

Name	
Company	
E-Mail	

Order #	1234
Received	03/15/16
Reported	03/16/16

Well	Strain Name	Sample Name	Flp	Cyp2r 1	Cre
A1	Strain A	1	Wild	Het	
A2	Strain A	2	Wild	Het	
A3	Strain A	3	Wild	Het	
A4	Strain A	4	Wild	Hom	
A5	Strain A	5	Wild	Wild	
A6	Strain A	6	Het	Hom	
A7	Strain B	1		Wild	Wild
A8	Strain B	2		Wild	Wild
A9	Strain B	3		Wild	Het
A10	Strain B	4		Het	Wild
A11	Strain C	1		Het	
A12	Strain C	2		Wild	
B1	Strain C	3		Wild	
B2	Strain C	4		Het	
B3	Strain C	5		Het	
B4	Strain C	6		Het	
B5	Strain C	7		Wild	
B6	Strain C	8		Het	
B7					
B8					
B9					
B10					
B11					
B12					
C1					

Key 86

Method 1

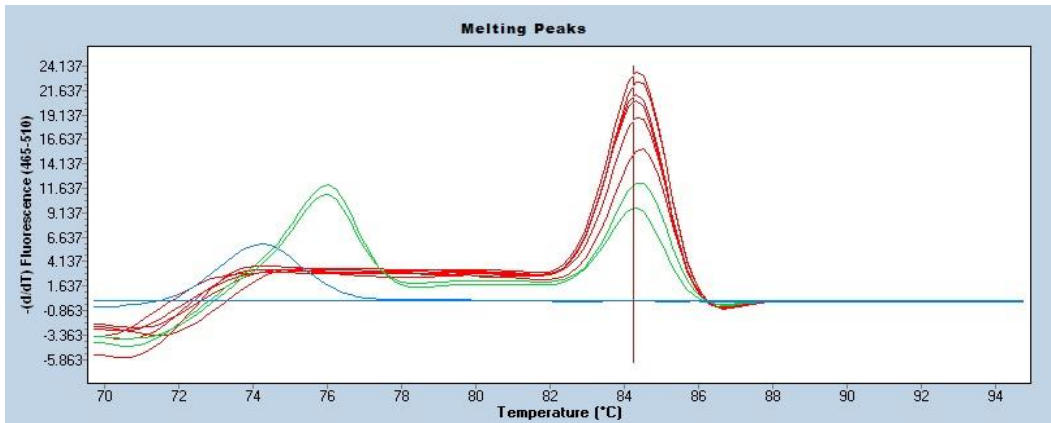
Name	Tm1	Area1	Tm2	Area2	Call
331	84.24	43.88			Wild
332	84.42	32.72			Wild
333	84.26	39.49			Wild
334	84.28	42.47			Wild
335	84.34	47.1			Wild
336	75.99	27.68	84.13	24.59	Het
wt	84.29	49.32			Wild
flp	76.05	19.98	84.31	29.6	Het
ntc					

Key 86

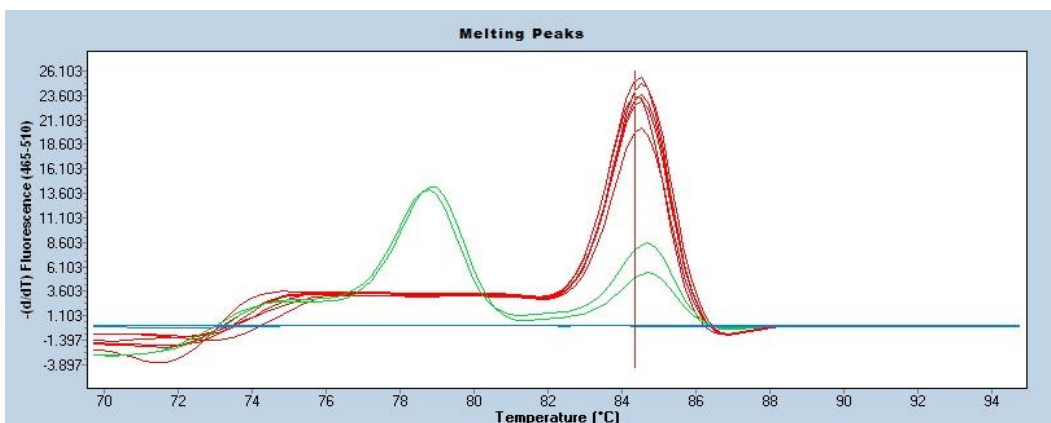
Method 2

Name	Tm1	Area1	Tm2	Area2	Call
331	84.33	48.21			Wild
332	84.4	52.48			Wild
333	84.43	41.25			Wild
334	84.4	47.12			Wild
335	84.45	47.23			Wild
336	78.79	32.94	84.65	12.55	Het
wt	84.46	52.18			Wild
flp	78.69	31.88	84.6	19.84	Het
ntc					

Method 1. Left peak is Flp. Right peak is internal control.



Method 2. Left peak is Flp. Right peak is internal control.



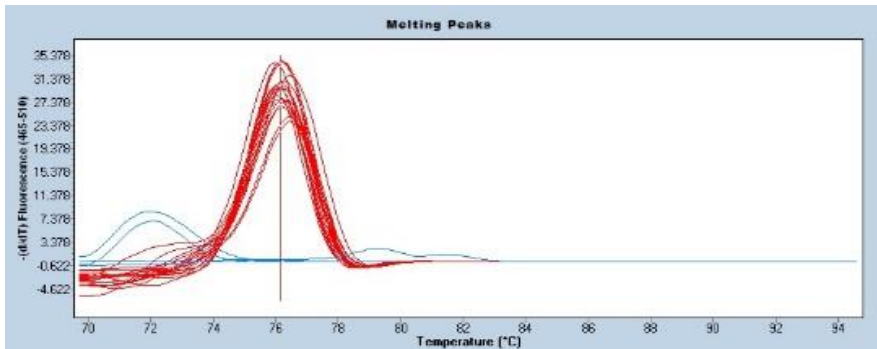
Method 1

Name	Wild		Mutant		Call
	Tm1	Area1	Tm1	Area1	
331	76.14	59.87	75.79	49.67	Het
332	76.4	56.4	76	44.78	Het
333	76.42	53.53	76.02	42.96	Het
334			75.98	53.79	Hom
335	76.19	68.4			Wild
336			75.87	53.29	Hom
315	76.31	64.71			Wild
316	76.22	75.79			Wild
317	76.41	76.82			Wild +
318	76.14	75.86	75.81	54.14	Het +
ntc					
279	76.16	63.72	75.82	51.36	Het +
280	76.2	66.82			Wild +
281	76.2	65.27			Wild +
282	76.06	64.23	75.61	48.97	Het +
283	76.09	56.14	75.5	45.07	Het +
284	76.04	64.43	75.52	53.79	Het +
285	76.08	62.2			Wild +
286	75.92	75.32	75.42	58.35	Het +
wt	76.15	76.01			Wild +
het	76.23	75.52	75.69	64.53	Het +
hom			74.71	22.89	Hom

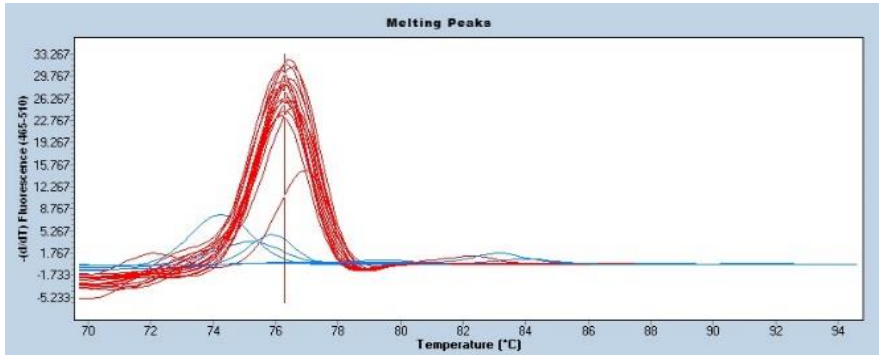
Method 2

Name	Wild		Mutant		Call
	Tm1	Area1	Tm1	Area1	
331	76.28	58.86	75.79	58.11	Het
332	76.91	27.65	75.99	52.07	Het
333	76.52	55.09	75.7	33.41	Het
334			75.78	56.7	Hom
335	76.23	53.63			Wild
336			76.06	46.44	Hom
315	76.3	65.32			Wild
316	76.27	74.09			Wild
317	76.51	66.88			Wild
318	76.45	61.64	75.86	58.46	Het
ntc					
279	76.37	57.21	75.86	54.81	Het
280	76.06	61.25			Wild
281	76.39	59.46			Wild
282	76.23	53.27	75.72	48.24	Het
283	76.1	49	75.54	56.42	Het
284	76.21	51.74	75.68	52.96	Het
285	76.24	60.43			Wild
286	76.06	65.24	75.48	57.86	Het
wt	76.13	59.89			Wild
het	76.51	64.91	75.72	56.05	Het
hom			75.7	17.47	Hom

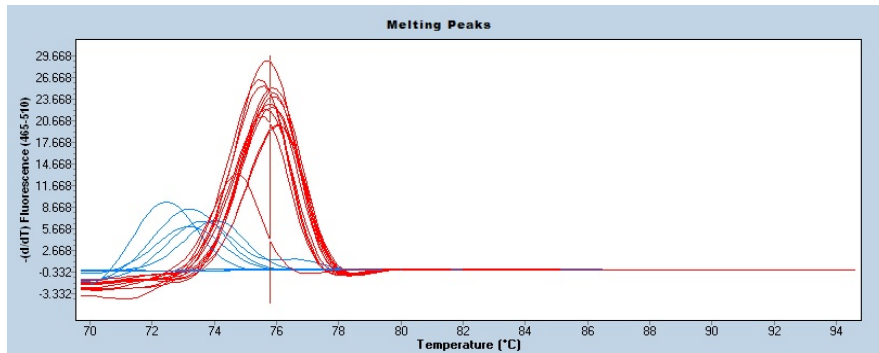
Method 1. Separated. Red peak is wild type.



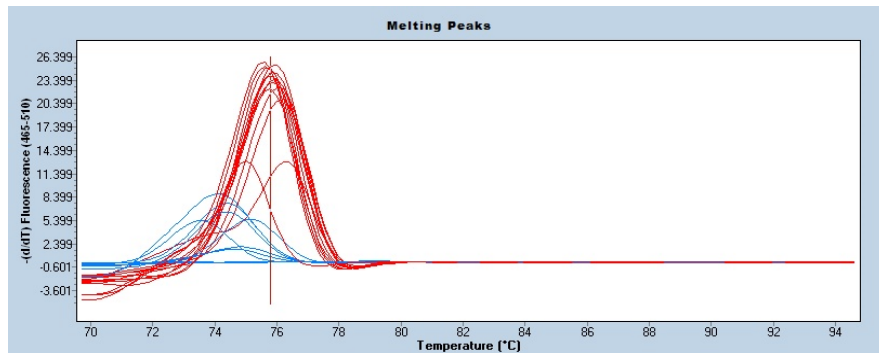
Method 1. Separated. Red peak is mutant.



Method 2. Separated. Red peak is wild type.



Method 2. Separated. Red peak is mutant.



Key	86
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Method 1

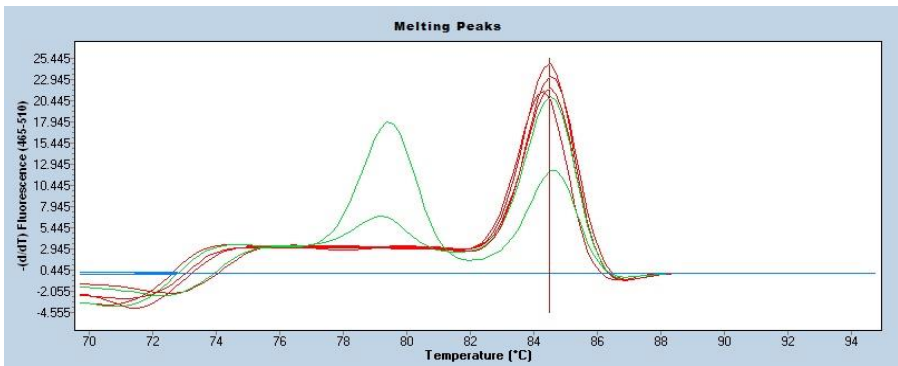
Name	Tm1	Area1	Tm2	Area2	Call
315	84.48	44.9			Wild
316	84.41	50.72			Wild
317	78.81	27.68	84.48	41.94	Het
318	84.23	43.68			Wild
wt	84.52	48.5			Wild
cre	79.38	42.65	84.56	26.53	Het
ntc					

Key	86
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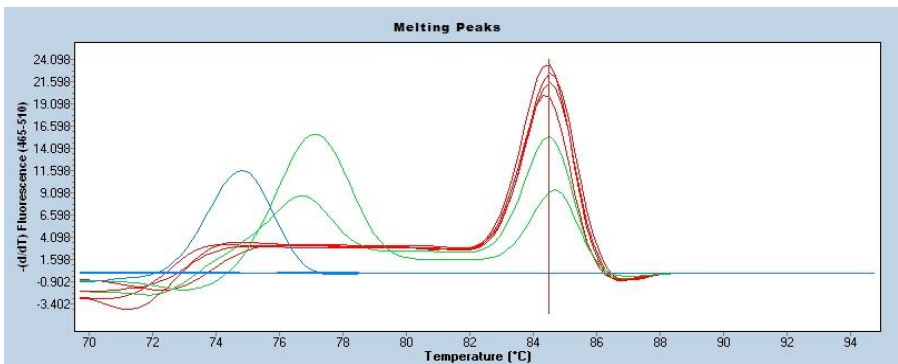
Method 2

Name	Tm1	Area1	Tm2	Area2	Call
315	84.48	42.02			Wild
316	84.48	46.1			Wild
317	76.87	20.1	84.57	37.77	Het
318	84.48	39.52			Wild
wt	84.48	46.5			Wild
cre	76.87	55.7	84.57	19.36	Het
ntc					

Method 1. Left peak is Cre. Right peak is internal control.



Method 2. Left peak is Cre. Right peak is internal control.



Method 1	Cre was detected utilizing SYBR Green melting curve analysis. The Cre mutant allele and gt(Rosa) control were amplified using SYBR Green PCR Mix (Cre forward primer CGAGTGATGAGGTTCCGCAAG, Cre reverse primer GGCAAACGGACAGAAGCATT, gt(Rosa)Forward primer GTTATCAGTAAGGGAGCTGCAGTGG, gt(Rosa) Reverse primer AGTTGCAGATCACGAGGGAA) and run on a Roche LightCycler 480 with conditions suggested for SYBR Green amplification. Analysis was performed using the Tm calling module of the Roche LightCycler 480 Software, release 1.5.1. Cre amplicon was detected at a melting point of 78°C and the melting point of the gt(Rosa) control amplicon was 85°C. Homozygotes and Heterozygotes not differentiated by assay.
Method 2	Cre was detected utilizing SYBR Green melting curve analysis. The Cre mutant allele and gt(Rosa) control were amplified using SYBR Green PCR Mix (Cre forward primer ACCGTACACAAAATTTGCCT, Cre reverse primer CTTGCGAACCTCATCACTCG, gt(Rosa)Forward primer GTTATCAGTAAGGGAGCTGCAGTGG, gt(Rosa) Reverse primer AGTTGCAGATCACGAGGGAA) and run on a Roche LightCycler 480 with conditions suggested for SYBR Green amplification. Analysis was performed using the Tm calling module of the Roche LightCycler 480 Software, release 1.5.1. Cre amplicon was detected at a melting point of 80°C and the melting point of the gt(Rosa) control amplicon was 85°C. Homozygotes and Heterozygotes not differentiated by assay.
Method 1	Flp was detected utilizing SYBR Green melting curve analysis. The Flp mutant allele and gt(Rosa) control were amplified using SYBR Green PCR Mix (Flp forward primer TGCCGGTCTATTTACTCGT, Flp reverse primer TACTTCTTTAGCGCAAGGGGTAG, gt(Rosa) forward primer GTTATCAGTAAGGGAGCTGCAGTGG, gt(Rosa) reverse primer AGTTGCAGATCACGAGGGAA) and run on a Roche LightCycler 480 with conditions suggested for SYBR Green amplification. Analysis was performed using the Tm calling module of the Roche LightCycler 480 Software, release 1.5.1. Flp amplicon was detected at a melting point of 76.2°C and the melting point of the gt(Rosa) control amplicon was 84.4°C. Homozygotes and Heterozygotes <u>not</u> differentiated by assay.
Method 2	Flp was detected utilizing SYBR Green melting curve analysis. The Flp mutant allele and gt(Rosa) control were amplified using SYBR Green PCR Mix (Flp forward primer TGTTGTGGGAAATTGGAGCG, Flp reverse primer AGTGCGAAGTAGTGATCAGGT, gt(Rosa) forward primer GTTATCAGTAAGGGAGCTGCAGTGG, gt(Rosa) reverse primer AGTTGCAGATCACGAGGGAA) and run on a Roche LightCycler 480 with conditions suggested for SYBR Green amplification. Analysis was performed using the Tm calling module of the Roche LightCycler 480 Software, release 1.5.1. Flp amplicon was detected at a melting point of 76.2°C and the melting point of the gt(Rosa) control amplicon was 84.4°C. Homozygotes and Heterozygotes <u>not</u> differentiated by assay.
Method 1	Cyp2r1 was detected utilizing SYBR Green melting curve analysis. The wild type and mutant alleles were amplified in <u>separate</u> reactions using SYBR Green PCR Mix (wild forward primer CAGTAAGGTATAGCATGCATTGTTTC, wild reverse primer CAGCTTGAAGTGAGGGGAGA, mutant forward CAGTAAGGTATAGCATGCATTGTTTC and mutant reverse TCGTGGTATCGTTATGCGCC) and run on a Roche LightCycler 480 with conditions suggested for SYBR Green amplification. Analysis was performed using the Tm calling module of the Roche LightCycler 480 Software, release 1.5.1. Wild type amplicon was detected at a melting point of 76.5°C and the melting point of the mutant amplicon was 76°C. All Genotypes should be detectable, however, no homozygotes have been detected at this time.
Method 2	Cyp2r1 was detected utilizing SYBR Green melting curve analysis. The wild type and mutant alleles were amplified in <u>separate</u> reactions using SYBR Green PCR Mix (wild forward primer CCAGTAAGGTATAGCATGCATTG, wild reverse primer TACCAGCTTGAAGTGAGGGG, mutant forward CCAGTAAGGTATAGCATGCATTG and mutant reverse TCGTGGTATCGTTATGCGCC) and run on a Roche LightCycler 480 with conditions suggested for SYBR Green amplification. Analysis was performed using the Tm calling module of the Roche LightCycler 480 Software, release 1.5.1. Wild type amplicon was detected at a melting point of 76.6°C and the melting point of the mutant amplicon was 76°C. All Genotypes should be detectable, however, no homozygotes have been detected at this time.

