Single cell genomic profiling of circulating tumor cells (CTCs) from metastatic colorectal cancer (mCRC) identify tumor heterogeneity and rare somatic driver alterations

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Background

- Tumor heterogeneity is frequently observed in metastatic late stage disease
- Specific genomic driver alterations are often associated with resistance/sensitivity to targeted therapies
- mCRC is often characterized as a heterogeneous and multi-focal disease
- Targeted therapies are often used to treat mCRC (e.g. VEGFi, EGFRi, TKIs)
- The presence of subclonal populations with separate driver alterations may affect response to these therapies (BRAF, EGFR, KRAS)
- Circulating tumor cells (CTCs) have been shown to reflect tumor heterogeneity in a liquid biopsy
- To this end, we developed a single cell targeted resequencing assay targeting 578 pan-cancer genes
- CTCs were characterized using the Epic CTC platform
- Single CTCs from a late stage mCRC patient were assayed to characterize the subclonal diversity of genomic alterations in a patient's CTCs

The Epic CTC Platform Workflow

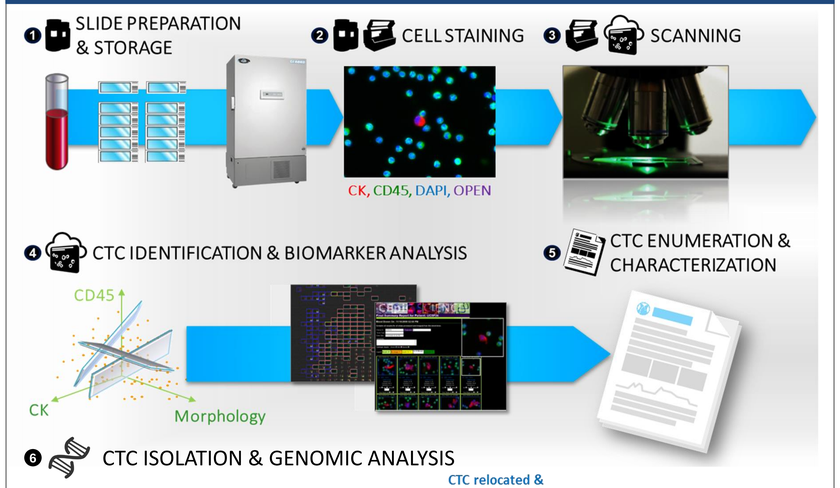
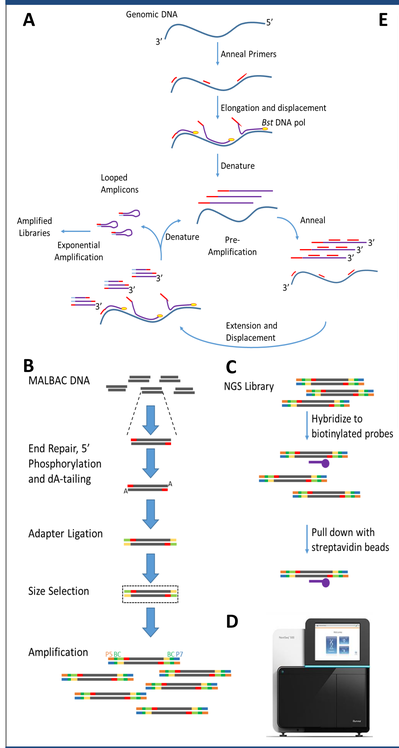


Figure 1. Epic CTC Assay and Cell Recovery Workflow

1) Nucleated cells from blood sample placed onto slides and stored at -80C
2) Slides stained with Cytokeratin (CK), CD45, DAPI and a biomarker of interest
3) Slides scanned for IF marker expression
4) Proprietary CTC identification analysis
5) CTC enumeration and biomarker analysis
6) Identified CTCs are recovered from slide surface for single cell genomic analysis

Assay Methods



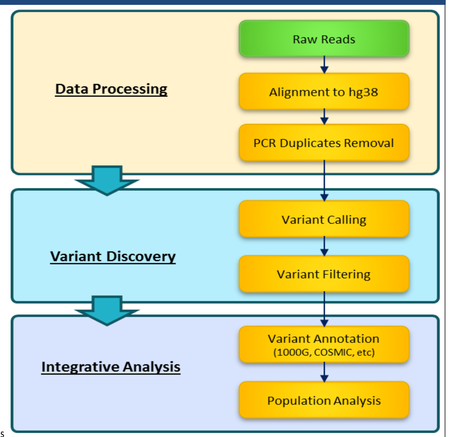
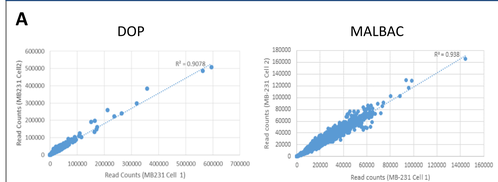


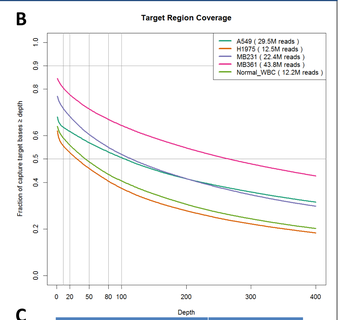
Figure 2. Targeted Resequencing Assay Workflow

A. Recovered CTCs are lysed and individually whole genome amplified using MALBAC WGA methods.
B. Amplified genomes are shotgun library constructed with molecular barcodes.
C. Libraries are pooled by compatible barcodes and enriched by target capture for coding regions of 578 pan cancer genes.
D. Enriched libraries are sequenced by 2 x 150bp PE reads on an Illumina NextSeq 500.
E. Raw FASTQ files are aligned to the hg38 reference genome, and PCR duplicates removed. Variant analysis was performed using VarScan. Variants were annotated and filtered for damaging effect (SIFT, PolyPhen2) & <1% 1000g using GenePool.

Method Development



Coverage statistic	MALBAC	DOP
Reads (M)	173	201
Coverage depth (10X target)	9	8
% genome coverage at (1X)	64	32
% genome coverage at (10X)	25	17
% uniformity	53	28



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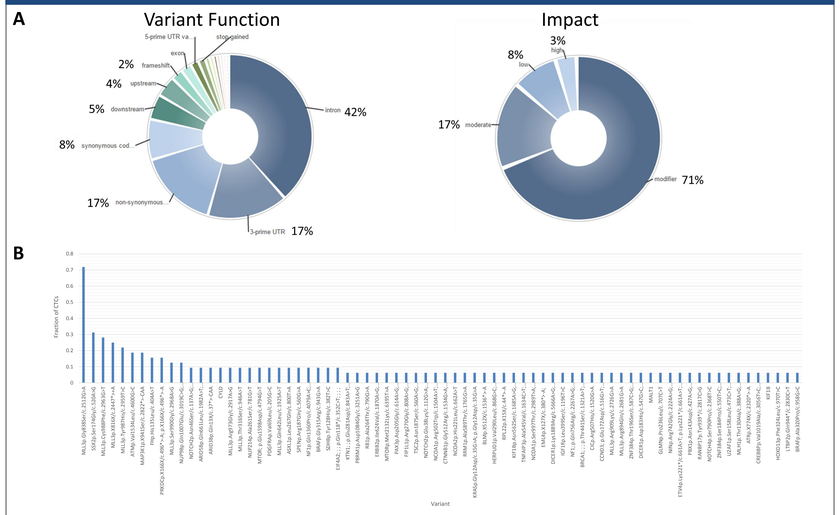
Test Sample	% On-target
A549	56%
H1975	55%
MB231	60%
MB361	59%
Donor WBC	57%

Figure 3. Assay Development Data

A. Comparison of pre-enrichment WGA method coverage MALBAC vs. DOP from MB-231 whole genome sequencing
B. Comparison of post-enrichment target region coverage across multiple cell lines
C. Comparison of post-enrichment on-target rate across development test samples and healthy donor white blood cell (WBC) samples

Patient Feasibility



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Gene	Mutation	% of CTCs	COSMIC ID	IMPACT	Comment
MLL3	p.Gly838Ser/c.2512G>A	69.70%		moderate	~20% in CRC associated with tumor progression and microsatellite instability
SSX2	p.Ser174Gly/c.520A>G	30.30%		moderate	
MLL3	p.X816X/c.2447*>+A	24.24%	289942	high	
ATM	p.Val1534Leu/c.4600G>C	18.18%		moderate	Homologous recombination deficiency~ PARP inhibitors
EIF4A2	p.Gln118*/c.352C>T	9.09%		high	Co-occurance with MLL3 clonal alteration
NOTCH2	p.Asn46Ser/c.137A>G	9.09%		moderate	Co-occurance with MLL3 clonal alteration
BRCA1	p.X607X/c.1820*>-A	9.09%	1383519	high	Homologous recombination deficiency~ PARP inhibitors
KRAS	p.Gly12Asp/c.35G>A	6.06%	521	moderate	Associated with resistance to EGFR TKIs
EML4	p.X127X/c.380*>-A	6.06%	1408084	high	
ABL2	p.X508X/c.1522*>-C	6.06%		high	
PIK3CA	p.X27X/c.79*>-C	3.03%		high	resistance to EGFR-TKIs
TP53	p.Cys190*/c.570T>A	3.03%	45394	high	Tumor suppressor inactivation

Figure 4. Patient feasibility data

A. Comparison of called variant function (left) and impact (right) across all patient CTCs
B. Frequency of damaging somatic variants observed across all patient CTCS
C. Moderate and high impact clonal and subclonal alterations detected across all patient CTCs

Conclusions

- ✓ Development of single cell targeted resequencing assay with reproducible genomic coverage
- ✓ Detection of damaging somatic mutations in single CTCs
- ✓ Detection of both clonal and subclonal alterations in mCRC patient CTCs