Electronic Supplementary Material (ESI) for Lab on a Chip.
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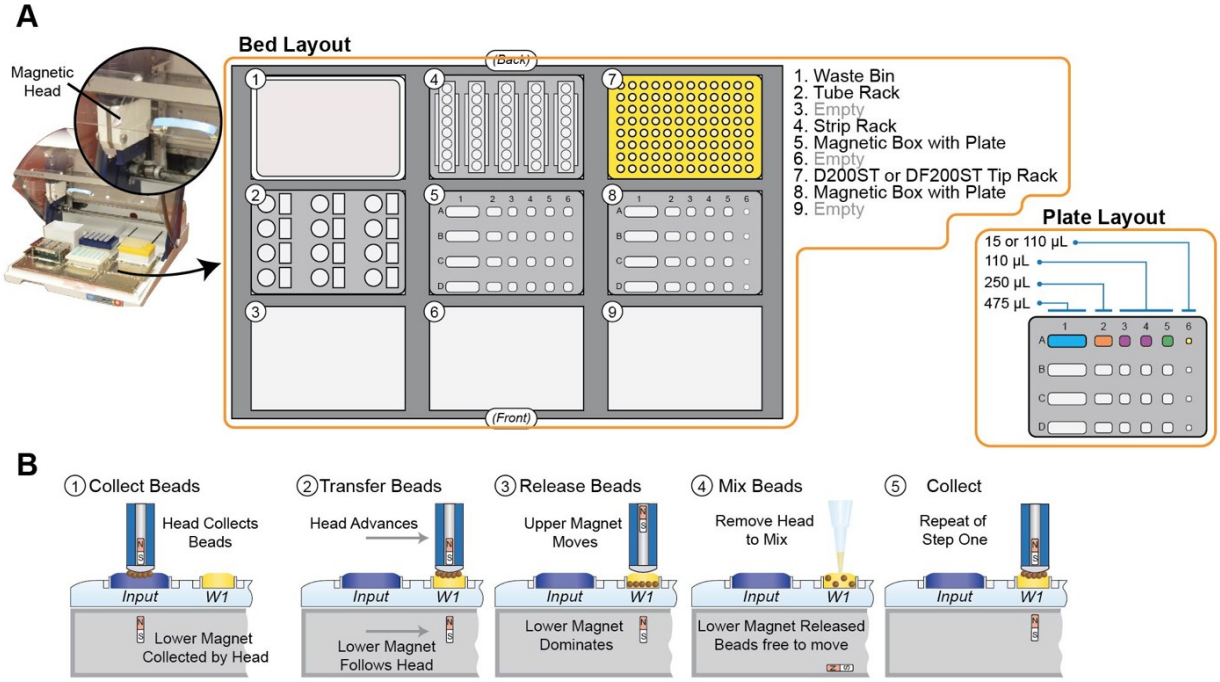
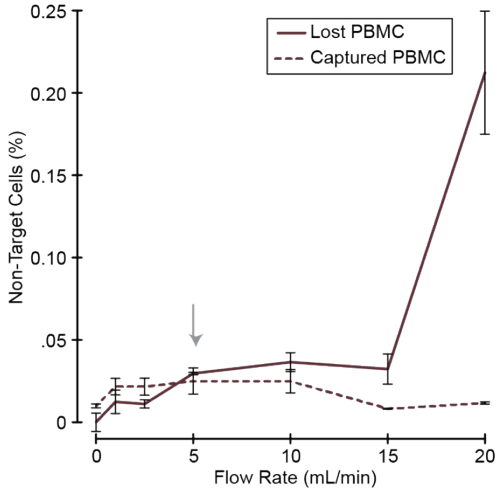
Supplemental Information

Automated ESP Platform Overview

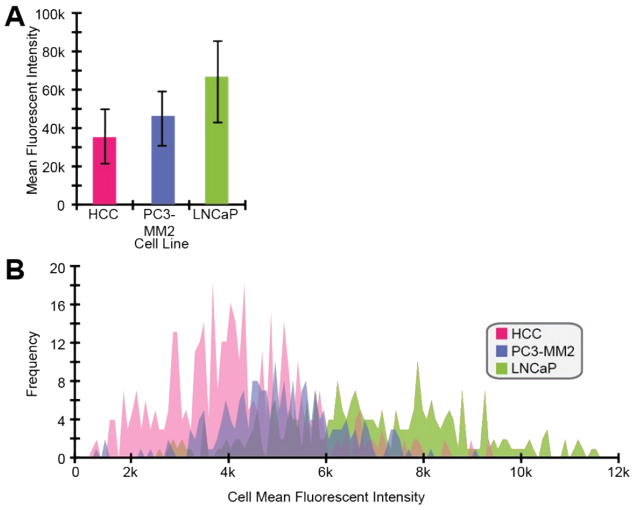
The PIPETMAX is equipped with: (1) a p200 pipette head, (2) a magnetic head, which is a p200 pipette head retrofitted with magnets (D36-N52, K&J Magnetics) and a rapid prototyped core (Midwest Prototyping) to fit the PMP collection strips, (3) a rapid prototyped rack for five the PMP collection strips (4) two magnet boxes[35], (5) a rack for 1.5 mL tubes, and (6) a rack of p200 tips (#DSF200ST, Gilson). Extraction plates (#22100008, Gilson), positioned on top of each magnetic box, contain four rows of wells, each row comprised of a sequence of six wells: one sample well (~475 µL), one large wash well (~250 µL), three small wash wells (110 µL each), and one output/elution well (110 µL for CTC samples or 15 µL for the NA samples). Buffy coat and PMPs are loaded into the sample well and allowed to bind for ~30 min and specific buffers (e.g., wash, stain, lysis buffer) are loaded into the remaining well at the predefined volumes. The magnetic head collects PMPs from the sample well by lowering over the well until the PMP collection strip contacts the fluid. The head then traverses to an adjacent well and the PMPs are released, mixed (either by pipette or magnetically), and recollected using the magnetic mechanism described by Guckenberger et al.[35]; this process is repeated until the PMPs reach the output well. Delays are added to specific wells (after the mixing step) to accommodate staining, fixing, and similar procedure. Once isolated, whole cells were either removed for microscopy or transferred to a second extraction plate for NA isolation. Cells were released directly in lysis buffer, allotted time for the NA to bind to PMPs, the PMPs carried through a series of washes and released into the elution buffer.



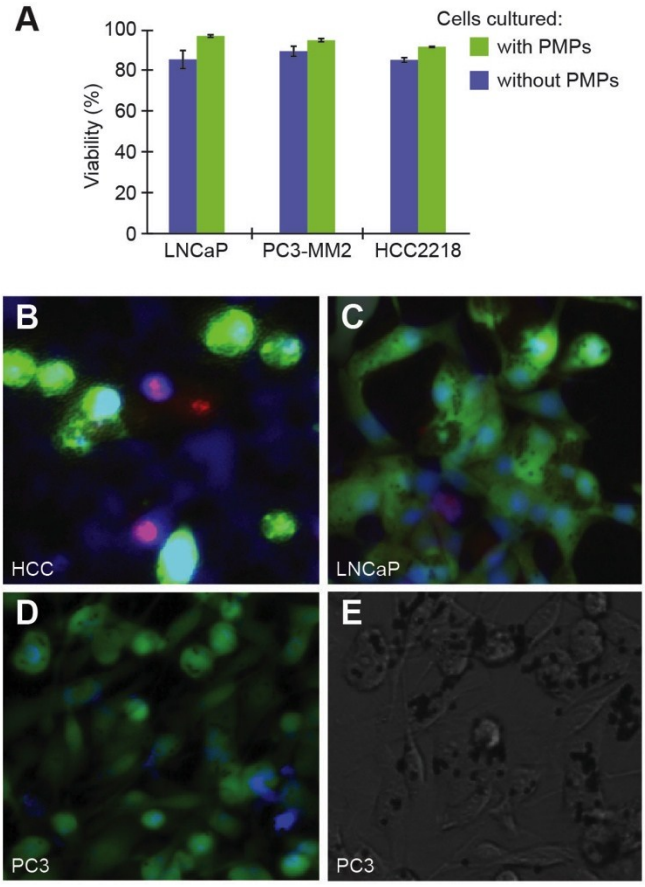
Supplemental Figure 1: Automated platform overview and magnetic system for bead manipulation. (A) A schematic overview of the automated platform and positions on the platform. (B) Relationship between the upper magnets, lower magnets, and magnetic beads.



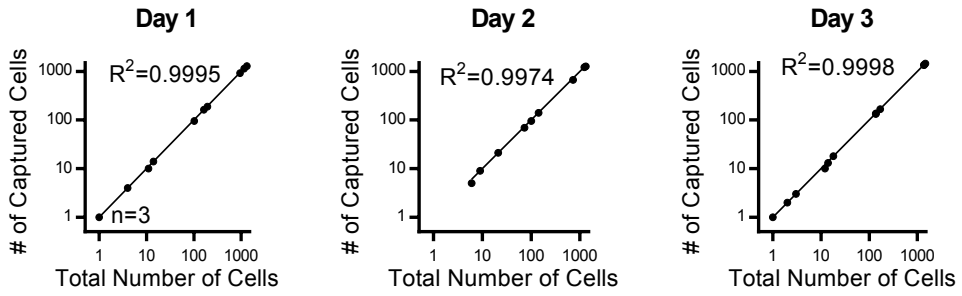
Supplemental Figure 2: Impact of shear mixing by pipette on non-target PBMCs including both PBMCs released from the PMPs during mixing (lost PBMCs) and PBMCs that still remained bound to the PMPs after mixing (captured PBMCs).



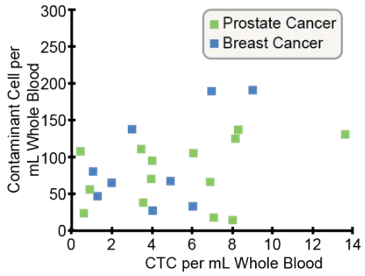
Supplemental Figure 3: EpCAM protein expression (A) Average of each cell line's EpCAM mean fluorescent intensity. Error bars represent standard deviation (n=3). (B) Histogram of the each cell's means fluorescent EpCAM intensity.



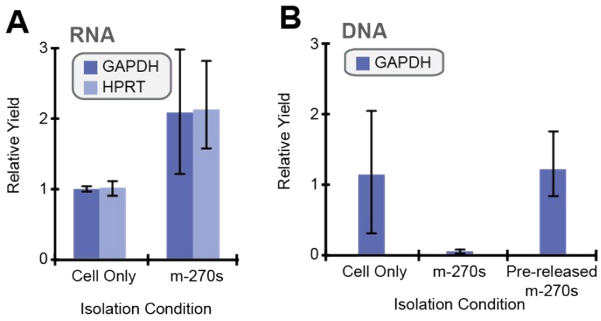
Supplemental Figure 4: Impact of prolonged PMP exposure to cell viability. (A) Viability of cells cultured with PMPs for 5 days, following isolation from a PBMC background. (B-D) Representative images of Live/Dead staining of each of the cell lines following culture with PMPs (blue-Hoechst, green-live, red-dead) (B-HCC, C-LNCaP, D-PC3-MM2). (E) Corresponding brightfield image of PC3-MM2 showing PMPs still attached to cells (black dots).



Supplemental Figure 5: Capture consistency of variable numbers of SK-RC52 cells on mTAE across three different days (captured with EpCAM and CAIX antibodies). Results highlight the consistency both across variable inputs and day-to-day performance of the platform.



Supplemental Figure 6: Captured contaminant cells normalized per mL of whole blood plotted against the number of CTCs captured per mL of whole blood in positive selection samples. Results indicate no relationship between contaminant cells and CTCs isolated from a sample.



Supplemental Figure 7: Impact of PMP on NA extraction. (A) Impact of M-270s on RNA extraction in the automated system. On average, M-270s improve RNA yields when included in with the RNA extraction PMPs. RNA quantification was done using two housekeeping genes, GAPDH and HPRT. (B) Impact of M-270s on DNA extraction in the automated system. Due to negative impact of M-270s on DNA extraction, a pre-lyse step was first performed, the M-270s removed, and DNA lysis buffer and PMPs added. Using this approach, DNA yields are consistent cell only DNA extraction yields.

AR	AR 1/2	Hs00907242_m1
	AR 4/5	Hs00171172_m1
	AR_V1	Custom (AI39R60)
	AR_V7	Hs04260217_m1
	AR_V9	Custom (AI6RPM5)
Prostate Cancer Specific Transcripts	KLK2	Hs00428383_m1
	KLK3 (PSA)	Hs02576345_m1
	TMPRSS2	Hs01120965_m1
	NKX3.1	Hs00171834_m1
	FOHL1 (PSMA)	Hs00379515_m1
	FOXA1	Hs04187555_m1

Epithelial	EpCAM	Hs00901885_m1
	KRT8 (cytokeratin 8)	Hs01595539_g1
Stem cell	PROM1 (CD133)	Hs01009250_m1
	PSCA	Hs04177224_g1
Blood	PTPRC (CD45)	Hs04189704_m1
Housekeeping	POLR2A	Hs00172187_m1
	P0	4333761F

Supplemental Figure 8: Overview of the primers used for quantifying specific target transcripts in both cell lines and CTCs. All primers were Taqman (ThermoFisher).

Prostate CTC Cell Isolation Patients					Metastasis				
Patient	Age (yrs)	Gleason Score	Current Treatment	Time since diagnosis (Months)	Bone	Lymph Nodes	Liver	Brain	Lung parenchyma /lymphagitic spread/pleural based
1*	73	3+4	Surveillance off ADT	14	X				
2*	80	4+5	Enzalutamide	3	X	X			
3	76	9	Enzalutamide	16		X			
4	69	3+3	ADT	12		X			X
5	59	4+5	ADT	2	X	X			
6	71	4+5	ADT	2	X				
7*	75	4+5	Clinical trial with 52 cycles of ARN-509	8		X			
8	67	4+3	Between treatments after radium 223	7	X	X			
9	70	3+4	Enzalutamide	16	X	X			
10*	76	5+4	Abiraterone	1	X	X			
11	77	4+5	Enzalutamide	2	X	X			
12	67	Poorly differentiated	Palliative radiation	12	X				
13	66	4+5	Docetaxel	6	X	X			X
14	81	4+3	Enzalutamide	5	X	X			
15	80	5+4	Phase I trial	6	X	X	X		
16**									

Supplemental Figure 9: Table of prostate cancer patient samples evaluated for CTC capture (* indicates multiple samples were obtained from the specified sample across multiple dates; ** patient data unknown).

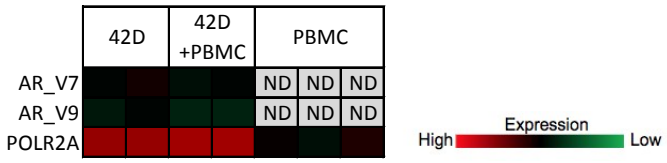
Breast CTC Cell Isolation Patients						Metastasis				
Patient	Age (yrs)	Tumor Stage at Diagnosis	Primary Therapy (Primary site)	Current Therapy (Metastatic)	Time since diagnosis	Bone	Lymph Nodes	Liver	Brain	Lung

17	60	II	Docetaxel, pertuzumab/ trastuzumab,	Capecitabine (Xeloda), zoledronic acid (Zometa)	19	X	X			X
18*	60	N/A	Anastrozole plus pamidronate, multiple lines of endocrine therapy	Exemestane plus entinostat or placebo, radiation	11					
19**										
20	75	IV	Radiation	Faslodex and zoledronic acid	2	X		X		
21	75	IV	Arimidex	Nab/paclitaxel and zoledronic acid	13	X				
22	70	IA	Tamoxifen	Dexamethasone, Zoledronic acid, T-DM1	9	X		X	X	
23*	60	III	Doxorubicin and Cyclophosphamide, followed by Paclitaxel and Trastuzumab, tamoxifen	Trastuzumab (Herceptin), everolimus (Afinitor)	5	X	X	X	X	X
24*	51	IIA	AC, dd-paclitaxel	Eribulin and pembrolizumab	11		X			
25	60	II	Adriamycin and Cytoxan, Tamoxifen	Capecitabine (Xeloda), zoledronic acid (Zometa)	19	X	X			X

Supplemental Figure 10: Table of breast cancer patient samples evaluated for CTC capture (* indicates multiple samples were obtained from the specified sample across multiple dates; ** patient data unknown).

Prostate CTC mRNA Analysis Samples					Metastasis				
Patient Number	Age (yrs)	Gleason Score	Current Treatment	Time since diagnosis	Bone	Lymph Nodes	Liver	Brain	Lung parenchyma/lymphagitic spread/pleural based
26	76	4+5	Phase II Trial	9		X			
27	70	4+3	Enzalutamide	13	X	X			
28*	68	4+5	Surveillance	20	X	X			

Supplemental Figure 11: Table of prostate cancer patient samples evaluated for CTC capture and subsequent mRNA extraction and analysis.



Supplemental Figure 12: Demonstration of the specificity of a subset of evaluated AR variant primers as well as undetected expression in PBMCs from a healthy donor. AR V7 and AR V9 were detected from RNA extracted from the prostate cancer line 42D and 42D with PBMCs, but not in PBMCs alone.