Targeted RNAseq for gene expression using unique molecular indexes (UMIs): Introduction to QIAseq Targeted RNA Panels.

Samuel Rulli, Ph. D, Global Product Manager QIAseq Targeted RNA Panels

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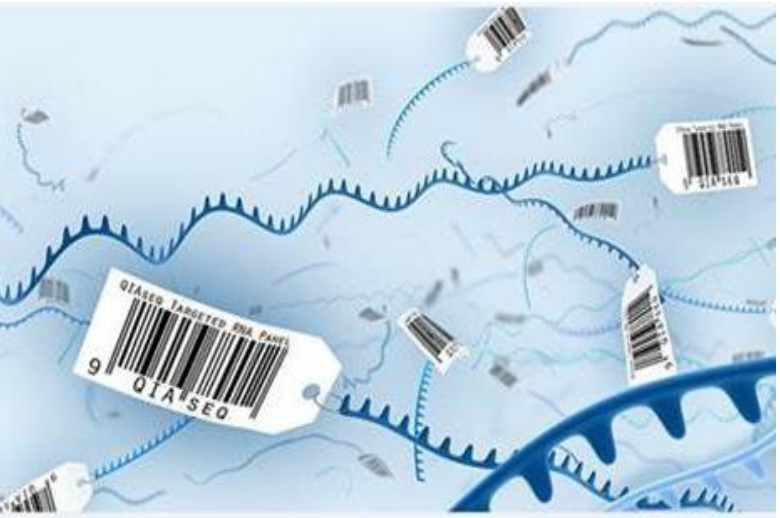
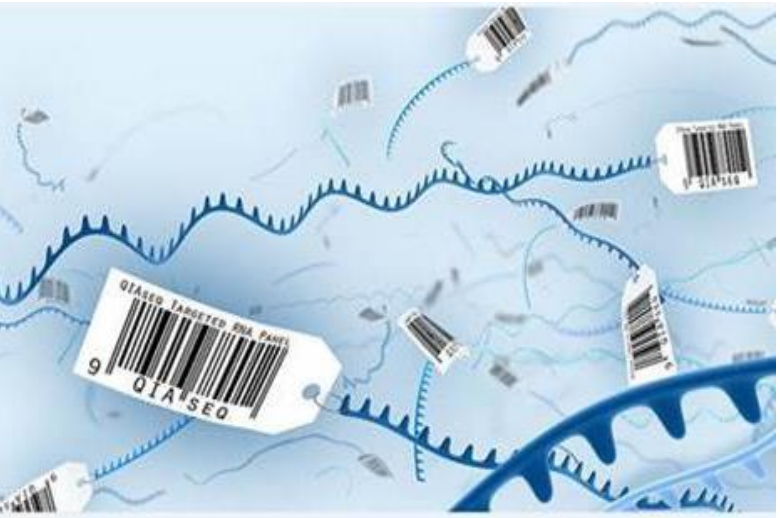
Agenda



1. Using NGS approaches for gene expression analysis
2. Principle of QIAseq Targeted RNAseq
   - 2.1 Molecular Barcodes
   - 2.2 QIAseq RNA workflow
3. An application of the QIAseq RNA system
   - 3.1 QIAseq data analysis
   - 3.2 Ingenuity IPA
4. Uses of UMIs in other types of targeted sequencing
5. Summary and Discussion

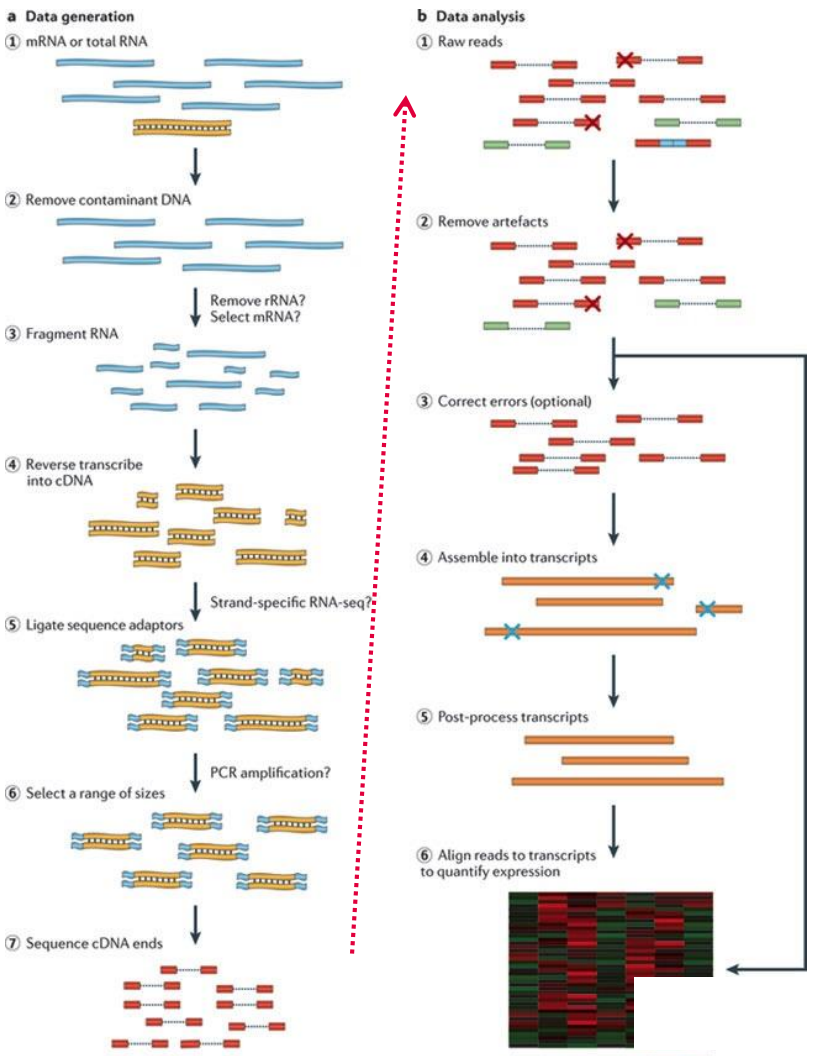
Agenda



Are “reads” the best we can do with sequencing?

Whole transcriptome sequencing for gene expression

- Quantifies and characterizes all RNA
- Final data point in READS per target



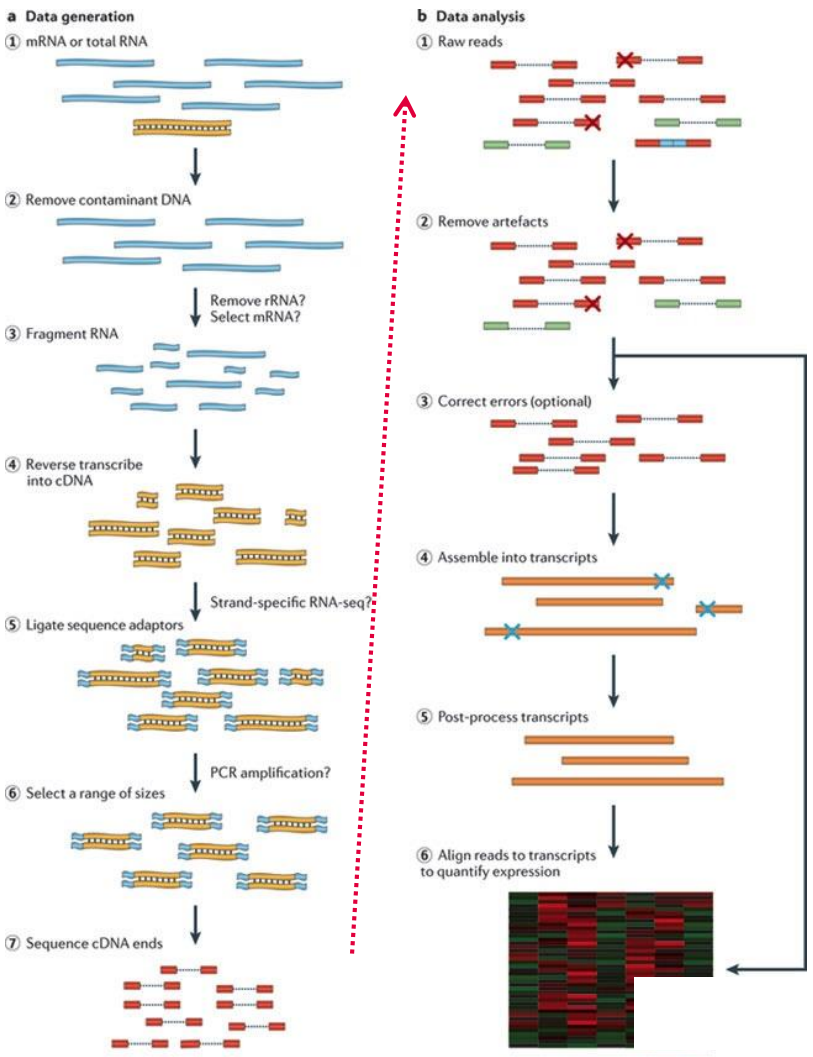
Are “reads” the best we can do with sequencing?

Whole transcriptome sequencing for gene expression

- Quantifies and characterizes all RNA
- Final data point in READS per target

Drawbacks

- Complex library construction
- 1µg of total RNA
- Fails on FFPE & fragmented RNA
- Large computational requirements
  - Massive amount of data generated
  - Filtering, alignment, assembly, curation
  - Aggressive normalization for quantification
  - Not at all straightforward
  - Requires skilled bioinformatics scientists
- Cost
  - Large read budget = money
  - Limits sample numbers in studies
- Only runs on HT instruments
  - Limits accessibility to core labs



Targeted expression analysis by NGS (Targeted Discovery/validation)

What are the advantages of applying ***targeted*** **gene profiling** to NGS?

- Use read budget only for genes of interest
  - Cost
  - Time (quick prep, run, analysis)
  - Sample throughput - multiplex many samples
- Desktop platforms can now be used for RNA analysis (500,000 reads per sample instead of 20,000,000 or more)
- Simplified bioinformatics (no assembly required)
  - Don’t need that bioinformatics guy down the hall
- Minimal sample pre-processing
  - No ribosomal depletion or blocking
  - No polyA selection
  - Only nanogram quantities of RNA required
  - 6 hour sample prep - only need thermocycler and magnet.

When? Who?

- Scientists with known gene list or pathway
- Follow up on WTS or microarray
- Alternative to digital PCR/ Nanostring/Taqman qPCR/ Fluidigm/OpenArray/Wafergen qPCR

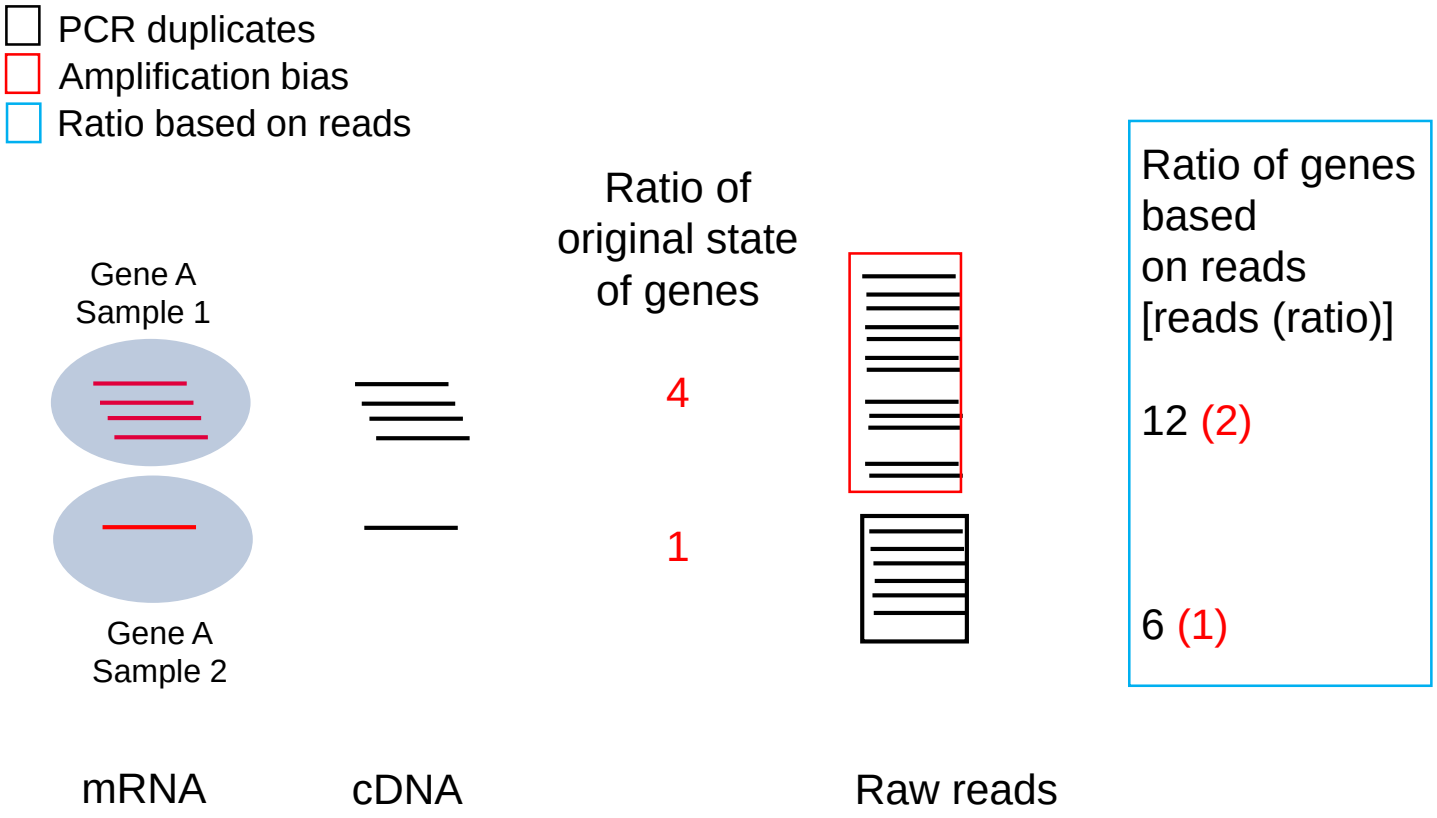
But we still need an improvement….

Targeted RNAseq still is a “read” based approach to understanding gene expression.

How do we go from “reads” to counting transcripts?

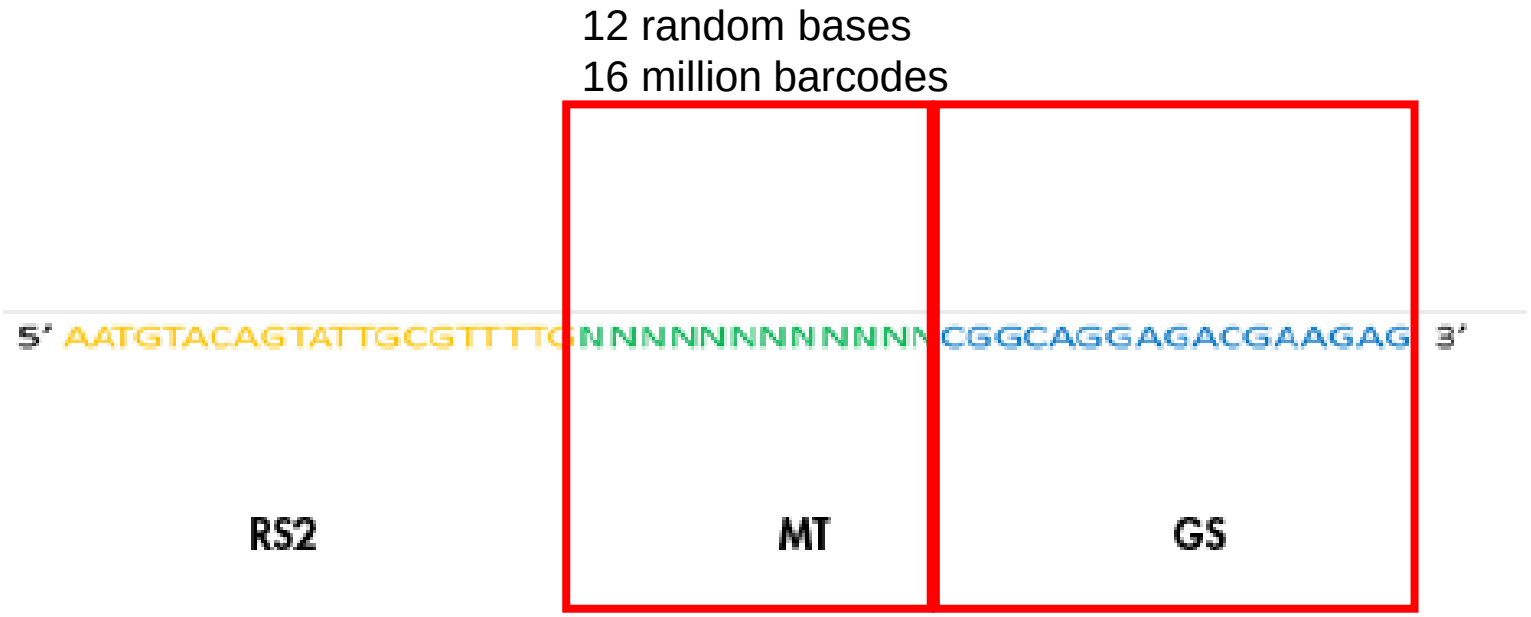
Counting reads during sequencing

PCR duplicates and amplification bias are major issues in current RNAseq workflows, as they result in biased and inaccurate gene expression profiles



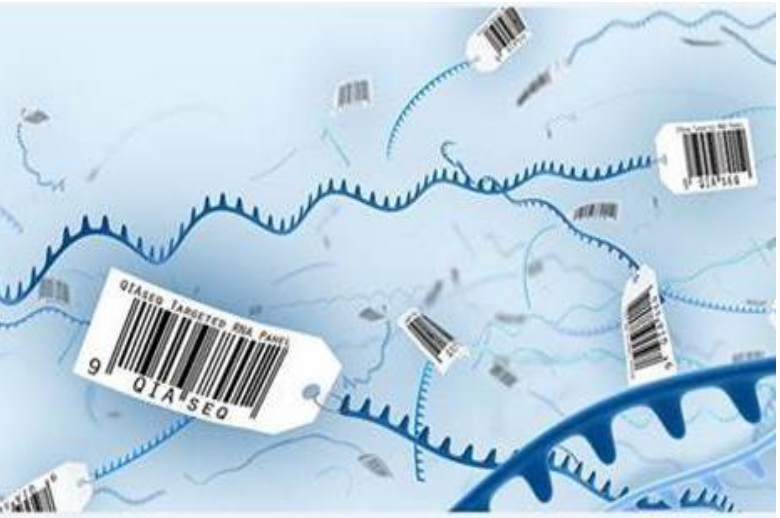
But we still need an improvement….

Molecular barcodes each “capture” event



Use approximately 1/20th of total number of primers in a reaction - each gene specific primer is statistically unique!

Agenda

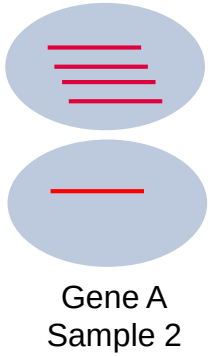


Digital sequencing by molecular barcodes for accuracy

Molecular barcodes allow the counting of original gene levels instead of PCR duplicates, thereby enabling digital sequencing and resulting in unbiased and accurate gene expression profiles

Tag each gene with unique molecular barcodes

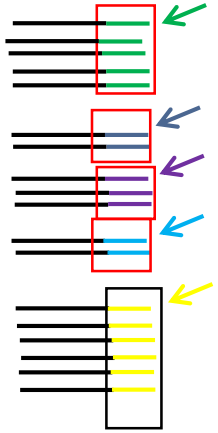
Count unique barcodes, not reads



Ratio of original state of genes

4

1



Ratio of genes based on barcodes

4

1

Ratio of genes based on reads [reads (ratio)]

12 (2)

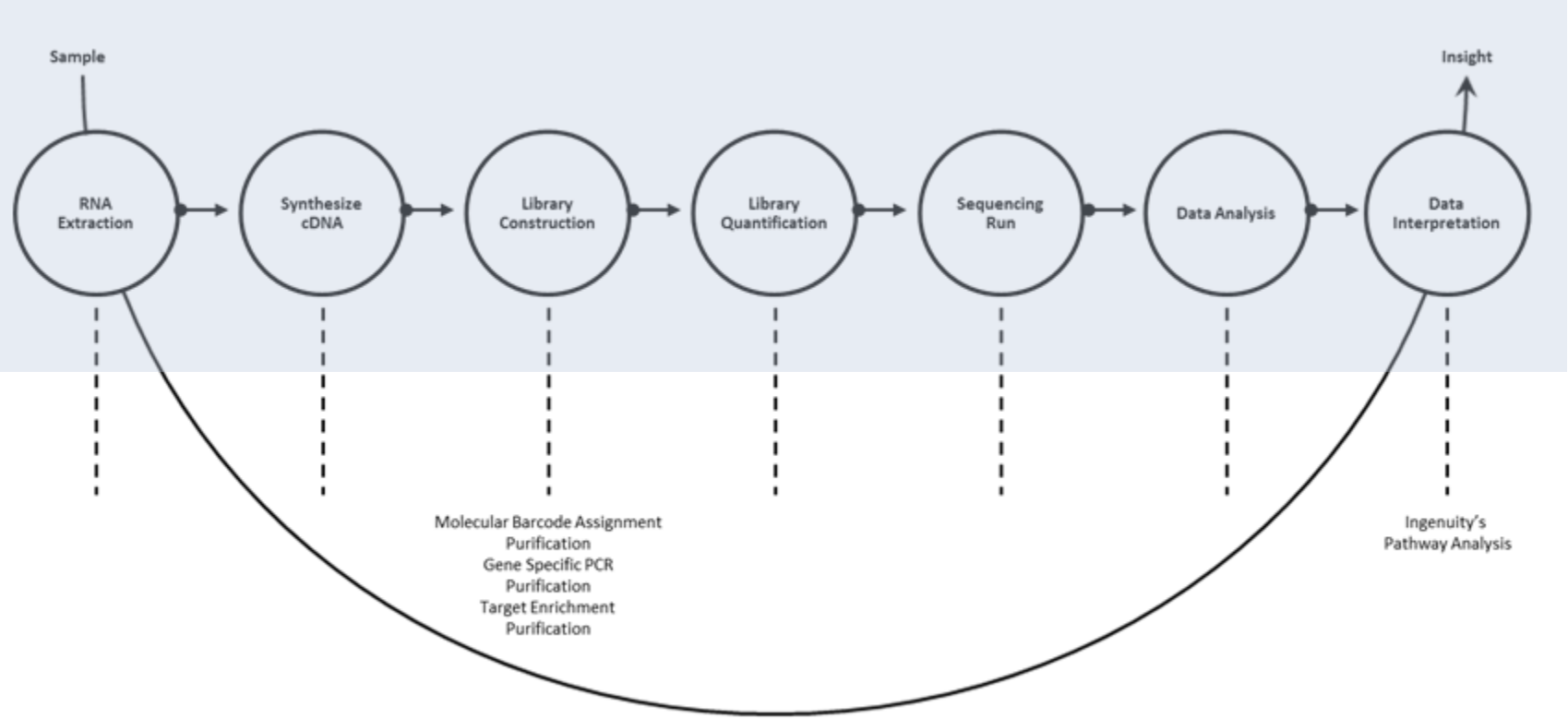
6 (1)

mRNA

cDNA

Barcode reads

QIAseq workflow: *From Sample to Insight*



"Leave no scientist behind"….

QIAseq workflow: *From Sample to Insight*

Included in Panel Kit

Index Kit

Library Quant Kit

Included in cloud

Sample

RNA Extraction

Synthesize cDNA

Library Construction

Library Quantification

Sequencing Run

Data Analysis

Data Interpretation

Insight

Molecular Barcode Assignment
Purification
Gene Specific PCR
Purification
Target Enrichment
Purification

Ingenuity's Pathway Analysis

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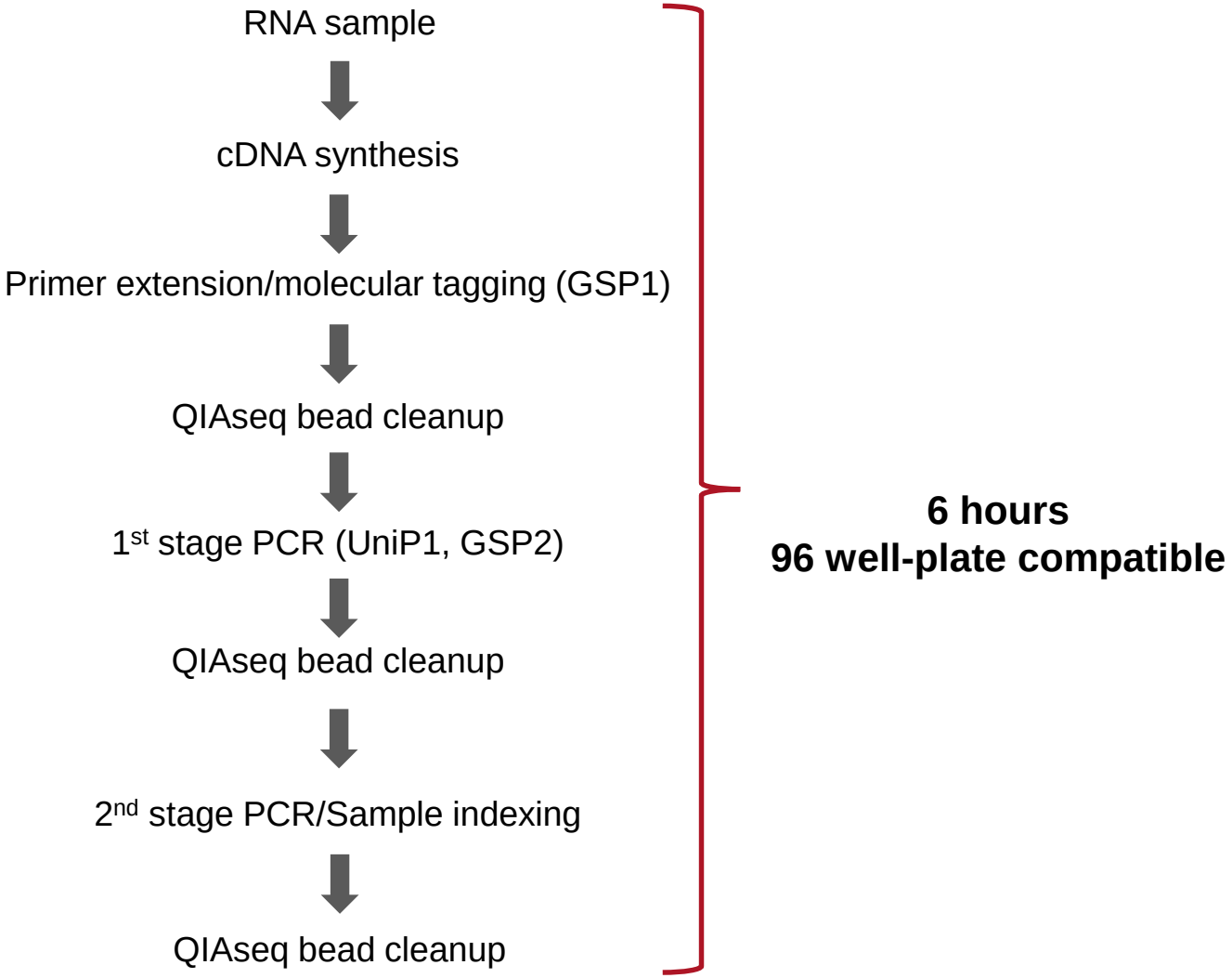
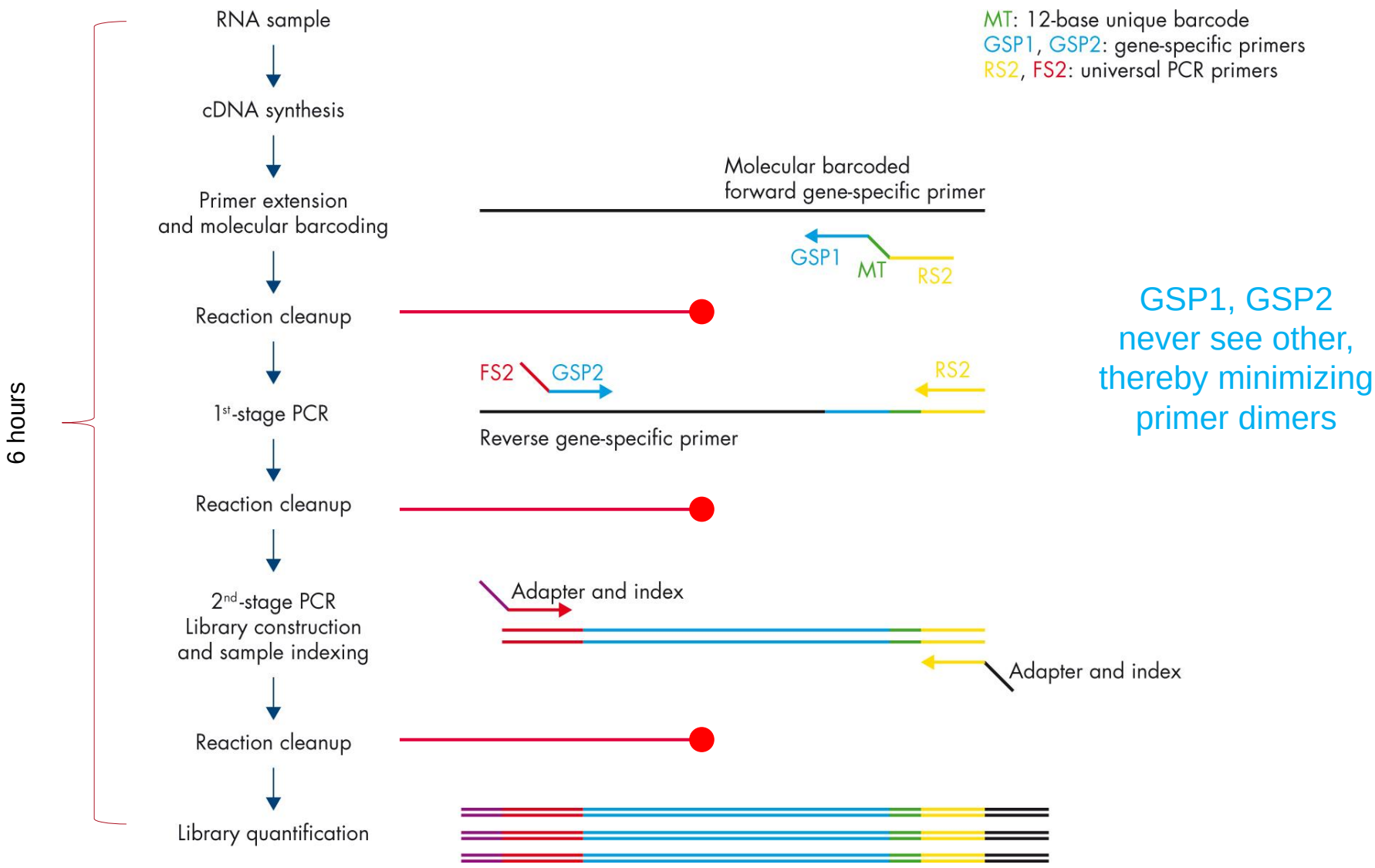
Ingenuity's Pathway Analysis

QIAseq targeted RNA 2-stage PCR workflow

Everything needed to go from RNA → Library in <u>one kit</u>, <u>one day</u>!



‘Simple’ procedure, integrated library preparation

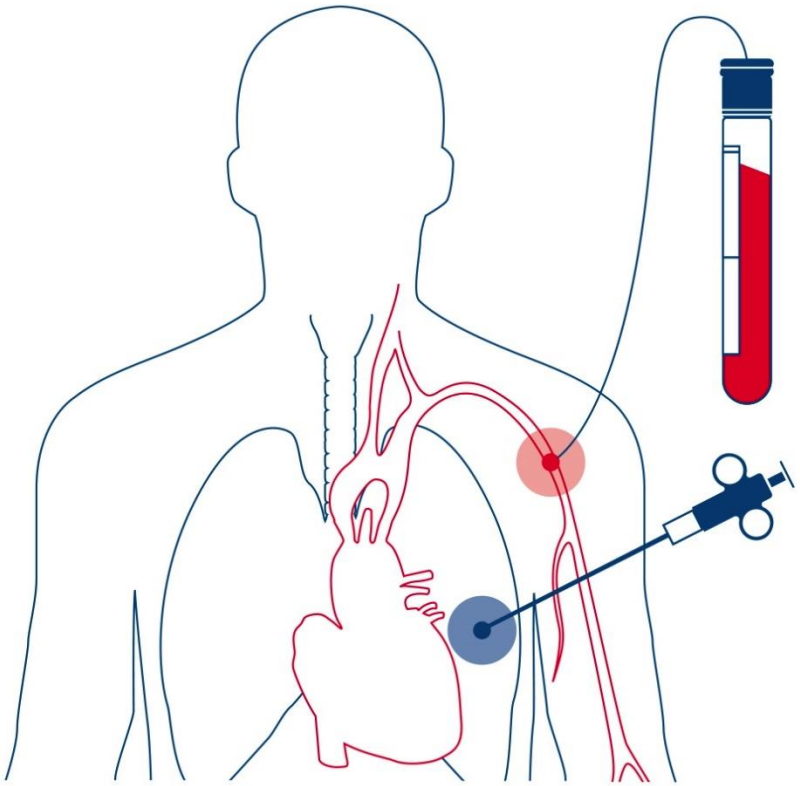


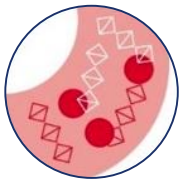
GSP1, GSP2 never see other, thereby minimizing primer dimers

Experimental setup & things to consider

Customer Criteria	Differential Gene Expression By QIAseq NGS
Species coverage	Human - Catalog, Extended, Virtual, Custom panels Mouse + Rat - Custom
Biological replicates	Essential for robustness of experimental design (and statistics!)
Short reads for FFPE, and Exosomal RNA	Average amplicon 97 bps'; range 95-130 bases
Coverage across the transcript (i.e. cover every exon)	We are counting single common regions per gene. Same design philosophy as RT2 PCR Arrays
Depth of sequencing	High enough to infer accurate statistics *determined by Smcounter - ~2-5 reads per random barcode*
Role of sequencing depth	Capture enough unique tags of each transcript such that statistical inferences can be made (>10 tags per gene)
Stranded library prep	Not required, amplicons do not overlap lncRNA
Type of reads (paired or Unpaired?)	Not necessary, 150 base single reads more than enough for accurate data
mRNA and lncRNAs	Qiaseq was designed against database containing lncRNA and mRNA. Assay are specific for lncRNA or mRNA. Currently 54,881 genes from Ensembl version 81

Access RNA from any sample





Free circulating nucleic acids

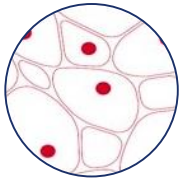
RNA and DNA from dead cells shed into the bloodstream, can contain cancer-related mutations.

Exosomes

Tiny microvesicles found in body fluids that transport RNA between cells.

Circulating tumor cells

Tumor cells shed from a tumor into the bloodstream carrying genetic information.

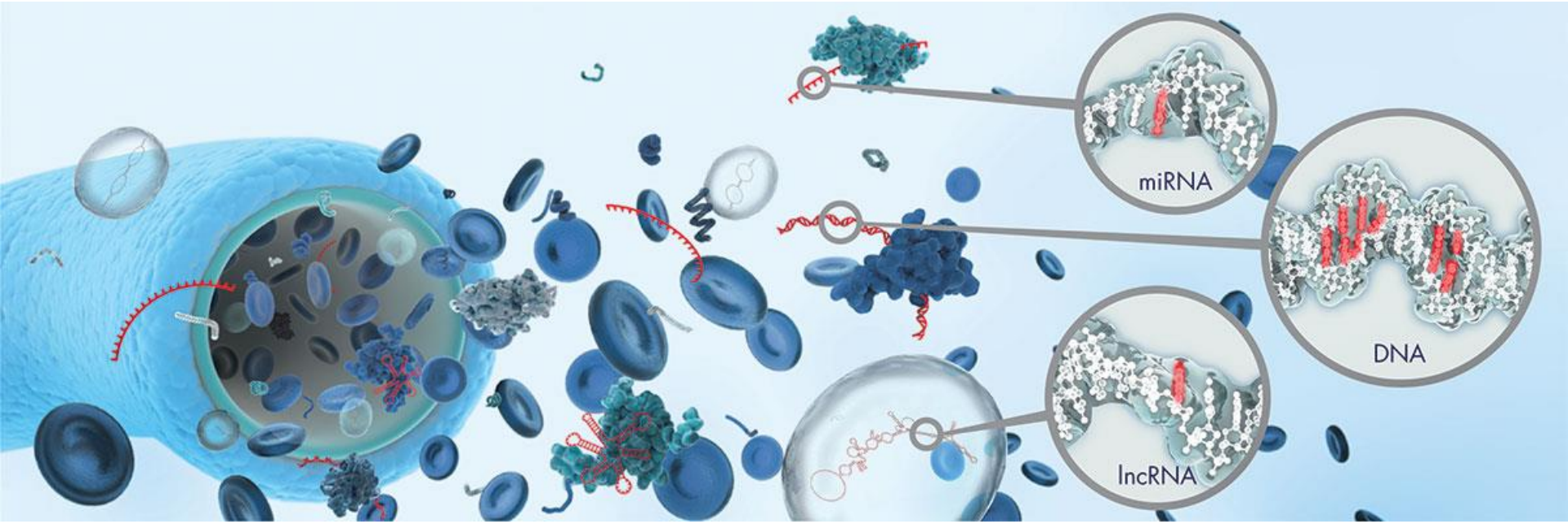


Tissue samples

Fresh tissue or archived **FFPE** samples

QIAGEN comprehensive sample isolation portfolio compatible with QIAseq RNA

Liquid biopsy: Non-invasive approaches to diagnostics



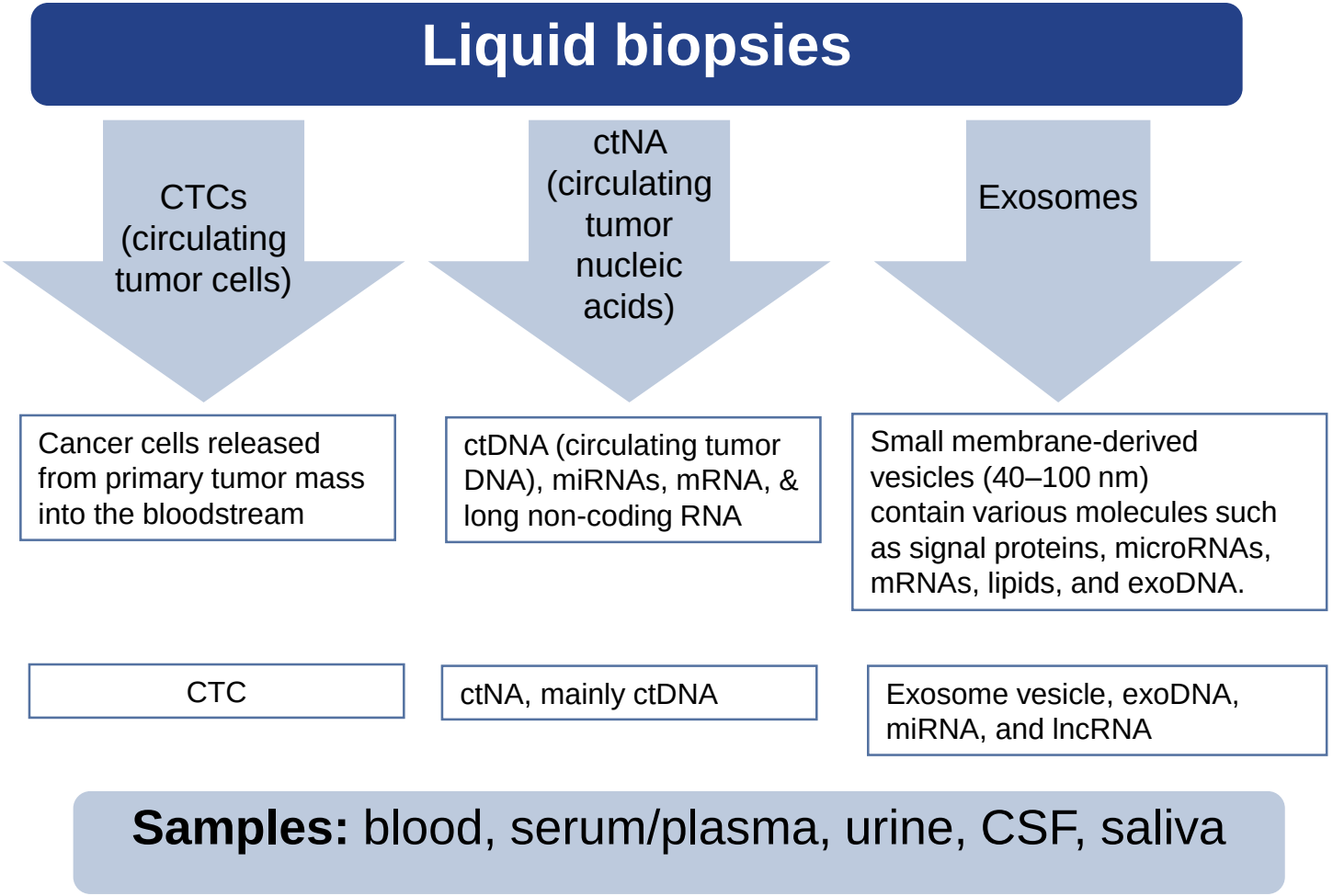
A liquid biopsy is a liquid biomarker that can be isolated from body fluids, such as blood, saliva, urine, ascites, or pleural effusion. Like a tissue biopsy, it is a representative of the tissue from which it has spread.

Liquid biopsies have become more clinically useful in recent years due to the ability to pair tests on circulating tumor cells with genomic tests.

Diaz, Jr., L.A. and Bardelli, A. (2014) “Liquid biopsies: genotyping circulating tumor DNA.” Am. Soc. Clin. Oncol. **32**, 579.

Liquid biopsy: Circulating biomarkers for cancer

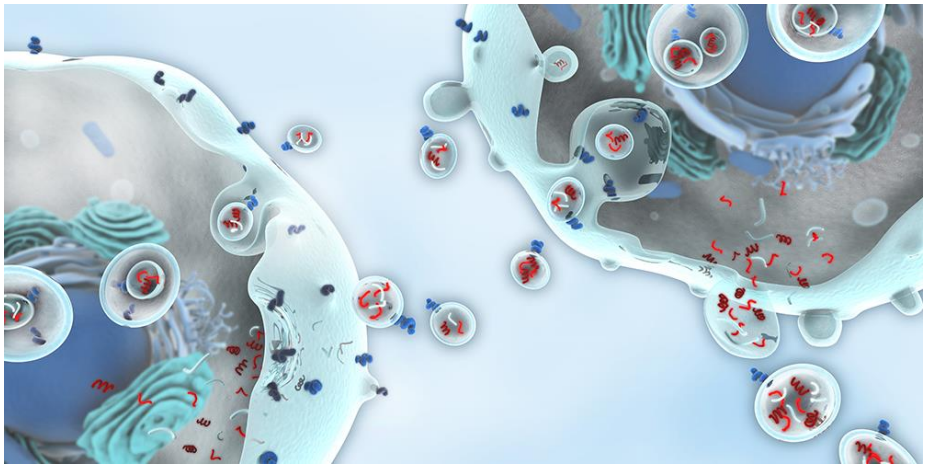
Tumors shed both intact cells (resulting in circulating tumor cells) as well as cellular components, such as nucleic acids (resulting in cell-free DNA or RNA).



Diaz, Jr., L.A. and Bardelli, A. (2014) “Liquid biopsies: genotyping circulating tumor DNA.” Am. Soc. Clin. Oncol. **32**, 579.

Exosomes as circulating cancer biomarkers

Exosomes: Small membrane vesicles (30–100 nm), secreted by most cell types into the bloodstream.



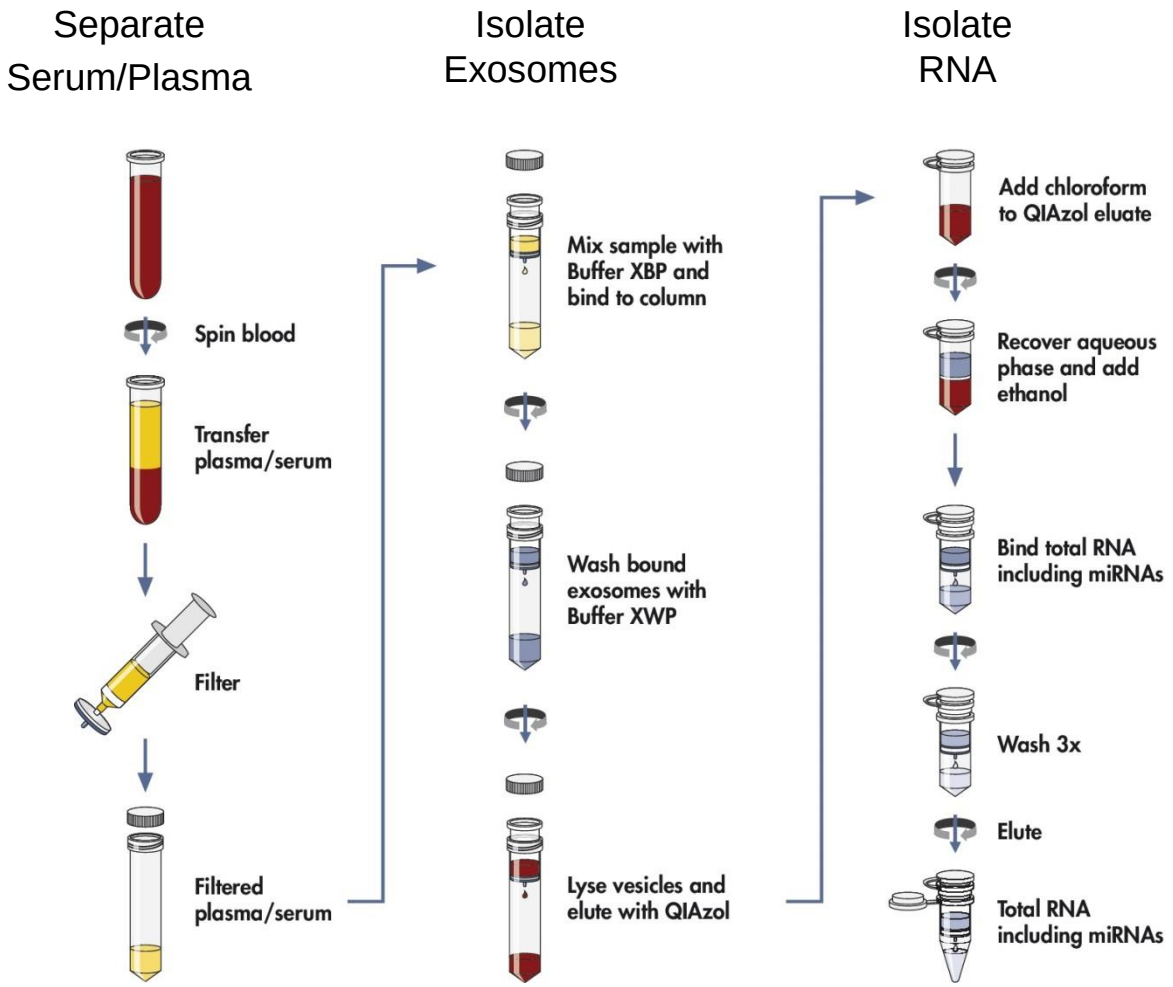
Functional biomolecules:

- DNA fragments (exosomal DNA, exoDNA)
- Proteins and/or peptides
- mRNA
- microRNA (miRNA)
- Lipids

- Exosomes play a central role in cell-to-cell communication
- The majority of DNA associated with tumor exosomes is double-stranded, representing whole genomic DNA
- Biological molecules (protein, RNA, and miRNA) contained in exosomes are well protected by a lipid bilayer membrane that confers a high degree of stability

Rolfo, C. et al. (2014) “Liquid biopsies in lung cancer: the new ambrosia of researchers.” Biochimica et Biophysica Acta **1846**, 539.
Klevebring, D. et. al. (2014) “Evaluation of exome sequencing to estimate tumor burden in plasma.” PLOS One **9**, e104417.

From sample to extracellular vesicle RNA isolation in just 1 hour

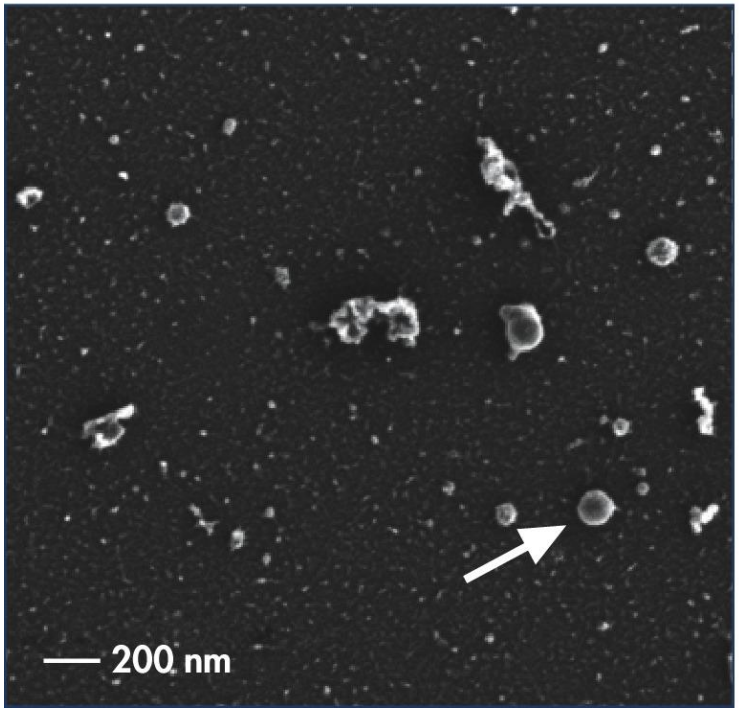


- Microvesicle isolation
  - 20 minutes
- RNA isolation
  - 35 minutes

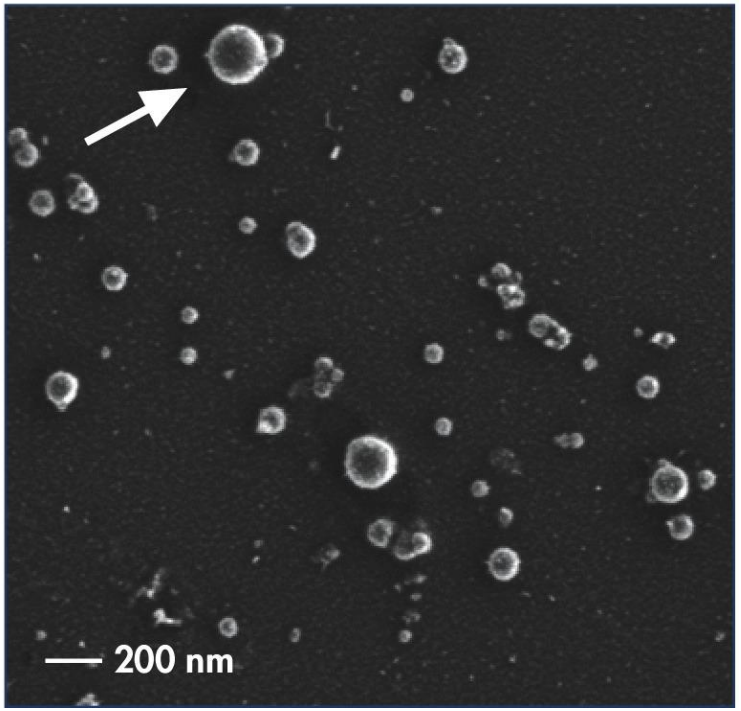
Specificity for vesicular RNA

Co-precipitation of large protein complexes

Ultracentrifugation (UC)



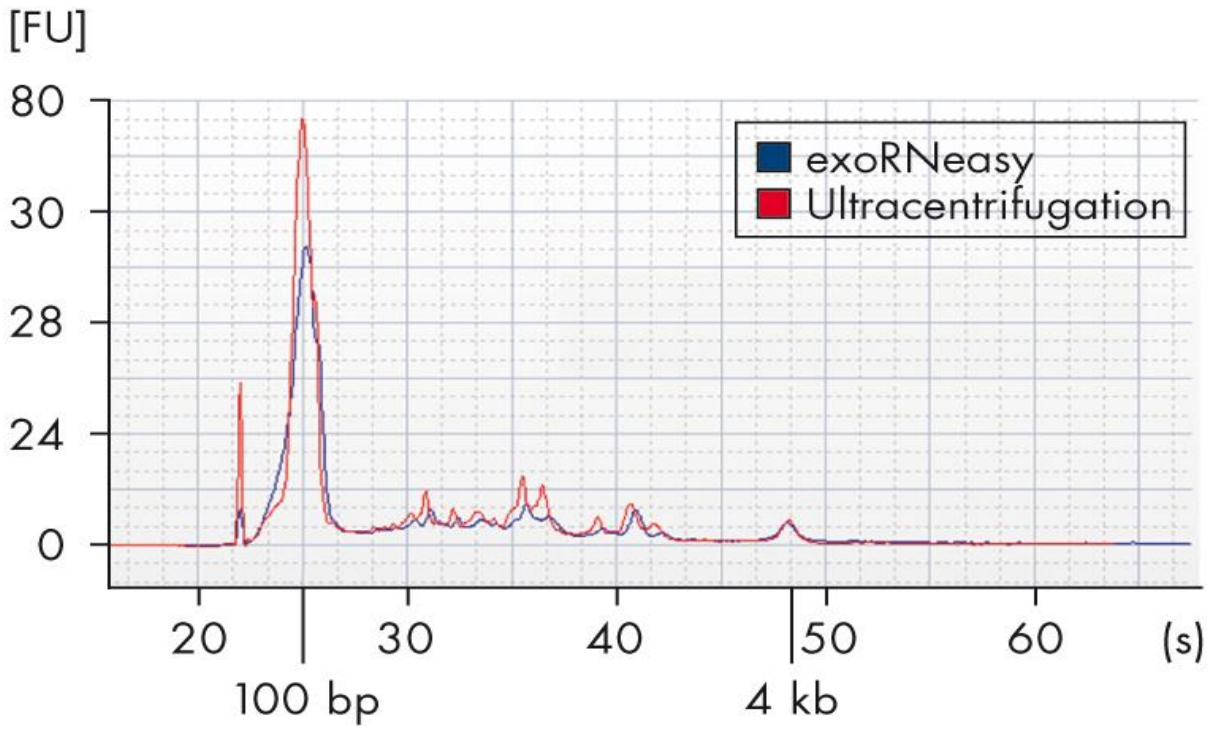
Eluate from exoEasy



- Both preparations contain vesicle-shaped structures within an expected size range
- **UC:** Many smaller, unidentified structures/particles that do not match the expected size
- **exoEasy:** Intact vesicles with higher purity

exoRNeasy isolates small and large RNAs from EV

Bioanalyzer sizing



2 ml plasma was pre-filtered (0.8 µm) to exclude larger particles.

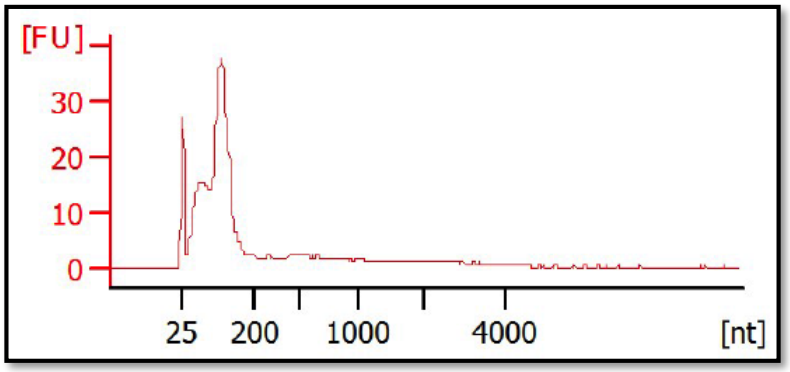
Both methods purify RNA of similar size and yield

EVs contain both small and large RNAs

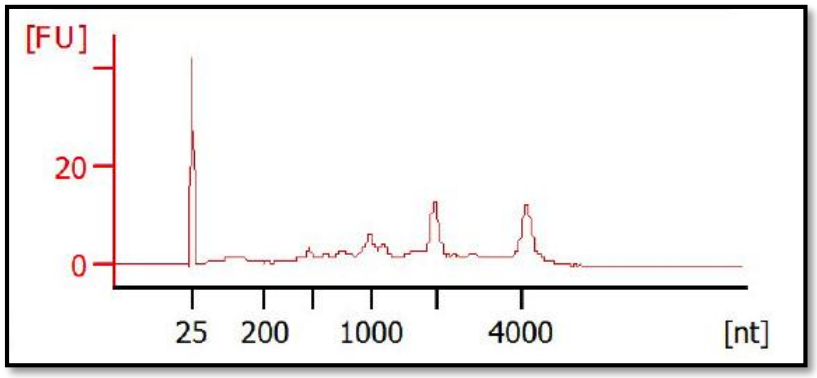
Purification of large, intact, non-degraded RNAs from EVs

exoRNeasy total RNA

Small RNA fraction

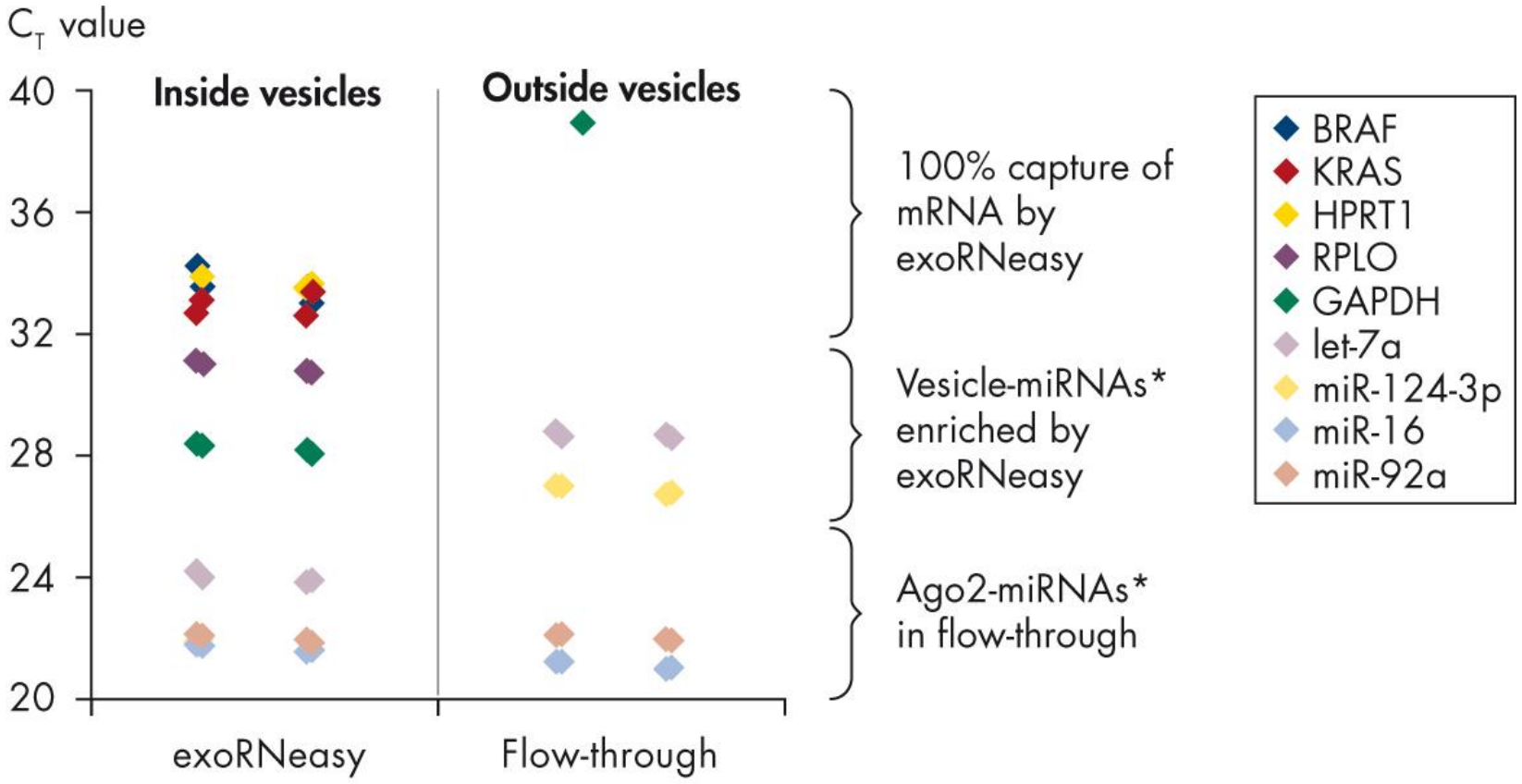


Large RNA fraction



exoRNeasy captures all mRNA and vesicle-specific miRNAs

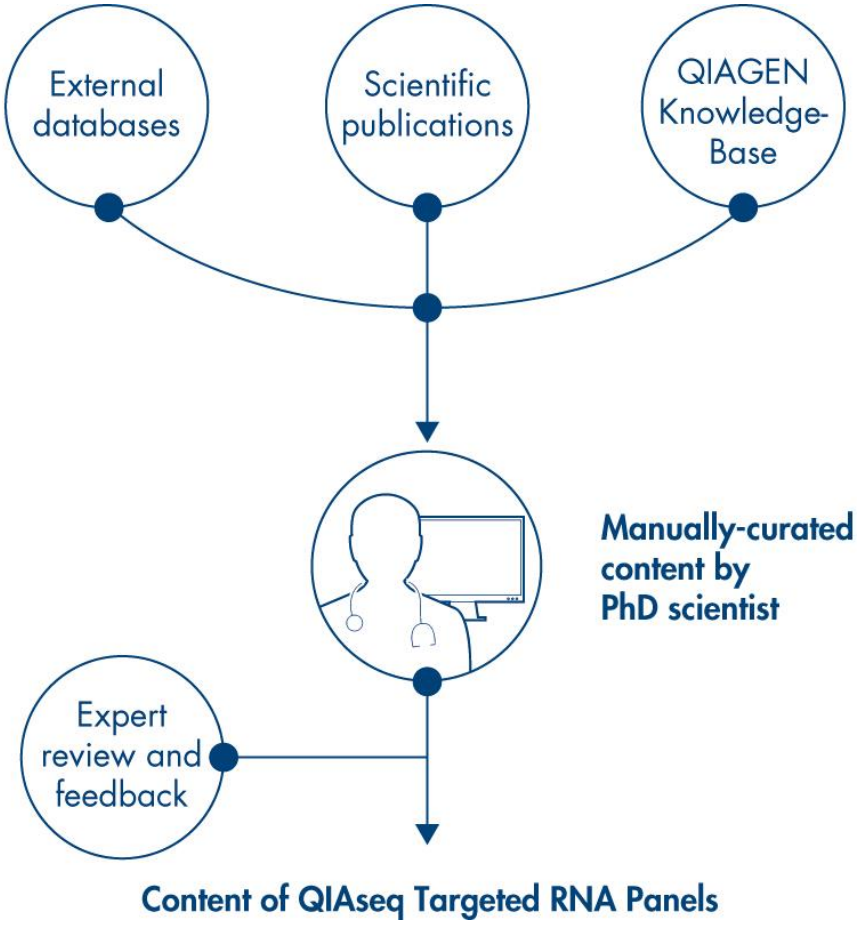
mRNA exclusively within vesicles – near 100% bound



miRNA in vesicular and non-vesicular fractions
(e.g. free Ago2 complexes)

** Arroyo, J.D. et al. (2011) Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc. Natl. Acad. Sci. USA **108**, 5003.*

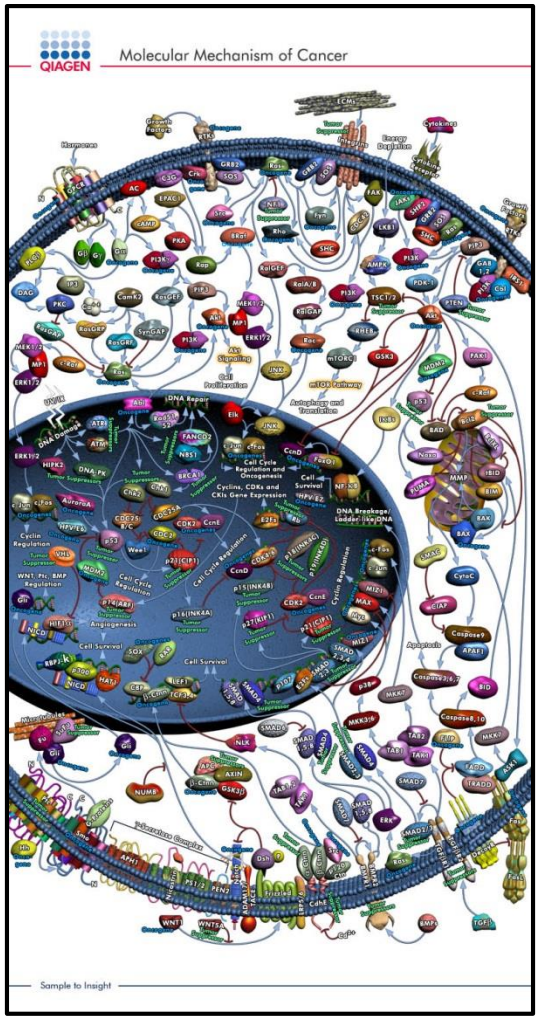
Content: Laboratory verified comprehensive panels



Flexible experiment design for any Catalog Panel options:

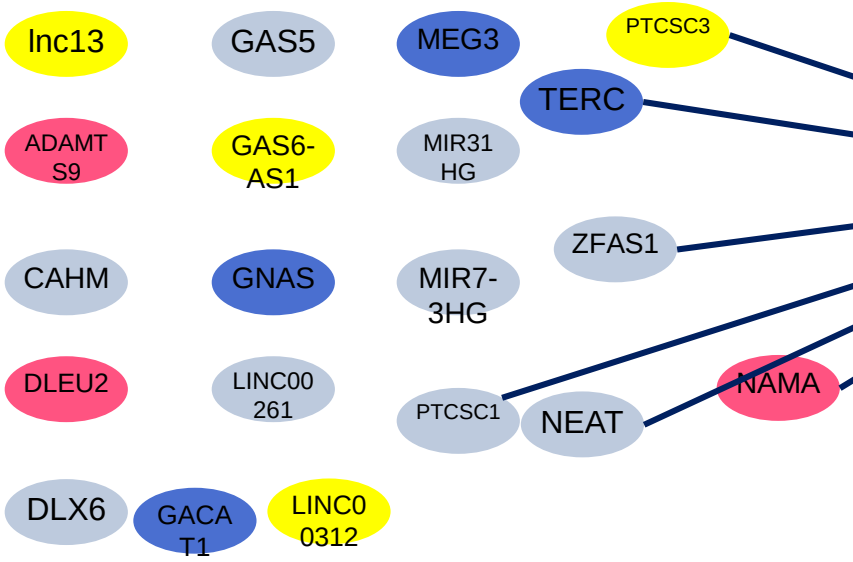
Comprehensive Panels (available for 12, 96 or 384 samples)

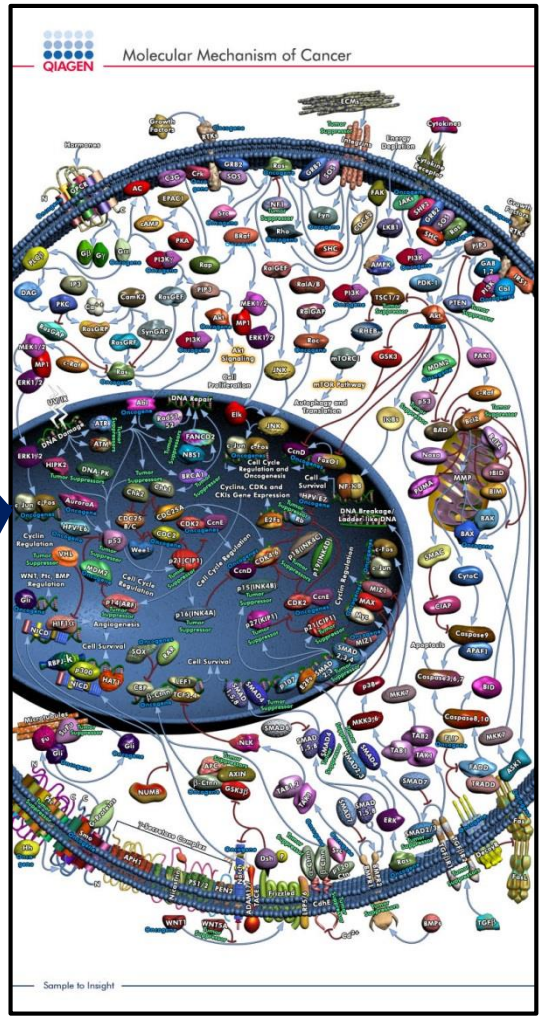
- Cancer Transcriptome (395)
- Inflammation & Immunity Transcriptome (475)
- Signal Transduction PathwayFinder (406)
- Stem Cell & Differentiation Markers (293)
- Molecular Toxicology Transcriptome (370)
- Angiogenesis & Endothelial Cell Biology (340)
- Apoptosis & Cell Death (264)
- ECM & Adhesion Molecules (421)



Flexible experiment design for any Extended Panel options:

Add 25 of your favorite targets (mRNAs or lncRNAs) to QIAGEN's comprehensive panel



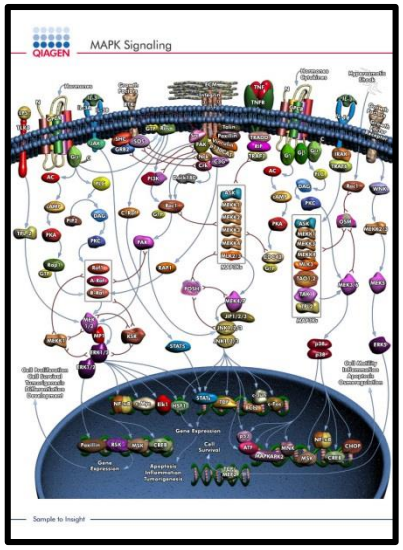


What is the role of tumor suppressor lncRNAs? Find out!

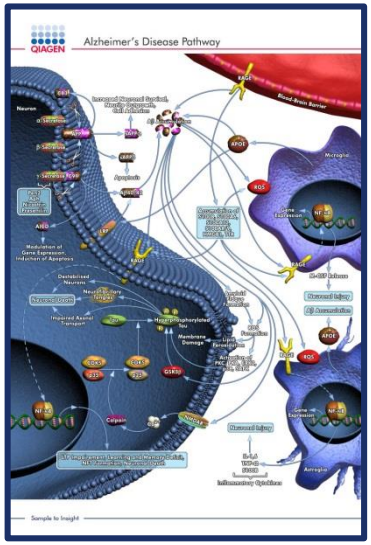
Flexible experiment design for any pathway panel options:

QIAseq Targeted RNA pathway panels (available for 12, 96 or 384 samples)

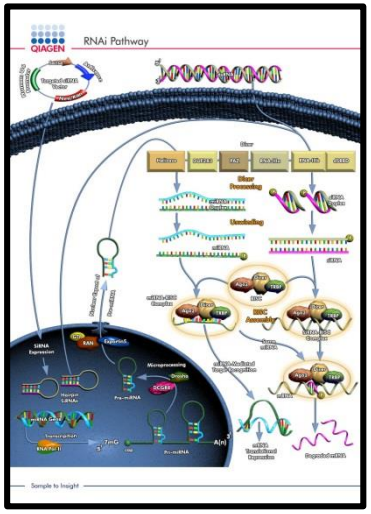
Each panel contains 84 genes + controls and housekeeping genes.
Choose from over 180 panels!



Pathways



Diseases

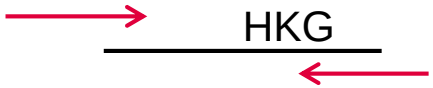


miRNA Targets

Controls: Take the guesswork out of your analysis!

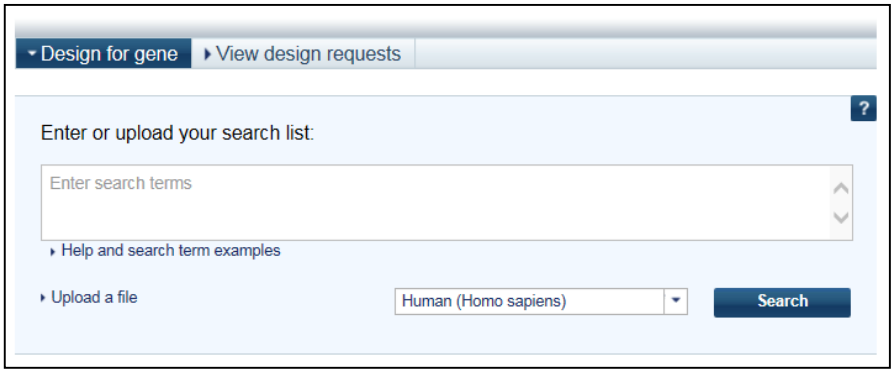
Built-in controls:

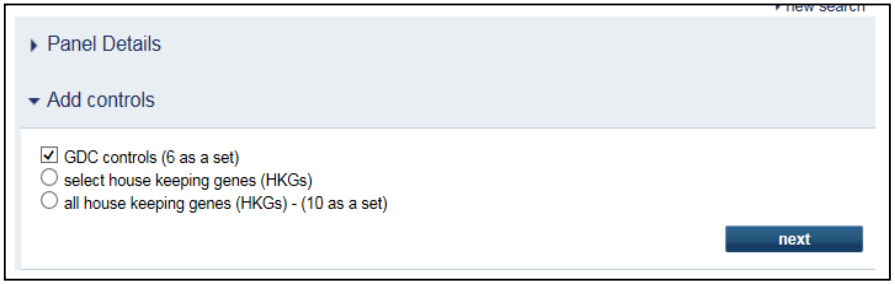
- gDNA assays to control for any gDNA contamination in the RNA sample
- Mean tags per target calculated and mRNAs near this number are flagged during analysis as ‘close to noise level’



- Multiple HKG assays to normalize data to make sample-to-sample and run-to-run comparisons possible
- Flexible – use one, two, all, none or any other genes as normalizers
- HKG efficacy evaluation built into secondary data analysis

Online custom builder:





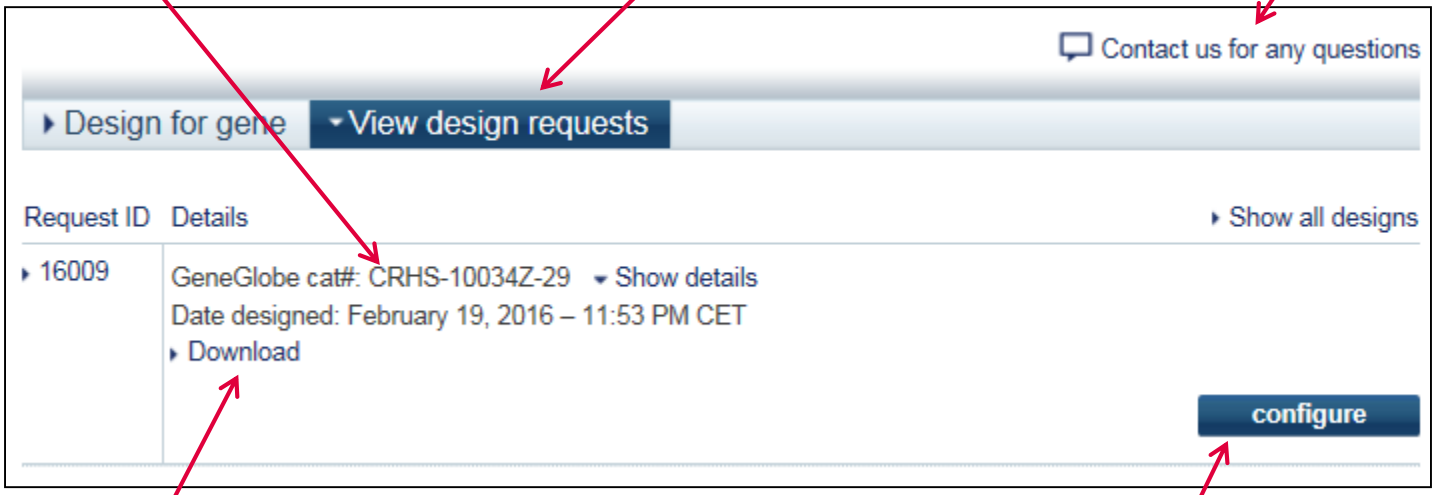
- Choose your own gene content from 54,881 human genes and lncRNA
- Easy to use online Custom Panel Builder to tailor panel to your research needs
  - Input list of genes
  - Select proper controls (genomic DNA contamination control, HKGs, or your own)
  - Output: list of genomic coordinates for primers designed specifically for your genes of interest

Custom builder

Custom panel number

All your custom designs are saved for easy retrieval

Have questions? Easily contact us



Download zip file containing:

- Summary file
- Bed file

Configure and order

Custom builder

Gene ID and symbol

Strand of the genome the gene is on

Amplicon coordinates

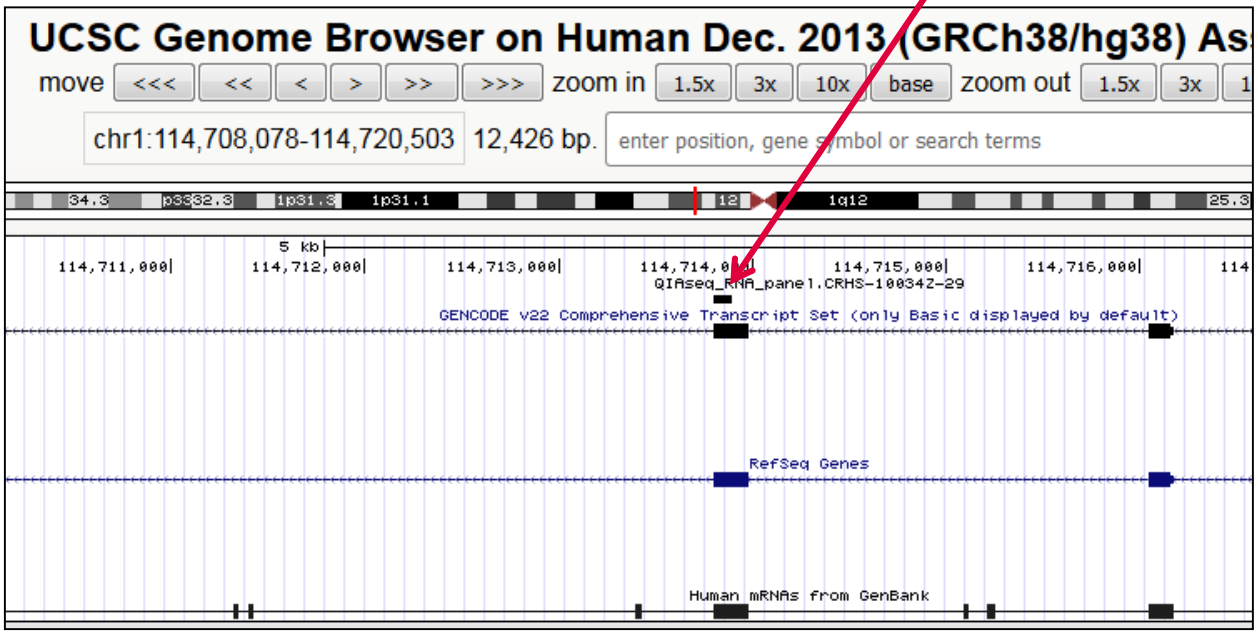
Designated controls are shown here

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	gene id	gene symbol	gene strand	chrom	GRCh38 loc 5	GRCh38 loc 3	control type	single exon	# Gencode Basic RNAs	# Gencode Basic RNAs matched	# off target genes	off target genes	amplicon not genome unique
2	ENSG00000041988	THAP3	1	chr1	6628529	6628647	reference_gene	1	3	3	0		0
3	ENSG00000084072	PPIE	1	chr1	39743264	39743891	reference_gene	0	4	4	0		0
4	_GDC_CONTROL_07_	_GDC_CONTROL_07_	1	chr1	104793033	104793127	gDNA_control	1	0	0	0		0
5	ENSG00000213281	NRAS	-1	chr1	114713799	114713893		1	1	1	0		0
6	ENSG00000174775	HRAS	-1	chr11	532251	532355		1	5	4	0		0

- Single exon (1) means both primers are within one exon
- **# Gencode basic RNAs:** Total number of RNA transcripts found for the gene in Gencode
- **# Gencode basic RNAs matched**: # of RNA transcripts targeted by the designed amplicon
- **# off target genes:** Rough prediction of # of off target genes that will also get enriched by the primer pair for the target gene
- **Amplicon not genome unique**: Reads that will not be able to be uniquely mapped to the genome; Some MT counts might come from another loci

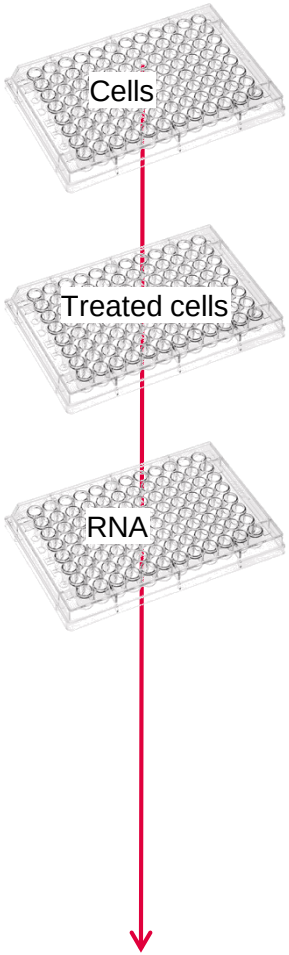
Bed file

Location of designed amplicon



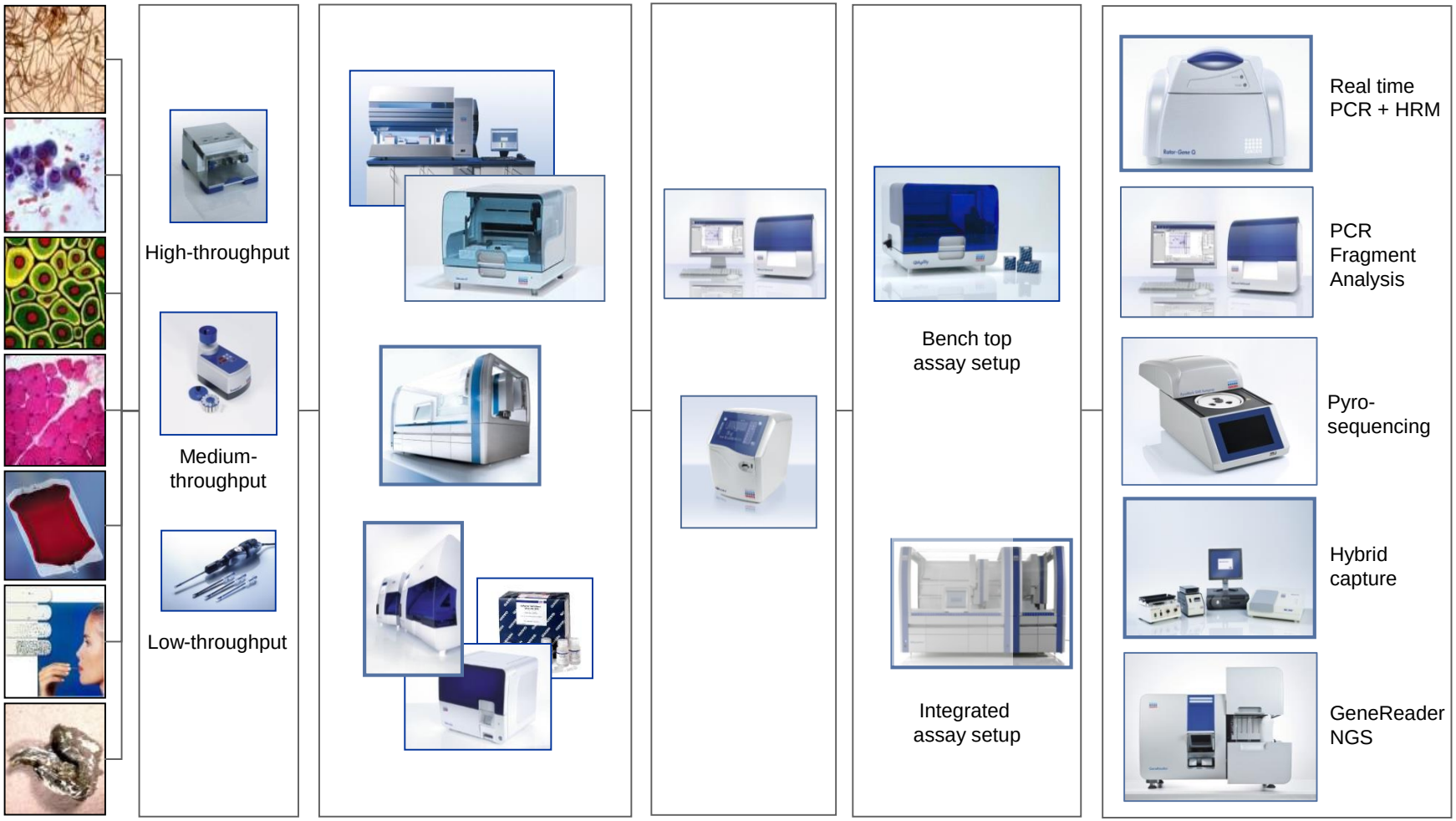
Agenda

Small Molecules – Signal Transduction Application



- HEK293T Cells were treated with 90 different chemical inhibitors
- The 421 Signal Transduction Gene QIAseq Panel was interrogated
- In one day we went from total RNA to sequence ready libraries for 96 samples. The final libraries were quantified, normalized, and pooled. Prior to loading onto a NextSeq, the denatured libraries were diluted to the appropriate input concentration to obtain to generate suitable clusters on the NextSeq

QIAGEN: Automating Sample to Insight

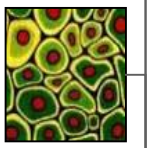


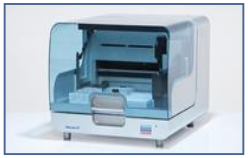
QIAGEN: Automating Sample to Insight

Purification

Quality control

Cells in 96 well plates



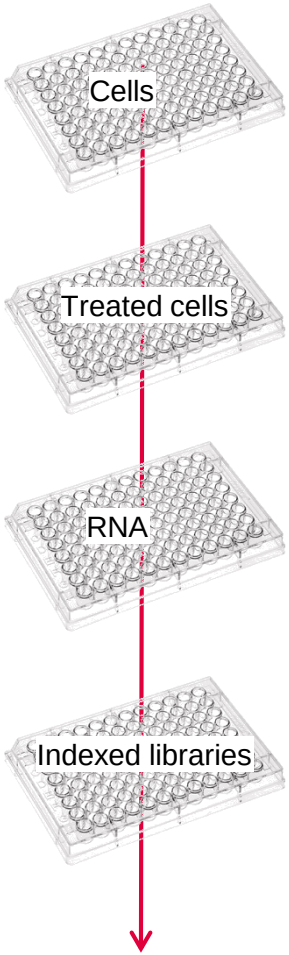


RNA isolation of 96 samples

RNA Integrity (96 samples done automatically while at lunch)

RNA quantification (16 samples/ 90 secs)

Small Molecules – Signal Transduction Application



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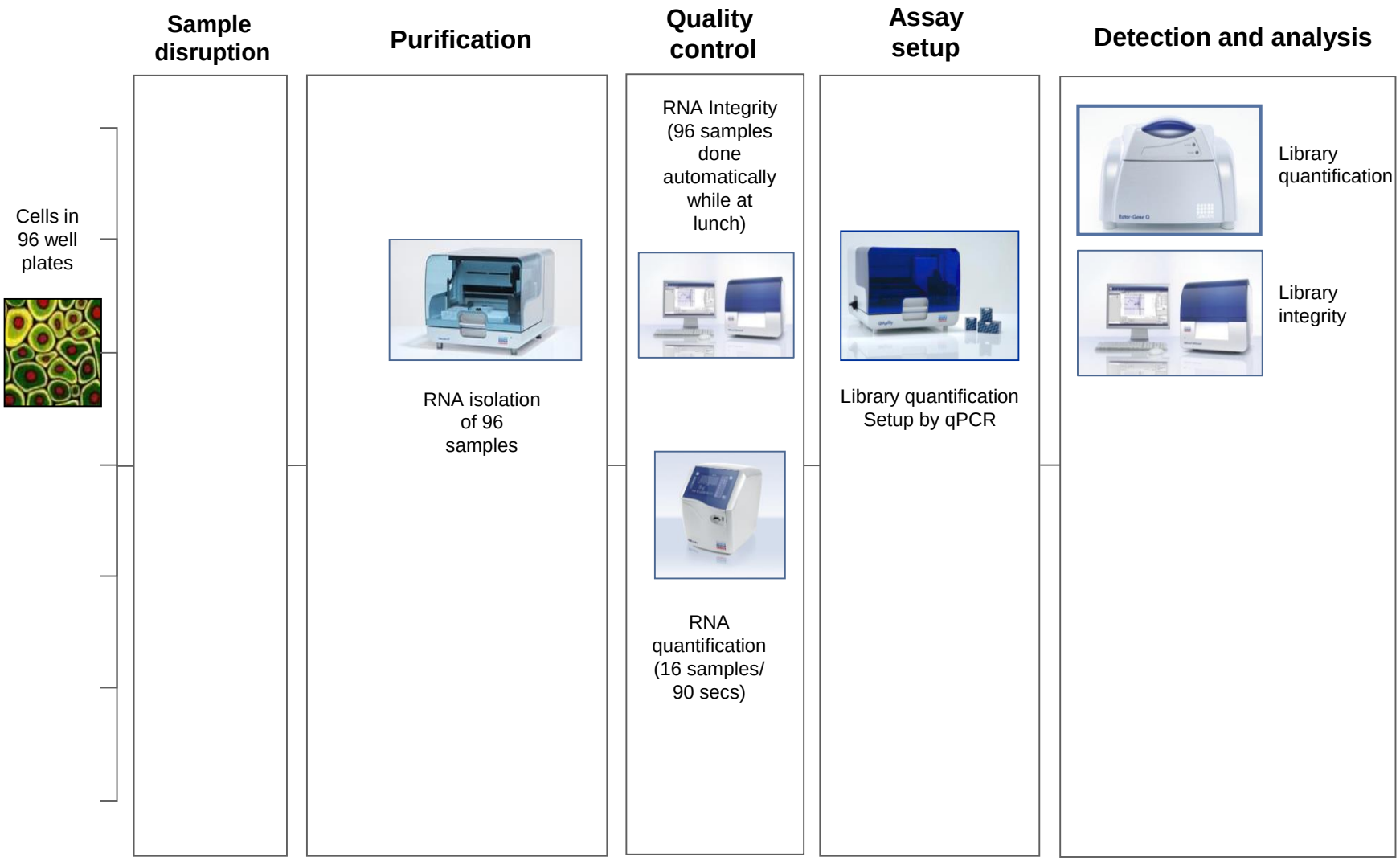
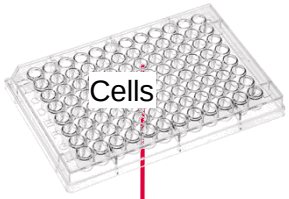
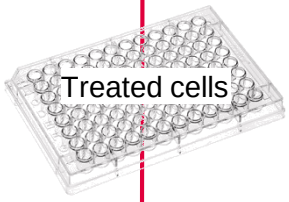
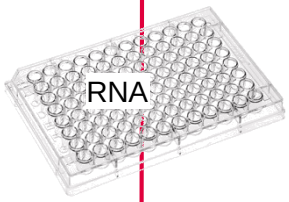
QIAGEN: Automating Sample to Insight

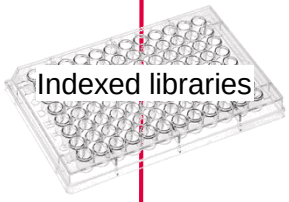


Small Molecules – Signal Transduction Application





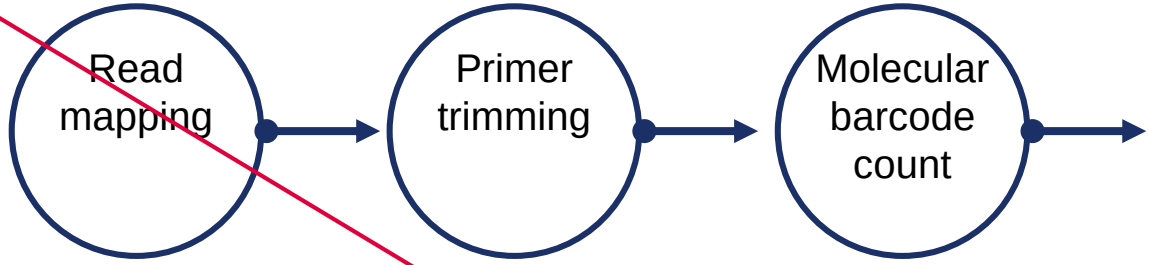




Normalized, pooled libraries

- HEK293T Cells were treated with 90 different chemical inhibitors
- The 421 Signal Transduction Gene QIAseq Panel was interrogated
- In one day we went from total RNA to sequence ready libraries for 96 samples. The final libraries were quantified, normalized, and pooled. Prior to loading onto a NextSeq, the denatured libraries were diluted to the appropriate input concentration to obtain to generate suitable clusters on the NextSeq
- The parameters of the NextSeq sequencing run were; Single 151 bp read, with a Custom Sequencing Primer (included in kit)

QIAseq Targeted RNA Data Analysis automated workflow



- **Read Mapping**
  - Identify the possible position of the read within the reference genome
  - Align the read sequence to reference sequences
- **Primer Trimming**
  - Remove the primer sequences from the reads
- **Molecular Barcode Counting**

Go get coffee

Primary data analysis for QIAseq Targeted RNA Sequencing

	A	B	C	D	E	F	G	H	I	J	K	L
1	read set	16693.ADCY5_S67	16693.AKT_S89	16693.AMPK_S3	16693.ATM_S36	16693.AURORA_S4	16693.BCR-ABL_S41	16693.Ca2-ATPase_S73	16693.Ca2-channel_S77	16693.CaMK-II_S6	16693.CASP1_S50	16693.CASP3-6-7-8-10_S52
2	reads total	2,121,847	903,510	2,339,304	1,963,219	2,213,291	1,309,441	953,046	2,132,040	2,303,187	1,212,167	1,948,38
3	reads dropped, < 55 bp	38,535	46,417	79,134	55,341	122,499	16,812	57,348	48,492	40,956	14,611	19,01
4	reads dropped, MT has >1 N base	0	0	0	0	0	0	0	0	0	0	
5	reads dropped, not mapped	6,577	4,676	13,526	8,591	14,814	4,149	5,595	7,834	6,528	2,940	5,41
6	reads dropped, < 60 bp aligned	51	31	137	48	163	40	47	80	65	27	7
7	reads dropped, off-target	2,029	1,623	6,819	2,726	5,097	1,482	1,597	2,313	2,033	1,185	2,14
8	reads dropped, poor alignment near MT	62,481	26,200	69,538	62,415	65,313	42,108	29,258	64,636	70,070	37,000	59,77
9	reads dropped, high reads per MT	969,174	399,563	1,664,150	1,199,098	1,679,405	0	562,201	1,045,685	637,535	0	
10	reads used for MT counting	1,043,000	425,000	506,000	635,000	326,000	1,244,850	297,000	963,000	1,546,000	1,156,404	1,861,96
11	MTs	68,726	27,897	35,963	41,171	22,704	83,902	20,957	63,003	103,094	106,252	126,20
12	reads per MT mean	15.2	15.2	14.1	15.4	14.4	14.8	14.2	15.3	15.0	10.9	14.
13												
14	reads dropped, < 55 bp	1.8%	5.1%	3.4%	2.8%	5.5%	1.3%	6.0%	2.3%	1.8%	1.2%	1.0%
15	reads dropped, MT has >1 N base	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
16	reads dropped, not mapped	0.3%	0.5%	0.6%	0.4%	0.7%	0.3%	0.6%	0.4%	0.3%	0.2%	0.3%
17	reads dropped, < 60 bp aligned	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
18	reads dropped, off-target	0.1%	0.2%	0.3%	0.1%	0.2%	0.1%	0.2%	0.1%	0.1%	0.1%	0.1%
19	reads dropped, poor alignment near MT	2.9%	2.9%	3.0%	3.2%	3.0%	3.2%	3.1%	3.0%	3.0%	3.1%	3.1%
20	reads dropped, high reads per MT	45.7%	44.2%	71.1%	61.1%	75.9%	0.0%	59.0%	49.1%	27.7%	0.0%	0.0%
21	reads used for MT counting	49.2%	47.0%	21.6%	32.3%	14.7%	95.1%	31.2%	45.2%	67.1%	95.4%	95.6%

Primary data analysis for QIAseq Targeted RNA Sequencing

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	gene id	gene symbol	gene strand	chrom	loc 5' GRCh38	loc 3' GRCh38	control type	single exon	16693.ADCY5_S67	16693.AKT_S89	16693.AMPK_S3	16693.ATM_S36	16693.AURORA_S4	16693.BCR-ABL_S41	16693.Ca2-ATPase_S73	16693.Ca2-channel_S77	16693.CaMK-II_S6	16693.CASP1_S50	16693.CASP3-6-7-8-10_S52	16693.CDK_S9
397	ENSG00000115596	WNT6	1	chr2	2.19E+08	2.19E+08		1	5	0	0	3	1	2	0	3	4	3	2	6
398	ENSG00000136936	XPA	-1	chr9	97687154	97687255		1	64	37	42	48	49	105	27	72	122	111	141	64
399	ENSG00000154767	XPC	-1	chr3	14156348	14156448		1	154	62	86	92	53	241	42	169	230	265	298	178
400	ENSG00000073050	XRCC1	-1	chr19	43552797	43552888		1	40	14	23	21	13	38	8	36	58	50	58	32
401	ENSG00000196419	XRCC6	1	chr22	41621991	41622084		1	940	303	474	450	273	1,188	263	739	1,411	1,435	1,741	886
402	ENSG00000060138	YBX3	-1	chr12	10699501	10699596		1	977	421	429	619	321	1,345	401	1,082	1,523	1,535	1,961	753
403	ENSG00000109906	ZBTB16	1	chr11	1.14E+08	1.14E+08		1	4	2	3	1	3	2	1	0	6	5	6	1
404	ENSG00000128016	ZFP36	1	chr19	39408035	39408161		1	10	3	3	3	2	9	1	5	12	7	5	7
405	ENSG00000185650	ZFP36L1	-1	chr14	68788691	68788787		1	51	20	31	16	22	65	8	32	57	75	81	43
406	ENSG00000162702	ZNF281	-1	chr1	2E+08	2E+08		1	207	99	116	112	67	250	57	190	338	364	415	256
407	_GDC_CONTROL_(	_GDC_CO	1	chr3	74811135	74811230	gDNA_control	1	0	0	0	0	0	0	0	0	0	0	0	0
408	_GDC_CONTROL_(	_GDC_CO	1	chr1	1.05E+08	1.05E+08	gDNA_control	1	0	0	0	0	0	0	0	0	0	0	0	0
409	_GDC_CONTROL_:	_GDC_CO	1	chr21	20087007	20087106	gDNA_control	1	0	1	0	0	0	0	0	0	0	0	1	0
410	_GDC_CONTROL_2	_GDC_CO	1	chr18	66284567	66284662	gDNA_control	1	0	0	0	0	0	0	0	0	0	0	0	0
411	_GDC_CONTROL_2	_GDC_CO	1	chr15	94718874	94718967	gDNA_control	1	0	0	0	0	0	0	0	0	0	0	0	0
412	_GDC_CONTROL_3	_GDC_CO	1	chr15	46219595	46219688	gDNA_control	1	0	0	0	0	0	0	0	0	0	0	0	0
413	ENSG00000130731	C16orf13	-1	chr16	634501	634621	reference_gene	1	113	35	58	50	23	139	36	98	187	158	166	94
414	ENSG00000112787	FBRSL1	1	chr12	1.33E+08	1.33E+08	reference_gene	1	172	76	91	95	52	173	43	143	266	235	326	159
415	ENSG00000100578	KIAA0586	1	chr14	58465870	58465964	reference_gene	1	133	45	68	94	43	146	30	109	162	168	221	114
416	ENSG00000169967	MAP3K2	-1	chr2	1.27E+08	1.27E+08	reference_gene	1	114	58	89	78	46	147	40	121	182	202	270	123
417	ENSG00000084072	PPIE	1	chr1	39743264	39743891	reference_gene	0	199	84	139	140	89	210	66	201	284	284	289	165
418	ENSG00000100023	PPIL2	1	chr22	21684767	21684860	reference_gene	1	205	98	122	107	71	253	57	177	331	324	407	192
419	ENSG00000132005	RFX1	-1	chr19	13961800	13961891	reference_gene	1	54	25	29	21	20	59	10	45	82	74	117	41
420	ENSG00000041988	THAP3	1	chr1	6628529	6628647	reference_gene	1	37	13	14	13	16	36	15	31	44	40	41	25
421	ENSG00000236104	ZBTB22	-1	chr6	33314794	33314894	reference_gene	1	26	20	15	27	15	33	12	32	48	47	37	31
422	ENSG00000083838	ZNF446	1	chr19	58480869	58480974	reference_gene	1	69	20	40	34	12	56	10	53	68	62	75	52

Differential gene expression inter- and intra-samples

QIAseq secondary data analysis setup

Upload data | Analysis setup | Analysis | Plots & charts | Export data

Plot Home | Scatter Plot | Volcano Plot | Clustergram

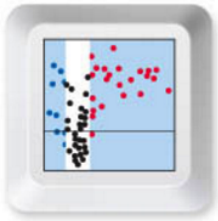
Select a Plot or Chart Icon below to launch a separate window.

Notes:

1. Before launching a plot or chart, please upload your readout data or take a test run.
2. Please disable any Pop-Up Window Blockers in your web browser.
3. To save the figure:
   a. Using Windows: Right-click on the figure and save the image/picture
   b. Using OS X: Hold down the Control key & Click and save the image/picture

Scatter Plot

The scatter plot compares the normalized expression of every gene on the array between two groups by plotting them against one another to quickly visualize large gene expression changes. The central line indicates unchanged gene expression. Set the boundary (fold regulation cut-off) and the experimental groups to compare. Then, export the lists of genes whose expression changes are greater than the selected boundary.



Volcano Plot

The volcano plot helps quickly identify significant gene expression changes. The volcano plot displays statistical significance versus fold-change on the y- and x-axes, respectively. The volcano plot combines a p-value statistical test with the fold regulation change enabling identification of genes with both large and small expression changes that are statistically significant. Select the experimental groups and the boundaries or the fold regulation cutoffs and p-value cutoff values. Then, export the lists of genes whose expression changes and p-values are beyond the selected boundaries.

* NOTE: This plot requires three or more replicates in each group.

Clustergram

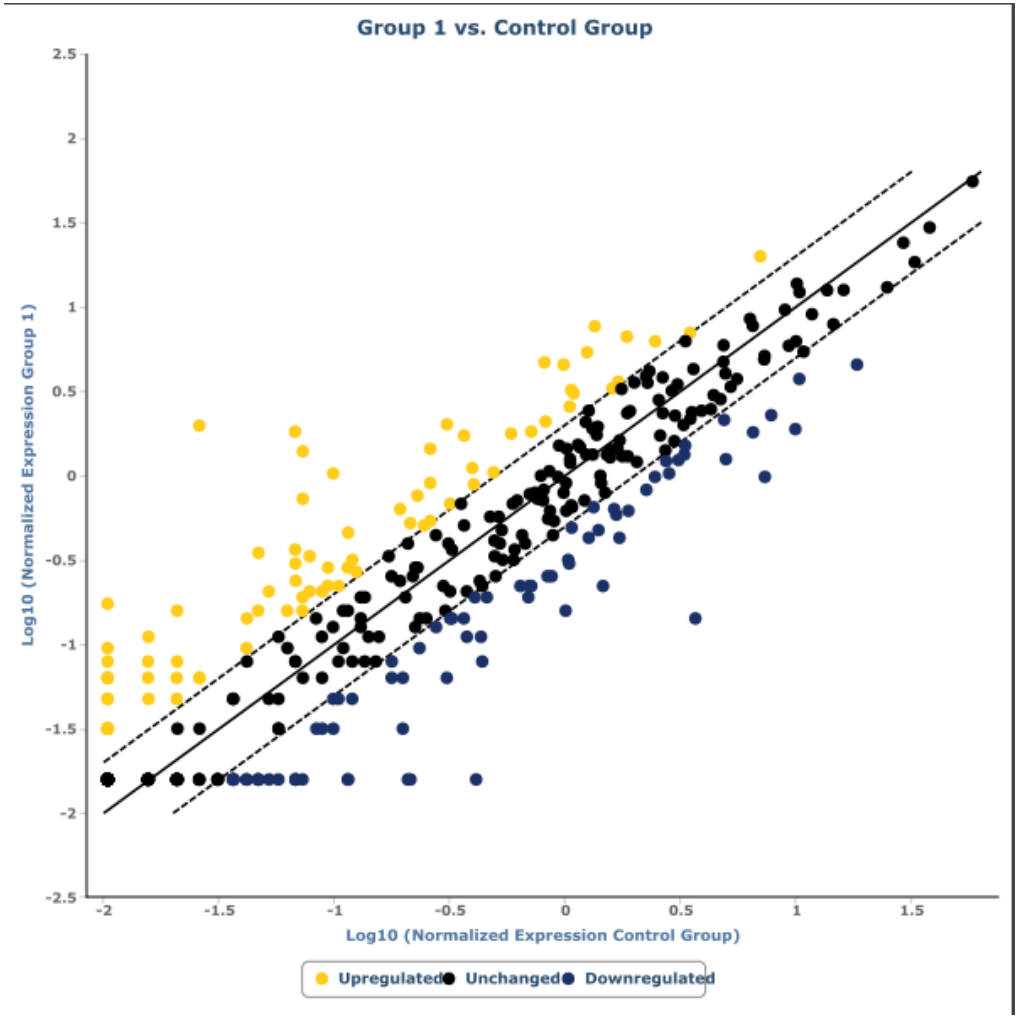
The clustergram performs non-supervised hierarchical clustering of the entire dataset to display a heat map with dendrograms indicating co-regulated genes across groups or individual samples.

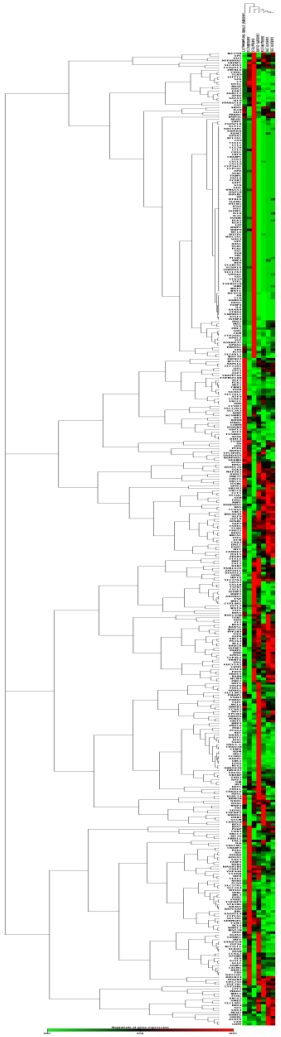
Analysis: What kinds of things get flagged?
Low tag #, high gDNA, poor normalizer performance

Changes in gene expression due to chemical perturbation and quantified by QIAseq RNA NGS were characterized.

	A	B	C	D	E	F
1	**Symbol**	**Group 1 vs Control Group FC**	**Group 2 vs Control Group FC**	**Group 3 vs Control Group FC**	**Group 4 vs Control Group FC**	**Group 5 vs Control Group FC**
2	ABCC4	0.347303984	0.599495235	0.913120058	1.007402609	0.706931657
3	ABCG2	0.789834324	0.545345791	0.593316022	0.865141275	0.229670718
4	ACACA	0.703476499	0.623639875	0.704593128	0.751523724	0.613677886
5	ACSL3	2.545269175	0.985252008	0.475569118	1.821679828	0.959904487
6	ACSL4	0.607090553	0.954206469	1.16790896	0.920871511	0.783629237
7	ACSL5	1.524483462	0.818680055	8.016242204	1.298761113	3.103061498
8	ACTC1	1.524483462	0.818680055	8.016242204	0.432920371	3.103061498
9	ADA	0.524762261	0.507255299	0.827812466	1.072952252	0.534072559
10	ADCYAP1	1.018793394	0.547113729	5.357155262	0.289315316	2.073737521
11	ADIPOQ	1.524483462	0.818680055	8.016242204	0.432920371	3.103061498
12	ADM	1.287226367	1.814578361	0.846083164	7.265196567	1.637580318
13	ADM2	0.759478555	0.407856143	3.993591403	0.215675503	1.54590635
14	ADORA1	1.524483462	0.818680055	8.016242204	3.896283339	3.103061498
15	AFAP1L2	0.553991157	0.446257558	1.456535084	1.33723293	0.563820032
16	AKR1C2	3.048966924	0.818680055	8.016242204	0.865840742	3.103061498
17	ALAS1	1.862272419	1.072901265	0.998259378	2.259144279	1.490488657
18	ALDOC	5.55235194	1.507248701	0.962509361	7.502548214	0.496779025
19	ANGPTL4	7.097042262	2.177864398	5.331230557	2.015406691	4.127404302
20	ANKRD37	0.712116512	0.600948414	2.139740566	0.866681597	0.828286671
21	APEX1	0.277482896	0.718928251	0.80634339	0.785226601	0.61931136
22	APOA1	0.509396697	0.547113729	2.678577631	0.867945947	1.03686876
23	APOA5	1.524483462	0.818680055	8.016242204	0.432920371	3.103061498
24	APOC3	0.435114402	0.233665691	6.863929684	0.123563091	0.885668346
25	APOE	3.048966924	0.818680055	8.016242204	1.731681484	3.103061498
26	AR	1.018793394	0.547113729	5.357155262	0.578630631	2.073737521
27	ARNT	0.631616725	1.136589464	1.281886459	0.714315259	1.060093787
28	ASNS	2.022391069	1.635229597	0.744168392	1.155112866	4.943570253
29	ATF3	5.781443893	1.395043461	2.464926417	3.610862055	11.4102239
30	ATF4	1.180830418	1.230428098	0.579847466	1.021648438	1.780069371
31	ATM	0.460883341	0.766464495	0.703590455	0.8063953	0.968382561
32	AXIN2	1.161108884	1.402965395	0.381593597	1.154054443	1.329422854
33	BAG2	0.302770298	1.006050942	0.895537771	0.816812128	0.731837318
34	BAX	0.622825729	1.169554298	0.513729458	0.753716486	0.778880146

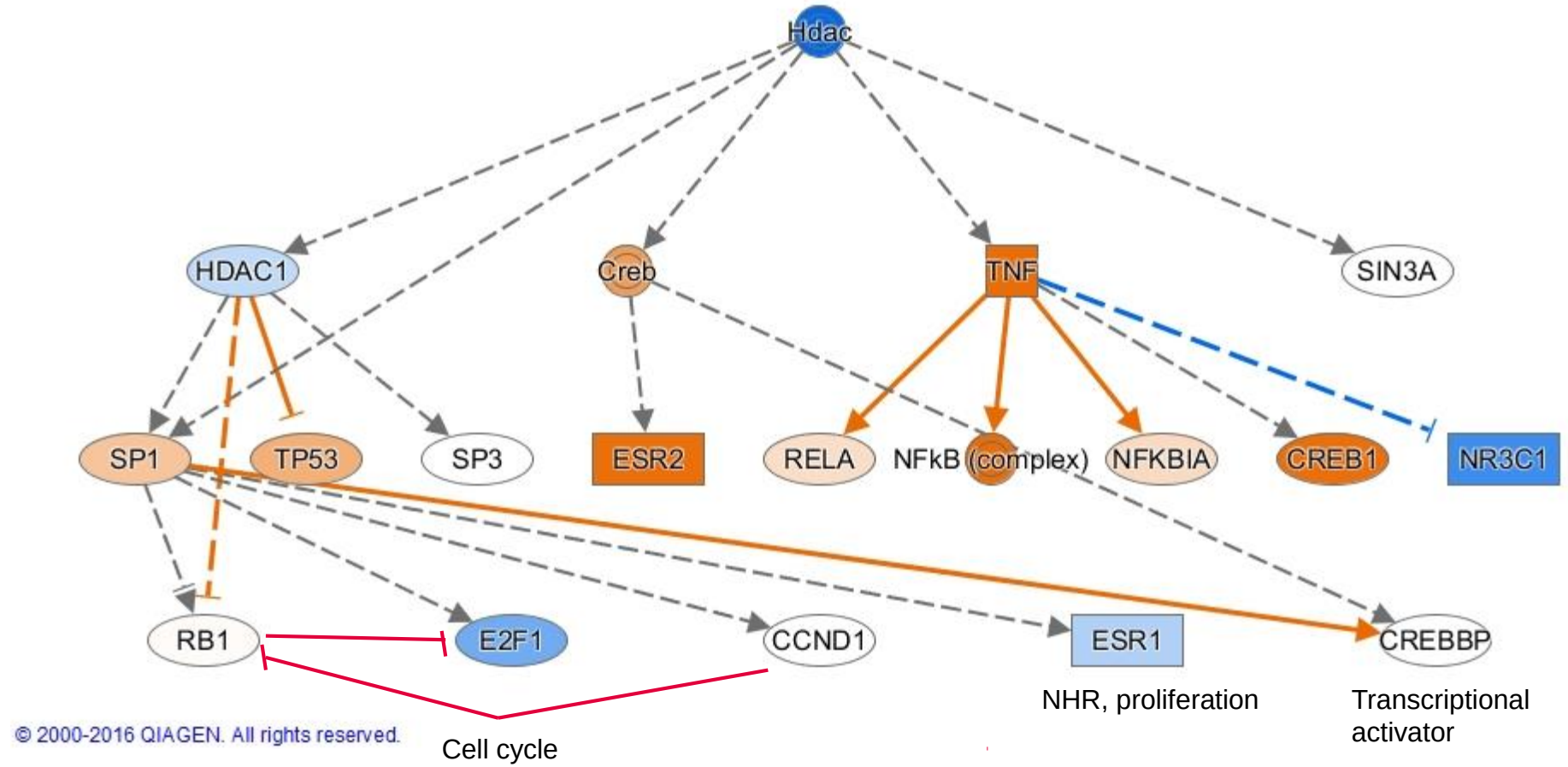
Scatter plot and clustergram (HDAC Sample compared to Control)





HDAC inhibitor network in HE293T Cells

HDAC is predicted to be inhibited by Trichostatin A and drives a mechanistic network with 18 other regulators.



Where can you run this?

QIAseq sample multiplexing guidelines on NGS platforms

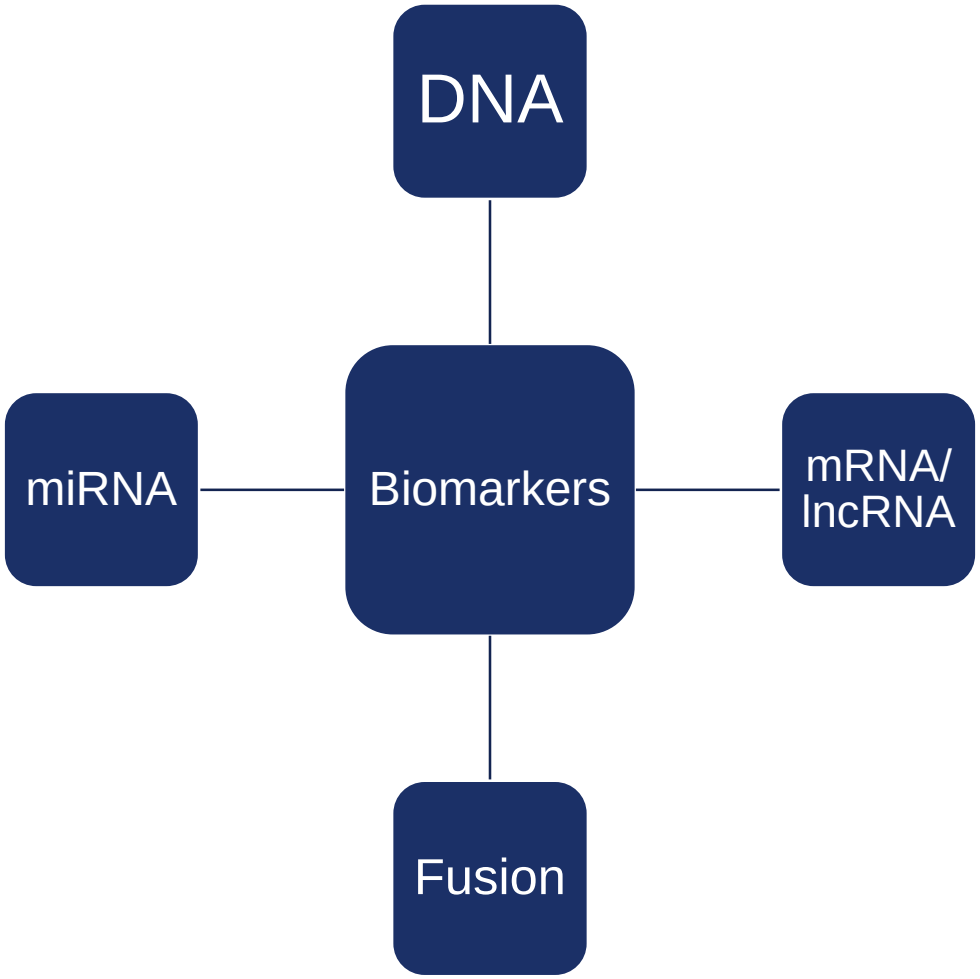
			# Samples_Moderate Read depth			
			average 5000 reads/gene			
			average 2-5 reads per MT			
Instrument	Version	Capacity	100 genes	250 genes	500 genes	1000 genes
MiSeq	V2	15 M	30	12	6	3
MiSeq	V3	25 M	50	20	10	5
NextSeq 500	High Output	400 M	800	320	160	80
NextSeq 500	Mid Output	130 M	260	104	52	26
Ion Torrent PGM	Ion 314 Chip v2	400-550 K	1	0	0	0
Ion Torrent PGM	Ion 316 Chip v2	2-3 M	4	2	1	0
Ion Torrent PGM	Ion 318 Chip v2	4-5.5 M	8	3	2	1
Ion S5	Ion 520 Chip	3-5 M	6	2	1	1
Ion S5	Ion 530 Chip	15-20 M	30	12	6	3
Ion S5	Ion 540 Chip	60-80 M	120	48	24	12

Unparalleled efficiency and flexibility vs PCR

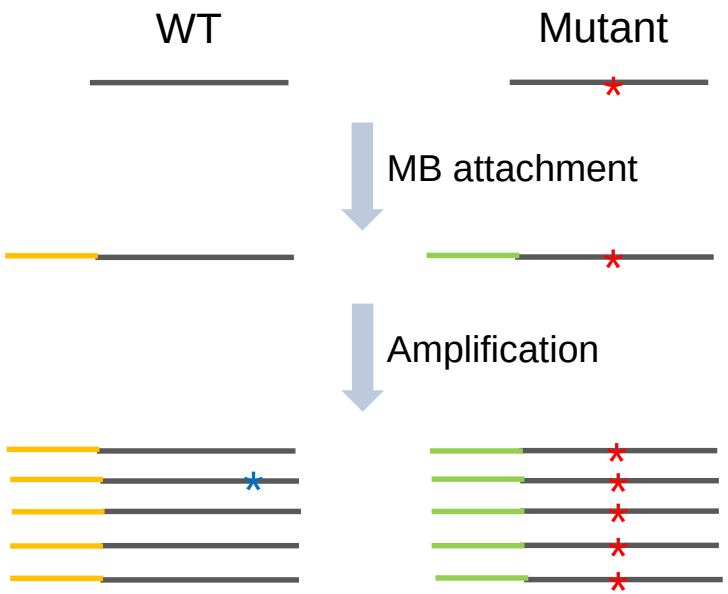
An example: 96 samples, 421 genes

Parameter	QIAseq targeted RNA panels	RT-PCR
Material required	One pool of primers	105 384-well plates
Run time	14 hours for NextSeq run	310 hours (2 hours per plate)
Hands-on time	3 hours (for 96 samples)	105 hours (one hour per plate)
Cost per sample	$65 (exclusive of sequencing run)	$239
Sample	10 ng each sample	4 µg each sample

Many forms of biomarkers



QIAseq Targeted DNA Panel: Principle



False variant is present in some fragments carrying the same MB

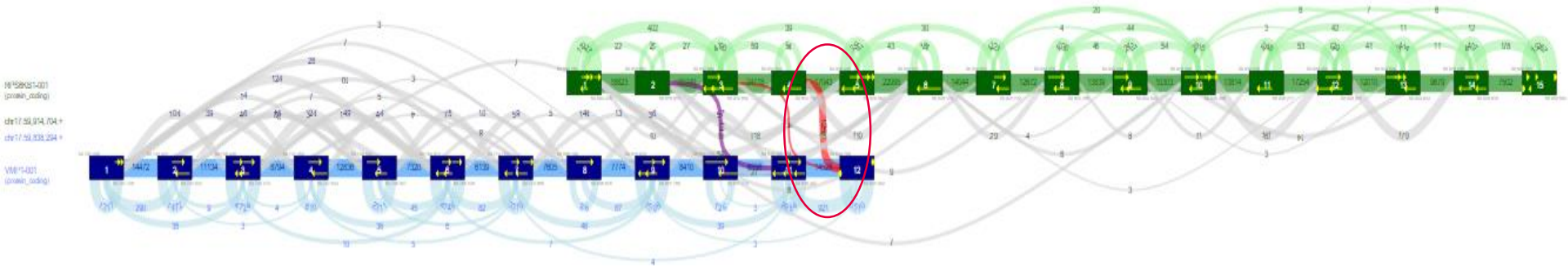
True variant is present in all fragments carrying the same MB

Molecular barcoding allows for traceability of variants, AND CNV analysis.

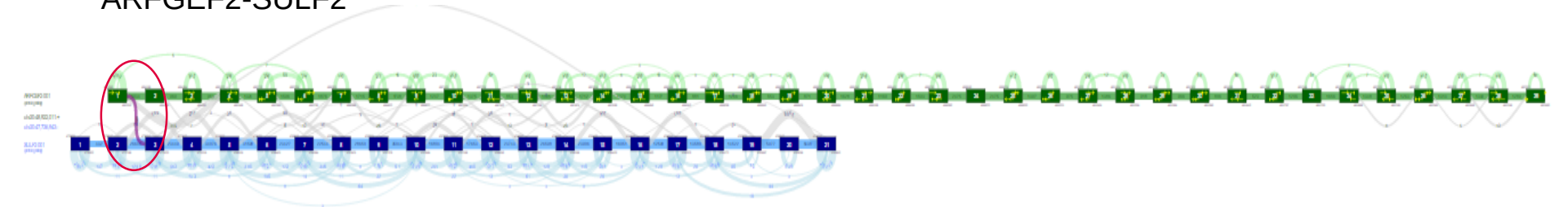
QIASeq Targeted RNAscan is a RNA target enrichment method that allows verification of known fusions and discovery of novel fusions with next-generation sequencing (NGS).

1. NCOA3--SULF2, *score:* 3806.00
2. RPS6KB1--VMP1, *score:* 1136.00
3. ARFGEF2--SULF2, *score:* 184.00
4. SULF2--PRICKLE2, *score:* 56.00
5. CLTC--VMP1, *score:* 6.00

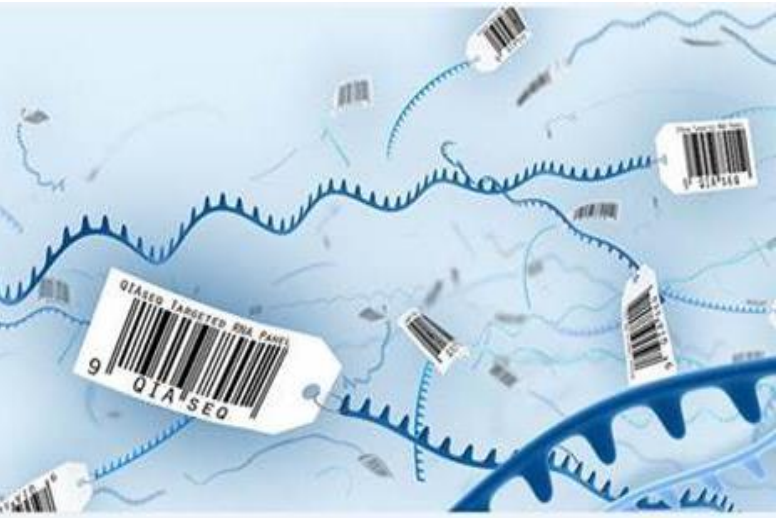
RPS6KB1-VMP1



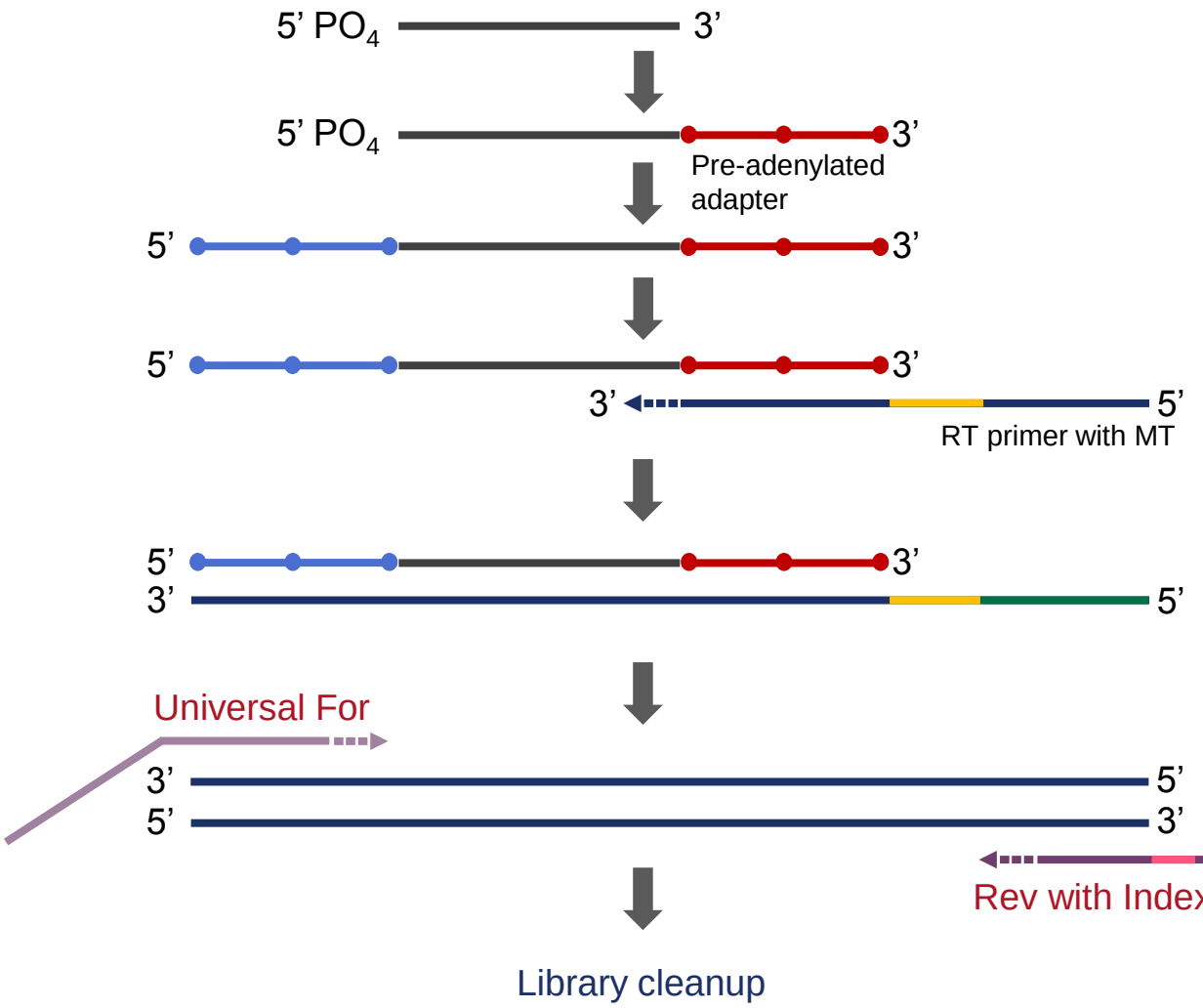
ARFGEF2-SULF2



Agenda



QIAseq miRNA Sequencing Kit: One-day Workflow



Sample to sequencer in 7 hours! Other protocols can take **2 days**!

Agenda

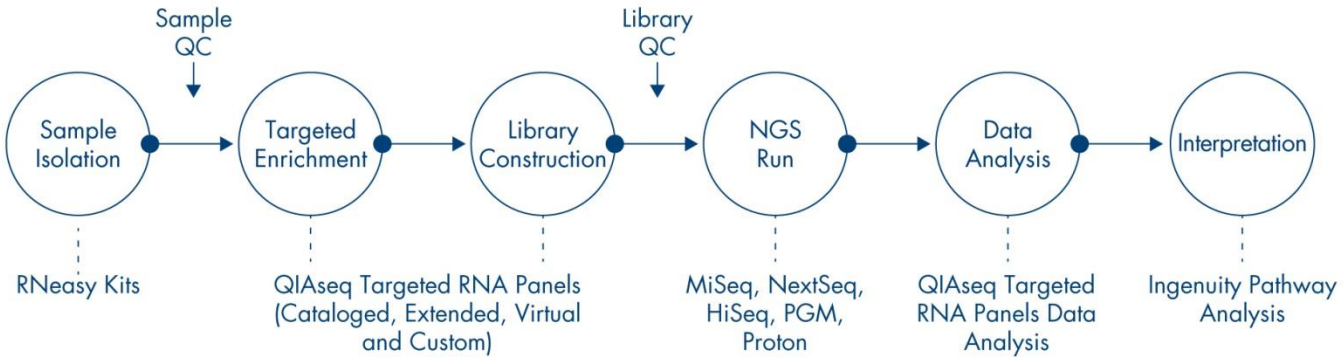
QIAseq Targeted RNAseq summary

- Extremely sensitive expression profiling, >1 copy per cell
- Highly flexible in experimental design, from 12 to 1000 or more targets, 1 to 96 samples

- High specificity, ~97-99% maintained through all panels
- Extremely high primer uniformity ~0.98 at 20% mean
- Random molecular barcoding for quantification

- Requires no rRNA depletion or blocking or dT selection
  - Only requires ~1ng-20ng total RNA
- Makes best use of limited NGS read budget
- System optimized for best possible performance with FFPE samples

- Leverage QIAGEN content know-how for NGS
  - Disease and pathway specific collections
  - Extended panels and fully custom gene content 12-1000 genes



QIAseq Targeted RNA Products

QIAseq Targeted RNA Panel (12 or 96 samples)
Kit containing reagents for first strand synthesis, Smcounter tagging, and gene-specific amplification for targeted RNA sequencing

QIAseq Targeted RNA Extended Panel (12 or 96 samples) (up to 25 additional targets)
Kit containing reagents for first strand synthesis, Smcounter tagging, and gene-specific amplification for targeted RNA sequencing;

QIAseq Targeted RNA Custom Panel (12, 96 or 384 samples)
Kit containing reagents for first strand synthesis, Smcounter tagging, and gene-specific amplification for targeted RNA sequencing

QIAseq Targeted RNA sample Indexing(12-plex or 96-plex HT) for ion torrent

QIAseq Targeted RNA sample Indexing (12-plex or 96-plex or HT) for illumina

Library Quant Assay/Array Kit
Assays and master mix for library quantification prior to NGS

Initial Content:comprehensive 250 – 500 gene panels and *ALL* human RT2 panel content (200 panels)

Immunity and Inflammation	Angiogenesis and Endothelial
Cell Death	Cancer Pathway
Signal Transduction	ECM and Cell Adhesion
Molecular Toxicology	Stem Cells

Questions?

Contact QIAGEN Technical Service

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