Evaluating Variability of Allergens in Commodity Crops (Peanuts)

Peggy Ozias-Akins Laura Ramos Paola Faustinelli Ye Chu *University of Georgia Manel Jordana Katherine Arias *McMaster University Soheila Maleki &USDA-ARS-SRRC James Huntley &NCGR Jay Thelen &University of Missouri

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The Primary Allergens of Peanut Are the Seed Storage Proteins

- Ara h 1 75 vicilin-type globulin (conarachin)
- Ara h 2 conglutin-type 25 albumin
 Other conglutins Ara h 6, Ara h 7
- Ara h 3/4 115 legumin-type globulin (arachin)

Other Allergenic Seed Proteins

Arah 5 - profilin

Arah 8 - pathogenesis-related (PR-10)

Oleosin

Lipid transfer protein

Agglutinin

Eight Allergenic Proteins?

How many genes for each protein class?

How many alleles for each gene?

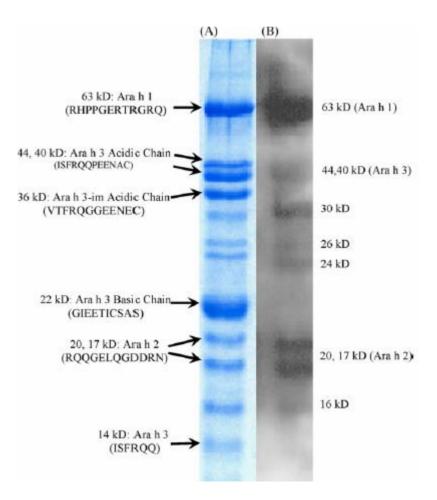
Peanut Seed Storage Proteins peanut seeds are ~25% protein

Ara h 1 and Ara h 2 Both are recognized by serum IgE from >90% patients with peanut allergies. Ara h 1 - 7-18.5% of total protein (Kang et al. 2007. Crop Sci. 47:997) Ara h 2 - 6.2-13.2%

Ara h 3 Recognized by ~50% of patient sera 21.8-38.5% of total protein

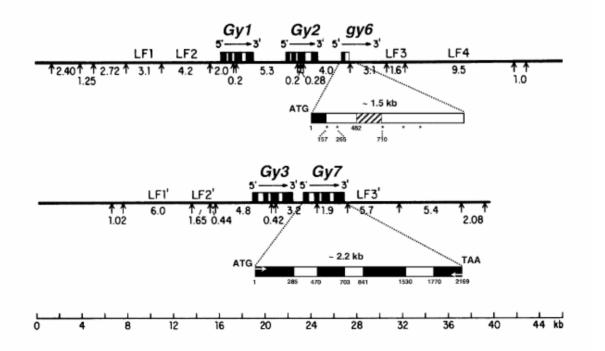
Ara h 6 Recognized by ~70% of patient sera (Koppelman et al. 2005. Clin. Exper. Allergy 35:490)

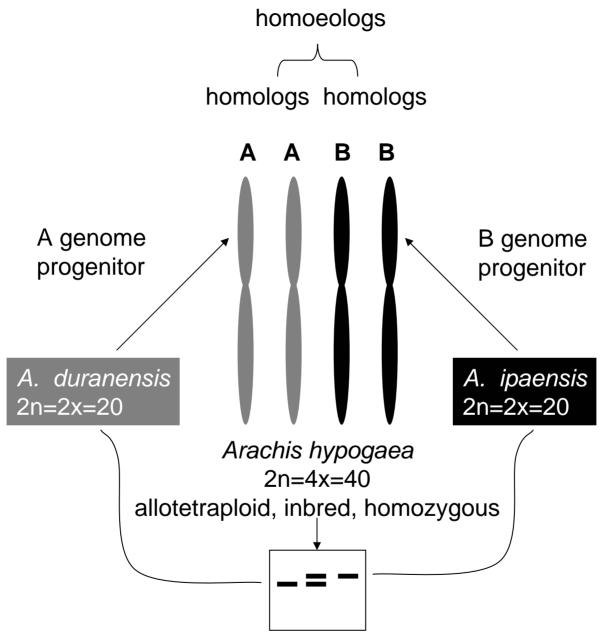
Glycinin Proteins in Peanut Kang and Gallo. 2007. Plant Sci. 172:345



Glycinin Genes in Soybean Beilinson et al. 2002. TAG 104:1132

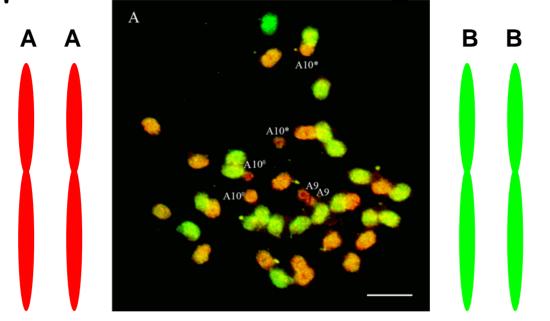
Class 1 - Gy 1, 2, 3 - linkage groups N & L Class 2 - Gy 4, 5 - linkage groups O & F gy6, Gy 7





Ara h 2 Southern blot

Peanut is an allotetraploid species with two subgenomes



A subgenome (A. duranensis)
B subgenome (A. ipaensis)

Ramos et al., 2006. Mol Gen Genomics. 275: 578-592.

Partial Nucleotide Sequence Alignment of A- and B-genome ara h 2 Genes

(105)	105	110	120	13	80	140	156
L77197 (Stanley, J.S.) (97)		CTCGAGA	GGGCGAAI	CTCAGGC	CCTGCGAG	CAACATCTC	ATGCAGAA
AY722689 (CON G2-Arah2) (105)	CCAG	CTCGAGA	GGGCGAA	CTGAGGC	CCTGCGAG	CAACATCTC	ATGCAGAA
Ara h2 cD NA (A-genome) (105)	CCAG	CTCGAGA	GGGCGAAI	CTGAGGC	CCTGCGAG	CAACATCTC	ATGCAGAA
AY158467 (Chatel et al.) (105)	CCAG	CTCGAGA	GGGCGAA	CTGAGGC	CCTGCGAG	CAACATCTC	ATGCAGAA
Ara h2 cD NA (B-genome) (105)	CCAG	CTCGAGA	GGGCGAA	CTTAGGC	CCTGCGAG	CAACATCTC	ATGCAGAA
4Y581853(CONG1-Arah2.02) (105)	CCAG	CTCGAGA	GGGCGAA	CTTAGGC(CCTGCGAG	CAACATCTC	ATGCAGAA
AY117434(Becker et al.) (96)	CCAG	CTCGAGA	GGGCGAA	CTTAGGC(CCTGCGAG	CAACATCTC	ATGCAGAA
C onsensus (105)	CCAG	CTCGAGA	GGGCGAA	CTGAGGC	CCTGCGAG	CAACATCTC	ATGCAGAA
(157)	157		170	180		90	20
L77197 (Stanley, J.S.) (149)	ATCC	AACGTGA	CGAGGAT	CATATGA.	ACGGGACC	CGTACAGCC	CTAGTCAG
AY722689 (CON G2-Arah2) (157)							
Ara h2 cD NA (A-genome) (157)	ATCC	AACGTGA	CGAGGAT	CATATGA.	ACGGGACC	CGTACAGCC	CTAGTCAG
AY158467 (Chatel et al.) (157)	ATCC	AACGTGA	CGAGGAT	CATATG <mark>G</mark>	ACGGGACC	CGTACAGCC	CTAGTCAG
Ara h2 cD NA (B-genome) (157)	ATCC	AACGTGA	CGAGGAT:	ICATATG <mark>G</mark>	ACGGGACC	CGTACAGCC	CTAGTCAG
Ara h2 cD NA (B-genome) (157) AY581853(C ONG1-Arah2.02) (157)	ATCC ATCC	AACGTGA AACGTGA	CGAGGAT' CGAGGAT'	ICATATG <mark>G</mark> . ICATATG <mark>G</mark> .	ACGGGACC ACGGGACC	CGTACAGCC CGTACAGCC	CTAGTCAG CTAGTCAG
Ara h2 cD NA (B-genome) (157)	ATCC ATCC ATCC	AACGTGA AACGTGA AACGTGA	CGAGGAT" CGAGGAT" CGAGGAT"	FCATATG <mark>G</mark> FCATATG <mark>G</mark> FCATATG <mark>G</mark>	ACGGGACC ACGGGACC ACGGGACC	CGTACAGCC CGTACAGCC CGTACAGCC	CTAGTCAG CTAGTCAG CTAGTCAG
Ara h2 cD NA (B-genome) (157) AY581853(C ONG1-Arah2.02) (157) AY117434 (Becker et al.) (148)	ATCC ATCC ATCC	AACGTGA AACGTGA AACGTGA	CGAGGAT" CGAGGAT" CGAGGAT"	FCATATG <mark>G</mark> FCATATG <mark>G</mark> FCATATG <mark>G</mark>	ACGGGACC ACGGGACC ACGGGACC	CGTACAGCC CGTACAGCC CGTACAGCC	CTAGTCAG CTAGTCAG CTAGTCAG
Ara h2 cD NA (B-genome) (157) AY581853(C ONG1-Arah2.02) (157) AY117434(Becker et al.) (148) C onsensus (157)	ATCC ATCC ATCC	AACGTGA AACGTGA AACGTGA	CGAGGAT" CGAGGAT" CGAGGAT"	FCATATG <mark>G</mark> FCATATG <mark>G</mark> FCATATG <mark>G</mark>	ACGGGACC ACGGGACC ACGGGACC	CGTACAGCC CGTACAGCC CGTACAGCC	CTAGTCAG CTAGTCAG CTAGTCAG CTAGTCAG
Ara h2 cD NA (B-genome) (157) AY581853(C ONG1-Arah2.02) (157) AY117434(Becker et al.) (148) C onsensus (157)	ATCC ATCC ATCC ATCC	AACGTGA AACGTGA AACGTGA AACGTGA	CGAGGAT CGAGGAT CGAGGAT CGAGGAT	CATATGG, CATATGG, CATATGG, CATATGG, 230	ACGGGACC ACGGGACC ACGGGACC ACGGGACC 240	CGTACAGCC CGTACAGCC CGTACAGCC CGTACAGCC 250	CTAGTCAG CTAGTCAG CTAGTCAG CTAGTCAG
Ara h2 cD NA (B- genome) (157) AY581853(C ONG1-Arah2.02) (157) AY117434 (Becker et al.) (148) C onsensus (157) (200 L77197 (Stanley, J.S.) (201 AY722689 (CON G2-Arah2) (209	ATCC ATCC ATCC ATCC 0 209 0) 209 0) ATC 0) ATC	AACGTGA AACGTGA AACGTGA AACGTGA	CGAGGAT CGAGGAT CGAGGAT CGAGGAT 220	CATATGG, CATATGG, CATATGG, CATATGG, 230	ACGGGACC ACGGGACC ACGGGACC ACGGGACC 240	CGTACAGCC CGTACAGCC CGTACAGCC CGTACAGCC 250 250 250 250 250 250 250 250 250 250	CTAGTCAG CTAGTCAG CTAGTCAG CTAGTCAG
Ara h2 cD NA (B-genome) (157) AY581853(C ONG1-Arah2.02) (157) AY117434 (Becker et al.) (148) C onsensus (157) L77197 (Stanley, J.S.) (20 AY722589 (C ON G2-Arah2) (20 Ara h2 cD NA (A-genome) (20	ATCC ATCC ATCC ATCC ATCC 0 200 0 200 0 0 0	AACGTGA AACGTGA AACGTGA AACGTGA	CGAGGAT CGAGGAT CGAGGAT CGAGGAT 220	CATATGG, CATATGG, CATATGG, CATATGG, 230	ACGGGACC ACGGGACC ACGGGACC ACGGGACC 240	CGTACAGCC CGTACAGCC CGTACAGCC CGTACAGCC 250 250 250 250 250 250 250 250 250 250	CTAGTCAG CTAGTCAG CTAGTCAG CTAGTCAG
Ara h2 cD NA (B-genome) (157) AY581853(C ONG1-Arah2.02) (157) AY117434 (Becker et al.) (148) C onsensus (157) L77197 (Stanley, J.S.) (20 AY722089 (CON G2-Arah2) (20 Ara h2 cD NA (A-genome) (20 AY158467 (Chatel et al.) (20	ATCC ATCC ATCC ATCC 0 200 0) 200 000 000 000 000 000 0000000000	AACGTGA AACGTGA AACGTGA C C CGTACAGO	CGAGGAT CGAGGAT CGAGGAT CGAGGAT 220	CATATGG, CATATGG, CATATGG, 230 230 AGGACCCG	ACGGGACC ACGGGACC ACGGGACC ACGGGACC 240 	CGTACAGCC CGTACAGCC CGTACAGCC CGTACAGCC GTA GTA GTA TGATCCGTA	CTAGTCAG CTAGTCAG CTAGTCAG CTAGTCAG CAGCCCTA CAGCCCTA CAGCCCTA CAGCCCTA
Ara h2 cD NA (B- genome) (157) AY581853(C ONG1-Arah2.02) (157) AY117434 (Becker et al.) (148) C onsensus (157) (209 L77197 (Stanley, J.S.) (20) AY722089 (CON G2-Arah2) (20) Ara h2 cD NA (A- genome) (20) Ara h2 cD NA (B- genome) (20)	ATCC ATCC ATCC ATCC) 200) ATCC) ATC) ATC) ATC) ATC) ATC	AACGTGA AACGTGA AACGTGA C C CGTACAGO CGTACAGO	CGAGGAT CGAGGAT CGAGGAT 220 220 CCTAGTC CCTAGTC	CATATGG. CATATGG. CATATGG. 230 230 230 230 230 230 230 230 230 230	ACGGGACC ACGGGACC ACGGGACC ACGGGACC 240 240 GACAGACG GACAGACG	CGTACAGCC CGTACAGCC CGTACAGCC CGTACAGCC CGTACAGCC CGTACAGCC CGTACAGCC CGTACAGCC TGATCCGTA TGATCCGTA	CTAGTCAG CTAGTCAG CTAGTCAG CTAGTCAG CAGCCCTA CAGCCCTA CAGCCCTA CAGCCCTA
Ara h2 cD NA (B- genome) (157) AY581853(C ONG1-Arah2.02) (157) AY117434 (Becker et al.) (148) C onsensus (157) (203 L77197 (Stanley, J.S.) (203 AY722089 (CON G2-Arah2) (203 Ara h2 cD NA (A- genome) (203 AY168467 (Chatel et al.) (203 AY581853(C ONG1-Arah2.02) (203	ATCC ATCC ATCC ATCC) 200) ATCC) ATC) ATC) ATC) ATC) ATC) ATC	AACGTGA AACGTGA AACGTGA AACGTGA C C CGTACAGC CGTACAGC CGTACAGC	CGAGGAT CGAGGAT CGAGGAT 220 220 CCTAGTC CCTAGTC	CATATGG. CATATGG. CATATGG. 230 230 230 230 230 230 230 230 230 230	ACGGGACC ACGGGACC ACGGGACC ACGGGACC 240 240 GACAGACG GACAGACG GACAGACG	CGTACAGCC CGTACAGCC CGTACAGCC CGTACAGCC GTA GTA GTA TGATC CGTA TGATC CGTA	CTAGTCAG CTAGTCAG CTAGTCAG CTAGTCAG CAGCCCTA CAGCCCTA CAGCCCTA CAGCCCTA CAGCCCTA
Ara h2 cD NA (B- genome) (157) AY581853(C ONG1-Arah2.02) (157) AY117434 (Becker et al.) (148) C onsensus (157) (200 L77197 (Stanley, J.S.) (201 AY722089 (CONG2-Arah2) (200 Ara h2 cD NA (A- genome) (200 Ara h2 cD NA (B- genome) (200 Ara h2 cD NA (B- genome) (200 AY581853(C ONG1-Arah2.02) (200 AY117434 (Becker et al.) (200	ATCC ATCC ATCC ATCC 0) 200 0) ATC 0) ATC 0) ATC 0) ATC 0) ATC 0) ATC	AACGTGA AACGTGA AACGTGA AACGTGA C C CGTACAGC CGTACAGC CGTACAGC	CGAGGAT CGAGGAT CGAGGAT 220 220 CCTAGTC CCTAGTC CCTAGTC CCCTAGTC	CATATGG. CATATGG. CATATGG. 230 230 AGGACCCG AGGACCCG AGGACCCG AGGACCCG	ACGGGACC ACGGGACC ACGGGACC ACGGGACC 240 240 GACAGACG GACAGACG GACAGACG GACAGACG GACAGACG	CGTACAGCC CGTACAGCC CGTACAGCC CGTACAGCC CGTACAGCC GTA GTA GTA TGATCCGTA TGATCCGTA CGATCCGTA	CTAGTCAG CTAGTCAG CTAGTCAG CTAGTCAG CAGCCCTA CAGCCCTA CAGCCCTA CAGCCCTA CAGCCCTA CAGCCCTA CAGCCCTA
Ara h2 cD NA (B- genome) (157) AY581853(C ONG1-Arah2.02) (157) AY117434 (Becker et al.) (148) C onsensus (157) (203 L77197 (Stanley, J.S.) (203 AY722089 (CON G2-Arah2) (203 Ara h2 cD NA (A- genome) (203 AY168467 (Chatel et al.) (203 AY581853(C ONG1-Arah2.02) (203	ATCC ATCC ATCC ATCC 0) 200 0) ATC 0) ATC 0) ATC 0) ATC 0) ATC 0) ATC	AACGTGA AACGTGA AACGTGA AACGTGA C C CGTACAGC CGTACAGC CGTACAGC	CGAGGAT CGAGGAT CGAGGAT 220 220 CCTAGTC CCTAGTC CCTAGTC CCCTAGTC	CATATGG. CATATGG. CATATGG. 230 230 AGGACCCG AGGACCCG AGGACCCG AGGACCCG	ACGGGACC ACGGGACC ACGGGACC ACGGGACC 240 240 GACAGACG GACAGACG GACAGACG GACAGACG GACAGACG	CGTACAGCC CGTACAGCC CGTACAGCC CGTACAGCC CGTACAGCC GTA GTA GTA TGATCCGTA TGATCCGTA CGATCCGTA	CTAGTCAG CTAGTCAG CTAGTCAG CTAGTCAG CAGCCCTA CAGCCCTA CAGCCCTA CAGCCCTA CAGCCCTA CAGCCCTA CAGCCCTA

(201)					
L77107 (Stanley, J.S.) (217)	TCCATA	FGATCGGAG	AGGCGCTGGATC	TCTCAGCACCA	AGAGAGGTGTTGC
AY722699 (CON 62-Arah2) (225)	TCCATA	FGATCGGAG	AGGCGCTGGATC	TCTCAGCACCA	AGAGAGGTGTTGC
Ara h2 oD NA (A-genome) (225)					
AY159467 (Chatel et al.) (261)					
Ara h2 cDNA (B-genome) (261)	TCCATA:	FGATCGGAG	AGGCGCTGGATC	TCTCAGCACCA	AGAGAGGTGTTGC
AY561853(CONG1-Arah2.02) (201)	TCCATA:	FGATEGGAG	AGGCGCTGGATC	TCTCAGCACCA	GGAGAGGTGTTGC
AY117434 (Becker et al.) (252)	TCCATA	FGATEGGAG	AGGCGCTGGATC	TCTCAGCACCA	AGAGAGGTGTTGC
Consensus (201)	TCCATA	FGATCGGAG	AGGCGCTGGATC	CTCTCAGCACCA	AGAGAGGTGTTGC
					——————————————————————————————————————

Partial Amino Acid Sequence Alignment of A- and B-genome ara h 2 Genes

		<u> </u>					– Section 1
	(1)	1 1	D	20	30		49
Tsl L77197 (Stanley, J.S.)	(1)		ALFLLAA	HASARQO	WELQGDRRC	QSQLERANL	RPCEQHL
Tsl cDNA Ara h2 (A-genome)	(1)	MAK <mark>LTILVA</mark> L	ALFLLAA	HASARQO	WELQGDRRC	QSQLERANL	RPCEQHL
Tsl cDNA Ara h2 (B-genome)	(1)	MAK <mark>LTILVA</mark> L	ALFLLAA	HASARQQ	WELQGDRRC	QSQLERANL	RPCEQHL
TsI cDNA AY117434 (Becker et al)	(1)				WELQGDRRC		
Tsl cDNA AY158467 (Chatel et al)		MAK <mark>LTILVA</mark> I					
Consensus	(1)	MAKLTILVAL	ALFLLAA	AHASARQO	WELQGDRRC	QSQLERANL	
		50		70			- Section 2
T-11 774 07 (Ob-select 1-0.)	(50) (47)		60	70	80		98
Tsl L77197 (Stanley, J.S.) Tsl oDNA Are 52 (A. renome)		MQKIQRDEDS					RGAGSSQ
Tsl cDNA Ara h2 (A-genome) Tsl cDNA Ara h2 (B-genome)		MQKIQRDEDS MQKIQRDEDS					RGAGSSQ
Tsi cDNA AY117434 (Becker et al)		MQKIQRDEDS					
Tsl cDNA AY158467 (Chatel et al)		MQKIQRDEDS					
Consensus	· ·	MQKIQRDEDS					
	(/						– Section 3
	(99)	99	,110	,120	,130)	147
Tsl L77197 (Stanley, J.S.)		HQERCCNELN	EFENNQF	RCMCEALC	QIMENQSDR	LQGRQQEQQ	FKRELRN
Tsl cDNA Ara h2 (A-genome)	(87)	HQERCCNELN	EFENNQF	RCMCEALQ	QIMENQSDR	LQGRQQEQQ	FKRELRN
Tsl cDNA Ara h2 (B-genome)		HQERCCNELN					
TsI cDNA AY117434 (Becker et al)		HQERCCNELN					
Tsl cDNA AY158467 (Chatel et al)	· ·	HQERCCNELN					
Consensus	(99)	HQERCCNELN	EFENNQF	RCMCEALQ	QIMENQSDR	LQGRQQEQQ	
	(4.40)	140	160 *		173		– Section 4
Tsi L77197 (Stanley, J.S.)	(148)						
Tsl cDNA Ara h2 (A-genome)							
Tsl cDNA Ara h2 (B-genome)							
Tsl cDNA AY117434 (Becker et al)							
Tsl cDNA AY158467 (Chatel et al)							
		TROODETRAD					

Consensus (148) LPQQCGLRAPQRCDLEVESGGRDRY

•There are 2 copies of *ara h 2* in tetraploid peanut •*ara h 2* is similar to another allergen gene, *ara h 6*

arah2-arah6 (8A2a).apr

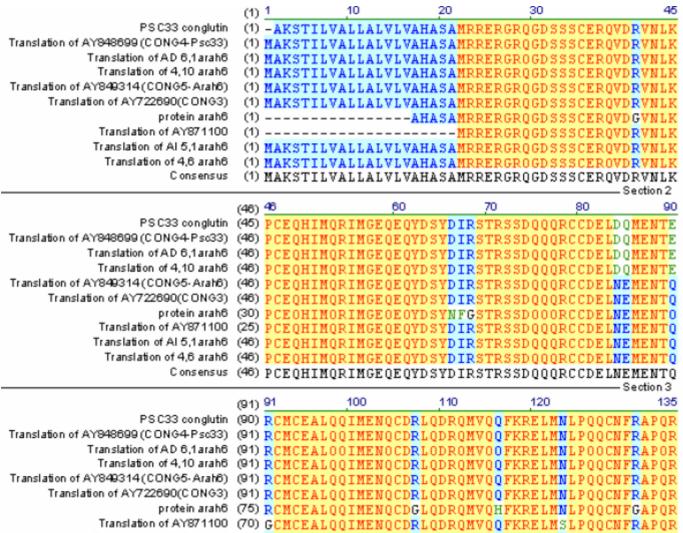
																							Section 1
	(1)	1		10		2	0		,30			40		50			60		,70	0			88
Arah6 ORF (8A2a)	(1)	ATG	GCCAA	GTCC	ACCA	тссі	GGTI	GCT	стсс	TTGC	CCT	CGTC	CTG <mark>G</mark> 1	r g <mark>gc</mark> a	CACG	CCTC	CGC	AAT <mark>G</mark>	AGGC	GCG <mark>2</mark>	GAGG	<mark>g</mark> gga	.G <mark>ACA</mark> G <mark>G</mark>
Arah2 ORF (8A2a)	(1)	ATG	GCCAA	GCTC	ACCA	TACI	AGT	AGC C	CTCG	ссст				CT <mark>GC</mark> C	CACG	CATO	TGC-	<mark>G</mark>	AGGC	AGC <mark>2</mark>	GTGG	<mark>3</mark> AAC	TACAAG
Consensus	(1)	ATG	GCCAA	.G C	ACCA	т ст	GTA	AGC (СТС		Т	C TC	CT G	GC	CACG	C TC	; GC	G	AGGC	P	G GG	-	ACA G
																							Section 2
	(89)				00		,110			120		13			140		15			160			176
Arah6 ORF (8A2a)	(89)	GGGI	ACTCA	TC <mark>AA</mark>	.GCTG	CGAG	AGG	CAGG	TAGA	CAGG	GTT.	AACC	TCAAC	SCCCI	GCGA	GCAG	CAC A	ATAA	TGCA	GAGG	ATC-		TGACGA
																			TGCA. TGCA			AACG	TGACGA
Consensus	(89)	GG	AC	AA	GIG	C AG	AG (AG .	I GA	AGG	G.	AACC	TAO	50001	GCGA	GCA	CA	IA	IGCA	GA	AIC		Section 3
	(177)	177			100		2	000		210	1		220		230	n		.240		2	50		264
(Arah6 ORF (8A2a)	(166)			ATGG	GCGZ	G					AAG	AGCA	GTAC-				AC				-TCC		204
Arah2 ORF (8A2a) ((171)	GGA	TCAT	ATGG	ACGG	GACO	CGT	ACAG	ссст	AGT	AGG	ATCC	GTAC	AGCCO	TAGT	CAG	1110				100		AGCCCT
Consensus (ATGG								A C					AC				TCC		
																							Section 4
((265)	265	270		2	80		290			300		31	0		320			30		340		352
Arah6 ORF (8A2a) (Arah2 ORF (8A2a) ((193)		<mark>T</mark> A	.C <mark>GAT</mark>	ATT <mark>A</mark>	.GGA	TAC1	CGA:	гсст	CCGA	CCA.	G <mark>CAA</mark>	CAGAC	GGTGC	CTGCG	ATGA	GCT	GAAC	GAGA	TGGA	GAAC	ACA <mark>C</mark>	AGAGAT
		AGT																					AAAGGT
Consensus ((265)		ΤA	GAT	A	.G G	; C1	GA?	гсст	С А	CA	CAA	AGAG	GGTG	TGC	ATGA	GCT	GAAC	GAG	T GA	GAAC		A AG T
	(0.5.0)			~~~		0.70								400					400				Section 5
	(353)			60		370			380	C 2 2 C	39			400		410	-		420	0000	43	-	440
Arah6 ORF (8A2a) (Arah2 ORF (8A2a) ((275)	GCA.	LGIGC	GAGG	CALL	GCAG	CAGA	AIAA.	IGGA TGGA	GAAC	CAG	NGCG.	ATAGO	STIGU	AGGA	CAGO	CAA		ACCA	GCAG ACAG	TTCA	AGAG	AGAGUI
Consensus (GAGCI
001001000	(000)	0011		onoo	onii	001	onor		10011	011110	ono	000	nino.			noc	John		000	0.000	1101		Section 6
((441)	441		450		4	60		470)		.480		49	0		500		5	10		522	
Arah6 ORF (8A2a)			GAACT		CCAA			ACTT:			CAC.		TTGC		-	GTG-		GTGG	-		G <mark>C</mark>		
Arah2 ORF (8A2a)																						ATAC	
Consensus ((441)	CA (GAACT	TGCC	CAA	CAGI	G	СТ	AGG	GCAC	CAC.	AGCG	TTGC	JA TI	GGA	GΤ	AC	GTGG	CGGC	AGA	С		

•There are 3 copies of *ara h 6* in tetraploid peanut

Arah6 genes.apr

												— Section 1
	(1) 1	,10	20	,30	40	,50	,60	70	,80	90	,100	119
AD 6,1arah6	(1) ATGGO	CAAGTCCACCA	ICCTGGTAGCTC	TCCTTGCCC:	TCGTCCTGGT	GGCACACGCC	TCCGC <mark>G</mark> ATGA(GECECEAEAE	GGGG <mark>C</mark> GACAAG	GGGACTCAT	CAAGCTGCGAGAG	GCAGGTAGA
AI 5,2+5,5arah6											CAAGCTGCGAGAG	
Al 5,1arah6											CAAGCTGCGAGAG	
Consensus	(1) ATGGG	CAAGTCCACCA	ICCTGGTAGCTC	TCCTTGCCC	TCGTCCTGGT	GGCACACGCC	TCCGCAATGA	GECECEYEYE	SECENENCIES	GGGACTCAT	CAAGCTGCGAGAG	GCAGGTAGA — Section 2
	(100) 100	120	140	150	100	.170	.180	.190	200	210	220	
	(120) 120	,130	,140	150	,160						TCCGACCAGCAAC	238
											TCCGACCAGCAAC	
											TCCGACCAGCAAC	
											TCCGACCAGCAAC	
	,											Section 3
((239) 239	250	260	270	280	290	300	310	320	,330	340	357
AD 6,1arah6 (AATGGTGCAGCAG	
Al 5,2+5,5arah6 (AATGGTGCAGCAG	
											AATGGTGCAGCAG	
Consensus ((239) GCGA1	GAGCTGAACGA	GATGGAGAACAC	ACAGAGATG	CATGTGCGAG	GCATTGCAGC	AGATAATGGA	GAACCAGTGC	SATAGGTTGCA	GGACAGGCA	AATGGTGCAGCAG	
	(050) 050	070	202	202	400	440	100	400	440	450	400	Section 4
AD 6,1arah6 ((358) 358	370	380	390	400	410	420	,430	440	450	460 CTGTGCCAAAAGA	476
											CTGTGCCAAAAGA CTGTGCCAAAA <mark>C</mark> A	
	(/										CIGIGCCAAAACA	
											CTGTGCCAAAACA	
	. ,											
((477) 477	490	500	,510	,520	530	540	,550	,560	570	580	595
AD 6,1arah6 ((477) GAAG1	AGCTTAT	GAGCTATTATG	TATECTTET	TTCGTTAATA	ATAAA <mark>T</mark> ATCA	TCACTGTATG	AATGTGGTGA	AGGTAP	GGTTATATG	AGCACCTTCGGTG	TGCTCTTAT
		AGCTTAT									AGCACCTTCGGTG	
											AGCACCTTCGGTG	
Consensus ((477) GAAG1	CAGCTTAT	GAGCTATTATG	TATECTTET	TTCGTTAATA	ATAAATATCA	TCACTGTATG	AATGTGGTGAI	TAG GTAR	GGTTATATG	AGCACCTTCGGTG	TGCTCTTAT ——Section 6
		610	000		639							Section 6
AD 6.1arah6 ((596) 596 (597) 596	TACCTATGTTT										
		TACCTGTGTTT:										
		TACCTATGTTT:										
		TACCTATGTTT										

Three Amino Acid Changes Distinguish A- and B-Genome *ara h 6* Genes



Translation of AY871100 (70) GCMCEALQQIMENQCDRLQDRQMVQQFKRELMSLPQQCNFRAPQR Translation of Al 5,1arah6 (91) RCMCEALQQIMENQCDRLQDRQMVQQFKRELMNLPQQCNFRAPQR Translation of 4,6 arah6 (91) RCMCEALOOIMENOCDRLODROMVOOFKRELMNLPOOCNFRAPOR Consensus (91) RCMCEALQQIMENQCDRLQDRQMVQQFKRELMNLPQQCNFRAPQR

Targeting Induced Local Lesions IN Genomes

(McCallum et al. 2000. Plant Physiol. 123:439)

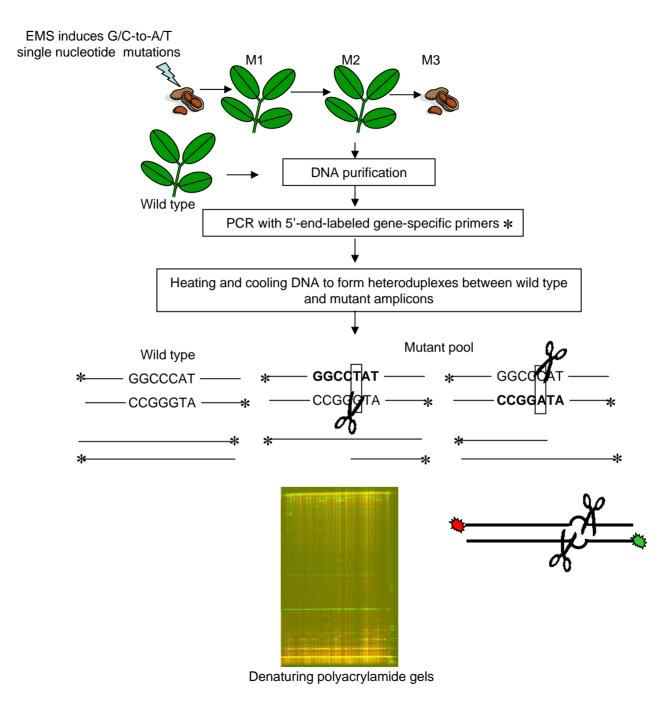
Functional Genomics Strategy

Forward genetics: Trait → Gene Ex: mutant phenotype

Reverse genetics: Gene → Trait Ex: RNAi; gene knockout

Advantages of TILLING

- Yields point mutations
- Mutation can be in any gene (random)
- Can target screening to gene of interest
- Phenotype can be determined later



Screening Is Carried Out By Mismatch Detection With Cel I

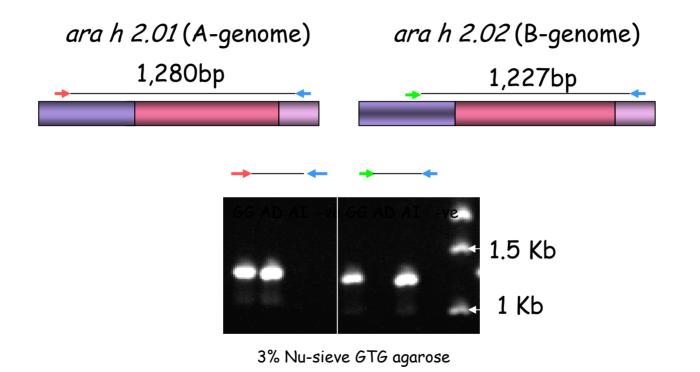
Screen pools of DNA from multiple plants by PCR —

atggcttgt taccgaaca

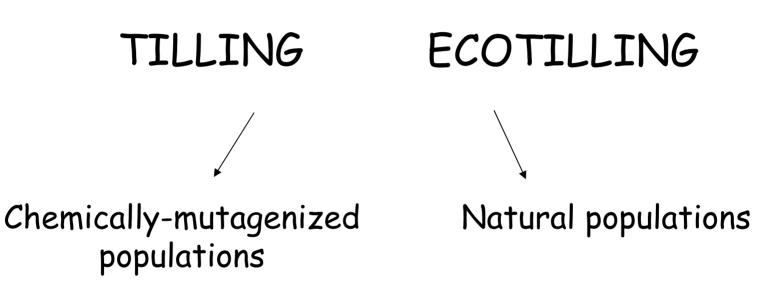
atgacttgt tactgaaca

taccgaaca atgacttgt atggcttgt taccgaaca taccgaaca atgacttgt tačtgaača tactgaaca atgačttgt tactgaaca atggcttgt taccgaaca atggcttgt

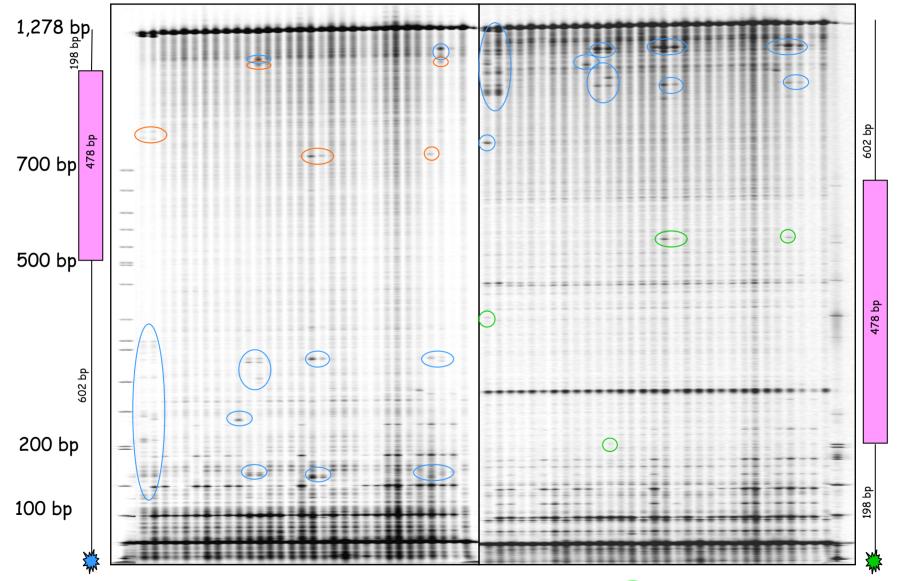
Gene-specific PCR for A- and B-Genome Ara h 2 Genes



Ozias-Akins *et al.*, 2006. Hypoallergenic foods beyond infant formulas. In: Food Allergy. ASM Press, Herndon, VA.



Ecotilling Arachis duranensis accessions

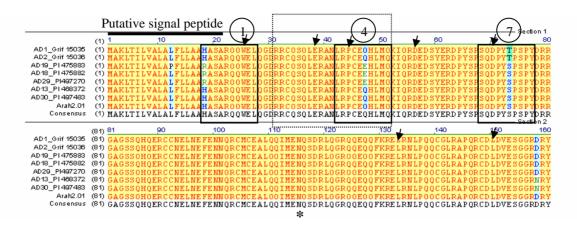


 \bigcirc Putative mutations in ORF \bigcirc Putative mutations in 5'UTR \bigcirc Putative mutations in 3'UTR

Ecotilling of Arachis duranensis

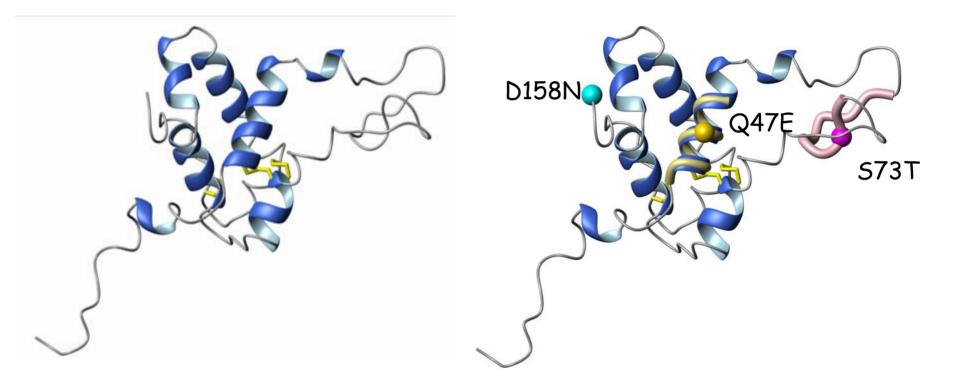
- Screened 30 accessions
- Seven showed variation from the WT coding region
- •Eight SNPs total 3 silent, 5 missense
- •Indels in non-coding regions

Amino Acid Changes Detected in ara d 2.01

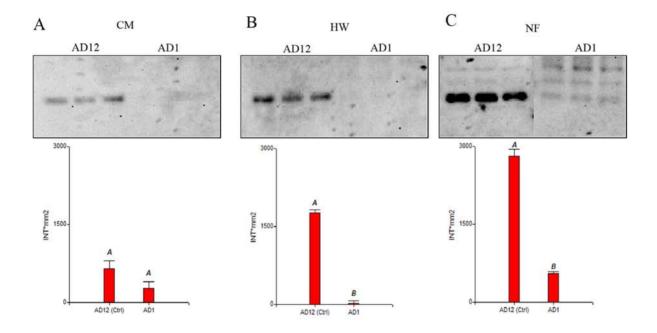


SNP position	aa change	Nature of the mutation	IgE epitope affected	T-cell epitope affected [#]	SIFT score	A. duranensis accession	Short ID
35	L12P	apolar \rightarrow apolar			0.11	PI 475883	AD19
53	H18R	polar [†] → charged polar	1 AHASARQQWEL A R ASARQQWEL		1.00	PI 475882 PI 475883	AD18 AD19
139	Q47E	polar → charged polar	4 LRPCEQHLMQ LRPCE E HLMQ	RRCQSQLERANLRPCEQHLM RRCQSQLERANLRPCE E HLM	1.00	PI 475882 PI 475883 PI 497270	AD18 AD19 AD29
218	S73T	polar → polar	7 SQDPYSPSY SQDPY T PSY		0.28	Grif 15035 Grif 15036	AD1 AD2
472	D158N	charged polar → polar			0.92	PI 468372 PI 497483	AD13 AD30

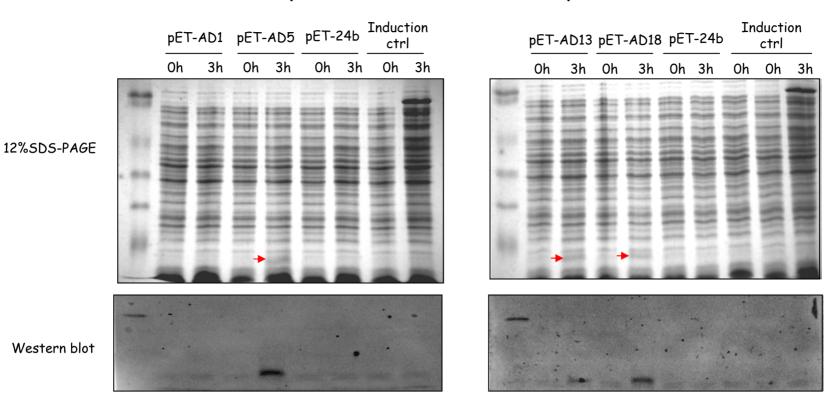
Modeling Arah 2



Immunoblot (IgE Binding) and Densitometric Analysis of Accession Carrying S73T Mutation



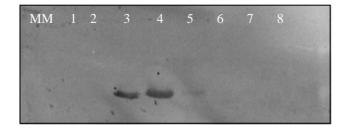
Reduction in IgE binding capacity 56.2% (A), 98.93% (B) and 80.4%(C)



Expression of recombinant proteins

Purification of recombinant proteins

Immunoblot analysis of different aliquots from purification process of rAD5 (wt)



Summary of Ecotilling

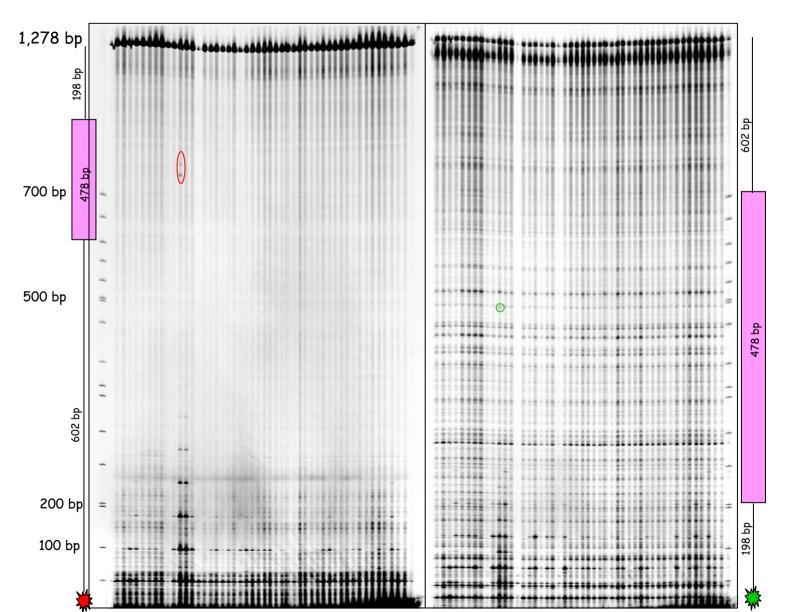
•Ecotilling allowed the identification of seven out of thirty *A. duranensis* accessions with putative mutations in the ORF of *ara d 2.01*

•The natural mutation rate found for this particular gene was 1 SNP/420 bp

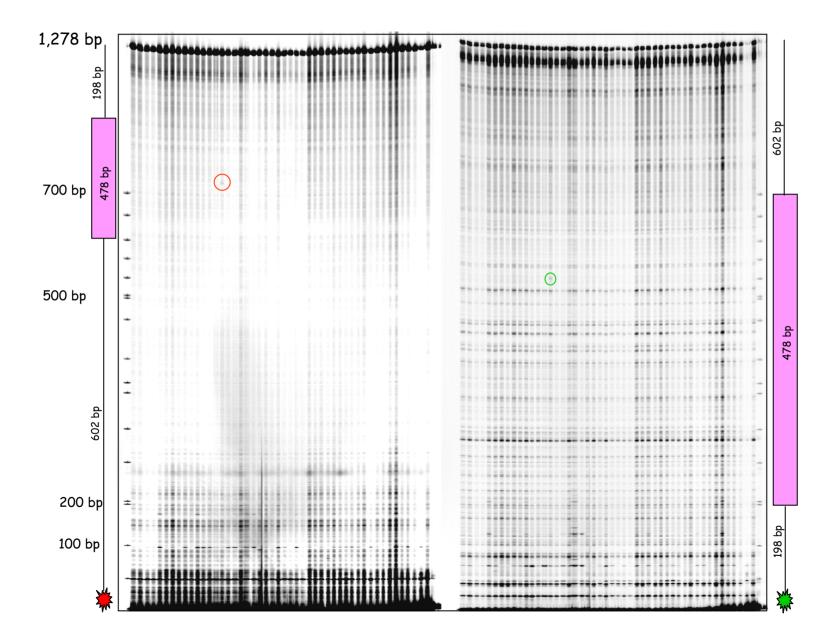
•Missense and silent, but no non-sense mutations were detected

•Reduction in IgE binding capacity of 56.2% (A), 98.93% (B) and 80.4%(C) was observed for one isoform

TILLING in EMS-mutagenized peanut population for ara h 2.01 mutations

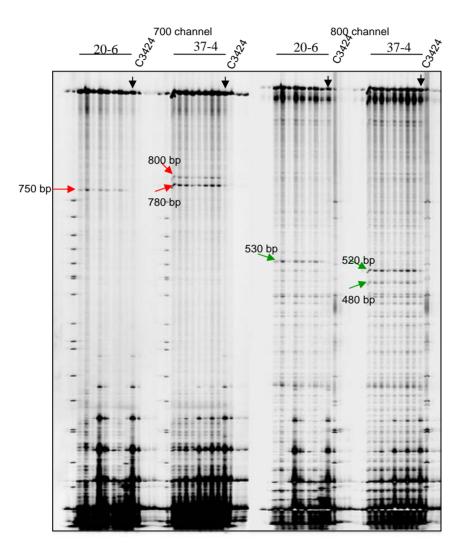


ara h 2.01



Identification of individuals for ara h 2.01 37-4 20-6 20-6 37-4 1,278 bp g | 602 bp 478 bp . 700 bp (\cdot) 500 bp • 478 bp 602 bp 198 bp

Detection of mutations in M3 generation of 20-6 and 37-4



Nucleotide and Amino Acid Sequences for Arah 2.01 Mutants

_																			Section 1
		(1)			10			20		30		40		50		60			75
	ORF 20-6 (temp) ORF 37-4		ATG	GCCA	AGCI	CACO	CATA CATA	CTAG CTAG	TAGCO	CTCG	CCCTT	FTCCTC	CTCG	CTGCC	CACGO CACGO	ATCT	GCGA	GGCA	CAGTGG
	arah2 ORF (AD)		ATG	GCCA	AGCI	CAC	CATA	CTAG	TAGC	CTCG	CCCTT:	FTCCTC	CTCG	CIGCC	CACGO	ATCT	GCGA	GGCA	SCAGIGG SCAGIGG
	Consensus	(1)	ATG	GCCA	AGCI	CACO	CATA	CTAG	TAGCO	CTCG	CCCTT	TTCCT	CTCG	CTGCC	CACGO	ATCT	GCGA	GGCA	CAGTGG Section 2
-		(76)	76			ç	90		100		110		120	1	13	0		140	5ection 2 150
	ORF 20-6 (temp)	(76)	GAA	стсс	AAGG			AGAT		GAGCC.		GAGAGO				-	GAGC	10.00	TTCATG
	ORF37-4				AAGG	AGAG	CAGA				AGCTCO								r <mark>c</mark> tcatg
	arah2 ORF (AD) Consensus										AGCTC(AGCTC(CTCATG
-				0100			onon		0004										Section 3
		(151)			160			170		180		190		,200			10		225
	ORF 20-6 (temp) ORF 37-4																CCGI	TACAGO	CCTAGT
	arah2 ORF (AD)															GGAT	CCGI	ACAG	CCTAGT
	Consensus	(151)	CAG	AAGA	TCCA	ACGI	TGAC	GAGG	ATTCA	ATATG.	AACGGG	SACCCO	TACA	GCCCT	AGTCA	GGAT	CCGI		CCTAGT Section 4
-		(226)	226			2	240		250		260		270)	28	0		290	300 300
	ORF 20-6 (temp)			TATG	ATCG	GAG	AGGC	GCTG	GATC	CTCTC.	AGCAC	CAAGAG	AGGT	GTTGC	AATGA	GCTG	AACG	AGTT	FGAGAAC
	ORF37-4					GAG	AGGC										AACG	AGTT	FGAGAAC
	arah2 ORF (AD) Consensus																AACG AACG	AGTT: AGTT'	FGAGAAC FGAGAAC
-									UALU,										Section 5
		(301)			310			320		330		340		350			30		375
	ORF 20-6 (temp)			C & & &	GGTG	CC & TFG	നേരന	GBGG								CTTC			
							STGC												CAACAG
	ORF37-4 arah2 ORF (AD)	(301) (301)	AAC AAC	СААА СААА	GGTG GGTG	CATO CATO	GTGC GTGC	GAGG GAGG	CATT(CAAC.	AGATC <i>I</i> AGATC <i>I</i>	ATGGAG ATGGAG	FAACC FAACC	AGAGC AGAGC	GATAG GATAG	GTTG GTTG	CAGO CAGO	GGAG GGAG	GCAACAG GCAACAG
	ORF37-4	(301) (301)	AAC AAC	СААА СААА	GGTG GGTG	CATO CATO	GTGC GTGC	GAGG GAGG	CATT(CAAC.	AGATC <i>I</i> AGATC <i>I</i>	ATGGAG ATGGAG	FAACC FAACC	AGAGC AGAGC	GATAG GATAG	GTTG GTTG	CAGO CAGO	GGAG GGAG	GCAACAG GCAACAG
	ORF37-4 arah2 ORF (AD)	(301) (301)	AAC AAC AAC	CAAA CAAA CAAA	IGGTG IGGTG IGGTG	CATO CATO CATO	GTGC GTGC GTGC	GAGG GAGG GAGG	CATT(CAAC.	AGATC <i>I</i> AGATC <i>I</i>	ATGGAG ATGGAG	AACC AACC AACC	<mark>AGAGC</mark> AGAGC AGAGC	<mark>GATAG</mark> GATAG GATAG	GTTG GTTG GTTG	CAGO CAGO CAGO 5	GGAG GGAG GGAG	GCAACAG GCAACAG
	ORF37-4 arah2 ORF (AD)	(301) (301) (301)	aac aac aac P	CAAA CAAA CAAA	GGTG GGTG GGTG Ve si	CATO CATO CATO gnal	GTGC GTGC GTGC	GAGG GAGG GAGG	CATT(CAAC. CAAC.	AGATCI AGATCI AGATCI	ATGGAG ATGGAG ATGGAG	AACC AACC AACC Epi	AGAGC AGAGC AGAGC itope#	gatag gatag gatag 4 Epi	GTTG GTTG GTTG	CAGO CAGO CAGO 5	GGAGO GGAGO GGAGO ection 1	GCAACAG GCAACAG
	ORF37-4 arah2 ORF (AD) Consensus	(301) (301) (301) (301)	AAC AAC AAC P	caaa caaa caaa Putati	IGGTG IGGTG IGGTG IVE SI	CATO CATO CATO CATO gnal	erge erge erge pept	GAGG GAGG GAGG tide 20	CATT(CATT) CATT(SCAAC. SCAAC. SCAAC. SCAAC.	AGATC AGATC AGATC	ATGGAG ATGGAG ATGGAG	FAACC FAACC FAACC Epi	AGAGC AGAGC AGAGC itope#	gatag gatag gatag gatag 4 Epi 0	GTTG GTTG GTTG tope#	CAGO CAGO CAGO 5 S	GGAG GGAG GGAG GGAG ection 1 67	GCAACAG GCAACAG
	ORF37-4 arah2 ORF (AD)	(301) (301) (301) (301) (301) (301)	AAC AAC AAC P	CAAA CAAA CAAA Putati	IGGTG IGGTG IGGTG IVE SI IVE SI 10 LVAL	CATO CATO CATO gnal	erec erec erec pept	GAGG GAGG GAGG tide 20 HASA	CATTO CATTO CATTO	SCAAC. SCAAC. SCAAC. 30	AGATCI AGATCI AGATCI RRCQS(ATGGAG ATGGAG ATGGAG 40 QLERAI	SAACC SAACC SAACC Epi	AGAGC AGAGC AGAGC itope# 5 EQHFM	GATAG GATAG GATAG 4 Epi 0	GTTG GTTG GTTG tope#	CAGO CAGO 5 SYER	GGAG GGAG GGAG GGAG ection 1 67 DPYSP	GCAACAG GCAACAG
Translation	ORF37-4 arah2 ORF (AD) Consensus f ORF 20-6 (temp)	(301) (301) (301) (301) (301) (1) (1) (1) (1) (1)	AAC AAC AAC P 1 MAF MAF	CAAA CAAA CAAA Putati (LTI) (LTI)	GGTG GGTG VE SI 10 LVAL LVAL LVAL	CATO CATO CATO SCATO SCATO SCATO ALFL ALFL	STGC STGC STGC Pept	GAGG GAGG GAGG tide 20 HASA HASA	CATTO CATTO CATTO RQQWH RQQWH RQQWH	SCAAC SCAAC SCAAC SCAAC SCAAC	AGATCJ AGATCJ AGATCJ RRCQS(RRCQS(RRCQS(ATGGAG ATGGAG ATGGAG 40 QLERAI QLERAI QLERAI	SAACC SAACC Epi Epi NLRPC NLRPC	AGAGC AGAGC AGAGC itope# 5 5 80HF 80HL 80HL	GATAG GATAG GATAG 4 Epi 0 0 10 10 10 10 10 10 10 10 10 10 10 10	GTTG GTTG GTTG tope# RDEDS RDEDS HDEDS	CAGO CAGO CAGO 5 5 5 5 5 7 5 7 5 7 5 7 5 7 5 7 5 7 5	GGAG GGAG GGAG GGAG C C C C C C C C C C	GCAACAG GCAACAG
Translation	ORF37-4 arah2 ORF (AD) Consensus f ORF 20-6 (temp) of Ara h2sa cD NA	(301) (301) (301) (301) (301) (1) (1) (1) (1) (1)	AAC AAC AAC P 1 MAF MAF	CAAA CAAA CAAA Putati (LTI) (LTI)	GGTG GGTG VE SI 10 LVAL LVAL LVAL	CATO CATO CATO SCATO SCATO SCATO ALFL ALFL	STGC STGC STGC Pept	GAGG GAGG GAGG tide 20 HASA HASA	CATTO CATTO CATTO RQQWH RQQWH RQQWH	SCAAC SCAAC SCAAC SCAAC SCAAC	AGATCJ AGATCJ AGATCJ RRCQS(RRCQS(ATGGAG ATGGAG ATGGAG 40 QLERAI QLERAI QLERAI	SAACC SAACC Epi Epi NLRPC NLRPC	AGAGC AGAGC AGAGC itope# 5 5 80HF 80HL 80HL	GATAG GATAG GATAG 4 Epi 0 0 10 10 10 10 10 10 10 10 10 10 10 10	GTTG GTTG GTTG tope# RDEDS RDEDS HDEDS	CAGO CAGO CAGO 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	GGAG GGAG GGAG GGAG GGAG G DPYSP DPYSP DPYSP DPYSP	GCAACAG GCAACAG
Translation	ORF37-4 arah2 ORF (AD) Consensus f ORF 20-6 (temp) of Ara h2sa cD NA lation of ORF37-4	(301) (301) (301) (301) (1) (1) (1) (1) (1) (1) (1) (1)	AAC AAC AAC P 1 MAF MAF MAF	CAAA CAAA CAAA Putati (LTI) (LTI)	GGTG GGTG VE SI 10 LVAL LVAL LVAL	CATO CATO CATO SCATO SCATO SCATO ALFL ALFL	STGC STGC STGC Pept	GAGG GAGG GAGG tide 20 HASA HASA	CATTO CATTO CATTO RQQWH RQQWH RQQWH	SCAAC SCAAC SCAAC SCAAC SCAAC	AGATCJ AGATCJ AGATCJ RRCQS(RRCQS(RRCQS(ATGGAG ATGGAG ATGGAG 40 QLERAI QLERAI QLERAI	SAACC SAACC Epi Epi NLRPC NLRPC	AGAGC AGAGC AGAGC itope# 5 5 80HF 80HL 80HL	GATAG GATAG GATAG 4 Epi 0 0 10 10 10 10 10 10 10 10 10 10 10 10	GTTG GTTG GTTG tope# RDEDS RDEDS HDEDS	CAGO CAGO CAGO 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	GGAG GGAG GGAG GGAG C C C C C C C C C C	SCAACAG SCAACAG SCAACAG
Translation Trans	ORF37-4 arah2 ORF (AD) Consensus fORF 20-6 (temp) of Ara h2sa cD NA lation of ORF37-4 Consensus	(301) (301) (301) (301) (1) (1) (1) (1) (1) (1) (1) (1) (68)	AAC AAC AAC P MAP MAP MAP MAP	CAAA CAAA CAAA Putati (LTII (LTII (LTII (LTII	GGTG GGTG GGTG LVE SI 10 LVAL LVAL LVAL LVAL	CATC CATC CATC CATC CATC CATC CATC CALFL ALFL ALFL 80	STGC STGC STGC pept LAAI LAAI LAAI	GAGG GAGG GAGG I I I I I I I I I I I I I	CATTO CATTO CATTO ROQUE ROQUE ROQUE ROQUE ROQUE	GCAAC GCAG G	AGATCI AGATCI AGATCI RRCQS(RRCQS(RRCQS(RRCQS)	ATGGAG ATGGAG ATGGAG DLERAI DLERAI DLERAI	SAACC SAAC SAACC SAA SAA	AGAGC AGAGC AGAGC itope# 5 5 5 80HFF 80HFF 80HLF 80HLF	GATAG GATAG GATAG GATAG 4 Epi 0 10 10 10 10 10 10 10 10 120 120	GTTG GTTG GTTG tope# RDEDS RDEDS HDEDS RDEDS	CAGO CAGO SYER SYER SYER SYER	GGAGG GGAGG GGAGG GGAGG GGAGG 67 DPYSP DPYSP DPYSP DPYSP DPYSP ection 2 134	SCAACAG SCAACAG SCAACAG
Translation Trans Translation o	ORF37-4 arah2 ORF (AD) Consensus f ORF 20-6 (temp) of Ara h2sa cD NA lation of ORF37-4	(301) (301) (301) (301) (1) (1) (1) (1) (1) (1) (1) (1) (1) (AAC AAC AAC P MAF MAF MAF 68 SQL	CAAA CAAA CAAA Putati (LTI) (LTI) (LTI) (LTI)	GGTG GGTG GGTG VE SI 10 LVAL LVAL LVAL LVAL	CATC CATC CATC CATC CATC CALFL ALFL ALFL ALFL 80 D RRG	STGC STGC GTGC Pept LAAI LAAI LAAI	GAGG GAGG GAGG Ide 20 HASA HASA HASA HASA	CATTO CATTO CATTO ROQUI ROQUI ROQUI ROQUI ROQUI ROQUI ROQUI ROQUI ROQUI ROQUI	GCAAC. GCAG. GCAAC. GCAG. GC	AGATCI AGATCI AGATCI RRCQS(RRCQS(RRCQS(RRCQS)	ATGGAG ATGGAG 40 212 RAI 212 RAI 212 RAI 212 RAI 212 RAI	SAACC SAAC SAACC SAACC SAA SAA	AGAGC AGAGC AGAGC itope# 5 EQHFF EQHLF EQHLF EQHLF EQHLF EQHLF	GATAG GATAG GATAG GATAG 4 Epi 0 10 10 10 10 10 10 120 120 120 120 120	GTTG GTTG GTTG TG CDED RDED RDED RDED RDED SRQQI	CAGO CAGO CAGO 5 SYER: S	GGAGG GGAGG GGAGG GGAGG GGAGG GGAGG C DPYSP DPYSP DPYSP DPYSP ection 2 134 KRELR	SCAACAG SCAACAG SCAACAG
Translation Trans Translation o Translation	ORF 37-4 arah2 ORF (AD) Consensus f ORF 20-6 (temp) of Ara h2sa cD N/ Lation of ORF 37-4 Consensus f ORF 20-6 (temp) of Ara h2sa cD N/ Lation of ORF 37-4	(301) (301) (301) (301) (1) (1) (1) (1) (1) (1) (1) (1) (1) (AAC AAC AAC P MAF MAF MAF SQI SQI SQI	CAAA CAAA CAAA Putati (LTI) (LTI) (LTI) (LTI) (LTI) (LTI) (LTI) (LTI) (LTI) (LTI) (LTI) (LTI)	GGTG GGTG GGTG (VC SI) (VC SI) (VAL) LVAL LVAL LVAL LVAL LVAL LVAL PSPYI PSPYI PSPYI	CATC CATC CATC CATC CATC CATC CATC CATC	GTGC GTGC GTGC Pept LAAI LAAI LAAI LAAI LAAI LAAI SAGS SAGS	GAGG GAGG GAGG HASA HASA HASA HASA SQHQ SQHQ SQHQ	CATTO CATTO CATTO RQQWI RQQWI RQQWI 90 ERCCN ERCCN ERCCN	GCAAC. GCAC. G	AGATCI AGATCI RRCQS(RRCQS(RRCQS(RRCQS) 100 FENNQI FENNQI	ATGGAC ATGGAC ATGGAC 40 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI	ACC ACC FAACC Epi VLRPC VLRPC VLRPC 110 ALQQI ALQQI	AGAGC AGAGC AGAGC AGAGC Itope# 5 EQHF EQHF EQHL EQHL EQHL EQHL EQHL MENQS MENQS	GATAG GATAG GATAG 4 Epi 0 10 10 10 10 10 10 10 10 10 10 10 10 1	GTTG GTTG GTTG TOPC# RDEDS RDEDS RDEDS RDEDS SRQQI GRQQI GRQQI GRQQI	CAGG CAGG CAGG SYER: SYE	GGAG GGAG GGAG GGAG C GGAG GGAG C C C C	GCAACAG GCAACAG GCAACAG
Translation Trans Translation o Translation	ORF 37-4 arah2 ORF (AD) Consensus f ORF 20-6 (temp) f ORF 20-6 (temp) Consensus f ORF 20-6 (temp) of Ara h2sa cD NA	(301) (301) (301) (301) (1) (1) (1) (1) (1) (1) (1) (1) (1) (AAC AAC AAC P MAF MAF MAF SQI SQI SQI	CAAA CAAA CAAA Putati (LTI) (LTI) (LTI) (LTI) (LTI) (LTI) (LTI) (LTI) (LTI) (LTI) (LTI) (LTI)	GGTG GGTG GGTG (VC SI) (VC SI) (VAL) LVAL LVAL LVAL LVAL LVAL LVAL PSPYI PSPYI PSPYI	CATC CATC CATC CATC CATC CATC CATC CATC	GTGC GTGC GTGC Pept LAAI LAAI LAAI LAAI LAAI LAAI SAGS SAGS	GAGG GAGG GAGG HASA HASA HASA HASA SQHQ SQHQ SQHQ	CATTO CATTO CATTO RQQWI RQQWI RQQWI 90 ERCCN ERCCN ERCCN	GCAAC. GCAC. G	AGATCI AGATCI RRCQS(RRCQS(RRCQS(RRCQS) 100 FENNQI FENNQI	ATGGAC ATGGAC ATGGAC 40 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI	ACC ACC FAACC Epi VLRPC VLRPC VLRPC 110 ALQQI ALQQI	AGAGC AGAGC AGAGC AGAGC Itope# 5 EQHF EQHF EQHL EQHL EQHL EQHL EQHL MENQS MENQS	GATAG GATAG GATAG 4 Epi 0 10 10 10 10 10 10 10 10 10 10 10 10 1	GTTG GTTG GTTG TOPC# RDEDS RDEDS RDEDS RDEDS SRQQI GRQQI GRQQI GRQQI	CAGG CAGG CAGG CAGG SYER: SYER	GGAG GGAG GGAG GGAG C GGAG GGAG C C C C	GCAACAG GCAACAG GCAACAG
Translation Trans Translation o Translation	ORF 37-4 arah2 ORF (AD) Consensus f ORF 20-6 (temp) of Ara h2sa cD N/ Lation of ORF 37-4 Consensus f ORF 20-6 (temp) of Ara h2sa cD N/ Lation of ORF 37-4	(301) (301) (301) (301) (1) (1) (1) (1) (1) (1) (1) (1) (1) (AAC AAC AAC AAC AAC AAC AAC AAC AAC AAC	CAAA CAAA CAAA Putati (LTI) (LTI) (LTI) (LTI) (LTI) (LTI) (LTI) (LTI) (LTI) (LTI) (LTI) (LTI)	GGTG GGTG GGTG VC SI; 10 LVAL LVAL LVAL LVAL LVAL PSPYI PSPYI PSPYI	CATC CATC CATC CATC CATC CATC CATC CATC	GTGC GTGC GTGC Pept LAAI LAAI LAAI LAAI LAAI LAAI SAGS SAGS	GAGG GAGG GAGG Tide 20 HASA HASA HASA HASA SQHQ SQHQ SQHQ	CATTO CATTO CATTO CATTO RQQWI RQQWI RQQWI 90 ERCCN ERCCN ERCCN	GCAAC. GCAC. G	AGATCI AGATCI RRCQS(RRCQS(RRCQS(RRCQS) 100 FENNQI FENNQI	ATGGAC ATGGAC ATGGAC 40 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI	ACC ACC FAACC Epi VLRPC VLRPC VLRPC 110 ALQQI ALQQI	AGAGC AGAGC AGAGC AGAGC Itope# 5 EQHF EQHF EQHL EQHL EQHL EQHL EQHL MENQS MENQS	GATAG GATAG GATAG 4 Epi 0 10 10 10 10 10 10 10 10 10 10 10 10 1	GTTG GTTG GTTG TOPC# RDEDS RDEDS RDEDS RDEDS SRQQI GRQQI GRQQI GRQQI	CAGG CAGG CAGG CAGG SYER: SYER	GGAG GGAG GGAG GGAG GGAG GGAG C C DPYSP DPYSP DPYSP DPYSP ection 2 134 KRELR KRELR KRELR	GCAACAG GCAACAG GCAACAG
Translation Trans Translation o Translation Trans Trans	ORF 37-4 arah2 ORF (AD) Consensus f ORF 20-6 (temp) of Ara h2sa oD NA lation of ORF37-4 Consensus f ORF 20-6 (temp) of Ara h2sa oD NA lation of ORF37-4 Consensus f ORF 20-6 (temp)	(301) (301) (301) (301) (1) (1) (1) (1) (1) (135) (135) (135)	AACC AACC AACC P MAF MAF MAF 68 SQI SQI SQI SQI SQI 135 NLI	CAAA CAAA CAAA Putati (LTI) (L	GGTG GGTG GGTG VE SI; 10 LVAL LVAL LVAL LVAL LVAL LVAL LVAL DSPYI PSPYI PSPYI PSPYI D GLRA	CATC CATC CATC CATC CATC CATC CATC CATC	GTGC GTGC GTGC Dept LAAI LAAI LAAI LAAI LAAI LAAI SAGS GAGS GAGS GAGS	GAGG GAGG GAGG Tide 20 HASA HASA HASA HASA SQHQ SQHQ SQHQ SQHQ	CATTO CATTO CATTO CATTO CATTO RQQWI RQQWI RQQWI 90 ERCCN ERCCN ERCCN ERCCN ERCCN	30 30 30 30 30 30 30 30 30 30	AGATCI AGATCI RRCQS(RRCQS(RRCQS(RRCQS) 100 FENNQI FENNQI	ATGGAC ATGGAC ATGGAC 40 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI	ACC ACC FAACC Epi VLRPC VLRPC VLRPC 110 ALQQI ALQQI	AGAGC AGAGC AGAGC AGAGC Itope# 5 EQHF EQHF EQHL EQHL EQHL EQHL EQHL MENQS MENQS	GATAG GATAG GATAG 4 Epi 0 10 10 10 10 10 10 10 10 10 10 10 10 1	GTTG GTTG GTTG TOPC# RDEDS RDEDS RDEDS RDEDS SRQQI GRQQI GRQQI GRQQI	CAGG CAGG CAGG CAGG SYER: SYER	GGAG GGAG GGAG GGAG GGAG GGAG C C DPYSP DPYSP DPYSP DPYSP ection 2 134 KRELR KRELR KRELR KRELR	GCAACAG GCAACAG GCAACAG
Translation Translation o Translation o Translation Translation Translation o Translation	ORF 37-4 arah2 ORF (AD) Consensus f ORF 20-6 (temp) of Ara h2sa oD Né lation of ORF37-4 Consensus f ORF 20-6 (temp) of Ara h2sa oD Né Lation of ORF37-4 Consensus f ORF 20-6 (temp) of Ara h2sa oD Né	(301) (301) (301) (301) (1) (1) (1) (1) (1) (1) (1) (1) (1) (AACC AACC AACC P MAP MAP MAP MAP MAP MAP MAP MAP MAP M	CAAA CAAA CAAA Putati (LTI) (L	GGTG GGTG GGTG VE SI; 10 LVAL LVAL LVAL LVAL LVAL LVAL LVAL DSPYI PSPYI PSPYI D GLRA GLRA	CATC CATC CATC CATC gnal) ALFL ALFL ALFL ALFL BO DRRG DRRG DRRG DRRG PQRC PQRC	STGC STGC STGC Pepti LLAAI LLAAI LLAAI LLAAI LLAAI LLAAI LLAAI SAGS SAGS SAGS (150 CD LD CD LD	GAGG GAGG GAGG GAGG Tide 20 HASA HASA HASA HASA SQHQ SQHQ SQHQ SQHQ	CATTO CATTO CATTO CATTO CATTO CATTO CATTO RQQWI RQQWI RQQWI 90 ERCCN ERCCN ERCCN ERCCN ERCCN ERCCN ERCCN	30 30 30 30 30 30 30 30 30 30	AGATCI AGATCI RRCQS(RRCQS(RRCQS(RRCQS(100 FENNQI FENNQI	ATGGAC ATGGAC ATGGAC 40 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI	ACC ACC FAACC Epi VLRPC VLRPC VLRPC 110 ALQQI ALQQI	AGAGC AGAGC AGAGC AGAGC Itope# 5 EQHF EQHF EQHL EQHL EQHL EQHL EQHL MENQS MENQS	GATAG GATAG GATAG 4 Epi 0 10 10 10 10 10 10 10 10 10 10 10 10 1	GTTG GTTG GTTG TOPC# RDEDS RDEDS RDEDS RDEDS SRQQI GRQQI GRQQI GRQQI	CAGG CAGG CAGG CAGG SYER: SYER	GGAG GGAG GGAG GGAG GGAG GGAG C C DPYSP DPYSP DPYSP DPYSP ection 2 134 KRELR KRELR KRELR KRELR	GCAACAG GCAACAG GCAACAG
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Translation Translation o Translation o Translation Translation Translation o Translation	ORF 37-4 arah2 ORF (AD) Consensus f ORF 20-6 (temp) of Ara h2sa oD Né lation of ORF37-4 Consensus f ORF 20-6 (temp) of Ara h2sa oD Né Lation of ORF37-4 Consensus f ORF 20-6 (temp) of Ara h2sa oD Né	(301) (301) (301) (301) (301) (301) (11) (301) (11) (11) (113) (113) (113) (113) (113) (113) (113) (113)	AACC AACC AACC P MAP MAP 68 SQI SQI SQI SQI SQI 135 NLI NLI	CAAA CAAAA CAAAA Putati KLTII KLTII OPYSI OPYSI OPYSI OPYSI 0PYSI 14 QQQCC	GGTG GGTG GGTG VC SI LVAL LVAL LVAL LVAL LVAL LVAL GLRA GLRA GLRA	CATCCATC CATCCATC gnal) ALFL ALFL ALFL 80 D RRG D RRG D RRG D RRG D RRG D RRG D RRG D RRG D RRG	STGC STGC STGC pept LAAN LAAN LAAN LAAN LAAN LAAN SAGS SAGS SAGS (160 D DD D DD D DD D DD	GAGG GAGG GAGG GAGG GAGG GAGG GAGG GAG	CATTO CATTO CATTO CATTO CATTO CATTO RQQWH RQQWH RQQWH RQQWH RQQWH RQQWH BRCCM BRCCM BRCCM BRCCM BRCCM BRCCM CATTO CATO CA	CAAC CAAC CAAC CAAC CAAC CAAC CAAC CAA	AGATCI AGATCI RRCQS(RRCQS(RRCQS(RRCQS(100 FENNQI FENNQI	ATGGAC ATGGAC ATGGAC 40 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI 2LE RAI	ACC ACC FAACC Epi VLRPC VLRPC VLRPC 110 ALQQI ALQQI	AGAGC AGAGC AGAGC AGAGC Itope# 5 EQHF EQHF EQHL EQHL EQHL EQHL EQHL MENQS MENQS	GATAG GATAG GATAG 4 Epi 0 10 10 10 10 10 10 10 10 10 10 10 10 1	GTTG GTTG GTTG TOPC# RDEDS RDEDS RDEDS RDEDS SRQQI GRQQI GRQQI GRQQI	CAGG CAGG CAGG CAGG SYER: SYER	GGAG GGAG GGAG GGAG GGAG GGAG C C DPYSP DPYSP DPYSP DPYSP ection 2 134 KRELR KRELR KRELR KRELR	GCAACAG GCAACAG GCAACAG

Conclusions of TILLING

2,020 individuals screened

•Recovered two validated *ara h 2.01* missense mutations and two putative mutants

•All mutants so far from EMS-mutagenized populations and for *ara h 2.01*

Mutation rate 1 SNP/212 kb

Silencing Allergens

Silence by mutation (knockout)

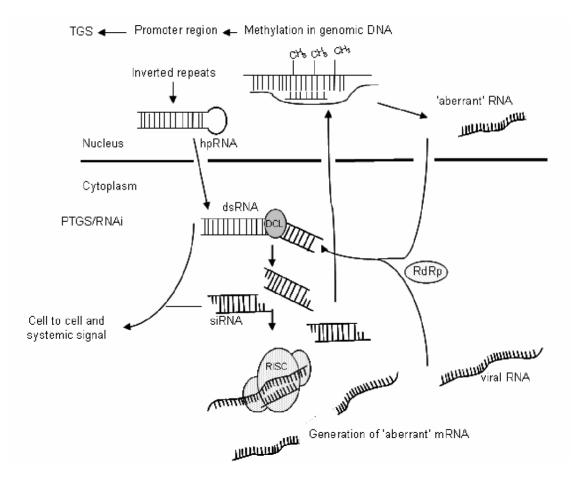
- Stable phenotype without genetic engineering
- Knockout is the less frequent mutation
- Only one gene targeted at one time

Silence post-transcriptionally – at the level of expression (knockdown)

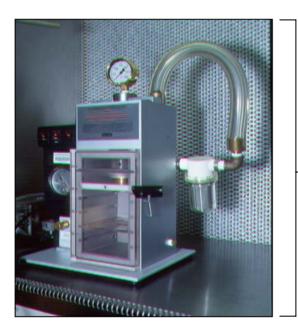
- Target multiple genes at one time
- Requires genetic engineering
- More variable in outcome and potentially unstable

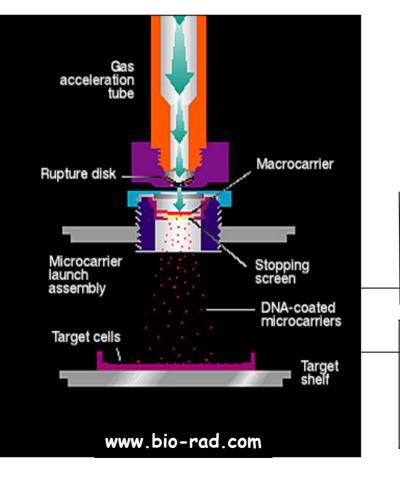
RNA Interference

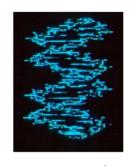
Ozias-Akins *et al.*, 2006. Hypoallergenic foods beyond infant formulas. In: Food Allergy. ASM Press, Herndon, VA

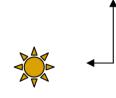


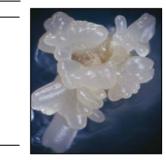
Direct DNA Transformation







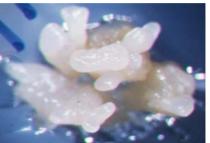




TRANSFORMATION CYCLE 12-18 months

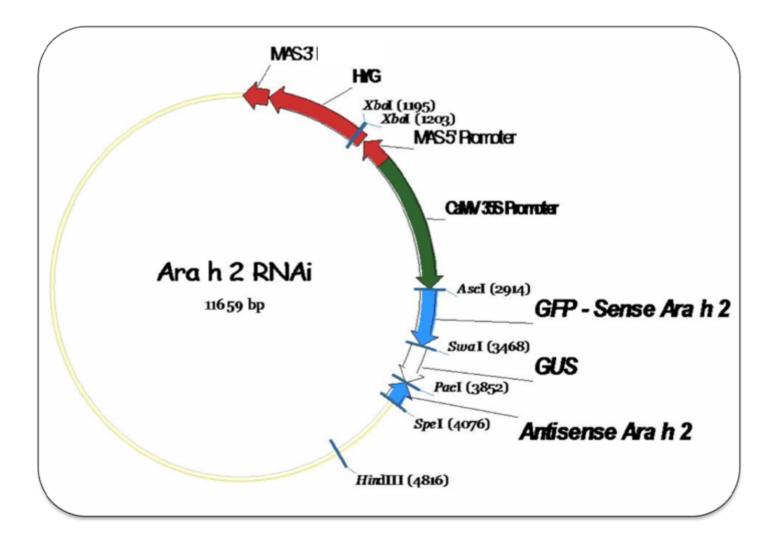






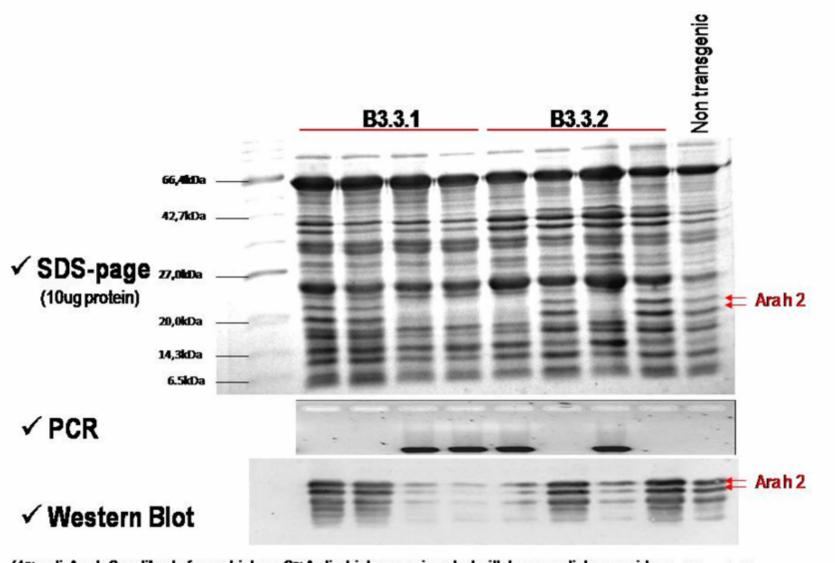


Construct for RNA Interference of ara h 2



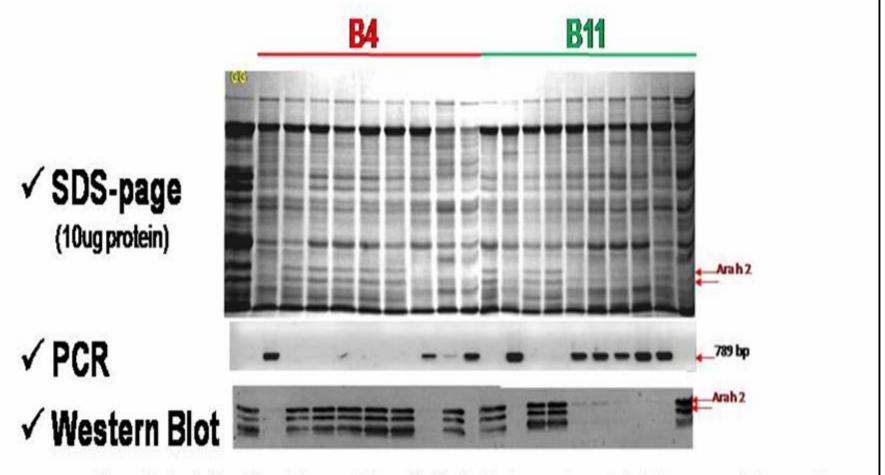
GENETIC	SELECTION	TRANSGENIC
TRANSFORMATION		LINES
via biolistics	Hygromycin resistant	→ 4 lines: no sufficient seeds.
	8 hygromycin	2 lines: B3.3.1 & B3.3.2
	resistant lines	reduced Ara h 2 protein
		→ 2 lines: B4 & B11
		barely detectable Ara h
		2.

Ara h 2 is reduced by ~90% in some transgenic lines



(1" anti-Ara h 2 antibody from chicken; 2" Anti-chicken conjugated with horseradish peroxidase - 10ug protein)

Ara h 2 is barely detectable in other transgenic lines

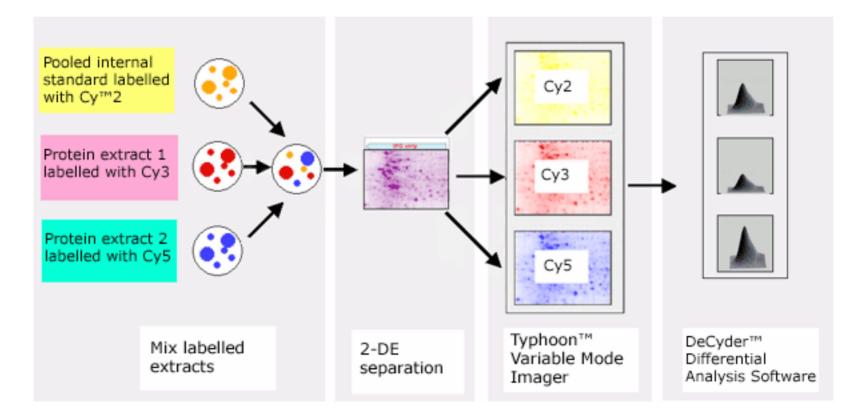


(1^{ry} anti-Ara h 2 antibody from chicken; 2^{ry} Anti-chicken conjugated with horseradish peroxidase - 10ug protein)

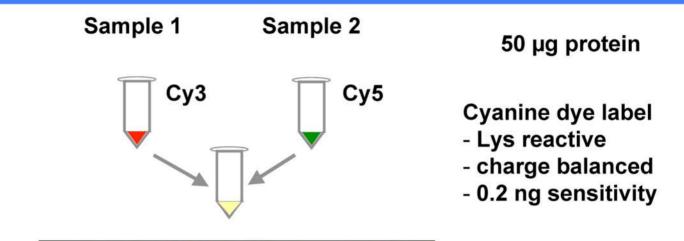
New variation of 2-D gels...Difference Gel Electrophoresis (DIGE)

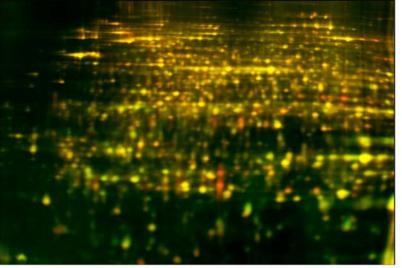
Differential Gel Electrophoresis (DIGE)

- samples resolved in the same gel
- simplifies spot matching



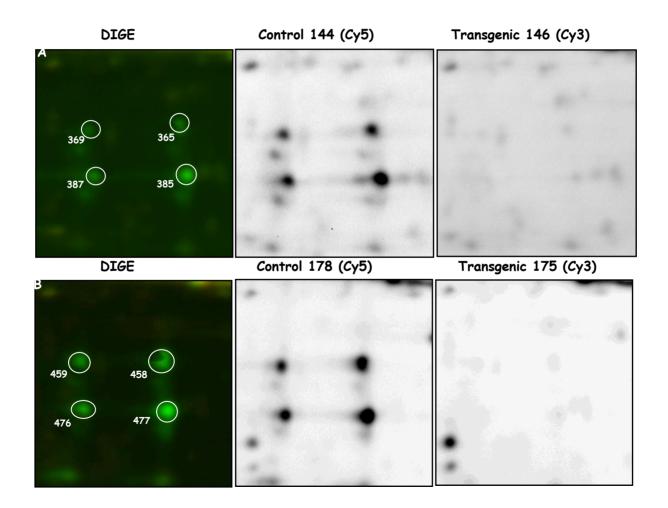
Difference Gel Electrophoresis - DIGE



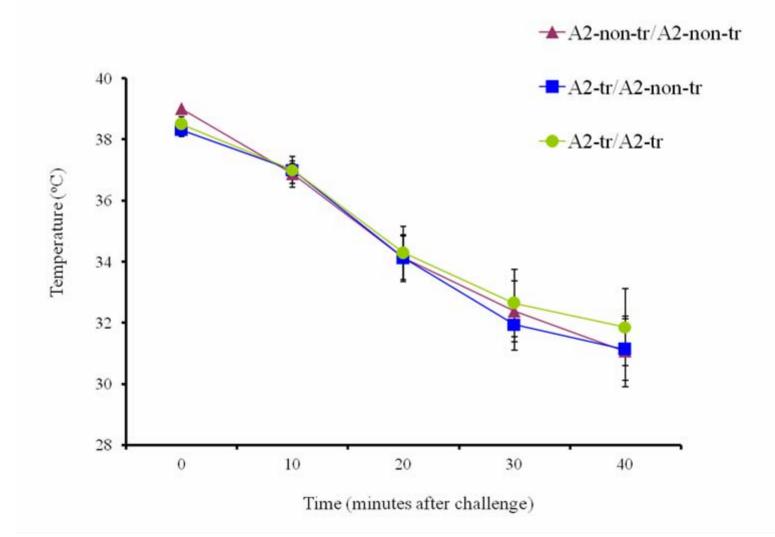


Arabidopsis seed

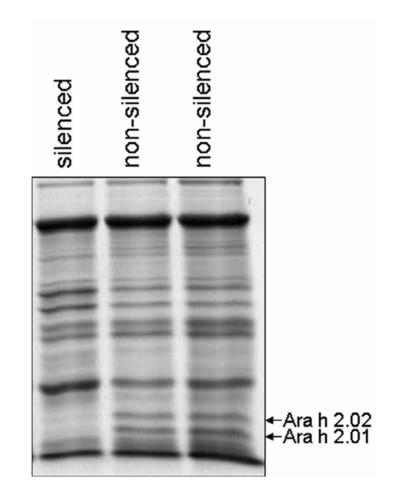
Difference Gel Electrophoretic Analysis Coupled with Tandem Mass Spectrometry



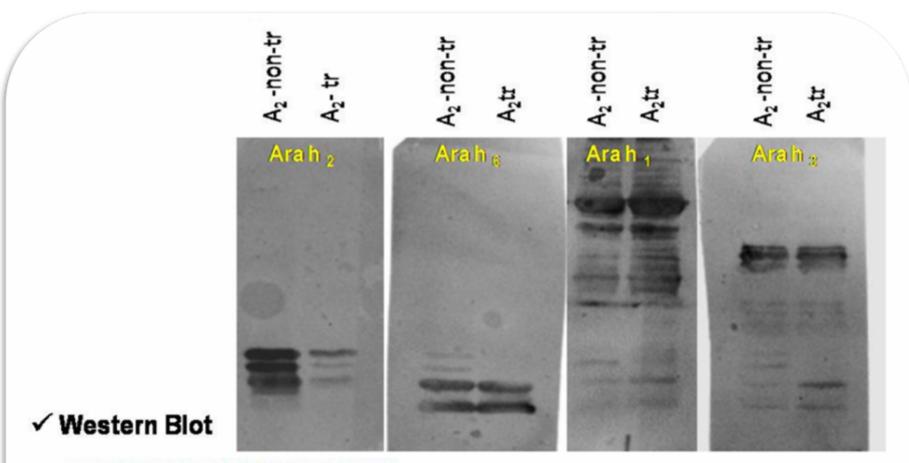
A 90% reduction in Ara h 2 is not sufficient to alter allergic reaction in a mouse model of anaphylaxis



RNA Interference Is Specific to Ara h 2 . . .

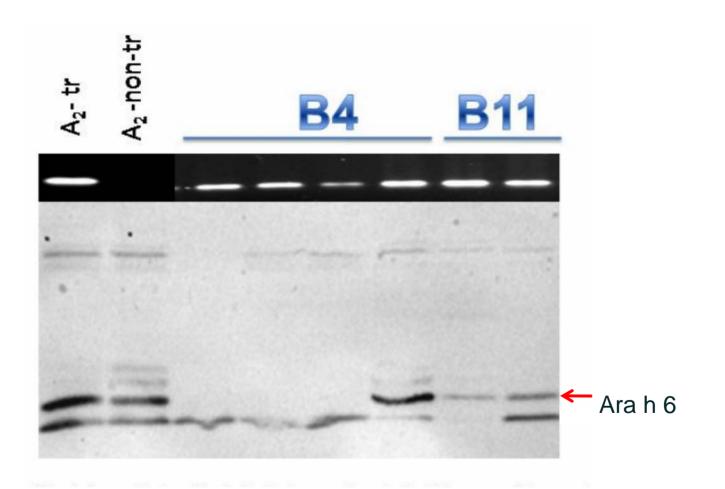


RNA Interference Is Specific to Ara h 2 . . .



(1^{ry} anti-Ara h 2, Ara h 6, Ara h 1 and Ara h 3 antibody from chicken; 2^{ry} Anti-chicken conjugated with horseradish peroxidase - 10ug protein)

RNA Interference Is Specific to Ara h 2 Except for Ara h 6 in Some Lines



Conclusions

RNA silencing is an effective method to silence allergen genes at least for the purpose of testing the effect of their absence on seed growth and development.

RNA silencing may be unstable under certain circumstances, and instability cannot be tolerated in a commercial crop.

TILLING to recover mutant allergen genes is likely to result in more stable reduction of allergens, but will require many steps including mutant production, mutant screening, and traditional breeding to combine mutant A- and B-genome genes.

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