Asuragen®

QuantideX®

NGS RNA Lung Cancer Kit

Protocol Guide

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

REF 49602

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Purpose

The QuantideX® NGS RNA Lung Cancer Kit is intended for the detection of clinically relevant content for RNA targets common to lung cancer including both fusions as well as mRNA expression profiling for relevant genes from RNA or TNA purified from human tissue, formalin-fixed paraffin embedded (FFPE) or cell-lines.

The kit covers 107 specific RNA fusions, MET exon 14 skipping, 3'-5' imbalance ratios for 4 RNA expression markers (see Appendix 3: Other Targets) involved in oncogenic translocations, and 23 expression markers that are semi-quantitatively evaluated against a set of low variation reference genes.

The kit supports multiplex next-generation sequencing analysis with an Illumina® MiSeq® instrument.

The kit includes software (QuantideX NGS Reporter, P/N 49562) that analyzes MiSeq data files for the identification of fusion targets using a locally integrated bioinformatic pipeline and companion data visualization tools.

Fusion Targets

3' Gene	5' Gene	COSMIC ID
0.21	CLTC	COSF470
ALK	CLTC	COSF434 / 435
ALK	CLTC	COSF472
ALK	DCTN1	
ALK	EML4	COSF1065 / 1128
ALK	EML4	COSF1368
ALK	EML4	COSF412
ALK	EML4	COSF476
ALK	EML4	COSF408 / 463
ALK	EML4	COSF1062 / 1063
ALK	EML4	COSF1539 / 1540
ALK	EML4	COSF462
ALK	EML4	COSF410
ALK	EML4	COSF414
ALK	EML4	COSF1541 / 1542
ALK	EML4	COSF1064
ALK	EML4	COSF1127
ALK	EML4	COSF477 / 491
ALK	EML4	COSF413
ALK	EML4	COSF475
ALK	EML4	COSF1367
ALK	EML4	
ALK	EML4	COSF487 / 1376
ALK	EML4	COSF488
ALK	EML4	COSF409 / 465
ALK	EML4	COSF730 / 731
ALK	EML4	COSF490
ALK	EML4	COSF464
ALK	EML4	COSF478 / 1543
ALK	EML4	COSF479 / 480
ALK	EML4	COSF1296 / 1297
ALK	EML4	COSF411/734
ALK	EML4	COSF1544/1545
ALK	EML4	
ALK	EML4	COSF474

3' Gene	5' Gene	COSMIC ID
ALK	EML4	COSF493
0.21	KIF5B	COSF1381 / 1382
ALK	KIF5B	COSF1060
ALK	KIF5B	COSF1061
ALK	KIF5B	COSF1257 / 1258
ALK	KIF5B	COSF1058 / 1059
ALK	KLC1	COSF1277
ALK	SQSTM1	
ALK	STRN	COSF1430 / 1431
ALK	STRN	COSF1669
ALK	STRN	COSF1670
ALK	STRN	COSF1538
ALK	TFG	COSF430
ALK	TFG	COSF432
ALK	TFG	COSF428 / 429
ALK	TFG	COSF424 / 425
ALK	TFG	COSF426
ALK	TPM3	COSF439
CIT	FGFR2	
FGFR1	BAG4	
MBIP	AXL	
NRG1	CD74	COSF1636
NRG1	CD74	COSF1666
NTRK1	CD74	
NTRK1	MPRIP	
NTRK1	TFG	COSF1328
NTRK1	TPM3	COSF1329 / 1330
NTRK3	ETV6	COSF571
NTRK3	ETV6	COSF1534
NTRK3	ETV6	COSF823
PDGFRA	SCAF11	
RET	CCDC6	COSF1533
RET	CCDC7	COSF1271
RET	CCDC8	COSF1532
RET	CCDC9	COSF1515
RET	KIF5B	COSF1232
RET	KIF5B	COSF1230

3' Gene	5' Gene	COSMIC ID
RET	KIF5B	COSF1253
RET	KIF5B	COSF1234
RET	KIF5B	COSF1241
RET	KIF5B	COSF1262
RET	NCOA4	COSF1340
RET	TRIM33	
ROS1	CCDC6	
ROS1	CD74	COSF1478
ROS1	CD74	COSF1202
ROS1	CD74	COSF1200
ROS1	CLTC	
ROS1	EZR	COSF1267
ROS1	EZR	COSF1396
ROS1	GOPC	COSF1139
ROS1	GOPC	COSF1140
ROS1	GOPC	COSF1188
ROS1	GOPC	COSF1243
ROS1	LRIG3	COSF1269
ROS1	SDC4	
ROS1	SDC4	COSF1265
ROS1	SDC4	COSF1278
ROS1	SDC4	COSF1280
ROS1	SLC34A2	COSF1261
ROS1	SLC34A2	COSF1259
ROS1	SLC34A2	COSF1196
ROS1	SLC34A2	COSF1198
ROS1	TPM3	COSF1273
ROS1	TPM3	
ACC3	FGFR3	COSF1434
TACC3	FGFR3	COSF1353
TACC3	FGFR3	COSF1348
TACC3	FGFR3	COSF1350
TACC3	FGFR3	COSF1349
TACC3	FGFR3	COSF1352
TACC3	FGFR3	COSF1357

Note: Shading is intended to assist with visual grouping of the common 3' Gene Partners.

Limitations

- This kit is intended for research use only. Not for use in diagnostic procedures.
- The QuantideX® NGS library preparation method is based on PCR amplification of targeted regions of cancer-associated genes. The presence of rare SNPs in the primer-binding region may result in reduced amplicon yield or allele dropout.
- The kit has been verified for use on the Applied Biosystems GeneAmp® PCR System 9700 and Applied Biosystems Veriti™ 96-Well Thermal Cycler instruments with default ramp rates. **Note:** Use of other instrument platforms other than those mentioned here are not supported.*
- The RNA Assay and Library Quant qPCR protocol has been verified for use on the Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument (Fast or Standard mode) and on the Roche **cobas z** 480 Analyzer. **Note:** Analysis in fast mode or use of other instrument platforms other than those mentioned here are not supported.*
- This kit has been verified using the Illumina® MiSeq® instrument.
- The reagents should be used within their labeled expiry and stored as per label recommendations (see the Reagents Supplied with this Kit section).
- Reagents stored frozen (-15 to -30°C) are formulated to support eight (8) freeze-thaw cycles.
- The concentration of the cDNA sample must be determined as functional copies per microliter (cp/µL).
    - Use the QuantideX NGS RNA Assay to obtain copy number concentrations for each RT product sample.
    - Samples with less than 100 cp/µL may be analyzed but are at-risk for inaccuracies due to the reduced template diversity available within that sample for amplification.
    - For best results, load ≥ 100 cp/µL (Total: 400 amplifiable copies) but < 6000 cp/µl into the Gene-Specific PCR enrichment

Warnings and Precautions

- Use proper personal protective equipment. Wear appropriate protective eyeglasses, protective gloves, and protective clothing when working with these materials.
- Follow Universal Precautions in compliance with OSHA 1910:1030, CLSI M29, or other applicable guidance when handling human samples.
- Use nuclease-free filter pipette tips and nuclease-free tubes.
- Seal plates in a safe and appropriate manner to reduce likelihood of evaporation or cross contamination of wells during library preparation. Automated heat-sealing using peelable foil seals is recommended.
    - The Bio-Rad PX1 Heat Sealer (185°C, 3 seconds) with Eppendorf Twin-Tec 96 well plates and peelable foil heat seals (Bio-Rad P/N 1814045), or equivalent, has been verified for efficacy.

* Contact Asuragen Technical Support for guidance.

- PCR carryover contamination can result in false positive signals. Use appropriate precautions in sample handling, workflow, and pipetting. **Note:** Separation of template (i.e. sample handling) and non-template (i.e. master mix formulation) handling is highly recommended to reduce the risk of contamination.
- Do not let beads dry out during Library Purification to mitigate risk of sample loss/low library yield.
    - To reduce drying effects, divide the plate into sets of 2 or 3 columns at a time when purifying for large sample batches.
- Do not combine kit components from different reagent lots.
- Prior to use, ensure that all instruments are calibrated according to the manufacturer's instructions.
- When working with less than 8 samples, it is recommended to repeat Library Quant after pooling/normalizing libraries prior to loading the MiSeq.

Principle of the Procedure

The kit includes reagents for RT, cDNA QC, targeted enrichment, index codes, library purification, quantification, and an easy-to-use bioinformatics software solution. Both wet and dry bench processes are integrated within a simple workflow optimized for use with low-quality and low-quantity RNA/TNA samples isolated from FFPE (Formalin-Fixed, Paraffin-Embedded), FNA (Fine Needle Aspiration) tumor biopsies, fresh frozen (FF) tissue, and cell lines. Each kit component serves an essential role in the library prep workflow:

- QuantideX® NGS RNA Assay (Lung): Reagents for functional RNA RT, subsequent quantification, and sample QC assessment. Additionally, the box contains the RNA Fusion Positive Ctrl (Lung).
- QuantideX NGS RNA Lung Panel: PCR primers and reagents that support single-well multiplex PCR enrichment across all targets.
- QuantideX NGS Codes: Set A/B/C/D: Dual Index code oligonucleotide mixtures specific for the MiSeq. These dual-index code primer mixes are formulated in racks of 48 codes.
- QuantideX NGS Library Pure Prep: A proprietary magnetic bead chemistry that provides size selection and purification of the amplified libraries.
- QuantideX NGS Library Quant: An assay that enables accurate assessment of purified libraries using a quantitative real-time PCR method.
- QuantideX NGS Sequencing Reagents: Custom sequencing primer mixtures for QuantideX NGS and standard Illumina library analysis.
- QuantideX NGS Reporter: An easy to use, integrated informatics processing and data reporting pipeline based on proprietary alignment and variant scoring algorithms.

Workflow

Reverse Transcription (RT)

- QuantideX® NGS RNA Assay (Lung) [Box 1]
  - 4 µL QuantideX® RT Buffer
  - 1 µL QuantideX® RT Enzyme Mix
  - 5 µL RNA/TNA sample, NTC or RNA Positive Fusion Ctrl (provided)

Temp	Time
42°C	45 min
93°C	10 min
4°C	hold

RNA Assay

- QuantideX NGS RNA Assay (Lung) [Box 1]
  - 5 µL 2X qPCR Master Mix
  - 2.5 µL Primer/Probe Mix (Lung)
  - 0.5 µL 20X ROX (if needed)
  - 2 µL RT product or qPCR Standards 1 - 4 (Lung)

Temp	Time	Cycling
95°C	5 min	-
95°C	5 sec	40 cycles
60°C	60 sec	

qPCR using FAM channel

Gene-Specific PCR

- QuantideX NGS RNA Lung Panel [Box 2]
  - 5 µL 2X Amplification Master Mix
  - 1 µL RNA Lung Primer Mix
  - 4 µL RT product
    - (Suggested input ≥ 400 functional copies)

Temp	Time	Cycling
95°C	5 min	-
95°C	15 sec	2 cycles
60°C	4 min	
95°C	15 sec	23 cycles
72°C	4 min	
72°C	10 min	-
4°C	hold	-

Tagging PCR

- QuantideX Codes - Set A/B/C/D [Box 3] & QuantideX NGS RNA Lung Panel [Box 2]
  - 7.5 µL 2X Index Master Mix
  - 5.5 µL Index Code (AIL001-AIL192)
  - 2 µL Gene-Specific PCR product

Temp	Time	Cycling
95°C	5 min	-
95°C	30 sec	10 cycles
55°C	30 sec	
72°C	60 sec	
72°C	10 min	-
4°C	hold	-

Library Purification

- QuantideX NGS Library Pure Prep [Box 4]
  - 11 µL Library Pure Prep Beads
  - 10 µL Tagging PCR Product

  } Mix, pellet beads on magnet, remove supernatant
  - 2 x 100 µL washes w/EtOH-diluted Wash Buffer, on magnet
  - Elute in 20 µL Elution Buffer, 5 min

Library Quant

- QuantideX NGS Library Quant [Box 5]
  - 5 µL 2X qPCR Master Mix
  - 2.5 µL LQ Primer/Probe Mix
  - 0.5 µL 20X ROX (if needed)
  - 2 µL purified product (diluted 10,000X in LQ Diluent) or LQ Standards 1 - 4

Temp	Time	Cycling
95°C	5 min	-
95°C	5 sec	35 cycles
60°C	60 sec	

qPCR using FAM channel

Pool, Denature, Dilute, Load

- QuantideX NGS Sequencing Reagents [Box 6] & Illumina® MiSeq® Reagent Kit
  - 15 µL 2.5 nM normalized library pool from stock purified products
  - 3 µL 1 nM PhiX Control V3, 2 µL 1N NaOH; denature for 5 min at room temp, snap cool on ice
  - Dilute 8 µL of the denatured library into 992 µL pre-chilled HT1 buffer
  - Dilute 4 µL Read 1, Index Read, & Read 2 Primers in 636 µL HT1 buffer individually, load MiSeq

Reagents Supplied with this Kit

Item #	Description	Cap Color	Storage Temp
49589	**QuantideX® NGS RNA Assay (Lung) [Box 1]**		
145435	QuantideX RT Enzyme Mix	green	-15 to -30°C
145436	QuantideX RT Buffer	green	-15 to -30°C
145442	RNA Fusion Positive Control (Lung)	clear	-15 to -30°C
145437–145440	qPCR Standards 1–4 (Lung)	white	-15 to -30°C
145444 145510*	2X qPCR Master Mix	yellow	-15 to -30°C
145443	Primer/Probe Mix (Lung)	blue	-15 to -30°C
145441 145543*	20X ROX	amber	2 to 8°C (after 1st use)
49588	**QuantideX NGS RNA Lung Panel [Box 2]**		
145348 145533*	2X Amplification Master Mix	green	-15 to -30°C
150016	RNA Lung Primer Mix	green	
145361 145534*	2X Index Master Mix	yellow	
49553–49556	**QuantideX NGS Codes - Set A/B/C/D [Box 3]**		
150004/150005/150006/150007 150011/150005/150006/150007*	Index Codes (ILM) - Set A/B/C/D	rack of codes	2 to 8°C (after 1st use)
49551	**QuantideX NGS Library Pure Prep [Box 4]**		
145351 145541*	Library Pure Prep Beads	amber	2 to 8°C
145352 145542*	Wash Buffer	bottle	
145353 145540*	Elution Buffer	blue	
49590	**QuantideX NGS Library Quant [Box 5]**		
145445 145550*	LQ Diluent	bottle	2 to 8°C (after 1st use)
145444 145510*	2X qPCR Master Mix	yellow	-15 to -30°C
145447 145551*	LQ Primer/Probe Mix	violet	-15 to -30°C
145441 145543*	20X ROX	amber	2 to 8°C (after 1st use)
145449–145452 145552–14555*	LQ Standards 1–4	blue	-15 to -30°C

Item #	Description	Cap Color	Storage Temp
49649	**QuantideX NGS Sequencing Regents [Box 6]**		
145365 145562*	Sequencing Diluent	blue	2 to 8°C
150001 150008*	Read 1 Sequencing Primers	green	
150002 150009*	Index Read Sequencing Primers	green	
150027 150010*	Read 2 Sequencing Primers	green	

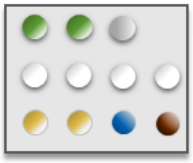
* Equivalent parts
See Appendix 1: Index Codes, for more details regarding QuantideX NGS Codes.

Note: Box numbers are included on box labels. Reactions supported assumes approximately using 15% overage when preparing reaction master mixes.

The QuantideX® NGS Reporter, a bioinformatics solution for analyzing MiSeq® output files, is included in the purchase of the kit and can be obtained at http://software.asuragen.com. See the QuantideX NGS Reporter User Guide (00002266) available for download at http://asuragen.com/ruo.

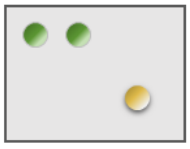
Kit Configuration

Box 1: QuantideX® NGS RNA Assay (Lung)



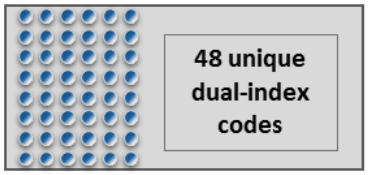
- QuantideX® RT Buffer
- QuantideX® RT Enzyme Mix
- RNA Fusion Positive Control
- qPCR Standards 1-4 (Lung)
- 2X qPCR Master Mix
- Primer/Probe Mix (Lung)
- 20X ROX

Box 2: QuantideX® NGS RNA Lung Panel

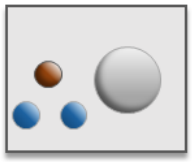


- 2X Amplification Master Mix
- RNA Lung Primer Mix
- 2X Index Master Mix

Box 3: QuantideX® NGS Codes - Set A to D

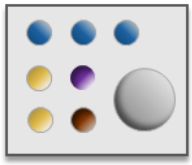


Box 4: QuantideX® NGS Library Pure Prep



- Library Pure Prep Beads
- Elution Buffer
- Wash Buffer

Box 5: QuantideX® NGS Library Quant



- LQ Primer/Probe Mix
- 2X qPCR Master Mix
- LQ Standards 1-4
- LQ Diluent
- 20X ROX

Box 6: QuantideX® NGS Sequencing Reagents

- Read 1 Sequencing Primers
- Index Read Sequencing Primers
- Read 2 Sequencing Primers
- Sequencing Diluent

Reagents Required but not Provided

- Suggested part numbers have been provided for reagents, but equivalent products may be used.
- Consult the MiSeq System User Guide for additional materials required to use Illumina® MiSeq instrument.
    - Nuclease-Free Water (not DEPC-Treated) (Thermo Fisher Scientific™, P/N AM9937)
    - Ethanol absolute ≥99.5% ACS (200 Proof) (VWR™, P/N 200004-484)
    - 2 N Sodium Hydroxide (VWR, P/N BDH7223-1)
    - PhiX Control v3 (Illumina, P/N 15017666)
    - Illumina MiSeq Reagent Kit and optimal number of samples for use by MiSeq Reagent Kit:

Illumina® MiSeq® Reagent Kits.

Reagent Kit	Manufacture/ Part Number	Number of Single Reads PF‡	Recommended Number of Libraries with Kit*
MiSeq Reagent Kit v3, 600 cycles	Illumina, P/N MS-102-3003	25 million	8–48
MiSeq Reagent Kit v2, 500 cycles	Illumina, P/N MS-102-2003	15 million	4–24

‡ The number of single reads passing filter is provided by Illumina. For more information, consult Illumina for MiSeq® System Specification.
* The number of libraries for each MiSeq reagent kit is recommended to achieve the optimized QuantideX® NGS RNA Lung data output. Consult Asuragen Technical Support for further assistance.

Consumables & Equipment Required but not Provided

Suggested part numbers have been provided for consumables and equipment, but equivalent products may be used. Item Numbers, where provided, may vary by region. Please contact Asuragen Technical Support for assistance.

- General laboratory equipment (e.g. pipettes, pipette tips, non-template laminar flow, HEPA filtered hood) and workspace to perform qPCR (one-way workflow utilizing template, template-free and post-amplification areas)
- Clear, low-retention 1.5 mL flip-top microcentrifuge tubes
- Centrifuge capable of spinning a 96-well plate
- PCR Plate Seals
- 96-well PCR Plates for use in thermal cycling
- Untreated 96-well microplates, round U-bottom, no lids, non-sterile (Evergreen, P/N 290-8117-01R)
- Magnetic Stand-96 (Thermo Fisher Scientific™, P/N AM10027)
- Applied Biosystems™ GeneAmp® PCR System 9700, or Applied Biosystems Veriti™ 96-Well Thermal Cycler
- Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument or Roche **cobas z** 480 Analyzer
    - Required: Detection of FAM and Cy5
- For Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument

- MicroAmp™ Optical 96-Well Reaction Plate with Barcode (Thermo Fisher Scientific, P/N 4306737)
- MicroAmp™ Optical Adhesive Film (Thermo Fisher Scientific, P/N 4311971)

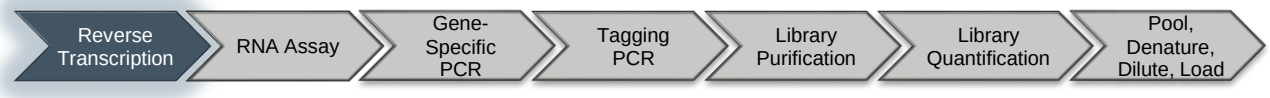
- For Roche **cobas z** 480 Analyzer†
  - LightCycler® 480 Sealing Foil (Roche, P/N 04729757001)
  - LightCycler 480 Multiwell Plate 96, white (Roche, P/N 04729692001)
- MiSeq® Desktop Sequencer (Illumina, P/N SY-410-1003)
- Decapper (automated or manual) for removal of 96-well format screw caps, for example, LabElite DeCapper (Hamilton, P/N 193600 or equivalent)
- Optional: Flatbed or handheld scanner with Data Matrix 2D barcode reading capabilities

Storage & Handling

- Store frozen reagents in the dark and in a freezer at -30°C to -15°C, as suggested in the Reagents Supplied with this Kit section
- Store non-frozen reagents at 2 to 8°C, as suggested in the Reagents Supplied with this Kit section.
- Do not freeze Library Pure Prep Beads (P/N 145351, Library Pure Prep Beads)
- Do not centrifuge Library Pure Prep Beads (P/N 145351, Library Pure Prep Beads)
- Minimize exposure of the Primer/Probe Mixes (P/N 145443, 145447) and ROX (P/N 145441) to light as these reagents are photosensitive
- The reagents have been verified for up to 8 batch uses through volumetric and freeze-thaw studies

Procedural Steps

Reverse Transcription (RT) [Box 1]



Instrument run time: ~55 min

1. Prepare an RT reaction master mix in a clean microcentrifuge tube.
   a. Add the reagents to the tube in the order listed; volumes are shown per reaction.
   b. Prepare sufficient master mix for the total number of samples tested, including any necessary controls (NTC and ● RNA Fusion Positive Control).
   c. Mix the master mix with gentle flicking/vortexing; briefly centrifuge to collect contents.

   RT Master Mix Calculation

	Per well
● QuantideX® RT Buffer	4.0 µL
● QuantideX RT Enzyme Mix	1.0 µL
Total Volume	**5.0 µL**

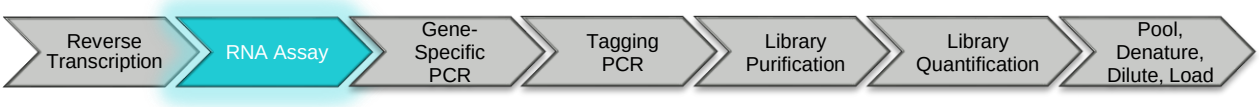
2. Aliquot 5 µL of RT master mix to separate wells in a 96-well reaction plate.

3. Add 5 µL of each RNA/TNA sample of interest to separate wells containing RT master mix; mix by pipetting.
4. Add 5 µL of each the following controls to separate wells containing RT master mix, mix by pipetting.
   a. RNA Fusion Positive Control
   b. NTC (nuclease-free water)

   Note: Including the suggested controls in each library prep batch helps to ensure run validity, monitor operator proficiency, and track performance trends over time.
5. Seal the plate and briefly centrifuge to collect contents. **Note**: Do not vortex the plate to reduce risk of contamination.
6. Perform the 10 µL Reverse Transcription (RT) reaction on a thermal cycler with the following conditions:

Temperature	Time
42 °C	45 minutes
93 °C	10 minutes
4 °C	hold

RNA Assay [Box 1]



Instrument run time: ~1.5 hours

1. Prepare a qPCR master mix in a clean microcentrifuge tube.
   a. Add the reagents to the tube in the order listed; volumes are shown per reaction.
   b. Prepare sufficient master mix for the total number of RT products, plus the 4 qPCR standards in duplicate (# of RT products + 8).
   c. Mix the master mix with gentle flicking/vortexing; briefly centrifuge to collect contents.
   d. After first use, store 20X ROX at 2 to 8°C.

   qPCR Master Mix Calculation

	Per well
2X qPCR Master Mix	5.0 µL
Primer/Probe Mix	2.5 µL
20X ROX	0.5 µL
Total Volume	**8.0 µL**

   Note: If using a qPCR instrument that does not utilize a passive reference (20X ROX), substitute this volume with nuclease-free water.

2. Aliquot 8 µL of qPCR master mix to separate wells in a 96-well optical PCR plate.
3. Add 2 µL of each qPCR Standard to separate wells in duplicate (8 wells total) and mix by pipetting.
4. Add 2 µL of each RT product to separate wells and mix by pipetting.

Example of a 6-reaction plate layout:

	1	2	3
A	qPCR Std. 1	Sample1	
B	qPCR Std. 1	Sample2	
C	qPCR Std. 2	Sample3	
D	qPCR Std. 2	Sample4	
E	qPCR Std. 3	RNA Fusion Pos. Ctrl.	
F	qPCR Std. 3	NTC	
G	qPCR Std 4		
H	qPCR Std 4		

5. Seal plate with optical seal compatible with the qPCR instrument used and briefly centrifuge to collect contents. **Note:** Do not vortex plate to reduce risk of contamination.
6. Perform the RNA Assay qPCR reaction on a qPCR instrument with the following conditions:

Temperature	Time	Cycling
95 °C	5 minutes	-
95 °C	5 seconds	40 cycles
60 °C	60 seconds	
Collect data during 60°C step		

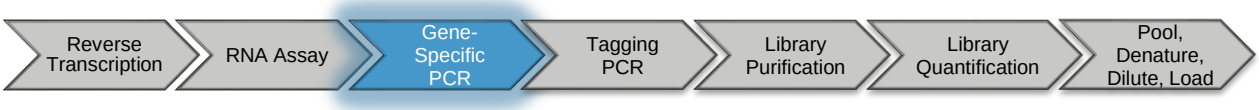
   a. For ABI 7500 instruments, select the following:
      i. Standard mode
      ii. FAM detector, no quencher
      iii. ROX Passive Reference
      iv. Auto Baseline
      v. Manual Threshold: 0.3
   b. For Roche **cobas z** 480 Analyzer, select the following:
      i. FAM detector
      ii. Fit Points analysis
      iii. Suggested analysis setting (verify and adjust per run): Background 3–9, Noise band = STD Multiplier 36, Threshold = Auto
7. Analysis
   a. Determine Copy Input:
      i. Plot a standard curve of the qPCR Standards, using log (concentration) as the x-axis, and FAM Cq values for each standard as the y-axis.

Description	Conc. (cp/µL)
qPCR Standard 1 (Lung)	6250
qPCR Standard 2 (Lung)	1250
qPCR Standard 3 (Lung)	250
qPCR Standard 4 (Lung)	50

      ii. Using the equation of the best-fit linear line, calculate concentration (cp/µL) for each sample. **Note:** Record all sample copy numbers for analysis by the QuantideX® NGS Reporter.

b. Input recommendations into QuantideX NGS RNA Lung library prep:
   i. **< 100 cp/µL:** may proceed to library prep, but at-risk for call inaccuracies due to potentially limiting functional template quantities
   ii. **≥ 100 cp/µL:** recommended input for library prep
   iii. **> 6000 cp/µL:** dilute an aliquot of the sample 10-fold in nuclease-free water for use in library prep (e.g. 2 µL sample in 18 µL water).

Gene-Specific PCR [Box 2]



Instrument run time: ~2.5 hours

1. Prepare a Gene-Specific (GS) PCR master mix in the order listed in a clean microcentrifuge tube.
   a. Add the reagents to the tube in the order listed; volumes are shown per reaction.
   b. Create enough master mix for all RT products (samples of interest plus any controls included).
   c. Gently vortex to mix, and briefly centrifuge to collect contents.

   GS PCR Master Mix Calculation

	Per well
2X Amplification Master Mix	5.0 µL
RNA Lung Primer Mix	1.0 µL
Total Volume	**6.0 µL**

2. Aliquot 6 µL of the GS PCR master mix into separate wells of a clean 96-well plate.
3. Add 4 µL of each RT product to separate wells containing GS PCR master mix, mix by pipetting. **Note**: Recommended cDNA input range is > 400 copies (>100 cp/µL)
4. Seal the plate and briefly centrifuge to collect contents. **Note:** Automated heat-sealing using peelable foil seals is recommended. Do not vortex plate to reduce risk of contamination.
5. Perform the 10 µL GS PCR reaction on a thermal cycler with the following conditions:

Temperature	Time	Cycling
95 °C	5 minutes	-
95 °C	15 seconds	2 cycles
60 °C	4 minutes	
95 °C	15 seconds	23 cycles
72 °C	4 minutes	
72 °C	10 minutes	-
4 °C	Hold	-

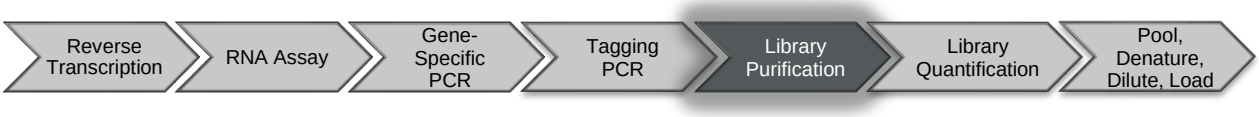
Tagging PCR [Box 2 and Box 3]

Instrument run time: ~45 min

1. Preparation:
   a. Centrifuge the rack of index codes for 1 minute at 2000 x g prior to each use.
   b. If needed, scan rack of codes with a barcode scanner to ensure correct positions of index codes. Refer to the provided COA as needed.
   c. Assign each sample/control from the GS PCR a unique Index Code (AIL001-AIL192), and record this information by plate location and sample ID. **Critical**: Each sample must be assigned (and receive) a different index code.
2. In a new 96-well plate, for each GS PCR product:
   a. Aliquot 7.5 µL 2X Index Master Mix
   b. Add 5.5 µL Index Code (AIL###, unique to each well)
3. Briefly centrifuge to collect contents, transfer 2 µL GS PCR product to corresponding wells containing Index Codes and 2X Index Master Mix. Mix by pipetting.
   Note: Ensure the correct Index Code was added to the appropriate well according to the previously made assignments and that each well received a different Index Code.
4. Seal the plate and briefly centrifuge to collect contents. **Note:** Automated heat-sealing using peelable foil seals is recommended. Do not vortex plate to reduce risk of contamination.
5. Perform the 15 µL Tagging PCR reaction on a thermal cycler with the following conditions:

Temperature	Time	Cycling
95 °C	5 minutes	-
95 °C	30 seconds	10 cycles
55 °C	30 seconds	
72 °C	60 seconds	
72 °C	10 minutes	-
4 °C	Hold	-

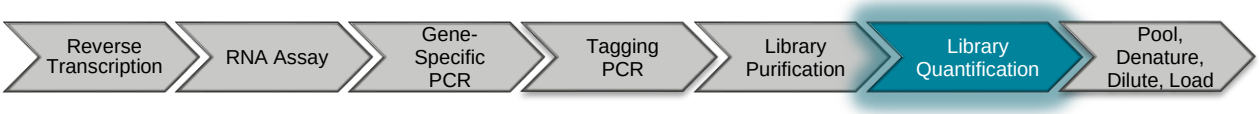
Library Purification [Box 4]



1. Initial Setup:
   a. If this is the first use of the kit, add 10 mL of 100% Ethanol to Wash Buffer bottle, cap the bottle, and mix well by inverting the bottle several times. **Note:** 10 mL of 100% ethanol must be added to the Wash Buffer bottle. Failure to add ethanol to the Wash Buffer will result in sample and reagent loss.
   b. Bring ● Library Pure Prep Beads to room temperature prior to use.
   c. Fully re-suspend ● Library Pure Prep Beads with gentle vortexing for 30–45 seconds. **Note:** DO NOT centrifuge the ● Library Pure Prep Beads.
   d. Ensure reagents and consumables are readily available before starting the purification. It is important to not let the beads dry out until the very end of the procedure. **Note:** To reduce drying effects, divide the plate into sets of 2 or 3 columns at a time when doing the purification for large sample batches.
2. Bind the Tagging PCR product to the magnetic beads:
   a. Aliquot 11 µL ● Library Pure Beads to each well in a clear, round-bottom, 96-well plate (Evergreen Scientific P/N 290-8117-01R). Prime pipette tip to ensure full volume dispense. **Note:** To keep bead concentration consistent across the plate while aliquotting, intermittently cap the ● Library Pure Prep Beads after every 4 wells and gently pulse vortex.
   b. Briefly centrifuge to collect contents and add 10 µL Tag PCR products to the wells of beads; mix by pipetting until mixture appears homogenous. 5 µL Tag PCR product will remain in Tag PCR plate. **Note:** Avoid creating bubbles while mixing. It is recommended to use a multichannel for ease of workflow from this point onwards.
3. Wash the bead-bound libraries:
   a. Place plate on the magnetic stand (Thermo Fisher Scientific P/N AM10027) to pellet beads out of solution (bead pelleting takes approximately 15 seconds).
   b. Leaving the plate on the magnetic stand, slowly remove and discard the clear supernatant (approximately 20 µL) from each well, taking care to avoid the bead pellet.
   c. Leaving the plate on the magnetic stand, add 100 µL of Wash Buffer containing Ethanol to each well.
   d. Wait for beads to re-pellet if they were disrupted when adding the Wash Buffer (approximately 15 seconds).
   e. Leaving the plate on the magnetic stand, slowly remove and discard the clear supernatant from each well, taking care to avoid the bead pellet.
   f. Repeat the wash (steps c to e) to perform a total of two washes, removing as much buffer as possible after the second wash.
4. Elute the purified libraries:
   a. After removal of all the Wash Buffer, incubate plate on the magnetic stand at room temperature for 1 minute to dry the beads. **Note**: Excessive bead drying (> 2 minutes) may lead to loss of product.
   b. Remove the plate from the magnetic stand.
   c. Add 20 µL of ● Elution Buffer to each well and mix by pipetting until bead pellets are fully re-suspended and elution reaction appears homogenous. **Note:** Use pipette tip in excess of 20 µL to avoid aspirating mixture into tip filters. Beads may clump or appear granular in solution during this step. This is acceptable, and does not affect the elution.

d. Incubate the plate for 5 minutes at room temperature once all bead pellets are re-suspended.
e. Place the plate on magnetic stand to pellet beads (approximately 15 seconds).
f. Transfer 18 µL of the clear supernatant containing the purified libraries into a clean 96-well plate. **Note:** Take care to avoid the beads.
g. This resultant plate contains the purified libraries to be pooled. An aliquot of the purified libraries will be quantified in the next section, Library Quantification [Box 5], prior to pooling.

Library Quantification [Box 5]



Instrument run time: 1.5 hours

1. Dilute an aliquot of purified library 10,000-fold (via two 100-fold serial dilutions) in LQ Diluent using the following procedure. **Note**: Thaw LQ Diluent at least 30 minutes prior to use. Both dilutions are discarded after the products have been quantified.
   a. Transfer 198 µL LQ Diluent to each well in a 96-well plate.
   b. Add 2 µL of the purified library, pipette up and down 5 times to ensure a full dispense.
   c. Mix by pipetting 10–20 times with a pipet set to 150 µL.
   d. Add 198 µL LQ Diluent to clean wells in a second 96-well plate.
   e. Transfer 2 µL of 1:100 diluted library to second plate, pipette up and down 5 times to ensure a full dispense.
   f. Mix by pipetting 10–20 times with a pipet set to 150 µL.
   g. After first use, store LQ Diluent at 2 to 8°C.
2. Prepare an LQ qPCR master mix in a clean microcentrifuge tube.
   a. Add the reagents to the tube in the order listed; volumes are shown per reaction.
   b. Create enough master mix for all 10,000-fold diluted purified products, plus the 4 LQ standards in duplicate (# of diluted products + 8).
   c. Gently vortex to mix, and briefly centrifuge the LQ qPCR master mix to collect contents.
   d. After first use, store 20X ROX at 2 to 8°C.

   LQ qPCR Master Mix

	Per well
2X qPCR Master Mix	5.0 µL
LQ Primer/Probe Mix	2.5 µL
20X ROX	0.5 µL
Total Volume	**8.0 µL**

   Note: If using a qPCR instrument that does not utilize a passive reference, substitute this volume with nuclease-free water.

3. Combine LQ qPCR master mix with standards and diluted samples in a 96-well optical plate.
   a. Aliquot 8 µL LQ qPCR master mix into a 96-well optical plate.
   b. Add 2 µL of each ● LQ Standard to separate wells in duplicate (8 wells total) and mix by pipetting.
   c. Add 2 µL of 10,000-fold diluted purified products to separate wells and mix by pipetting.
   d. Seal the plate with an optical seal compatible with the qPCR instrument used and briefly centrifuge to collect contents.

   Example of a 6-reaction plate layout:

	1	2	3
A	LQ Std. 1	Sample1	
B	LQ Std. 1	Sample2	
C	LQ Std. 2	Sample3	
D	LQ Std. 2	Sample4	
E	LQ Std. 3	Sample5	
F	LQ Std. 3	Sample6	
G	LQ Std. 4		
H	LQ Std. 4		

4. Perform 10 µL LQ PCR on a qPCR instrument with the following conditions:

Temperature	Time	Cycling
95 °C	5 minutes	-
95 °C	5 seconds	35 cycles
60 °C	60 seconds	
Collect data during 60 °C step		

   a. For ABI 7500 instruments select the following:
      i. Standard mode
      ii. FAM detector, no quencher
      iii. ROX Passive Reference
      iv. Auto Baseline
      v. Manual Threshold: 0.3
   b. For Roche **cobas z** 480 Analyzer select the following:
      i. FAM detector
      ii. Fit Points analysis
      iii. Suggested analysis setting (verify and adjust per run): Background 3-9, Noise band = STD Multiplier 36, Threshold = Auto

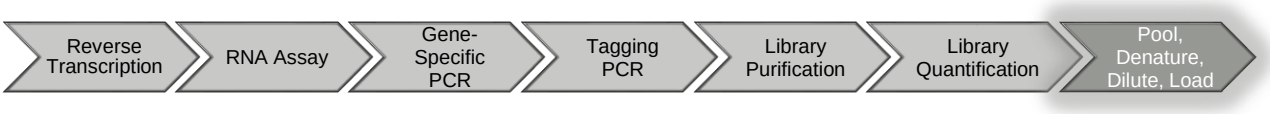
5. Analysis
    a. Determine the Concentration:
        i. Plot a standard curve of the LQ Standards, using log (concentration) as the x-axis, and FAM Cq values for each standard as the y-axis.

Description	Conc. (pM)
LQ Standard 1	20.00
LQ Standard 2	2.00
LQ Standard 3	0.20
LQ Standard 4	0.02

        ii. Using the equation of the best-fit linear line, calculate concentration (pM) for diluted purified products.
        iii. Multiply concentrations by the dilution factor (10,000) to derive the concentrations of the stock purified products.
        iv. Divide the concentrations by 1,000 to convert from pM to nM.
        v. Use these concentrations in nM of the purified products for future steps.

Note: Discard all dilution plates before proceeding to the next step.

Pool, Denature, Dilute, Load [Box 6]



Use the following instructions in conjunction with the Illumina MiSeq instructions for loading and running the instrument.

1. Setup:
    a. Follow the Illumina MiSeq instructions for thawing and preparing the MiSeq Reagent cartridge for loading.
    b. If needed, dilute stock PhiX control to 1 nM in ● Sequencing Diluent.
    c. If needed, dilute stock NaOH to 1 N in nuclease-free water.
2. Pooling/normalizing the libraries based on their respective concentrations from LQ PCR:
    a. Determine the median concentration of all the prepared libraries ($Conc_{median}$).
    b. Use the formula below to determine how much volume of each individual sample library to add to the pool. The formula targets 5 µL per library. **Note:** For ease of pipetting, round the calculated volume to the nearest microliter.

$$Vol_{lib} = \frac{Conc_{median}}{Conc_{lib}} * 5\ \mu L$$

    c. Transfer the calculated, rounded volume of each library to a clean microcentrifuge tube. **Note:** Do not pipet less than 2 µL of any one library and no more than 15 µL.
    d. For NTCs, transfer only 4 µL of purified product, regardless of the calculated concentration.

3. Using the rounded volumes used of each library, calculate the total concentration of the pool. **Note:** When working with less than 8 samples, it is recommended to repeat Library Quant after pooling/normalizing libraries prior to loading the MiSeq.

$$Conc_{pool} = \frac{(Conc_{lib} * Vol_{lib})_1 + (Conc_{lib} * Vol_{lib})_i}{Vol_{pool}}$$ where "i" is each library

4. Dilute the library pool to 2.5 nM in ● Sequencing Diluent in a clean microcentrifuge tube.
5. Transfer 992 µL of the HT1-hyb buffer to a clean microcentrifuge tube, place on ice.
6. Denature the pooled library:
   a. Transfer 15 µL of the 2.5 nM pool to a clean microcentrifuge tube at room temperature.
   b. Add 3 µL of 1 nM PhiX control, vortex to mix, and briefly centrifuge.
   c. Add 2 µL of 1 N NaOH, vortex to mix, and briefly centrifuge.
   d. Incubate the tube for 5 minutes at room temperature.
   e. Place the tube on ice immediately for at least 2 minutes. This tube now holds the denatured library.
7. Dilute the denatured library mixture:
   a. Transfer 8 µL of the denatured library to the pre-chilled 992 µL aliquot of HT1-hyb buffer.
   b. Vortex to mix, briefly centrifuge to collect contents, and place on ice. This 1000 µL is the sample to be loaded into the MiSeq cartridge in position 17.
8. Dilute the sequencing primers for in 3 separate microcentrifuge tubes on ice:
   a. Add 4 µL ● Read 1 Sequencing Primers to 636 µL HT1-hyb buffer.
   b. Add 4 µL ● Index Read Sequencing Primers to 636 µL HT1-hyb buffer.
   c. Add 4 µL ● Read 2 Sequencing Primers to 636 µL HT1-hyb buffer.
   d. Vortex all to mix, briefly centrifuge, place on ice until ready to load into MiSeq cartridge.
9. Load the MiSeq cartridge:
   a. Add 600 µL of the pooled, denatured, diluted library to position 17.
   b. Add 600 µL diluted Read 1 Sequencing Primers to position 18.
   c. Add 600 µL diluted Index Read Sequencing Primers to position 19.
   d. Add 600 µL diluted Read 2 Sequencing Primers to position 20.

   Note: Follow the Illumina MiSeq instructions for loading and running the MiSeq instrument.

Generation of MiSeq Sample Sheet

1. Download the Illumina IEM software at Illumina.com.
2. Add the “ASGN QuantideX NGS RNA Lung.txt” protocol file into the appropriate location to integrate with the IEM software (e.g. C:\Program Files\Illumina\Illumina Experiment Manager\SamplePrepKits).
3. Open the “Generate FASTQ.txt” file found in <C:\Program Files\Illumina\Illumina Experiment Manager\Applications> with the Asuragen-provided version of the .txt file. **Note**: The .txt files mentioned above are included with the purchase of the QuantideX® NGS RNA Lung Cancer Kit and available by contacting Asuragen Technical Support.
4. Follow the Illumina instructions to generate the sample sheet in the IEM software, adhering to the following parameters:

- Sample Prep Kit Type: ASGN QuantideX NGS RNA Lung
- Index Reads: 2
- Assign Index Codes (AIL001–AIL192) I-5 and I-7 to correct sample IDs, making sure they match the assignments made during Tagging PCR. Refer to QuantideX NGS Codes COA as needed.
- Instrument: MiSeq
- Application Category: Other
- Application: FASTQ only
- FASTQ only run settings:
    - Cycles Read 1: 201
    - Cycles Read 2: 201
- FASTQ only Workflow-Specific settings:
    - Custom Primer for Read 1
    - Custom Primer for Index
    - Custom Primer for Read 2

5. Transfer the resulting .csv file to the MiSeq instrument and follow the Illumina instructions to run the MiSeq instrument.

QuantideX NGS Reporter

Data analysis and interpretation is conducted using a completely integrated bioinformatics suite, the QuantideX® NGS Reporter software (49562). The software is available with purchase of the kit at http://software.asuragen.com. The QuantideX NGS Reporter User Guide (00002265) provides detailed instructions for using the software and are provided separately with the download package. This requires the copy number input for each sample determined from the RNA Assay [Box 1] to instruct the variant caller.

Quality Control Procedures

Controls

- RNA Assay and Library Quant Standards: coefficient of determination (r2) > 0.96
- RNA Fusion Positive Ctrl (Lung): EML4_ALK fusion detected (annotated as Synthetic)

Disclaimers

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References

1. Hadd, A.G., et al. Targeted, high-depth, next-generation sequencing of cancer genes in formalin-fixed, paraffin-embedded and fine-needle aspiration tumor specimens. J Mol Diagn 2013; 15(2): 234-47.

Glossary of Symbols

Symbol	Description
REF	Catalog number
LOT	Batch code
Σ	Contains sufficient for <n> tests
	Consult instructions before use
	Use by
	Manufacturer

Appendix 1: Index Codes

QuantideX® NGS Codes Set A 49553; 150004			QuantideX NGS Codes Set B 49554; 150005			QuantideX NGS Codes Set C 49555; 150006			QuantideX NGS Codes Set D 49556; 150007		
Set ID	**I7 Index ID**	**I5 Index ID**	**Set ID**	**I7 Index ID**	**I5 Index ID**	**Set ID**	**I7 Index ID**	**I5 Index ID**	**Set ID**	**I7 Index ID**	**I5 Index ID**
AIL001	I7-001	I5-01	AIL049	I7-007	I5-01	AIL097	I7-013	I5-01	AIL145	I7-019	I5-01
AIL002	I7-001	I5-02	AIL050	I7-007	I5-02	AIL098	I7-013	I5-02	AIL146	I7-019	I5-02
AIL003	I7-001	I5-03	AIL051	I7-007	I5-03	AIL099	I7-013	I5-03	AIL147	I7-019	I5-03
AIL004	I7-001	I5-04	AIL052	I7-007	I5-04	AIL100	I7-013	I5-04	AIL148	I7-019	I5-04
AIL005	I7-001	I5-05	AIL053	I7-007	I5-05	AIL101	I7-013	I5-05	AIL149	I7-019	I5-05
AIL006	I7-001	I5-06	AIL054	I7-007	I5-06	AIL102	I7-013	I5-06	AIL150	I7-019	I5-06
AIL007	I7-001	I5-07	AIL055	I7-007	I5-07	AIL103	I7-013	I5-07	AIL151	I7-019	I5-07
AIL008	I7-001	I5-08	AIL056	I7-007	I5-08	AIL104	I7-013	I5-08	AIL152	I7-019	I5-08
AIL009	I7-002	I5-01	AIL057	I7-008	I5-01	AIL105	I7-014	I5-01	AIL153	I7-020	I5-01
AIL010	I7-002	I5-02	AIL058	I7-008	I5-02	AIL106	I7-014	I5-02	AIL154	I7-020	I5-02
AIL011	I7-002	I5-03	AIL059	I7-008	I5-03	AIL107	I7-014	I5-03	AIL155	I7-020	I5-03
AIL012	I7-002	I5-04	AIL060	I7-008	I5-04	AIL108	I7-014	I5-04	AIL156	I7-020	I5-04
AIL013	I7-002	I5-05	AIL061	I7-008	I5-05	AIL109	I7-014	I5-05	AIL157	I7-020	I5-05
AIL014	I7-002	I5-06	AIL062	I7-008	I5-06	AIL110	I7-014	I5-06	AIL158	I7-020	I5-06
AIL015	I7-002	I5-07	AIL063	I7-008	I5-07	AIL111	I7-014	I5-07	AIL159	I7-020	I5-07
AIL016	I7-002	I5-08	AIL064	I7-008	I5-08	AIL112	I7-014	I5-08	AIL160	I7-020	I5-08
AIL017	I7-003	I5-01	AIL065	I7-009	I5-01	AIL113	I7-015	I5-01	AIL161	I7-021	I5-01
AIL018	I7-003	I5-02	AIL066	I7-009	I5-02	AIL114	I7-015	I5-02	AIL162	I7-021	I5-02
AIL019	I7-003	I5-03	AIL067	I7-009	I5-03	AIL115	I7-015	I5-03	AIL163	I7-021	I5-03
AIL020	I7-003	I5-04	AIL068	I7-009	I5-04	AIL116	I7-015	I5-04	AIL164	I7-021	I5-04
AIL021	I7-003	I5-05	AIL069	I7-009	I5-05	AIL117	I7-015	I5-05	AIL165	I7-021	I5-05
AIL022	I7-003	I5-06	AIL070	I7-009	I5-06	AIL118	I7-015	I5-06	AIL166	I7-021	I5-06
AIL023	I7-003	I5-07	AIL071	I7-009	I5-07	AIL119	I7-015	I5-07	AIL167	I7-021	I5-07
AIL024	I7-003	I5-08	AIL072	I7-009	I5-08	AIL120	I7-015	I5-08	AIL168	I7-021	I5-08
AIL025	I7-004	I5-01	AIL073	I7-010	I5-01	AIL121	I7-016	I5-01	AIL169	I7-022	I5-01
AIL026	I7-004	I5-02	AIL074	I7-010	I5-02	AIL122	I7-016	I5-02	AIL170	I7-022	I5-02
AIL027	I7-004	I5-03	AIL075	I7-010	I5-03	AIL123	I7-016	I5-03	AIL171	I7-022	I5-03
AIL028	I7-004	I5-04	AIL076	I7-010	I5-04	AIL124	I7-016	I5-04	AIL172	I7-022	I5-04
AIL029	I7-004	I5-05	AIL077	I7-010	I5-05	AIL125	I7-016	I5-05	AIL173	I7-022	I5-05
AIL030	I7-004	I5-06	AIL078	I7-010	I5-06	AIL126	I7-016	I5-06	AIL174	I7-022	I5-06
AIL031	I7-004	I5-07	AIL079	I7-010	I5-07	AIL127	I7-016	I5-07	AIL175	I7-022	I5-07
AIL032	I7-004	I5-08	AIL080	I7-010	I5-08	AIL128	I7-016	I5-08	AIL176	I7-022	I5-08
AIL033	I7-005	I5-01	AIL081	I7-011	I5-01	AIL129	I7-017	I5-01	AIL177	I7-023	I5-01
AIL034	I7-005	I5-02	AIL082	I7-011	I5-02	AIL130	I7-017	I5-02	AIL178	I7-023	I5-02
AIL035	I7-005	I5-03	AIL083	I7-011	I5-03	AIL131	I7-017	I5-03	AIL179	I7-023	I5-03
AIL036	I7-005	I5-04	AIL084	I7-011	I5-04	AIL132	I7-017	I5-04	AIL180	I7-023	I5-04
AIL037	I7-005	I5-05	AIL085	I7-011	I5-05	AIL133	I7-017	I5-05	AIL181	I7-023	I5-05
AIL038	I7-005	I5-06	AIL086	I7-011	I5-06	AIL134	I7-017	I5-06	AIL182	I7-023	I5-06
AIL039	I7-005	I5-07	AIL087	I7-011	I5-07	AIL135	I7-017	I5-07	AIL183	I7-023	I5-07
AIL040	I7-005	I5-08	AIL088	I7-011	I5-08	AIL136	I7-017	I5-08	AIL184	I7-023	I5-08
AIL041	I7-006	I5-01	AIL089	I7-012	I5-01	AIL137	I7-018	I5-01	AIL185	I7-024	I5-01
AIL042	I7-006	I5-02	AIL090	I7-012	I5-02	AIL138	I7-018	I5-02	AIL186	I7-024	I5-02
AIL043	I7-006	I5-03	AIL091	I7-012	I5-03	AIL139	I7-018	I5-03	AIL187	I7-024	I5-03
AIL044	I7-006	I5-04	AIL092	I7-012	I5-04	AIL140	I7-018	I5-04	AIL188	I7-024	I5-04
AIL045	I7-006	I5-05	AIL093	I7-012	I5-05	AIL141	I7-018	I5-05	AIL189	I7-024	I5-05
AIL046	I7-006	I5-06	AIL094	I7-012	I5-06	AIL142	I7-018	I5-06	AIL190	I7-024	I5-06
AIL047	I7-006	I5-07	AIL095	I7-012	I5-07	AIL143	I7-018	I5-07	AIL191	I7-024	I5-07
AIL048	I7-006	I5-08	AIL096	I7-012	I5-08	AIL144	I7-018	I5-08	AIL192	I7-024	I5-08

Index region sequences of the Index Codes can be found in the ASGN QuantideX NGS RNA Lung.txt file distributed with the purchase of the kit and available by contacting Asuragen Technical Support.

QuantideX® NGS Codes Rack Lay-out

QuantideX NGS Codes - Set A (49553; 150004 or 150011)

		I7 Index ID											
		I7-001	*I7-002*	*I7-003*	*I7-004*	*I7-005*	*I7-006*						
I5 Index ID		1	2	3	4	5	6	7	8	9	10	11	12
I5-01	A	AIL001	AIL009	AIL017	AIL025	AIL033	AIL041						
I5-02	B	AIL002	AIL010	AIL018	AIL026	AIL034	AIL042						
I5-03	C	AIL003	AIL011	AIL019	AIL027	AIL035	AIL043						
I5-04	D	AIL004	AIL012	AIL020	AIL028	AIL036	AIL044						
I5-05	E	AIL005	AIL013	AIL021	AIL029	AIL037	AIL045						
I5-06	F	AIL006	AIL014	AIL022	AIL030	AIL038	AIL046						
I5-07	G	AIL007	AIL015	AIL023	AIL031	AIL039	AIL047						
I5-08	H	AIL008	AIL016	AIL024	AIL032	AIL040	AIL048						

QuantideX NGS Codes - Set B (49554; 150005)

		I7 Index ID											
		I7-007	*I7-008*	*I7-009*	*I7-010*	*I7-011*	*I7-012*						
I5 Index ID		1	2	3	4	5	6	7	8	9	10	11	12
I5-01	A	AIL049	AIL057	AIL065	AIL073	AIL081	AIL089						
I5-02	B	AIL050	AIL058	AIL066	AIL074	AIL082	AIL090						
I5-03	C	AIL051	AIL059	AIL067	AIL075	AIL083	AIL091						
I5-04	D	AIL052	AIL060	AIL068	AIL076	AIL084	AIL092						
I5-05	E	AIL053	AIL061	AIL069	AIL077	AIL085	AIL093						
I5-06	F	AIL054	AIL062	AIL070	AIL078	AIL086	AIL094						
I5-07	G	AIL055	AIL063	AIL071	AIL079	AIL087	AIL095						
I5-08	H	AIL056	AIL064	AIL072	AIL080	AIL088	AIL096						

QuantideX NGS Codes - Set C (49555; 150006)

		I7 Index ID											
		I7-013	*I7-014*	*I7-015*	*I7-016*	*I7-017*	*I7-018*						
I5 Index ID		1	2	3	4	5	6	7	8	9	10	11	12
I5-01	A	AIL097	AIL105	AIL113	AIL121	AIL129	AIL137						
I5-02	B	AIL098	AIL106	AIL114	AIL122	AIL130	AIL138						
I5-03	C	AIL099	AIL107	AIL115	AIL123	AIL131	AIL139						
I5-04	D	AIL100	AIL108	AIL116	AIL124	AIL132	AIL140						
I5-05	E	AIL101	AIL109	AIL117	AIL125	AIL133	AIL141						
I5-06	F	AIL102	AIL110	AIL118	AIL126	AIL134	AIL142						
I5-07	G	AIL103	AIL111	AIL119	AIL127	AIL135	AIL143						
I5-08	H	AIL104	AIL112	AIL120	AIL128	AIL136	AIL144						

QuantideX NGS Codes - Set D (49556; 150007)

		I7 Index ID											
		I7-019	*I7-020*	*I7-021*	*I7-022*	*I7-023*	*I7-024*						
I5 Index ID		1	2	3	4	5	6	7	8	9	10	11	12
I5-01	A	AIL145	AIL153	AIL161	AIL169	AIL177	AIL185						
I5-02	B	AIL146	AIL154	AIL162	AIL170	AIL178	AIL186						
I5-03	C	AIL147	AIL155	AIL163	AIL171	AIL179	AIL187						
I5-04	D	AIL148	AIL156	AIL164	AIL172	AIL180	AIL188						
I5-05	E	AIL149	AIL157	AIL165	AIL173	AIL181	AIL189						
I5-06	F	AIL150	AIL158	AIL166	AIL174	AIL182	AIL190						
I5-07	G	AIL151	AIL159	AIL167	AIL175	AIL183	AIL191						
I5-08	H	AIL152	AIL160	AIL168	AIL176	AIL184	AIL192						

Note: The codes are based on separate dual index code combinations and are interchangeable for analysis.

Appendix 2: Target Regions

3' Gene	5' Gene	Transcript Definition	Type
ALK	CLTC	CLTC{ENST00000269122}:r.1_5101_insGGUG_ALK{ENST00000389048}:r.4080-120_6220	cDNA
ALK	CLTC	CLTC{ENST00000269122}:r.1_5177_ALK{ENST00000389048}:r.4080_6220	cDNA
ALK	CLTC	CLTC{ENST00000269122}:r.1_5177+2010_ALK{ENST00000389048}:r.4080-48_6220	cDNA
ALK	DCTN1	DCTN1{ENST00000361874}:r.1_3514_ALK{ENST00000389048}:r.4080_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_1903_ALK{ENST00000389048}:r.4080_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_2229+2522_ALK{ENST00000389048}:r.4126_6220	gDNA
ALK	EML4	EML4{ENST00000318522}:r.1_929_EML4{ENST00000318522}:r.903+188_903+220_ALK{ENST00000389048}:r.4080_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_929+(7320)_ALK{ENST00000389048}:r.4080_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_1751_ALK{ENST00000389048}:r.4080_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_1751_ALK{ENST00000389048}:r.4080-69_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_1751_ALK{ENST00000389048}:r.4080-90_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_1751+(3600)_ALK{ENST00000389048}:r.4080-297_6220	gDNA
ALK	EML4	EML4{ENST00000318522}:r.1_1751+1485_ALK{ENST00000389048}:r.4080-1254_6220	gDNA
ALK	EML4	EML4{ENST00000318522}:r.1_1751+2575_ALK{ENST00000389048}:r.4080-203_6220	gDNA
ALK	EML4	EML4{ENST00000318522}:r.1_1903_ALK{ENST00000389048}:r.4080-124_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_1903_ALK{ENST00000389048}:r.4094_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_1903_ALK{ENST00000389048}:r.4118_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_1903_insAUAUGCUGGAU_ALK{ENST00000389048}:r.4129_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_1969_ALK{ENST00000389048}:r.4151_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_2029_ALK{ENST00000389048}:r.4080_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_2229_EML4{ENST00000318522}:r.2229+2517_2229+2522_ALK{ENST00000389048}:r.4126_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_2229_insCAUACUAUGUAUACAAGGGAGUU_ALK{ENST00000389048}:r.4126_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_2318_ALK{ENST00000389048}:r.4080_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_2318+654_insU_ALK{ENST00000389048}:r.4080-172_6220	gDNA
ALK	EML4	EML4{ENST00000318522}:r.1_2504_ALK{ENST00000389048}:r.4080_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_2504_ALK{ENST00000389048}:r.4080-18_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_2504+182_ALK{ENST00000389048}:r.4080-67_6220	gDNA
ALK	EML4	EML4{ENST00000318522}:r.1_2504+545_ALK{ENST00000389048}:r.4080-232_6220	gDNA

3’ Gene	5’ Gene	Transcript Definition	Type
ALK	EML4	EML4{ENST00000318522}:r.1_470_ALK{ENST00000389048}:r.4080_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_470_ALK{ENST00000389048}:r.4080-117_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_929_ALK{ENST00000389048}:r.3975_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_929_ALK{ENST00000389048}:r.4080_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_929_ALK{ENST00000389048}:r.4080-18_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_929_insCAAAAAUGUCAACUCGCAAAAAAAACAGCCAAG_ALK{ENST00000389048}:r.4080_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_929+220_ALK{ENST00000389048}:r.4080_6220	cDNA
ALK	EML4	EML4{ENST00000318522}:r.1_929+805_insAA_ALK{ENST00000389048}:r.4080-115_6220	gDNA
ALK	KIF5B	KIF5B{ENST00000302418}:r.1_2183_ALK{ENST00000389048}:r.4080_6222	cDNA
ALK	KIF5B	KIF5B{ENST00000302418}:r.1_2183_ALK{ENST00000389048}:r.4094_6222	cDNA
ALK	KIF5B	KIF5B{ENST00000302418}:r.1_2183+2477_ALK{ENST00000389048}:r.4005_6222	gDNA
ALK	KIF5B	KIF5B{ENST00000302418}:r.1_2490_ALK{ENST00000389048}:r.4080_6222	cDNA
ALK	KIF5B	KIF5B{ENST00000302418}:r.1_3219_ALK{ENST00000389048}:r.4080_6222	cDNA
ALK	KLC1	KLC1{ENST00000348520}:r.1_1580_ALK{ENST00000389048}:r.4080_6220	cDNA
ALK	SQSTM1	SQSTM1{ENST00000514093}:r.1_842_ALK{ENST00000389048}:r.4080_6220	cDNA
ALK	STRN	STRN{ENST00000263918}:r.1_421_ALK{ENST00000389048}:r.4080_6222	cDNA
ALK	STRN	STRN{ENST00000263918}:r.1_421+2363_ALK{ENST00000389048}:r.4080-440_6220	gDNA
ALK	STRN	STRN{ENST00000263918}:r.1_421+2483_insUGU_ALK{ENST00000389048}:r.4080-580_6220	gDNA
ALK	STRN	STRN{ENST00000263918}:r.1_421+6813_ALK{ENST00000389048}:r.4080-1756_6220	gDNA
ALK	TFG	TFG{ENST00000240851}:r.1_755+430_ALK{ENST00000389048}:r.4080-1687_6220	gDNA
ALK	TFG	TFG{ENST00000240851}:r.1_920+2559_insU_ALK{ENST00000389048}:r.4080-940_6220	gDNA
ALK	TFG	TFG{ENST00000240851}:r.1_1061_ALK{ENST00000389048}:r.4080_6220	cDNA
ALK	TFG	TFG{ENST00000240851}:r.1_755_ALK{ENST00000389048}:r.4080_6220	cDNA
ALK	TFG	TFG{ENST00000240851}:r.1_920_ALK{ENST00000389048}:r.4080_6220	cDNA
ALK	TPM3	TPM3{ENST00000368533}:r.1_717_ALK{ENST00000389048}:r.4080_6220	cDNA
CIT	FGFR2	FGFR2{ENST00000358487}:r.1_2574_CIT{ENST00000392521}:r.2961_8708	cDNA
FGFR1	BAG4	BAG4{ENST00000521282}:r.1_192_FGFR1{ENST00000397091}:r.1680_5702	cDNA
MBIP	AXL	AXL{ENST00000301178}:r.1_2767_MBIP{ENST00000416007}:r.563_1648	cDNA
NRG1	CD74	CD74{ENST00000009530}:r.1_627_NRG1{ENST00000356819}:r.963_3083	cDNA
NRG1	CD74	CD74{ENST00000009530}:r.1_882_NRG1{ENST00000356819}:r.963_3083	cDNA
NTRK1	CD74	CD74{ENST00000353334}:r.1_868_NTRK1{ENST00000392302}:r.1262_2609	cDNA
NTRK1	MPRIP	MPRIP{ENST00000395811}:r.1_3048_NTRK1{ENST00000392302}:r.1421_2609	cDNA

3’ Gene	5’ Gene	Transcript Definition	Type
NTRK1	TFG	TFG{ENST00000240851}:r.1_920_NTRK1{ENST00000392302}:r.1262_2609	cDNA
NTRK1	TPM3	TPM3{ENST00000368533}:r.1_717_NTRK1{ENST00000392302}:r.1262_2609	cDNA
NTRK3	ETV6	ETV6{ENST00000396373}:r.1_1283_NTRK3{ENST00000394480}:r.1908_19984	cDNA
NTRK3	ETV6	ETV6{ENST00000396373}:r.1_737_NTRK3{ENST00000394480}:r.1719_19984	cDNA
NTRK3	ETV6	ETV6{ENST00000396373}:r.1_737_NTRK3{ENST00000394480}:r.1908_19984	cDNA
PDGFRA	SCAF11	SCAF11{ENST00000369367}:r.1_213_PDGFRA{ENST00000257290}:r.320_6576	cDNA
RET	CCDC6	CCDC6{ENST00000263102}:r.1_535+1054_RET{ENST00000355710}:r.2369-?_5659	gDNA
RET	CCDC6	CCDC6{ENST00000263102}:r.1_535_RET{ENST00000355710}:r.2369_5659	cDNA
RET	CCDC6	CCDC6{ENST00000263102}:r.1_535+1111_RET{ENST00000355710}:r.2369-807_5659	gDNA
RET	CCDC6	CCDC6{ENST00000263102}:r.1_685_RET{ENST00000355710}:r.2369_5659	cDNA
RET	KIF5B	KIF5B{ENST00000302418}:r.1_2183_RET{ENST00000355710}:r.2369_5629	cDNA
RET	KIF5B	KIF5B{ENST00000302418}:r.1_2372_RET{ENST00000355710}:r.2369_5629	cDNA
RET	KIF5B	KIF5B{ENST00000302418}:r.1_2897_RET{ENST00000355710}:r.2369_5629	cDNA
RET	KIF5B	KIF5B{ENST00000302418}:r.1_3002_RET{ENST00000355710}:r.2369_5629	cDNA
RET	KIF5B	KIF5B{ENST00000302418}:r.1_3002+152_insCUUU_RET{ENST00000355710}:r.2369-12_5629	gDNA
RET	KIF5B	KIF5B{ENST00000302418}:r.1_3219_RET{ENST00000355710}:r.2112_5629	cDNA
RET	NCOA4	NCOA4{ENST00000452682}:r.1_870_RET{ENST00000355710}:r.2369_5659	cDNA
RET	TRIM33	TRIM33{ENST00000358465}:r.1_2502_RET{ENST00000355710}:r.2369_5659	cDNA
ROS1	CCDC6	CCDC6{ENST00000263102}:r.1_1079_ROS1{ENST00000368508}:r.5841_7435	cDNA
ROS1	CD74	CD74{ENST00000009530}:r.1_627_ROS1{ENST00000368508}:r.5841_7435	cDNA
ROS1	CD74	CD74{ENST00000009530}:r.1_627_ROS1{NM_002944}:r.5448_7368	cDNA
ROS1	CD74	CD74{ENST00000009530}:r.1_627_ROS1{NM_002944}:r.5757_7368	cDNA
ROS1	CLTC	CLTC{ENST00000393043}:r.1_5177_ROS1{ENST00000368508}:r.5841_7435	cDNA
ROS1	EZR	EZR{ENST00000367075}:r.1_1259_ROS1{NM_002944}:r.5757_7368	cDNA
ROS1	EZR	EZR{ENST00000367075}:r.1_1259+207_oEZR{ENST00000367075}:r.1259+210_1259+244_ROS1{NM_002944}:r.5757-744_7368	gDNA
ROS1	GOPC	GOPC{ENST00000368498}:r.1_1334_ROS1{NM_002944}:r.5841_7368	cDNA
ROS1	GOPC	GOPC{ENST00000368498}:r.1_1334+2304_ROS1{NM_002944}:r.5841-2355_7368	gDNA
ROS1	GOPC	GOPC{ENST00000368498}:r.1_726_ROS1{NM_002944}:r.5977_7368	cDNA
ROS1	GOPC	GOPC{ENST00000368498}:r.1_726+822_insGAUAUGCUGAGUAUUUGCUCAAAGGAAAGUCACCUCU_ROS1{NM_002944}:r.5977-563_7368	gDNA
ROS1	LRIG3	LRIG3{ENST00000320743}:r.1_2982_ROS1{ENST00000368508}:r.5841_7435	cDNA
ROS1	SDC4	SDC4{ENST00000372733}:r.1_239_ROS1{ENST00000368508}:r.5757_7368	cDNA
ROS1	SDC4	SDC4{ENST00000372733}:r.1_239_ROS1{NM_002944}:r.5448_7368	cDNA
ROS1	SDC4	SDC4{ENST00000372733}:r.1_485_ROS1{NM_002944}:r.5448_7368	cDNA

3’ Gene	5’ Gene	Transcript Definition	Type
ROS1	SDC4	SDC4{ENST00000372733}:r.1_485_ROS1{NM_002944}:r.5757_7368	cDNA
ROS1	SLC34A2	SLC34A2{ENST00000382051}:r.1_2076_ROS1{NM_002944}:r.5757_7368	cDNA
ROS1	SLC34A2	SLC34A2{ENST00000382051}:r.1_2076_ROS1{NM_002944}:r.5448_7368	cDNA
ROS1	SLC34A2	SLC34A2{ENST00000382051}:r.1_429_ROS1{NM_002944}:r.5448_7368	cDNA
ROS1	SLC34A2	SLC34A2{ENST00000382051}:r.1_429_ROS1{NM_002944}:r.5757_7368	cDNA
ROS1	TPM3	TPM3{ENST00000368530}:r.1_968_ROS1{NM_002944}:r.5841_7368	cDNA
ROS1	TPM3	TPM3{ENST00000368533}:r.1_319_ROS1{ENST00000368508}:r.5977_7435	cDNA
TACC3	FGFR3	FGFR3{ENST00000440486}:r.1_2530_TACC3{ENST00000313288}:r.1943_2781	cDNA
TACC3	FGFR3	FGFR3{NM_000142}:r.1_2530_TACC3{ENST00000313288}:r.1751_2781	cDNA
TACC3	FGFR3	FGFR3{NM_000142}:r.1_2530_TACC3{ENST00000313288}:r.2048_2781	cDNA
TACC3	FGFR3	FGFR3{NM_000142}:r.1_2530+104_TACC3{ENST00000313288}:r.541_2781	cDNA
TACC3	FGFR3	FGFR3{NM_000142}:r.1_2530+27_TACC3{ENST00000313288}:r.2048-1511_2781	cDNA
TACC3	FGFR3	FGFR3{NM_000142}:r.1_2530+38_TACC3{ENST00000313288}:r.2048-1459_2781	gDNA
TACC3	FGFR3	FGFR3{NM_000142}:r.1_2530+63_TACC3{ENST00000313288}:r.1877_2781	cDNA

Appendix 3: Other Targets

Other Targets	Target Type
ABCB1	Expression Marker
BRCA1	Expression Marker
CD274	Expression Marker
CDKN2A	Expression Marker
CTLA4	Expression Marker
ERCC1	Expression Marker
ESR1	Expression Marker
FGFR1	Expression Marker
FGFR2	Expression Marker
IFNGR	Expression Marker
ISG15	Expression Marker
MET	Expression Marker
MSLN	Expression Marker
PDCD1	Expression Marker
PDCD1LG2	Expression Marker
PTEN	Expression Marker
RRM1	Expression Marker
TDP1	Expression Marker
TERT	Expression Marker
TLE3	Expression Marker
TOP1	Expression Marker
TUBB3	Expression Marker
TYMS	Expression Marker
ALK	3’-5’ Imbalance
NTRK1	3’-5’ Imbalance
RET	3’-5’ Imbalance
ROS1	3’-5’ Imbalance
Multiple Endogenous Targets	Endogenous Controls
MET exon 14 skipping	3

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