

Supplemental Information for

**Morphological phenotyping of organotropic brain- and bone-seeking triple negative
metastatic breast tumor cells**

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Supplemental Tables

Table S1: Results of D'Agostino-Pearson normality test (performed using Graphpad) for all figures from the main manuscript. Cell line clones are abbreviated P (MDA-MB-231 parental cells), BR (MDA-MB-231-BR brain-seeking cells), and BO (MDA-MB-231-BO bone-seeking cells). Table S1 spans the following two pages.

Figure 1B (diameter of suspended cells)

D'Agostino & Pearson test	P	BR	BO
K2	124.9	15.51	53.19
P value	<0.0001	0.0004	<0.0001
Passed normality test (alpha=0.05)?	No	No	No
P value summary	****	***	****

Figure 1C (area of suspended cells)

D'Agostino & Pearson test	P	BR	BO
K2	214.2	25.86	60.02
P value	<0.0001	<0.0001	<0.0001
Passed normality test (alpha=0.05)?	No	No	No
P value summary	****	****	****

Figure 2B (area)

	Passage 6				Passage 7				Passage 10				Passage 12		
D'Agostino & Pearson test	P	BR	BO	P	BR	BO	P	BR	BO	P	BR	BO	P	BR	BO
K2	198.8	42.64	71.53	119.9	171.7	138.4	82.46	69.45	99.01	111.5	73.53	107.9	111.5	73.53	107.9
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Passed normality test (alpha=0.05)?	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
P value summary	****	****	****	****	****	****	****	****	****	****	****	****	****	****	****

Figure 2C (inverse aspect ratio)

D'Agostino & Pearson test	P	BR	BO	P	BR	BO	P	BR	BO	P	BR	BO
K2	39.39	15.84	19.50	27.59	17.05	16.63	13.94	10.85	13.75	10.67	21.92	9.244
P value	<0.0001	0.0004	<0.0001	<0.0001	0.0002	0.0002	0.0009	0.0044	0.0010	0.0048	<0.0001	0.0098
Passed normality test (alpha=0.05)?	No	No	No	No	No	No	No	No	No	No	No	No
P value summary	****	***	****	****	***	***	***	**	**	**	****	**

Figure 2D (solidity)

D'Agostino & Pearson test	P	BR	BO	P	BR	BO	P	BR	BO	P	BR	BO
K2	21.95	43.33	17.65	5.958	46.00	19.31	15.64	39.15	12.97	9.003	19.73	58.17
P value	<0.0001	<0.0001	0.0001	0.0508	<0.0001	<0.0001	0.0004	<0.0001	0.0015	0.0111	<0.0001	<0.0001
Passed normality test (alpha=0.05)?	No	No	No	Yes	No	No	No	No	No	No	No	No
P value summary	****	****	***	ns	****	****	***	****	**	*	****	****

Figure 2E (circularity)

D'Agostino & Pearson test	P	BR	BO	P	BR	BO	P	BR	BO	P	BR	BO
K2	12.46	2.078	18.74	2.030	10.71	16.67	1.319	2.001	9.496	4.612	4.937	2.703
P value	0.0020	0.3538	<0.0001	0.3624	0.0047	0.0002	0.5170	0.3677	0.0087	0.0996	0.0847	0.2589
Passed normality test (alpha=0.05)?	No	Yes	No	Yes	No	No	Yes	Yes	No	Yes	Yes	Yes
P value summary	**	ns	****	ns	**	***	ns	ns	**	ns	ns	ns

Figure 3B (2D migration speed)

	Collagen I			Fibronectin			Poly – D – Lysine		
	P	BR	BO	P	BR	BO	P	BR	BO
D'Agostino & Pearson test									
K2	4.153	15.75	10.92	18.24	0.9394	13.72	18.55	8.190	5.951
P value	0.1254	0.0004	0.0043	0.0001	0.6252	0.0010	<0.0001	0.0167	0.0510
Passed normality test (alpha=0.05)?									
P value summary	ns	***	**	***	ns	**	****	*	ns

Figure 4B-D (chemotactic index in microchannels)

	P					BR					BO				
Channel Width (µm)	3	6	10	20	50	3	6	10	20	50	3	6	10	20	50
D'Agostino & Pearson test															
K2	4.760	14.86	17.16	44.00	22.54	4.801	44.95	81.83	69.46	24.86	3.721	48.26	62.83	144.4	29.70
P value	0.0925	0.0006	0.0002	<0.0001	<0.0001	0.0907	<0.0001	<0.0001	<0.0001	<0.0001	0.1556	<0.0001	<0.0001	<0.0001	<0.0001
Passed normality test (alpha=0.05)?	Yes	No	No	No	No	Yes	No	No	No	No	Yes	No	No	No	No
P value summary	ns	***	***	****	****	ns	****	****	****	****	ns	****	****	****	****

Figure 4E G (migration speed in microchannels)

Figure 4E-G (migration speed in microchannels)

	P					BR					BO				
Channel Width (µm)	3	6	10	20	50	3	6	10	20	50	3	6	10	20	50
D'Agostino & Pearson test															
K2	0.9259	70.51	6.088	22.40	1.408	9.826	21.22	4.760	9.933	240.2	2.904	57.88	7.280	95.57	26.76
P value	0.6294	<0.0001	0.0476	<0.0001	0.4945	0.0074	<0.0001	0.0925	0.0070	<0.0001	0.2341	<0.0001	0.0263	<0.0001	<0.0001
Passed normality test (alpha=0.05)?	Yes	No	No	No	Yes	No	No	Yes	No	No	Yes	No	No	No	No
P value summary	ns	****	*	****	ns	**	****	ns	**	****	ns	****	*	****	****

Figure 5B (Young’s modulus)

	Glass			194 kPa			1 kPa		
	P	BR	BO	P	BR	BO	P	BR	BO
D'Agostino & Pearson test									
K2	5.679	4.957	0.4733	14.20	41.94	7.013	50.18	12.10	10.19
P value	0.0585	0.0839	0.7893	0.0008	<0.0001	0.0300	<0.0001	0.0024	0.0061
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	No	No	No	No	No	No
P value summary	ns	ns	ns	***	****	*	****	**	**

Figure 5C (area)

	Glass			194 kPa			1 kPa		
	P	BR	BO	P	BR	BO	P	BR	BO
D'Agostino & Pearson test									
K2	513.1	371.5	1041	207.6	228.1	94.42	178.0	303.5	143.5
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Passed normality test (alpha=0.05)?	No	No	No	No	No	No	No	No	No
P value summary	****	****	****	****	****	****	****	****	****

Figure 5D (inverse aspect ratio)

	P	BR	BO	P	BR	BO	P	BR	BO
D'Agostino & Pearson test									
K2									
P value	102.3	124.4	204.1	5800	4763	269.3	102.1	83.87	118.7
Passed normality test (alpha=0.05)?	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
P value summary	No	No	No	No	No	No	No	No	No
	****	****	****	****	****	****	****	****	****

Figure 5E (circularity)

	P	BR	BO	P	BR	BO	P	BR	BO
D'Agostino & Pearson test									
K2	22.55	34.82	176.3	1189	4346	81.54	157.2	125.4	3261
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Passed normality test (alpha=0.05)?	No	No	No	No	No	No	No	No	No
P value summary	****	****	****	****	****	****	****	****	****

Figure 5F (solidity)

	P	BR	BO	P	BR	BO	P	BR	BO
D'Agostino & Pearson test									
K2	370.8	362.1	46.23	158.3	92.04	23.52	384.3	440.3	59.37
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Passed normality test (alpha=0.05)?	No	No	No	No	No	No	No	No	No
P value summary	****	****	****	****	****	****	****	****	****

Figure 6C (focal adhesion parameters)

PY-paxillin – area

	P	BR	BO
D'Agostino & Pearson test			
K2	107.5	242.0	393.3
P value	<0.0001	<0.0001	<0.0001
Passed normality test (alpha=0.05)?	No	No	No
P value summary	****	****	****

of PY-paxillin per cell

	P	BR	BO
D'Agostino & Pearson test			
K2	9.006	23.03	45.42
P value	0.0111	<0.0001	<0.0001
Passed normality test (alpha=0.05)?	No	No	No
P value summary	*	****	****

PY-paxillin density

	P	BR	BO
D'Agostino & Pearson test			
K2	3.324	7.856	12.31
P value	0.1898	0.0197	0.0021
Passed normality test (alpha=0.05)?	Yes	No	No
P value summary	ns	*	**

Phospho-FAK - area

	P	BR	BO
D'Agostino & Pearson test			
K2	333.0	257.2	292.3
P value	<0.0001	<0.0001	<0.0001
Passed normality test (alpha=0.05)?	No	No	No
P value summary	****	****	****

of Phospho-FAK per cell

	P	BR	BO
D'Agostino & Pearson test			
K2	4.865	9.194	14.76
P value	0.0878	0.0101	0.0006
Passed normality test (alpha=0.05)?	Yes	No	No
P value summary	ns	*	***

Phospho-FAK density

	P	BR	BO
D'Agostino & Pearson test			
K2	6.255	43.20	17.74
P value	0.0438	<0.0001	0.0001
Passed normality test (alpha=0.05)?	No	No	No
P value summary	*	****	***

Table S2: Statistical comparisons for morphological parameters of MDA-P, MDA-BR, and MDA-BO cells on collagen I-coated polyacrylamide gels of varying stiffness (corresponds to Figure 5). * indicates $P < 0.05$. ** indicates $P < 0.01$. *** indicates $P < 0.001$. **** indicates $P < 0.0001$.

Area							
Comparisons	Glass	194 kPa	1 kPa	Comparisons	MDA-P	MDA-BR	MDA-BO
P vs BR	**	**	ns	Glass vs 194 kPa	****	****	****
P vs BO	****	****	****	Glass vs 1 kPa	****	****	****
BR vs BO	****	****	****	194 kPa vs 1 kPa	****	****	****

Inverse Aspect Ratio							
Comparisons	Glass	194 kPa	1 kPa	Comparisons	MDA-P	MDA-BR	MDA-BO
P vs BR	**	ns	***	Glass vs 194 kPa	****	****	ns
P vs BO	**	ns	****	Glass vs 1 kPa	****	****	****
BR vs BO	****	ns	**	194 kPa vs 1 kPa	****	****	****

Solidity							
Comparisons	Glass	194 kPa	1 kPa	Comparisons	MDA-P	MDA-BR	MDA-BO
P vs BR	**	*	****	Glass vs 194 kPa	**	ns	****
P vs BO	****	****	****	Glass vs 1 kPa	****	****	****
BR vs BO	****	****	****	194 kPa vs 1 kPa	****	****	****

Circularity							
Comparisons	Glass	194 kPa	1 kPa	Comparisons	MDA-P	MDA-BR	MDA-BO
P vs BR	**	ns	**	Glass vs 194 kPa	***	***	**
P vs BO	****	****	****	Glass vs 1 kPa	****	****	****
BR vs BO	****	****	****	194 kPa vs 1 kPa	****	****	****

Cell line authentication

Upon arriving in our lab, the cells obtained from Dr. Toshiyuki Yoneda in Osaka, Japan were checked for authenticity using STR testing via Laragen, Inc. Results from the cell line authentication for the three cell lines, as provided by Laragen, Inc., are included on the following pages.

Sample 1 = MDA-MB-231 parental (MDA-P)

Sample 2 = MDA-MB-231 brain-seeking (MDA-BR)

Sample 3 = MDA-MB-231 bone-seeking (MDA-BO)

Report Date: May 9th, 2018

Celient: Marina Shumakovich
Institution: U of Maryland
Phone: 301-314-1813
Address: 8278 Paint Branch Dr. 3107A James Clark Hall

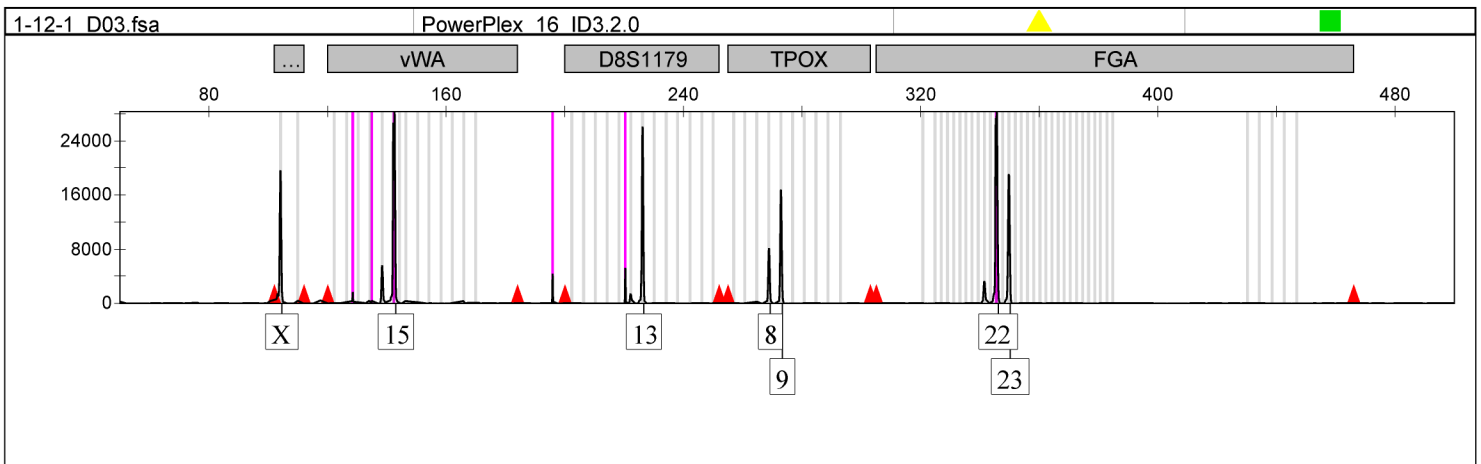
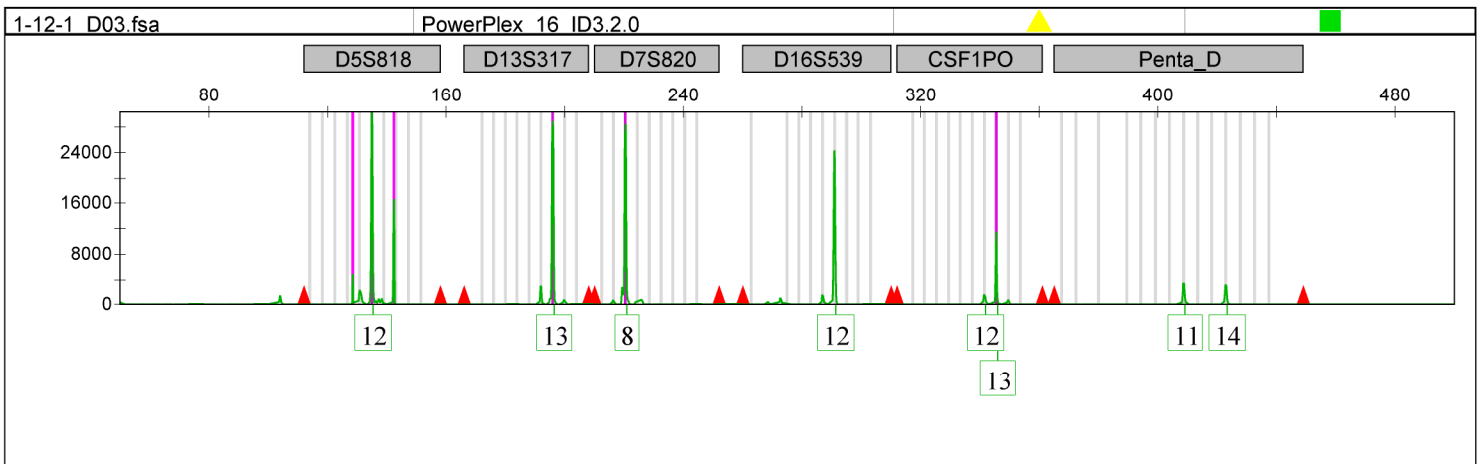
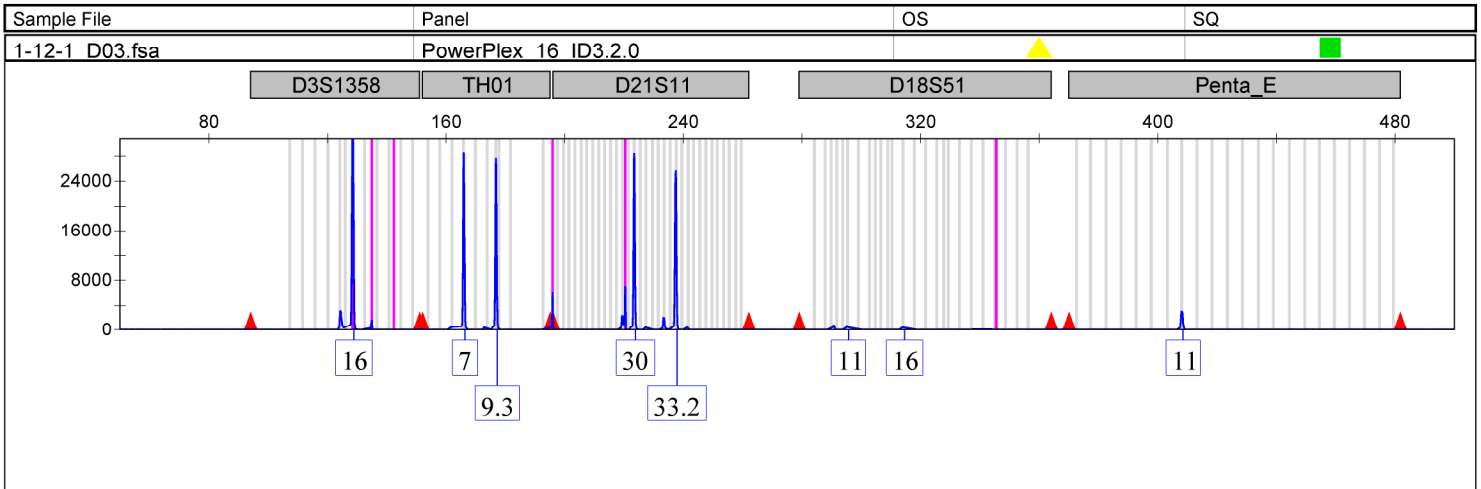
PI: Dr. Stroka
PO: CC
Email: mshumako@terpmail.umd.edu

Summary of the Report: Authentication for all cell lines used in this study was performed using the Promega powerplex16 System recommended by American Type Culture Collection. The STR alleles were searched either on ATTC or DSMZ databases depending on availability of the cell lines in the databases.

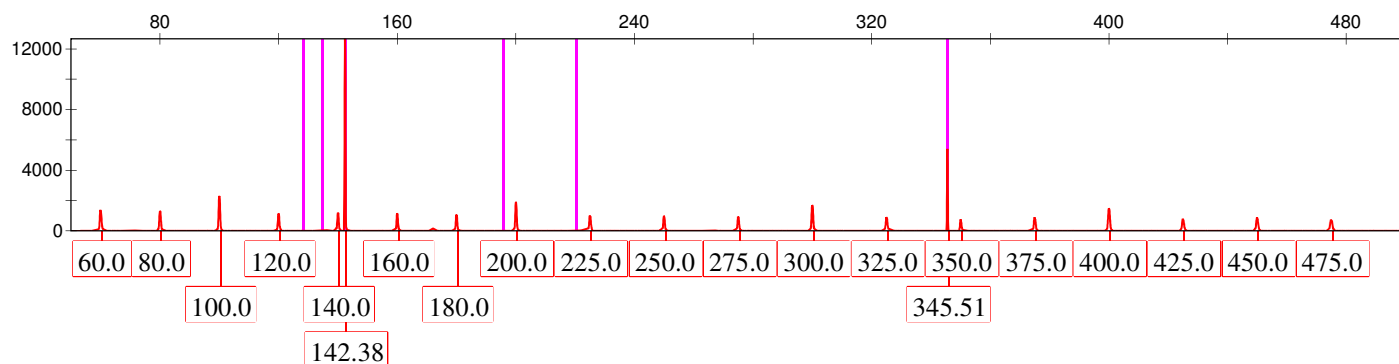
Sample ID: 1

Sample DSMZ#: HTB-26
DSMZ Database Best Match Score: 0.89
DSMZ Database Best Match Cell Line: MDA-MB-231

Marker	Dye	Allele 1	Allele 2	Allele 3	DSMZ
D5S818	G	12			12,12
D13S317	G	13			13,13
D7S820	G	8			8,9
D16S539	G	12			12,12
vWA	Y	15			15,18
TH01	B	7	9.3		7,9.3
AMEL	Y	X			X,X
TPOX	Y	8	9		8,9
CSF1PO	G	12	13		12,13
D18S51	B	11	16		
D21S11	B	30	33.2		
D3S1358	B	16			
D8S1179	Y	13			
FGA	Y	22	23		
Penta_D	G	11	14		
Penta_E	B	11			



Sample File	Panel	OS	SQ
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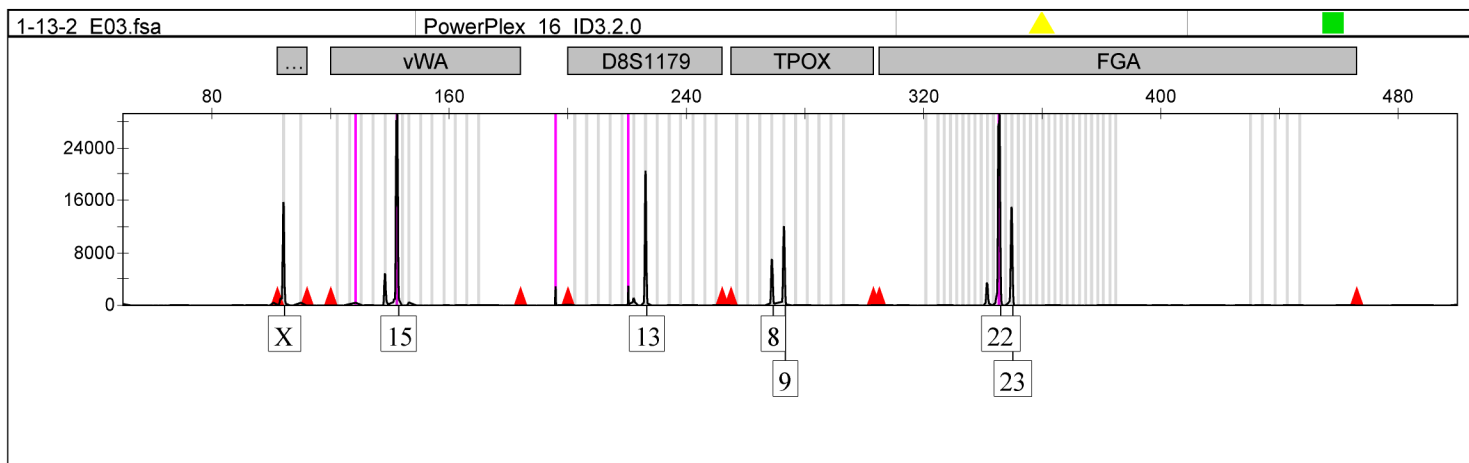
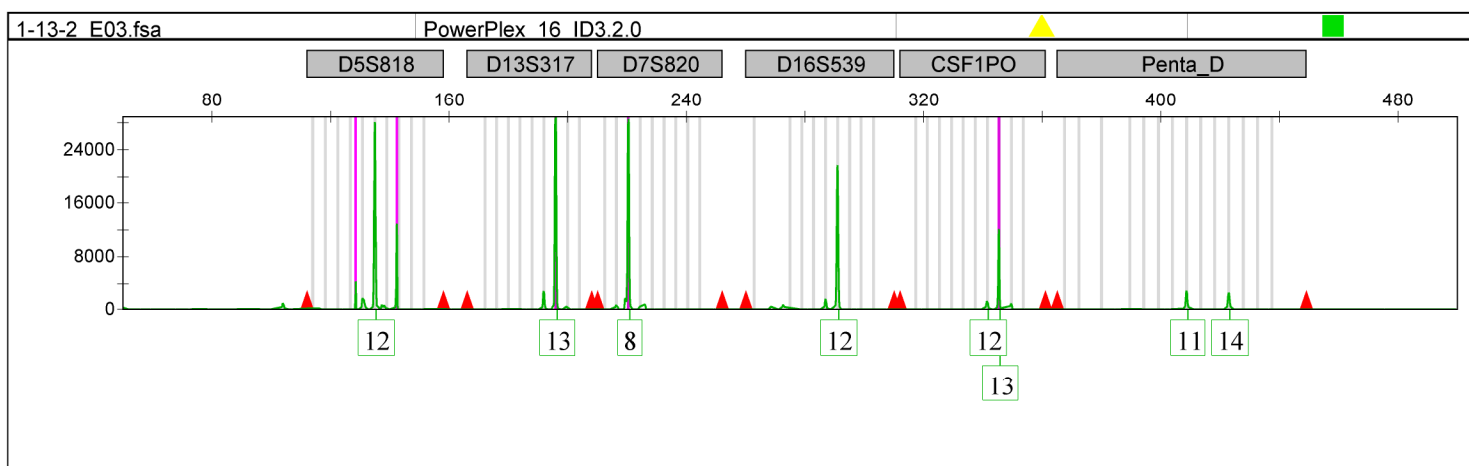
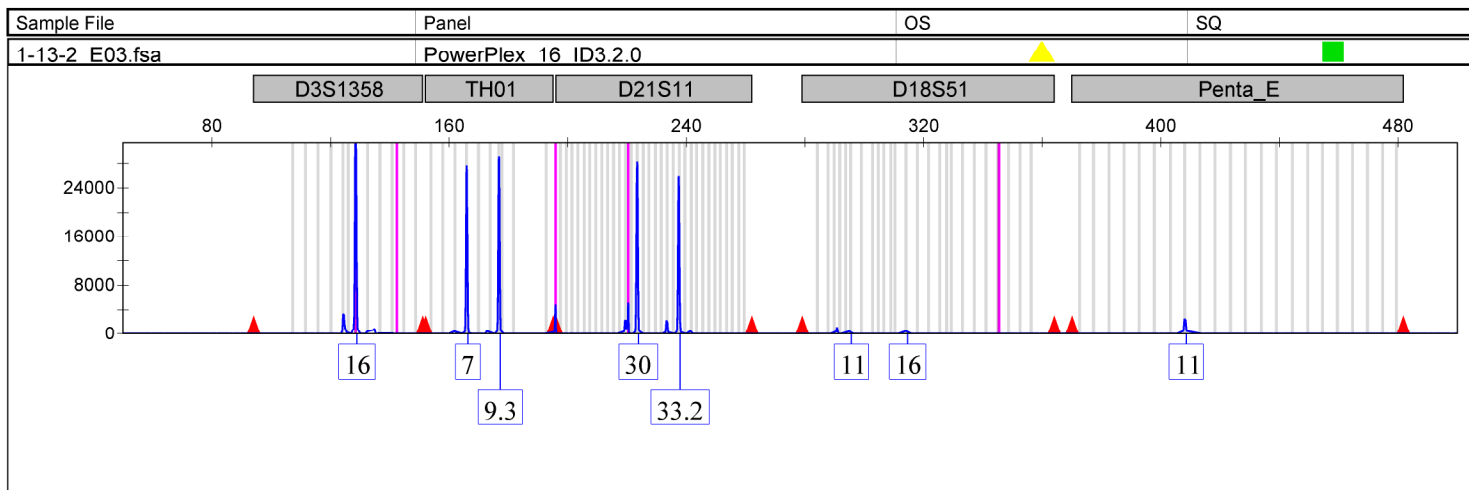
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Sample DSMZ#: HTB-26

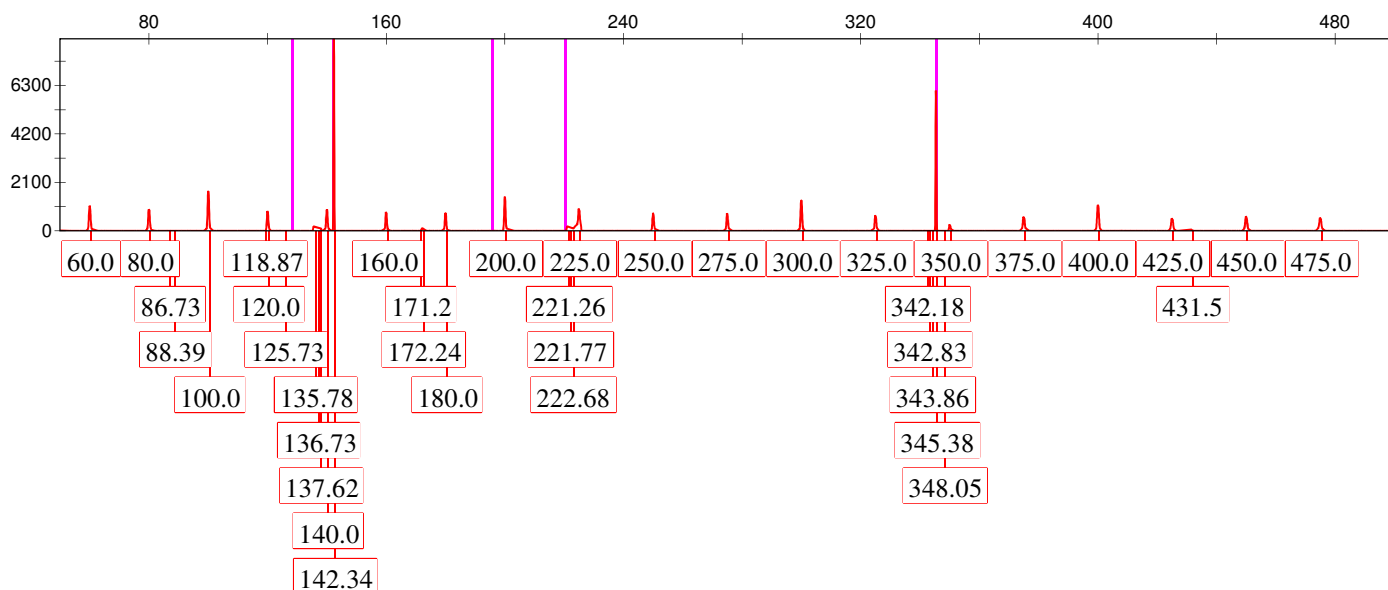
DSMZ Database Best Match Score: 0.89

DSMZ Database Best Match Cell Line: MDA-MB-231

Marker	Dye	Allele 1	Allele 2	Allele 3	DSMZ
D5S818	G	12			12,12
D13S317	G	13			13,13
D7S820	G	8			8,9
D16S539	G	12			12,12
vWA	Y	15			15,18
TH01	B	7	9.3		7,9.3
AMEL	Y	X			X,X
TPOX	Y	8	9		8,9
CSF1PO	G	12	13		12,13
D18S51	B	11	16		
D21S11	B	30	33.2		
D3S1358	B	16			
D8S1179	Y	13			
FGA	Y	22	23		
Penta_D	G	11	14		
Penta_E	B	11			



Sample File	Panel	OS	SQ
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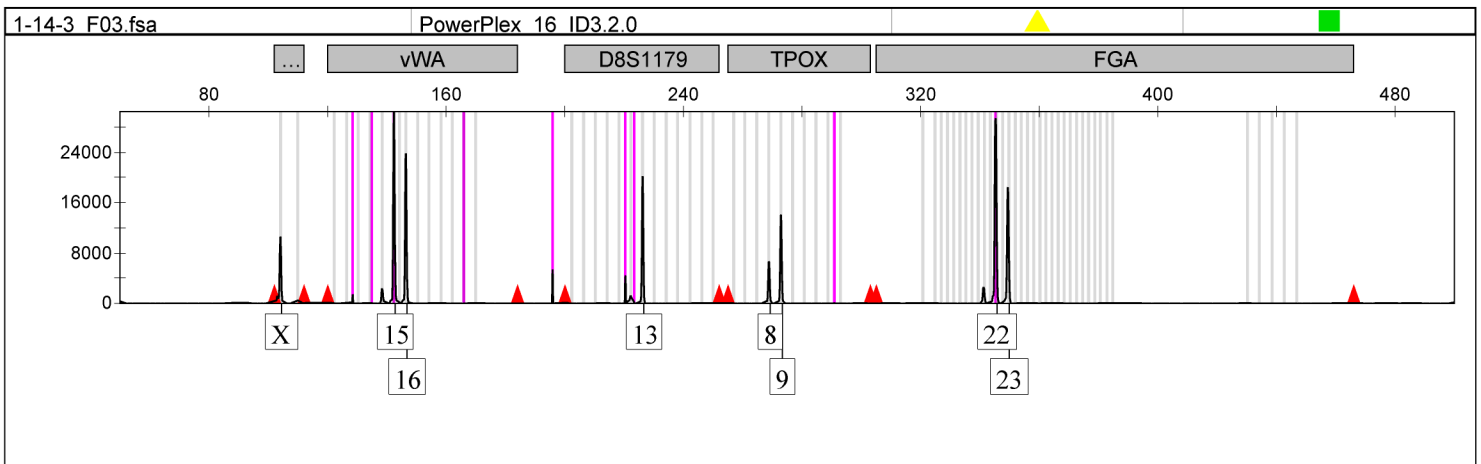
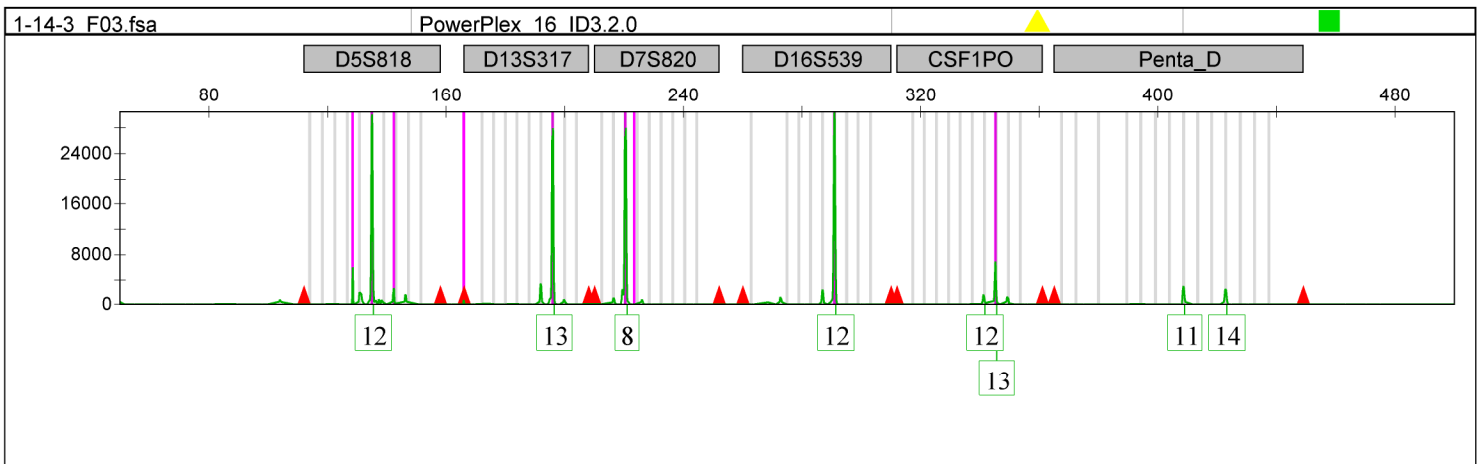
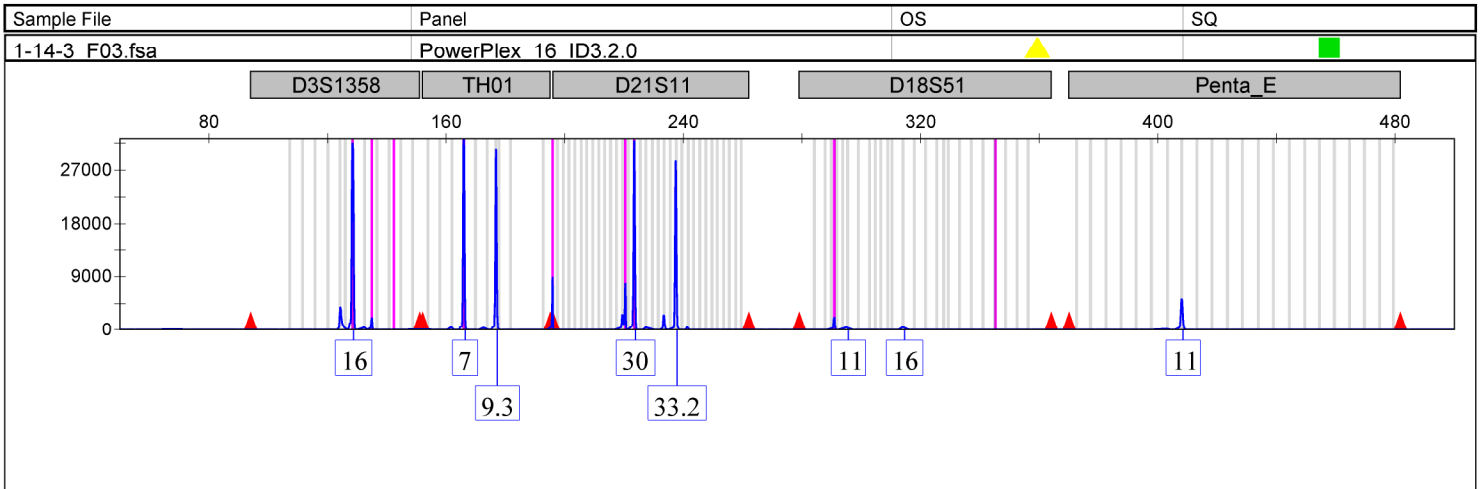
Sample ID: 3

Sample DSMZ#: HTB-26

DSMZ Database Best Match Score: 0.89

DSMZ Database Best Match Cell Line: MDA-MB-231

Marker	Dye	Allele 1	Allele 2	Allele 3	DSMZ
D5S818	G	12			12,12
D13S317	G	13			13,13
D7S820	G	8			8,9
D16S539	G	12			12,12
vWA	Y	15	16		15,18
TH01	B	7	9.3		7,9.3
AMEL	Y	X			X,X
TPOX	Y	8	9		8,9
CSF1PO	G	12	13		12,13
D18S51	B	11	16		
D21S11	B	30	33.2		
D3S1358	B	16			
D8S1179	Y	13			
FGA	Y	22	23		
Penta_D	G	11	14		
Penta_E	B	11			



Sample File	Panel	OS	SQ
1-14-3 F03.fsa	PowerPlex 16 ID3.2.0		

