Superprimers

A New Approach for Genotyping Degraded DNA

Biodynamics

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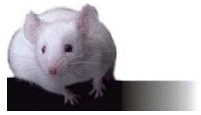
5'ccggaggtaaaggtgtcttaaagtgagaaagaataactgcatcttaacctattgggaggtcattgtaaagaggagagtgatggggtcagattgtacagagg
aggcacttcgtggtggtcaggagcacacactccagggcagtgttccaacctgagtctgccaaggactagcaggttgctaaccaccctgtgtccagttt 3’



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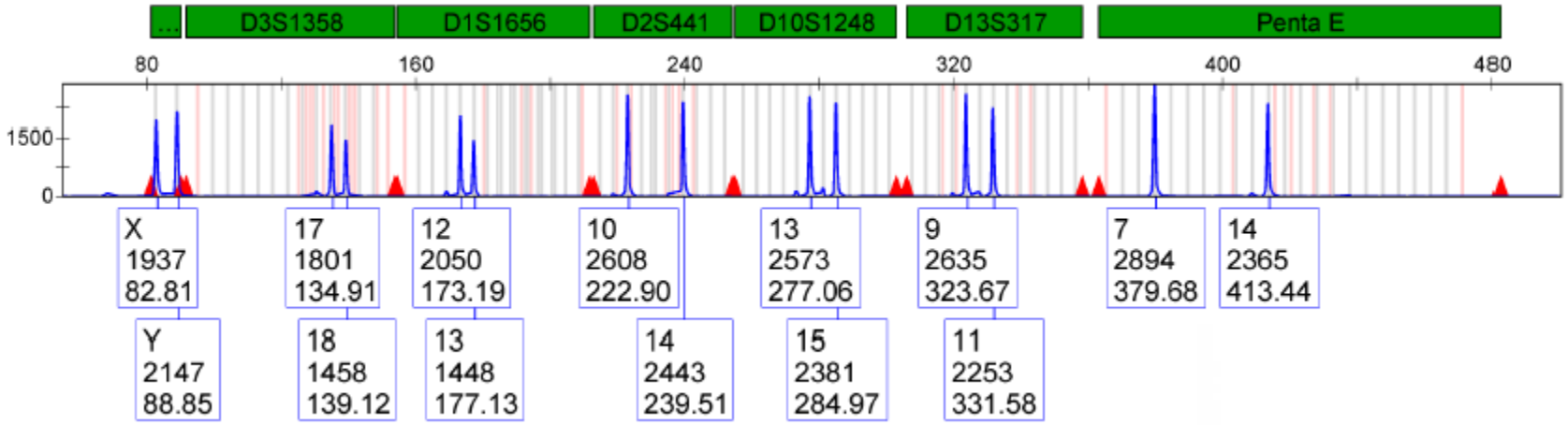
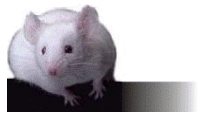
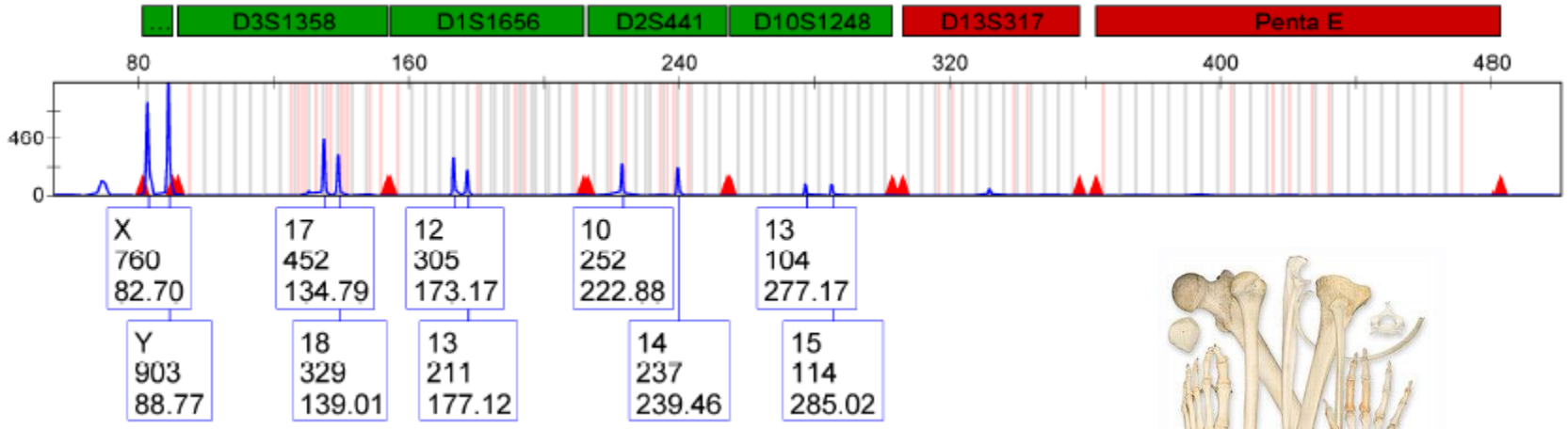
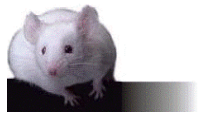
Current multiplex STR kit on intact DNA



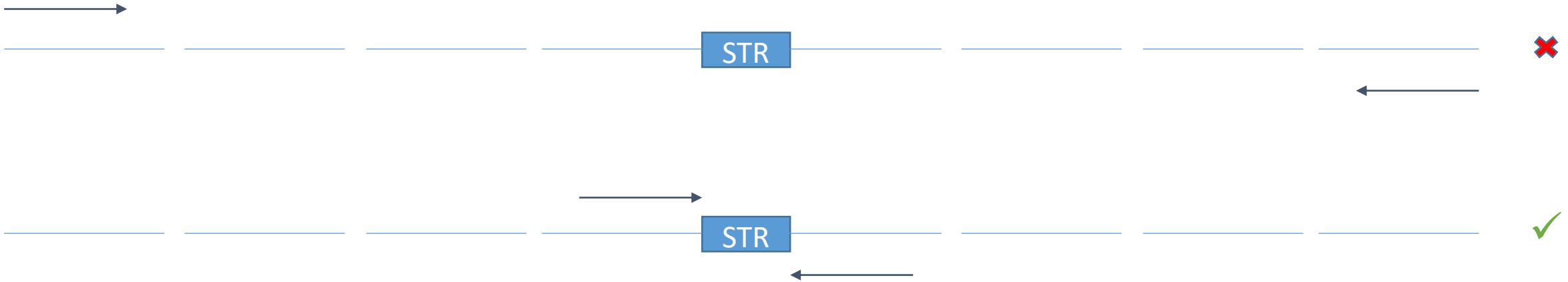


Current multiplex STR kit on degraded DNA

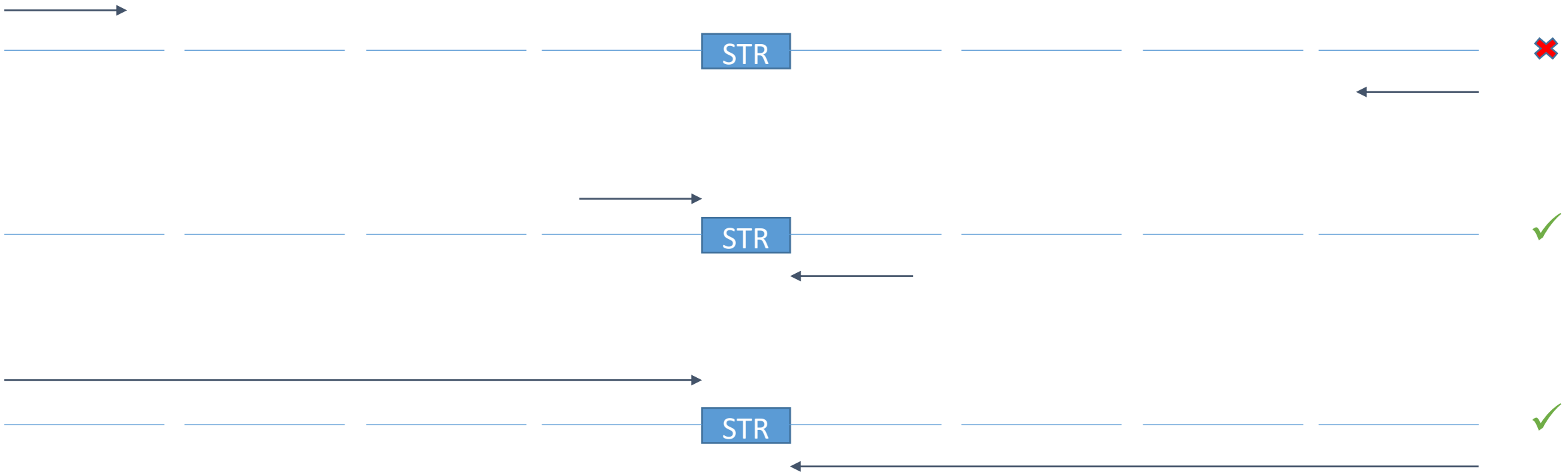




Superprimers on degraded DNA



Superprimers on degraded DNA



Amplifying with superprimers

120nt

ccggaggtaa aggtgtctta aagtgagaaa gaataactgc atcttaacct attgggaggt cattgtaaag aggagagtga tggggtcaga ttgtacagag gaggcacttc gtggtggtca

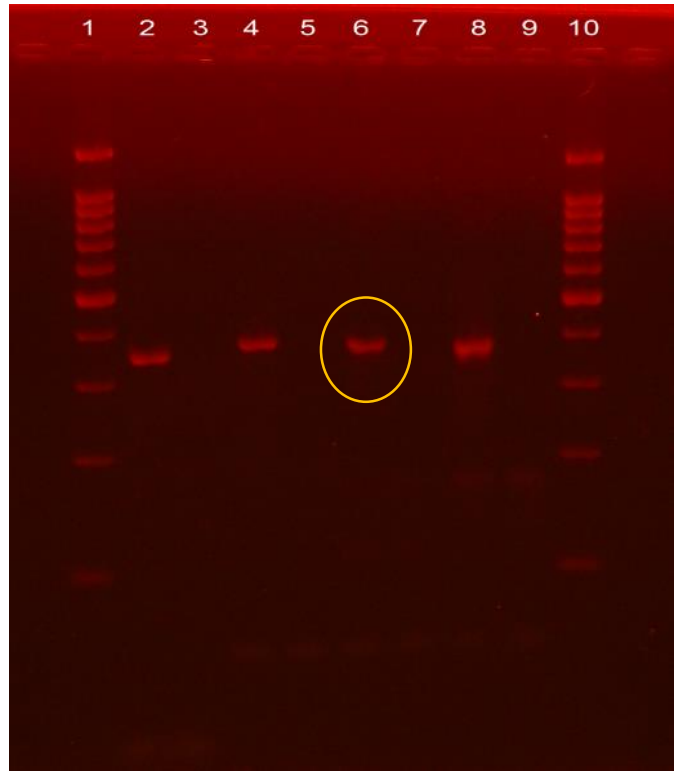
CSFFW2416HS (24nt)

CSFFW120 (120nt)

CSFFW200 (200nt)

CSFFW260 (260nt)

CSFFW300 (300nt)



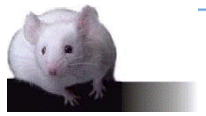
1: 100bp
4: CSFFW2416HS + CSFRV60J
8: CSFFW200 + CSFRV60J

2: CSFFW2416HSJ + CSFRV2216HS
6: CSFFW120 + CSFRV60J
10: 100bp

AGAT

CSFRV60J (60nt)

CSFRV2216HS (22nt)



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Amplifying with superprimers

200nt

ccggaggtaa aggtgtctta aagtgagaaa gaataactgc atcttaacct attgggaggt cattgtaaag aggagagtga tggggtcaga ttgtacagag gaggcacttc gtggtggtca
ggagcacaca ctccagggca gtgttccaac ctgagtctgc caaggactag caggttgcta accaccctgt gtctcagttt

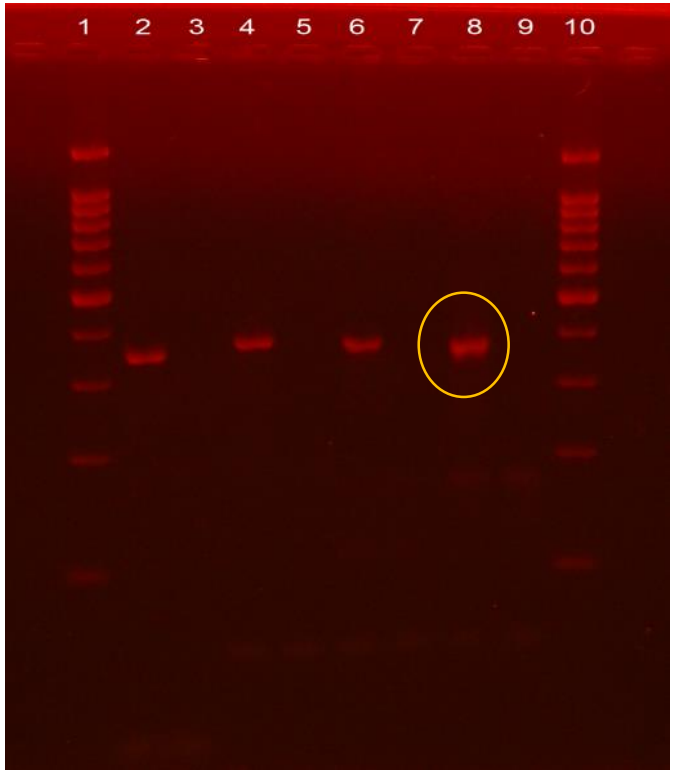
CSFFW2416HS (24nt)

CSFFW120 (120nt)

CSFFW200 (200nt)

CSFFW260 (260nt)

CSFFW300 (300nt)



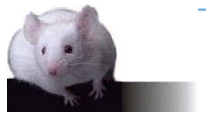
1: 100bp
4: CSFFW2416HS + CSFRV60J
8: CSFFW200 + CSFRV60J

2: CSFFW2416HSJ + CSFRV2216HS
6: CSFFW120 + CSFRV60J
10: 100bp

AGAT

CSFRV60J (60nt)

CSFRV2216HS (22nt)



Biodynamics

Amplifying with superprimers

260nt

5’ ccggaggtaa aggtgtctta aagtgagaaa gaataactgc atcttaacct attgggaggt cattgtaaag aggagagtga tggggtcaga ttgtacagag gaggcacttc gtggtggtca ggagcacaca ctccagggca gtgttccaac ctgagtctgc caaggactag caggttgcta accaccctgt gtctcagttt tcctacctgt aaaatgaaga tattaacagt aactgccttc atagatagaa gatagataga 3’

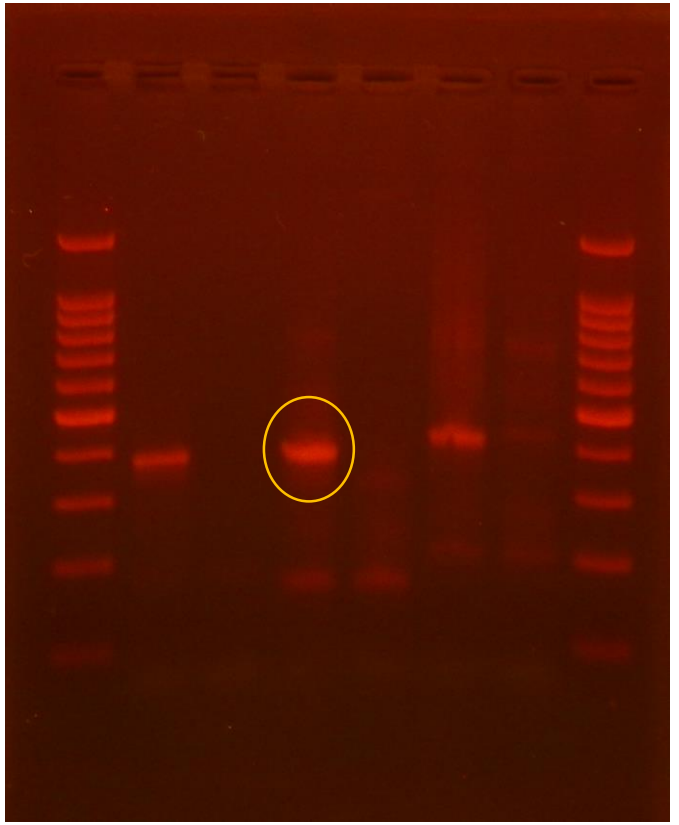
CSFFW2416HS (24nt) →

CSFFW120 (120nt) →

CSFFW200 (200nt) →

CSFFW260 (260nt) →

CSFFW300 (300nt) →



1: 100bp
2: CSFFW200 + CSFRV60J
4: CSFFW260 + CSFRV60J
6: CSFFW300 + CSFRV60J
8: 100bp

AGAT

← CSFRV60J (60nt)

← CSFRV2216HS (22nt)

Biodynamics

Amplifying with superprimers

300nt

5’ gtgaggagtg gccacagggg agcagaggag gtggcagaag ccggaggtaa aggtgtctta aagtgagaaa gaataactgc atcttaacct attgggaggt cattgtaaag aggagagtga tggggtcaga ttgtacagag gaggcacttc gtggtggtca ggagcacaca ctccagggca gtgttccaac ctgagtctgc caaggactag caggttgcta accaccctgt gtctcagttt tcctacctgt aaaatgaaga tattaacagt aactgccttc atagatagaa gatagataga 3’

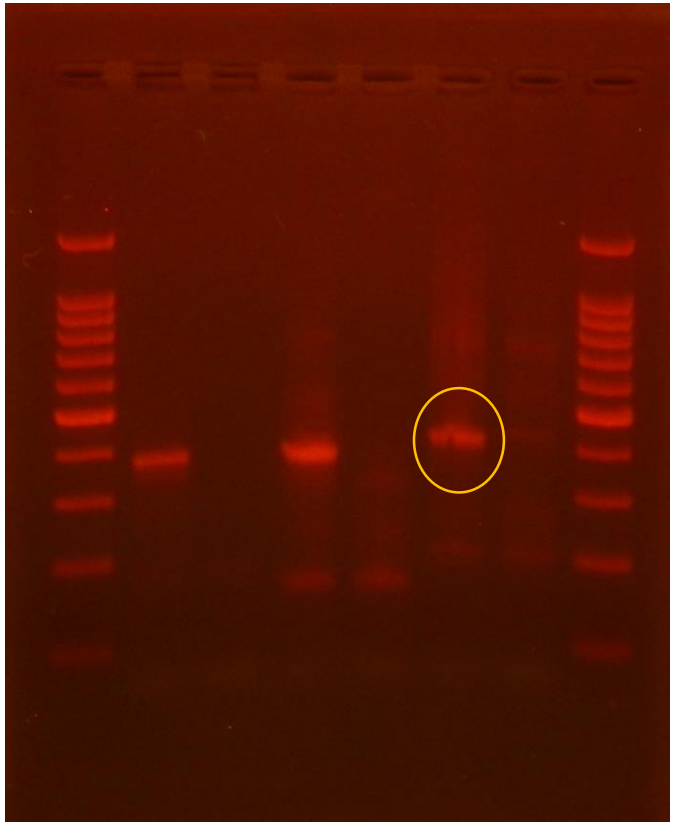
CSFFW2416HS (24nt) →

CSFFW120 (120nt) →

CSFFW200 (200nt) →

CSFFW260 (260nt) →

CSFFW300 (300nt) →

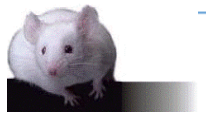


1: 100bp
2: CSFFW200 + CSFRV60J
4: CSFFW260 + CSFRV60J
6: CSFFW300 + CSFRV60J
8: 100bp

AGAT

← CSFRV60J (60nt)

← CSFRV2216HS (22nt)



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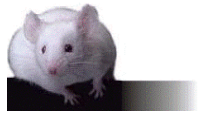
PCR conditions

✓ Same PCR reagents.

✓ Same cycling parameters.

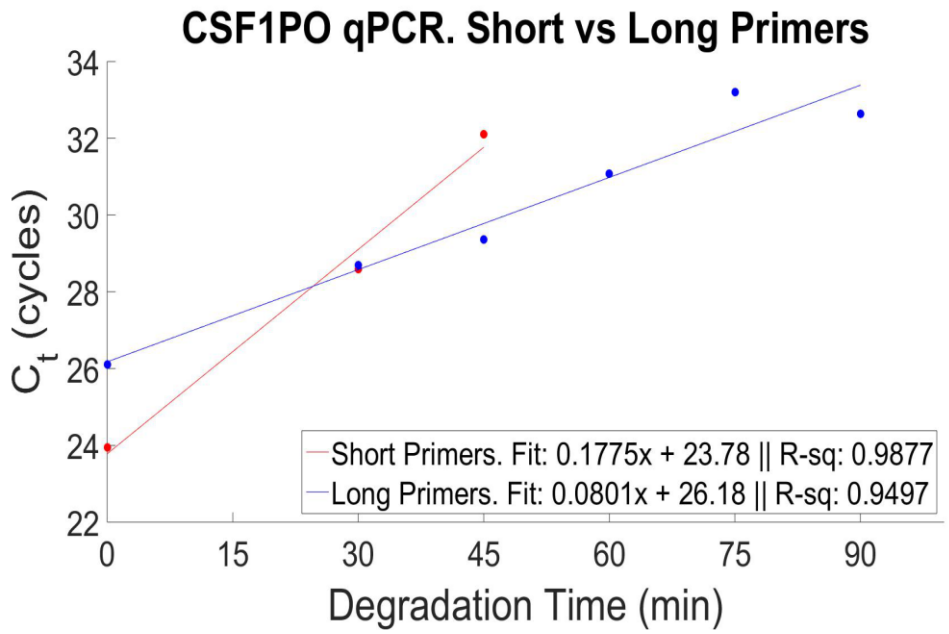
✓ Same primer concentrations.

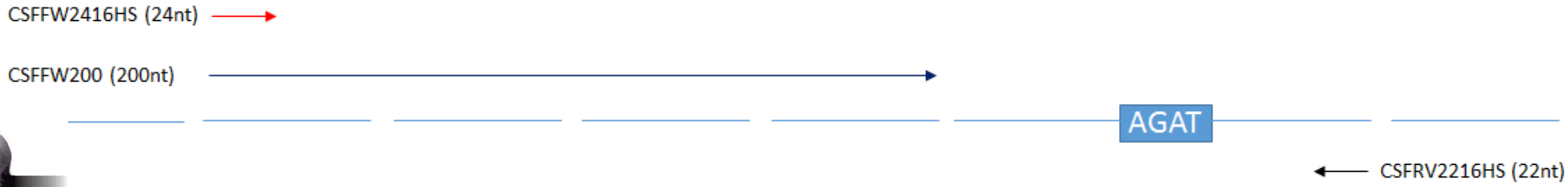
✓ Superprimers must be PAGE purified.



Superprimers amplify better on degraded DNA

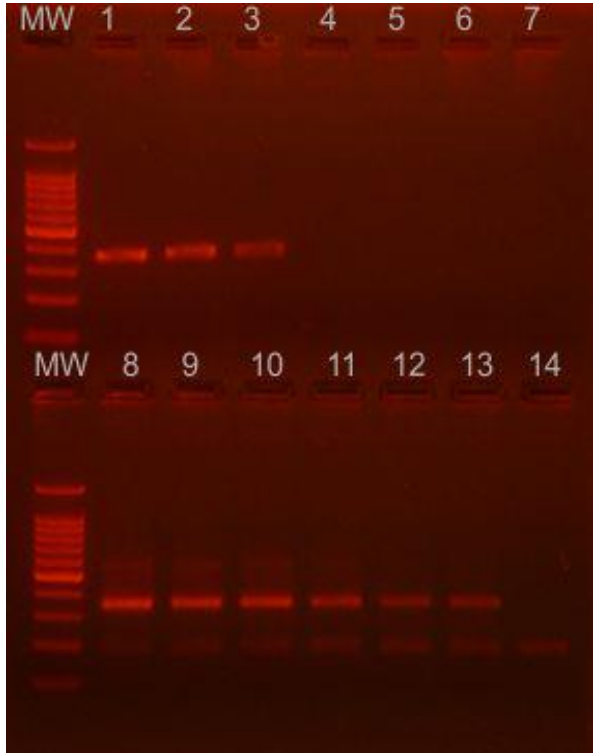
qPCR with primer pairs CSFFW2416HS+CSFRV2216HS *vs.* CSFFW200+CSFRV2216HS on degraded 2800M gDNA





Superprimers amplify better on degraded DNA

qPCR with primer pairs CSFFW2416HS+CSFRV2216HS *vs.* CSFFW200+CSFRV2216HS on degraded 2800M gDNA

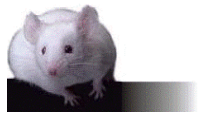


No	Heat-degradation(min)	Primer pair	Ct
1	0	CSFFW2416HS + CSFRV2216HS	23,95
2	30	CSFFW2416HS + CSFRV2216HS	28,59
3	45	CSFFW2416HS + CSFRV2216HS	32,11
4	60	CSFFW2416HS + CSFRV2216HS	
5	75	CSFFW2416HS + CSFRV2216HS	
6	90	CSFFW2416HS + CSFRV2216HS	
7	NTC	CSFFW2416HS + CSFRV2216HS	
8	0	CSFFW200 + CSFRV2216HS	26,10
9	30	CSFFW200 + CSFRV2216HS	28,70
10	45	CSFFW200 + CSFRV2216HS	29,37
11	60	CSFFW200 + CSFRV2216HS	31,07
12	75	CSFFW200 + CSFRV2216HS	33,20
13	90	CSFFW200 + CSFRV2216HS	32,64
14	NTC	CSFFW200 + CSFRV2216HS	

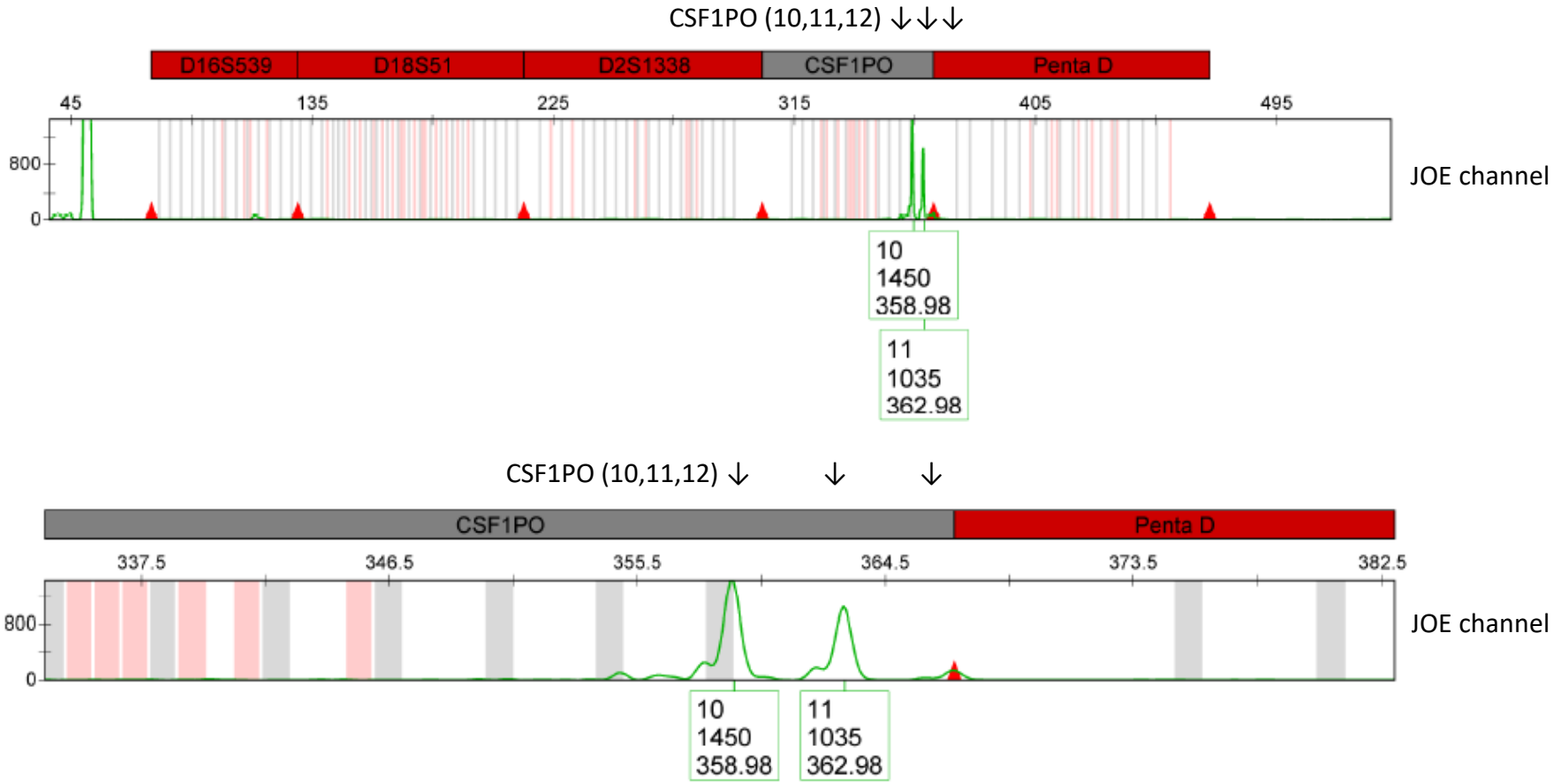
2% agarose gel electrophoresis of qPCR amplification products.

Upper panel: MW (100bp marker, Promega), samples 1-7

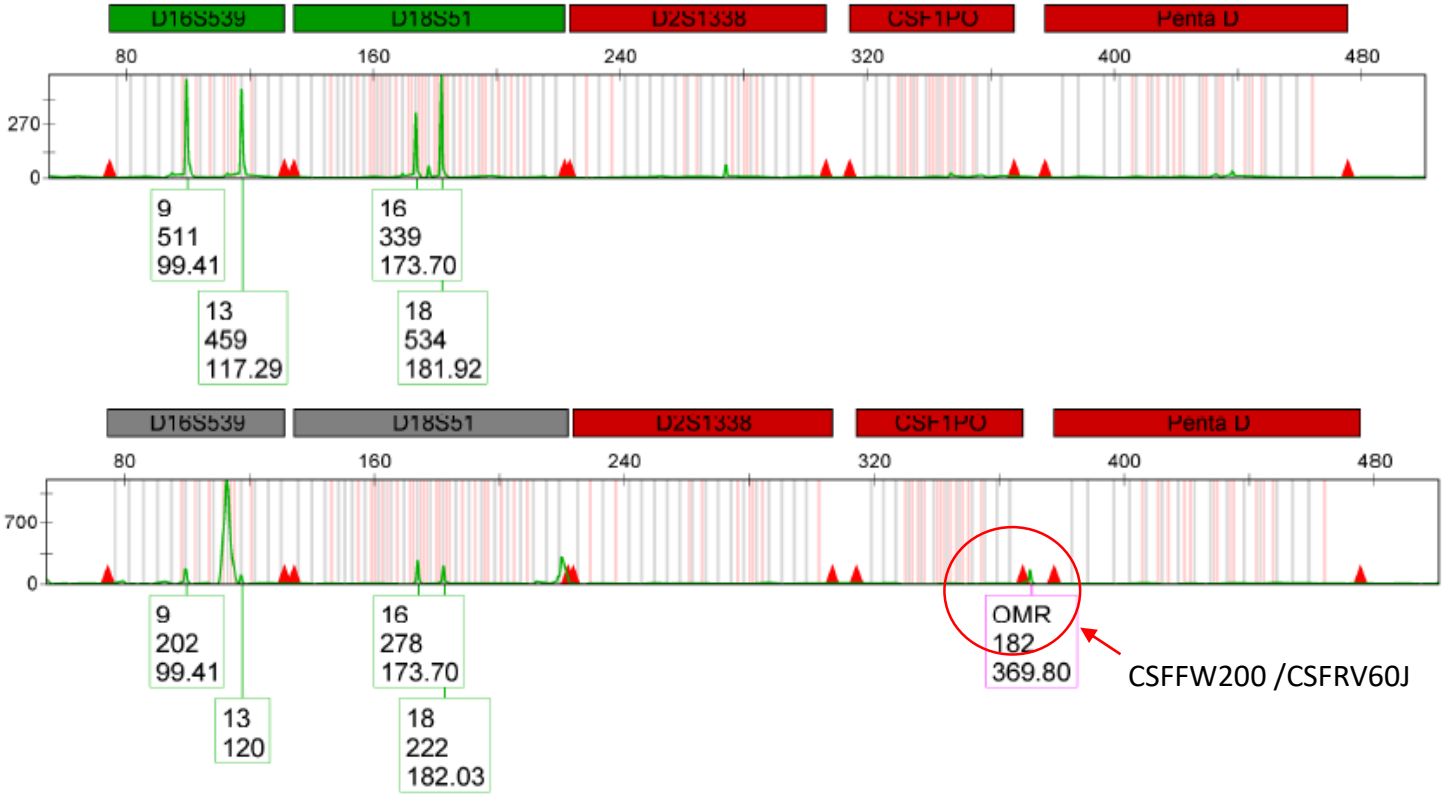
Lower panel: MW (100bp marker, Promega), samples 8-14.

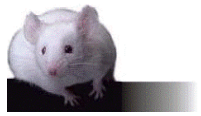


Distictive CSF1PO triplet on 9948 DNA



Spiking the superprimer pair CSFFW200 /CSFRV60J into PowerPlex™ Fusion 6C on degraded DNA (60 min)





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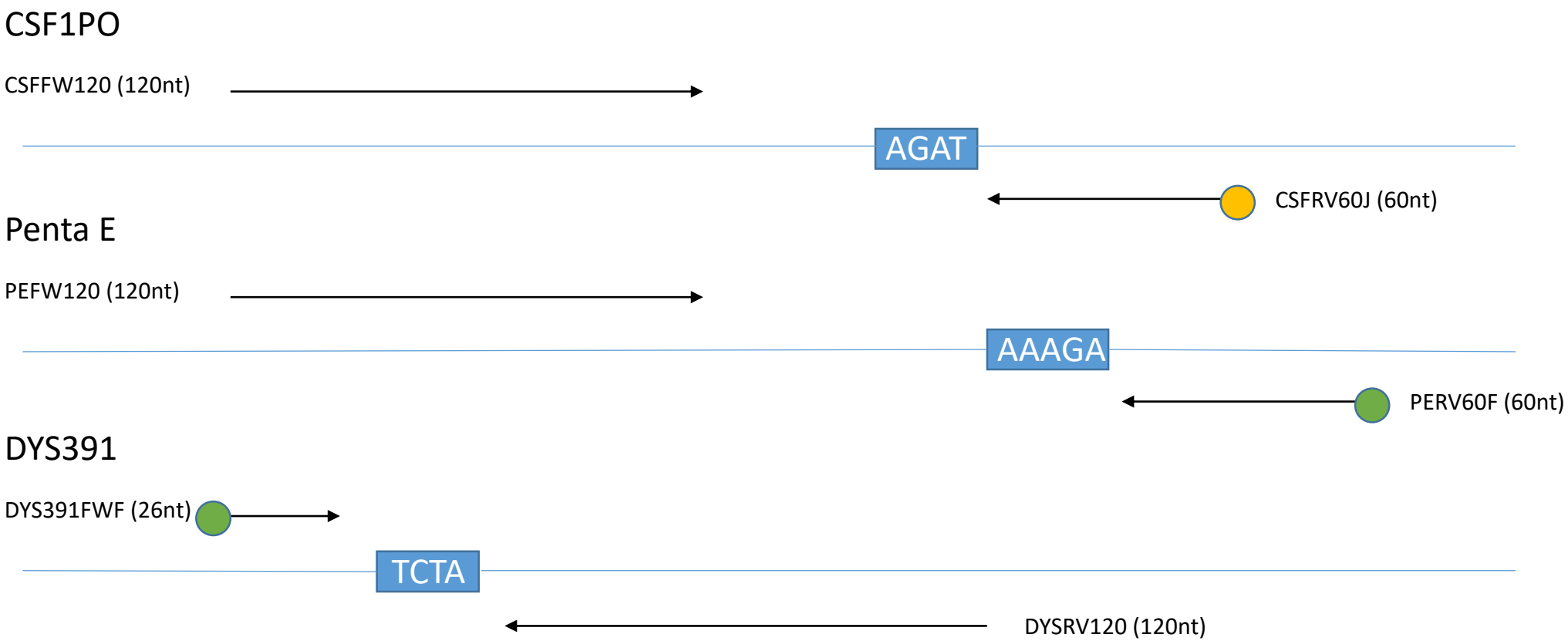
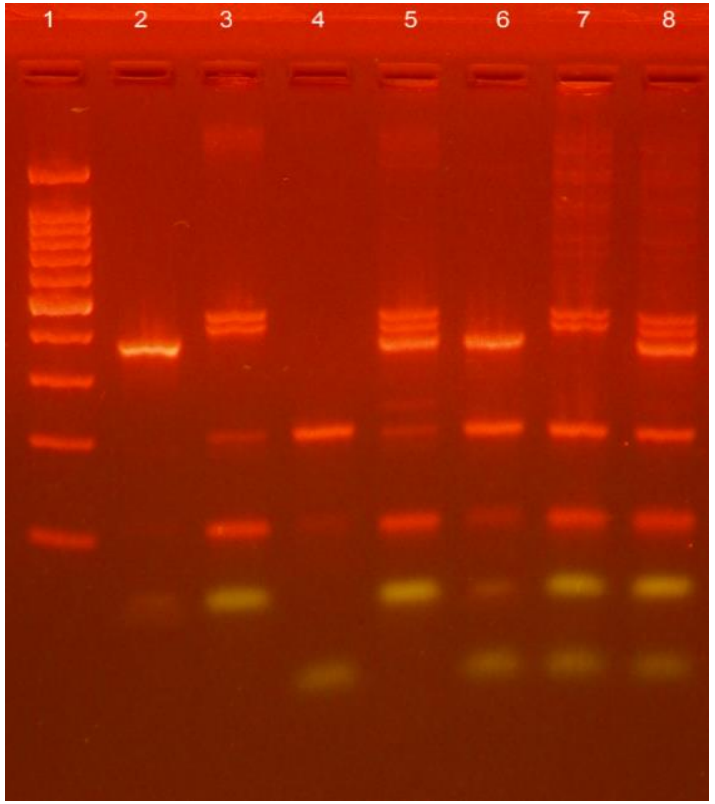
Other STR markers



Multiplex PCR



1: 100bp

2: CSF1PO

3: Penta E

4: DYS391

5: CSF1PO + Penta E

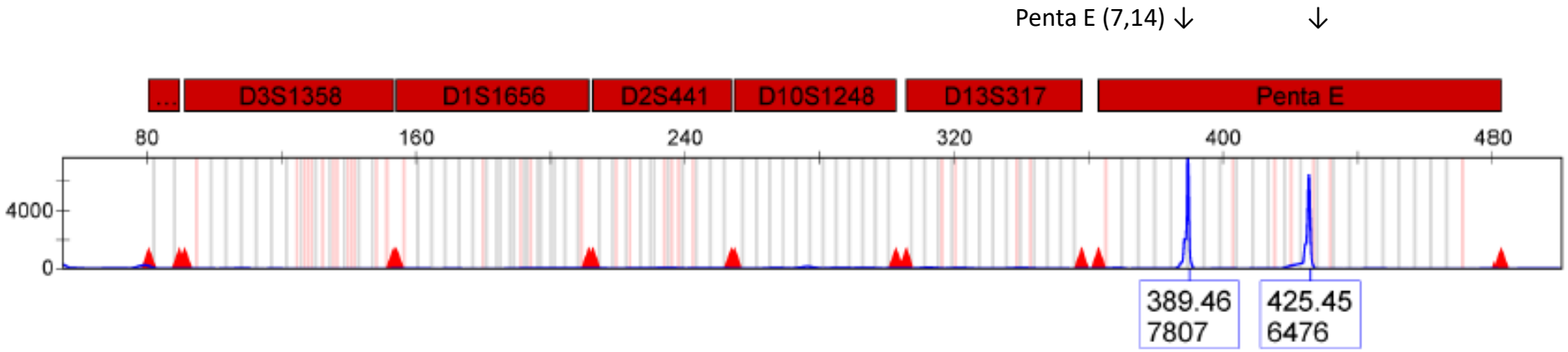
6: CSF1PO + DYS391

7:Penta E + DYS391

8: CSF1PO + Penta E + DYS391

DNA:2800M

Superprimers do not need to be fully complementary



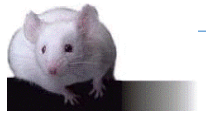
PEFW25+M13

M13 (175nt)

Penta E (25nt)

5’ ---------- 3’

gDNA

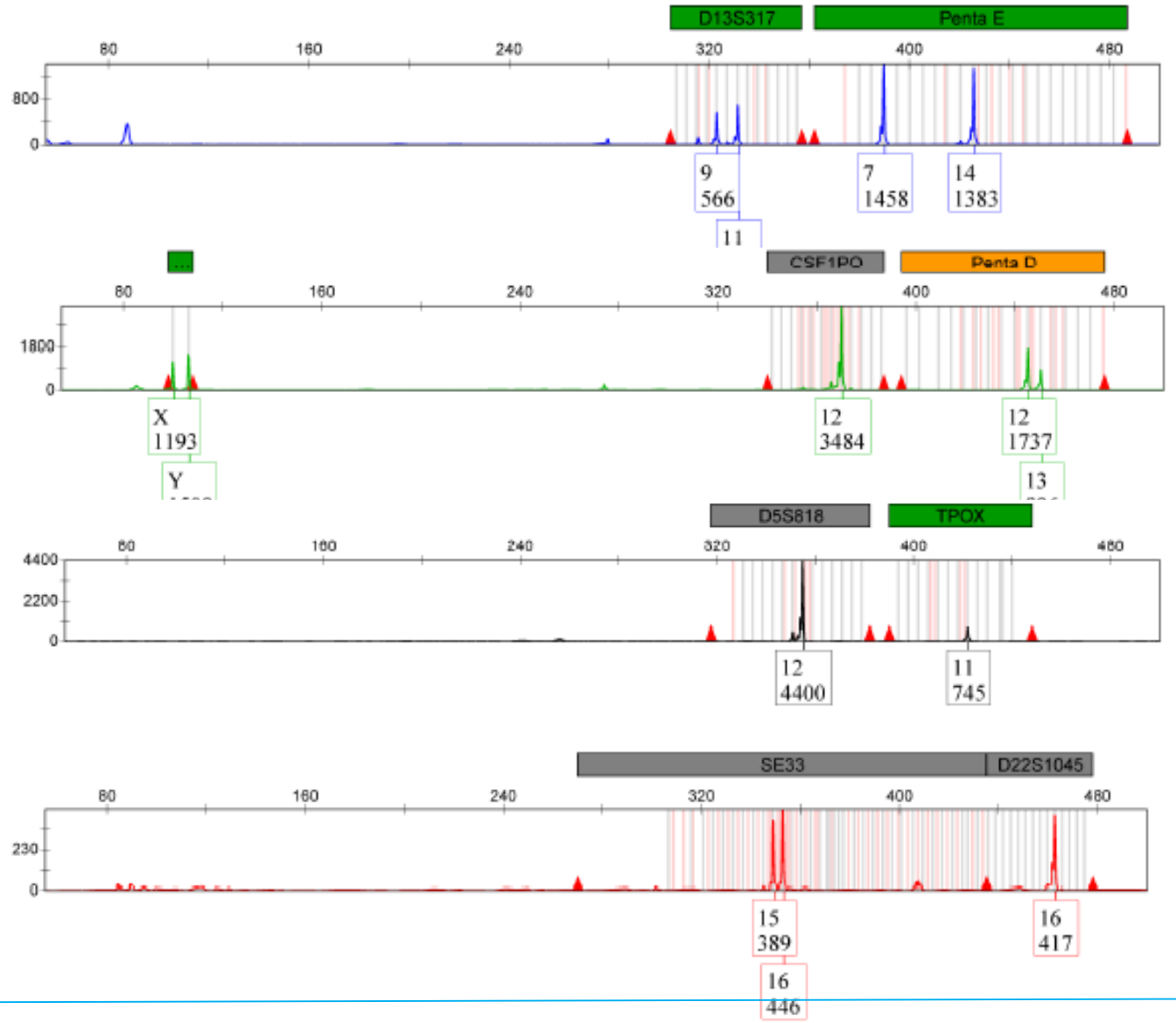


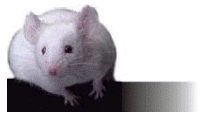
Biodynamics

Nonaplex system

Primer name	Direction	Label	Sequence	Target locus
CSF200FW	Forward	-	cggaggtaaaggtgtcttaaagtgagaaagaataactgcatcttaacctattgggaggtcattgtaaagaggagagtgatggggtcagattgtacagaggagg cacttcgtggtggtcaggagcacacactccagggcagtgttccaacctgagtctgccaaggactagcaggttgctaaccaccctgtgtctcagttt	CSF1PO
CSF60RVJOE	Reverse	NHS Ester-JOE	JOE/atctcctggtgcacacttggacagcatttcctgtgtcagaccctgttctaagtacttcct	CSF1PO
PEM1325FW	Forward	-	ccatttgcgaaatgtatctaatggtcaaactaaatctactcgttcgcagaattgggaatcaactgttacatggaatgaaacttccagacaccgtactttagttgcat atttaaaacatgttgagctacagcaccagattcagcaattaagctctaagccatccgcaaaaatgaccgctgggtgtggtggtaggcacctgt	Penta E
PE26RVFL	Reverse	56-FAM	6FAM/tgggttattaattgagaaaactccttacaattt	Penta E
D5S200FW	**Forward**	**-**	**ggcttacccccctcattttgaaaatacatgggagaaaataatacatagccacatttgtaattttctaattcaaaggagtatataattatgtaataattttaaaatta aatactgagacatgcatatgcttttaaagcttctaattaaagtggtgtcccagataatctgtactaataaaagtatattttaatagcaagtatg**	**D5S818**
D5S60RVTM	Reverse	NHS Ester-TAMRA	TAMRA/ccaatcatagccacagtttacaacatttgtatctttatctgtatccttatttatacctct	D5S818
D13M1325FW	Forward	-	acctgatttttgatttatggtcattctcgttttctgaactgtttaaagcatttgaggggattcaatgaatatttatgacgattccgcagtattggacgctatccagtct aaacattttactattacccccctctggcaaaacttcttttgcaaaagcctctcgctattttggttttaggcagcccaaaaagacagacaga	D13S317
D13M1325RVFL	Reverse	56-FAM	6FAM/ctggtaaacgagggttatgatagtgttgctcttaccatctaacgcctatctgtatttaca	D13S317
PDM1325FW	**Forward**	**-**	**ttgctcttactatgcctcgtaattccttttggcgttatgtatctgcattagttgaatgtggtattcctaaatctcaactgatgaatctttctacctgtaataatgttgtt ccgttagttcgttttattaacgtagatttttcttaaaatcgcataaggtaattcacaatgattaatacactccagcctaggtgacagagc**	**Penta D**
PDM1325RVJOE	Reverse	NHS Ester-JOE	JOE/tcttcccaacgtcctgactggtataatgagccagttaataggtcatgattttgtgatatc	Penta D
SE33M1329FWROX	Forward	NHS Ester-ROX	ROX/aattgatgccacctttttcagctcgcgccccaggaaggaaggaagaaaaagaaagaaaaag	SE33
SE33200RV	Reverse	-	ccttgcgcatgctggtgcagttgtcgacgacgacgagcgcggtgatagcatcatccatggtgagctggcggcgggtgcggacgcaaggcgcagcggcaaggac aaggttctgtgctcgctgggctgacgcggtctccgcggtgtaaggaggtttatatatatttctacaacatctcccctaccgctatagtaacttgctc	SE33
D22S200FW	**Forward**	**-**	**tccccctacagggtgactgcatctccgagtcctggcttgtcatgcctgacagagggctgccgagtgagcagcttaaggcatcctgccactgtgcagctgccaaccc tacagcccggcagccctgcgggaggaagctctagtgcaggcctcttaggatctggggtccaggatgctgatttcagggccgggaccttgggcac**	**D22S1045**
D22S60RVROX	Reverse	NHS Ester-ROX	ROX/agagtgcccggcacagtgtgagtgatcacgcgaatgtatgattggcaatatttttataac	D22S1045
TPOX60FWTM	Forward	NHS Ester-TAMRA	TAMRA/gatcactagcacccagaaccgtcgactggcacagaacaggcacttagggaaccctcactg	TPOX
TPOX200RV	Reverse	-	actgctgtgtcgtgactccccctgtgtgggaccctcgtggcggctcttacactgctgtgtcgtgactcctcctgtgtgctcacaccaagcactctcgtgtttgcgaccc caacgctcaaacgtgactcctcctgtactgtcctgggcgctcaggggaggaactgggaaccacacaggttaattaagagattcatccaaaa	TPOX
AmelFWJOE	Forward	NHS Ester-JOE	JOE/ccctgggctctgtaaagaa	Amelogenin
AmelRV	Reverse	-	atcagagcttaaactgggaagctg	Amelogenin

Nonaplex system



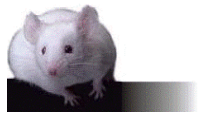


Biodynamics

Nonaplex on cadaveric samples

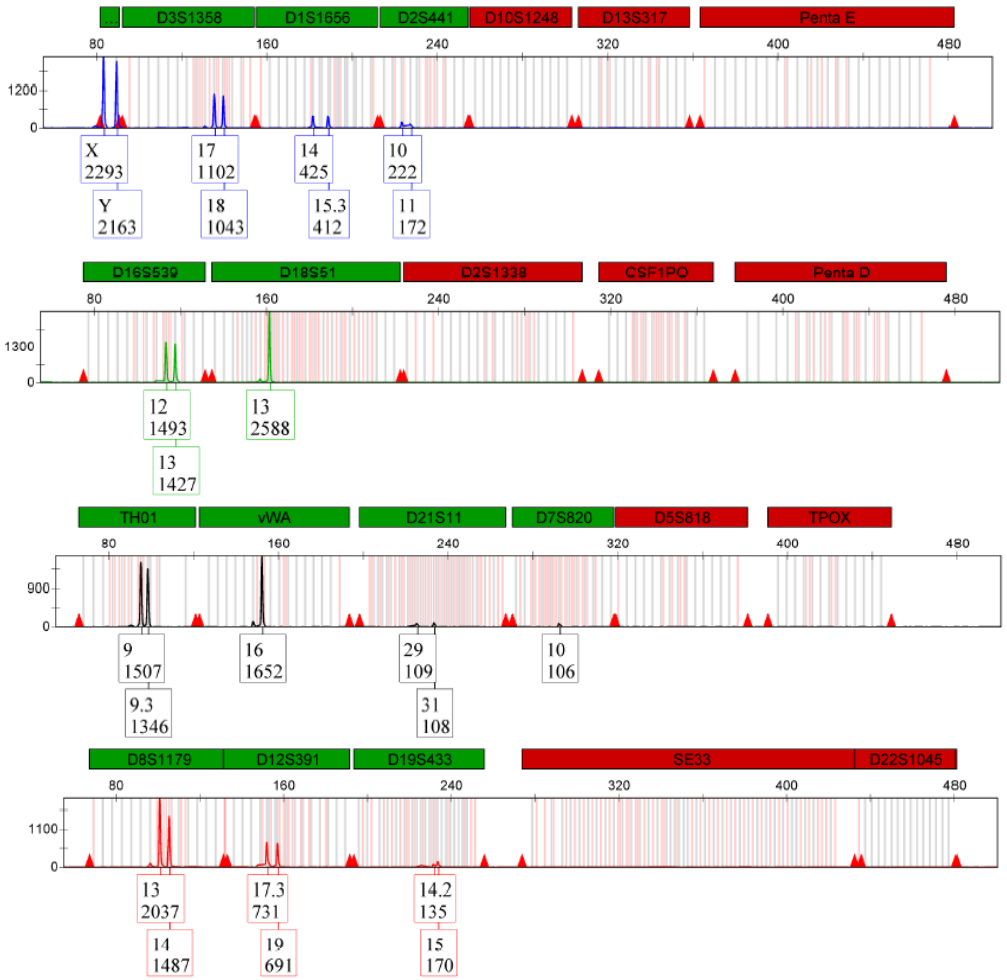
Assigned genotypes for D13S317, Penta E, CSF1PO, Penta D, Amelogenin, D5S818, TPOX, SE33 and D22S1045 in cadaveric samples with PPFusion 6C vs. Nonaplex.

	Sample A5000		Sample A5001		Sample A5004		Sample A5163		Sample A5165		Sample A5523		Sample A5570		SampleA5616	
	Fusion 6C	Nonaplex	Fusion 6C	Nonaplex	Fusion 6C	Nonaplex	Fusion 6C	Nonaplex	Fusion 6C	Nonaplex	Fusion 6C	Nonaplex	Fusion 6C	Nonaplex	Fusion 6C	Nonaplex
D13S317	-	14	-	10	11	14	-	9,12	-	9,12	-	9,12	-	8,12	-	11
Penta E	-	11,22	-	19	-	11,22	-	12,16	-	-	-	12	-	7,10	-	7,13
CSF1PO	-	10,13	-	10,11,12	13	10,13	-	10,12	-	10,12	-	11,12	-	10,12	-	11
Penta D	-	11	-	9	-	11,13		12		-	-	10	-	-	-	9,11
Amelogenin	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y
D5S818	-	9,11	-	11?	-	9,11	-	8,11	-	8,11	-	11	-	12,13	-	11,13
TPOX	-		-		-	8	-	8	-	-	-	8,10	-	-	-	-
SE33	-		-	-	-	19,31,2	-	20,28,2	-	20	-	17,26.2	-	22,27,2	-	16,23,2
D22S1045	-	-	-	-	-	15,16?	-	15	-	-	-	-	-	-	-	-

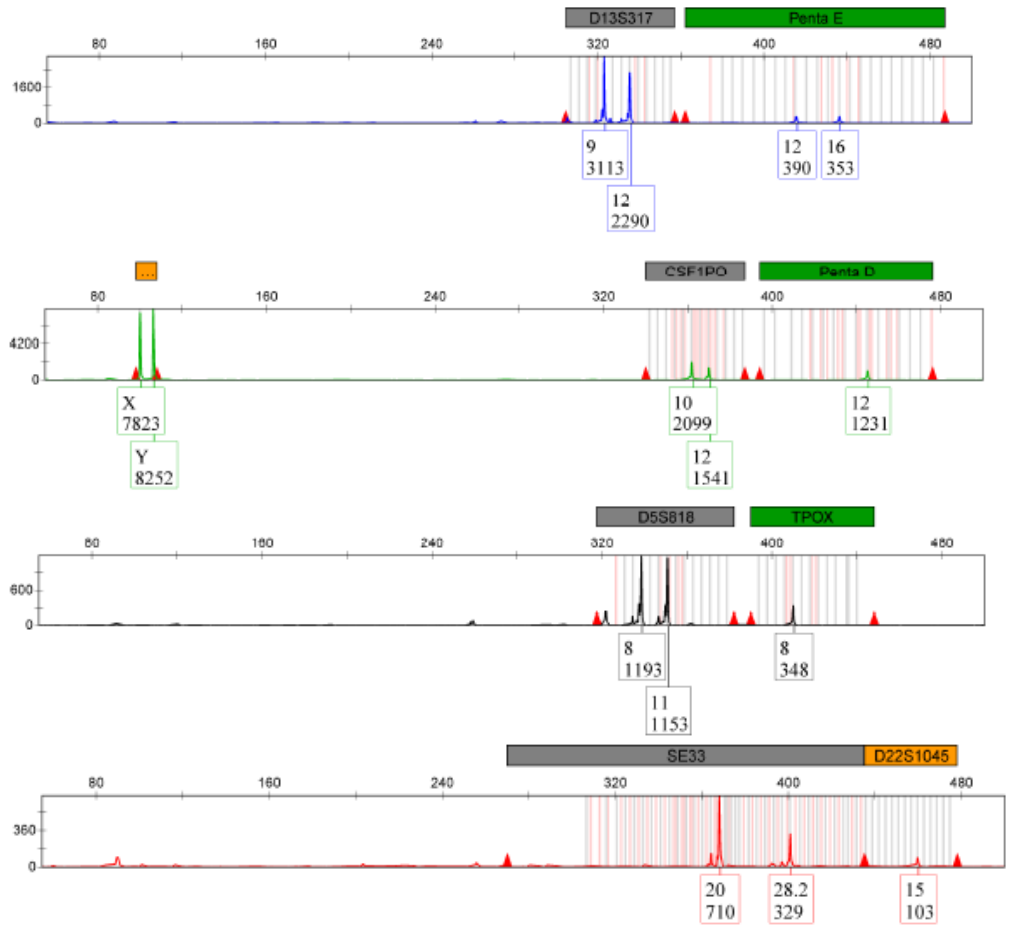


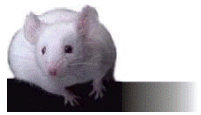
Nonaplex on cadaveric samples (A5163)

PPFusion 6C



Nonaplex



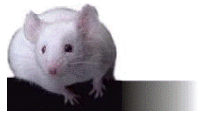


Biodynamics

Other applications

Reference samples: Preventing in/del miscalls

Prenatal and oncogenic circulating cell-free (ccfDNA) plasma samples (170 bp)



Summary

✓ Polynucleotide primers provide
more complete CE profiles for degraded DNA samples.