

Conclusions

We have developed two thaw methods for archival PBMCs, either using the proprietary MarrowMax™ medium or an RPMI based media with high FBS content. Statistical analysis showed that both methods were comparable, but with a possible preference for MarrowMax™. We demonstrated that these thawed cells were able to undergo DNA repair in a manner comparable to freshly drawn WB. To our knowledge, this is the first phenotypic assay of DNA repair functionality in cryopreserved PBMCs and represents a significant step forward for high impact, large-scale, demographic studies using cohorts such as the BCFR. Future work: This thawing protocol can be applied for any metabolic phenotypic assay using multi-color panels including γ -H2AX and a nuclear stain, as the image stream platform can accommodate 4-9 fluorescent channels. This assay technique and validated machine learning classifiers was developed to be scaled up in a large (>400) paired case-control cohort of breast cancer patients. Using this larger cohort, proper model testing could be done to further validate these machine learning classifiers and determine if the differences between apoptotic events (as classified by the edge staining and pan-nuclear staining) are significantly different at different time points or radiation doses. Ultimately, this cohort will be used to test for a correlation between DRC and breast cancer risk.

Supplemental Figures

Supplemental Table 1. Epidemiological data for cryogenically preserved PBMCs used in this study

Donor#	Donation Date	Age at Interview	BMI	Race/Ethnicity	Smoking Status
1	6/1/1998	43	24.9	Other	Non-Smoker
2	8/13/1999	36	20.5	Other	Non-Smoker
3	8/24/1999	47	30.1	Other	Smoker
4	9/15/1999	33	25.8	White	Smoker
5	9/23/1999	45	23.9	White	Non-Smoker
6	9/28/1999	42	29.2	White	Non-Smoker
7	3/13/2001	47	23.2	White	Non-Smoker
8	12/29/1999	42	27.3	Other	Non-Smoker
9	10/3/2001	67	28.3	White	Non-Smoker
10	10/5/2001	57	24.2	Hispanic	Non-Smoker
11	10/13/1999	44	23.2	Other	Non-Smoker
12	5/26/1999	45	19.5	Other	Smoker
13	7/29/1999	67	23.5	Other	Non-Smoker
14	5/22/1999	50	20.0	Other	Non-Smoker
15	7/27/2001	70	28.8	White	Non-Smoker
15	4/22/1999	43	28.9	Other	Non-Smoker
16	7/22/1999	47	18.7	Other	Smoker
17	10/4/1999	41	23.0	Other	Non-Smoker
18	8/19/1999	47	29.8	Other	Non-Smoker
19	12/29/1999	68	25.0	Other	Smoker

Supplemental Table 2. Net γ -H2AX fluorescence in PBMCs from 19 donors at serial time points (1-20h). Fluorescence values (arbitrary units) of cells after gating strategies discussed above

Time (hr)	MarrowMax™ Media					RPMI+30%FBS Media				
	1	2	3	5	20	1	2	3	5	20
Donor1	6459.135	9815.262	8964.727	NA	NA	959.7186	9955.634	8334.906	2746.41	541.4851
Donor2	7633.71	8473.178	8269.042	4955.196	495.9535	7643.71	7415.421	6619.759	4321.696	549.5778
Donor3	10250.88	11505.1	7978.822	4564.568	3614.442	9504.126	8270.196	7159.567	284.3621	397.9783
Donor4	14985.01	24126.95	10838.2	7101.092	253.7828	14144.12	24890.76	11744.34	7561.72	624.2804
Donor5	8839.927	6982.514	6117.49	3744.181	662.1821	8571.471	8702.745	6117.562	4011.613	516.3899
Donor6	8702.637	8866.777	7496.387	4718.03	494.7579	6448.448	6916.897	6286.755	4793.491	452.4978
Donor7	11541.68	12553.16	7931.49	5178.438	332.9373	13262.16	16795.59	NA	4831.572	517.1593
Donor8	6276.689	7289.746	5420.853	4770.318	694.3109	7001.545	7034.871	6021.267	4268.24	335.7537
Donor9	8123.593	8191.799	6279.553	4356.481	744.3651	7699.171	8809.242	6062.908	4863.975	1062.907
Donor10	11036.64	10235.38	7345.67	5831.504	-5.745	7371.352	6886.124	6250.757	4765.495	-186.018
Donor11	12619.21	11486.91	11079.31	5733.749	-54.4218	12626.07	11379.06	9230.248	5580.505	326.1971
Donor12	5627.747	9271.087	8060.925	5152.029	691.0786	7159.91	8403.8	7372.732	8222.152	1015.145
Donor13	15941.46	14861.38	15514.96	6700.826	984.279	20057.12	14252.48	17387.22	5401.886	1140.13
Donor14	9402.057	10383.07	8534.563	5414.031	1379.748	8133.236	9634.949	8981.046	5012.221	388.2656
Donor15	13037.85	13325.02	14672.38	10006.83	1152.478	15969.75	10873.53	13849.75	9839.599	878.159
Donor16	16677.52	20177.42	17241.21	6915.947	936.5819	12622.53	21752.81	14958.36	7797.924	813.1492
Donor17	11856.67	15213.4	12889	7475.517	653.3858	6693.838	11340.5	12044.23	6289.701	629.1842
Donor18	5072.061	6800.426	11458.13	9350.713	740.2701	NA	NA	NA	NA	NA
Donor19	7632.862	6743.039	7843.518	5208.459	938.7754	7985.202	7955.929	7451.11	7026.967	2799.032

are based on median fluorescence intensity of each irradiated time point after subtracting its appropriate un-irradiated control. Missing values (“NA”) indicate insufficient number of cells (<100 cells).

Conflict of Interest

The authors have no conflict of interest to declare.

Data availability

The data types associated with ImageStream data acquisition are Raw image file (.rif), compensated image file (.cif), and data analysis file (.daf) and require the IDEAS software for analysis. Data from these experiments are available upon request.

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