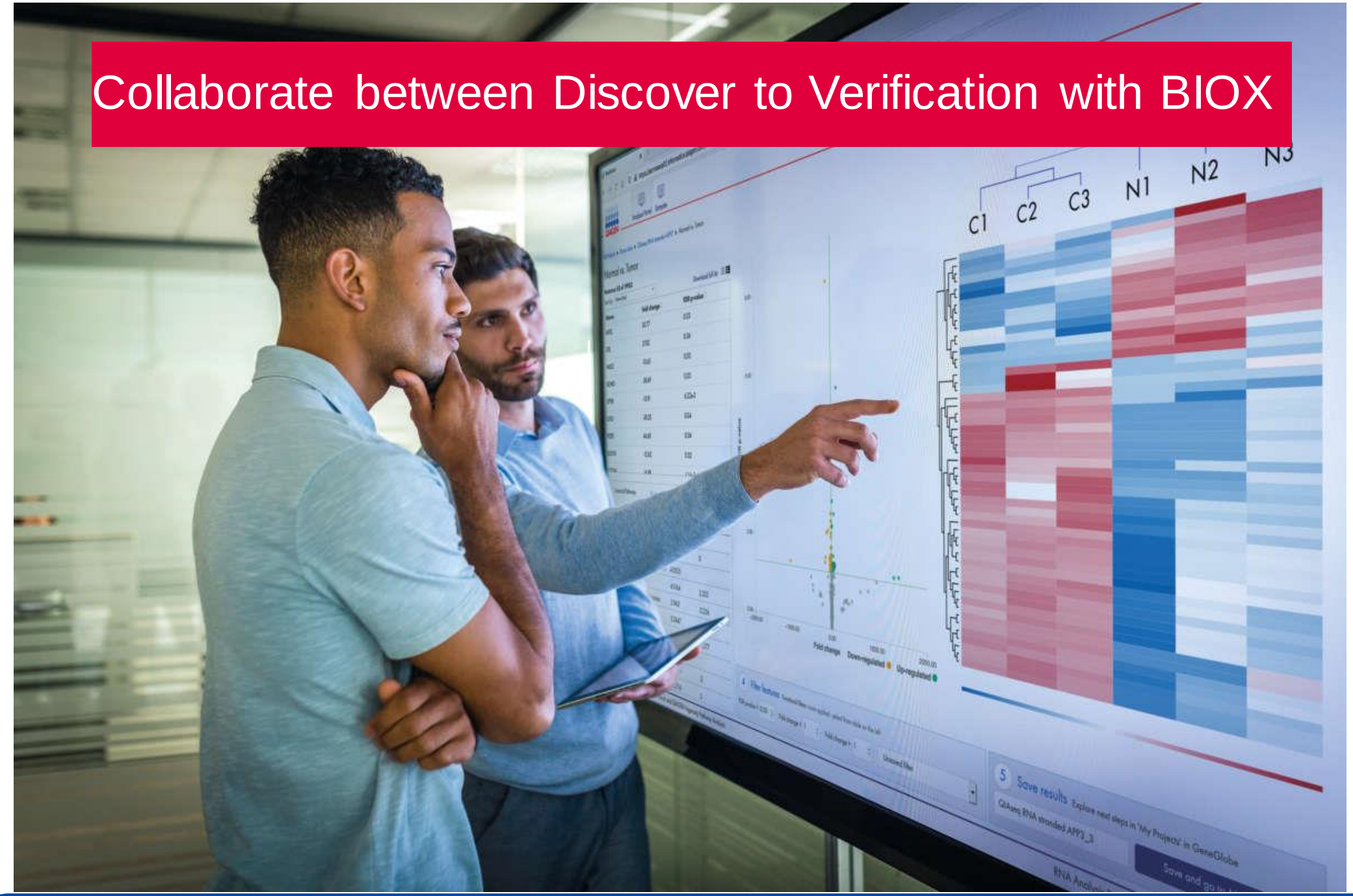
cDNA
Sample Barcoding



QIAseq FastSelect libraries can also be pooled after cDNA



Collaborate between Discover to Verification with BIOX



QIAGEN CLC Genomics Workbench for NGS analysis

CLC Genomics server:

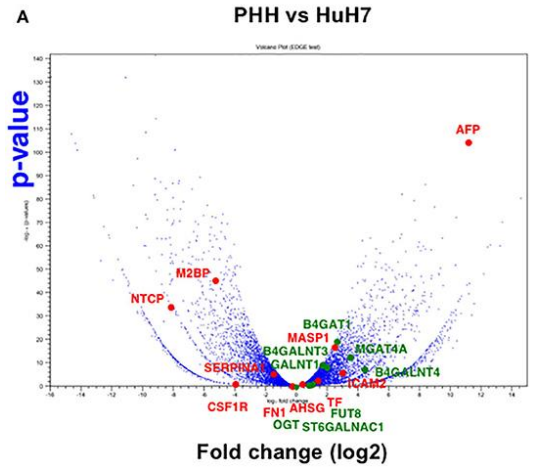
- Scale to any number of samples and any type of genomes
- Integrate any command line and parametrize for easy button for novice GUI users
- Control access via LDAP/Active directory using any scheduler out there (or ours)

CLC Cloud module:

- Instant scaling to any degree if you have Amazon account
- Eliminate need for costly hardware
- Eliminate need for hardware maintenance
- Pipe reads straight from base space to Amazon without pulling it down
- No idle costs

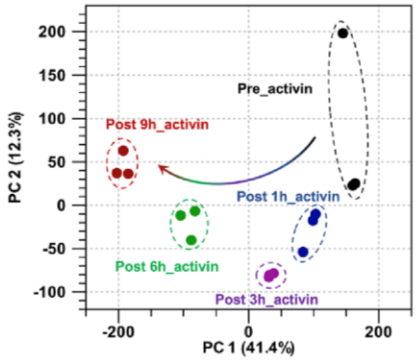
QIAGEN CLC Genomics Workbench for NGS analysis

Volcano Plots

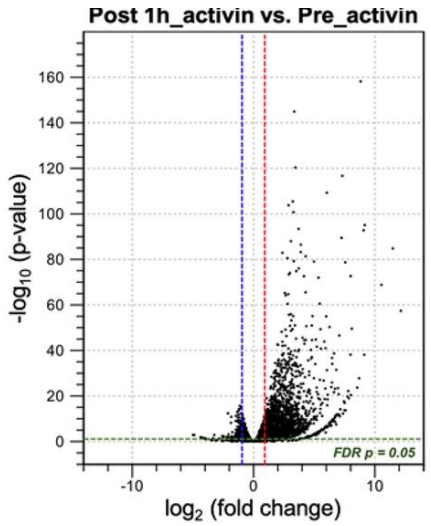


Angata K, et al. Glycogene Expression Profiling of Hepatic Cells by RNA-Seq Analysis for Glyco-Biomarker Identification. Front Oncol. (2020). PMC7402167.

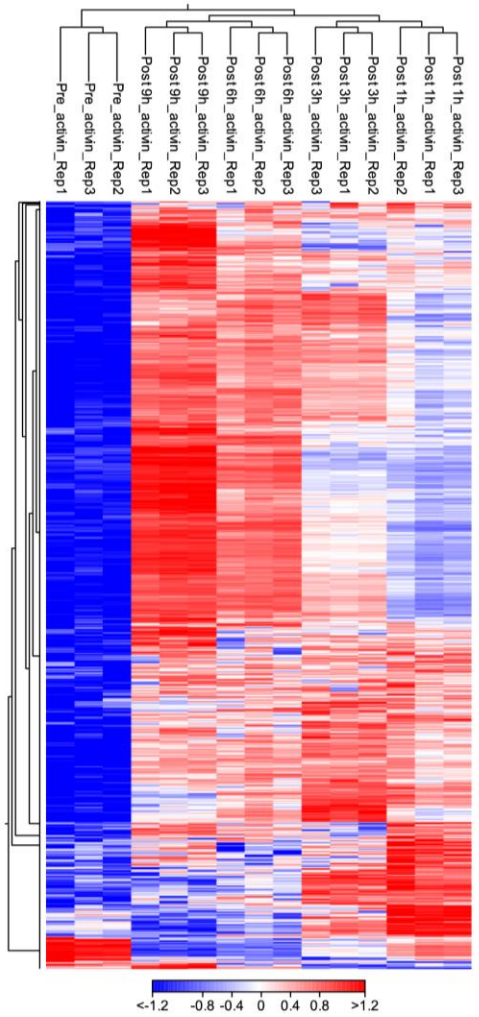
PCA Analysis



Volcano Plot

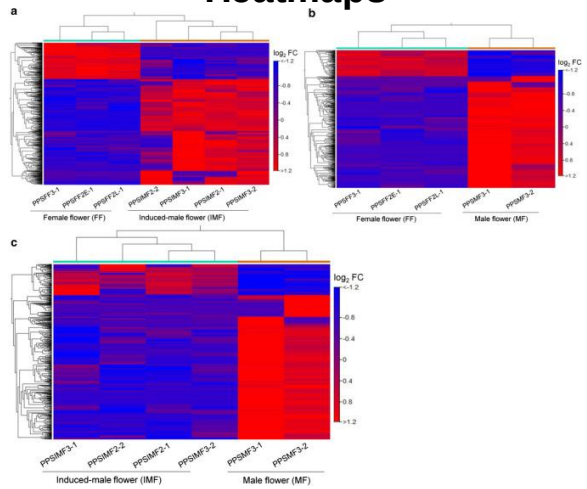


Heatmap

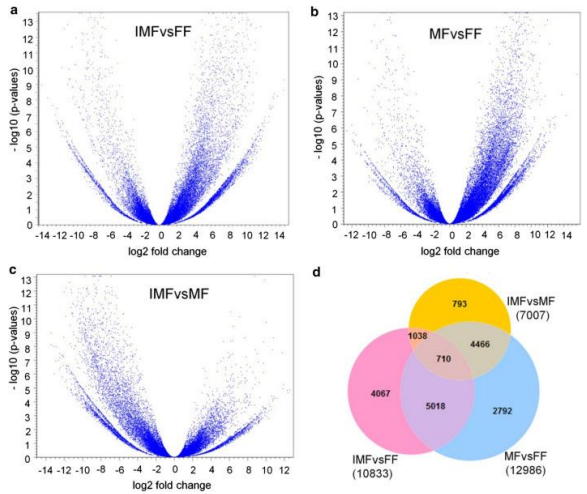


Satou-Kobayashi Y, *et al.* Temporal transcriptomic profiling reveals dynamic changes in gene expression of Xenopus animal cap upon activin treatment. Sci Rep. (2021). PMC8282838.

Heatmaps

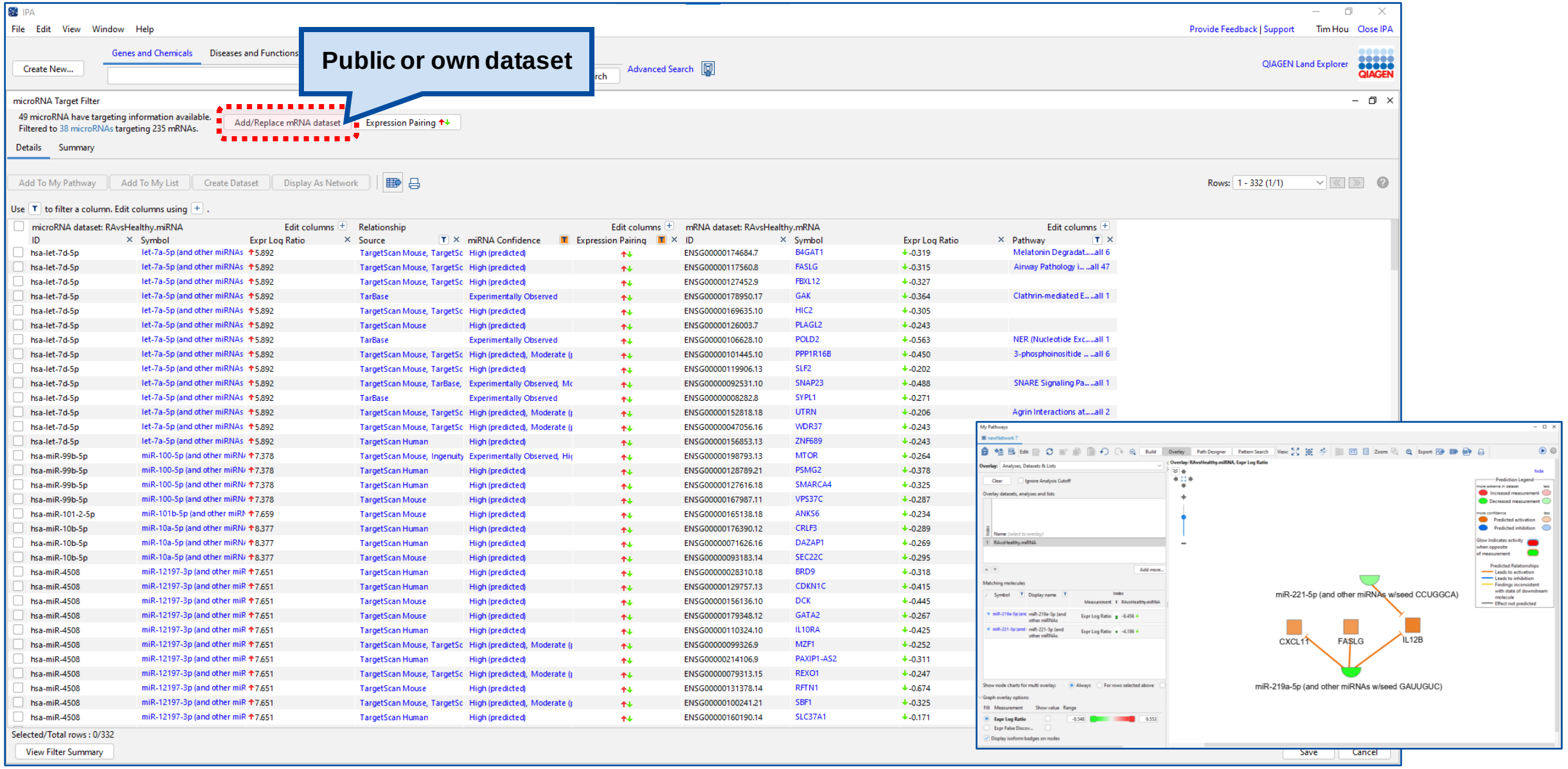


Volcano Plots & Venn Diagram



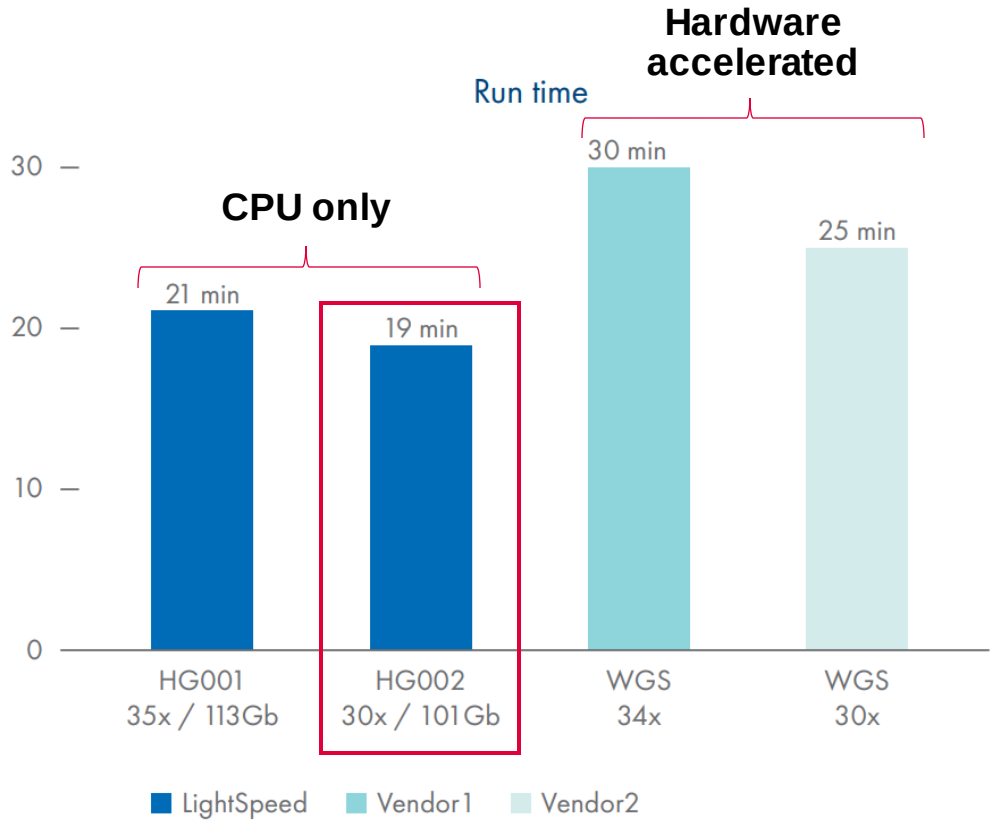
Adal AM, et al. Comparative RNA-Seq analysis reveals genes associated with masculinization in female Cannabis sativa. Planta. (2021). PMC7779414.

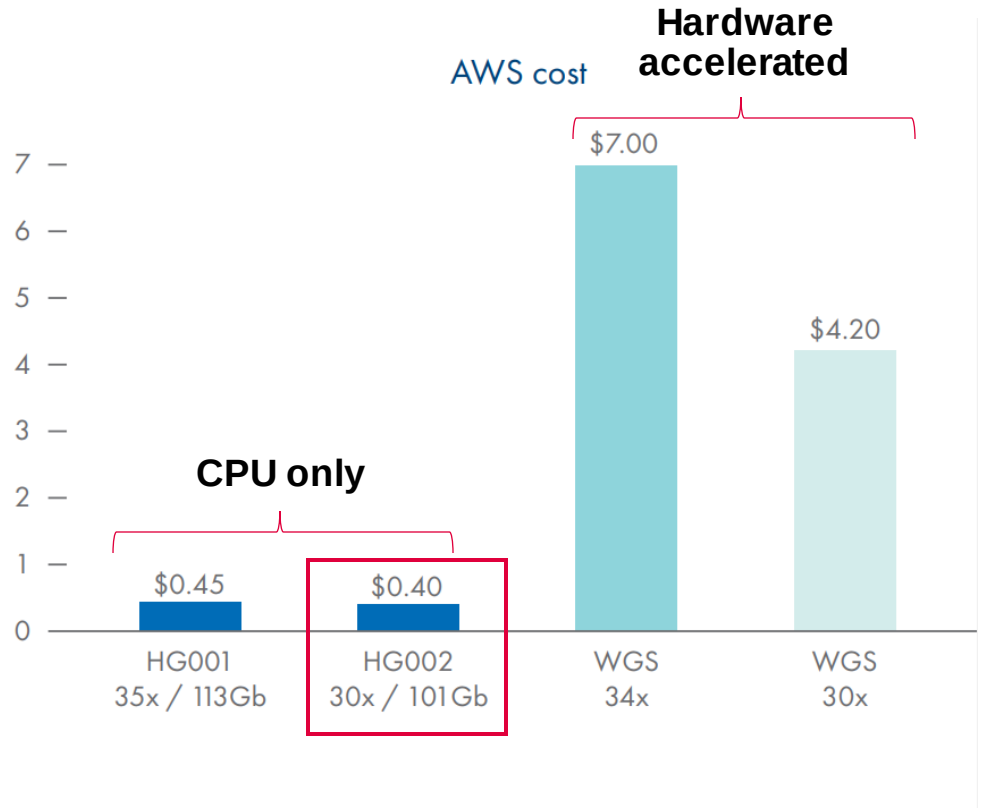
QIAGEN Ingenuity Pathway Analysis for NGS interpretation



Breaking speed and cost barriers with LightSpeed

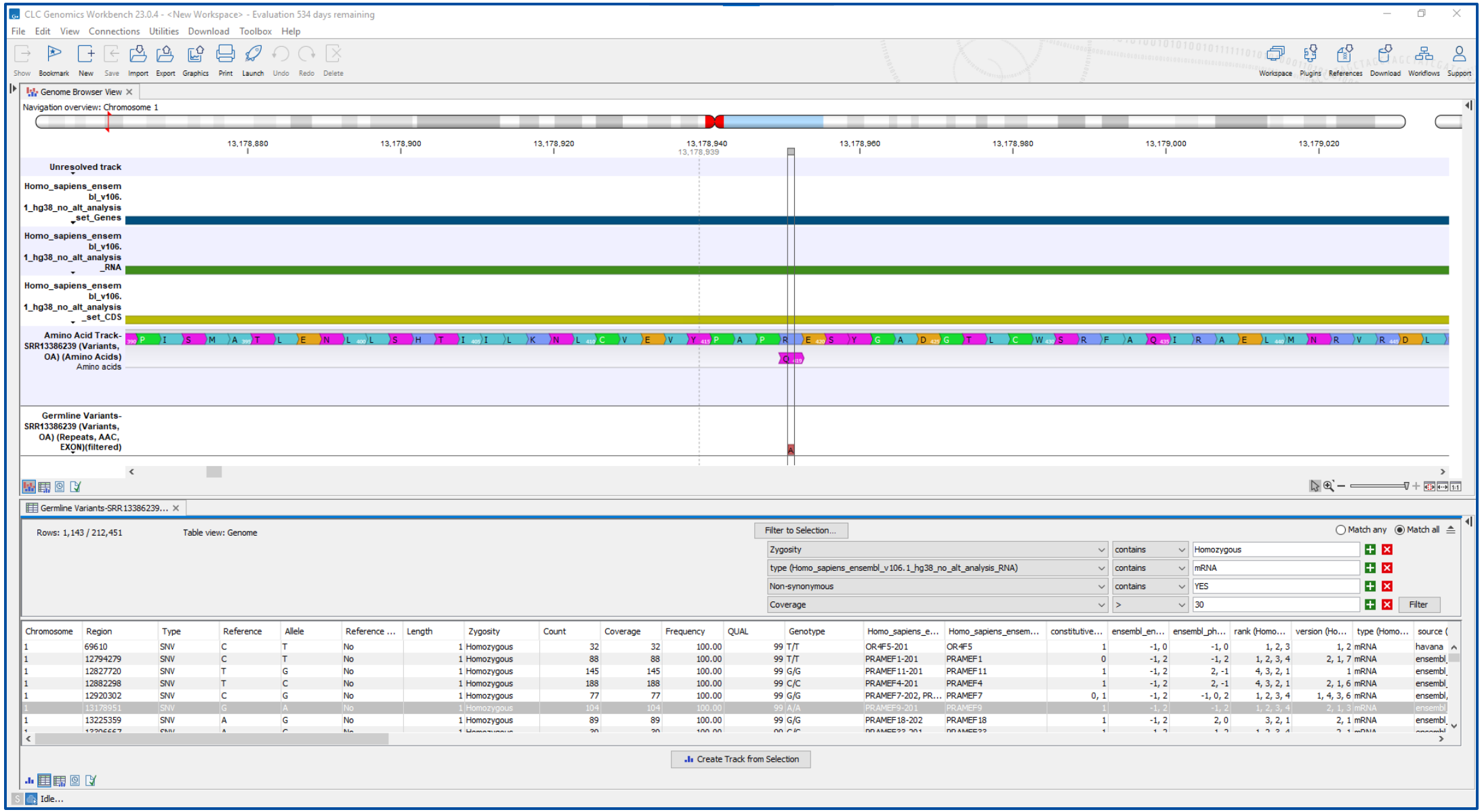
LightSpeed is the fastest and cheapest-to-run hereditary FASTQ-to-VCF WGS pipeline available





Less than 20 minutes and 40 cents for 30x WGS on AWS

Beyond exomes: Ultrafast FASTQ to interpretation



mRNA-seq	NGS	dPCR
lncRNAs	NGS	dPCR
miRNA	NGS	dPCR
Microbial DNA	NGS	dPCR
Genomes/ Exomes	NGS	dPCR

Collaborate across technologies for discovery to verification

Join QIAGEN to Speed up and Simplify

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RNA-seq Analysis portal is the bridge between NGS and digital PCR

Analysis Portal | Samples | Help

All Projects > Demo project v4.0

Demo project v4.0

Collection of demo data for exploring RNA-seq Analysis Portal views and reports

Secondary analysis is powered by QIAGEN CLC Genomics Workbench.
Visit our website to download a free trial and unleash the power of user-friendly bioinformatics.

109 samples — Created: 09-03-2023

6 experiments

QIAseq Stranded RNA - Cell line mixtures with FastSelect 12 samples	Done
QIAseq Stranded RNA - Tissue types with FastSelect 6 samples	Done
QIAseq miRNA - Tissue types 6 samples	Done
QIAseq Stranded RNA - FastSelect vs no rRNA depletion 6 samples	Done
QIAseq 3′ UPX - T-cell gene changes after gene knockout 48 samples	Done
QIAseq UPXome RNA Library Kit - Cell line mixtures with FastSelect 31 samples	Done

Create experiment | Send copy of project

QIAseq Stranded RNA - Cell line mixtures with FastSelect

Created: 09-03-2023

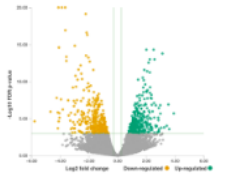
Stranded transcriptome libraries prepared from mixtures of two cell lines, UHRR (Universal Human Reference RNA) and HBRR (Human Brain Reference RNA), using QIAseq Stranded Total RNA Lib Kit. QIAseq FastSelect for depletion of rRNA. Three replicates for each sample mixture.

12 samples: QIAseq Stranded mRNA Select/Total RNA Lib Kit , Homo sapiens (GRCh38.103)

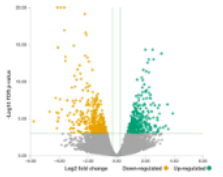
Experiment design: Test differential expression due to Mixture. Compare Against control group (UHRR)

5 analyses, organized by type — Search analyses

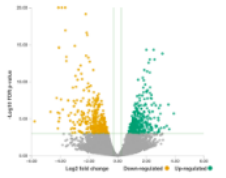
Differential expression: 3UHRR_1HBRR vs. UHRR — Done
Analysis of Mixture: 3UHRR_1HBRR vs. UHRR



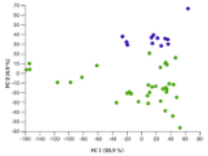
Differential expression: 1_UHRR_3_HBRR vs. UHRR — Done
Analysis of Mixture: 1_UHRR_3_HBRR vs. UHRR



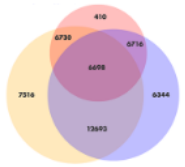
Differential expression: HBRR vs. UHRR — Done
Analysis of Mixture: HBRR vs. UHRR



Experiment summary and QC — Done
Experiment summary, sample review and quality control



Comparison: 3UHRR_1HBRR vs. UHRR, 1_UHRR_3_HBRR vs. UHRR, HBRR vs. UHRR — Done
Venn diagram of differentially expressed features across the differential expression analyses:
- 1_UHRR_3_HBRR vs. UHRR
- 3UHRR_1HBRR vs. UHRR
- HBRR vs. UHRR



Delete experiment | Delete analyses | Compare analyses

Powered by QIAGEN CLC Genomics Workbench and QIAGEN Ingenuity Pathway Analysis — RNA-seq Analysis Portal 4.0 — Samuel Rulli

Yes vs. No

Features: 863 of 36813

Sort by: Name (Asc)

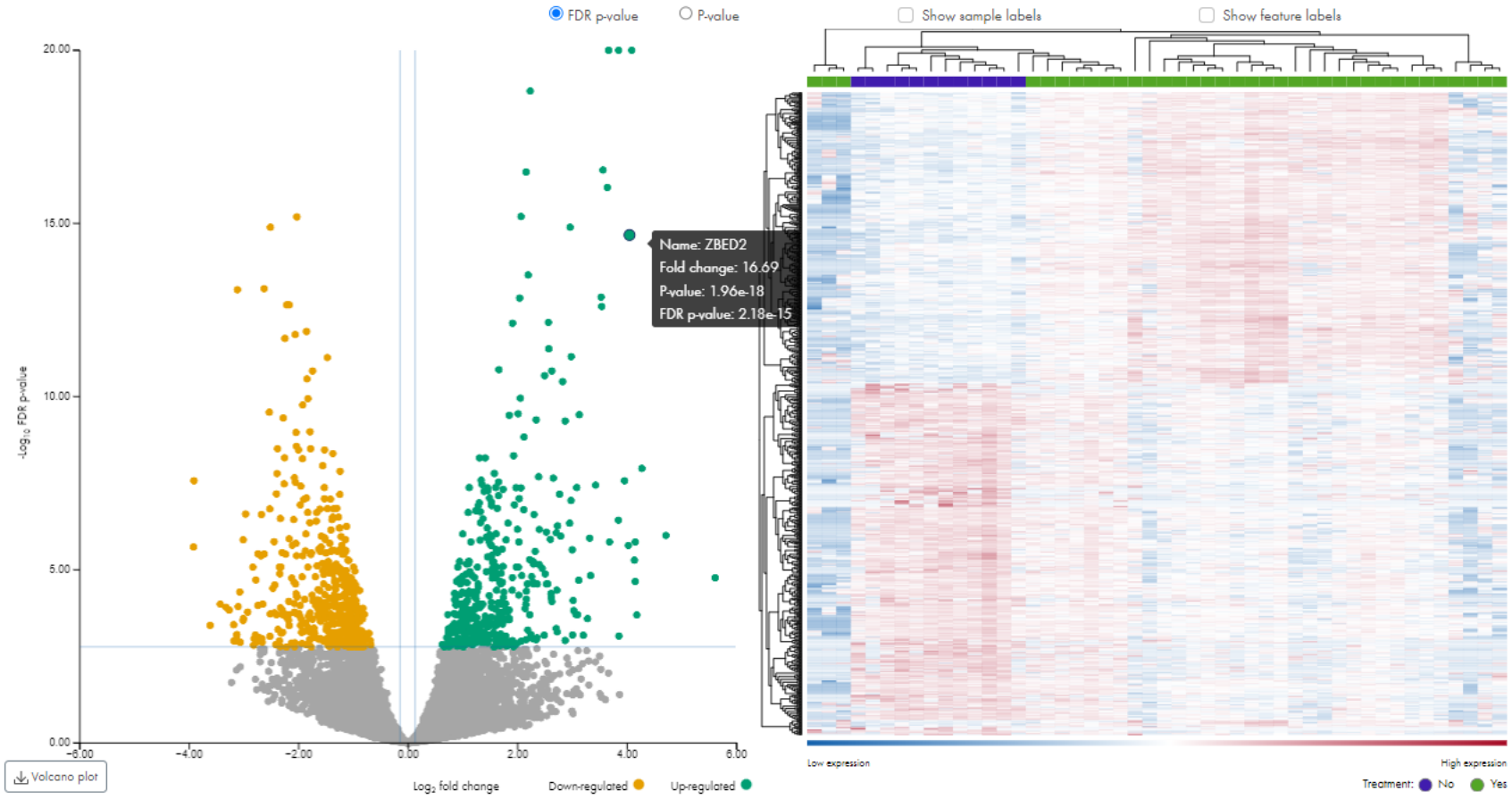
Full feature list

Name	Fold change	FDR p-value	P-value	Biotype
AAK1	-1.69	7.83e-4	3.95e-5	protein_coding
ABCE1	2.31	7.26e-4	3.56e-5	protein_coding
ABHD17A	-2.26	6.27e-4	2.97e-5	protein_coding
ABLIM1	-4.93	1.46e-4	4.94e-6	protein_coding
AC004882.3	-2.48	8.11e-4	4.12e-5	lncRNA
AC005944.1	-2.55	2.17e-5	4.80e-7	lncRNA
AC008875.3	-2.08	1.03e-3	5.56e-5	lncRNA
AC010247.2	-4.99	6.78e-4	3.30e-5	lncRNA
AC018653.3	-8.75	7.42e-4	3.66e-5	lncRNA
AC022706.1	-4.17	2.97e-8	1.58e-10	lncRNA

Biological insights: Canonical pathways

Pathway	-log10 of p-value	z-score
Pathogen Induced Cytokine Storm Signaling Pathway	6.955	2.082
Multiple Sclerosis Signaling Pathway	6.762	-
Differential Regulation of Cytokine Production in Intes...	5.622	2.828
NOD1/2 Signaling Pathway	5.405	1.043
Granulocyte Adhesion and Diapedesis	5.405	-
Role of Hypercytokinemia/hyperchemokinemia in the...	4.943	1.069
Erythropoietin Signaling Pathway	4.821	-2.236
Cell Cycle Control of Chromosomal Replication	4.799	3.317
HMGB1 Signaling	4.680	2.000
TNFR2 Signaling	4.434	1.000

View details



Filters

FDR p-value ≤ 1.71e-3 | Fold change < -1.1 | Fold change > 1.1 | Reset

Biological insights filter: None selected

Advanced filters

Filter not saved

Save

What's next?

Design follow-up experiments in GeneGlobe.

Start now

Differential Gene Expression & statistics

Yes vs. No

Features: 863 of 36813

Sort by: Name (Asc) Full feature list

Name	Fold change	FDR p-value	P-value	Biotype
AAK1	-1.69	7.83e-4	3.95e-5	protein_coding
ABCE1				ding
ABHD1				ding
ABLIM				ding
AC004				RNA
AC005				RNA
AC008				RNA
AC010247.2	-4.99	6.78e-4	3.30e-5	lncRNA
AC018653.3	-8.75	7.42e-4	3.66e-5	lncRNA
AC022706.1	-4.17	2.97e-8	1.58e-10	lncRNA

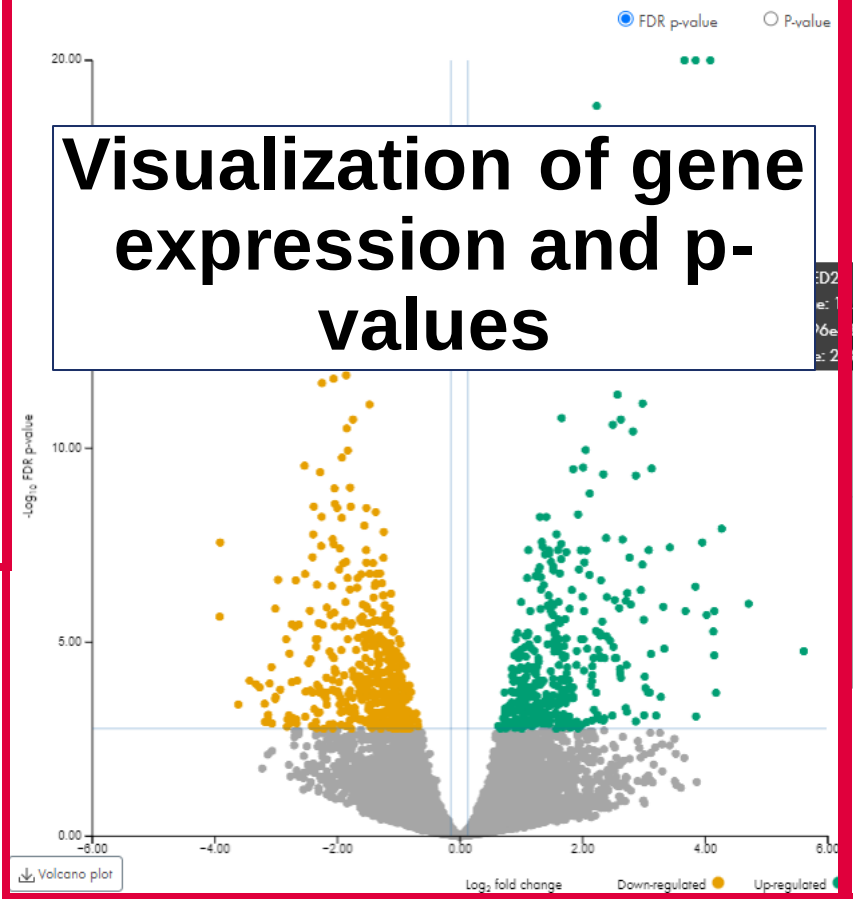
Biological insights Canonical pathways

Ingenuity Pathway Analysis

Pathway	-log10 of p-value	z-score
Role of Hypercytokinemia/hyperchemokinemia in the...	4.943	1.069
Erythropoietin Signaling Pathway	4.821	-2.236
Cell Cycle Control of Chromosomal Replication	4.799	3.317
HMGB1 Signaling	4.680	2.000
TNFR2 Signaling	4.434	1.000

View details

Visualization of gene expression and p-values

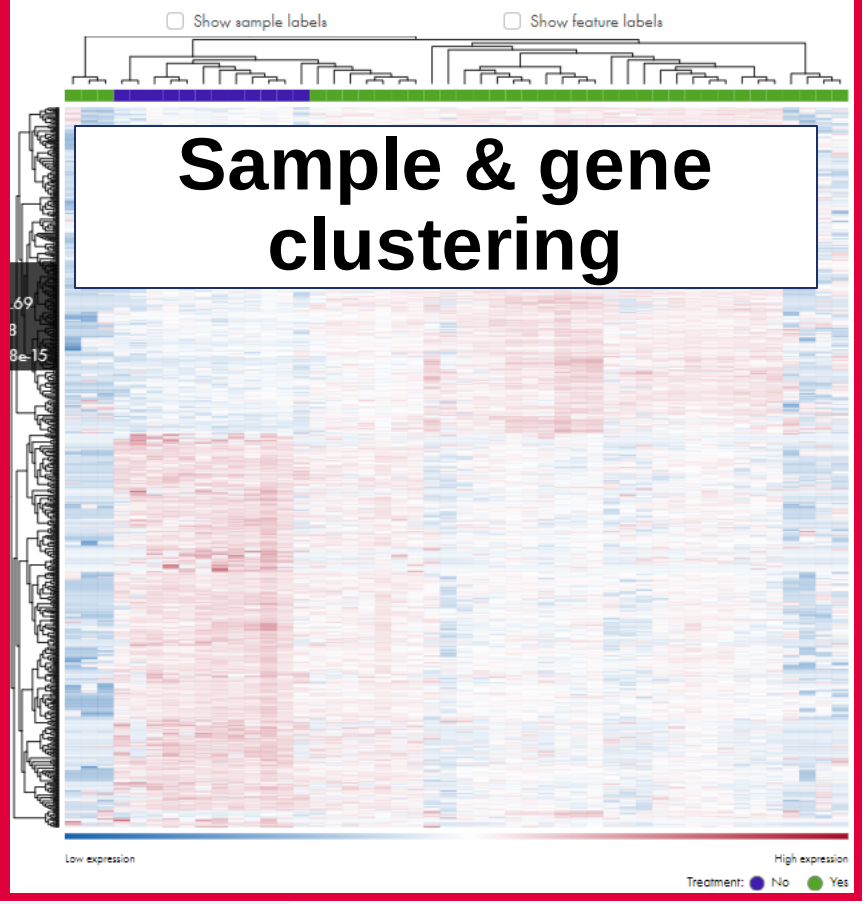


Filters FDR p-value ≤ 1.71e-3 Fold change < -1.1 Fold change > 1.1 Reset

Biological insights filter: None selected Advanced filters

Filter not saved Save

Sample & gene clustering

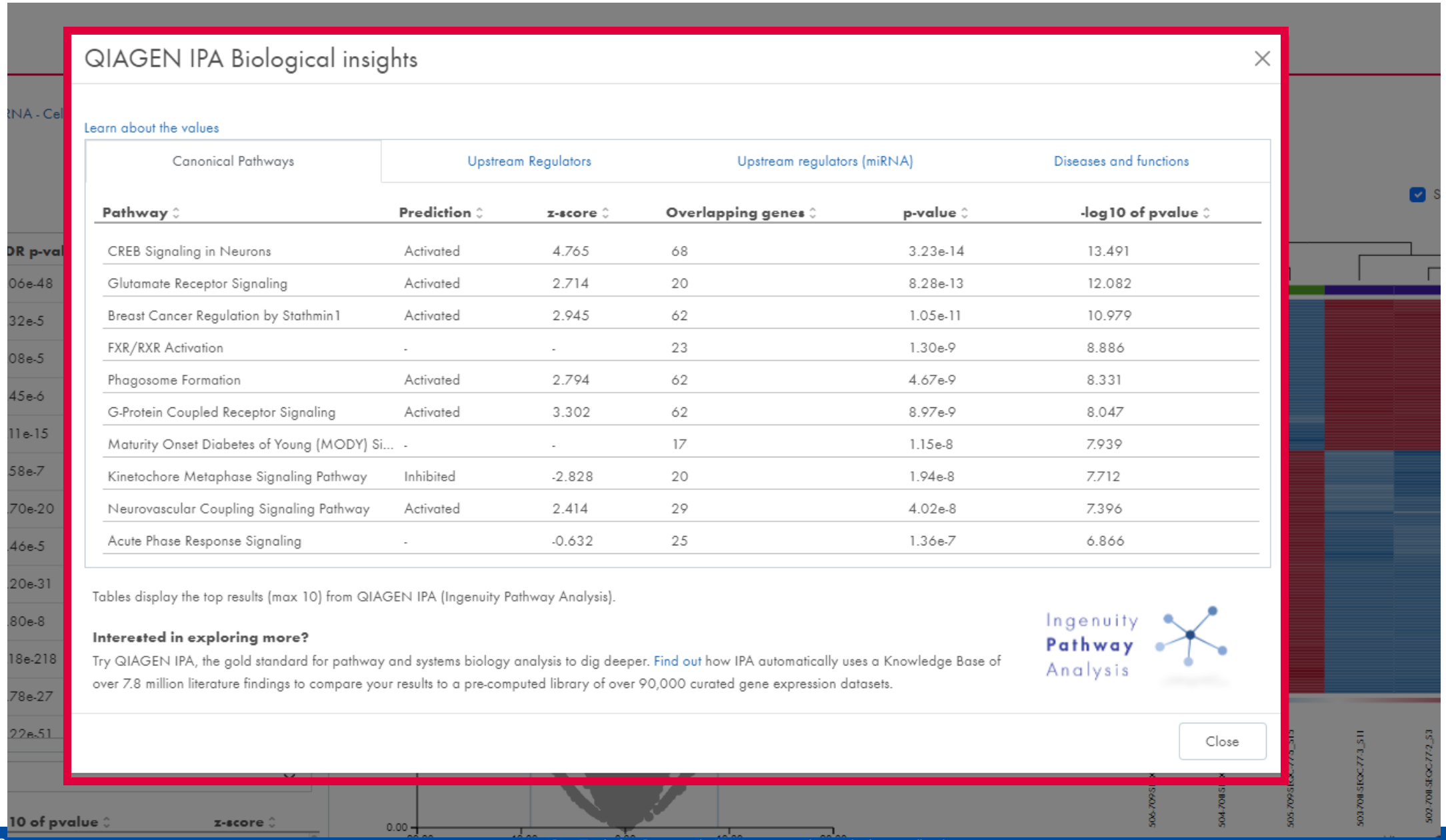


What's next?

Design follow-up experiments in GeneGlobe.

Start now

Use QIAGEN IPA results to pick better biomarkers



QIAGEN IPA Biological insights

Learn about the values

Canonical Pathways | Upstream Regulators | Upstream regulators (miRNA) | Diseases and functions

Pathway	Prediction	z-score	Overlapping genes	p-value	-log10 of pvalue
CREB Signaling in Neurons	Activated	4.765	68	3.23e-14	13.491
Glutamate Receptor Signaling	Activated	2.714	20	8.28e-13	12.082
Breast Cancer Regulation by Stathmin1	Activated	2.945	62	1.05e-11	10.979
FXR/RXR Activation	-	-	23	1.30e-9	8.886
Phagosome Formation	Activated	2.794	62	4.67e-9	8.331
G-Protein Coupled Receptor Signaling	Activated	3.302	62	8.97e-9	8.047
Maturity Onset Diabetes of Young (MODY) Si...	-	-	17	1.15e-8	7.939
Kinetochore Metaphase Signaling Pathway	Inhibited	-2.828	20	1.94e-8	7.712
Neurovascular Coupling Signaling Pathway	Activated	2.414	29	4.02e-8	7.396
Acute Phase Response Signaling	-	-0.632	25	1.36e-7	6.866

Tables display the top results (max 10) from QIAGEN IPA (Ingenuity Pathway Analysis).

Interested in exploring more?

Try QIAGEN IPA, the gold standard for pathway and systems biology analysis to dig deeper. Find out how IPA automatically uses a Knowledge Base of over 7.8 million literature findings to compare your results to a pre-computed library of over 90,000 curated gene expression datasets.

Ingenuity Pathway Analysis

Close

Yes vs. No

Features: 30 of 36813

Sort by: Name (Asc)

Full feature list

Name	Fold change	FDR p-value	P-value	Biotype
AC135048.1	-6.70	3.46e-6	4.97e-8	lncRNA
ADRB2	-15.07	2.68e-8	1.38e-10	protein_coding
CCDC112	-6.46	3.94e-6	5.97e-8	protein_coding
CCDC65	-7.15	8.37e-6	1.45e-7	protein_coding
CCL1	10.72	3.61e-8	2.00e-10	protein_coding
CCL3	16.96	4.80e-23	3.60e-27	protein_coding
CCL3L3	17.74	1.57e-6	1.92e-8	protein_coding
CCL4L2	11.50	1.34e-13	1.61e-16	protein_coding
CD83	16.25	1.99e-6	2.53e-8	protein_coding
EGR1	19.27	1.19e-8	5.33e-11	protein_coding

Biological insights: Canonical pathways

Pathway	-log10 of p-value	z-score
Pathogen Induced Cytokine Storm Signaling Pathway	6.955	2.082
Multiple Sclerosis Signaling Pathway	6.762	-
Differential Regulation of Cytokine Production in Intes...	5.622	2.828
NOD1/2 Signaling Pathway	5.405	1.043
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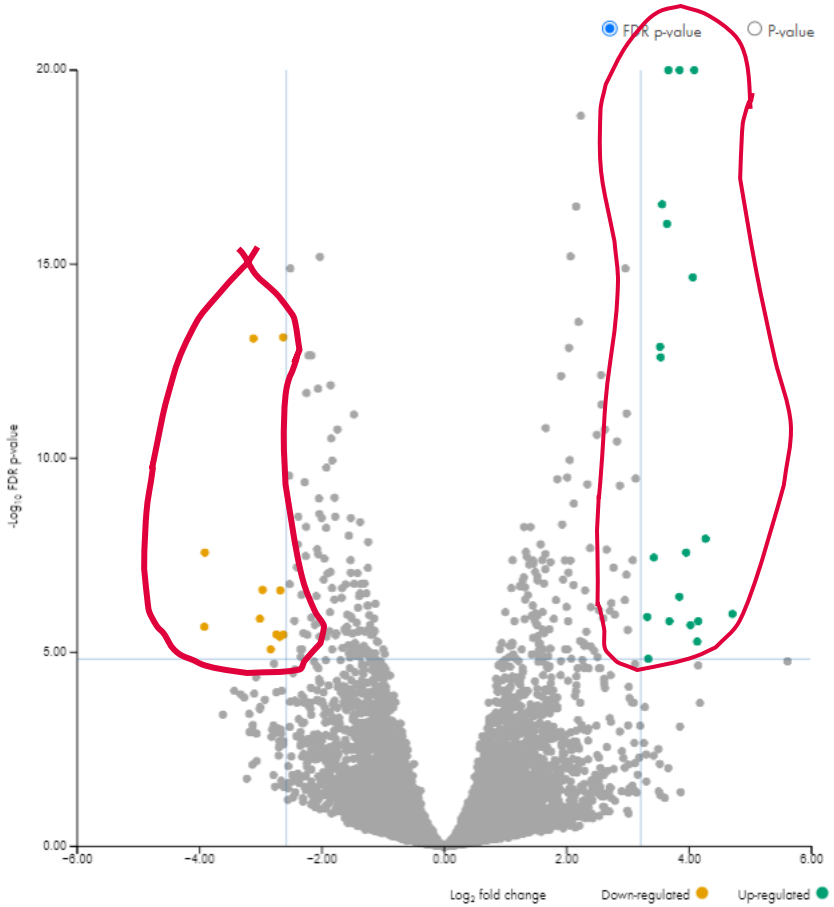
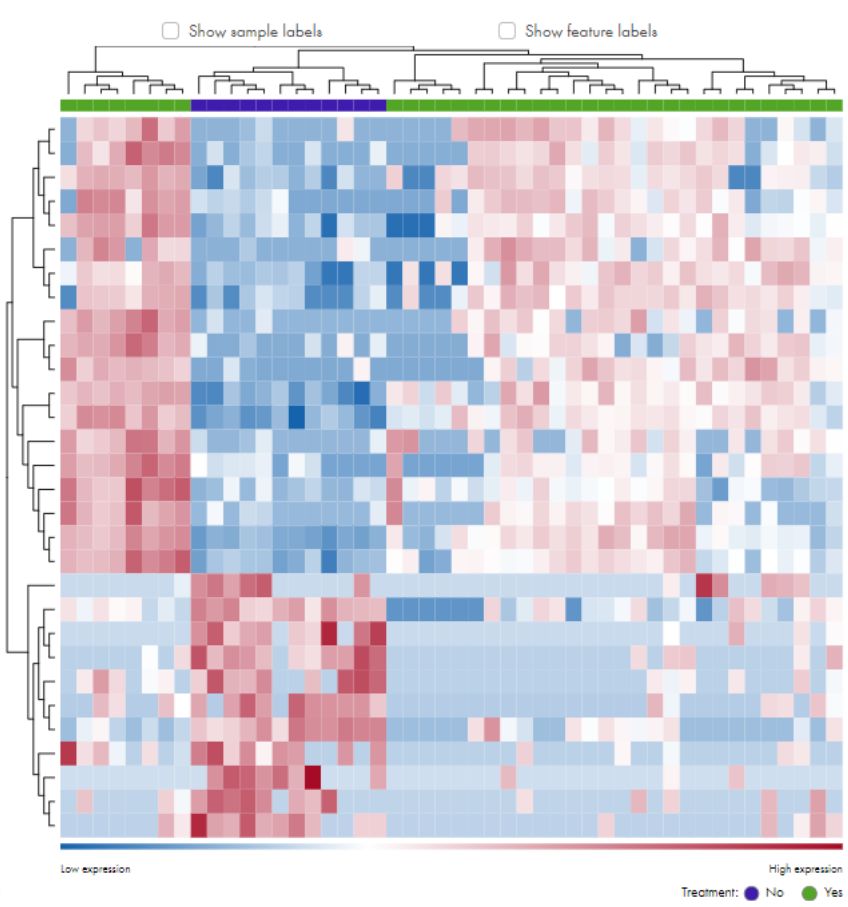
Cell Cycle Control of Chromosomal Replication	4.799	3.317
HMGB1 Signaling	4.680	2.000
TNFR2 Signaling	4.434	1.000

View details

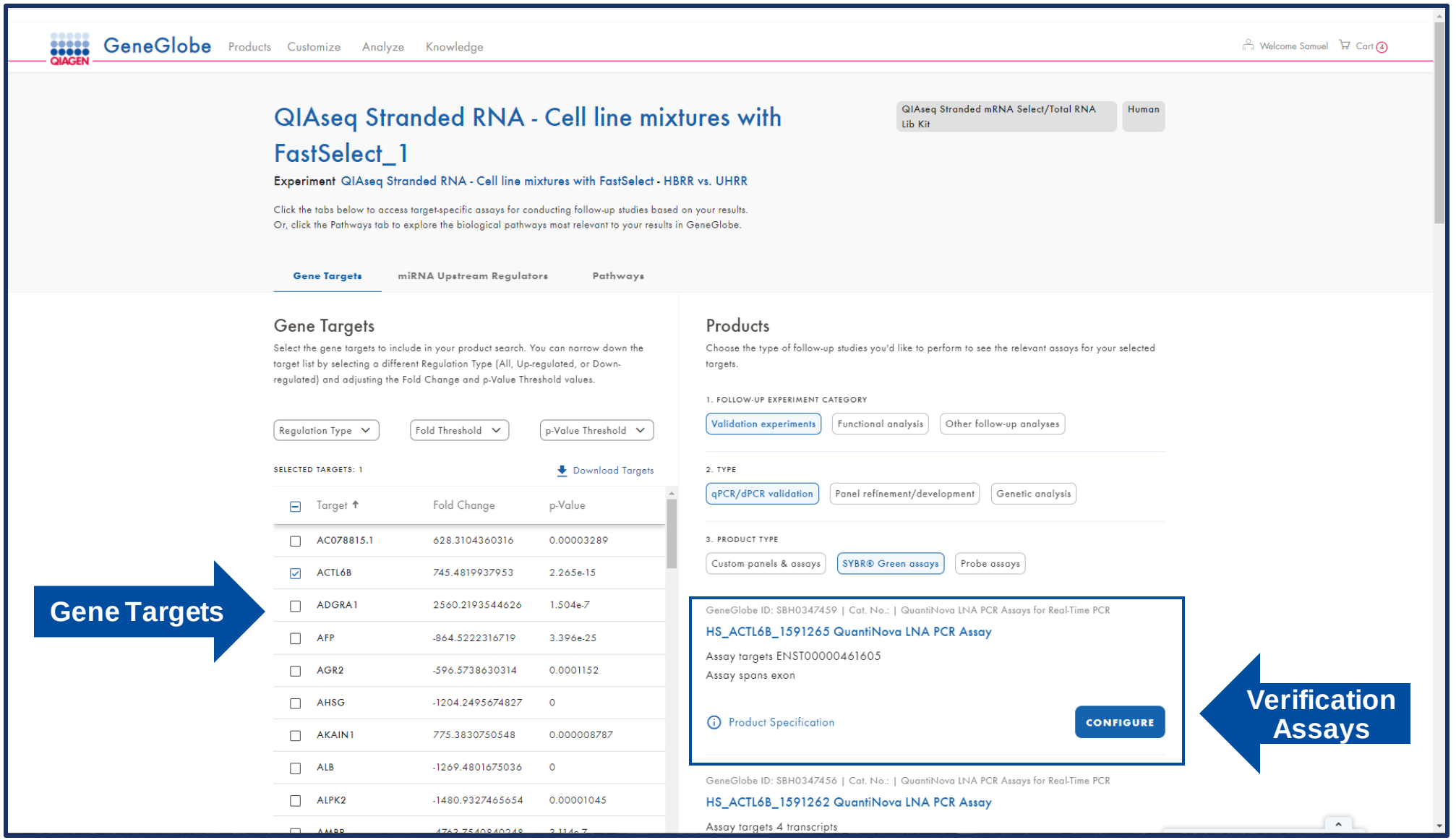




Filters
FDR p-value ≤ 1.53e-5
Fold change < -5.97
Fold change > 9.36
Reset
Biological insights filter: None selected
Advanced filters
Filter not saved
Save

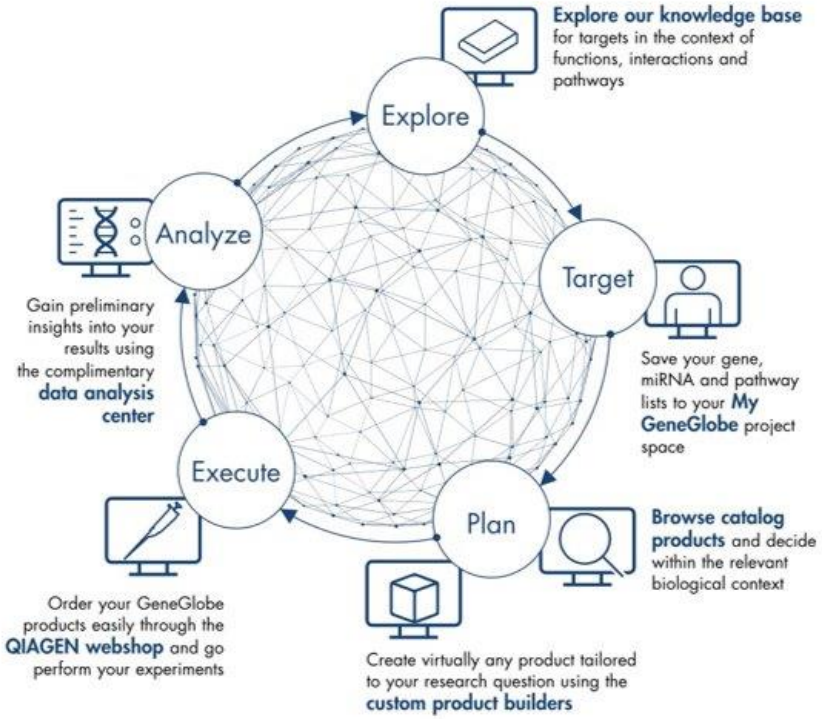
What's next?
Design follow-up experiments in GeneGlobe.
Start now

Return to GeneGlobe to find assays for dPCR or qPCR verifications



QIAcuity Consumables—Assay Content

GeneGlobe Design & Analysis Hub



QIAcuity Digital PCR Assays

- **Infectious Disease (>650)**
- **Mutation Detection (>250)**
- **miRNA/ RNA Expression (>1.3M)**
- **Copy Number Variation (>200)**
- **Microbial Detection**
- **AAV Backbone**
  10 backbone assays

microbialanalysis.qiagen.com/

QIAGEN

Microbial analysis | Samples | Help

All projects > My research project > This is the title I gave to the experiment > Taxonomic profiling

What goes in must come out

482 findings

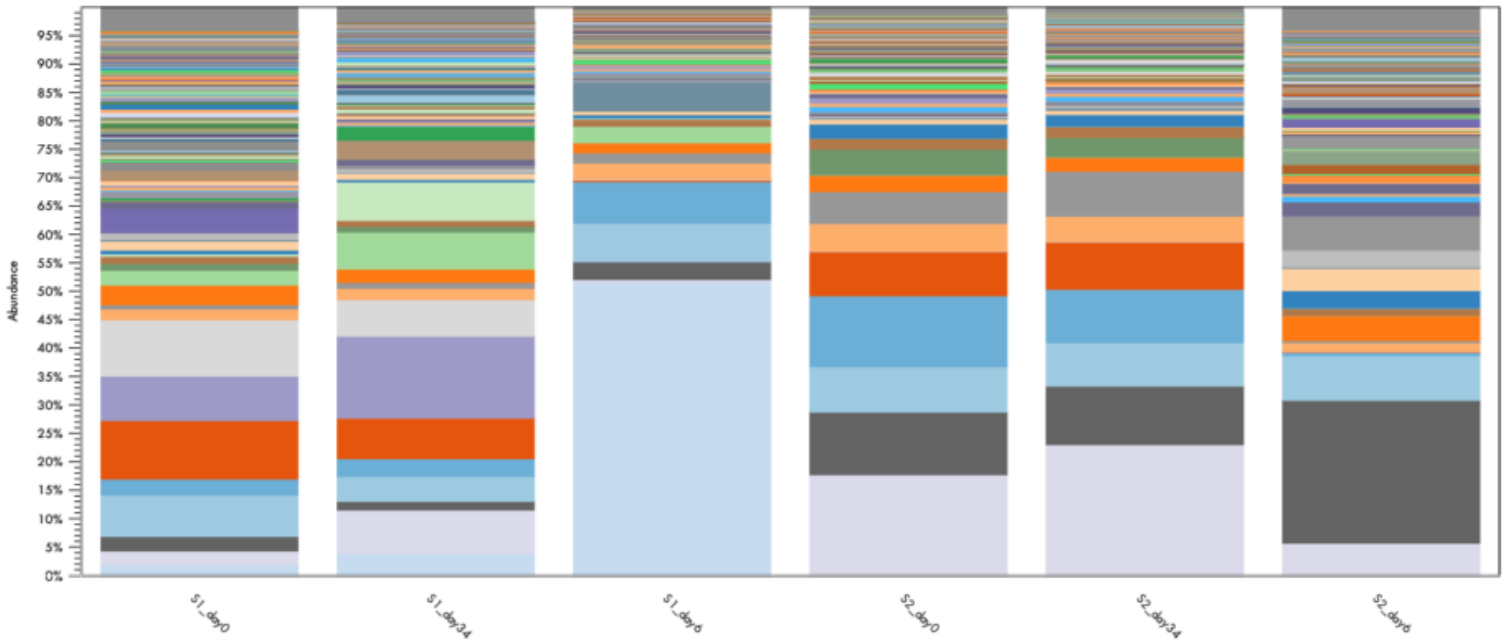
Sort by: Name (Asc)

Full feature list

Name	Assembly ID	Combined Abundance	Min
Alistipes putredinis	MGYG000001302.1	475786	866
Phocaeicola coprocola	MGYG000001306	250911	0
Barnesiella intestinihominis	MGYG000000144	337477	0
Bacteroidales (Unknown)	MGYG000000003	543104	6602
Alistipes shahii	MGYG000001378	65690	0
Bacteria (Unknown)	MGYG000000044	220285	2543
Bacteroides ovatus	MGYG000004797	478320	5222
Parabacteroides merdae	MGYG000001346	181257	422
Phocaeicola sartorii	MGYG000003681	601278	2315
Bacteroides uniformis	MGYG000000196	732217	8336
Bacteroides (Unknown)	MGYG000003844	233718	1405
Bacteroides stercoris	MGYG000004006	814512	540
Bacteroides thetaiotaomicron	MGYG000001361	29570	0
Lachnospiraceae (Unknown)	MGYG000002670	103909	8798
Ruminiclostridium_E sp900539195	MGYG000001415	29099	0
Alistipes sp000434235	MGYG000000254	22791	0
Bacteroidaceae (Unknown)	MGYG000000076	114248	1152
Sutterella wadsworthensis	MGYG000000203	133930	0
Clostridia (Unknown)	MGYG000001345	64240	3047
Agathobacter sp900546625	MGYG000001372	69259	3914
Alistipes communis	MGYG000004003	41761	0
Odoribacter splanchnicus	MGYG000003694	18677	0

Add filter

Taxonomic abundance: aggregated at Gene name/Name



Show legend

Sort samples by: Name

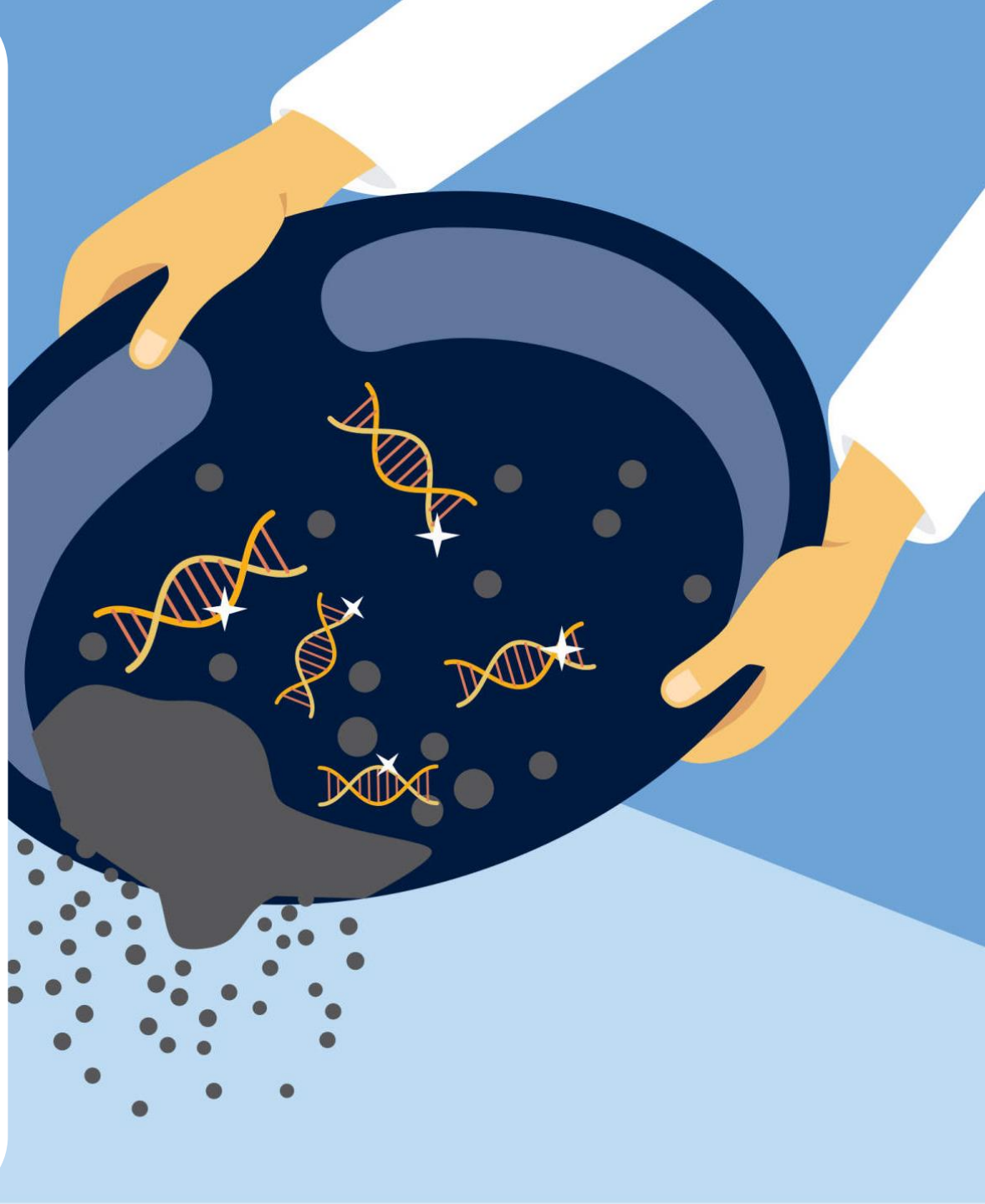
Aggregation

Feature level: Name/species

Sample level: Name

Saved views and filters

Name: Standard view

Save

Confidently detect ultra-rare cell-free DNA variants with QIAseq Targeted cfDNA Ultra Panels