

Abstract

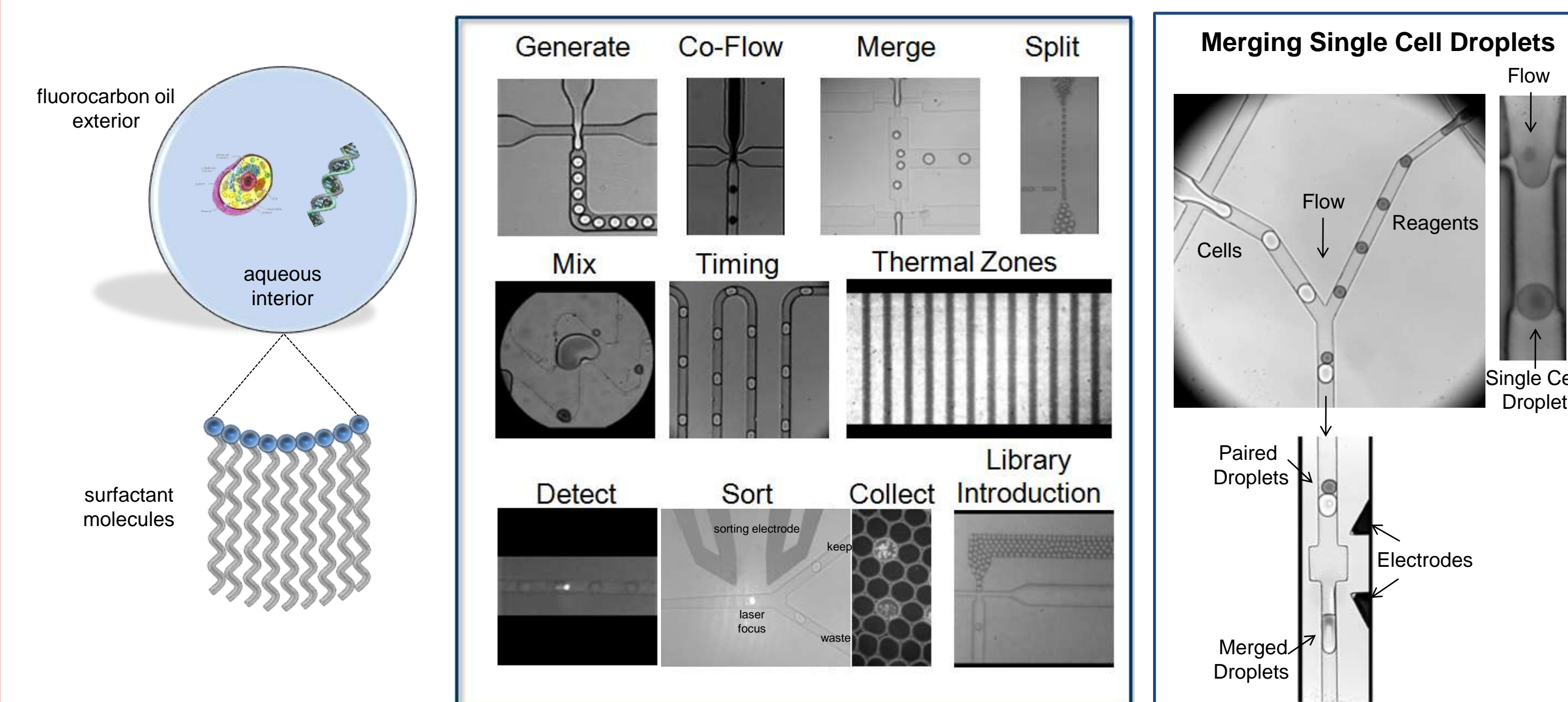
Cancerous cell growth can result from mutation of the underlying genome or from aberrant epigenomic regulation, altering transcript and protein expression levels and causing uncontrolled growth. The resulting tumors are often composed of heterogeneous mixtures of both clonally transformed tumor cells and other cell types. Here we describe extensions of our novel microdroplet technology that enable analysis of a DNA sample's methylome and allow detection of allelic variants in heterogeneous mixtures. We also present unique new capabilities for capturing both phenotypic and genomic information from droplet-encapsulated individual cells, enabling analysis of tumors with single-cell resolution.

RainDance Technologies' microfluidic technology produces uniform picoliter-scale aqueous microdroplets at rates up to 10 million droplets per hour. Each droplet is the functional equivalent of an individual test tube and can contain a single molecule, reaction, or cell. This versatile technology can adapt proven assays to high-speed workflows with a minimum of process-induced bias or errors. Our initial application provides targeted sequence enrichment to prepare samples for next-generation sequencing (illustrated below). The sequencing depth and reduced amplification bias provided by microdroplet technology enables accurate detection of sequence variants within a heterogeneous mix of sample DNA. In addition, we have extended this approach to enable targeted sequencing of a sample's methylome using bisulfite-treated templates, providing base-pair resolution of the methylated cytosines that have been associated with aberrant transcription in cancer.

The self-contained microdroplet environment can also help scientists study biological samples as a collection of individual cells. Single-cell droplet technology provides a cost-effective method to gain sequence information from individual cells that have been sorted by phenotype. We present data illustrating the workflow we are using to perform low-bias single-cell whole-genome or transcriptome amplification within the sorted microdroplets. The amplified single cell genomes can be used as the starting material for targeted sequence enrichment using the RDT1000, providing an automated sample preparation method for single cell analysis using any next-generation sequencing platform. Single-cell droplet technology is an exciting new tool that will allow for a more thorough examination of the variations that influence the predisposition and composition of cancer, and the cellular responses to therapeutic and prevention agents.

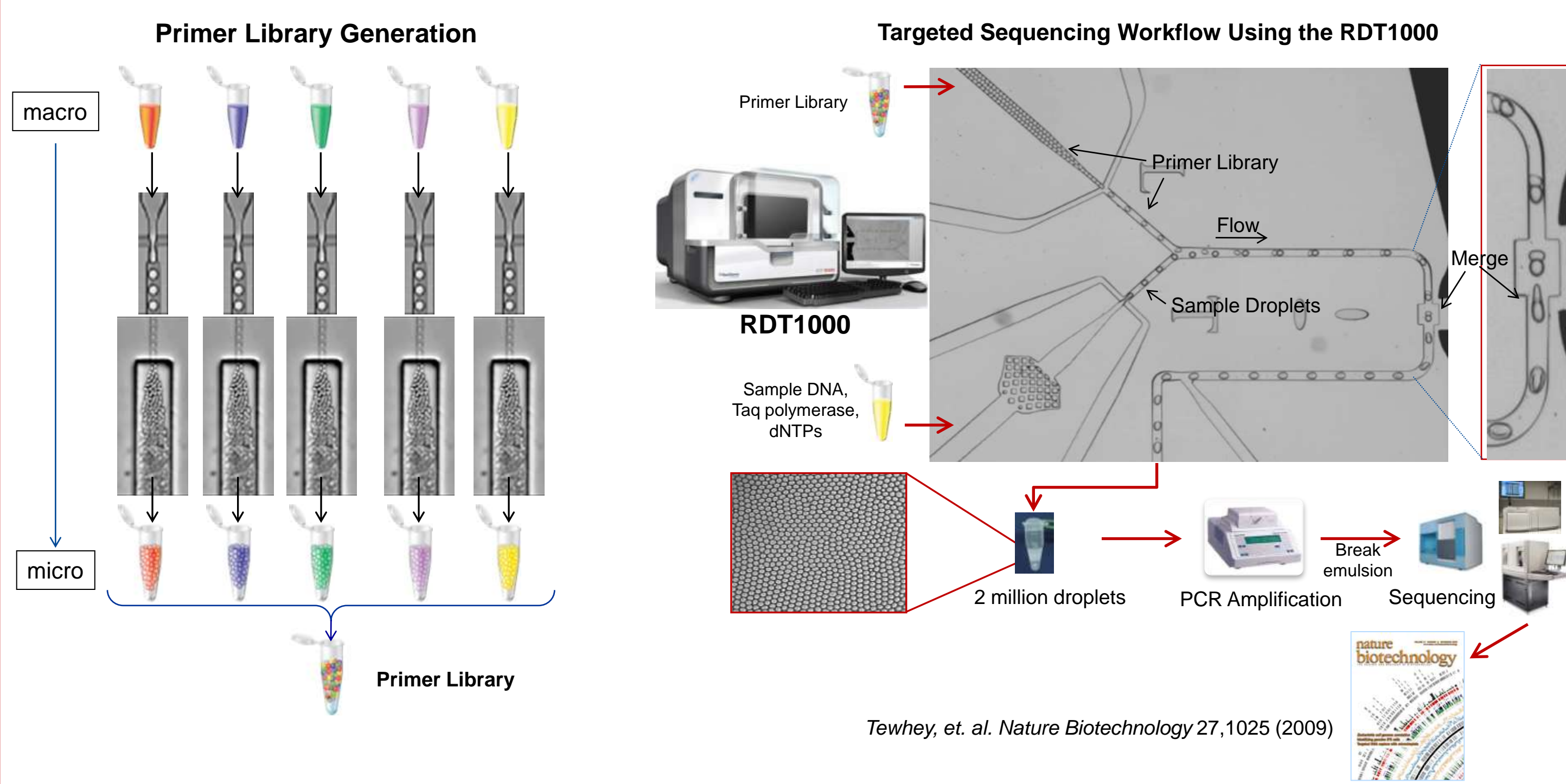
RainStorm™ Droplet-Based Microfluidics

Rapid and reproducible processing of millions of reactions is enabled by replacing traditional assay plates and automation systems with microscopic droplets and disposable fluidic chips. Aqueous samples (beads, cells, enzymes, antibodies, DNA) are encapsulated within each droplet, and surrounded by an immiscible carrier oil (Generate image on upper left of the panel). The droplets are stabilized with bio-compatible surfactants, allowing for robust manipulations both on and off chip. Examples of various modules are shown below.



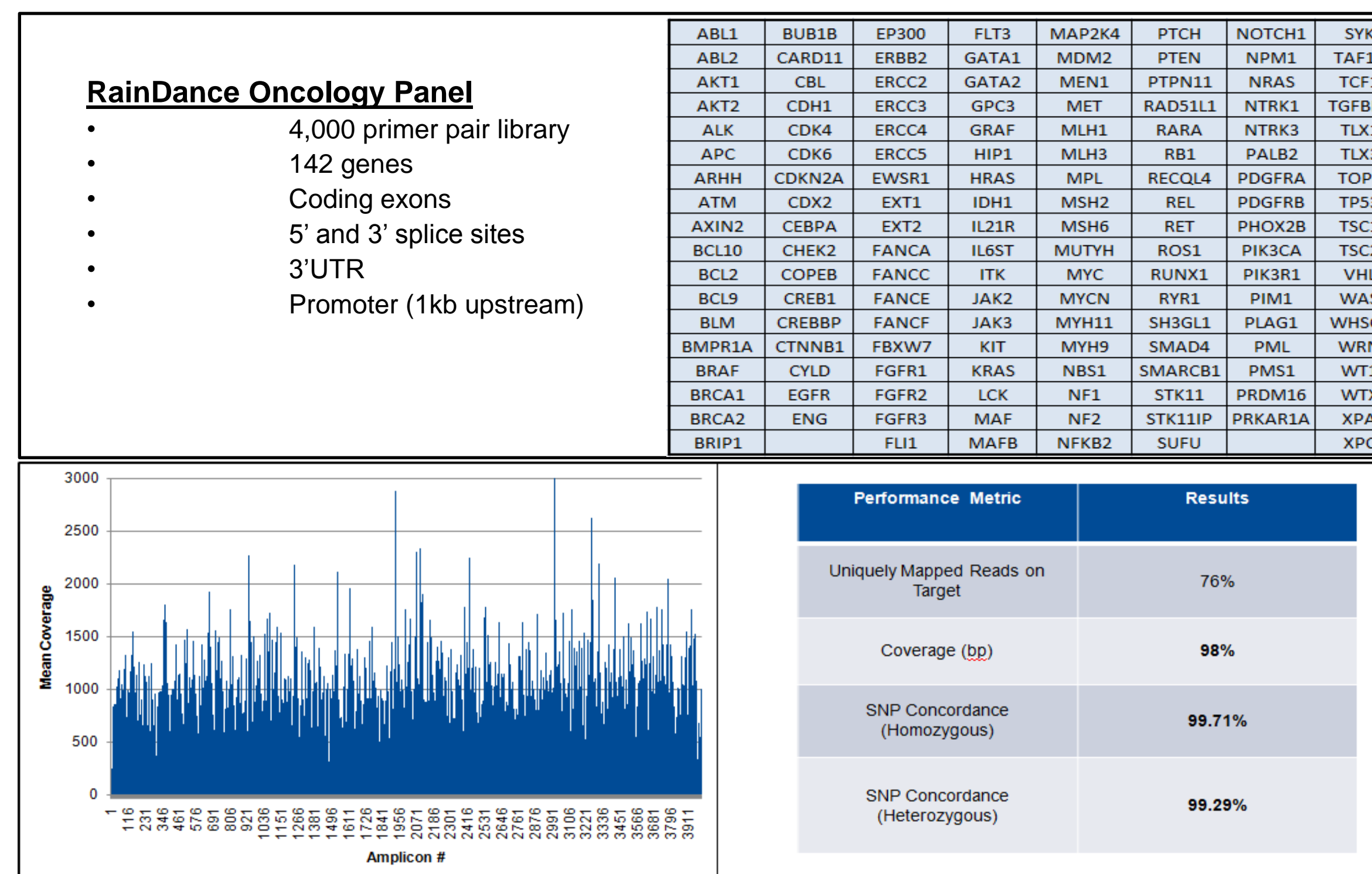
Enrichment of Resequencing Targets

RainDance Technologies' solution for targeted sequence enrichment uses the traditional 'gold standard' method of PCR, adapted to the microdroplet format, to amplify the genomic loci of interest with high coverage and low amplification bias. The loci of interest are targeted by standard PCR primers pairs that have been reformatted into a custom Primer Library, provided as a stable ready-to-use reagent (Library Generation, below left). Using the RDT1000, the DNA sample is converted into microdroplets. Each droplet (containing DNA, polymerase, and buffered dNTPs) is combined with one of the Primer Droplets, and the entire collection of merged 'PCR droplets' is placed in a standard thermocycler for amplification.



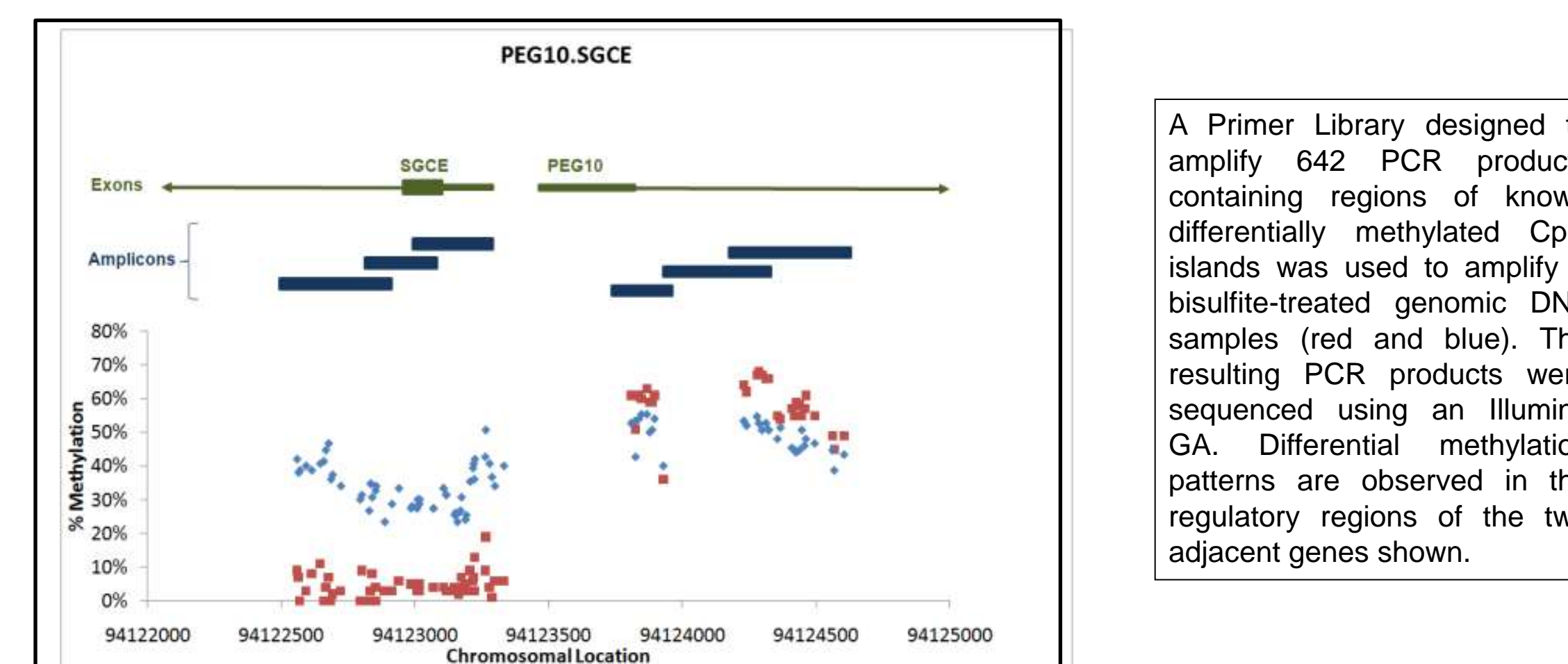
Pre-made droplet libraries enable the use of PCR for high-throughput targeted sequencing applications

Targeted Sequencing of Cancer Gene Networks



Results from targeted sequencing of 142 cancer genes demonstrate high accuracy of variant detection and uniform depth of coverage

Droplet Methyl-Seq With Bisulfite Treated DNA

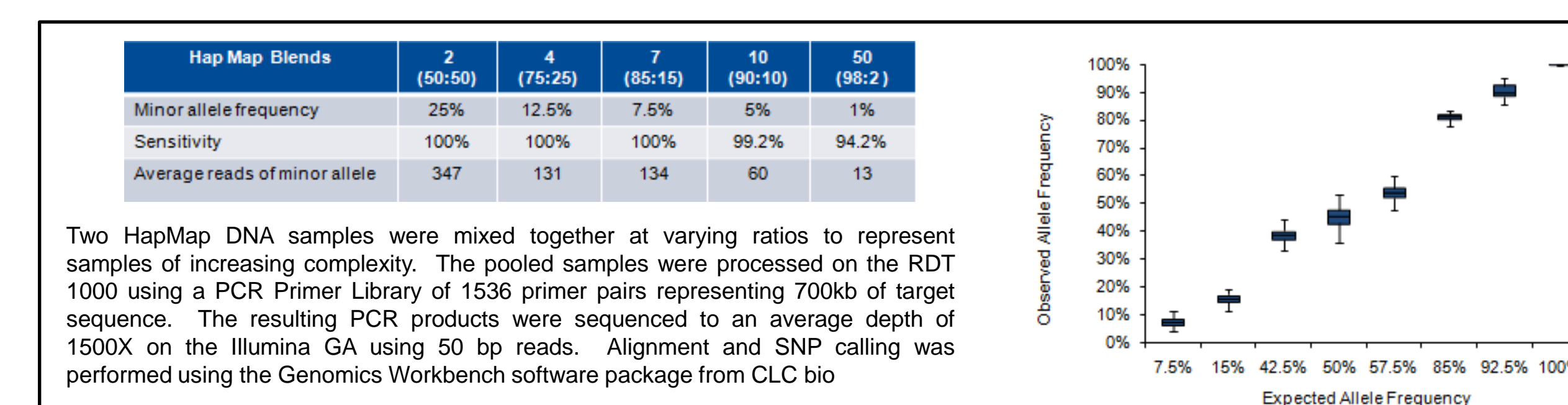


Targeted Methyl-Seq enables quantitative analysis of differential methylation with single base resolution

Sequencing of Heterogeneous Samples

The combination of single molecule PCR and large numbers of replicate independent reactions enabled by droplet PCR maintains the allelic representation of complex samples including highly heterogeneous samples such as tumors.

The ability to accurately detect sequence variants in pooled DNA samples enables large-scale targeted re-sequencing studies for population genetics studies, and characterization of allele frequencies as a follow-up to genome-wide association studies (GWAS). By adjusting the amount of genomic DNA and the complexity of the Primer Library, the researcher can tune the assay to the appropriate confidence interval to detect rare sequence variants.



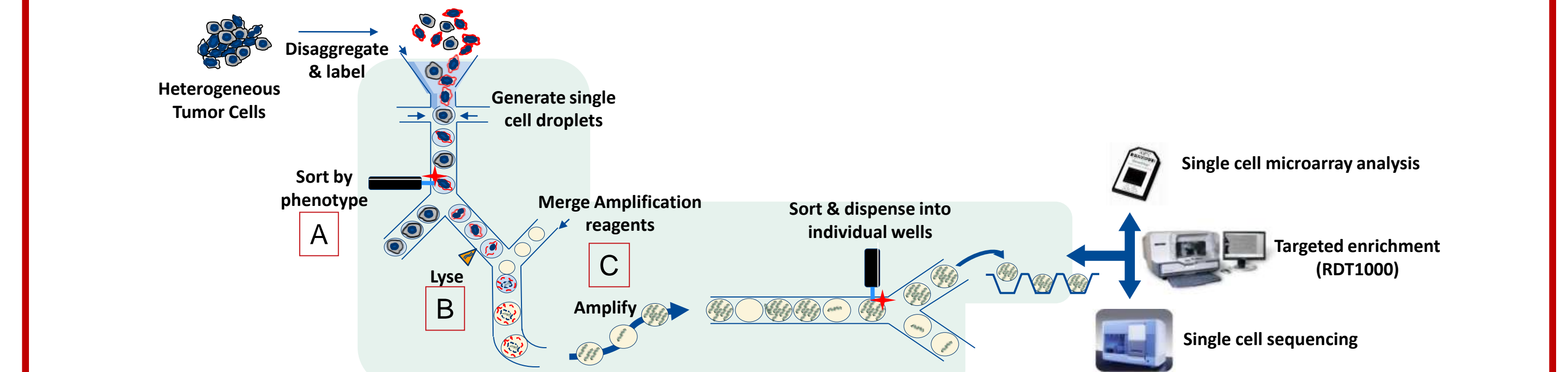
Two HapMap DNA samples were mixed together at varying ratios to represent samples of increasing complexity. The pooled samples were processed on the RDT 1000 using a PCR Primer Library of 1536 primer pairs representing 700kb of target sequence. The resulting PCR products were sequenced to an average depth of 1500X on the Illumina GA using 50 bp reads. Alignment and SNP calling was performed using the Genomics Workbench software package from CLC bio

Note: Recent enhancements to the RDT1000 sequence enrichment assay have increased the number of PCR droplets to two million droplets per sample (a 2X increase beyond the data presented here) enabling greater sensitivity to detect rare alleles and allowing the use of droplet libraries with up to 20,000 different primer pairs.

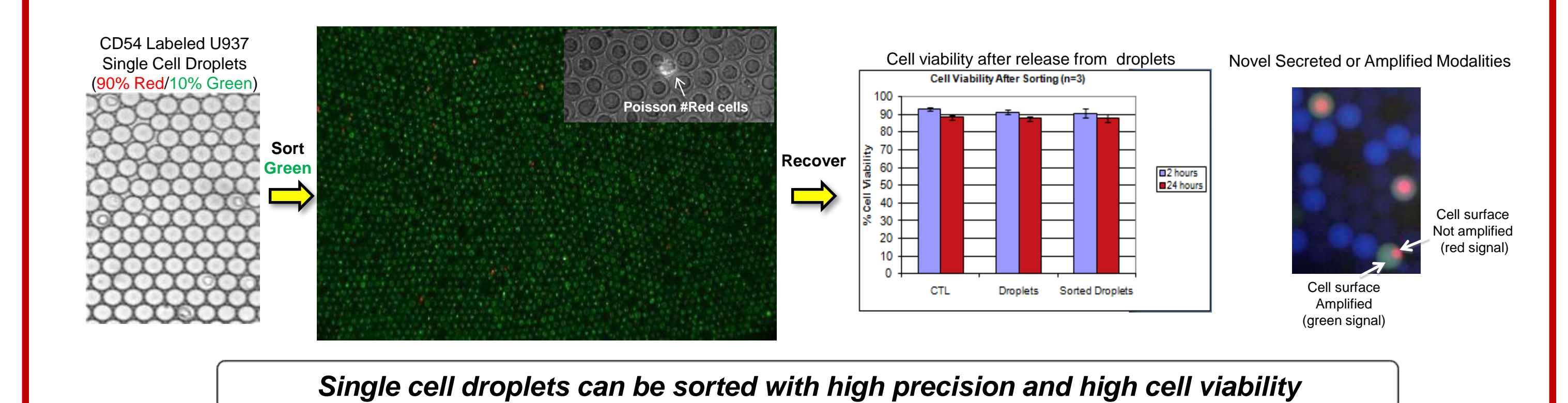
Microdroplet PCR maintains allelic representation in complex samples

Droplet-Based Single Cell Genomics

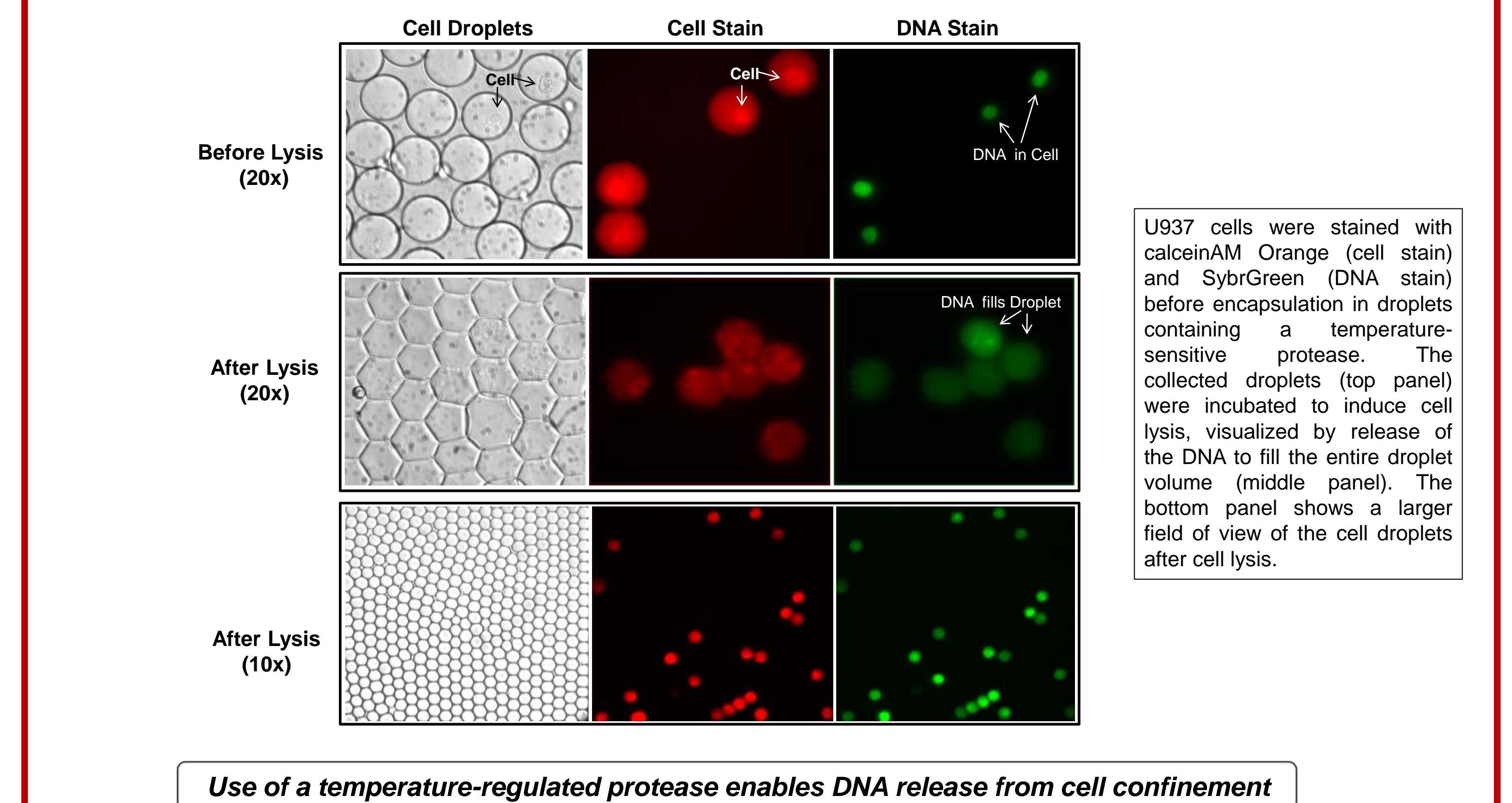
Droplet-Based Single Cell Genomics Workflow



A Sorting Cell Droplets with Conventional, Secreted, or Amplified Biomarkers

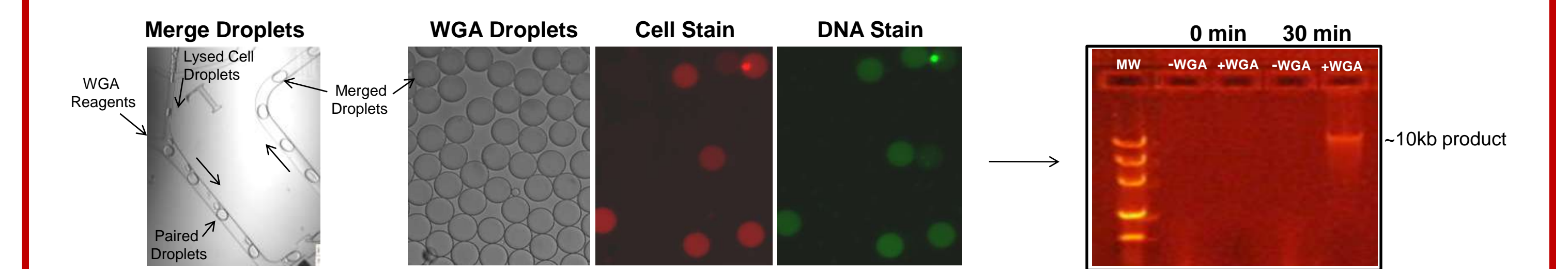


B Cell Lysis and Proteolysis Inside Droplets Releases Accessible DNA



C WGA Inside Droplets Amplifies Single Cell Genome

Following cell lysis and proteolysis to release the cell's genomic DNA, the cell droplets were combined with droplets filled with WGA reagents and incubated for amplification. For the images below, a DNA stain was included with the WGA reagents in order to visualize the amplified WGA product. The resulting material was released from the droplets and run on an agarose gel (panel on right).



Merging the lysed cell droplets with amplification reagents results in amplification of each individual cell genome

Summary

- Targeted Sequencing
 - The RDT 1000 and pre-made Primer Libraries provide >95% coverage, and high accuracy for discovery and validation of genomic and epigenomic variation in gene networks
- Droplet Single-Cell Genomics (in development)
 - Enabling genome and transcriptome sequencing from single cells
 - Utilizes conventional and novel sorting of phenotypes, genome and transcriptome amplification, and analysis by sequencing, digital PCR or other widely accepted genomics methodologies