Immunophenotyping of TCR and BCR Clonotypes

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Immunophenotyping of T and B cells

Genetic Immune ID
TCR, BCR > billion genes
HLA, KIR > dozens genes
eQTL > ~5,000 genes

Expression
T cells (Tn, Th, Tm, Tc, etc.)
B cells (Bn, Bm, plasma, etc.)
(other immune and APC cells)

TCR-BCR Immunophenotype
TCR/ BCR repertoire and expression of key T and B cell typing and activation marker genes

Bulk Analysis
TCR/BCR > (genDNA, RNA)
RNA-seq, 5' RACE/SMART
Multiplex RT-PCR
High Throughput (10K-500K)

Single-Cell Analysis
scRNA-seq, 5' RACE/SMART
Droplets > e.g. 10xGenomics,
Microwells > e.g. CellSee, BD
Medium throughput (1K-5K)

Single-Cell Analysis
5' RACE/SMART
Multiplex RT-PCR
FACS-sorted cells >96/384 plates
Low throughput (100-1,000)

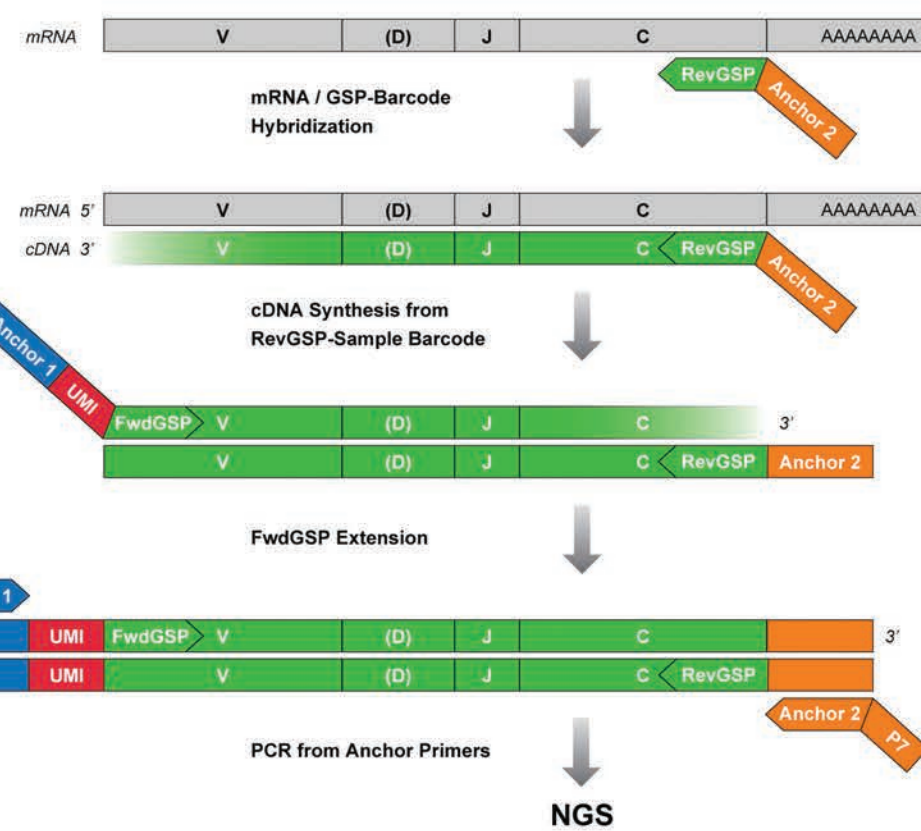## Targeted Multiplex RT-PCR: DriverMap™ Technology

- Combined TCR/BCR repertoire analysis and expression profiling of up to 19K genes
- Sensitivity down to single-cell level
- Defined amplicons for any mRNA portion, not limited to 3' or 5' end (CDR3, HLA SNP, homologous genes, e.g., IL family)
- Could be run directly in cell lysate (single cell, whole blood)
- Doesn't require rRNA, globin depletion

TCR Repertoire Bulk Profiling: SMART vs Multiplex RT-PCR (DriverMap)
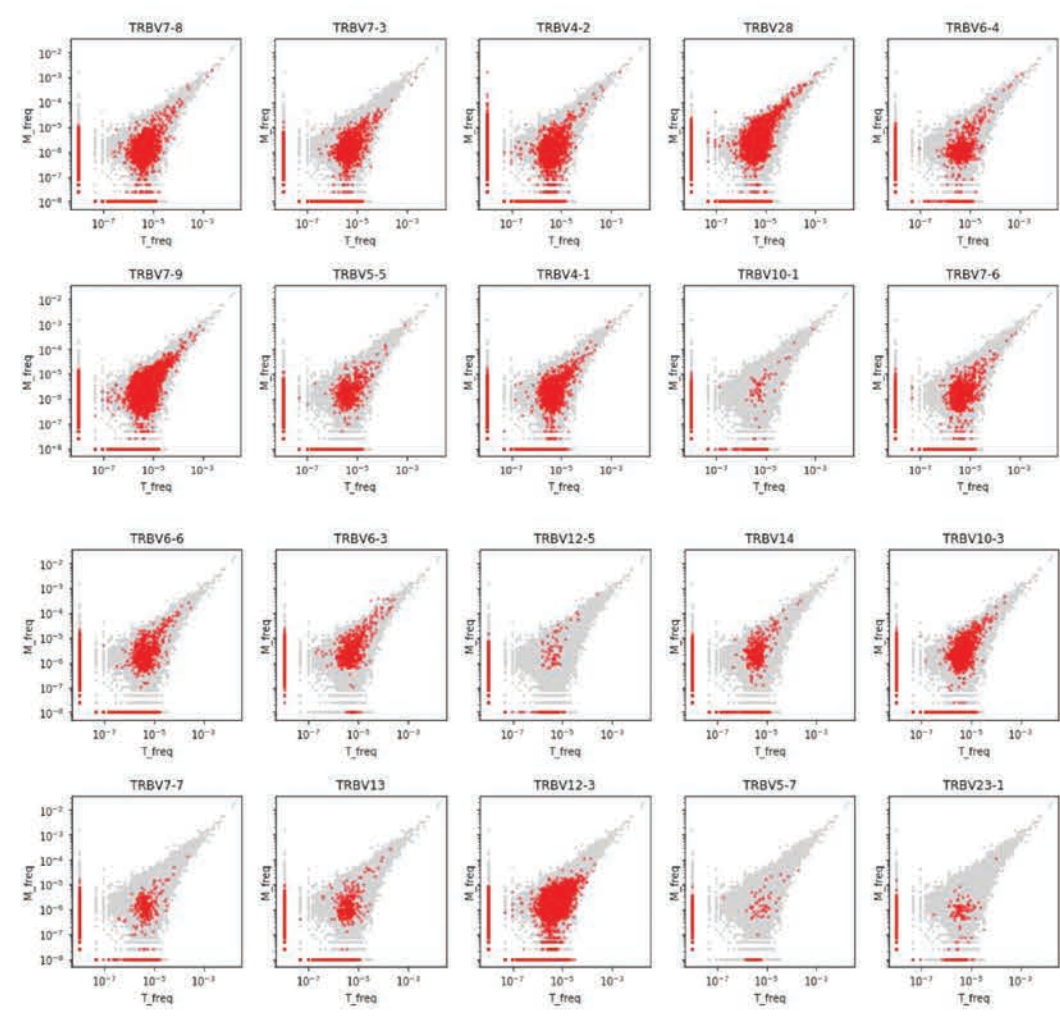
- Similar V gene usage
- DriverMap: Less biases, less background in PCR
- DriverMap: Shows 3x more clonotypes for both TRB and TRA (200K vs 70K clonotypes, 50ng PBMC RNA

T – SMART
M – Multiplex RT-PCR
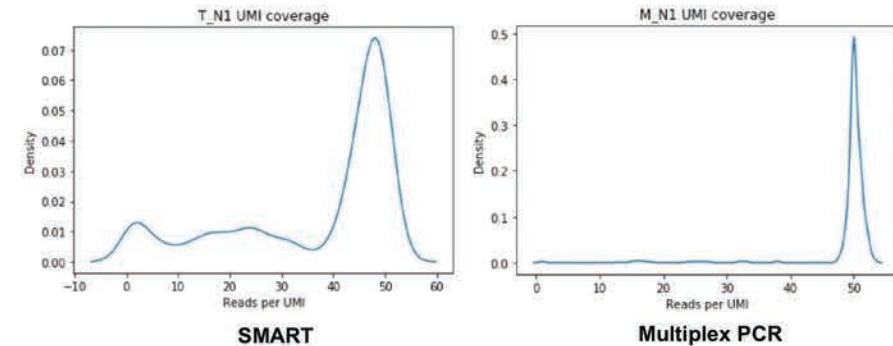
Advantages of Microsampling Technology

Blood Collection Tubes vs. Mitra® Collection Devices

With Mitra devices donors collect their own precise volumes of blood, with only minimal training, whenever and wherever it's convenient.
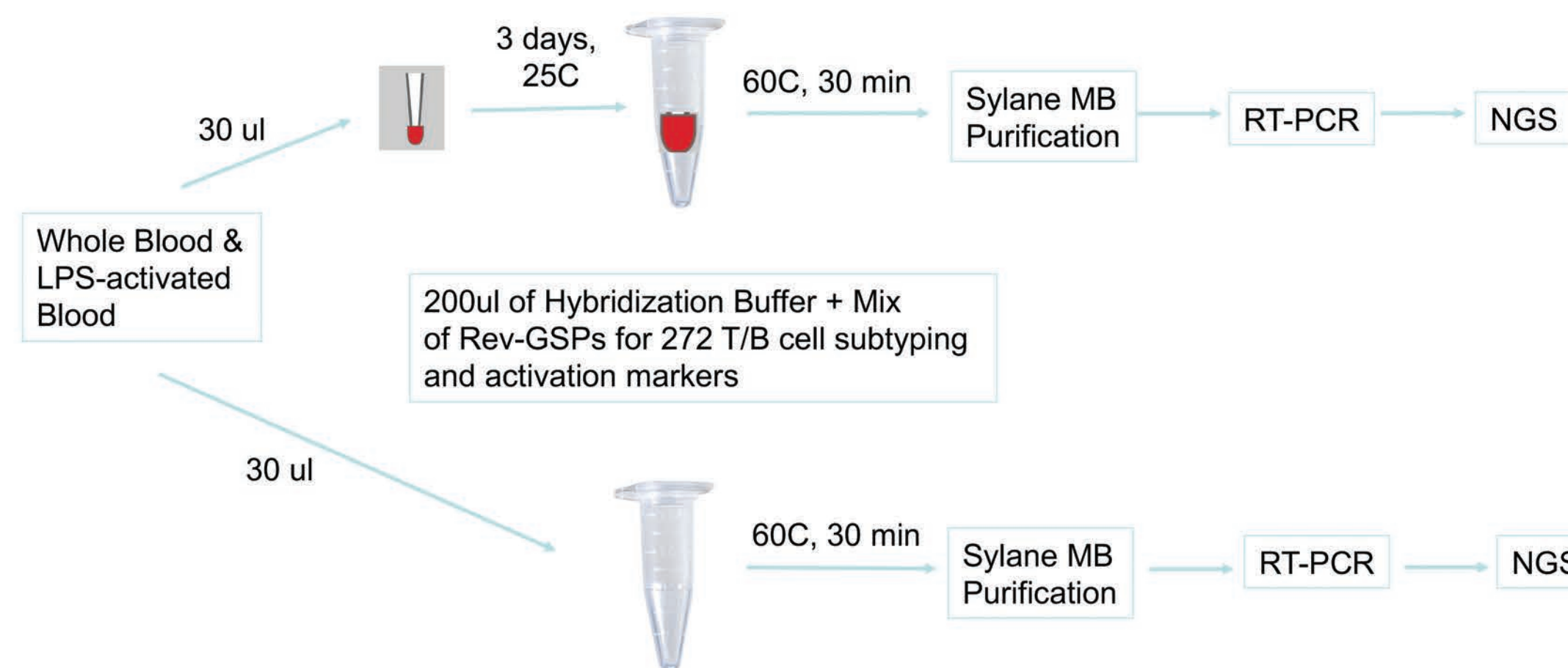
Forgo venipuncture and simply prick the finger.

Mitra Microsamples dry under ambient conditions - there is no need for centrifuge, transfer, or freezing.

Transport Mitra® dried blood specimens via post - eliminate cold-chain shipping and courier costs.
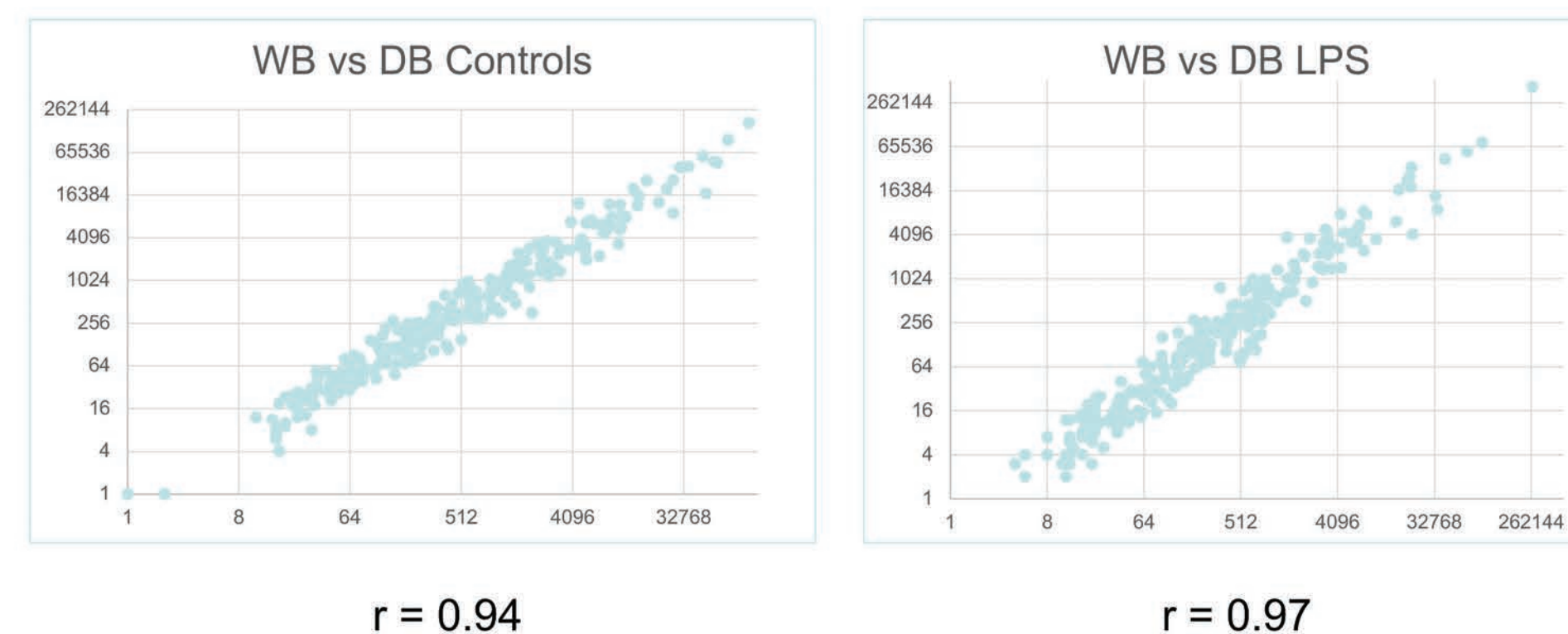
Image above courtesy of Neoteryx LLC

Processing Outline of Mitra/GenTegra Dried Blood Samples

Whole Blood & LPS-activated Blood

30 ul → 3 days, 25C → 60C, 30 min → Sylane MB Purification → RT-PCR → NGS

200ul of Hybridization Buffer + Mix of Rev-GSPs for 272 T/B cell subtyping and activation markers

30 ul → 60C, 30 min → Sylane MB Purification → RT-PCR → NGS
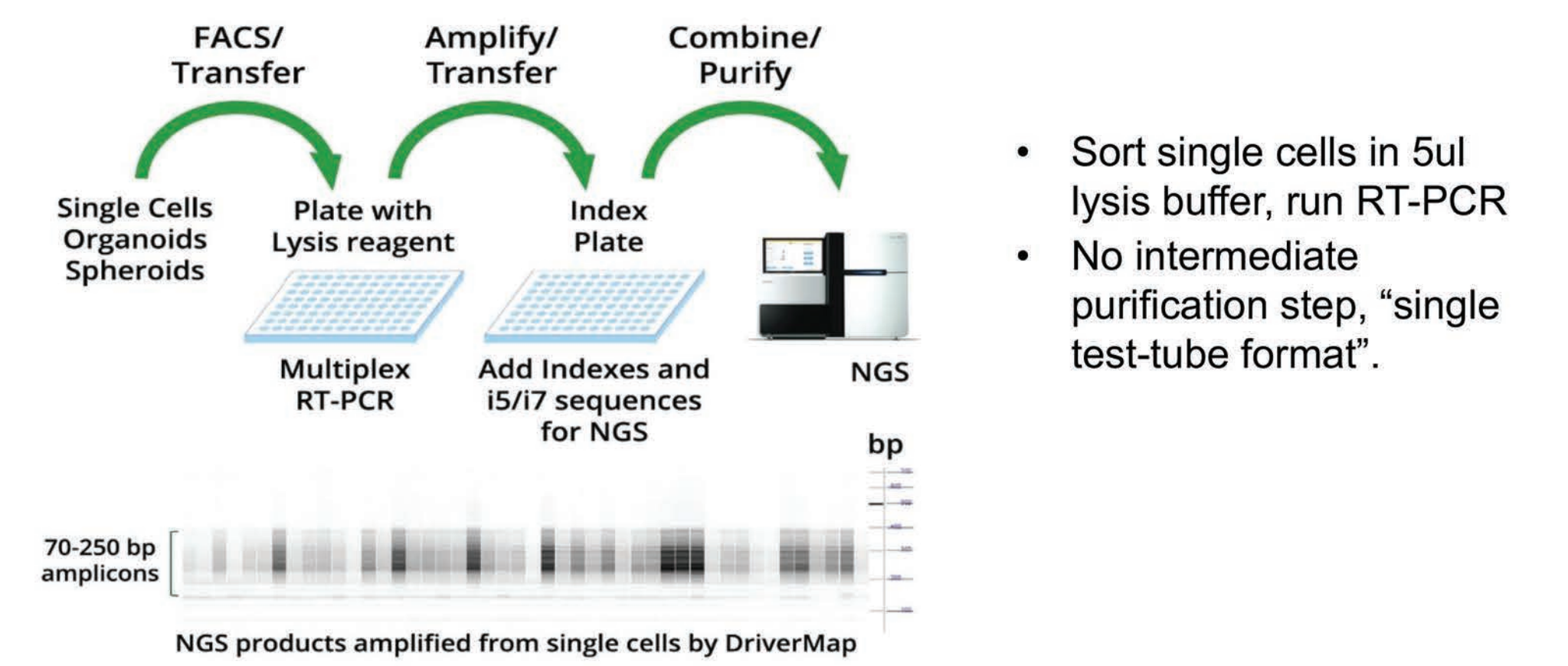
Mitra is a registered trademark of Neoteryx LLC. Gentegra is a registered trademark of GenTegra LLC.

Correlation of Whole Blood vs Dried Blood Gene Counts

WB vs DB Controls — r = 0.94

WB vs DB LPS — r = 0.97

Single-cell SMART vs DriverMap Profiling in 96/384-well Plates

FACS/Transfer → Amplify/Transfer → Combine/Purify

Single Cells Organoids Spheroids → Plate with Lysis reagent → Index Plate → NGS

Multiplex RT-PCR | Add Indexes and i5/i7 sequences for NGS

70-250 bp amplicons | bp

NGS products amplified from single cells by DriverMap

- Sort single cells in 5ul lysis buffer, run RT-PCR
- No intermediate purification step, "single test-tube format".

DriverMap versus SMART for Single-Cell Profiling

DriverMap PanCancer 1.4K assay in HCC1395 and HCC1395-BL Cells

Appr. 20% genes can't be detected by SMART technology
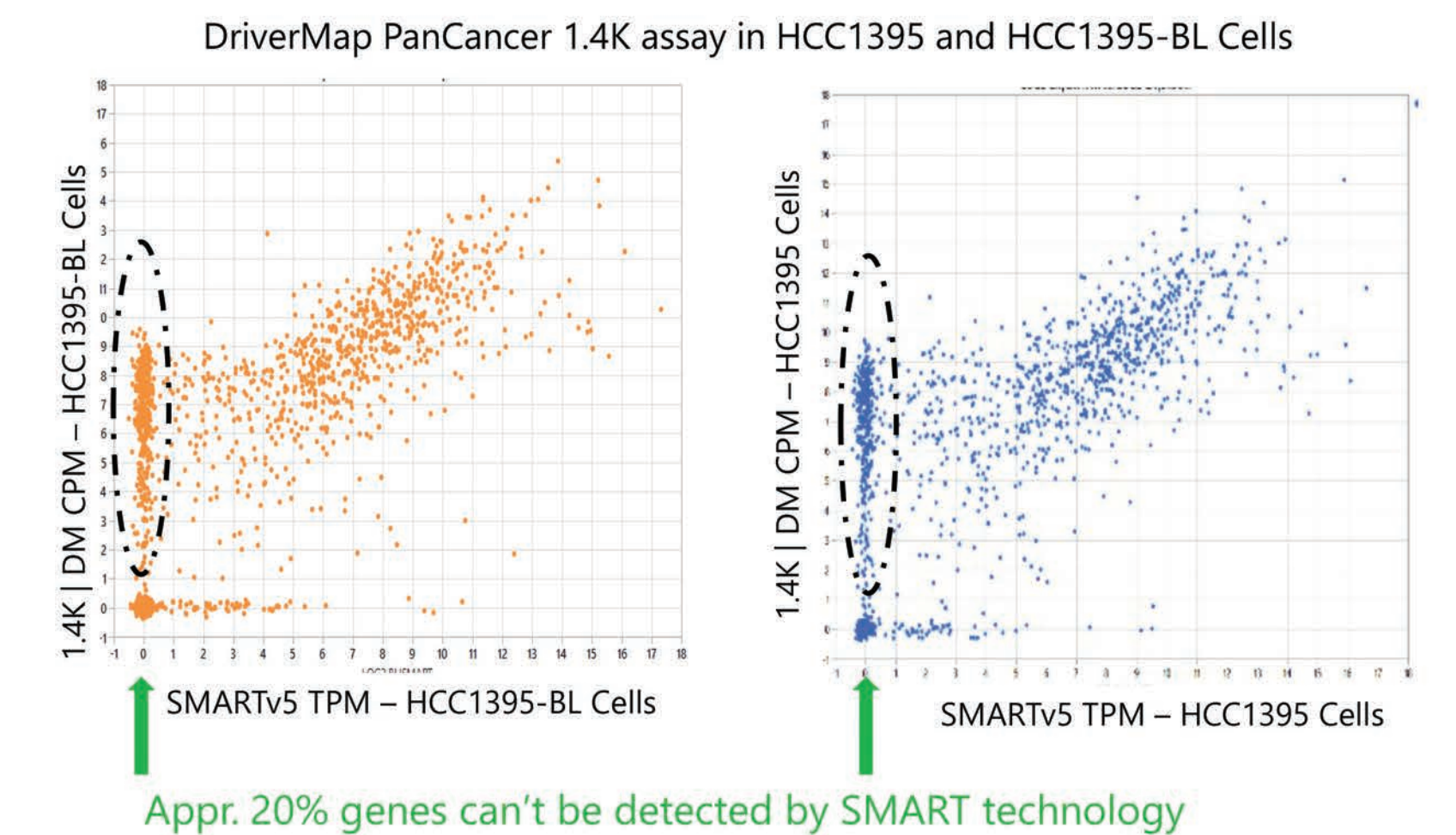
Summary

- Immunophenotyping > Integrated analysis of TCR/BCR clonotypes and expression profiling of cell typing, activation markers in T and B cells
- DriverMap multiplex RT-PCR is the most promising high-performance technology for immunophenotyping in both bulk and single-cell samples
- Bulk profiling > best strategy for large-scale TCR/BCR repertoire analysis and discovery of novel biomarkers
- Single-cell analysis > best strategy to link TCR/BCR clonotypes with cellular phenotypes for few samples, e.g., after bulk profiling studies
- DriverMap and Mitra/GenTegra Microsampling technology > promising strategy for large-scale TCR/BCR repertoire analysis and biomarker discovery in whole blood samples