

Supplementary data

Table S2

Common metabolic pathways for DF-1 cells in DMEM/F12 (1:1) and DMEM in pathway timing analysis

Style	Sub Pathway	Raw p		Impact
		DMEM/F12(1:1)	DMEM	
Carbohydrate	Glutathione metabolism	9.19E-05	1.13E-04	0.59
	Citrate cycle (TCA cycle)	1.66E-03	7.42E-03	0.26
	Fructose and mannose metabolism	9.13E-04	6.79E-03	0.24
	Arginine and proline metabolism	8.01E-03	8.68E-06	0.52
Amino Acid	Glycine, serine and threonine metabolism	1.45E-05	2.95E-05	0.59
	Alanine, aspartate and glutamate metabolism	8.89E-03	4.53E-03	0.78
	Histidine metabolism	1.34E-03	5.90E-03	0.27
	Cysteine and methionine metabolism	3.56E-03	7.04E-03	0.54
Nucleotide	Purine metabolism	3.99E-04	1.83E-04	0.42
	Pyrimidine metabolism	2.16E-04	4.39E-04	0.3
Others	Aminoacyl-tRNA biosynthesis	2.01E-03	4.91E-03	0.19
	Pantothenate and CoA biosynthesis	4.43E-03	3.41E-03	0.47

Table S3

Analysis of nucleic acid metabolism related pathways.

Sub Pathway	Component	A 72h	A 72h	A 72h	B 72h	B 72h	B 72h
		A 12h	A 24h	A 36h	B 12h	B 24h	B 36h
Purine Metabolism, Adenine containing	adenosine 5'-diphosphate (ADP)	0.61	0.66	0.77	0.77	0.52	0.7
	adenosine 5'-monophosphate (AMP)	0.55	0.71	0.82	0.75	0.82	0.84
	adenosine	1.36	1.35	1.46	0.96	1.08	1.37
	adenine	0.86	0.75	0.82	0.9	1	1.15
Purine Metabolism, Guanine containing	guanosine 5'- monophosphate (5'-GMP)	0.78	0.91	0.99	0.81	0.97	0.93
	guanosine	1.35	0.92	0.84	0.92	0.63	1.28
	guanine	2.96	2.72	1.31	1.66	1.15	2.09
Pyrimidine Metabolism, Uracil containing	uridine monophosphate (5' or 3')	0.29	0.81	0.62	0.47	0.99	1.07
	uridine	0.31	1.39	1.03	0.25	0.8	0.75
	uracil	1.16	2.44	2.11	0.29	0.7	1.58
Pyrimidine Metabolism, Cytidine containing	cytidine 5'-monophosphate (5'-CMP)	0.32	1.03	0.72	0.64	0.99	1.15
	cytidine	0.57	1.95	1.46	0.31	0.64	1.32
Pyrimidine Metabolism, Thymine containing	thymidine 5'-monophosphate	0.54	0.53	0.5	0.63	0.5	0.47

A means intracellular metabolite from DMEM/F12(1:1) and B means from DMEM. Green indicates that the ratio of 72h to other time points is less than 1, and the depth varies with the value of the value; red indicates that the ratio of 72h to other time points is greater than 1

Table S4

Matrix and experimental responses of PBD with N = 11

Run	Factor A	Factor B	Factor C	Factor D	Factor E	Factor F	Factor G	Factor H	Factor J	Factor K	Factor L	Response 1: max cell density		Response 2: specific growth rate	
	Amino Acids 1	Amino Acids 2	Amino Acids 3	Amino Acids 4	Amino Acids 5	Putrescine	Nucleotides	Vitamins	Inorganic Salts	Others	Dummy	10 ⁶ cells/ml		h ⁻¹	
1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	2.390	± 0.176	0.030	± 0.006
2	1	-1	1	1	-1	1	1	1	-1	-1	-1	2.287	± 0.078	0.030	± 0.007
3	-1	1	1	-1	1	1	1	-1	-1	-1	1	2.557	± 0.139	0.059	± 0.002
4	1	1	-1	1	1	1	-1	-1	-1	1	-1	2.420	± 0.116	0.051	± 0.003
5	1	-1	-1	-1	1	-1	1	1	-1	1	1	2.697	± 0.255	0.074	± 0.002
6	1	1	-1	-1	-1	1	-1	1	1	-1	1	0.377	± 0.028	0.007	± 0.004
7	1	-1	1	1	1	-1	-1	-1	1	-1	1	0.871	± 0.013	0.018	± 0.005
8	1	1	1	-1	-1	-1	1	-1	1	1	-1	0.036	± 0.037	0.019	± 0.002
9	-1	-1	-1	1	-1	1	1	-1	1	1	1	0.391	± 0.024	0.003	± 0.024
10	-1	1	-1	1	1	-1	1	1	1	-1	-1	1.723	± 0.077	0.020	± 0.006
11	-1	1	1	1	-1	-1	-1	1	-1	1	1	2.767	± 0.070	0.036	± 0.012
12	-1	-1	1	-1	1	1	-1	1	1	1	-1	2.127	± 0.025	0.037	± 0.000

Table S5

Response of one-factor-at-a-time design.

Term	Level	max cell density	specific growth rate
		10 ⁶ cells/ml	h ⁻¹
Control		2.367 ± 0.074	0.035 ± 0.004
	1	2.767 ± 0.054	0.038 ± 0.005
E-amino acids 5	1.5	3.340 ± 0.054	0.038 ± 0.009
	2	2.707 ± 0.019	0.039 ± 0.007
H-Vitamins	1	2.683 ± 0.082	0.038 ± 0.004
	1.5	3.007 ± 0.071	0.037 ± 0.003
K-others	2	2.373 ± 0.012	0.035 ± 0.012
	1	2.967 ± 0.048	0.037 ± 0.012
K-others	1.5	3.117 ± 0.074	0.035 ± 0.007
	2	2.710 ± 0.078	0.037 ± 0.004

Table S6

Matrix and experimental and predicted responses of BBD

run	E	H	K	maximum cell density (10 ⁶ cells/ml)		specific growth rate (h ⁻¹)	
	amino acids 5	vitamins	others	Experimental	Predicted	Experimental	Predicted
1	0	-1	1	2.240 ± 0.062	2.30	0.035 ± 0.0011	0.036
2	1	-1	0	2.753 ± 0.082	2.65	0.038 ± 0.0038	0.037
3	0	1	1	2.530 ± 0.071	2.44	0.032 ± 0.0055	0.033
4	-1	1	0	2.310 ± 0.022	2.41	0.037 ± 0.0022	0.038
5	-1	0	-1	2.317 ± 0.054	2.28	0.038 ± 0.0008	0.037
6	-1	-1	0	2.090 ± 0.080	2.03	0.032 ± 0.0039	0.032
7	0	1	-1	2.480 ± 0.008	2.42	0.040 ± 0.0032	0.04
8	1	0	1	2.617 ± 0.021	2.65	0.037 ± 0.0034	0.037
9	0	0	0	2.680 ± 0.079	2.66	0.035 ± 0.0056	0.035
10	-1	0	1	2.253 ± 0.070	2.24	0.035 ± 0.0030	0.033
11	1	0	-1	2.587 ± 0.088	2.60	0.034 ± 0.0033	0.035
12	1	1	0	2.447 ± 0.071	2.51	0.037 ± 0.0014	0.035
13	0	-1	-1	2.223 ± 0.019	2.31	0.033 ± 0.0056	0.033
14	0	0	0	2.653 ± 0.042	2.66	0.035 ± 0.0022	0.035
15	0	0	0	2.640 ± 0.099	2.66	0.036 ± 0.0028	0.035