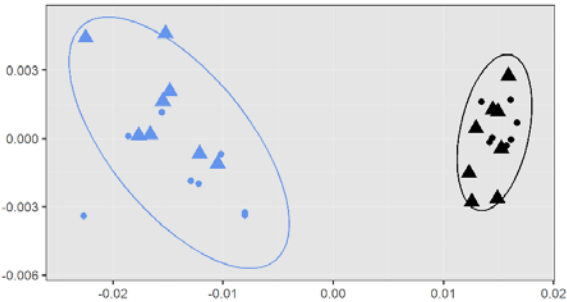


Fig. S1

Primer Utilizing Profiles



Temp
● 45
▲ 55

Method
● DePCR
▲ TAS

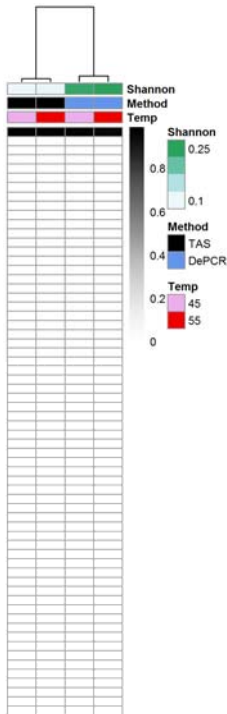
ANOSIM

DePCR vs TAS:
R=1, P=0.0001

TAS45 vs TAS55:
R=0.12; p=0.0012

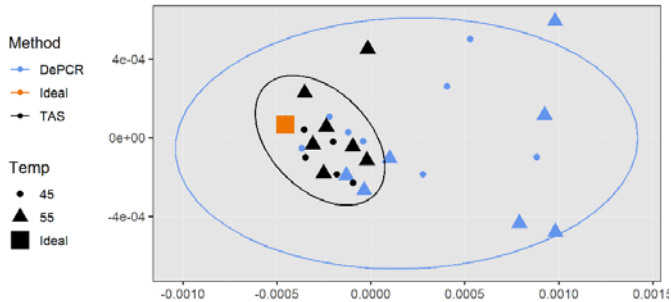
DePCR45 vs DePCR55:
R=0.099, P=0.109

Experiment: A1
Primers: 1
Templates: 1



Comparison	DePCR45	DePCR55	ANOVA
% Reads 0 MM	96.07	95.73	0.07
% Reads 1 MM	3.68	3.99	0.06
% Reads 2 MM	0.25	0.27	0.62
% Reads 3MM	0.00	0.01	0.28
% Reads with 3' MM	26.81	25.29	0.23
% Reads with Mid' MM	30.87	34.19	0.01
% Reads with 5' MM	42.32	40.51	0.17
Average Primer Tm	57.42	57.43	0.02
Average Shannon	0.26	0.27	0.41

Template Profiles



Method
● DePCR
▲ Ideal
● TAS

Temp
● 45
▲ 55
■ Ideal

ANOSIM

DePCR vs TAS:
R=0.201, P=0.0003

TAS45 vs TAS55:
R=-0.049; p=0.762

DePCR45 vs DePCR55:
R=-0.015, P=0.478

	Comparison	Average Ideal Score	ANOVA
1	TAS	0.06	0.00
	DePCR	0.17	
2	TAS45	0.05	0.39
	TAS55	0.06	
3	DePCR45	0.15	0.38
	DePCR55	0.20	

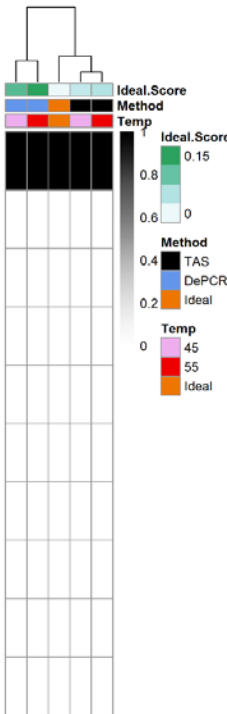
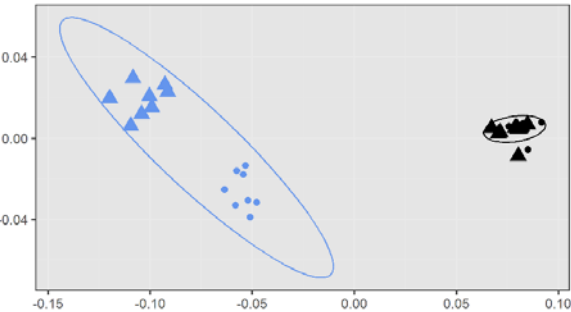


Fig. S2

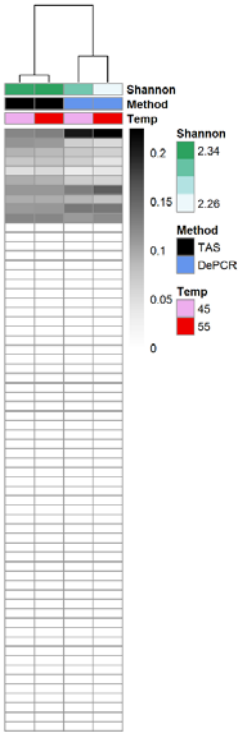
Primer Utilizing Profiles

Experiment: A2
Primers: 10
Templates: 1

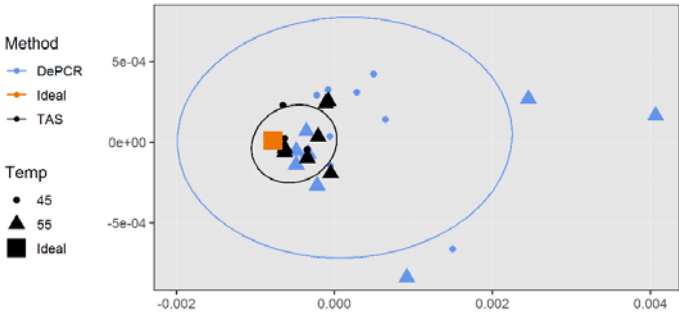


ANOSIM
DePCR vs TAS:
 $R=1, P=0.0001$
TAS45 vs TAS55:
 $R=0.425; p=0.0009$
DePCR45 vs DePCR55:
 $R=1, P=0.0002$

Comparison	DePCR45	DePCR55	ANOVA
% Reads 0 MM	20.51	22.40	0.00
% Reads 1 MM	77.21	75.57	0.00
% Reads 2 MM	2.21	1.99	0.03
% Reads 3MM	0.06	0.04	0.05
% Reads with 3' MM	22.46	19.63	0.00
% Reads with Mid' MM	34.31	36.69	0.00
% Reads with 5' MM	43.23	43.68	0.28
Average Primer Tm	57.79	57.84	0.00
Average Shannon	2.31	2.26	0.00



Template Profiles



ANOSIM
DePCR vs TAS:
 $R=0.169, P=0.0003$
TAS45 vs TAS55:
 $R=0.183; p=.031$
DePCR45 vs DePCR55:
 $R=0.063, P=0.00063$

Comparison	Average Ideal Score	ANOVA
1 TAS	0.06	0.00
DePCR	0.26	
2 TAS45	0.04	0.04
TAS55	0.09	
3 DePCR45	0.22	0.53
DePCR55	0.30	

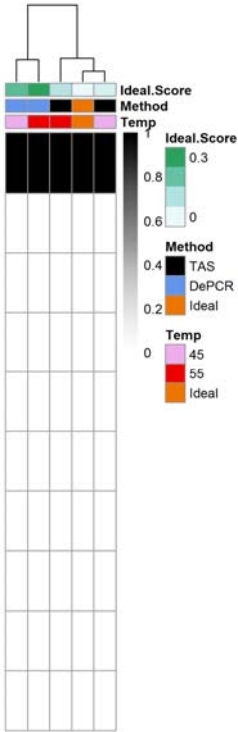
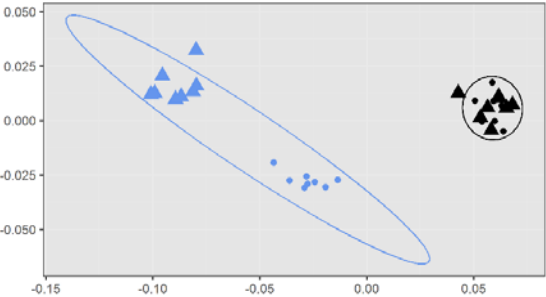


Fig. S3

Primer Utilizing Profiles



Temp
● 45
▲ 55

Method
● DePCR
▲ TAS

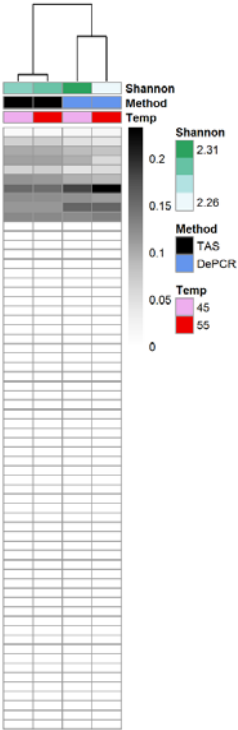
ANOSIM

DePCR vs TAS:
 $R=0.968$, $P=0.0001$

TAS45 vs TAS55:
 $R=0.207$; $p=0.02$

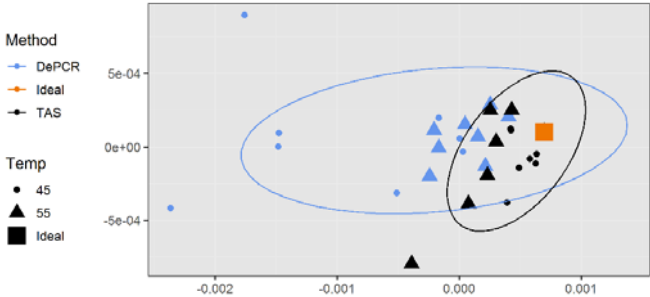
DePCR45 vs DePCR55:
 $R=1$, $P=0.0002$

Experiment: A3
Primers: 9
Templates: 1



Comparison	DePCR45	DePCR55	ANOVA
% Reads 0 MM	1.39	1.34	0.50
% Reads 1 MM	95.79	95.93	0.28
% Reads 2 MM	2.76	2.66	0.43
% Reads 3MM	0.06	0.07	0.59
% Reads with 3' MM	24.06	20.38	0.00
% Reads with Mid' MM	33.92	37.58	0.00
% Reads with 5' MM	42.03	42.04	0.98
Average Primer Tm	57.81	57.95	0.00
Average Shannon	2.31	2.25	0.00

Template Profiles



Method
● DePCR
● Ideal
● TAS

Temp
● 45
▲ 55
■ Ideal

ANOSIM

DePCR vs TAS:
 $R=0.25$, $P=0.0001$

TAS45 vs TAS55:
 $R=0.064$; $p=0.137$

DePCR45 vs DePCR55:
 $R=0.26$, $P=0.006$

Comparison	Average Ideal Score	ANOVA
1 TAS	0.08	0.00
DePCR	0.25	
2 TAS45	0.05	0.17
TAS55	0.10	
3 DePCR45	0.36	0.00
DePCR55	0.14	

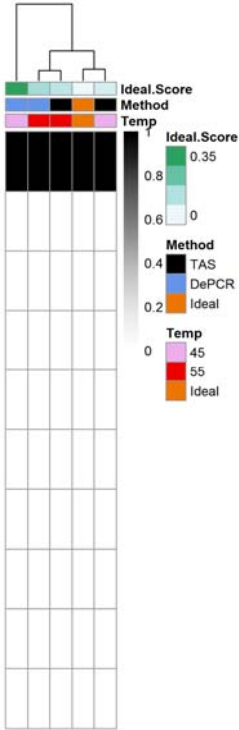
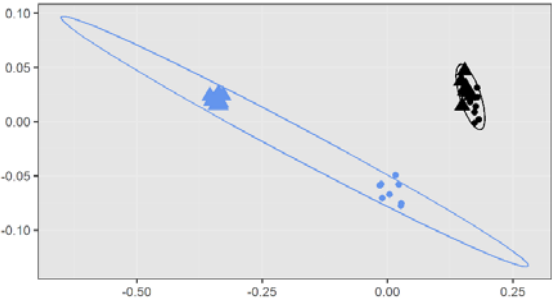


Fig. S4

Primer Utilizing Profiles



ANOSIM

DePCR vs TAS:
R=0.733, P=0.0001

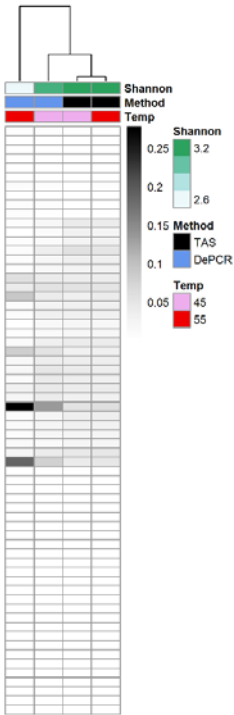
TAS45 vs TAS55:
R=0.894; p=0.0002

DePCR45 vs DePCR55:
R=1, P=0.0002

Temp
● 45
▲ 55

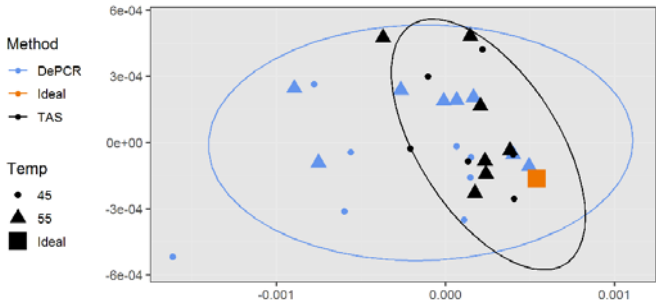
Method
● DePCR
● TAS

Comparison	DePCR45	DePCR55	ANOVA
% Reads 0 MM	0.48	0.39	0.12
% Reads 1 MM	2.07	2.45	0.01
% Reads 2 MM	96.12	95.82	0.04
% Reads 3MM	1.34	1.34	0.95
% Reads with 3' MM	25.84	17.10	0.00
% Reads with Mid' MM	37.01	44.30	0.00
% Reads with 5' MM	37.15	38.59	0.00
Average Primer Tm	58.56	58.97	0.00
Average Shannon	3.25	2.58	0.00



Experiment: A4
Primers: 27
Templates: 1

Template Profiles



ANOSIM

DePCR vs TAS:
R=0.108, P=0.013

TAS45 vs TAS55:
R=-0.048; p=0.71

DePCR45 vs DePCR55:
R=-0.01, P=0.469

Comparison	Average Ideal Score	ANOVA
1 TAS	0.10	0.02
DePCR	0.18	
2 TAS45	0.09	0.71
TAS55	0.11	
3 DePCR45	0.21	0.37
DePCR55	0.16	

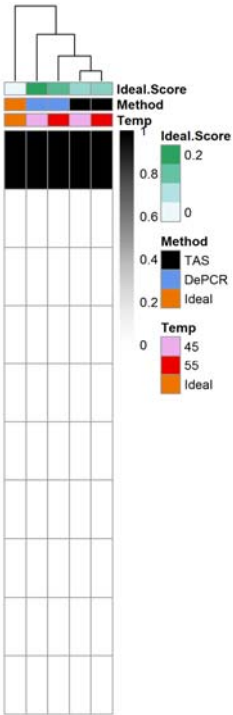
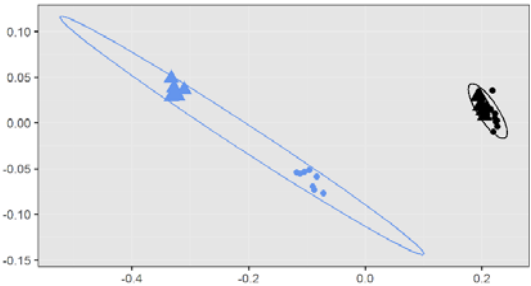


Fig. S5

Primer Utilizing Profiles



ANOSIM

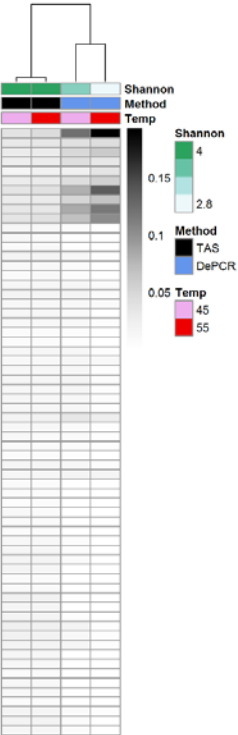
DePCR vs TAS:
R=1, P=0.0001

TAS45 vs TAS55:
R=1; p=0.0002

DePCR45 vs DePCR55:
R=1, P=0.0002

Temp
● 45
▲ 55

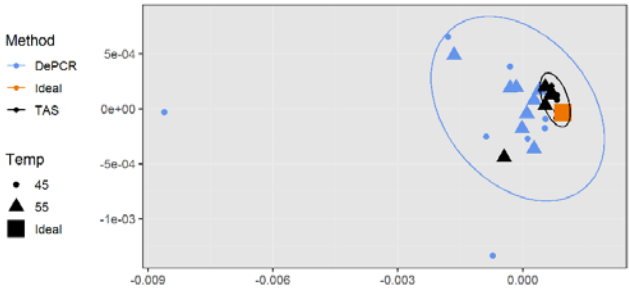
Method
◆ DePCR
◆ TAS



Comparison	DePCR45	DePCR55	ANOVA
% Reads 0 MM	12.16	19.31	0.00
% Reads 1 MM	47.68	65.16	0.00
% Reads 2 MM	29.86	12.81	0.00
% Reads 3MM	10.31	2.72	0.00
% Reads with 3' MM	27.41	21.24	0.00
% Reads with Mid' MM	34.60	37.10	0.00
% Reads with 5' MM	37.99	41.66	0.00
Average Primer Tm	58.18	57.99	0.00
Average Shannon	3.43	2.76	0.00

Experiment: A6
Primers: 64
Templates: 1

Template Profiles

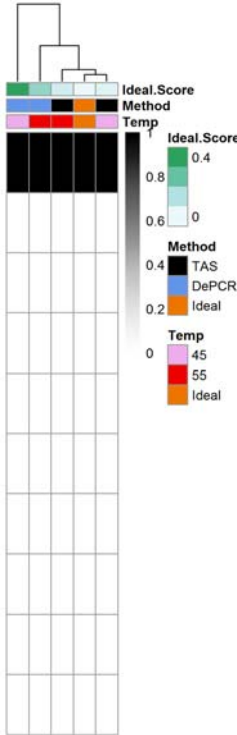


ANOSIM

DePCR vs TAS:
R=0.168, P=0.0002

TAS45 vs TAS55:
R=0.074; p=0.109

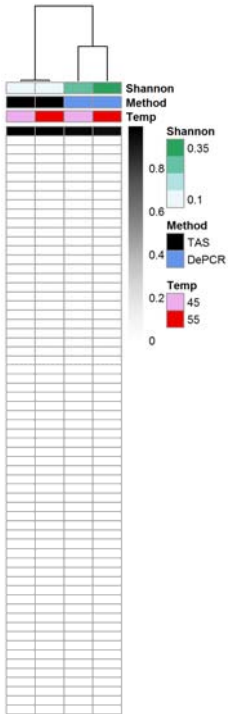
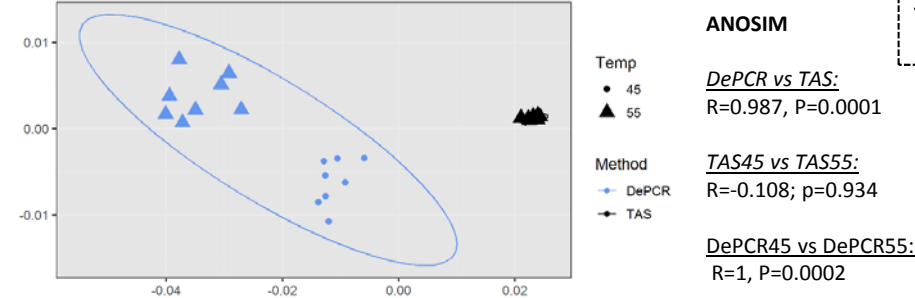
DePCR45 vs DePCR55:
R=-0.007, P=0.469



	Comparison	Average Ideal Score	ANOVA
1	TAS	0.05	0.01
	DePCR	0.36	
2	TAS45	0.04	0.31
	TAS55	0.07	
3	DePCR45	0.49	0.25
	DePCR55	0.22	

Fig. S6

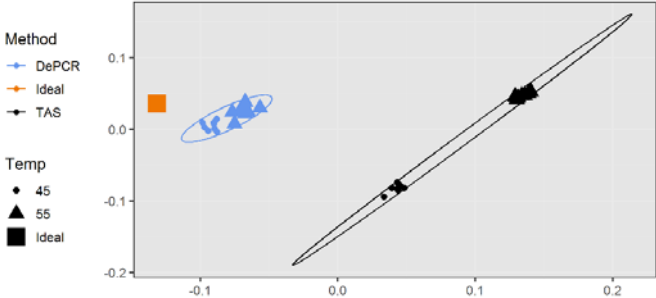
Primer Utilizing Profiles



Comparison	DePCR45	DePCR55	ANOVA
% Reads 0 MM	14.27	16.77	0.00
% Reads 1 MM	84.21	81.72	0.00
% Reads 2 MM	1.50	1.48	0.79
% Reads 3MM	0.02	0.03	0.36
% Reads with 3' MM	22.00	19.63	0.00
% Reads with Mid' MM	36.82	39.84	0.00
% Reads with 5' MM	41.18	40.52	0.02
Average Primer Tm	57.42	57.43	0.01
Average Shannon	0.27	0.36	0.00

Experiment: B1
Primers: 1
Templates: 10

Template Profiles



ANOSIM
DePCR vs TAS:
 $R=0.878$, $P=0.0001$
TAS45 vs TAS55:
 $R=1$; $p=0.0002$
DePCR45 vs DePCR55:
 $R=0.909$, $P=0.0002$

Comparison	Average Ideal Score	ANOVA
1 TAS	50.80	0.00
DePCR	22.82	
2 TAS45	45.63	0.00
TAS55	55.97	
3 DePCR45	22.42	0.12
DePCR55	23.23	

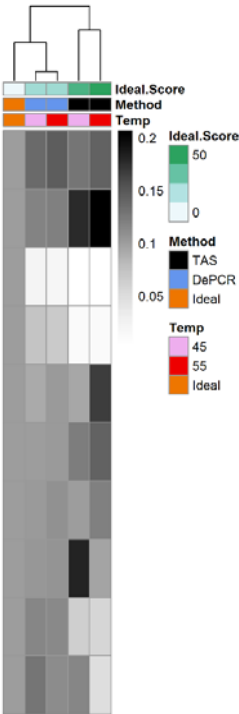
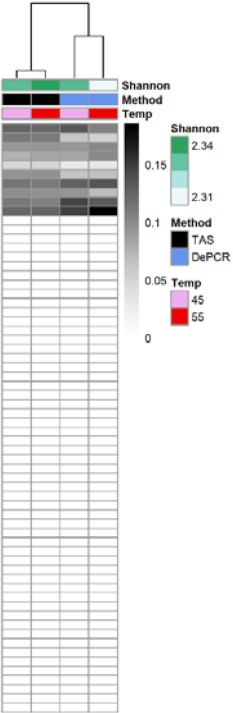
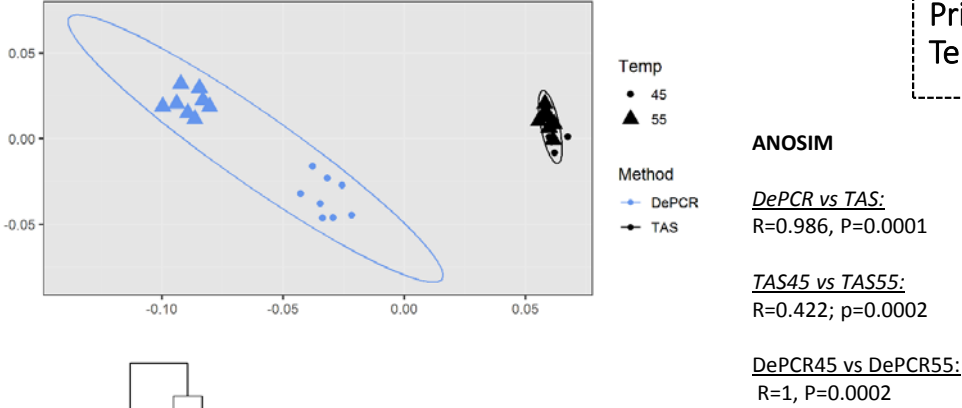


Fig. S7

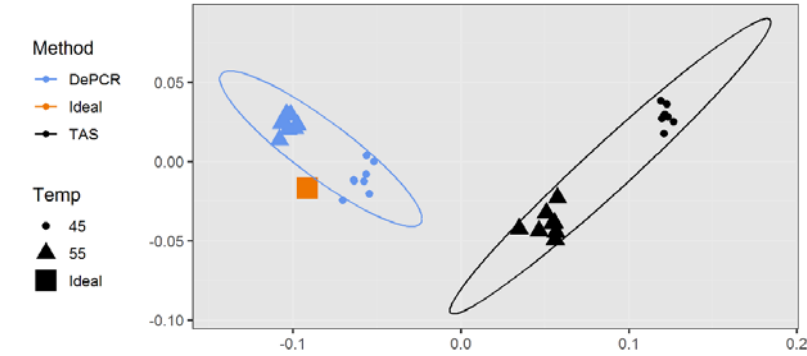
Primer Utilizing Profiles

Experiment: B2
Primers: 10
Templates: 10



Comparison	DePCR45	DePCR55	ANOVA
% Reads 0 MM	28.98	48.05	0.00
% Reads 1 MM	46.54	43.24	0.00
% Reads 2 MM	24.26	8.63	0.00
% Reads 3MM	0.22	0.08	0.00
% Reads with 3' MM	23.28	25.47	0.00
% Reads with Mid' MM	34.68	35.29	0.10
% Reads with 5' MM	42.04	39.24	0.00
Average Primer Tm	57.78	57.87	0.00
Average Shannon	2.33	2.31	0.00

Template Profiles



ANOSIM

DePCR vs TAS:
R=0.993, P=0.0001

TAS45 vs TAS55:
R=1; p=0.0002

DePCR45 vs DePCR55:
R=1, P=0.0002

	Comparison	Average Ideal Score	ANOVA
1	TAS	38.60	0.00
	DePCR	16.55	
2	TAS45	45.48	0.00
	TAS55	31.73	
3	DePCR45	18.00	0.00
	DePCR55	15.10	

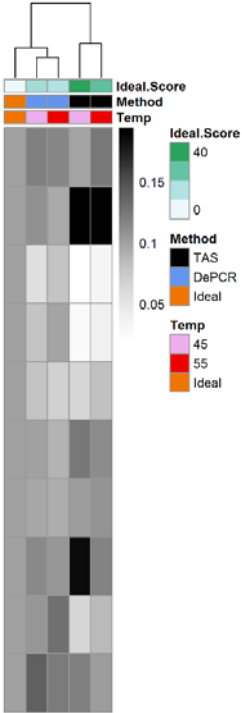


Fig. S8

Primer Utilizing Profiles

Experiment: B3
Primers: 9
Templates: 10

ANOSIM

DePCR vs TAS:
R=1, P=0.0001

TAS45 vs TAS55:
R=-0.051; p=0.671

DePCR45 vs DePCR55:
R=1, P=0.0002

Temp

● 45
▲ 55

Method

● DePCR
▲ TAS

Comparison	DePCR45	DePCR55	ANOVA
% Reads 0 MM	29.10	49.31	0.00
% Reads 1 MM	42.17	40.86	0.00
% Reads 2 MM	28.46	9.73	0.00
% Reads 3MM	0.27	0.10	0.00
% Reads with 3' MM	23.19	24.82	0.00
% Reads with Mid' MM	35.92	36.26	0.40
% Reads with 5' MM	40.89	38.91	0.00
Average Primer Tm	57.77	57.91	0.00
Average Shannon	2.26	2.23	0.00

Template Profiles

Method

● DePCR
● Ideal
● TAS

Temp

● 45
▲ 55
■ Ideal

ANOSIM

DePCR vs TAS:
R=0.916, P=0.0001

TAS45 vs TAS55:
R=1; p=0.0002

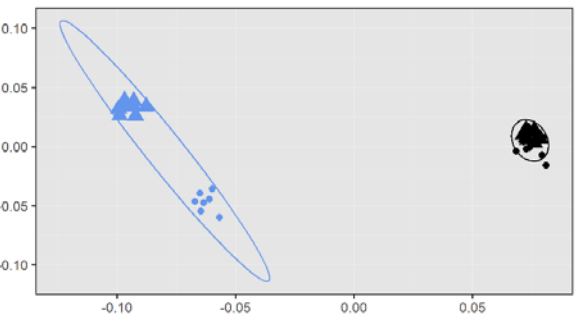
DePCR45 vs DePCR55:
R=1, P=0.0002

	Comparison	Average Ideal Score	ANOVA
1	TAS	38.38	0.00
	DePCR	16.69	
2	TAS45	45.24	0.00
	TAS55	31.52	
3	DePCR45	19.30	0.00
	DePCR55	14.41	

Fig. S9

Primer Utilizing Profiles

Experiment: B4
Primers: 27
Templates: 10



ANOSIM

DePCR vs TAS:
R=1, P=0.0001

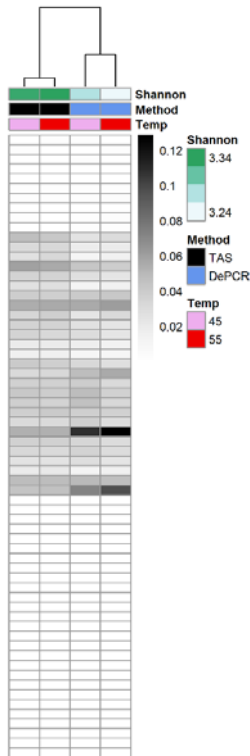
TAS45 vs TAS55:
R=0.47; p=0.0002

DePCR45 vs DePCR55:
R=1, P=0.0002

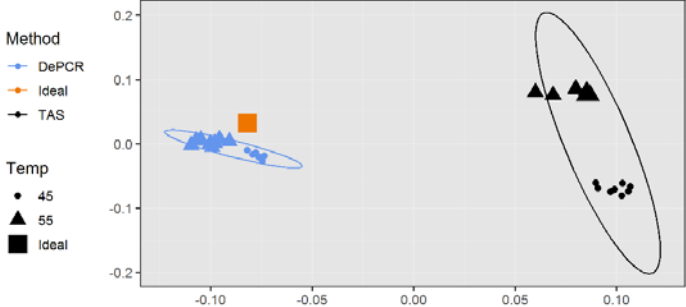
Temp
● 45
▲ 55

Method
● DePCR
▲ TAS

Comparison	DePCR45	DePCR55	ANOVA
% Reads 0 MM	0.56	0.76	0.00
% Reads 1 MM	49.53	74.13	0.00
% Reads 2 MM	45.13	22.37	0.00
% Reads 3MM	4.77	2.75	0.00
% Reads with 3' MM	26.39	22.68	0.00
% Reads with Mid' MM	36.84	40.90	0.00
% Reads with 5' MM	36.77	36.42	0.07
Average Primer Tm	58.59	58.76	0.00
Average Shannon	3.27	3.22	0.00



Template Profiles



ANOSIM

DePCR vs TAS:
R=1, P=0.0001

TAS45 vs TAS55:
R=1; p=0.0002

DePCR45 vs DePCR55:
R=1, P=0.0002

	Comparison	Average Ideal Score	ANOVA
1	TAS	38.13	0.00
	DePCR	11.53	
2	TAS45	42.14	0.00
	TAS55	34.13	
3	DePCR45	11.82	0.41
	DePCR55	11.28	

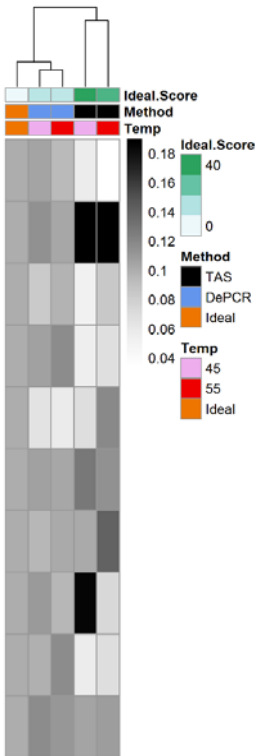
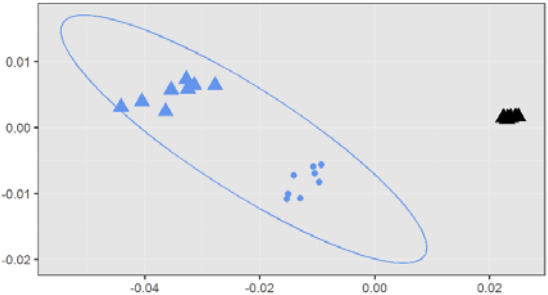


Fig. S10

Primer Utilizing Profiles

Experiment: C1
Primers: 1
Templates: 10



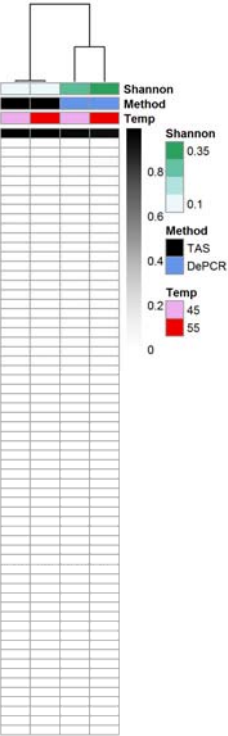
ANOSIM

DePCR vs TAS:
R=0.992, P=0.0001

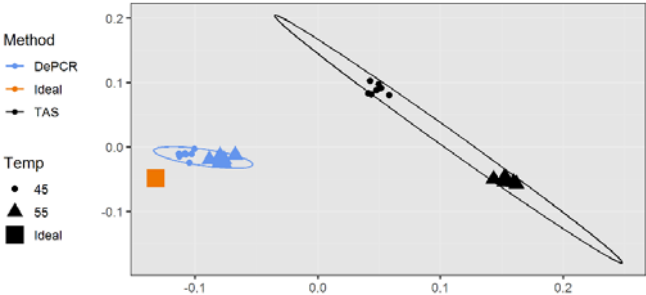
TAS45 vs TAS55:
R=0.104; p=0.117

DePCR45 vs DePCR55:
R=1, P=0.0002

Comparison	DePCR45	DePCR55	ANOVA
% Reads 0 MM	3.42	5.77	0.00
% Reads 1 MM	94.72	92.70	0.00
% Reads 2 MM	1.83	1.51	0.00
% Reads 3MM	0.02	0.02	0.35
% Reads with 3' MM	22.75	20.24	0.00
% Reads with Mid' MM	36.06	39.65	0.00
% Reads with 5' MM	41.19	40.11	0.02
Average Primer Tm	57.42	57.43	0.16
Average Shannon	0.28	0.36	0.00



Template Profiles



ANOSIM

DePCR vs TAS:
R=0.875, P=0.0001

TAS45 vs TAS55:
R=1; p=0.0002

DePCR45 vs DePCR55:
R=0.993, P=0.0002

	Comparison	Average Ideal Score	ANOVA
1	TAS	54.32	0.00
	DePCR	22.45	
2	TAS45	48.72	0.00
	TAS55	59.91	
3	DePCR45	21.04	0.00
	DePCR55	23.87	

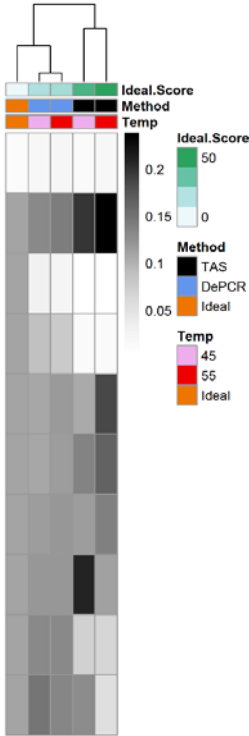
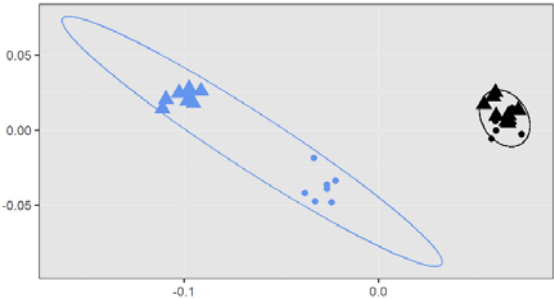


Fig. S11

Primer Utilizing Profiles



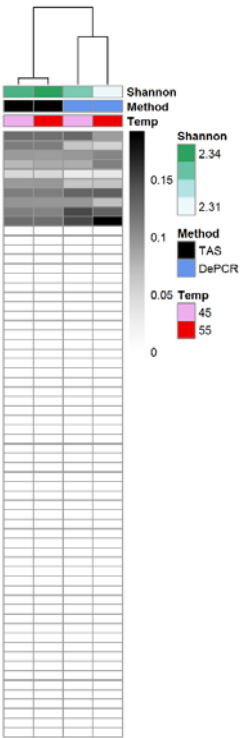
ANOSIM

DePCR vs TAS:
R=0.956, P=0.0001

TAS45 vs TAS55:
R=0.335; p=0.002

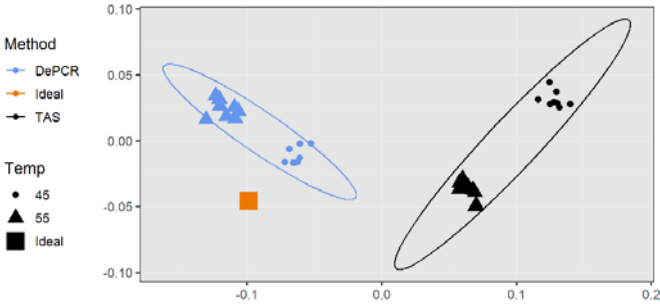
DePCR45 vs DePCR55:
R=1, P=0.0002

Comparison	DePCR45	DePCR55	ANOVA
% Reads 0 MM	29.58	51.32	0.00
% Reads 1 MM	42.98	38.78	0.00
% Reads 2 MM	27.22	9.83	0.00
% Reads 3MM	0.23	0.07	0.00
% Reads with 3' MM	23.43	26.64	0.00
% Reads with Mid' MM	34.80	34.94	0.65
% Reads with 5' MM	41.77	38.42	0.00
Average Primer Tm	57.79	57.89	0.00
Average Shannon	2.33	2.30	0.00



Experiment: C2
Primers: 10
Templates: 10

Template Profiles



ANOSIM

DePCR vs TAS:
R=1, P=0.0001

TAS45 vs TAS55:
R=1; p=0.0002

DePCR45 vs DePCR55:
R=1, P=0.0002

Comparison	Average Ideal Score	ANOVA
1 TAS	41.48	0.00
DePCR	17.56	
2 TAS45	49.17	0.00
TAS55	33.79	
3 DePCR45	19.07	0.00
DePCR55	16.04	

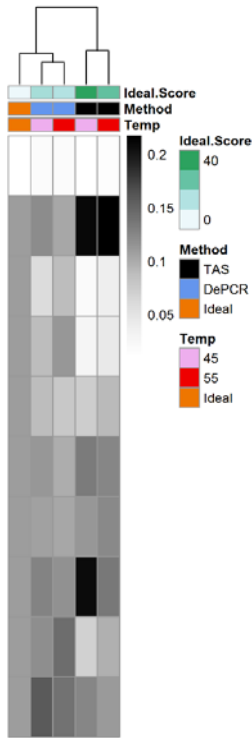
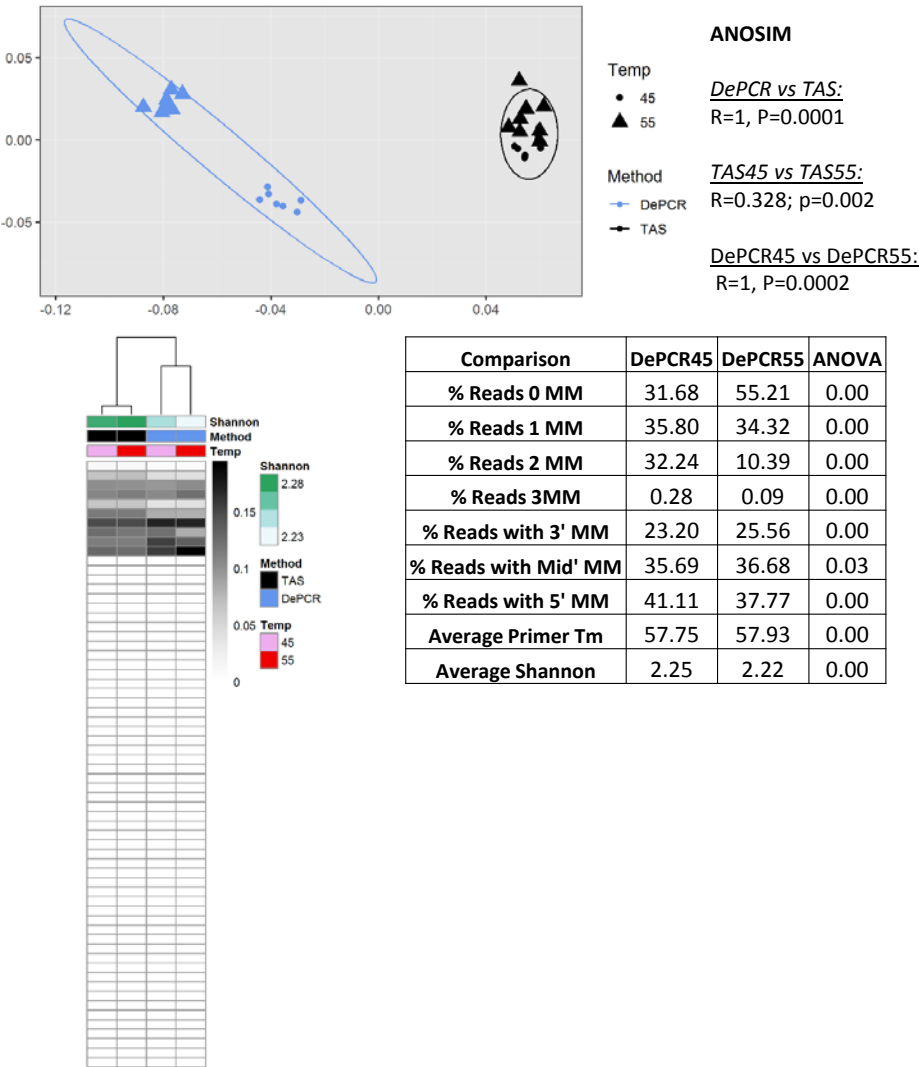


Fig. S12

Primer Utilizing Profiles



Experiment: C3
Primers: 9
Templates: 10

Template Profiles

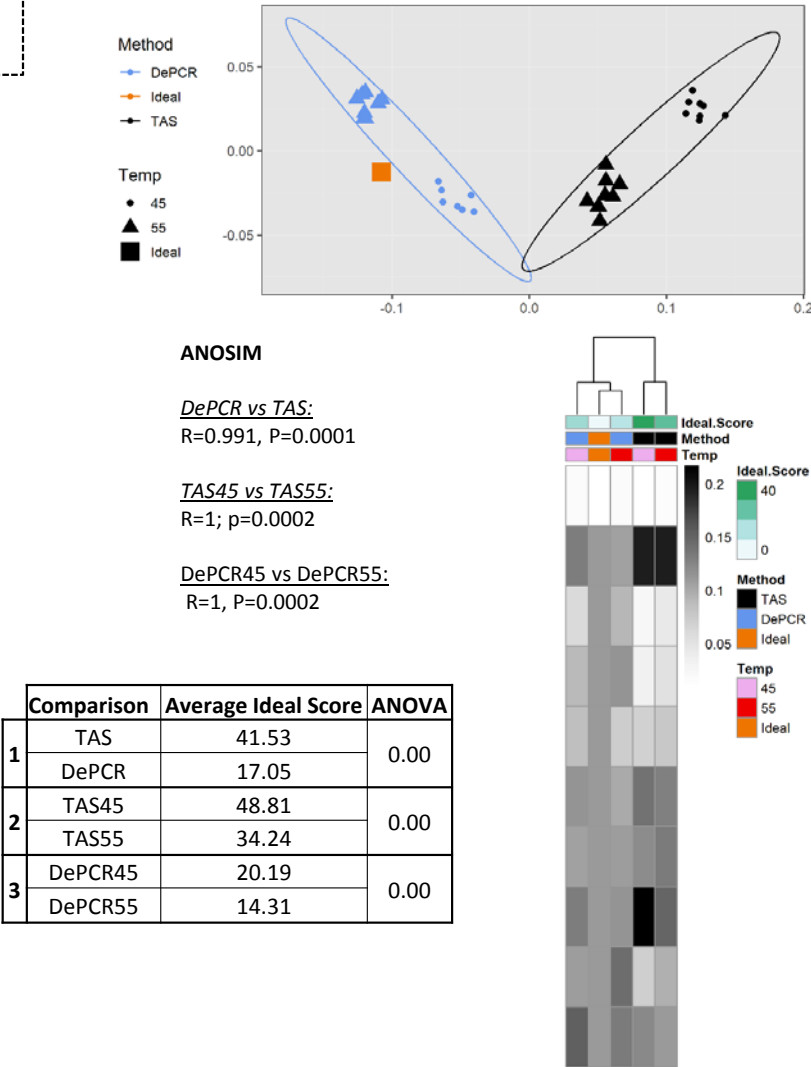
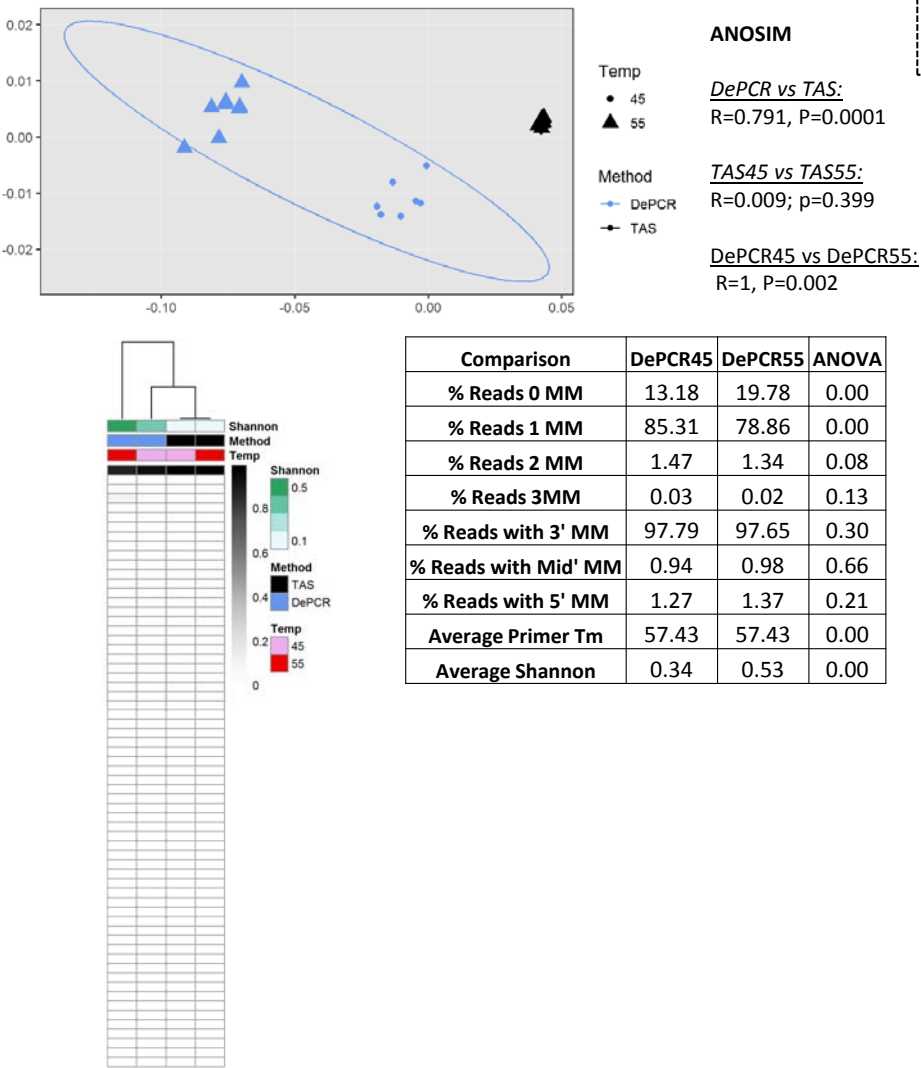


Fig. S13

Primer Utilizing Profiles



Experiment: D1
Primers: 1
Templates: 4

Template Profiles

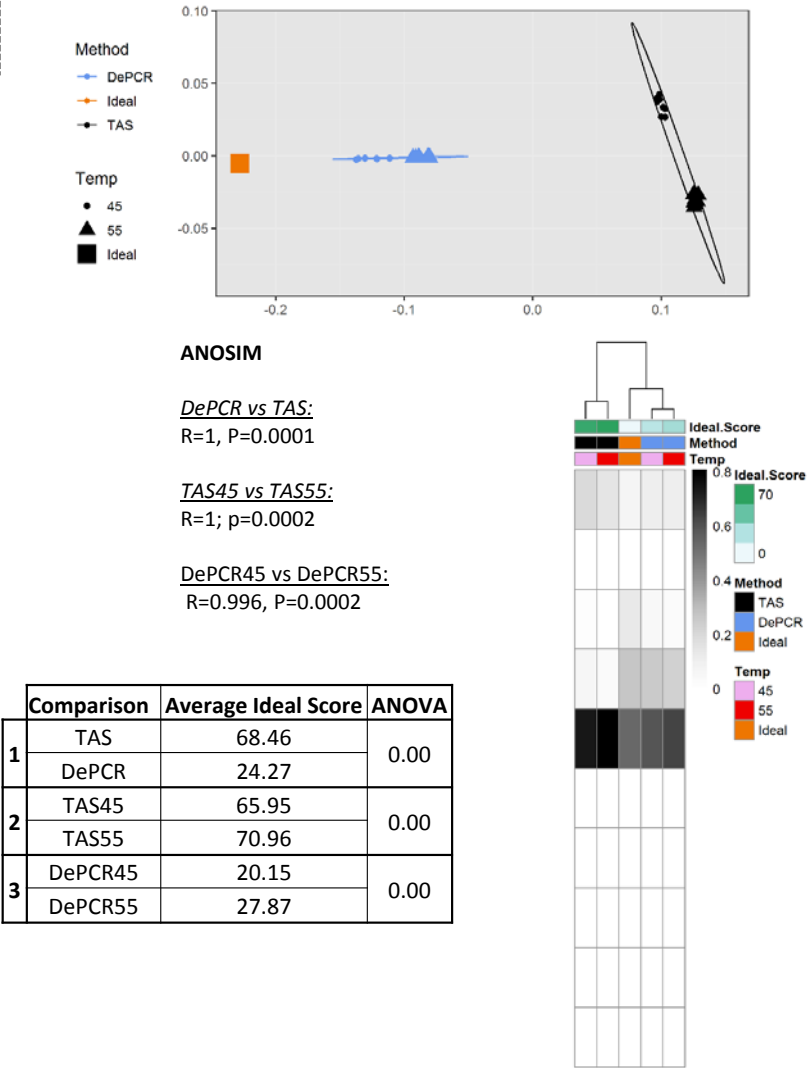
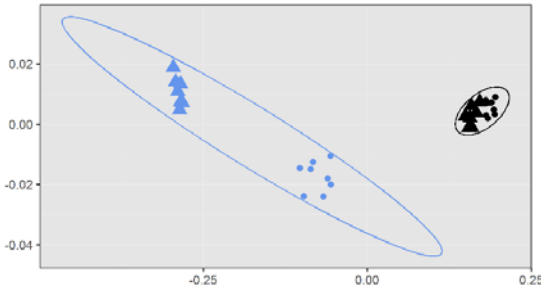


Fig. S14

Primer Utilizing Profiles

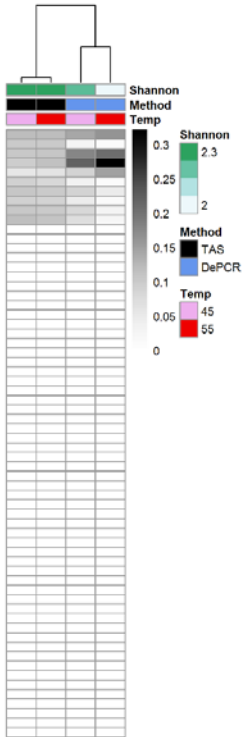


ANOSIM

DePCR vs TAS:
 $R=0.97$, $P=0.0001$

TAS45 vs TAS55:
 $R=0.866$; $p=0.0002$

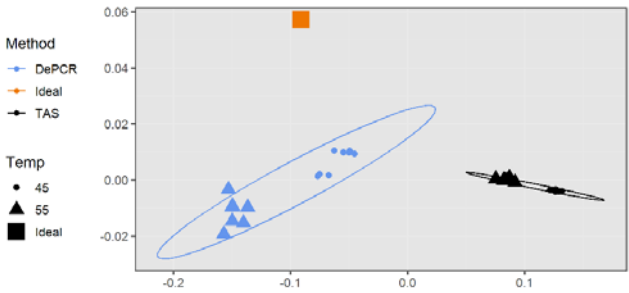
DePCR45 vs DePCR55:
 $R=1$, $P=0.0002$



Comparison	DePCR45	DePCR55	ANOVA
% Reads 0 MM	26.59	43.80	0.00
% Reads 1 MM	43.50	46.84	0.00
% Reads 2 MM	29.69	9.28	0.00
% Reads 3MM	0.22	0.08	0.00
% Reads with 3' MM	64.27	75.44	0.00
% Reads with Mid' MM	15.38	12.56	0.00
% Reads with 5' MM	20.35	12.00	0.00
Average Primer Tm	57.98	58.11	0.00
Average Shannon	2.23	1.90	0.00

Experiment: D2
Primers: 10
Templates: 4

Template Profiles



ANOSIM

DePCR vs TAS:
 $R=1$, $P=0.0001$

TAS45 vs TAS55:
 $R=1$; $p=0.0002$

DePCR45 vs DePCR55:
 $R=1$ $P=0.0002$

	Comparison	Average Ideal Score	ANOVA
1	TAS	41.16	0.00
	DePCR	13.23	
2	TAS45	45.17	0.00
	TAS55	36.58	
3	DePCR45	9.53	0.00
	DePCR55	17.45	

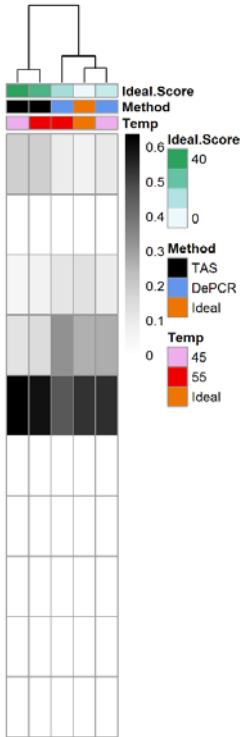
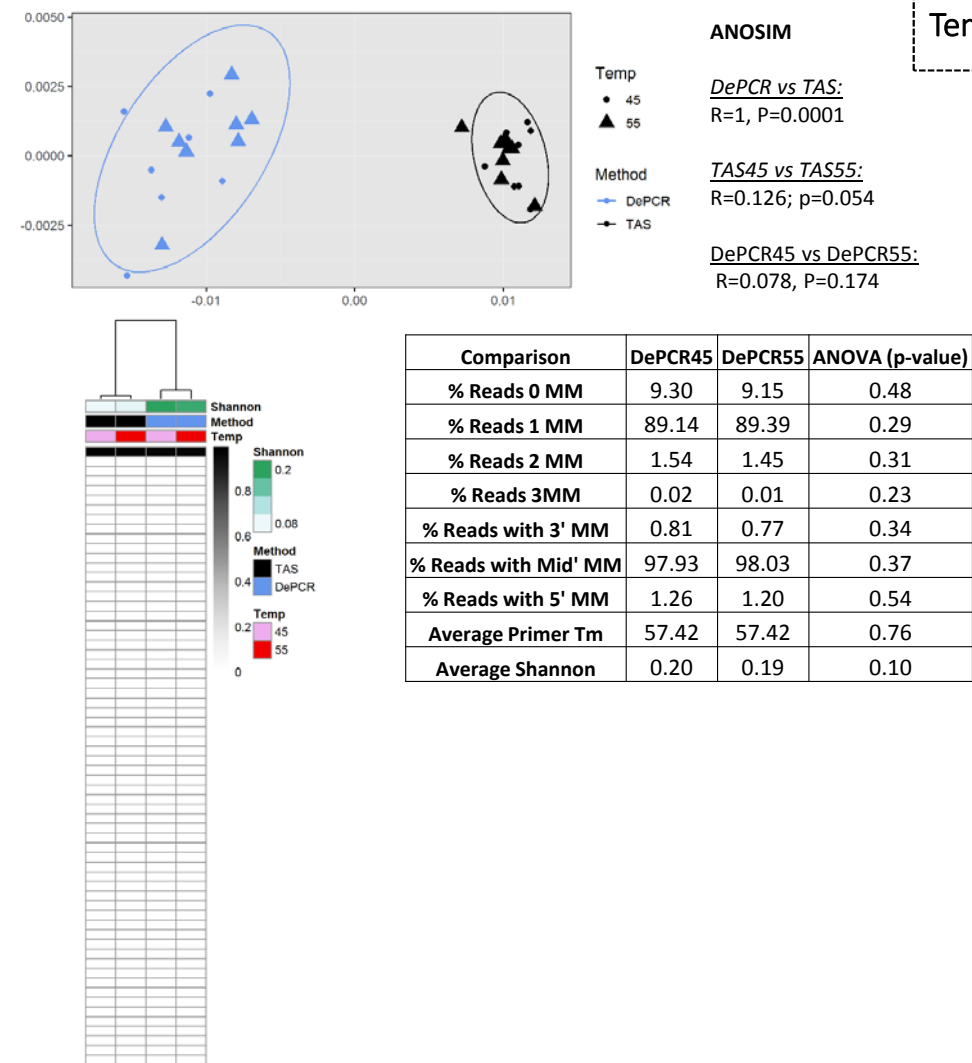


Fig. S15

Primer Utilizing Profiles



Experiment: E1
Primers: 1
Templates: 4

Template Profiles

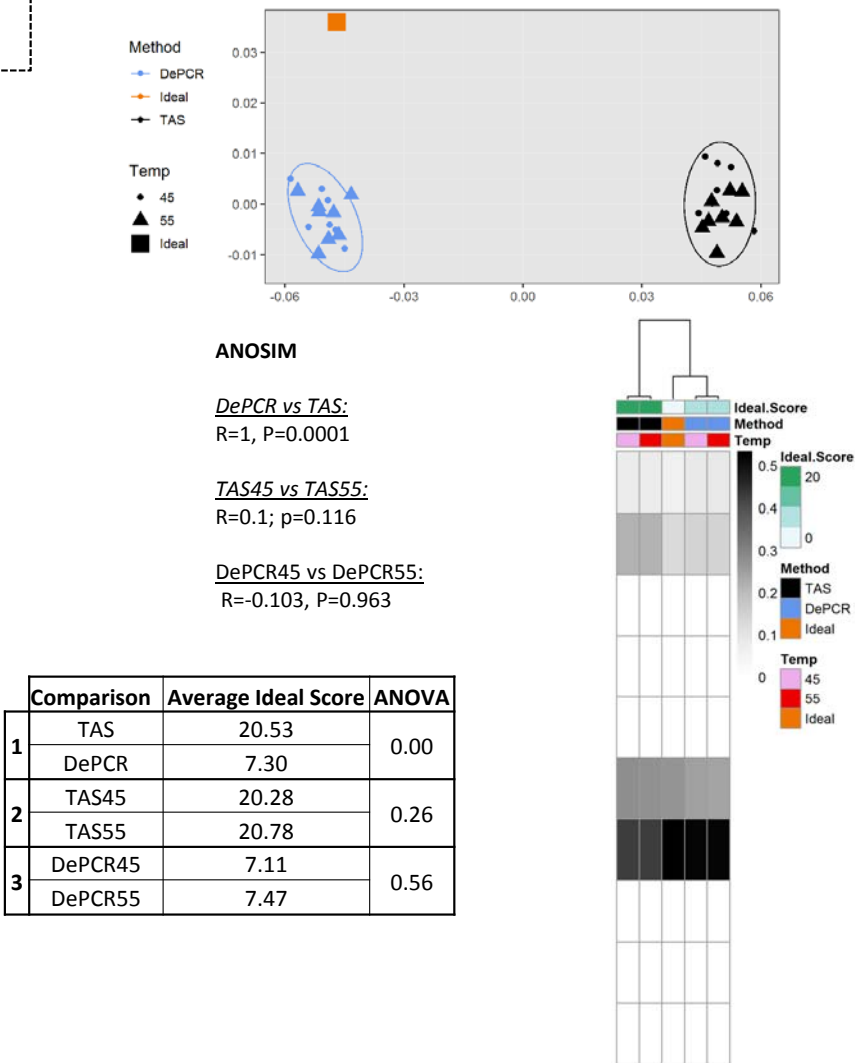
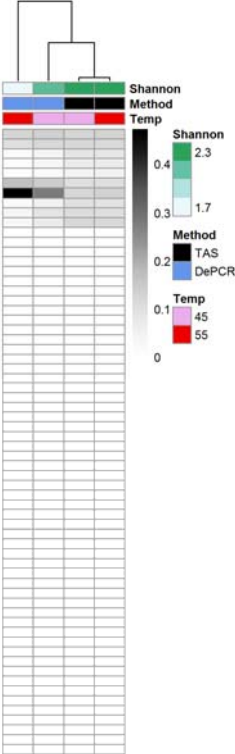
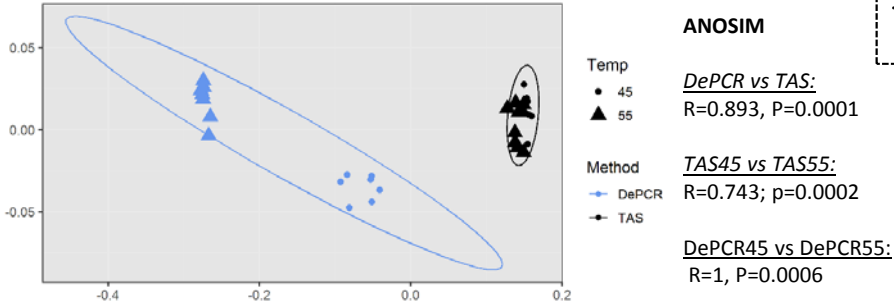


Fig. S16

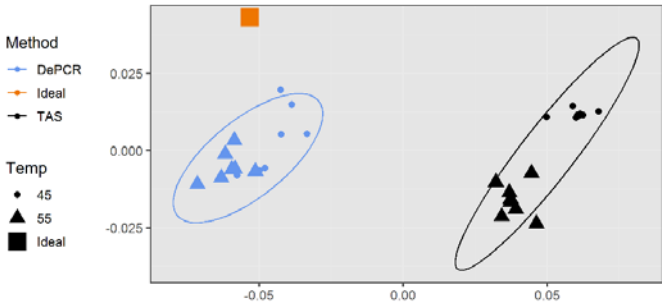
Primer Utilizing Profiles



Comparison	DePCR45	DePCR55	ANOVA
% Reads 0 MM	30.88	51.68	0.00
% Reads 1 MM	41.43	38.94	0.00
% Reads 2 MM	27.43	9.31	0.00
% Reads 3MM	0.25	0.07	0.00
% Reads with 3' MM	10.65	7.69	0.00
% Reads with Mid' MM	66.09	76.16	0.00
% Reads with 5' MM	23.26	16.15	0.00
Average Primer Tm	58.44	58.93	0.00
Average Shannon	2.16	1.70	0.00

Experiment: E2
Primers: 10
Templates: 4

Template Profiles



ANOSIM

DePCR vs TAS:
R=1, P=0.0001

TAS45 vs TAS55:
R=0.984; p=0.0002

DePCR45 vs DePCR55:
R=0.633, P=0.0001

	Comparison	Average Ideal Score	ANOVA
1	TAS	21.50	0.00
	DePCR	4.91	
2	TAS45	21.66	0.59
	TAS55	21.35	
3	DePCR45	5.95	0.00
	DePCR55	3.87	

