



Targeted Sequencing Using Droplet-Based Microfluidics

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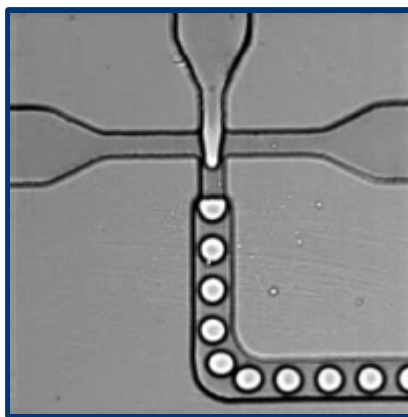


RainDance Technologies

Who we are:

RainDance Technologies is a Provider of **Microdroplet-based Solutions**

The Company's ***RainStorm™*** Technology Produces
Picoliter-sized Droplets at a Rate of **10 million Per Hour**



30 micron (16 pL) droplets: 3,000 / second

Each droplet is the functional equivalent of an individual test tube and can contain a single molecule, cell or reaction

Accelerating Human Health and Disease Research

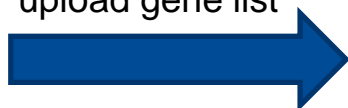


Custom Order Primer Libraries



customer

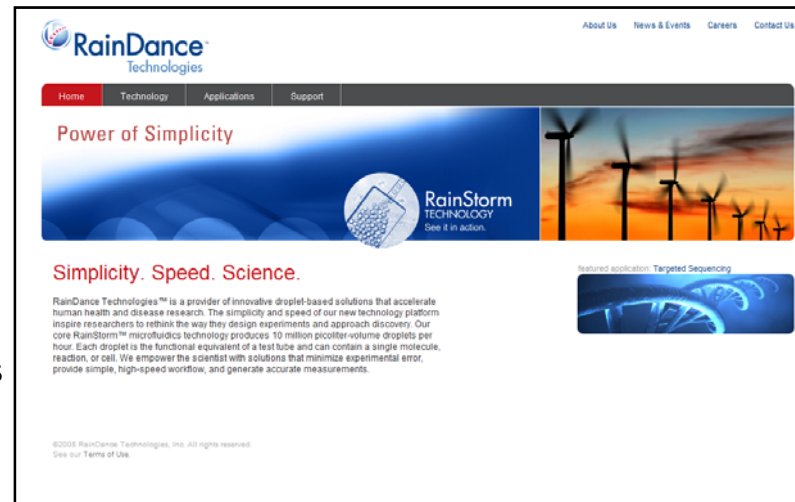
upload gene list



- RefSeq ID
- CCDS
- Genomic coordinates
- Sequence



Primer Library aliquots



- primer design
- oligo synthesis
- primer library production



Primer Design Criteria

Parameter	Spec	Notes
SNP Filter	Avoid known SNPs in primers	Using latest info in dbSNP
Amplicon Length (bp) 454 FLX 454 Titanium Illumina / SOLiD	150-250 200-600 200-600	
Amplicon GC Content (%)	30-70%	50% optimal
Padding Region	50 bp	Max. position for 3' end of primer outside of target region.
Overlap (for tiled amplicons)	20-100 bp	• Upstream of primer sequence of overlapping amplicons
Primer Tm (°C)	56-60	
isPCR 1 product 2 products 3-5 products Max product size (bp)	100% 4000	<ul style="list-style-type: none"> • Percent of amplicons with products predicted by isPCR • BLASTN word size of 7; >80% overall identity and perfect match of 4 bp at 3' end OR perfect match of 11 bp at 3' end • Max allowable product size for non-specific isPCR amplicon to be considered.



RainDance Primer Library Production

1. Primer Design

- Customer's regions of interest

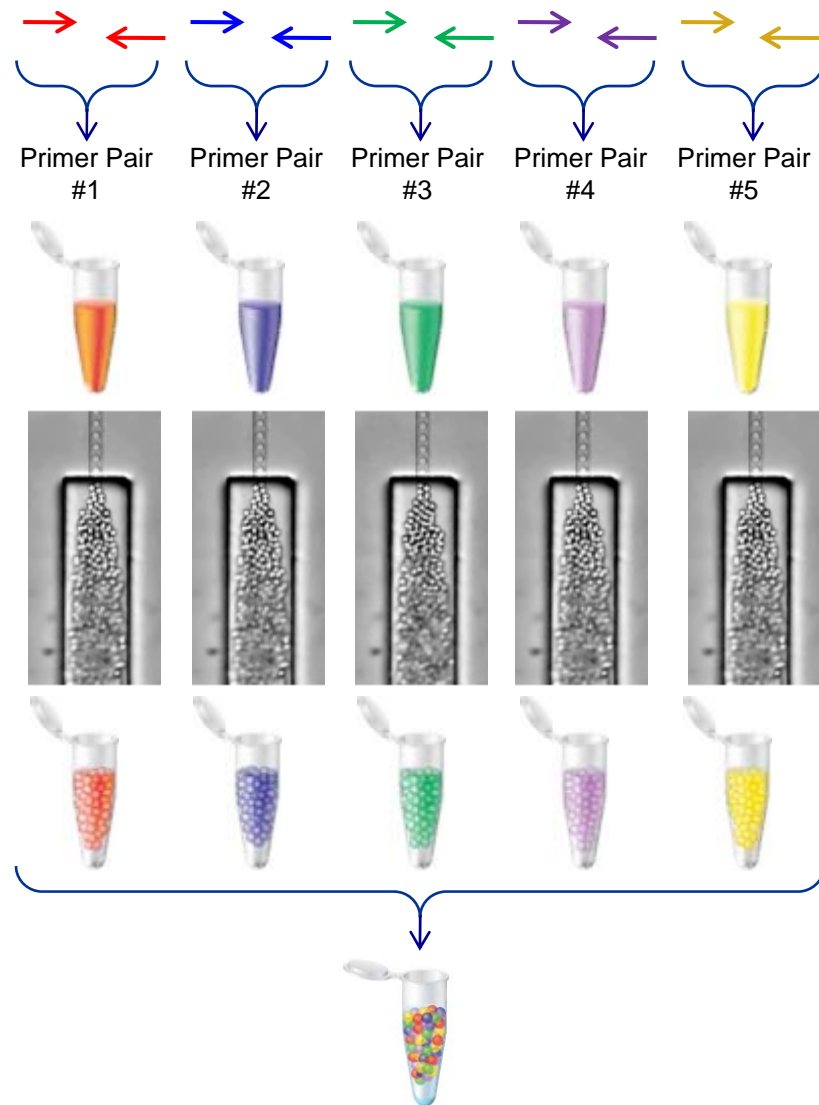
2. Primer Synthesis

- Forward and reverse primer pair

3. Primer Droplet Aliquot Manufacture

- Controlled droplet volume
- Controlled droplet count

4. Primer Library





Primer Library Quality Control

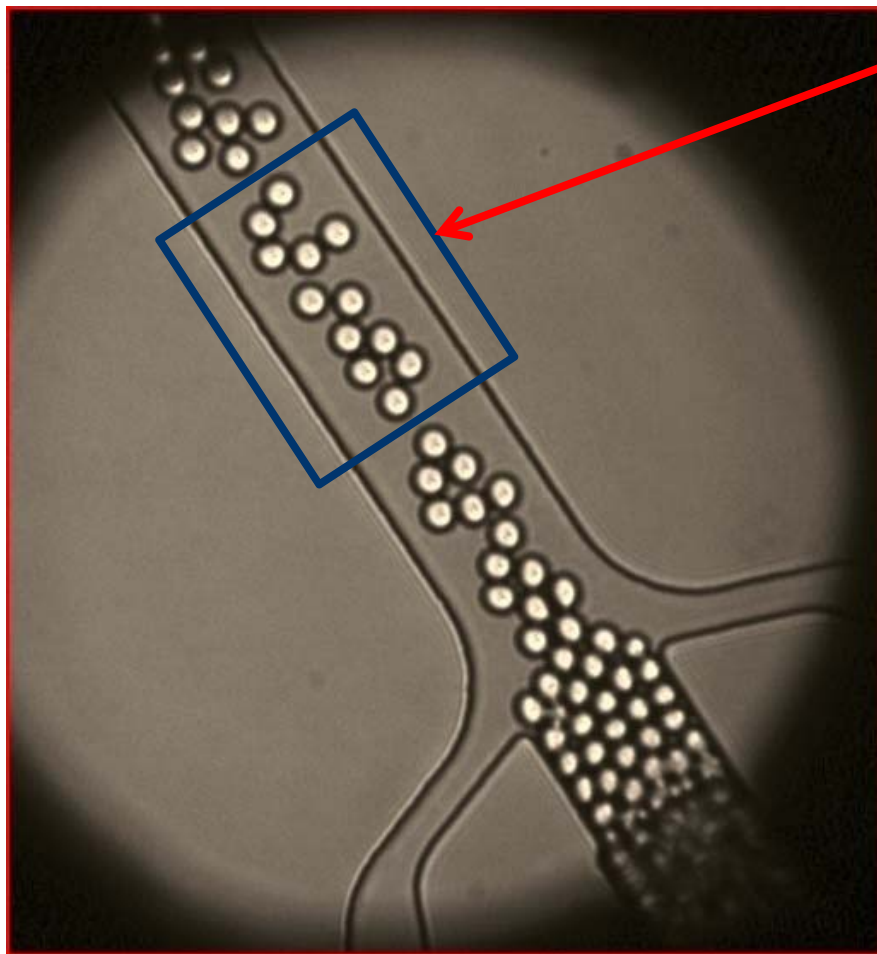


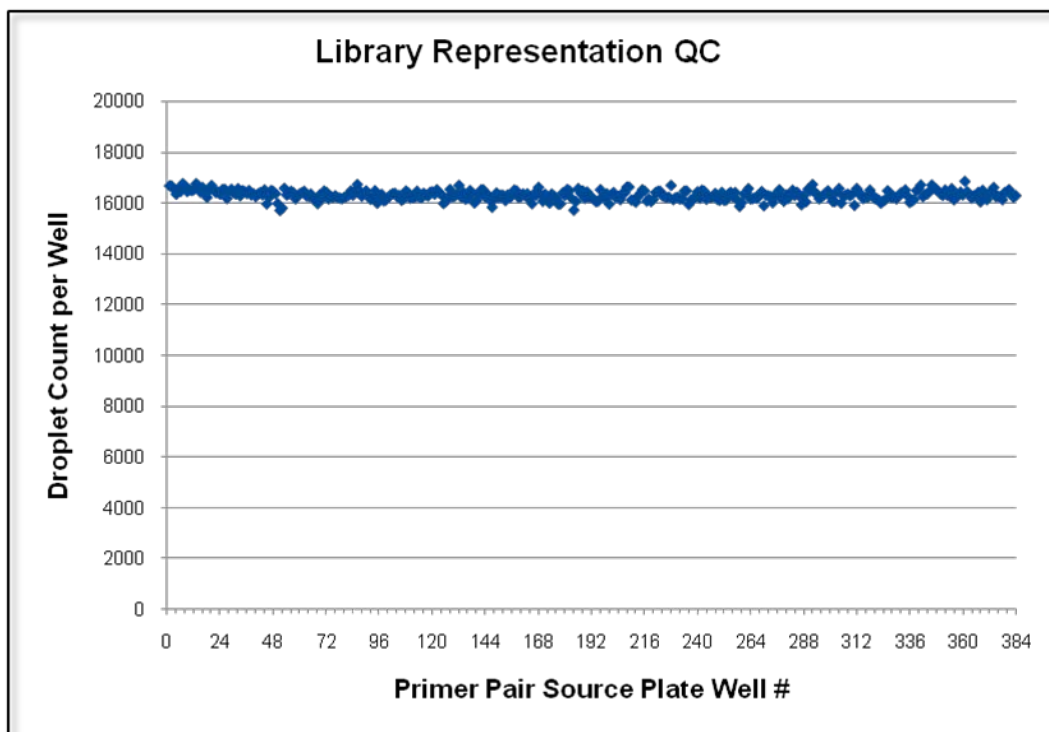
Image Analysis:

- Droplet size
- Uniformity



Primer Library Quality Control

- Real-time QC of droplet representation, ~ 6,266,496 droplets
 - Average Number of Droplets = 16,319*
 - Std Dev = 184

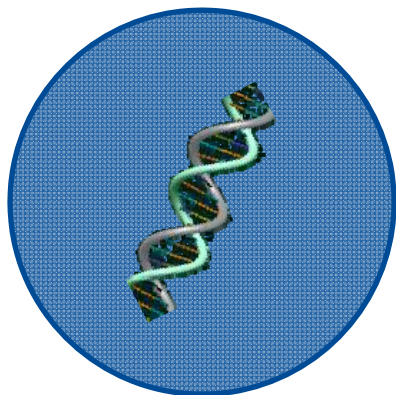


*representative of ~5% of all droplets

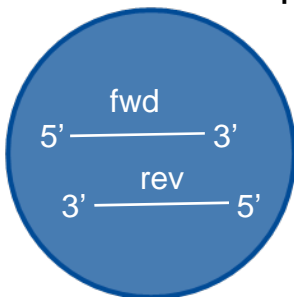


RDT Polymerase Chain Reaction

Inputs



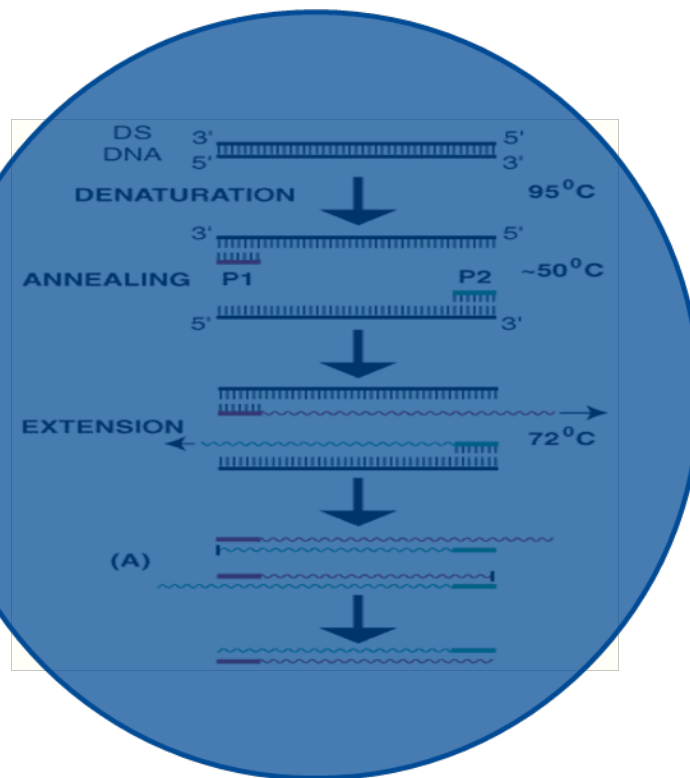
Genomic DNA Template Droplet
33 microns - 18 pL



Primer Pair Droplet
25 microns - 8 pL

Merge

Output



PCR Droplet
37 microns - 26 pL



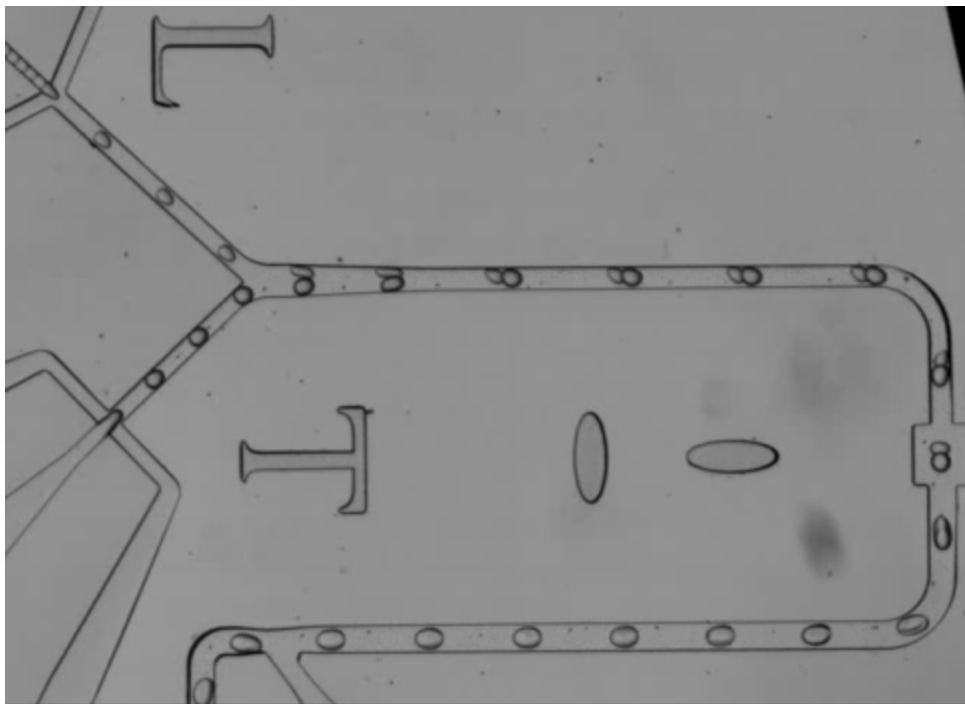
RDT 1000 Droplet-Based PCR



Primer
Library



Genomic DNA
Template Mix





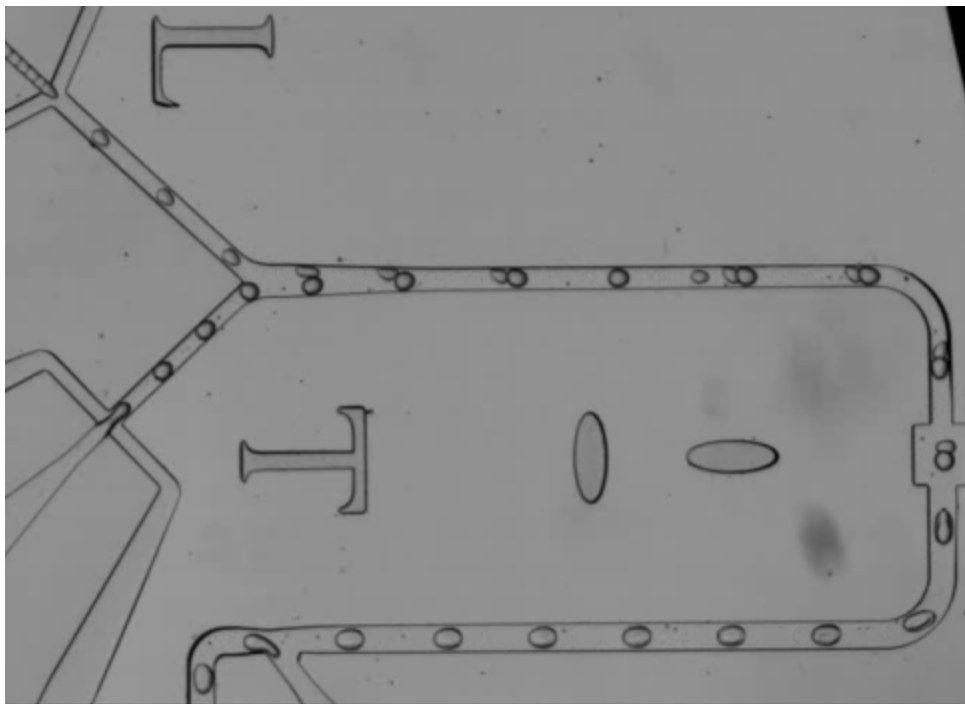
RDT 1000 Droplet-Based PCR



Primer
Library

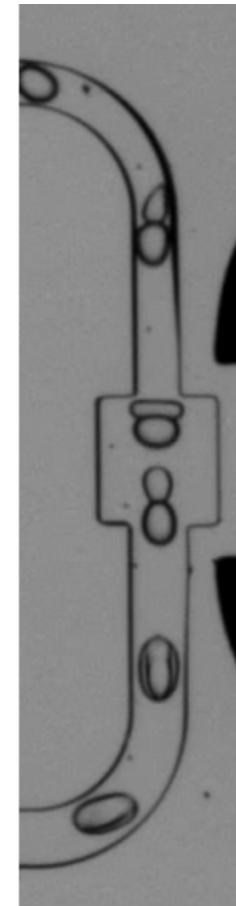


Genomic DNA
Template Mix



PCR Library

1 million droplets
45 minutes per run

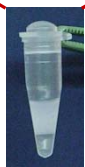
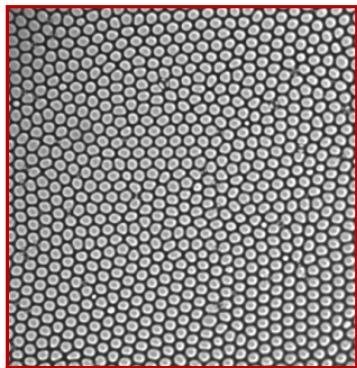


Droplet
Merge Area



Targeted Sequencing Workflow Off-Chip Process

50 uL of droplets per sample



PCR Library



PCR Amplification

10 minute process

Break
Emulsion



Purified
Amplicon
DNA



Sequencing

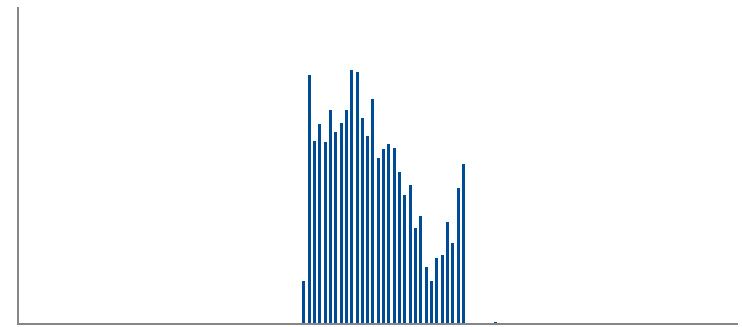




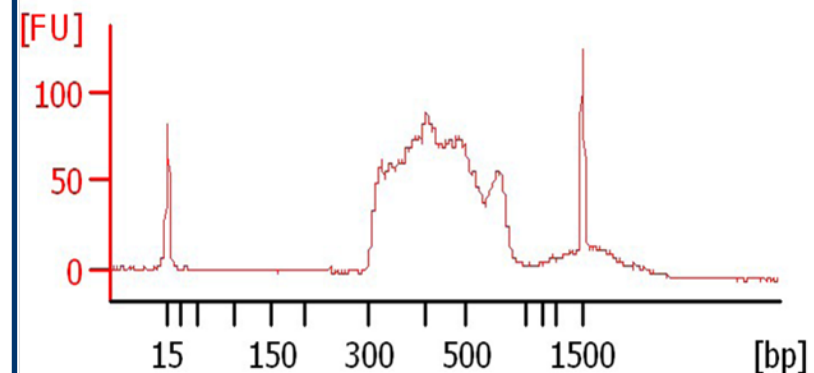
RDT 4000 Member Library

- Genes associated with core signaling pathways in pancreatic cancer
- Total Amplicon Bases = 1.63 Mb
- Total Targeted Region = 1.34 Mb
- Primer Library Specifications:
 - Primer Length 17-30 bp
 - Primer Tm 55.4-61.2°C
 - Amplicon Length 299-659 bp
 - Amplicon GC 25.1-81.5%
- HapMap Sample NA18858
- Sequenced using 454 and Illumina
 - ½ 454 FLX run
 - 2 lanes on Illumina GAII

Calculated Amplicon Peaks



Agilent Bioanalyzer Analysis





RDT 4000 Member Library

Exon Sequencing Results (1.34 Mb / 3976 exons)

Targeted Sequencing Results	454 FLX	Illumina GAI
Percent of Target Amplicons Covered (Completeness)	96.9%	99.7%
Target Representation Bias (<5-fold) (Sequencing Efficiency)	89%	81%
Target Representation Bias (<10-fold) (Sequencing Efficiency)	93%	93%
Targeted Bases Greater Than 10x Coverage	87%	86%

Sequenced using 454 and Illumina
½ 454 FLX run
2 lanes on Illumina GAI



RDT 4000 Member Library: SNP Analysis

	454 FLX	Illumina GAI
SNP's Detected (Coverage > 20x)	1675	1852
Concordant SNP's	1648	1824
Percent Concordant	98.4%	98.5%
Allelic ratios for SNPs	Mean - 49.5% STD - 9.8%	
Discordant calls with HapMap 18858 reference	27	28



Discordant SNP Analysis

	454 FLX	Illumina GAI	Likely Cause of Error
Primary discordant calls with HapMap 18858 reference	27	28	
1. SNPs not in database confirmed by both 454 and Illumina	13	13	Novel
2. SNP in database not found by either Illumina or 454	8	8	Database Error
3. SNP in database found in either 454 or Illumina but not both	4	4	Sequencing Platform Error
4. SNPs not in database confirmed by either 454 or Illumina but not both	2		Unknown
5. SNPs in database not found by Illumina with insufficient reads by 454		3	Unknown
Concordance after removing Database Errors and Sequencing Platform Errors	2/1675 (99.8%)	3/1852 (99.8%)	

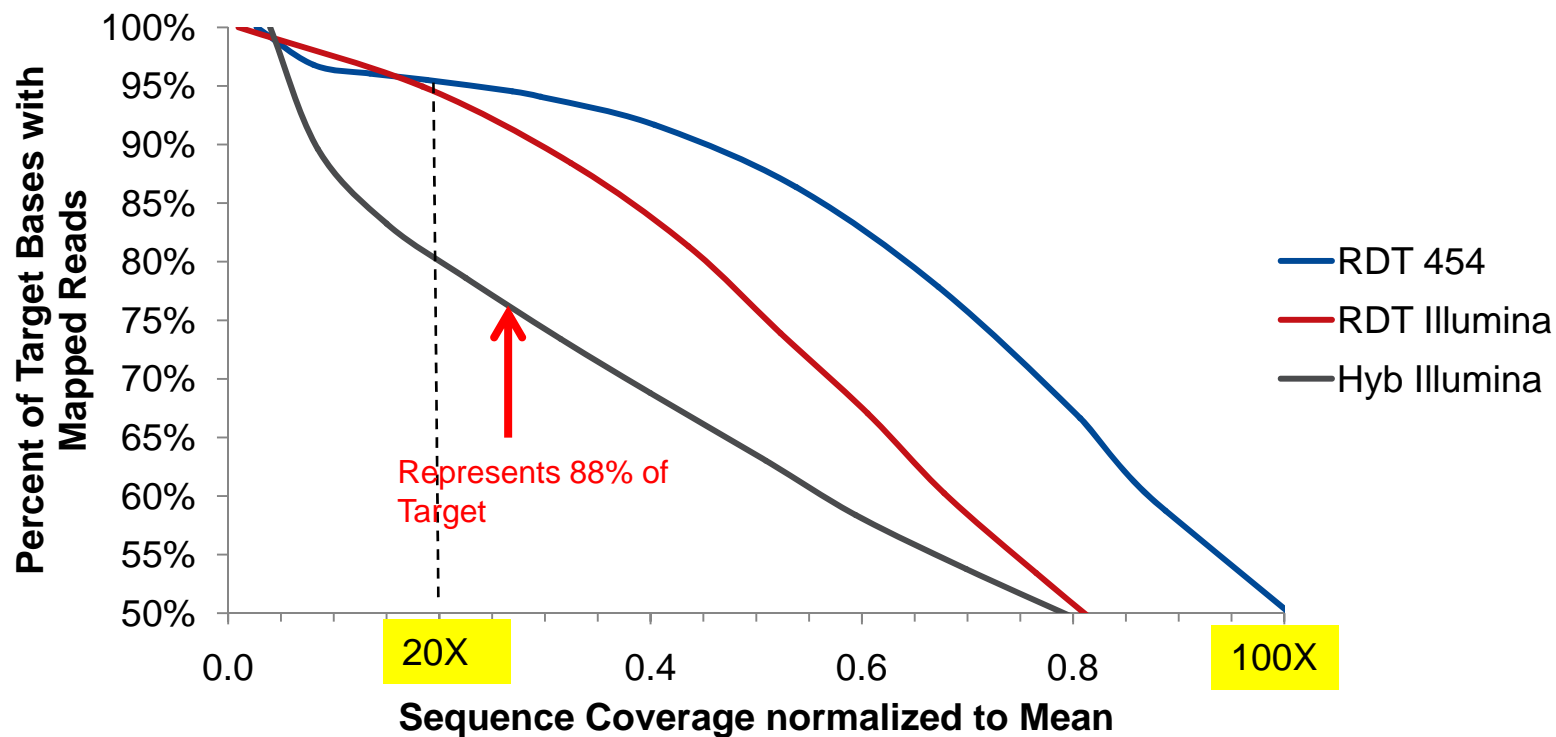


Points to Consider for Targeted Sequencing

- What percent of target bases are represented at a minimum coverage?
- How much sequencing capacity is required?



Cumulative Coverage Profile Comparison



Process	% of Bases with 0.2X of Mean
RDT (454)	96%
RDT (Illumina)	94%
Hyb (Illumina)	88% 64%

Hybridization (Hyb) data from Gnirke et al. 2009



RainDance Technologies RDT 1000

