

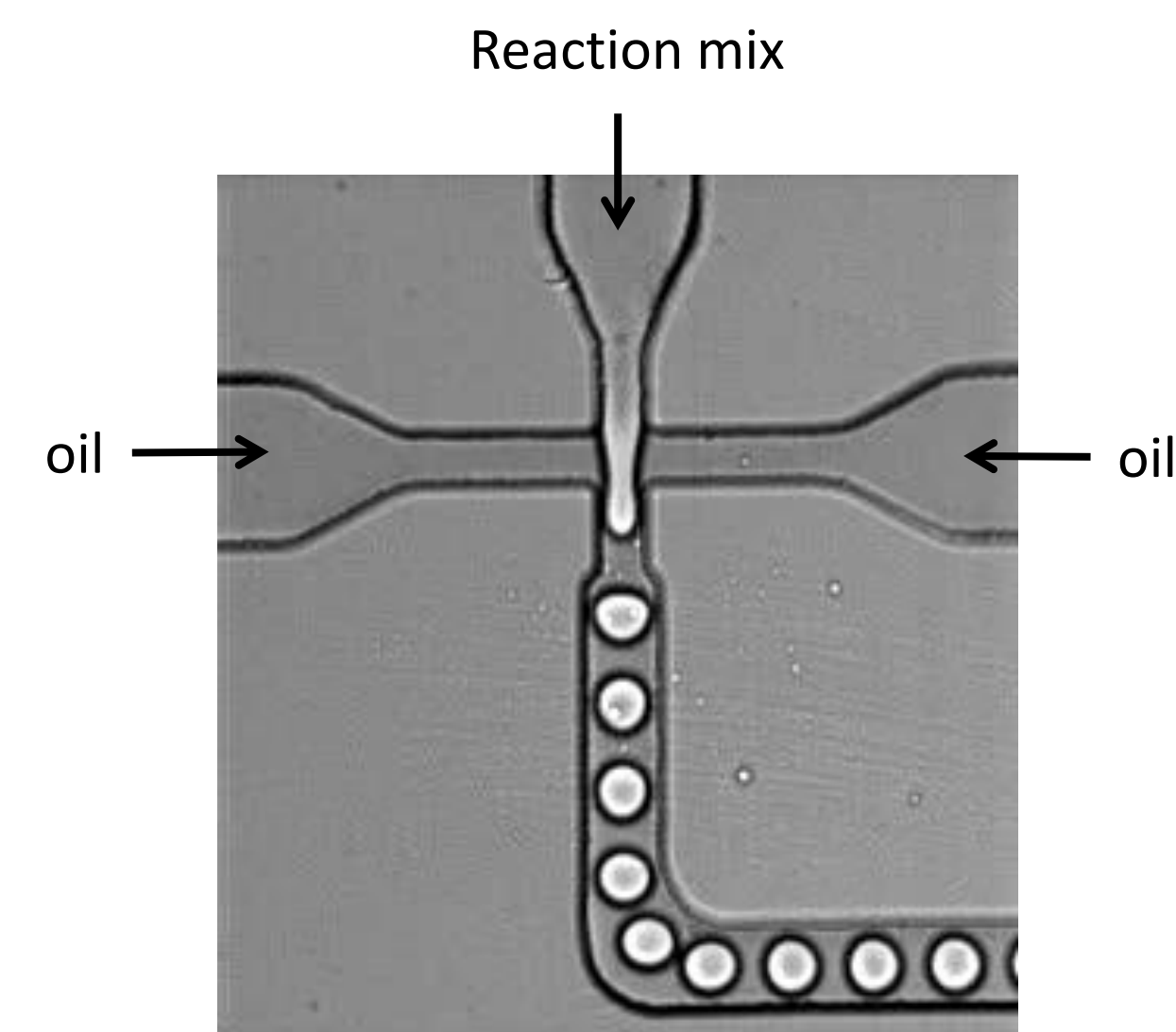
Introduction

Raindance Technologies has developed a technology platform to enable the use of PCR for high-throughput amplification of targeted regions from either contiguous loci or thousands of exons.

Recent advances in DNA sequencing technologies have improved accuracy and dramatically reduced the cost of DNA sequencing. However, even with the improved efficiency of these "second generation" systems, sequencing thousands of whole human genomes across various phenotypes is still economically unrealistic. Therefore, in order to exploit the full potential of these new sequencing techniques, a robust method for isolating biologically relevant genomic loci on the megabase scale will be required.

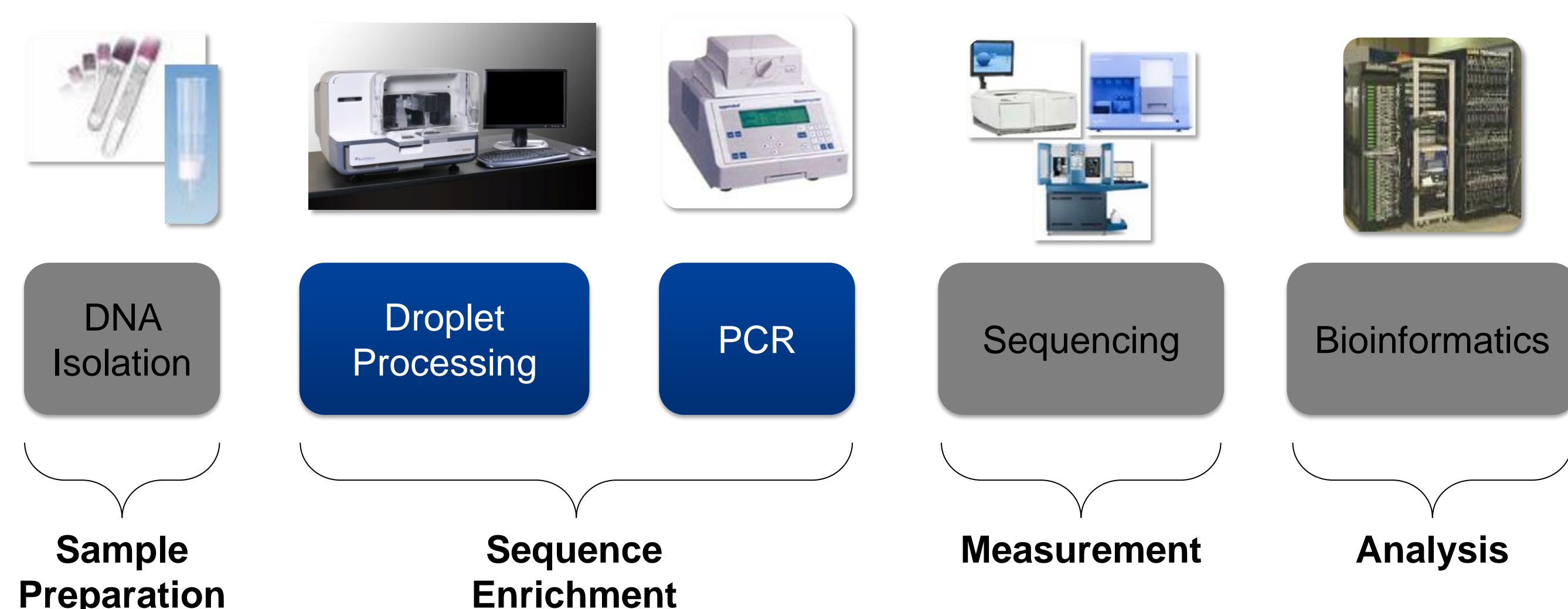
The Sequence Enrichment application from RainDance Technologies leverages the sensitivity and specificity of PCR in a novel microdroplet-based format which avoids limitations of traditional multiplex amplification or hybridization methods.

RainStorm™ Droplet-Based Microfluidics



Rapid and reproducible processing of millions of reactions is enabled by replacing traditional assay plates and automation systems with microdroplets and disposable fluidic chips. Aqueous genomic DNA samples are encapsulated within a droplet surrounded by a bio-compatible surfactant in an immiscible fluorinated carrier oil. Droplet integrity is maintained throughout the range of the Sequence Enrichment process.

Targeted Sequencing Workflow



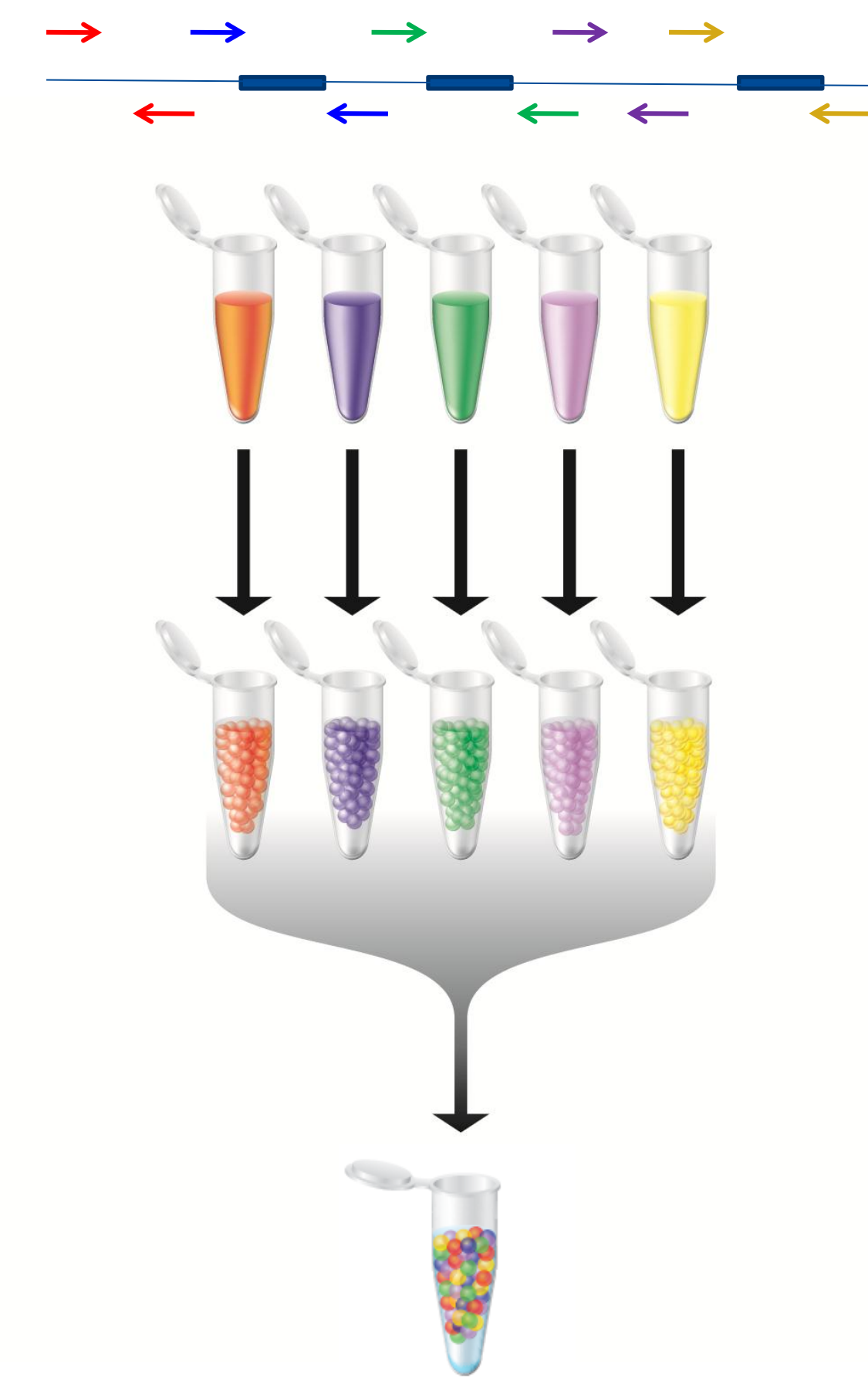
Primer Library Preparation

PCR primers are designed to amplify target DNA sequences using standard primer design parameters (avoiding complications introduced by traditional multiplex primer design).

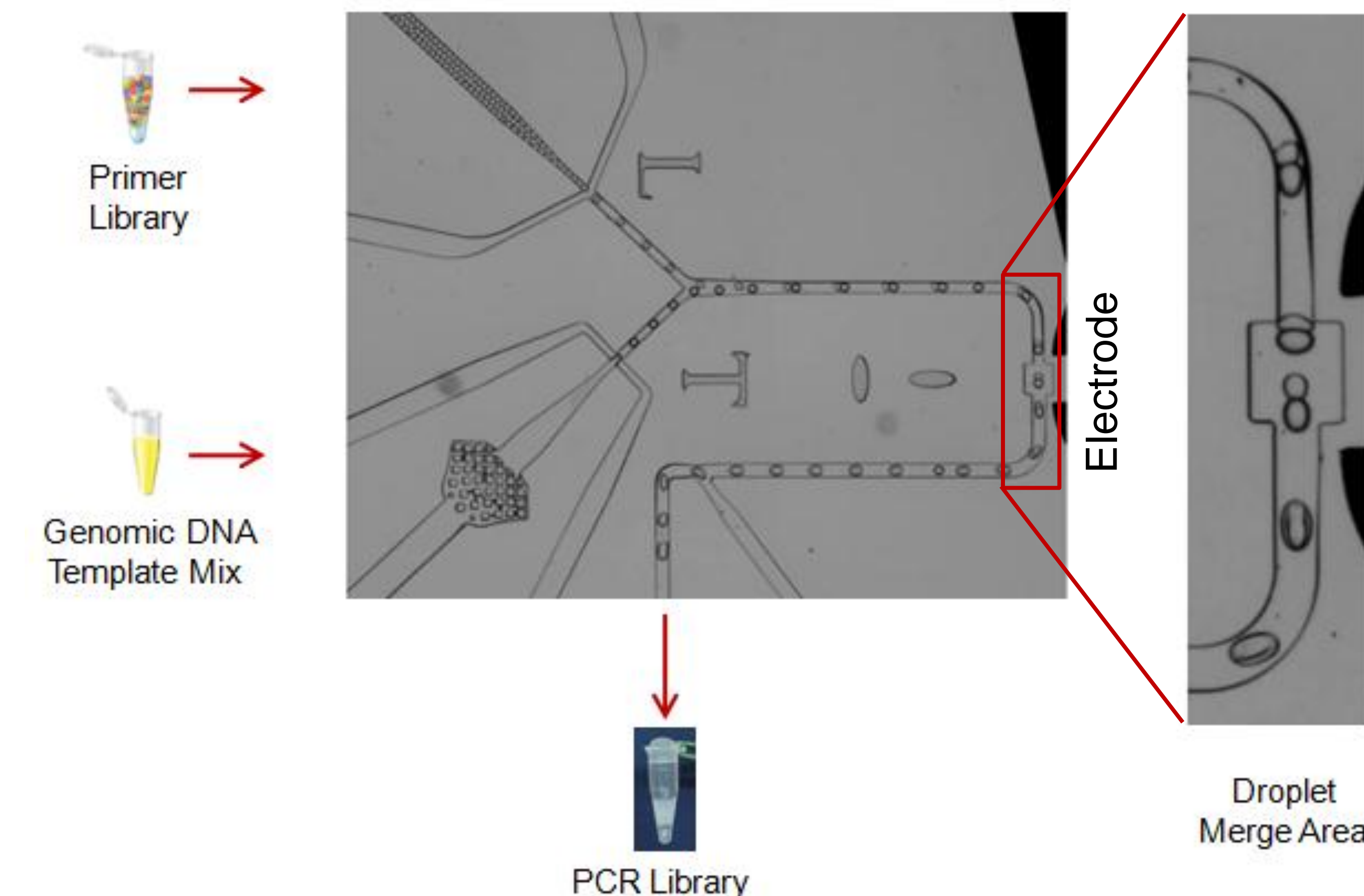
PCR primers are synthesized and individually reformatted into droplets with each droplet containing only a single primer pair.

Primer library droplets are subjected to real-time quality control to assay for uniform number of droplets of a consistent volume for each primer pair.

Primer droplets are pooled together to form a droplet library representing up to 4000 different PCR primer pairs.



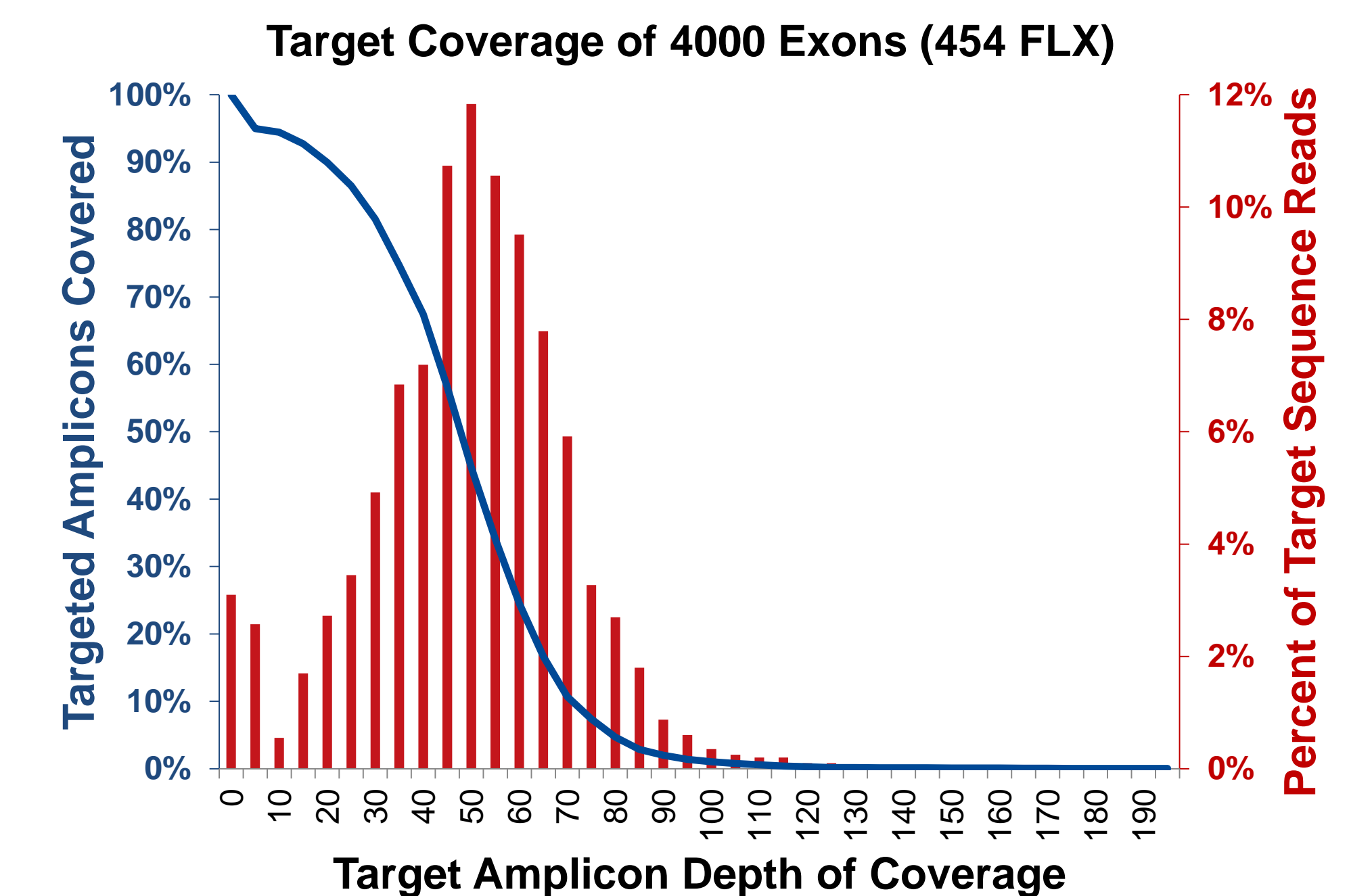
Droplet-Based PCR Process



The RDT 1000 instrument generates droplets containing genomic DNA and PCR reaction components (minus the forward and reverse primers). Primer Library droplets are introduced to the chip environment to achieve a 1:1 pairing of genomic DNA and Primer Library droplets. The paired droplets flow past the electrode and are instantly merged together to form a single PCR droplet. The merged droplets are then automatically dispensed into a PCR tube, which is transferred to a standard thermal cycler for PCR amplification of the targeted loci.

Targeted Sequencing of 4000 Exons

A library of PCR primers was designed to amplify 4000 exons representing a total of 1.64 Mb of target sequence. The library was designed to validate the performance specifications of the sequence enrichment application for a representative set of conditions such as amplicon length (299-659 bp), PCR primer T_m (55-61°C) and sequence content (25-82% GC). A HapMap DNA sample (NA18858) was processed through the sequence enrichment assay to evaluate SNP concordance and allelic bias. The resulting amplicons were sequenced using both the Illumina Genome Analyzer and 454 FLX sequencing platforms.



Targeted Sequencing Results	454 FLX	Illumina GA
Reads mapping to target regions	94%	78%
Percent of target amplicons covered	96.9%	99.7%
Target representation bias (<5-fold difference)	89%	81%
Target representation bias (<10-fold difference)	93%	93%
SNP concordance with HapMap	98.4%	98.5%
Allelic ratios for SNPs	Mean = 49.5%, STD = 9.8%	

	454 FLX	Illumina GAll	Likely Cause of Error
Primary discordant calls with HapMap 18858 reference	27	28	
SNPs not in database confirmed by both 454 and Illumina	13	13	Database error
SNP in database not found by either Illumina or 454	8	8	Database error
Unexplained discordant SNPs	6	10	
True Concordance	2/1675 (99.8%)	3/1852 (99.8%)	

Summary

Sequence enrichment using droplet-based PCR provides high specificity and uniform enrichment required to maintain an efficient targeted sequencing workflow and more importantly, provide high quality sequence data in order to provide high confidence in variant detection required for large-scale targeted resequencing applications. The RDT 1000 and Sequence Enrichment assay function to serve workflows for both short-read and long-read sequencing platforms.