

Discover

- Whole genome and transcriptome
- Solutions for cell-free and ultra-low samples

Target

- Epigenomics, exome and actionable variants
- Disease and custom targeted panels

Verify

- Digital PCR and custom assays
- Digital insights for data and pathway analysis

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





QIAseq portfolio by Analyte



m Viral /Bac Hum an DNA & RNA Analyte Viral teria DNA **RNA** Methylated DNA cfDNA R **RNA** RN A+D NA Ν A VVhw hole Tran scrip ome Ampl ificati on (WT A) Library, e Gen Amp ificat on (WG A) Tar rRN A Targ eted Sin gle Cel Hybrid Capture get ed Pan **Enrichment** & Libi ary Kit Library Targeted Targeted Panel Library Kits rem oval Targeted Panels gle Cell Pan Amplification Panels Panels el Kits **Kits** els QIA QIA seq Sing le Cell DN A Libr QIA QIA QIA seq Targ eted DN A Pan QIA seq mult i-mod al Pan QIA QIA QIA seq Targ eted cfD NA pan el QIA seq Stra nde d mR NA seq SAR S CO V2 Prim er Pan QIA seq xHy b QIA seq cfD NA libra ry Kit seq QIA seq miR NA Libr ary Kit QIA seq ULI Libr ary Kit seq xHY B Hu seq FX DN QIA QIA QIA QIA seq Fusi on XP QIA seq Stra nde d Sing le Cell RN A Lib QIA seq Fast Sele QIA seq Fast Sele seq Dire ct SAR S-C0V seq Targ eted RN A QIA seq 3' UPX QIA seq UPX ome Repl i-g WG A QIA seq Met hyl seq Met hyl Libr QIA seq TCR Repl i-g WT A QIA QIA seq 16S /IT S **Product Lines** seq UPX 3' seq xHY micr obia A Libr В man Exo -seq ct ct ary ary Pan ary Kits els els Kits me Kit els el



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 Ki Workflow Time	t
rRNA Removal + RNA fragmentation	16 minutes	
cDNA synthesis	90 minutes	
QIAseq bead cleanup	45 minutes	5 hours
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	RNA to library pooling
QIAseq bead cleanup	45 minutes	
Library Normalization with QIAseq Normalizer	30 minutes – 45 minutes	
Sequence Ready libraries	@ 4nM concentration	

QIAseq FastSelect supports model organisms and more





QIAGEN

QIAseq FastSelect supports model organisms and more





QIAGEN

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



QIAGEN

Fas use

0





Protocol

Application of Oxford Nanopore Technology to Plant Virus Detection

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

Plant Health and Environment Laboratory, Ministry for Primary Industries, P.O. Box 2095, Auckland 1140, New Zealand; David.Waite@mpi.govt.nz

* 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.

For molecular biology applications Not intended for diagonatic purposes

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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



QIΔGEN

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



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Sequence Ready libraries	@ 4nM concentration	

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



OIAGEN

QIAseq Normalizer: Bind, wash, elute





Modification is always required for normalization:



Beads contained in the Normalization Reagent

Limited bead capacity

library molecules

Wash away unbound

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

QIAGEN

Normalization of QIAseq FX DNA libraries

Library representation





- ----Sequence reads (%)
- Target reads (%)



*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



-Target read %

Library representation



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





		Before normalization (qPCR of native library)	Sequence reads (qPCR/dilute/pool)	Sequence reads (QIAseq Normalizer)
The QIAseq Normalizer matches the performance of the	CV%	36.4 %	14.8 %	12.4 %
"qPCR+dilute" gold standard with minimized hands-on time	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

Normalization library concentration (nmol/L)



Pre-normalization library concentration

- post-normalization library concentration (nmol/l, qPCR)
- ----- % sequence reads
- ----Target read %

Sequence reads (%)



Before normalization

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

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



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Sequence Ready libraries	@ 4nM concentration	

Simplify your library preparation with 1 kit for most applications



RNAseq Analysis Portal access



Simplify your library preparation with 1 kit for most applications



RNAseq Analysis Portal access

Low-input RNA



cDNA Sample Barcoding

The ultimate in RNA library prep versatility

> QIAseq FastSelect • RNA Library Kit

> > 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





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

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.

-12 -10 -8 -6 -4 -2 0 2 4 6 10 12 14 log2 fold change MEvsME

MEvsEE



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



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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

CIAGEN Analysis Portal Samples) Help
All Projects > Demo project v4.0 Demo project v4.0 @ Collection of demo data for exploring RNA-sea Analysis Portal views and reports @			Secondary and <u>Visit our website</u> to download a free t	lysis is powered by QIAGEN CLC Genomics Workbench. rial and unleash the power of user-friendly bioinformatics.
109 samples Created: 09-0	3-2023 QIAseq Stranded RNA - Cell lin	ne mixtures with FastSelect 🗹		Created: 09-03-2023
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Create experiment Send copy of project	Delete experiment Delete analyses			Compare analyses

owered by QIAGEN CLC Genomics Workbench and QIAGEN Ingenuity Pathway Analysis

All Projects > Demo project v4.0 > QIAseq 3' UPX - T-cell gene changes after gen... > Yes vs. No

Yes vs. No



Powered by QIAGEN CLC Genomics Workbench and QIAGEN Ingenuity Pathway Analysis



All Projects > Demo project v4.0 > QIAseq 3' UPX - T-cell gene changes after gen... > Yes vs. No



Powered by QIAGEN CLC Genomics Workbench and QIAGEN Ingenuity Pathway Analysis

Use QIAGEN IPA results to pick better biomarkers

QIAGEN IPA Biological insights

NA - Cel

20e-31

80e-8

. 78e-27

22e-51

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arn about the values					
Canonical Pathways	Upstree	im Regulators	Upstream regulators	Diseases and functions	
Pathway 🗘	Prediction 0	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) S	i	-	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).

0.00 -

Interested in exploring more?

z-score

 18e-218
 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.





Close

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October 30, 2023

All Projects > Demo project v4.0 > QIAseq 3' UPX - T-cell gene changes after gen... > Yes vs. No

Yes vs. No

Features: 30 of 36813 🕁 Full feature list 🛛 🏙 🖽 Sort by: Name (Asc) 🔻 Fold change FDR p-value Name P-value Biotype AC135048.1 -6.70 3.46e-6 4.97e-8 IncRNA -15.07 1.38e-10 protein_coding ADRB2 2.68e-8 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 17.74 1.57e-6 1.92e-8 CCL3L3 protein_coding 1.61e-16 CCL4L2 11.50 1.34e-13 protein_coding 2.53e-8 CD83 16.25 1.99e-6 protein_coding EGR1 19.27 1.19e-8 5.33e-11 protein codina Biological insights Canonical pathways \sim Pathway -log10 of p-value 0 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 4.434 1.000 TNFR2 Signaling View details



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	Gene Targets Select the gene targets to include in your produ- target list by selecting a different Regulation Ty- regulated) and adjusting the Fold Change and Regulation Type V Fold Threshold SELECTED TARGETS: 1 Target T Fold Char	uct search. You can narrow down the pe (All, Up-regulated, or Down- p-Value Threshold values.	Products Choose the type of follow-up studies you'd like to perform to see the relevant assays for your selected targets. 1. FOLLOW-UP EXPERIMENT CATEGORY Validation experiments Functional analysis Other follow-up analyses 2. TYPE QPCR/dPCR validation Panel refinement/development Genetic analysis	
Gene Targets	□ AC078815.1 628.31043 ☑ ACTL6B 745.48199 □ ADGRA1 2560.2193	360316 0.00003289 377953 2.265e-15 3544626 1.504e-7	3. PRODUCT TYPE Custom panels & assays SYBR® Green assays Probe assays GeneGlobe ID: SBH0347459 Cat. No.: QuantiNova LNA PCR Assays for Real-Time PCR	
J	AFP -864.5222	316719 3.396e-25 630314 0.0001152	HS_ACTL6B_1591265 QuantiNova LNA PCR Assay Assay targets ENST00000461605	
	AHSG -1204.249	5674827 0	Product Specification CONFIGURE	Verification
	AKAIN1 775.38303	750548 0.000008787 1675036 0		Assays
	ALPK2 -1480.932	7465654 0.00001045	GeneGlobe ID: SBH0347456 Cat. No.: QuantiNova LNA PCR Assays for Real-Time PCR HS_ACTL6B_1591262 QuantiNova LNA PCR Assay Assay targets 4 transcripts	

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What goes in must come out

482 findings

Sort by:	Name (Asc)	•		\mathbf{A}	Full feature list	
Name		Assembly ID		Combined Abundance Mir		
Alistipe	s putredinis	MGYG000001302.1		475786		866
Phocaeicola coprocola		MGYG000001306		2509	11	0
Barnesiella intestinihominis			GYG000000144	3374	77	0
Bactero	idales (Unknown)	M	GYG00000003	5431	04	6602
Alistipe	s shahii	M	GYG000001378	6569	0	0
Bacterie	a (Unknown)	M	GYG000000044	2202	85	2543
Bactero	ides ovatus	M	GYG000004797	4783	20	5222
Paraba	cteroides merdae	M	GYG000001346	1812.	57	422
Phocae	icola sartorii	M	GYG000003681	6012	78	2315
Bactero	ides uniformis	M	GYG000000196	7322	17	8336
Bactero	ides (Unknown)	MGYG00003844		2337	18	1405
Bactero	ides stercoris	MGYG000004006		8145	12	540
Bactero	ides thetaiotaomicron	MGYG000001361		2957	0	0
Lachno	spiraceae (Unknown)	M	GYG000002670	1039	09	8798
Ruminic	lostridium_E sp900539195	M	GYG000001415	2909	9	0
Alistipe	s sp000434235	M	GYG000000254	2279	1	0
Bactero	idaceae (Unknown)	М	GYG000000076	1142	48	1152:
Sutterel	la wadsworthensis	M	GYG000000203	1339	30	0
Clostric	ia (Unknown)	M	GYG000001345	6424	0	3047
Agatho	bacter sp900546625	M	GYG000001372	6925	9	3914
Alistipe	s communis	M	GYG000004003	4176	1	0
Odorib	acter splanchnicus	M	GYG000003694	1867	7	0

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Taxonomic abundance: aggregated at Gene name/Name



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