Use of the DEPArray platform to detect, isolate, and molecularly characterize pure tumor cells from peripheral blood samples enriched using the CellSearch® system

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Abstract #10616

Abstract

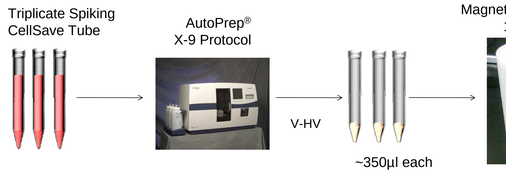
Background: Circulating tumor cells (CTC) offer the potential for serially monitoring the molecular profile of a tumor. However, enrichment techniques provide a level of purity problematic for most molecular analysis methods, and do not readily provide for analysis of tumor cell heterogeneity. We evaluate the use of DEPArray™ (Silicon Biosystems), an automated system enabling image-based cell sorting with single-cell resolution, for CTC isolation and characterization from enriched blood samples.

Methods: Experiments were carried out with healthy-donor blood (HB) collected in CellSave tubes, spiked with tumor cells (TC) and enriched on Veridex's AutoPrep with the CellSearch® Epithelial Cell Kit. DEPArray™ system was used for detection and multiple recoveries of single TCs (or control WBCs) and 5 cell batches. A comparison of blind enumeration results with Veridex's CellTracks Analyzer II® (CTAII) was carried out on replicate samples.

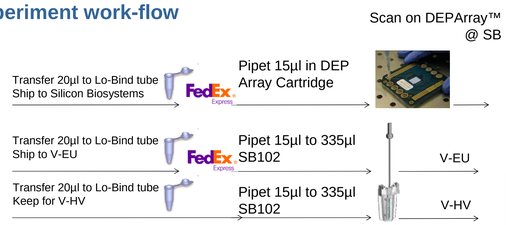
Results: No TCs were detected among negative controls (n=10). TC count (normalized to sample volume analyzed) was compared sample-wise for each replicate (n=20): DEPArray/CTAII count was, on average, 100% (standard deviation = 52%). Enriched mixtures of Her2+ and Her2- TCs spiked in HB samples (n=5), were sorted by DEPArray and recovered into separate tubes. By phenotypical re-analysis no Her2+ cells were detected among the Her2- cell fraction and vice versa, neither were donor WBCs found (100% purity). KRAS-mutated, A549 cells spiked in HB were enriched and loaded on DEPArray. Individual fractions containing either 1 to 5 tumor cells or donor WBCs were sorted. Whole Genome Amplification (*Ampli*1™ WGA, Silicon Biosystems), KRAS specific gene amplification and Capillary Electrophoresis sequencing were carried out. TCs successfully amplified showed only mutated KRAS, (WBCs were only wild-type).

Conclusions: DEPArray™ achieved 100% purity, eliminating all white blood cells (WBC), in the isolation of a mixed population of tumor cell lines downstream of CellSearch® enrichment. This enabled molecular profiling of pure tumor cells from whole blood spiked tumor cell lines. Detection of molecular heterogeneity of tumor cells is demonstrated through KRAS sequencing

Tumor Cell Detection



Experiment work-flow



DEPArray™ Cell Browser screenshot

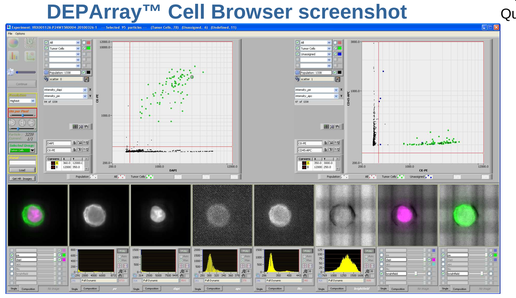
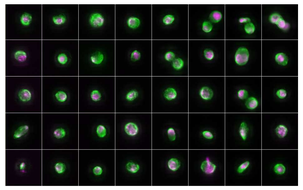
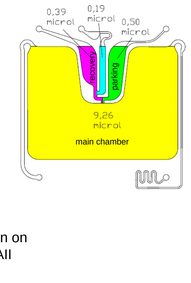


Image gallery of TCs from DEPArray™



Summary of results

Cells prepared in Veridex central lab, PA (V-HV); count performed in blind by remote labs: Veridex Enschede, NL (V-EU) and SIlicon Biosystems (SB) with DEPArray. Negative controls (only Healthy donor Blood, n=10), were negative in all platforms (data not shown). For spiking experiments (n=20, Table 1) of TCs (SKBr3), a normalization coefficient (9,26/15=61,7%) is used on Veridex counts to take into account volume effectively analyzed by DEPArray (Main Chamber volume). DEParray/CTAII remote count Ratio = SB/(V-EU)=100%, σ=52%. Average number of TC in remote labs [(SB+V-EU)/2]/(V-HV)=80% due to shipping loss, σ=16%, (<σ(SB/V-EU) due to negative correlation from sample splitting after pooling).



Cells (Normalized)				
V-HV	V-EU	SB	SB/EU	(EU+SB)/2
12	12	12	97%	99%
12	9	13	150%	88%
94	91	25	27%	62%
10	3	6	194%	43%
12	16	9	56%	107%
9	7	5	74%	64%
117	115	61	53%	75%
132	105	78	74%	69%
93	83	45	54%	69%
69	65	60	93%	90%
102	103	85	82%	92%
48	27	55	202%	85%
159	146	142	97%	90%
162	71	150	211%	68%
66	59	48	82%	81%
44	41	38	92%	91%
44	31	22	71%	59%
62	70	46	66%	93%
78	57	57	100%	73%
80	73	90	123%	102%

Table 1

mean	100%	80%
std-dev	52%	16%

Cell Separation

Experiment work-flow

Separation by Immunophenotype: Results evaluation by CTAII®

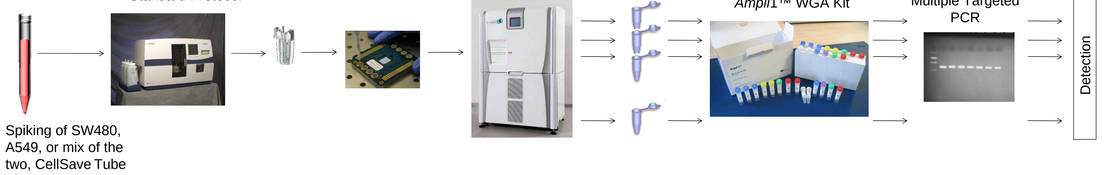
Duplicate preparations of Her2+/Her2- admixed cells (SKBr3/PC3-9) spiked in HB (n=5) were enriched with CellSearch® and scanned by CTAII® before and after DEPArray sorting of Her2+ and Her2- fractions. Sorted fractions re-injected in Veridex cartridges and analyzed with CTAII revealed 100% purity, with no WBCs or wrong type of tumor cells

CellTracks Analyzer II® Images

Replicate	Sorted by DEPArray™ (SB)		Analyzed by CTAII (V-HV)		
			Her2-	Her2+	Purity
1	Her2-	50	28	0	100%
	Her2+	47	0	17	100%
2	Her2-	6	3	0	100%
	Her2+	27	0	16	100%
3	Her2-	17	7	0	100%
	Her2+	26	0	16	100%
4	Her2-	5	1	0	100%
	Her2+	11	0	6	100%
5	Her2-	8	5	0	100%
	Her2+	16	0	11	100%

Cell Molecular Characterization

Experiment work-flow



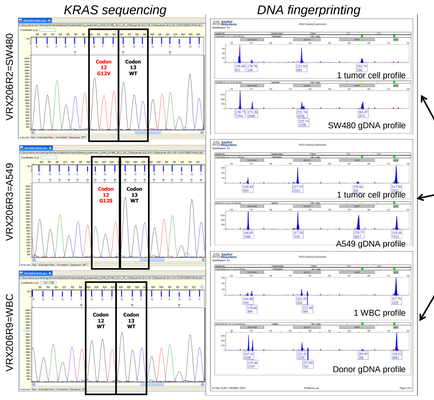
Molecular characterization for purity and mutational status

Across four Spiking experiments of viable (>90%) KRAS mutated cell lines (SW480 n=2, A549 n=1, mix SW480/A549 n=1) in HB, multiple recoveries (range 12-21 per experiment) of individual cells (n=56), 5 cell batches (n=5), or negative controls (n=8) were carried out. *Ampli*1™ WGA kit (Silicon Biosystems) products from each tube were DNA-fingerprinted (home-brewn 11 loci multiplex reaction, to confirm cell presence, identity and purity) and KRAS gene-specific amplification products were sequenced (on ABI 3730xl). In 91% (51/56) of single cell recoveries, cell presence was confirmed. The 5 tubes with no signal from STR and KRAS suggests that the cell has been removed during surnatant removal before WGA. All successfully amplified cells matched 100% KRAS mutational status and DNA fingerprint, no signals was detected in negative controls recoveries (buffer only). In the mixed tumor cells experiment, different KRAS mutations (and DNA fingerprints) were detected in different tumor cell recoveries, reflecting cell heterogeneity.

Molecular Analysis Results

Summary

	N	KRAS call	KRAS no call	KRAS call rate	# Alleles expected	# Alleles found	ACR % (non void)
Single cells	56	51	5	91%	857	588	68,6%
TC	38	36	2	95%	557	388	69,7%
WBC	18	15	3	83%	300	200	66,7%
5 cells	5	5	0	100%	97	82	84,5%
TC	1	1	0	100%	17	15	88,2%
WBC	4	4	0	100%	80	67	83,8%
Negative Controls	8	0	0	NA	0	0	NA



Sample ID	Recovery ID	# cells	cell type	K-ras PCR	K-Ras type	Notes	# Alleles expected	# Alleles found	Allele Call Rate %	Result Classification
VRX202	1	1	TC	1	G12S	allelic count refer to A549	12	11	92%	MATCH
	2	1	TC	1	G12S	allelic count refer to A549	12	12	100%	MATCH
	3	1	TC	1	G12S	allelic count refer to A549	12	9	75%	MATCH
	4	1	TC	1	G12S	allelic count refer to A549	12	11	92%	MATCH
	5	1	TC	1	G12S	allelic count refer to A549	12	9	75%	MATCH
	6	1	TC	1	G12S	allelic count refer to A549	12	12	100%	MATCH
	7	1	TC	0	NA	allelic count refer to A549	12	0	0%	NO-CALL
	8	1	TC	1	G12S	allelic count refer to A549	12	12	100%	MATCH
	9	1	TC	1	G12S	allelic count refer to A549	12	10	83%	MATCH
	10	1	TC	1	G12S	allelic count refer to A549	12	10	83%	MATCH
	B10	0	BUFFER	0	NA	negative control recovery	0	0NA		MATCH
	11	1	WBC	1	WT	allelic count refer to WBC	20	16	80%	MATCH
	12	1	WBC	1	WT	allelic count refer to WBC	20	13	65%	MATCH
	13	1	WBC	1	WT	allelic count refer to WBC	20	14	70%	MATCH
	14	1	WBC	1	WT	allelic count refer to WBC	20	11	55%	MATCH
	15	1	WBC	1	WT	allelic count refer to WBC	20	15	75%	MATCH
	16	1	WBC	1	WT	allelic count refer to WBC	20	19	95%	MATCH
	B16	0	BUFFER	0	NA	negative control recovery	0	0NA		MATCH
VRX203	1	1	TC	1	G12V	allelic count refer to SW480	17	14	82%	MATCH
	2	1	TC	1	G12V	allelic count refer to SW480	17	12	71%	MATCH
	3	1	TC	1	G12V	allelic count refer to SW480	17	11	65%	MATCH
	4	1	TC	1	G12V	allelic count refer to SW480	17	11	65%	MATCH
	5	1	TC	1	G12V	allelic count refer to SW480	17	11	65%	MATCH
	6	1	TC	1	G12V	allelic count refer to SW480	17	11	65%	MATCH
	7	1	TC	1	G12V	allelic count refer to SW480	17	14	82%	MATCH
	8	1	TC	1	G12V	allelic count refer to SW480	17	9	53%	MATCH
	9	1	TC	1	G12V	allelic count refer to SW480	17	10	59%	MATCH
	10	1	TC	1	G12V	allelic count refer to SW480	17	7	41%	MATCH
	11	1	TC	0	NA	allelic count refer to SW480	17	0	0%	NO-CALL
	12	1	TC	1	G12V	allelic count refer to SW480	17	10	59%	MATCH
	13	1	TC	1	G12V	allelic count refer to SW480	17	15	88%	MATCH
	B13	0	BUFFER	0	NA	negative control recovery	0	0NA		MATCH
VRX206	14	5	WBC	1	WT	allelic count refer to WBC	20	16	80%	MATCH
	15	1	WBC	1	WT	allelic count refer to WBC	20	13	65%	MATCH
	16	1	WBC	1	WT	allelic count refer to WBC	20	11	55%	MATCH
	17	1	WBC	1	WT	allelic count refer to WBC	20	14	70%	MATCH
	18	1	WBC	1	WT	allelic count refer to WBC	20	12	60%	MATCH
	19	1	WBC	1	WT	allelic count refer to WBC	20	12	60%	MATCH
	B19	0	BUFFER	0	NA	negative control recovery	0	0NA		MATCH
VRX207	1	1	TC	1	G12V	allelic count refer to SW480	17	15	88%	MATCH
	2	1	TC	1	G12S	allelic count refer to A549	12	12	100%	MATCH
	3	1	TC	1	G12S	allelic count refer to A549	12	11	92%	MATCH
	N4	0	BUFFER	0	NA	negative control recovery	0	0NA		MATCH
	5	5	WBC	1	WT	allelic count refer to WBC	20	16	80%	MATCH
	6	1	WBC	1	WT	allelic count refer to WBC	20	13	65%	MATCH
	7	1	WBC	0	NA	allelic count refer to WBC	20	0	0%	NO-CALL
	8	1	WBC	1	WT	allelic count refer to WBC	20	15	75%	MATCH
	9	1	WBC	1	WT	allelic count refer to WBC	20	11	55%	MATCH
	10	1	TC	0	NA	allelic count refer to WBC	20	0	0%	NO-CALL
	N10	0	BUFFER	0	NA	negative control recovery	0	0NA		MATCH
VRX208	1	1	TC	1	G12V	allelic count refer to SW480	17	13	76%	MATCH
	2	1	TC	1	G12V	allelic count refer to SW480	17	15	88%	MATCH
	3	1	TC	1	G12V	allelic count refer to SW480	17	11	65%	MATCH
	4	1	TC	1	G12V	allelic count refer to SW480	17	7	41%	MATCH
	5	1	TC	1	G12V	allelic count refer to SW480	17	7	41%	MATCH
	6	1	TC	1	G12V	allelic count refer to SW480	17	12	71%	MATCH
	7	1	TC	1	G12V	allelic count refer to SW480	17	14	82%	MATCH
	8	1	TC	1	G12V	allelic count refer to SW480	17	8	47%	MATCH
	9	1	TC	1	G12V	allelic count refer to SW480	17	7	41%	MATCH
	10	1	TC	1	G12V	allelic count refer to SW480	17	6	35%	MATCH
	11	1	TC	1	G12V	allelic count refer to SW480	17	7	41%	MATCH
	12	1	TC	1	G12V	allelic count refer to SW480	17	7	41%	MATCH
	N12	0	BUFFER	0	NA	negative control recovery	0	0NA		MATCH
	13	5	WBC	1	WT	allelic count refer to WBC	20	16	80%	MATCH
	14	1	WBC	1	WT	allelic count refer to WBC	20	13	65%	MATCH
	15	1	WBC	1	WT	allelic count refer to WBC	20	12	60%	MATCH
	16	1	WBC	0	NA	allelic count refer to WBC	20	0	0%	NO-CALL
	N16	0	BUFFER	0	NA	negative control recovery	0	0NA		MATCH