Sequential eDAR Isolation and FISH Identification of Rare Cells from Blood by Using Concentrated Peripheral Blood Mononuclear Cells

Shihan Xu[1,2], Li Wu[2,5], Yuling Qin[2,5], Yifei Jiang[2], Kai Sun[2], Chenee Holcomb[3], Michael G. Gravett[3], Lucia Vojtech[3], Perry G. Schiro[4] and Daniel T. Chiu*[,1,2]

[1] Department of Bioengineering, University of Washington, Seattle, United States

[2] Department of Chemistry, University of Washington, Seattle, United States

[3] Department of Obstetrics & Gynecology, University of Washington, Seattle, United States

[4] MiCareo Inc., Xing-Ai Road Ln. 77 No. 69 5F, Taipei City, Taiwan

[5] School of Public Health, Nantong University, Nantong, Jiangsu, 226019, P. R. China

* To whom correspondence should be addressed: chiu@uw.edu

Table S1. Recovery rate of spike-in MCF-7 cells from samples with different PBMC concentrations. In "L_S" samples, MCF-7 cells were first labeled with PE-anti-EpCAM antibody, then spiked into PBMCs. In the "S_L" sample, unlabeled MCF-7 cells were first spiked into a suspension of PBMCs, then labeled with PE-anti-EpCAM antibody.

PBMCs concentration (cells/mL)	2.5×10^7 (L_S)	5.0×10^7(L_S)	1.0×10^8(L_S)	2.5×10^7(S_L)
MCF-7 cells spiked	90	50	350	250
MCF-7 cells collected	80	43	299	218
Recovery rate	88.9%	86.0%	85.4%	87.2%

Table S2. Success rate of single-cell FISH analysis.

Slide No.	Counted cells	Cell with FISH signals	FISH success rate
1	100	80	80%
2	100	65	65%
3	25	16	64%
4	17	12	70.6%
5	19	14	73.7%
Total	261	187	71.6%

Table S3. The success rate of FISH analysis was improved by increasing the denaturation temperature.

Sample NO.	Temperature (℃)	FISH success rate
1	74	72.3%
2	76	80.0%
3	78	85.7%