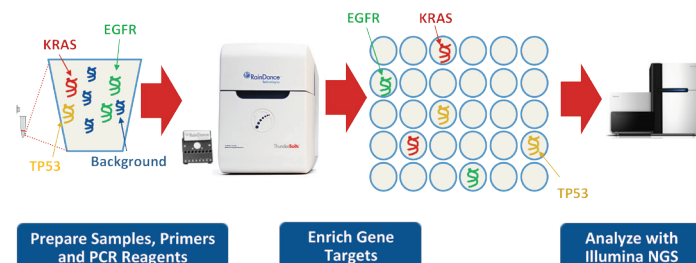


# RainDance ThunderBolts™ Cancer Panel

Sequence 10 ng DNA from FFPE, tissue and plasma for low overall cost per sample

## Lightning in a Droplet

The ThunderBolts™ Cancer Panel is a next-generation sequencing (NGS) panel for profiling informative driver mutations. The ThunderBolts Cancer Panel enables researchers to rapidly detect and cost-effectively analyze mutations in FFPE (Formalin-Fixed Paraffin-Embedded) and liquid biopsy (circulating tumor DNA) samples. The panel features NGS breakthroughs in sequence coverage and uniformity, as well as allelic sensitivity, sample flexibility, workflow, and cost.



**Image 1:**  
Simple Workflow for the ThunderBolts Cancer Panel

## Cancer Gene Content

The ThunderBolts Cancer Panel uses 230 amplicons to interrogate mutations/hotspots in 50 oncogenes, tumor suppressors and drug resistance markers (Table 1). The panel includes content found on the Ion Torrent AmpliSeq™ Cancer Hotspot Panel v2 and the Illumina TruSeq® Amplicon Cancer Panel products.

**Table 1:**  
ThunderBolts Cancer Panel Gene List

ABL1	EGFR	GNAQ	KRAS	PTPN11
AKT1	ERBB2	GNAS	MET	RB1
ALK	ERBB4	HNF1A	MLH1	RET
APC	EZH2	HRAS	MPL	SMAD4
ATM	FBXW7	IDH1	NOTCH1	SMARCB1
BRAF	FGFR1	IDH2	NPM1	SMO
CDH1	FGFR2	JAK2	NRAS	SRC
CDKN2A	FGFR3	JAK3	PDGFRA	STK11
CSF1R	FLT3	KDR	PIK3CA	TP53
CTNNB1	GNA11	KIT	PTEN	VHL

## True Single Molecule Amplifications

The ThunderBolts Cancer Panel utilizes the ThunderBolts System and RainDance's proprietary RainStorm™ single-molecule PCR technology to generate 8 million PCR droplet reactions. Each droplet is consistent in size (5 pL) and contains either zero or one target molecule. This true digital approach ensures high coverage, specificity and uniformity and therefore, optimizes sequencing performance for the Illumina MiSeq® System (Image 1). Every droplet counts.

## Key Advantages

- **Target relevant cancer content:** 230 amplicons interrogate mutations/hotspots in 50 cancer genes
- **Superior coverage:** Typical results include:
  - 100% of amplicons at 100x (2,500 average mean reads/target)
  - 98% of amplicons at 500x (2,500 average mean reads/target)
- **Minimal sample input required:** As low as 10 ng amplifiable DNA with no pre-amplification
- **Sample flexibility:** Compatible with all types of DNA, including FFPE, tissue and plasma
- **High overall uniformity:** 98% at 500x (0.2x of mean)
- **Streamlined workflow for Illumina NGS:** RainDance DirectSeq™ method eliminates sequencing library preparation steps
- **Reduced time to result:** <3 hours hands-on time enabling sample-to-results (TAT) in <48 hours
- **Highly sensitive:** Detects minor allele frequencies (MAF) as low as 1-5%
- **Low cost capital investment:** Panel runs on the compact, deployable ThunderBolts System

## High Coverage and Uniformity

The ThunderBolts Cancer Panel provides superior coverage, specificity and uniformity for both cell line and FFPE DNA with input amounts as low as 10 ng. The following table (Table 2) shows the standard sequencing metrics from a recent study using samples with 10 ng and 100 ng input amounts.

**Table 2:**  
Observed Sequencing Metrics for Cell and FFPE DNA

DNA Type	Input DNA ng	Mean Reads	1X	30X	100X	500X	Uniformity
Cell line	10 ng	4,210	100%	100%	100%	97.83%	97.83%
Cell line	10 ng	6,226	100%	100%	100%	97.39%	97.39%
Cell line	100 ng	4,033	100%	100%	100%	97.83%	97.83%
Cell line	100 ng	7,399	100%	100%	100%	97.39%	97.39%
FFPE	10 ng	4,223	100%	100%	100%	96.96%	96.96%
FFPE	10 ng	6,047	100%	100%	100%	96.09%	96.09%
FFPE	100 ng	4,108	100%	100%	100%	95.65%	95.65%
FFPE	100 ng	6,490	100%	100%	100%	95.65%	95.65%

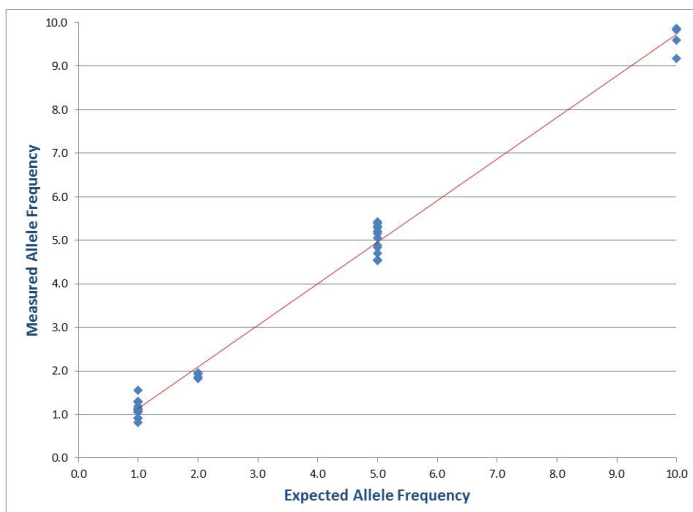
**Mean Reads:** Mean number of mapped reads per target

**1x/30x/100x/500x:** Percentage of the region of interest covered at x depth of coverage

**Uniformity:** Percentage of region of interest that has at least 20% of mean base coverage

## Accurate Low MAF Detection

The plot below (Figure 2) shows the correlation between expected and measured variant allele frequencies from replicate runs using the ThunderBolts Cancer Panel. Low minor allele variants were detected at 10%, 5%, 2% and 1% with excellent correlation ( $R^2 = .996$ ).



**Figure 2:**  
Correlation between Expected and Measured Minor Allele Frequencies in Replicates

## Precise Validation with Digital PCR

In a study profiling known mutations in pancreatic cancer samples, researchers from the University of Michigan sequenced DNA (8.4 ng to 50 ng) isolated from pancreatic cyst fluid biopsies on the Illumina MiSeq System using the ThunderBolts Cancer Panel. Known mutations in KRAS and GNAS, as well as in other genes (PIK3CA and KIT) were accurately detected (Table 3) and validated with the RainDrop Digital PCR System (Table 4). Current industry standard assay calls failed to detect these same mutations in two of the three samples.

**Table 3:**  
Mutation Detection Using Low Input Pancreatic Cyst Fluid Biopsy

Case	Pathology	KRAS (freq)	GNAS (freq)	PIK3CA (freq)	KIT (freq)	KDR
1	Invasive CA	G12D (29%)	R201C (32%)	I393M (58%)	M541L (42%)	
2	Dysplasia	G12D (40%)	R201H (36%)	-	-	Q472H V297I
3	Dysplasia	G12D (21%)	R201C (20%)	-	-	
4	NI	-	-	-	-	
5	NI	-	-	-	-	

**Table 4:**  
Validation and Concordance of KRAS Mutation with Digital PCR

Case	Pathology	ThunderBolts (freq)	RainDrop (freq)	Current Industry Standard Assay Call
1	Invasive CA	29%	27%	Absent
2	Dysplasia	40%	37%	Present
3	Dysplasia	21%	21%	Absent
4	NI	0%	0%	Absent
5	NI	0%	0%	Absent

## Ordering Information

Product	Description	Part Number
ThunderBolts™ Cancer Panel (24 sample) Kit	ThunderBolts Cancer Panel primers, Chips (6), Carrier Oil, Stabilizer Solution, Destabilizer Solution	20-07217
ThunderBolts™ Cancer Panel (48 sample) Kit	ThunderBolts Cancer Panel primers, Chips (12), Carrier Oil, Stabilizer Solution, Destabilizer Solution	20-07215
ThunderBolts™ System	Includes the ThunderBolts System instrument with computer and instrument control software	20-07700

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The RainDance ThunderBolts Cancer Panel and ThunderBolts System are for Research Use Only. Not for use in diagnostic procedures.

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