Supplementary Materials

Figure S1. Primer sequences that were used for amplification of the whole *psbA* gene in *A. artemisiifolia* by Sanger sequencing. Numbering of location is based on *in silico* identified mRNA sequence of psbA gene (MT425203).

Primer	Length (bp)	Ann. °C	Location (bp)	Sequence 5′-3′
AmbpsbA1F	574	59	1-24	ATGACTGCAATTTTAGAGAGACGC
AmbpsbA1R			554-574	ATTCCAGGCTGAGCACAACA
AmbpsbA2F	599	58	464-487	CTGCTGTTTTCTTGATCTACCCA
AmbpsbA2R			1036-1062	TTATCCATTTGTAGATGGAGCTTCAA

The schematic representation of the *psbA* gene in *A. artemisiifolia* indicating regions amplified by different primers. Vertical grey lines indicate amino acid positions that have been effected to herbicide resistance-conferring amino acid substitutions. The number after the amino acid refers to the amino acid position in the *psbA* gene *of A. artemisiifolia.* Colour lines show positions of two primer pairs which cover the whole *psbA* sequence. Numbers between round brackets are start and end positions of primers numbered by Sanger sequenced fragments of target enzyme (MT879746).

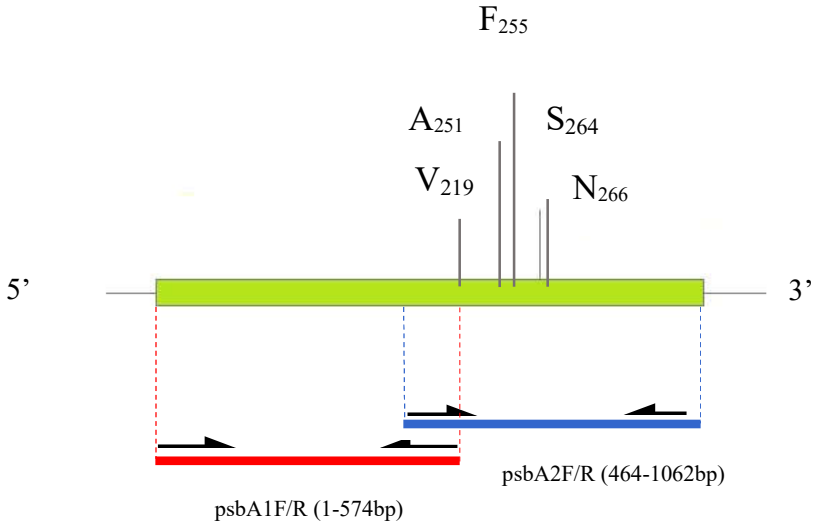
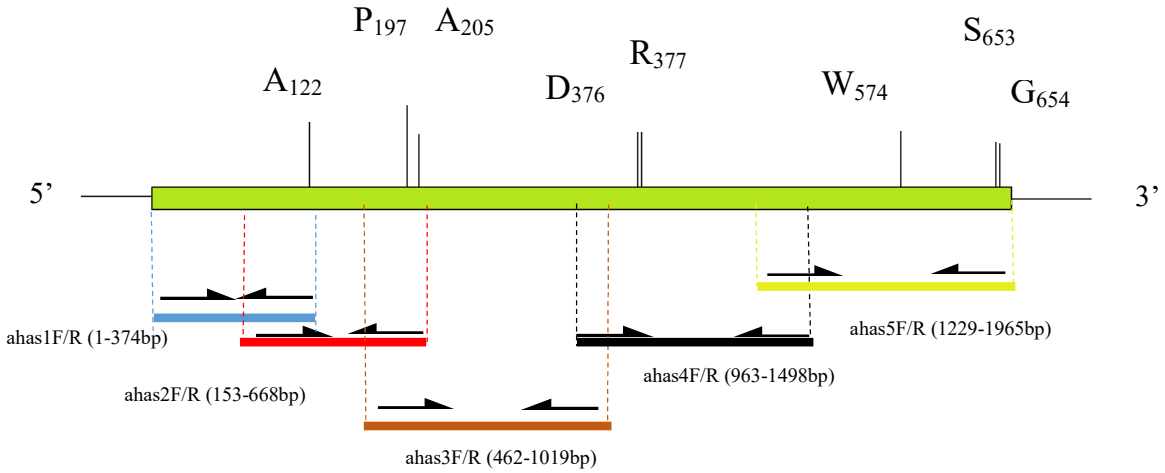


Figure S2. Primer sequences that were used for amplification of the whole *ahas* gene in *A. artemisiifolia* by Sanger sequencing. Numbering of location is based on *in silico* identified mRNA sequence of *ahas* gene (MK096760).

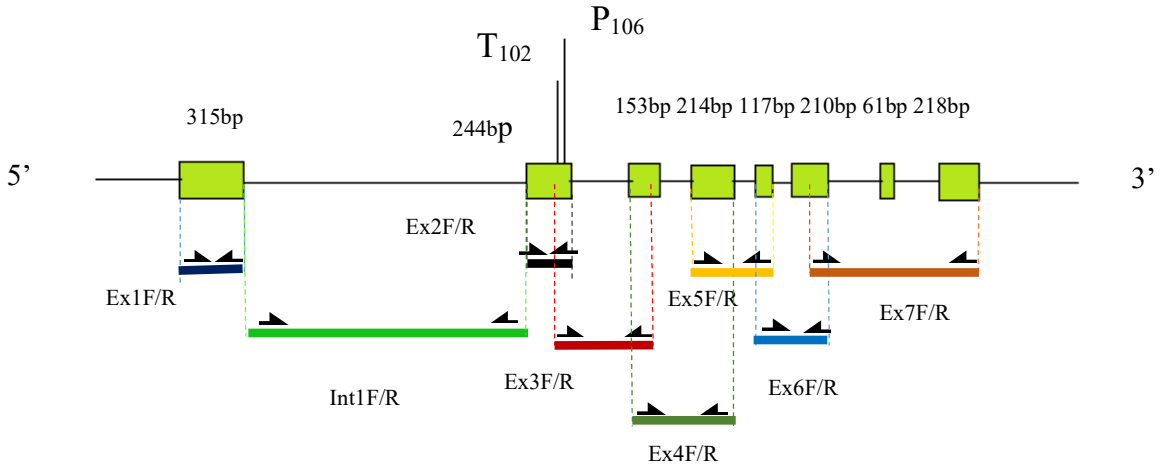
Primer	Length (bp)	Ann. °C	Location (bp)	Sequence 5′-3′
Ambahas1F	374	57	1-18	ATGGCGGCGGCTATCTCT
Ambahas1R			354-374	ACCATCCGAAACGTCCTTCC
Ambahas2F	516	60	153-173	CAACGTTCTCTCCGACCACA
Ambahas2R			646-668	CTAGGGTTGTTAGGGAGGCTTT
Ambahas3F	557	60	462-485	TGGAGCTACAAACCTAGTCAGTG
Ambahas3R			999-1019	GGGATGCATGGGACGGTTTA
Ambahas4F	535	60	963-986	GTACCCTGCTTCGAGTGATTTGT
Ambahas4R			1478-1498	ACTAGGGGCGATGGGTTTTG
Ambahas5F	736	62	1229-1253	TGGAGGAAAAGAATTCGGTGACTA
Ambahas5R			1941-1965	GGCGATGGCAGAACGAAATACTAA



The schematic representation of the *ahas* gene in *A. artemisiifolia* indicating regions amplified by different primers. Vertical grey lines indicate amino acid positions that have been effected to herbicide resistance-conferring amino acid substitutions. The number after the amino acid refers to the amino acid position in the *ahas* gene of *Arabidopsis thaliana.* Colour lines show positions of five primer pairs which cover the whole *ahas* sequence. Numbers between round brackets are start and end positions of primers numbered by Sanger sequenced fragments of target enzyme (MT415954).

Figure S3. Primer sequences that were used for amplification of the whole *epsps* gene in *A. artemisiifolia* by Sanger sequencing. Numbering of location is based on *in silico* identified mRNA sequence (Transcriptome analysis - TA) of *epsps* gene (MK096765) and amplified *epsps* gene by Sanger sequencing (SS) method (MT415955, MT409110).

Primer		Lengt	Locatio	Lengt	Locatio	Ann	Sequence 5′-3′
1	AmbEpspsEx1	315	1-24	315	1-24	60	ATGGCGATTCACCTTAACAACA
	AmbEpspsEx1		295-315		295-315		CTTTTAGCTGCTCTTGCTGAG
2	AmbEpspsInt	1043	287-308	47	287-308	56.6	GGATCCTTCTTTTAGCTGCTCT
	AmbEpspsInt		1311-		315-334		AGGGGACTACTGTTGTAGACA
3	AmbEpspsEx2	243	1311-	243	315-334	58	AGGGGACTACTGTTGTAGACA
	AmbEpspsEx2		1536-		540-558		GCTGCTGGTGGAAACTCA
4	AmbEpspsEx3	605	1370-	329	374-399	58	CTTTGAGAGCTCTAGGGTTAAA
	AmbEpspsEx3		1955-		683-703		AGCTGCCAATGGAGGTCTTC
5	AmbEpspsEx4	498	1831-	370	559-583	59	AGCTACATACTAGATGGTGTTC
	AmbEpspsEx4		2308-		908-929		CCGAGGTGGTCAAAAGTACAA
6	AmbEpspsEx5	408	2117-	327	717-742	56	CAAACTGTCGGGATCTATTAGT
	AmbEpspsEx5		2504-		1023-		GGTTGTGGGACAAGCAGTTTA
7	AmbEpspsEx6	455	2410-	327	930-953	58	ATCACCTGGAAATGCTTACGTA
	AmbEpspsEx6		2846-		1237-		GTCCCACAGCCATTAGAGAC
8	AmbEpspsEx7	942	2596-	471	1068-	59	GGTCCTTGGACAAATGGGTG
	AmbEpspsEx7		3515-		1515-		CTTCAGAGATTCACCAAGCATT

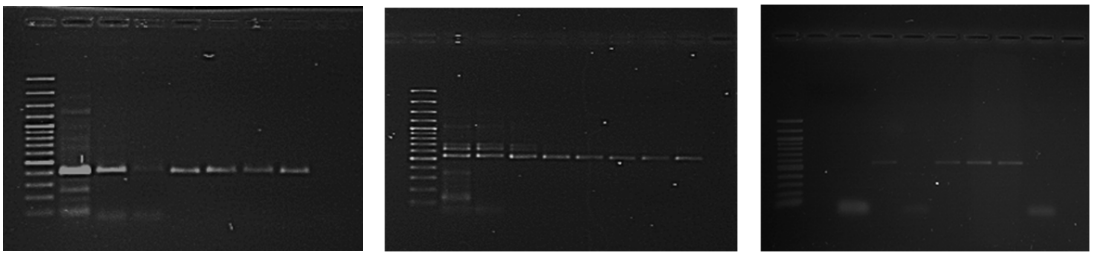


The schematic representation of the *epsps* gene in *A. artemisiifolia* indicating regions amplified by different primers. Green boxes indicate exons and grey lines between boxes indicate introns. Numbers over the boxes show the length of each individual exon. Vertical grey lines indicate amino acid positions that have been effected to herbicide resistance-conferring amino acid substitutions. The number after the amino acid refers to the amino acid position in the *epsps* gene of *Eleusine indica*. Coloured lines show positions of eight primer pairs which cover the whole epsps sequence.

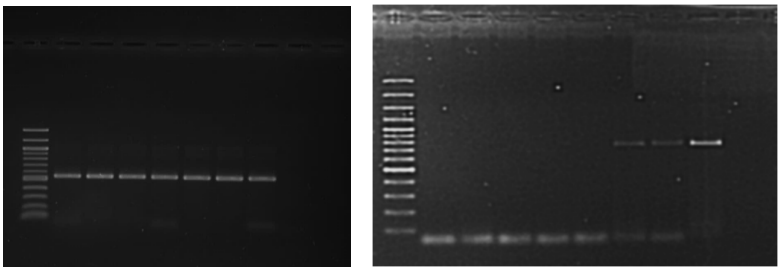
Profile of PCR: The PCR amplifications was performed in a final volume of 15 µL, containing 50 ng of template DNA, 10 × PCR buffer Dream Taq, 0.2mM dNTP, 1.2 µM of primers, and 1.25 U of Dream Taq DNA polymerase (Thermo Fisher Scientific, USA). PCR reaction was carried out in a Eppendorf Mastercycler ep384 (Eppendorf AG, Germany) with the following profile: 94°C for 3 min; 39 cycles of 94°C for 30 sec, at the primer annealing temperature for 1 min, 72°C for 1 min; and a final extension at 72°C for 7 min. The PCR products were separated on 1.5% agarose gel (Promega, USA) in 0.5 % TBE buffer (220 V; 40 min) and post-stained in ethidium bromide buffer. Samples were purified using NucleoSpin Gel and PCR Clean-up system (Macharey-Nagel GmbH &Co, Germany).

Figure S4. Gel pictures of Sanger sequencing

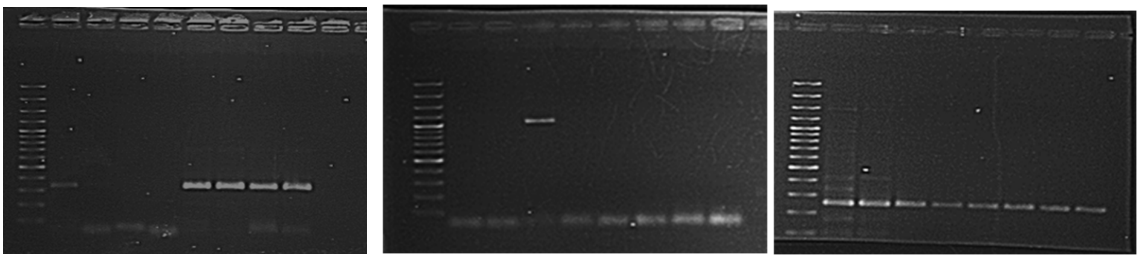
Ambahas1F/R (length 374bp) Ambahas2F/R (516bp) Ambahas3F/R (557bp)



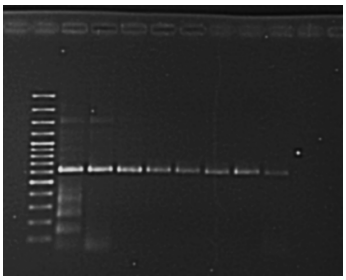
Ambahas4F/R (535bp) Ambahas5F/R (736bp)



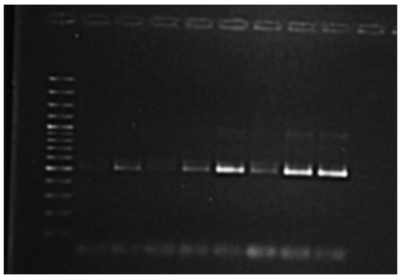
AmbEpspsEx1F (315bp) AmbEpspsInt1F (916bp) AmbEpspsEx2F (243bp)



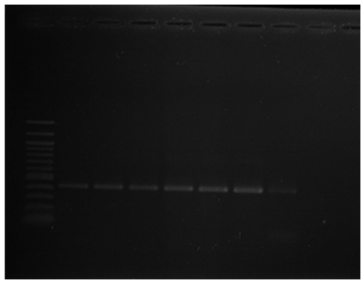
AmbEpspsEx3F (605bp)



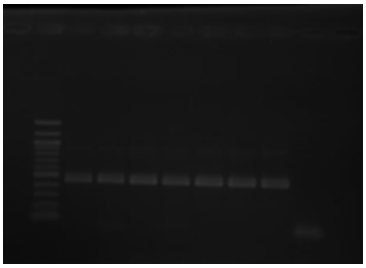
AmbEpspsEx4F (498 bp)



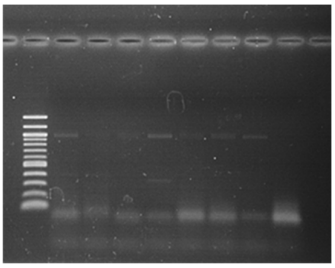
AmbEpspsEx5F (408bp)



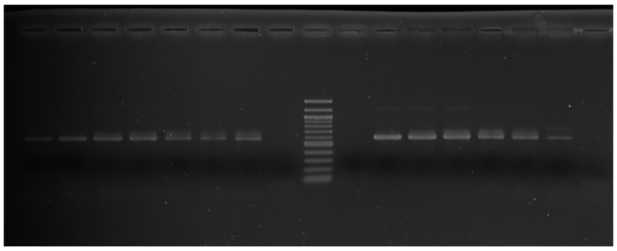
AmbEpspsEx6F (455bp)



AmbEpspsEx7F (942bp)



AmbpsbA1F/R and AmbpsbA2F/R (574 and 599 bp)



Ambppx2 amp 98F/R

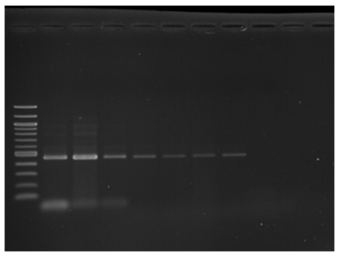
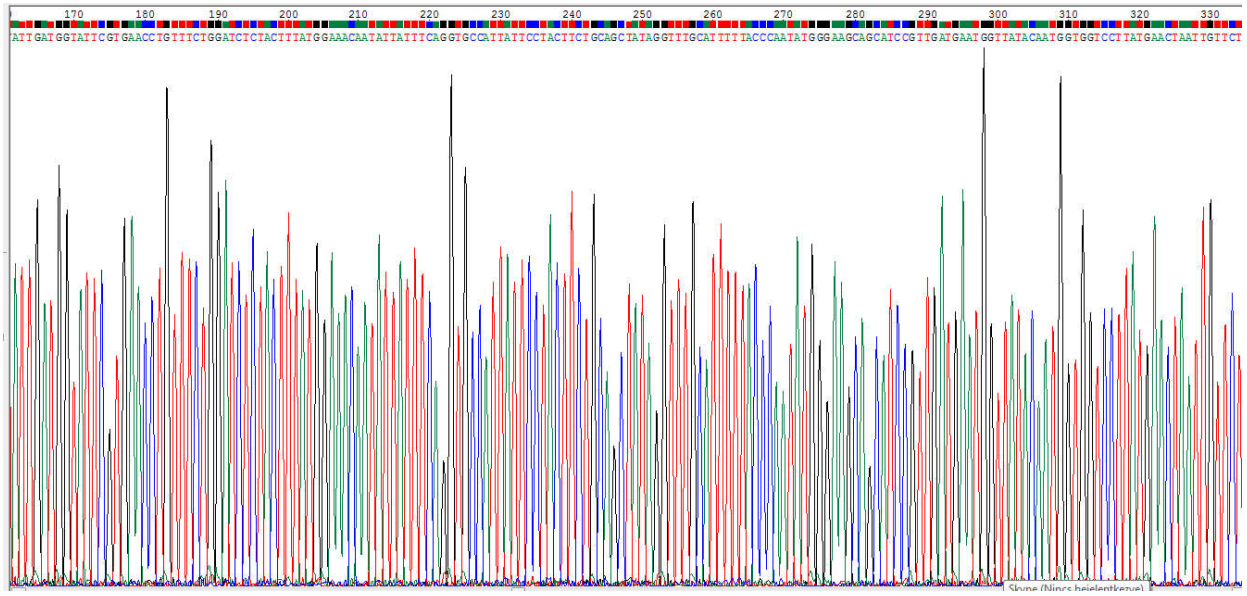
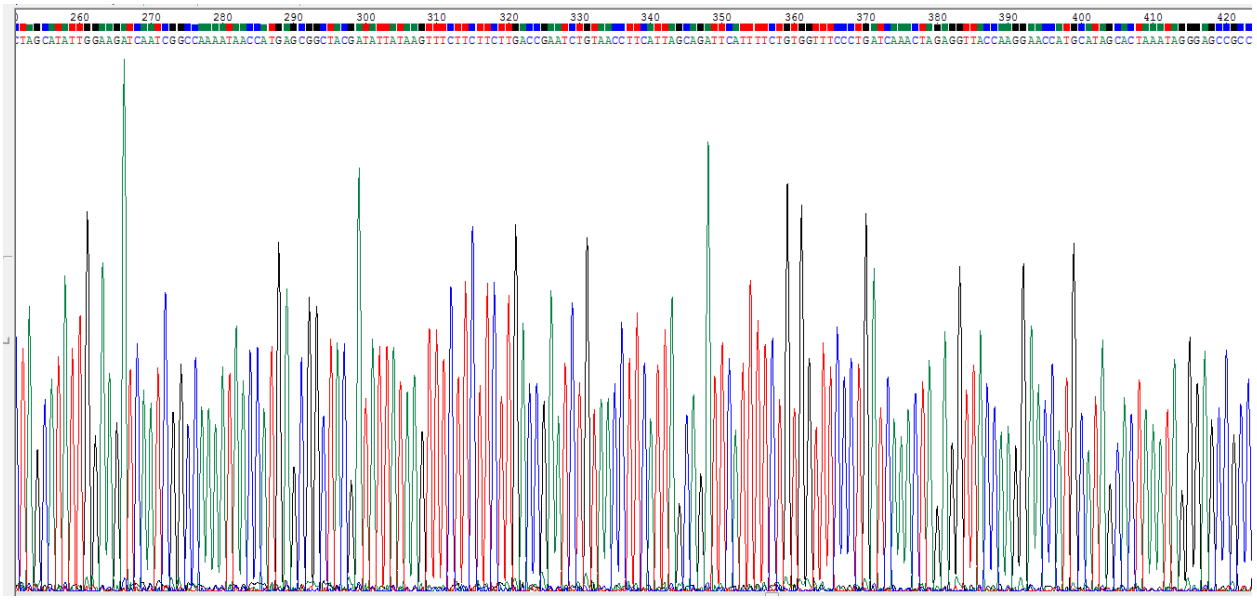


Figure S5. Electropherograms of Sanger sequencing

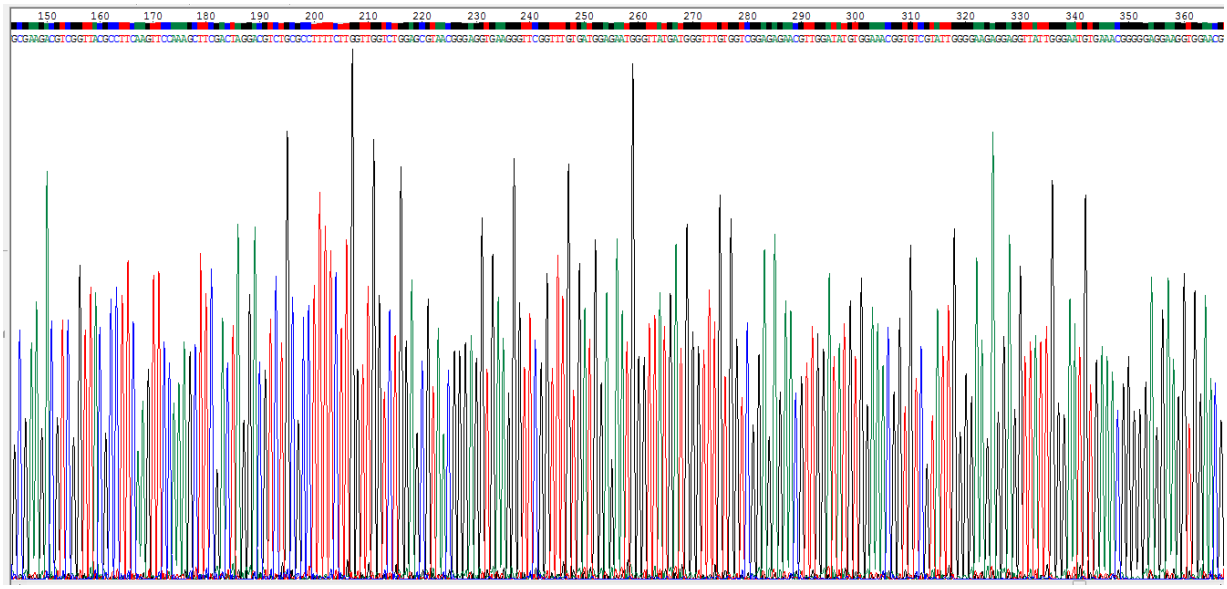
Electropherogram data of the fragment that was amplified with AmbpsbA1F/R primer pair in *A. artemisiifolia*.



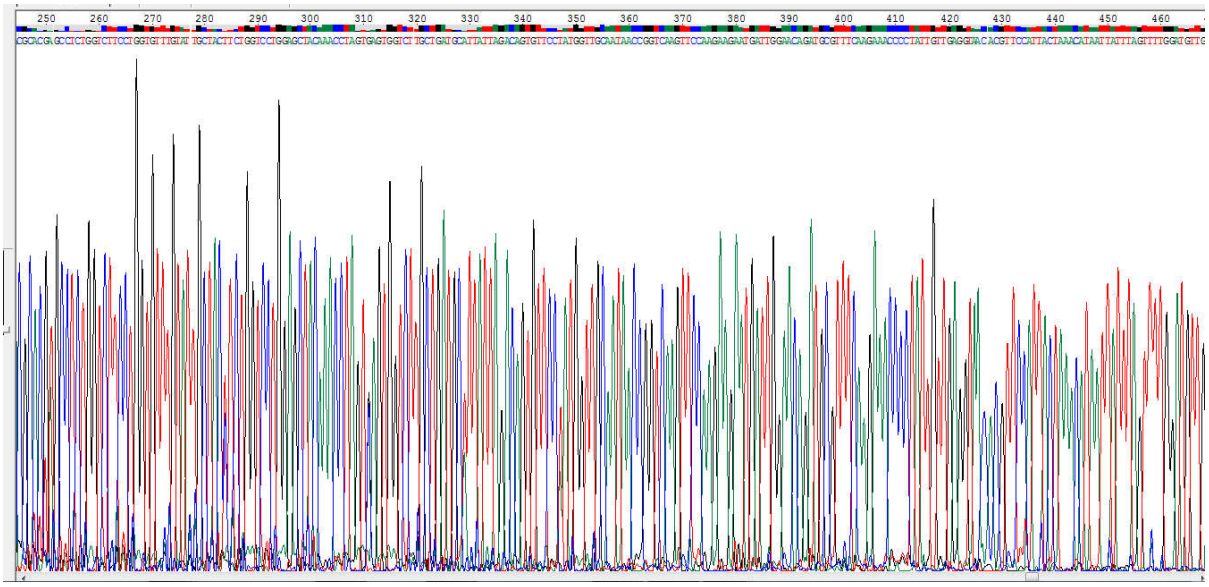
Electropherogram data of the fragment that was amplified with AmbpsbA2F/R primer pair in *A. artemisiifolia*.



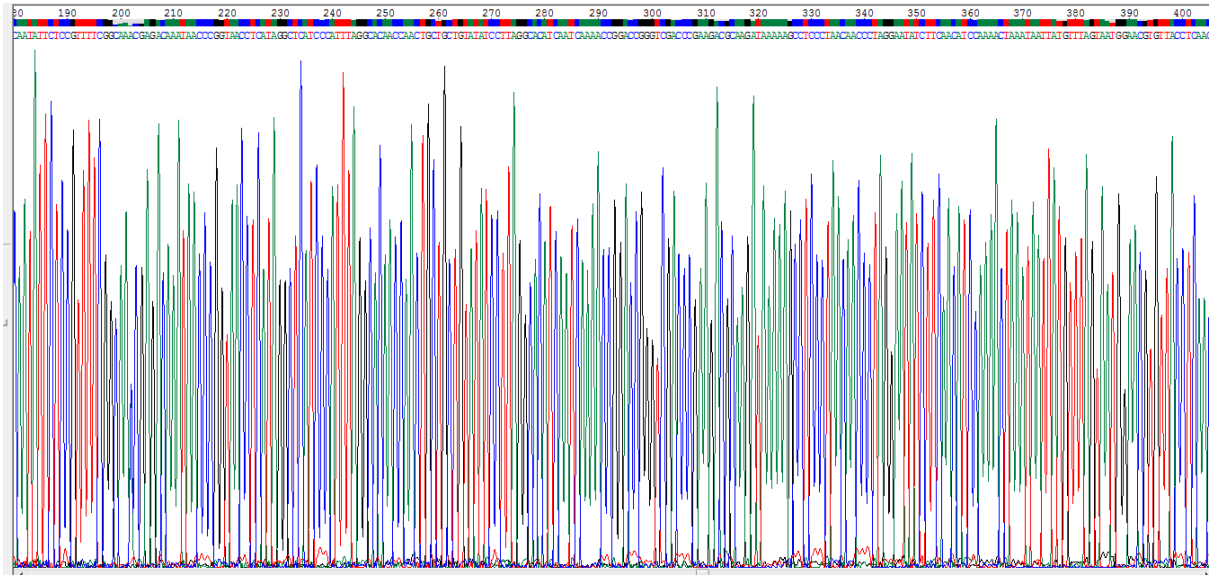
Electropherogram data of the fragment that was amplified with Ambahas1F/R primer pair in *A. artemisiifolia*.



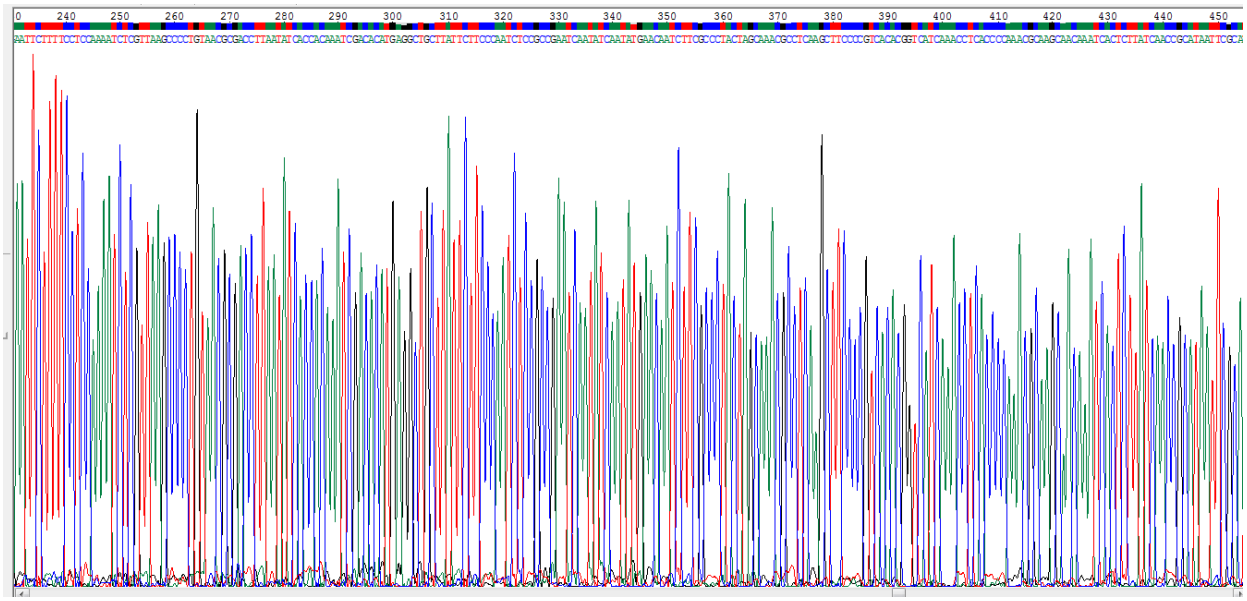
Electropherogram data of the fragment that was amplified with Ambahas2F/R primer pair in *A. artemisiifolia*.



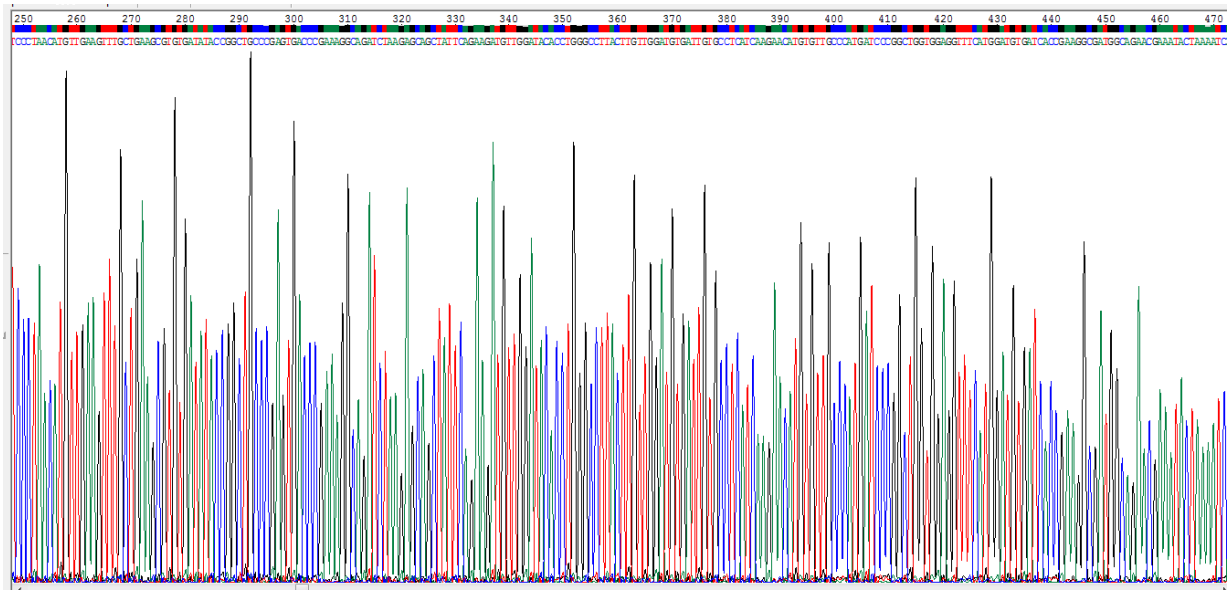
Electropherogram data of the fragment that was amplified with Ambahas3F/R primer pair in *A. artemisiifolia*.



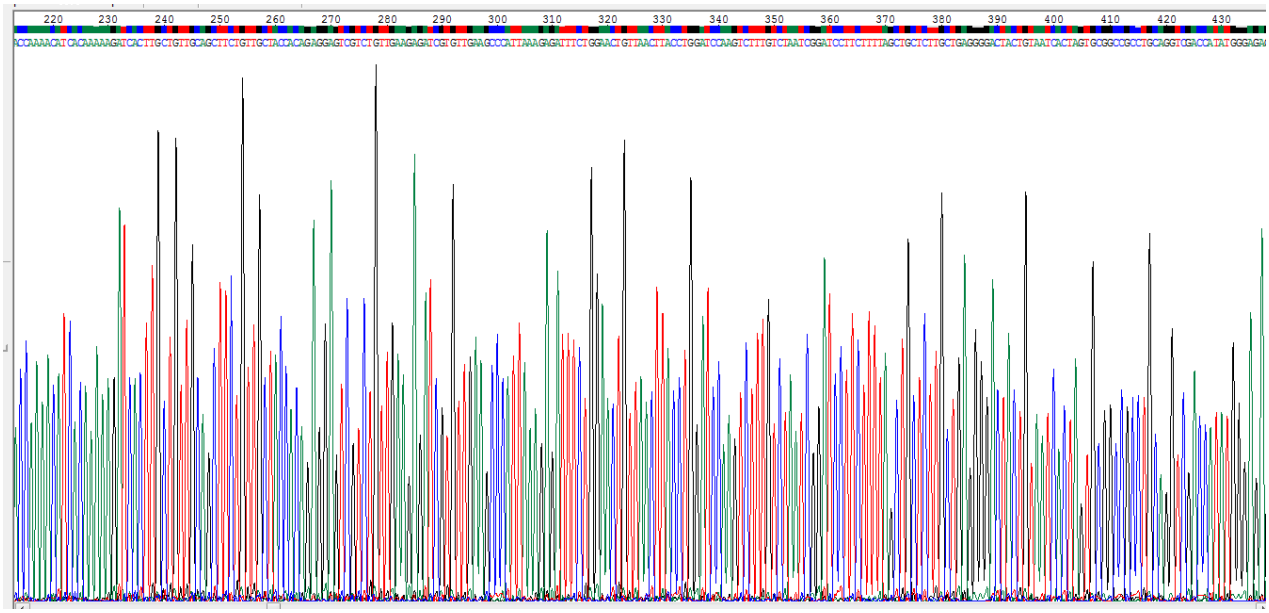
Electropherogram data of the fragment that was amplified with Ambahas4F/R primer pair in *A. artemisiifolia*.



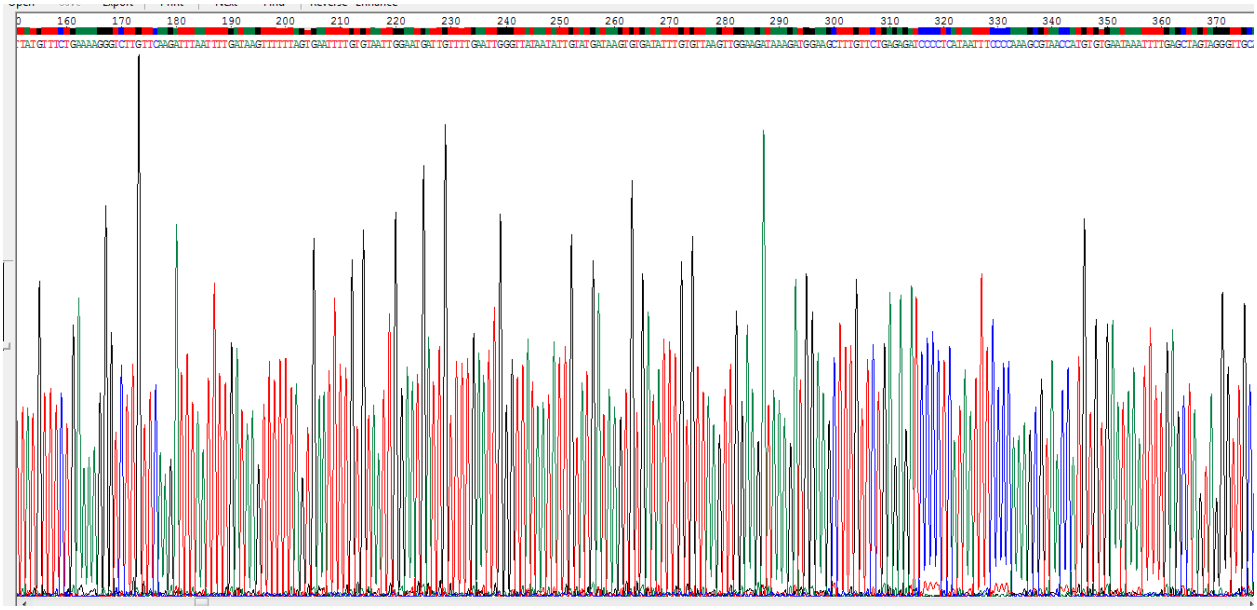
Electropherogram data of the fragment that was amplified with Ambahas5F/R primer pair in *A. artemisiifolia*.



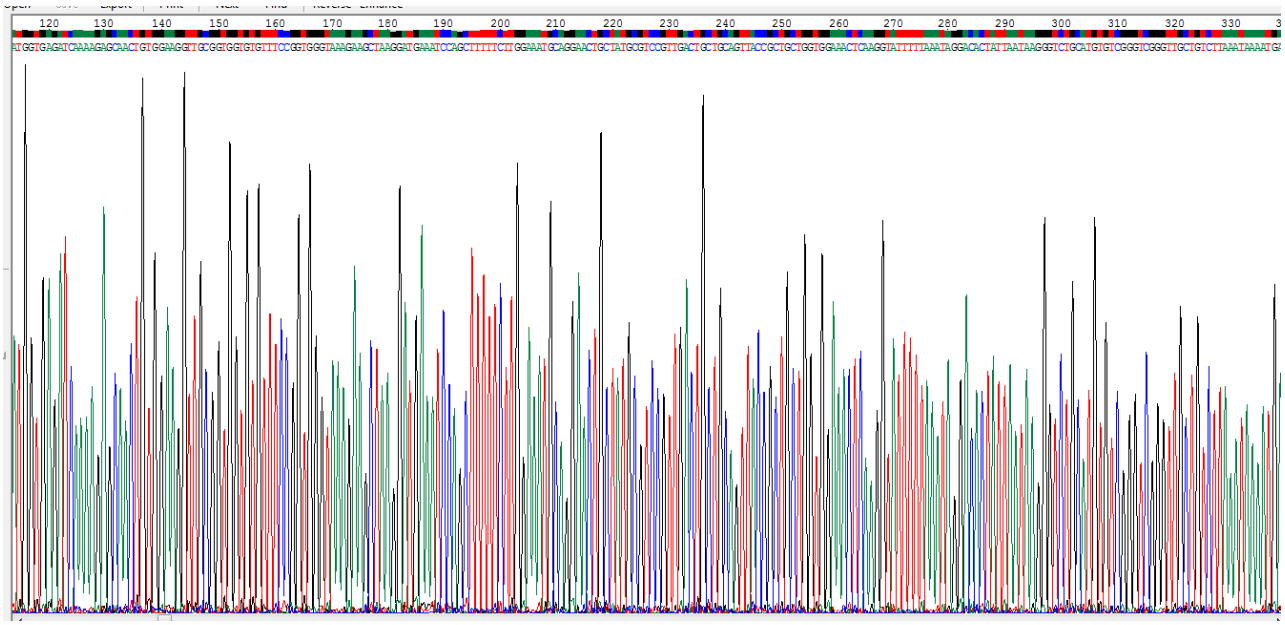
Electropherogram data of the fragment that was amplified with AmbEpspsEx1F/R primer pair in *A. artemisiifolia*.



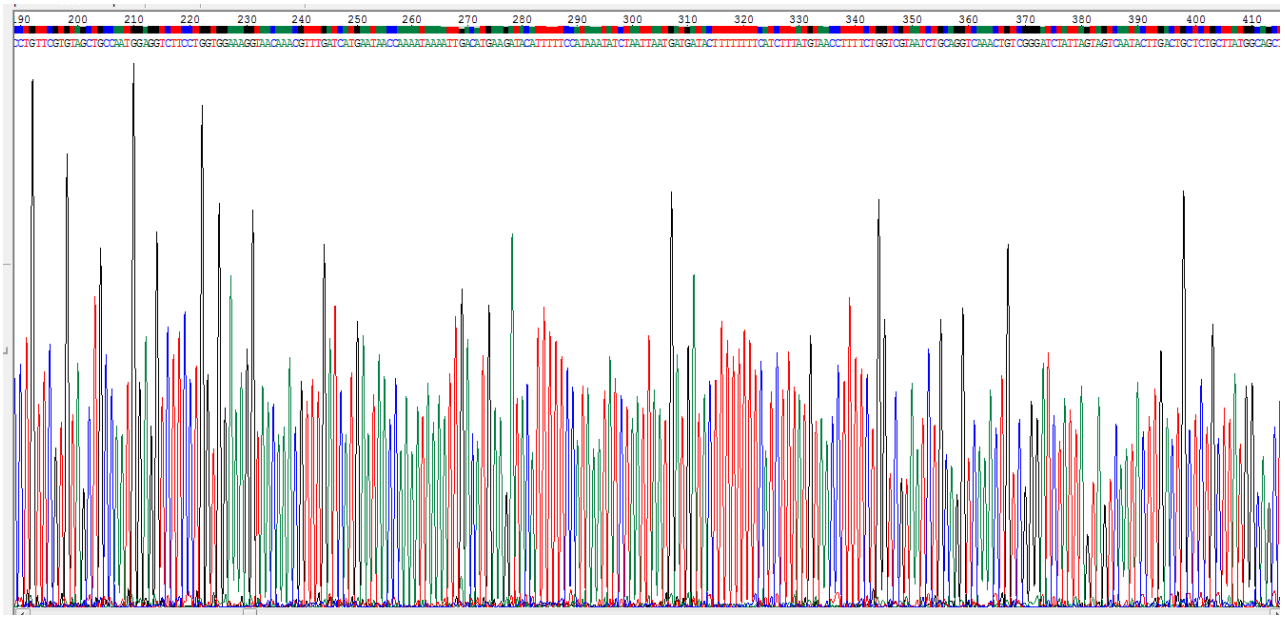
Electropherogram data of the fragment that was amplified with AmbEpspsInt1F/R primer pair in *A. artemisiifolia*.



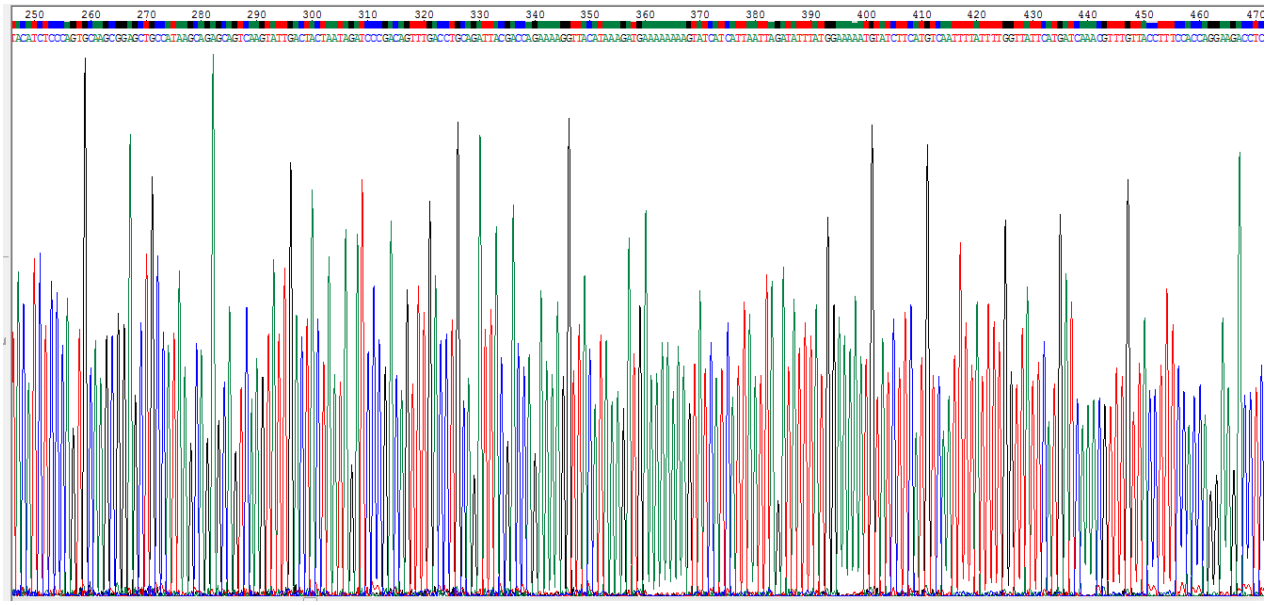
Electropherogram data of the fragment that was amplified with AmbEpspsEx2F/R primer pair in *A. artemisiifolia*.



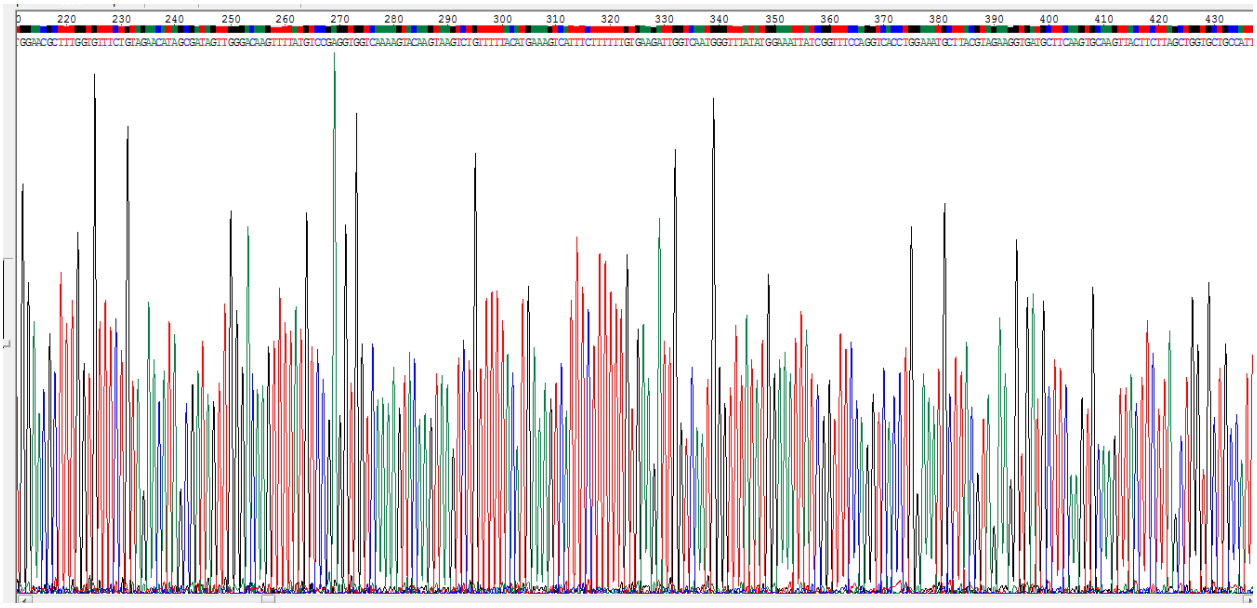
Electropherogram data of the fragment that was amplified with AmbEpspsEx3F/R primer pair in *A. artemisiifolia*.



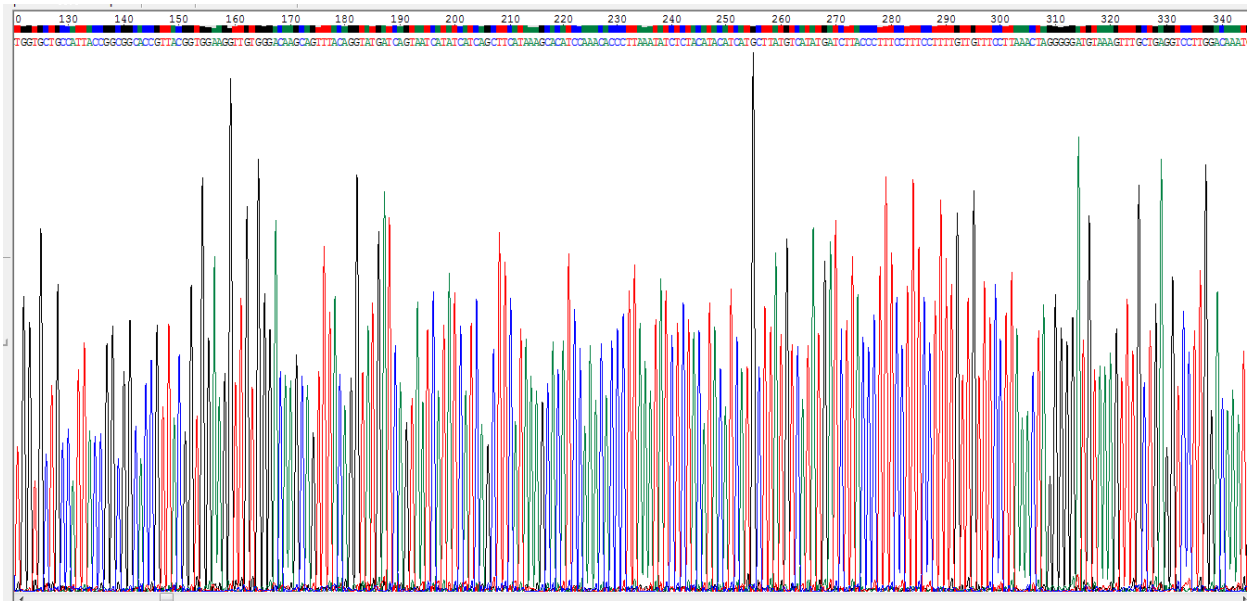
Electropherogram data of the fragment that was amplified with AmbEpspsEx4F/R primer pair in *A. artemisiifolia*.



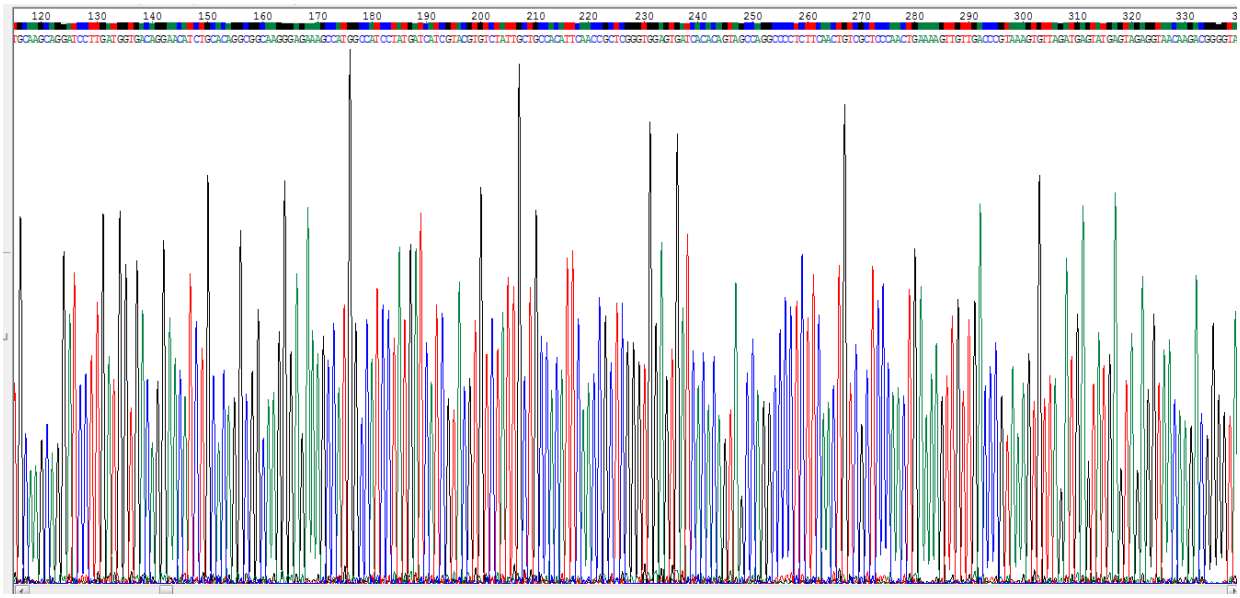
Electropherogram data of the fragment that was amplified with AmbEpspsEx5F/R primer pair in *A. artemisiifolia*.



Electropherogram data of the fragment that was amplified with AmbEpspsEx6F/R primer pair in *A. artemisiifolia*.



Electropherogram data of the fragment that was amplified with AmbEpspsEx7F/R primer pair in *A. artemisiifolia*.



Electropherogram data of the fragment that was amplified with ppx2 amp 98F/R primer pair in *A. artemisiifolia*.

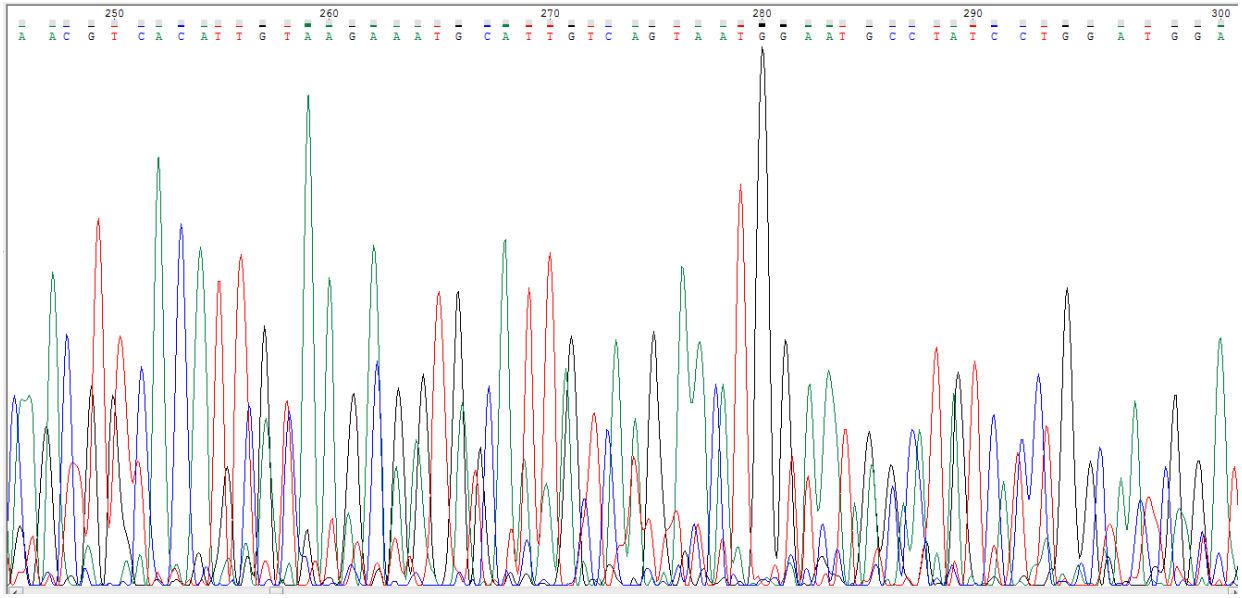


Figure S6. Comparison of protein sequences of a) *psbA* b) *epsps* c) *ahas* and d) *ppx2* genes in *A. artemisiifolia*. Abbreviations: Q, (query) coding sequences deriving from Sanger-sequenced data (SS), S (subject) coding sequences deriving from *in silico* predicted cds of Illumina data (Transcriptome analysis –TA).

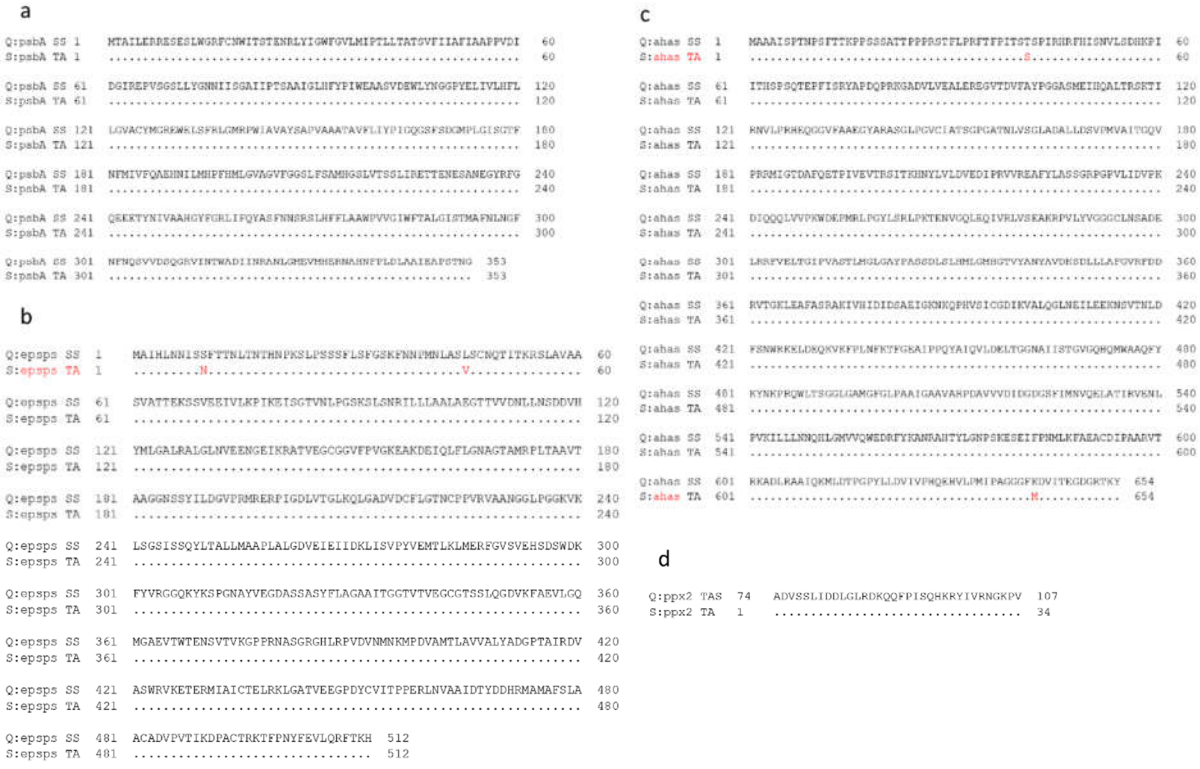


Figure S7. Primer sequences used for amplicon sequencing of *psbA, ahas, epsps*, and *ppx2* genes in *A. artemisiifolia.*

Gene	Primer name with resistance-conferring amino acid position	Length with adapter (bp)	Sequence 5′-3′
ahas1	ahas amp 122F	288	CAACGTTCTCTCCGA
	ahas amp 122R		ACCATCCGAAACGT
ahas2	ahas amp 197-205F	273	TGGAGCTACAAACCT
	ahas amp 197-205R		CTAGGGTTGTTAGGG
ahas3	ahas amp 376-377F	319	GGGATGCATGGGAC
	ahas amp 376-377R		TGGAGGAAAAGAAT
ahas4	ahas amp 574-653-654F	453	ATGAATGTTCAAGA
	ahas amp574-653-654R		GGCGATGGCAGAAC
epsps	epsps amp 102-106F	310	AGGGGACTACTGTTG
	epsps amp 102-106R		GCTGCTGGTGGAAA
ppx2	ppx2 amp 98F	390	GGCAGATGTTAGCA
	ppx2 amp 98R		CATTGTAAGAAATG
psbA	psbA amp 219-266F	377	ATGAAGGTTACAGA
	psbA amp 219-266R		TATCATTAACCGTGC
adapters	IlluminaNextera adapterF	33	TCGTCGGCAGCGTCA
	IlluminaNextera adapterR	34	CTGTCTCTTATACAC

Profil of PCR: The PCR amplifications were performed in a final volume of 25 µL, containing 100 ng of template DNA, 12.5 µL 2X KAPA HiFi HotStart ReadyMix (F. Hoffmann-La Roche, Switzerland) and 0.3 µM of both primers. The ReadyMix contains KAPA HiFi HotStart DNA Polymerase (0.5 U per 25 µL reaction) in a proprietary reaction buffer containing dNTPs (0.3 mM of each dNTP at 1X), MgCl2 (2.5 mM at 1X) and stabilizers. Amplification was carried out in a GeneAmp® PCR System 9700 Thermal Cycler (Ap-plied Biosystems, USA) with the following profile: 95°C for 3 min, 25 cycles of 98°C for 20 sec, 65°C for 15 sec, 72°C for 60 sec, and a final extension at 72°C for 3 min. The PCR products were separated on 1.5% agarose gel (Promega, USA) in 0.5 % TBE buffer (220 V; 40 min) and post-stained in ethidium bromide buffer. PCR products were con-trolled with Agilent 2100 Bioanalyzer (Agilent Technologies, Germany) using Agilent DNA 1000 Kit and were purified using NucleoSpin Gel and PCR Clean-up system (Macharey-Nagel GmbH &Co, Germany).

Figure S8. Concentrations of 3 technical repeat of AMI and AMU samples. The lowest concentration of sample signed with bf. numbers.

Fragments of samples	Amino acid subst.	AMI			AMU		
		S1	S2	S3	S1	S2	S3
psbA	Val219 Ala251 Phe255 Ser264 Asn266	72,1	33,8	**40,8**	48,3	54,1	53,8
ahas 1	Ala 122	60,3	37,3	48,1	**24,3**	**19,1**	26,2
ahas 2	Pro197 Ala 205	**50,3**	49	49,3	43,4	24,7	30
ahas 3	Asp 376 Arg 377	74,3	45,5	58	37	33,4	38,6
ahas 4	Trp 574 Ser 653 Gly 654	88,6	84,4	100,9	37,1	27,6	**19,9**
epsps	Thr102 Pro106	51,9	**32,8**	76,3	31,7	40,3	34,6
ppx2	Arg98	58,8	81,8	48,7	37,5	39,5	30,1

Figure S9. Gel electrophoresis of 7 gene fragments of *A. artemisiifolia* in three replicate (n=3) of a.)AMI and b.)AMU samples. This 7 gene fragments were used for amplicon sequencing procedure. These fragments include detected herbicide resistance-conferring mutation points of *ahas, epsps, ppx2* and *psbA* genes. During the workflow the second sample was used to make bulk and was sequenced on Illumina MiSeg550 platform.

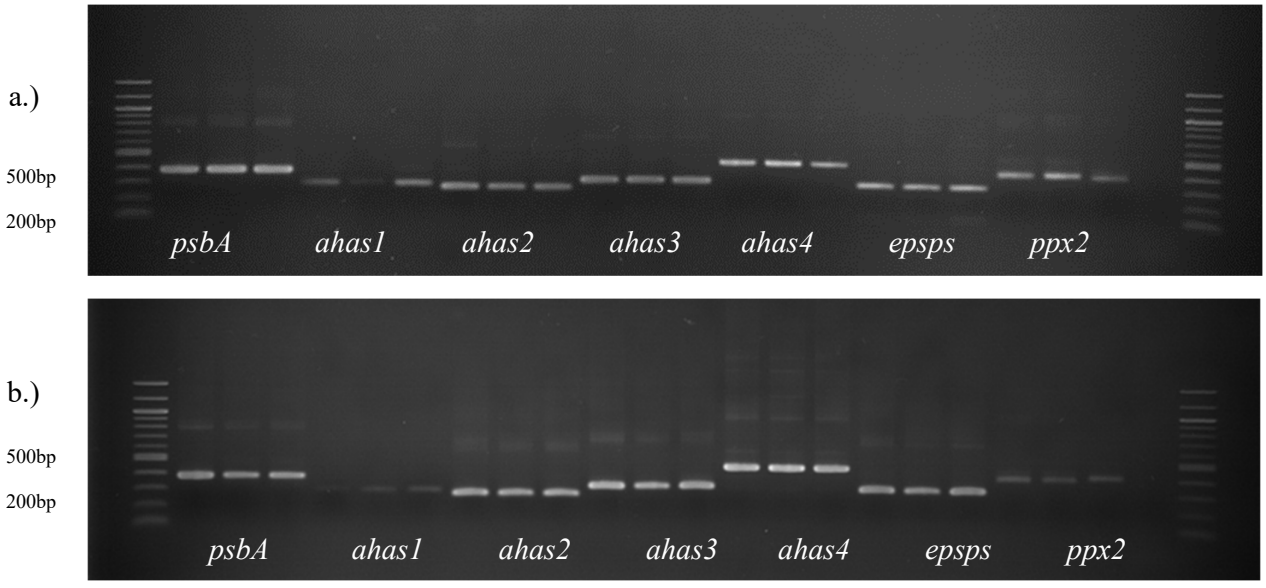
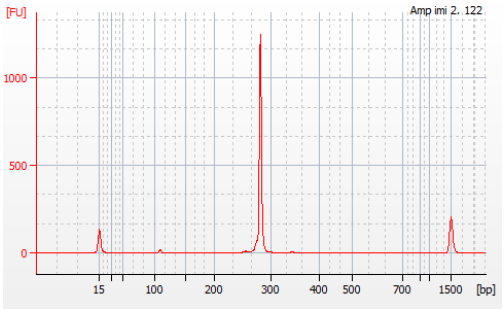
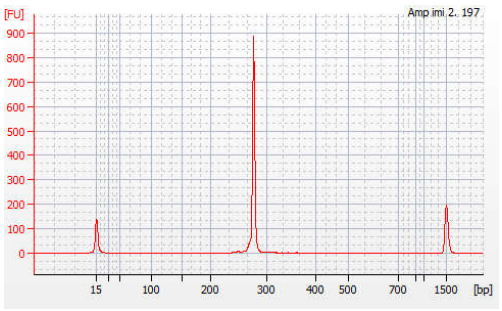
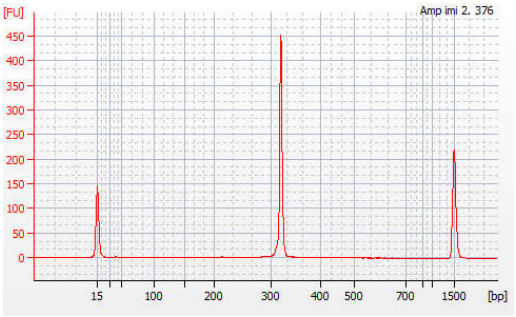
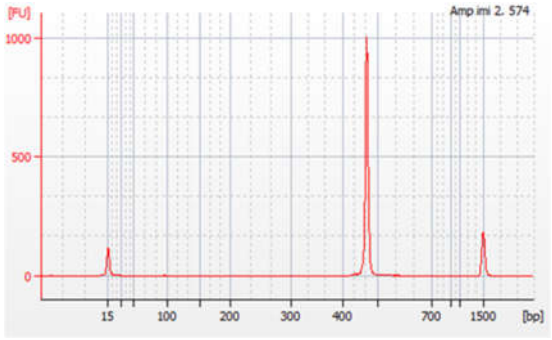


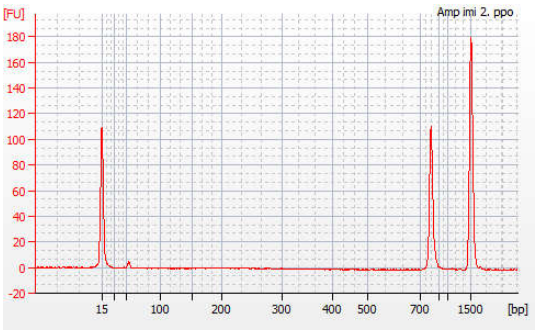
Figure S10. Electropherogram data of AMI samples

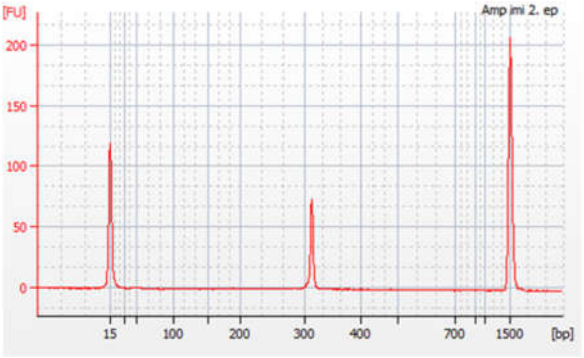












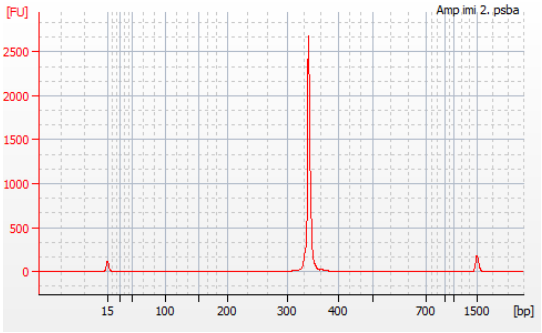
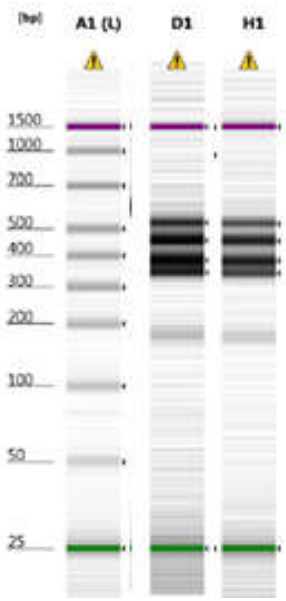
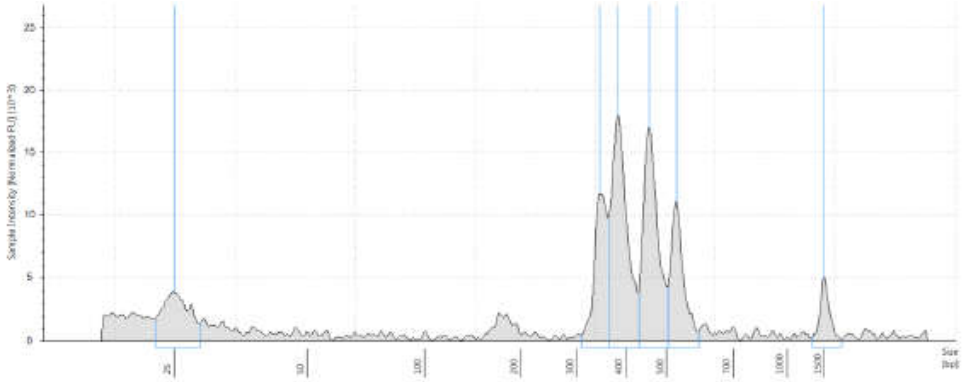


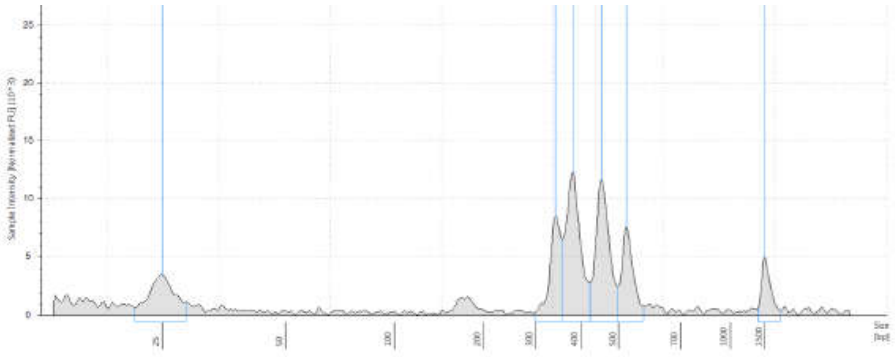
Figure S11. TapeStation Analysis Software A.02.02 (SR1) data of AMI and AMU samples





Sample Table

Well	Conc. [ng/µl]	Sample Description	Alert	Observations
D1	116	AMPool1	⚠	Caution! Expired ScreenTape device



Sample Table

Well	Conc. [ng/µl]	Sample Description	Alert	Observations
H1	75.2	AMPool2	⚠	Caution! Expired ScreenTape device

Figure S12. Bioinformatics analysis

Sample	Number of read	Assembled read with PEAR	Assembled read with PEAR%	Filtered contig with USEARCH	Filtered contig with USEARCH %	Number of groups	Number of singletons	Number of singletons %	Number of groups v10
AMI1	288214	286288	99,3	284236	99,3	20521	14269	69,5	15
AMI2	289034	282035	97,6	279818	99,2	19117	13163	68,9	18
AMI3	296772	263293	88,7	260582	99	16130	10949	67,9	20
AMI4	276462	274700	99,4	272079	99	20630	14398	69,8	16
AMU1	316465	268958	84,9	265410	98,7	16837	11350	67,4	11
AMU2	337224	262809	77,9	255357	97,9	17192	11982	69,7	12
AMU3	369748	272347	68,6	257993	94,7	16672	11418	68,5	10
AMU4	330820	290547	87,8	287197	98,8	18026	12414	68,9	11
Average	313092,375	275122,125	88,025	270334	98,325	18140,625	12492,875	68,825	14,125