

Soybean Allergens Characterization and Removal

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Agri-Food Canada

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Canada

Outline

Brief review of food allergies

Soybean proteomics

Strategies to eliminate allergens from soy



What is a Food Allergy?

An immunological reaction (IgE) resulting from the ingestion, inhalation, or atopic contact with a food or food ingredient

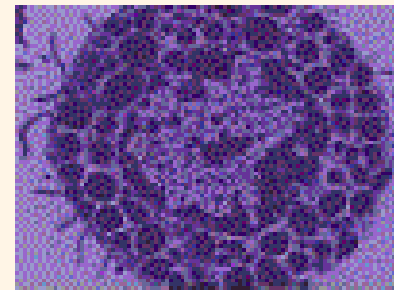


Hypersensitized Individual (follows 1st exposure)

Second Exposure
to Food/Protein

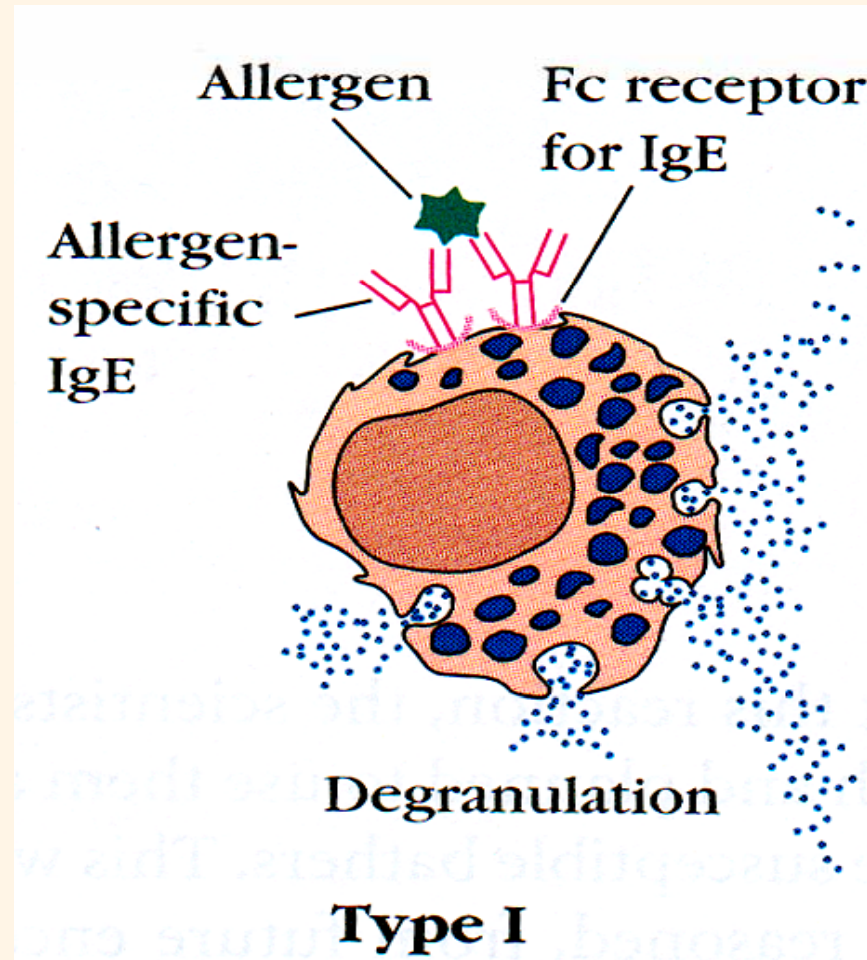


Adverse Reaction
possible
Anaphylaxis



Activated Mast Cells
React to Food/Protein

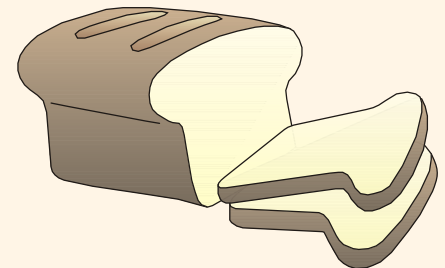
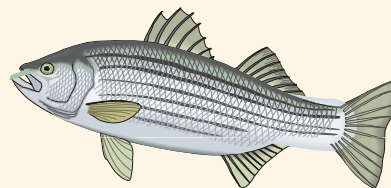
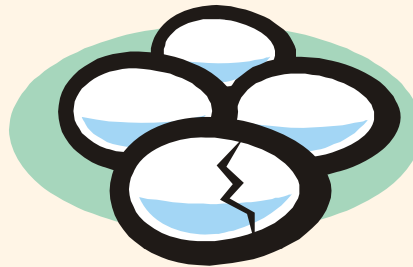
IgE-Mediated Hypersensitivity



Most common allergenic foods

(1992 US study on children with food allergies)

- Eggs 25%
- Peanuts 24%
- Milk 23%
- Tree nuts 10%
- **Soybeans 6%**
- Fish 3%
- Wheat 2.5%



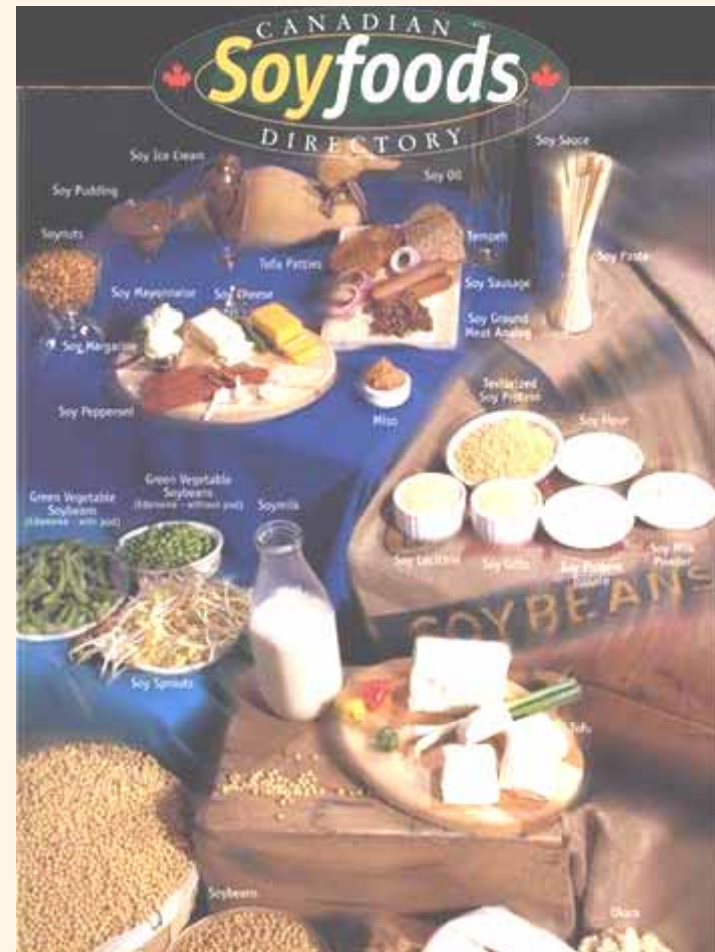
Soybean seed content

- 40% Protein
- 20% Oil
- 12% Carbohydrates
- Other (tannins, phytates, phytoestrogens, oligosaccharides, saponins)

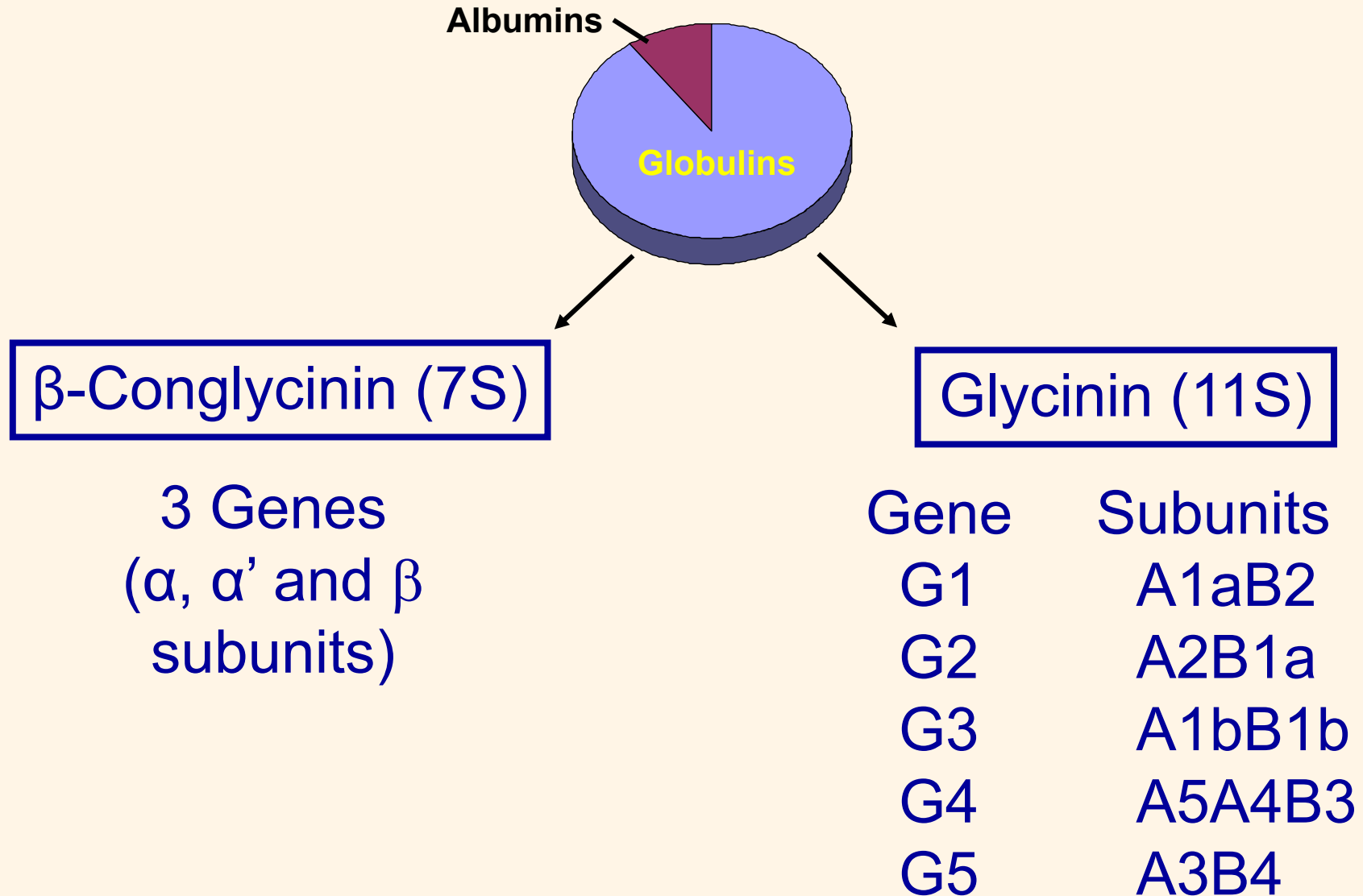


Soy in the food industry

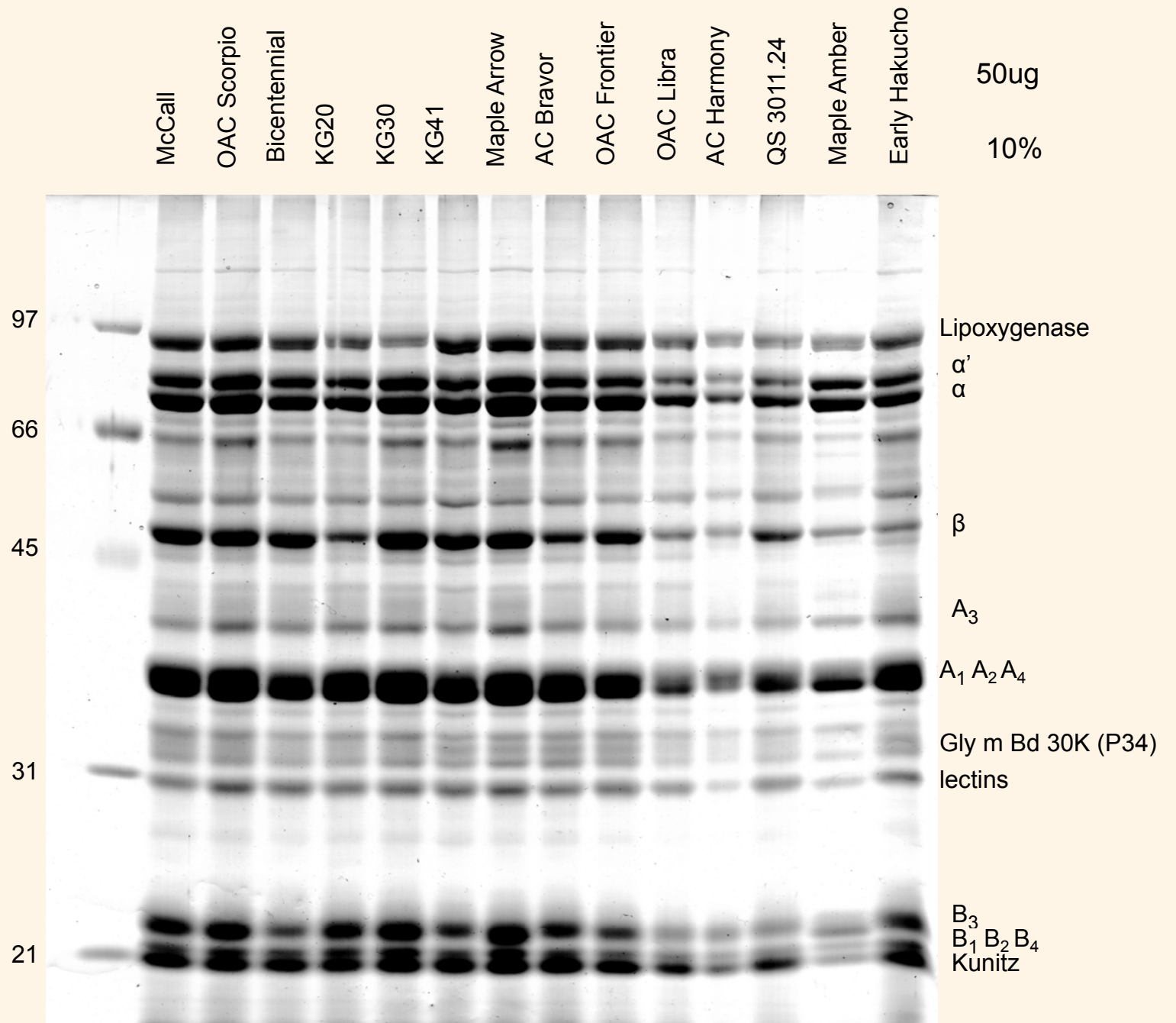
- Soy presence in foods increasing every year
- Health benefits
- Soy prevalence increases chances of exposure (allergic reactions)



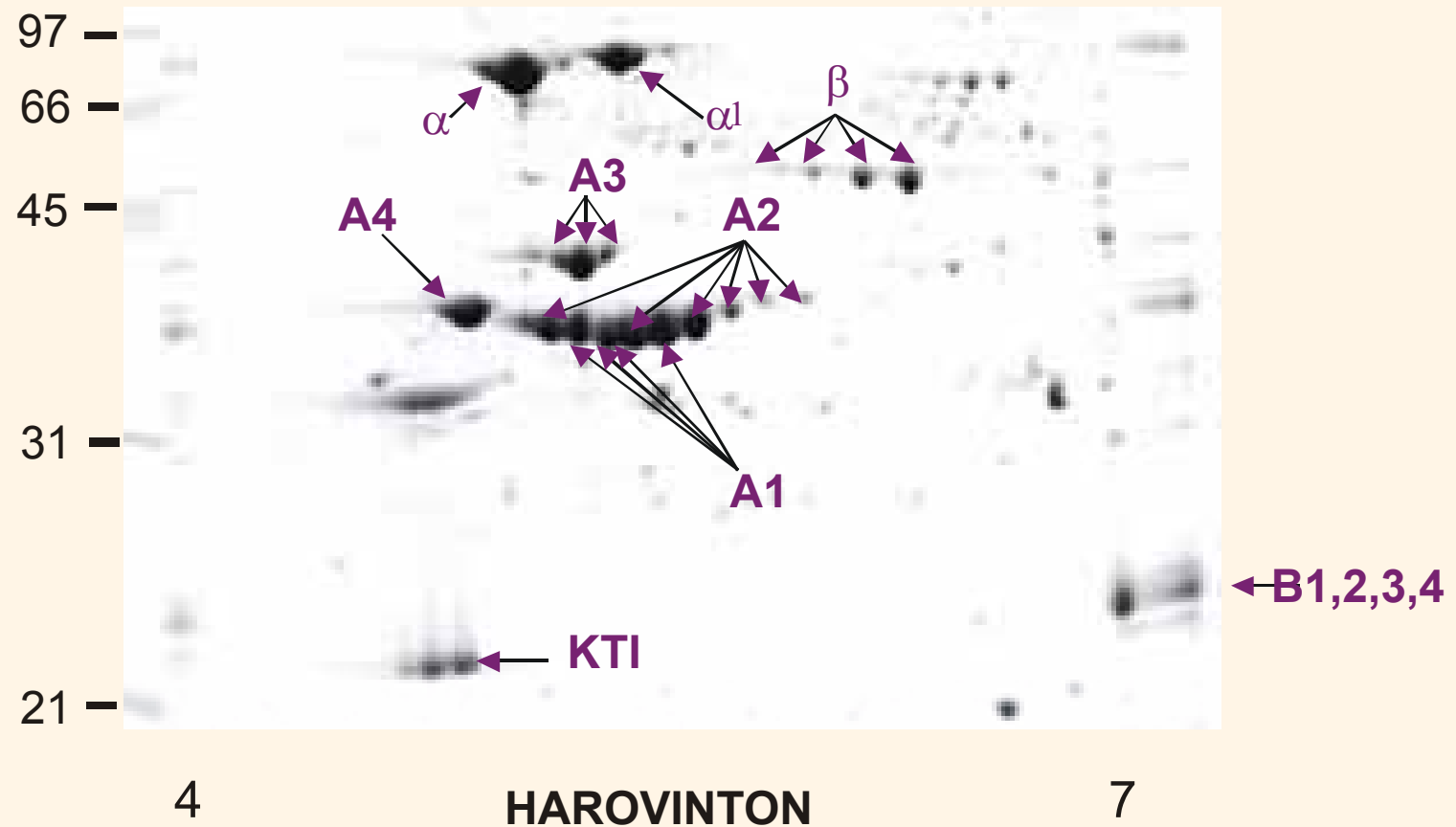
Soybean proteins



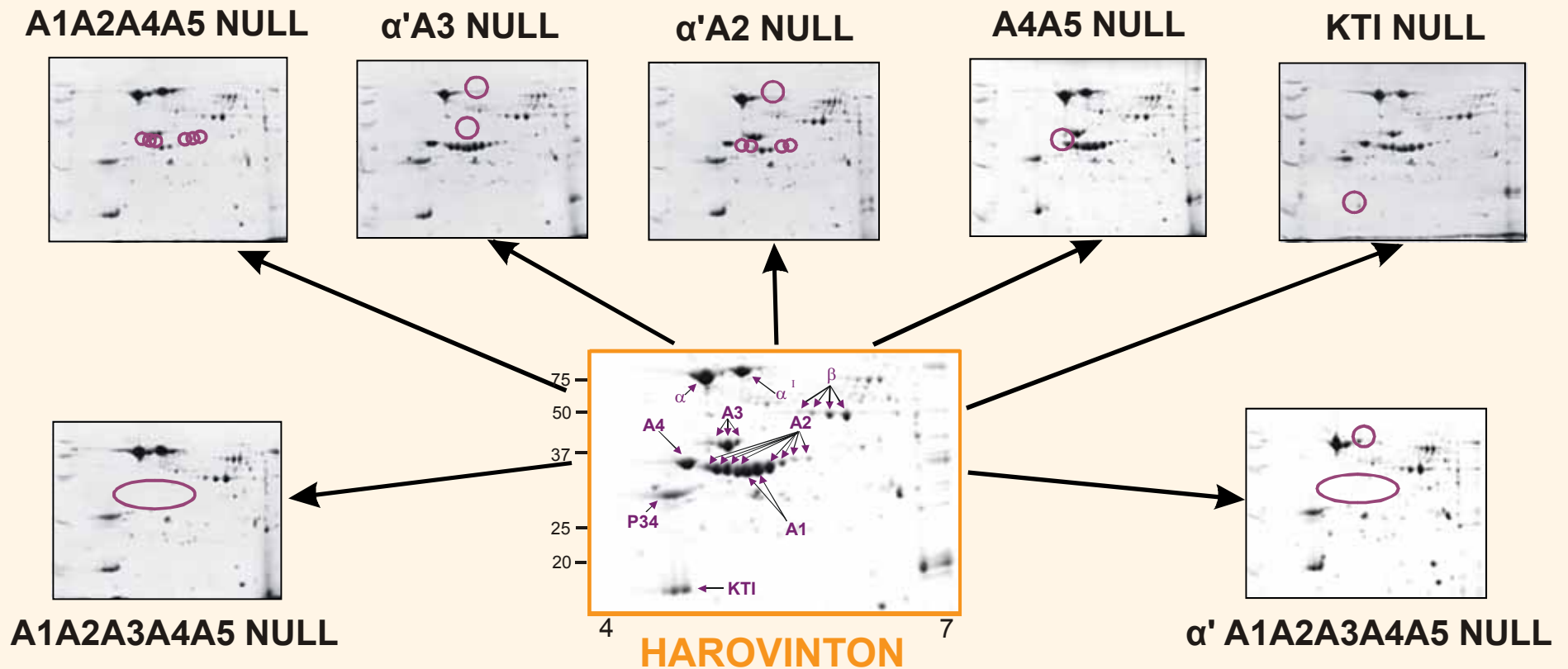
March09-04



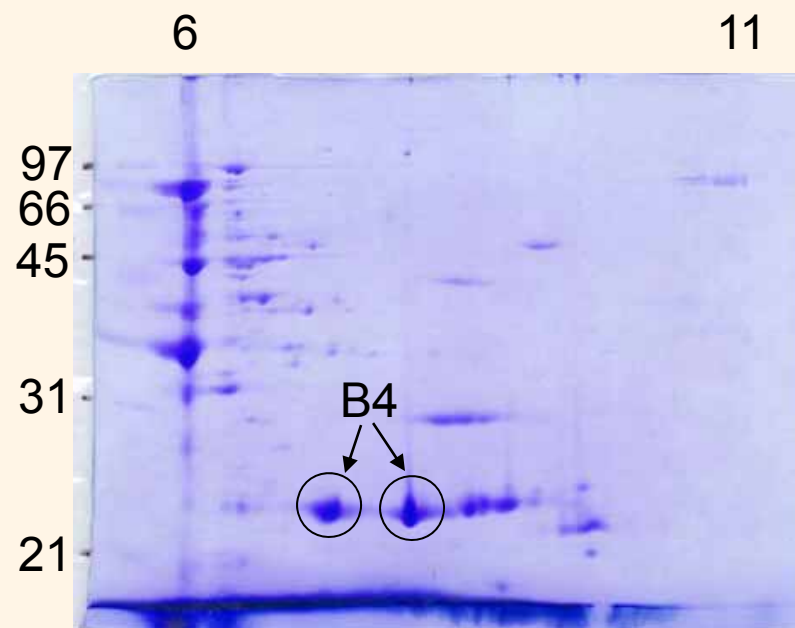
2D gel electrophoresis



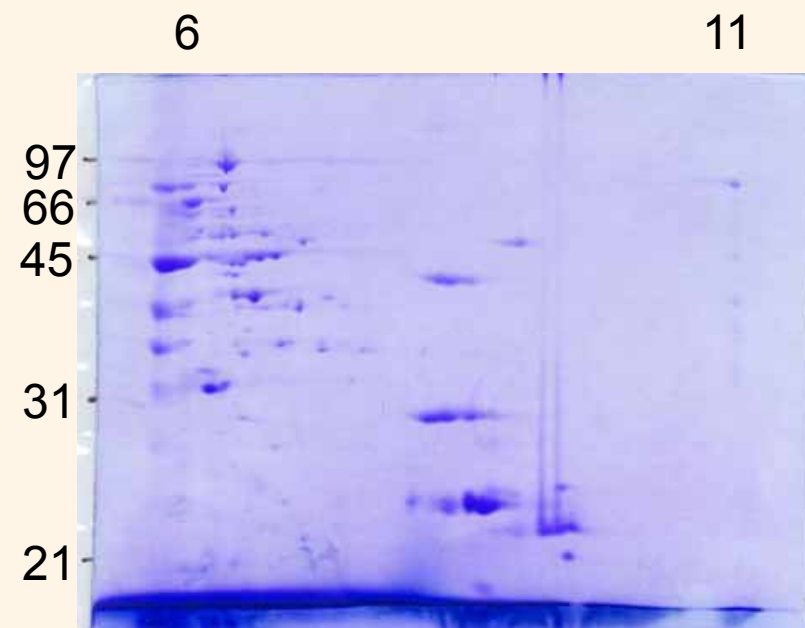
Identification of glycinin and β -conglycinin subunits



Identification of basic glycinin chains



Harovinton



A3 B4 null

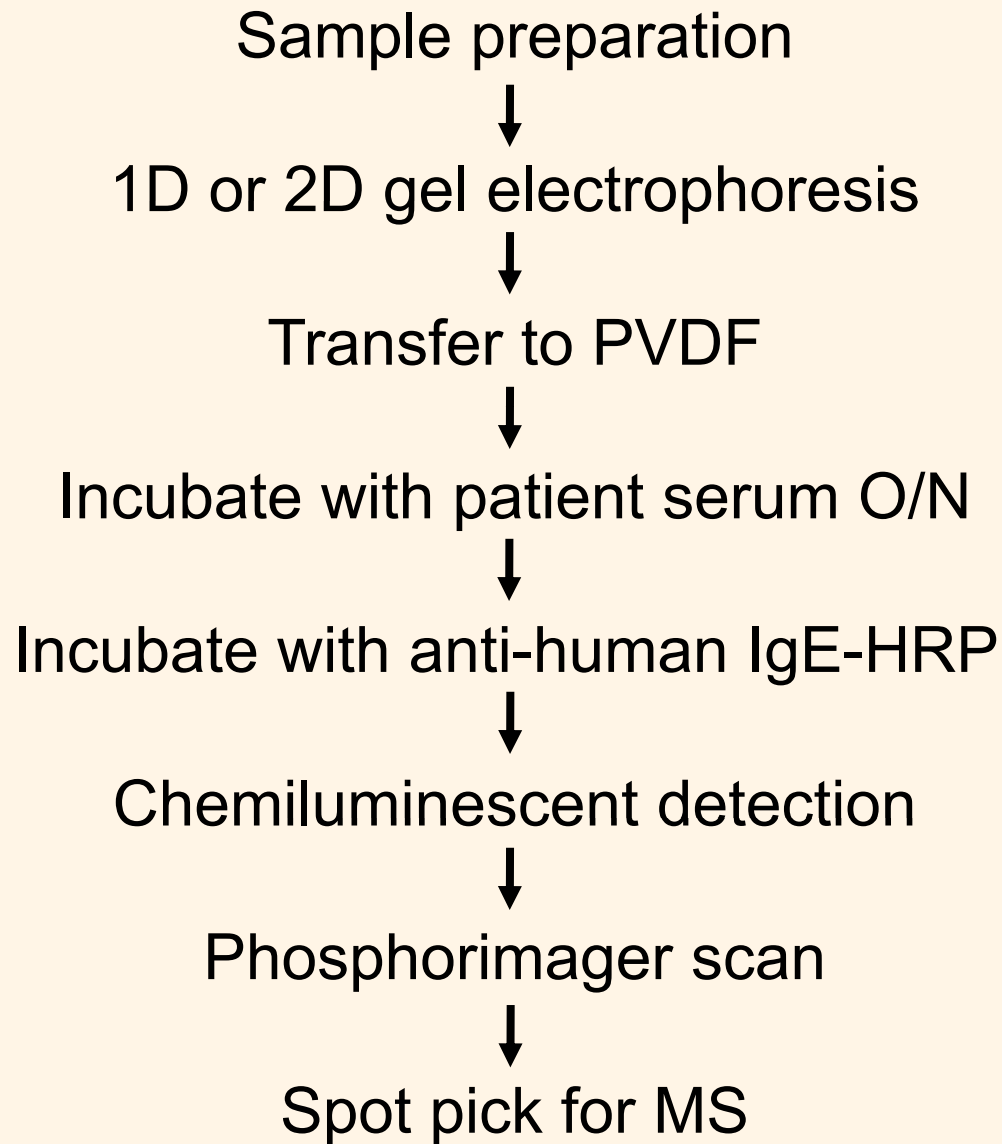
Protein Quality of null Soybeans

Zarkadas et al 2007. Food Res Intl.

- Total protein 33 – 37%
- EAA₉ 44.5 – 47.3%
- Physiologically normal seeds, plants



Methodology

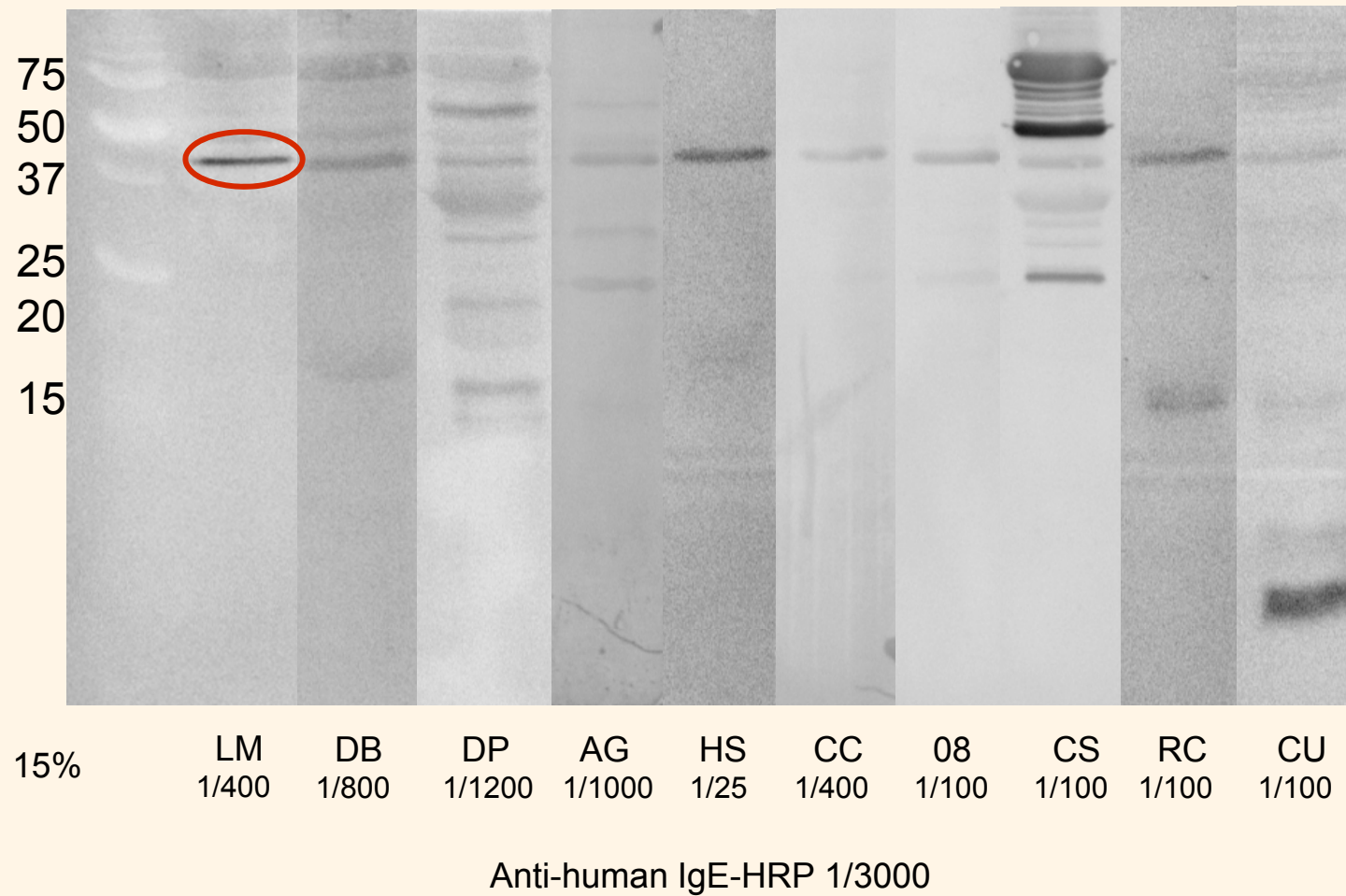


Characteristics of patients allergic to soybean

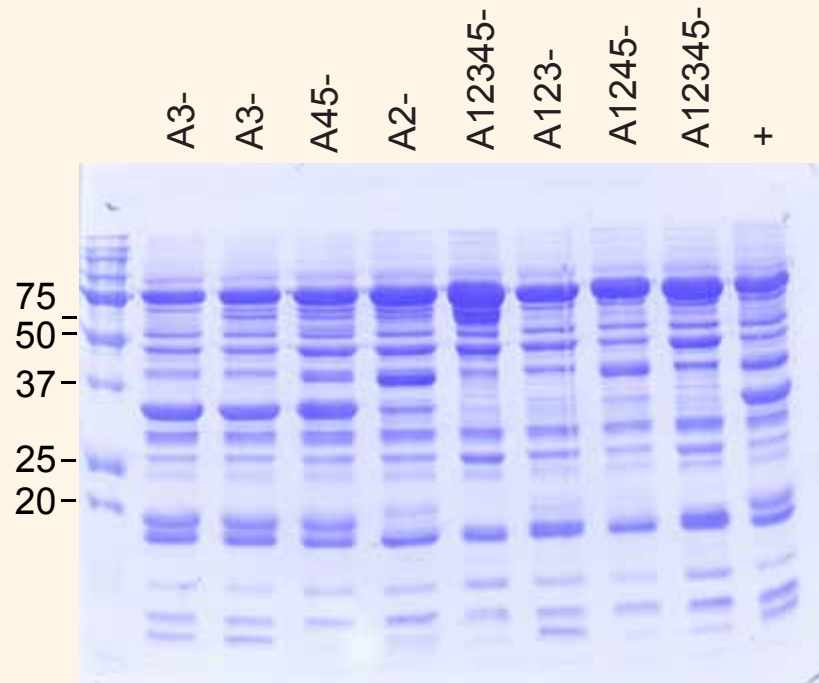
Code	Sex	Age	RAST	Symptoms
LM	F	23	68x ¹	laryngeal edema, bronchospasm, hives, itching, abdominal pain. Also allergic to peanuts
CU	F	42	66x	hives, asthma, watery eyes, sneezing, rhinitis. Lost allergy to peanut.
CS	F	25	5x	respiratory difficulty, bronchoconstriction, wheezing. Also allergic to peanuts
RC	M	54	3x	hives, localized swelling, bronchoconstriction, metallic taste. Not allergic to peanuts
08	M	28	ND	anaphylaxis, wheezing, generalized pruritus, throat tightness, redness, chest tightness. Also allergic to peanuts
DB	M	42	34.8kUA/L	anaphylaxis, blood pressure increase. Not allergic to peanuts
DP	F	25	NA	anaphylaxis, throat closure, total swelling of eyes, hives, vomiting. Also allergic to peanuts
AG	F	32	3.18kUA/L	breathing trouble, nasal congestion. Not allergic to peanuts.
HS	F	31	0.6kUA/L	eczema. Not allergic to peanuts
CC	F	35	11x	hives, swelling, difficulty breathing, diarrhea, lip edema. Also allergic to peanuts

¹ Internal RAST score. Indicates number of times great the counts were compared to negative serum

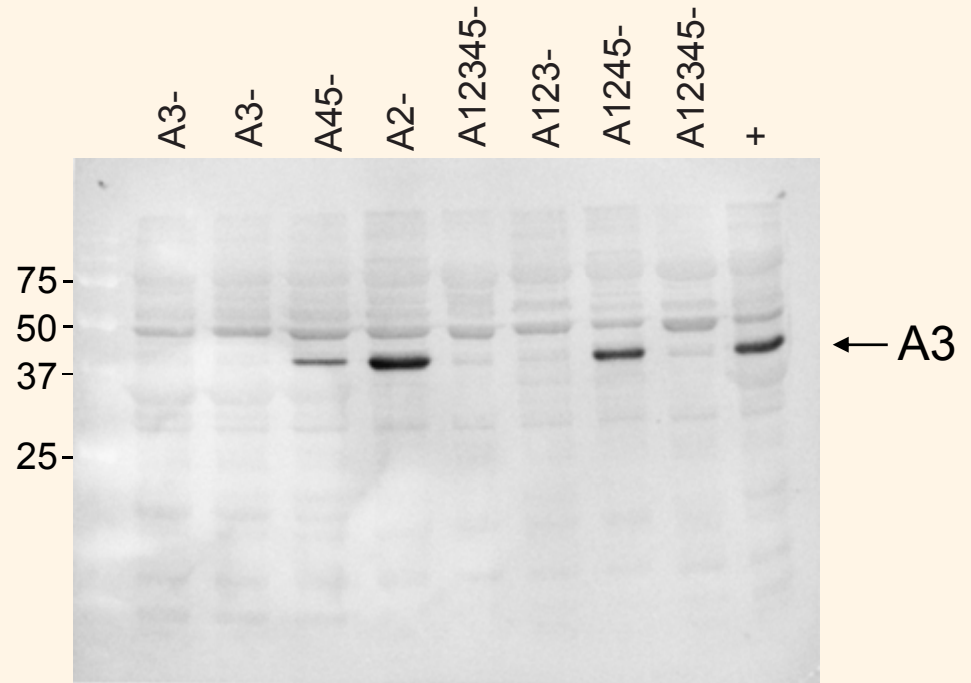
Patient reactions to soy proteins



Screening of glycinin null lines with LM serum

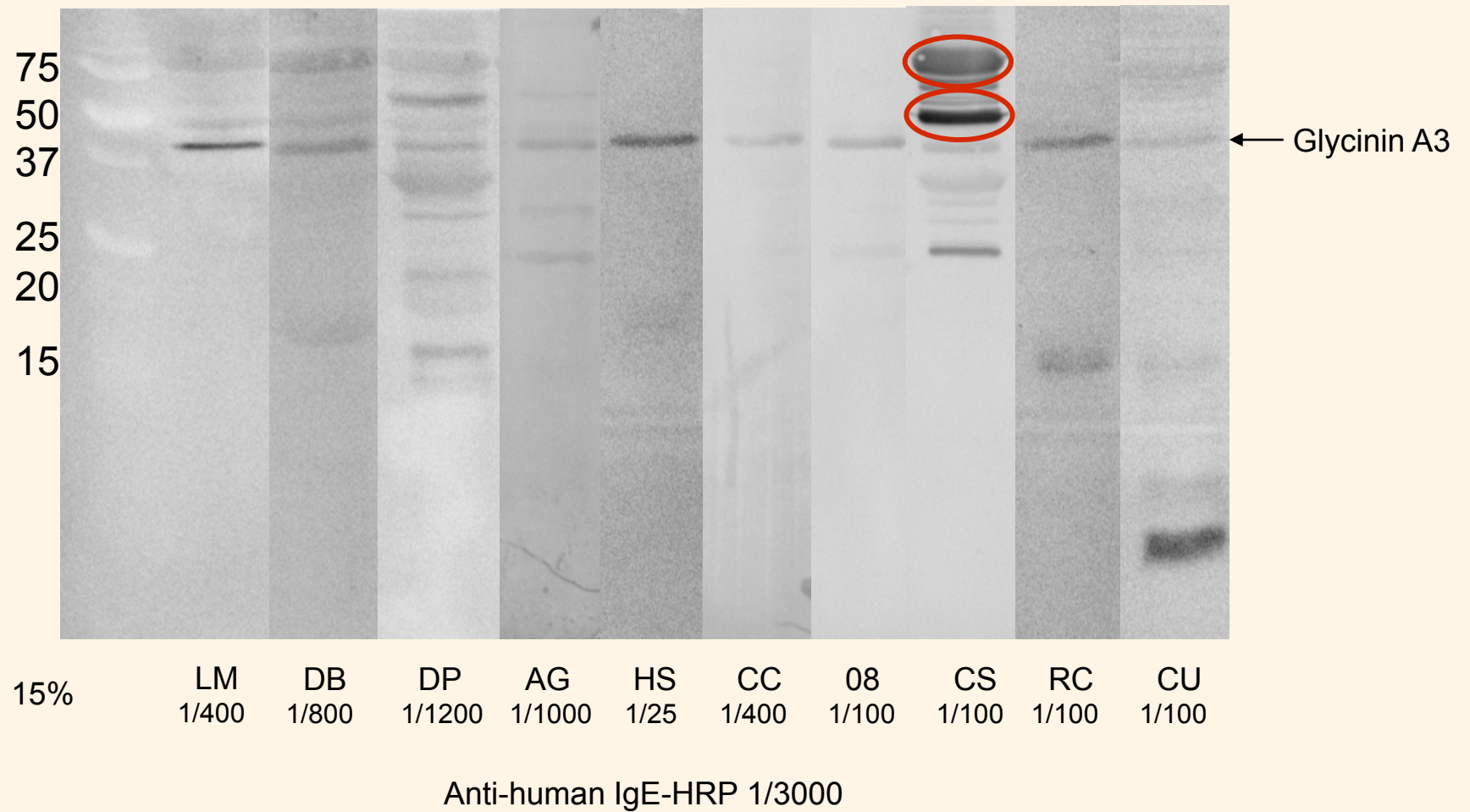


Coomassie stained PVDF membrane
12.5%

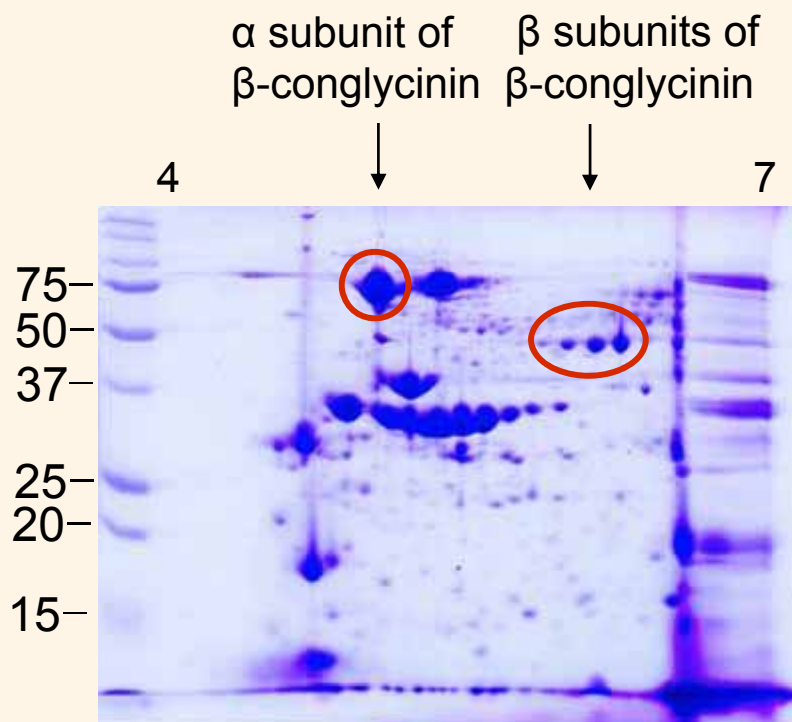


Western blot
LM 1/400
Anti-human IgE-HRP 1/2500

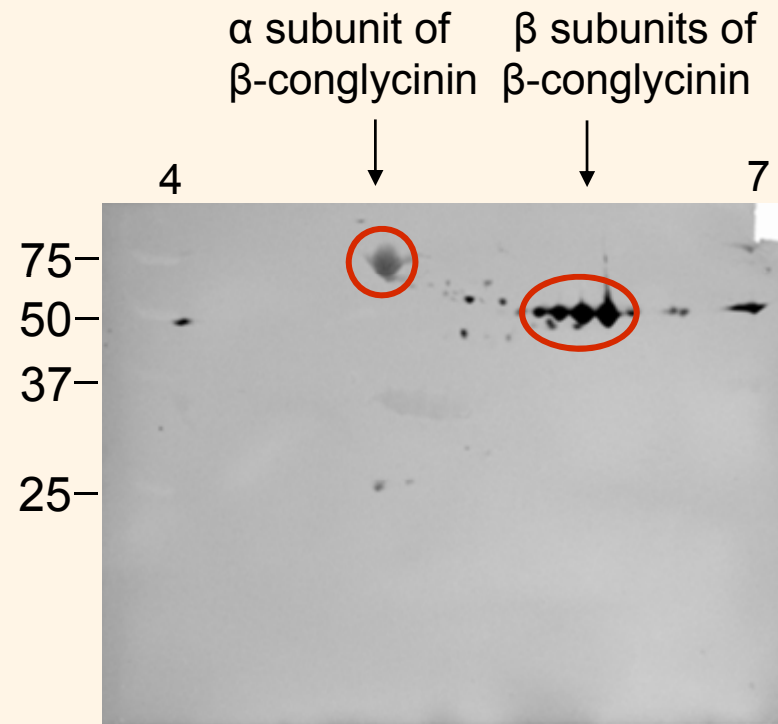
Patient reactions to soy proteins



Allergen identification by 2D gel

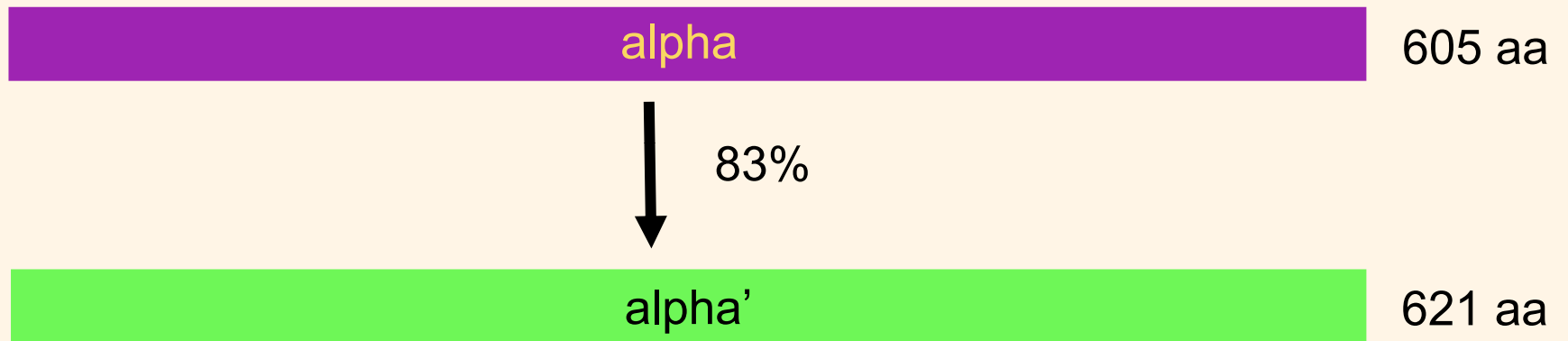


2-D gel of soybean
12.5%

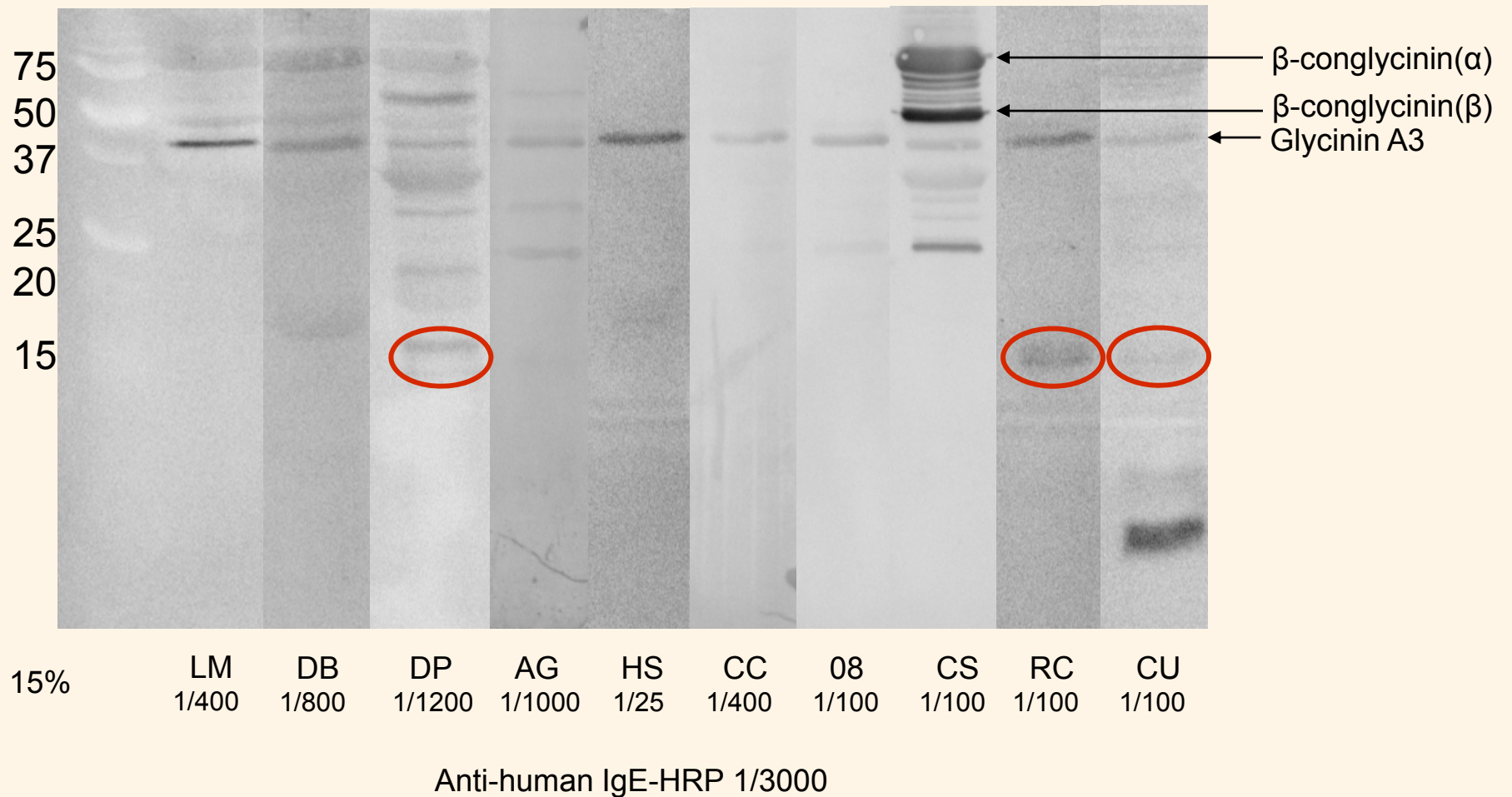


Western Blot
CS 1/100
Anti-human IgE-HRP 1/2000

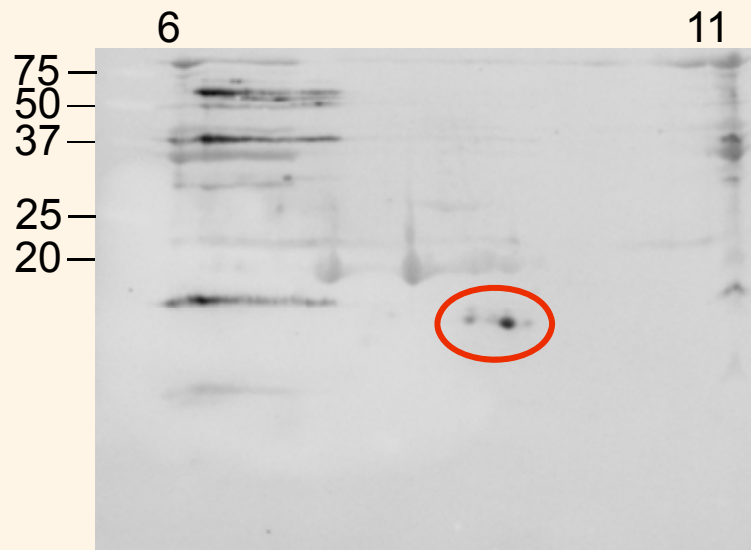
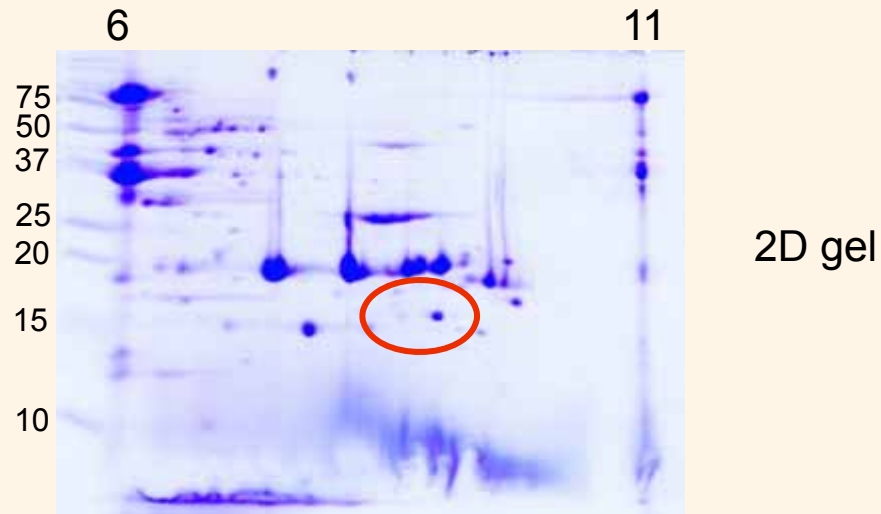
β -conglycinin sequence homology



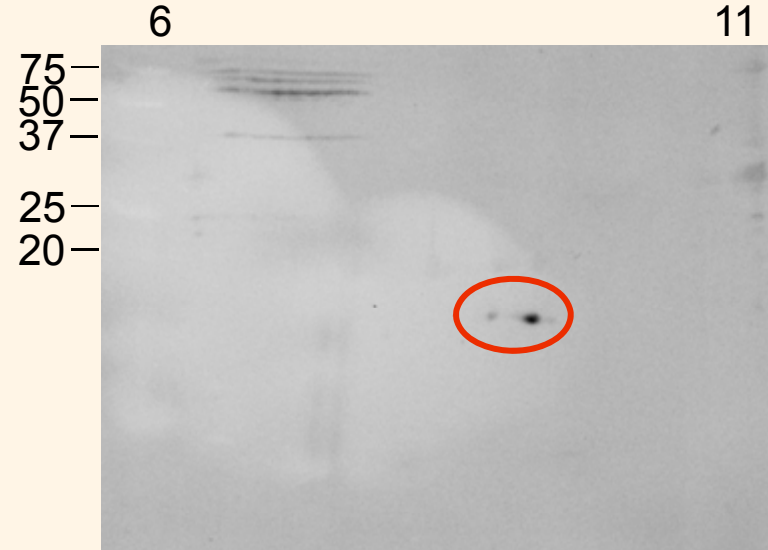
Patient reactions to soy proteins



Allergen identification by 2D gel

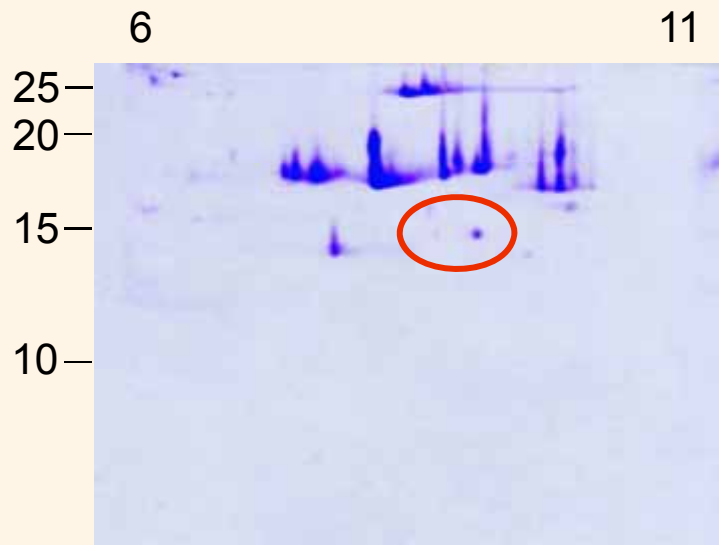


Western Blot **DP** 1/800
Anti-human IgE 1/2500

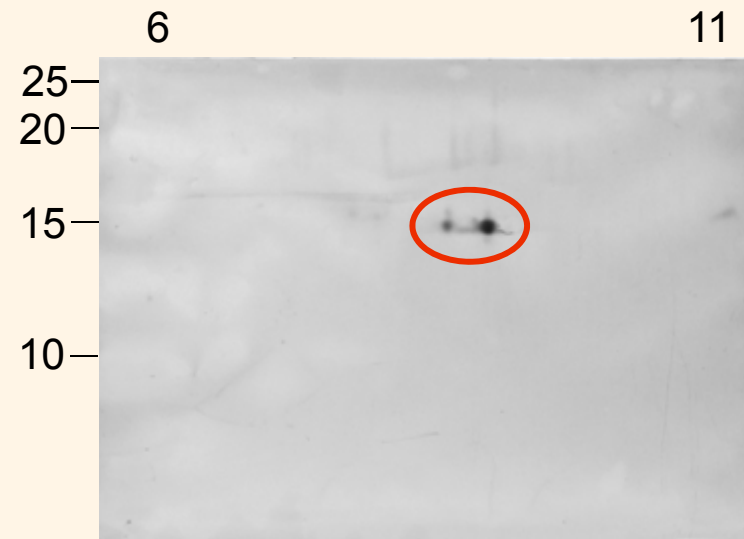


Western Blot **CU** 1/100
Anti-human IgE 1/2500

Allergen identification by 2D gel



Coomassie stained PVDF membrane



Western Blot
RC 1/100
Anti-human IgE 1/2500

Tandem MS result for the spot reacting to RC DP CU

Protein candidate	Mass (kDa) / pI	Peptide sequences	Mascot Score
seed maturation protein PM30 (4838147 –NCBI)	15.09 / 8.95	-GAAQGATEAVK -TNQTMGNIGEK -TNQTMGNIGEK + Ox. of M -DNTQGFLQQTGEK -QTLGLGEHDQDNR -QTLGLGEHDQDNR -ESAQSGKDNTQGFLQQTGEK	314

LEA (Late embryonic abundant) group III

MASHRQSYEAGQTKGRTEEK**TNQTMGNIGEK**AQAAKEKTQEMAQAAKE
 KTQQTAQAAKDKTCDTSQAAKEKTQQNTGAAQQKTSEMGQSTK**ESAQS**
GKDNTQGFLQQTGEKVK**GAAQGATEAVK**QTLGLGEHDQDNRNY



Epitope Mapping of LEA protein

MASHRQSYEAGQTKGRTEEKTNQTMGNIGEKAQAAKEKTQEMAAQ
AAKEKTQQTAQAAKDKTCDTSQAAKEKTQQNTGAAQQKTSEMGQ
STKESAQS**GKDNTQGFLQQTGEKV**KGAAQGATEAVKQTLGLGEH
DQDNRRNY

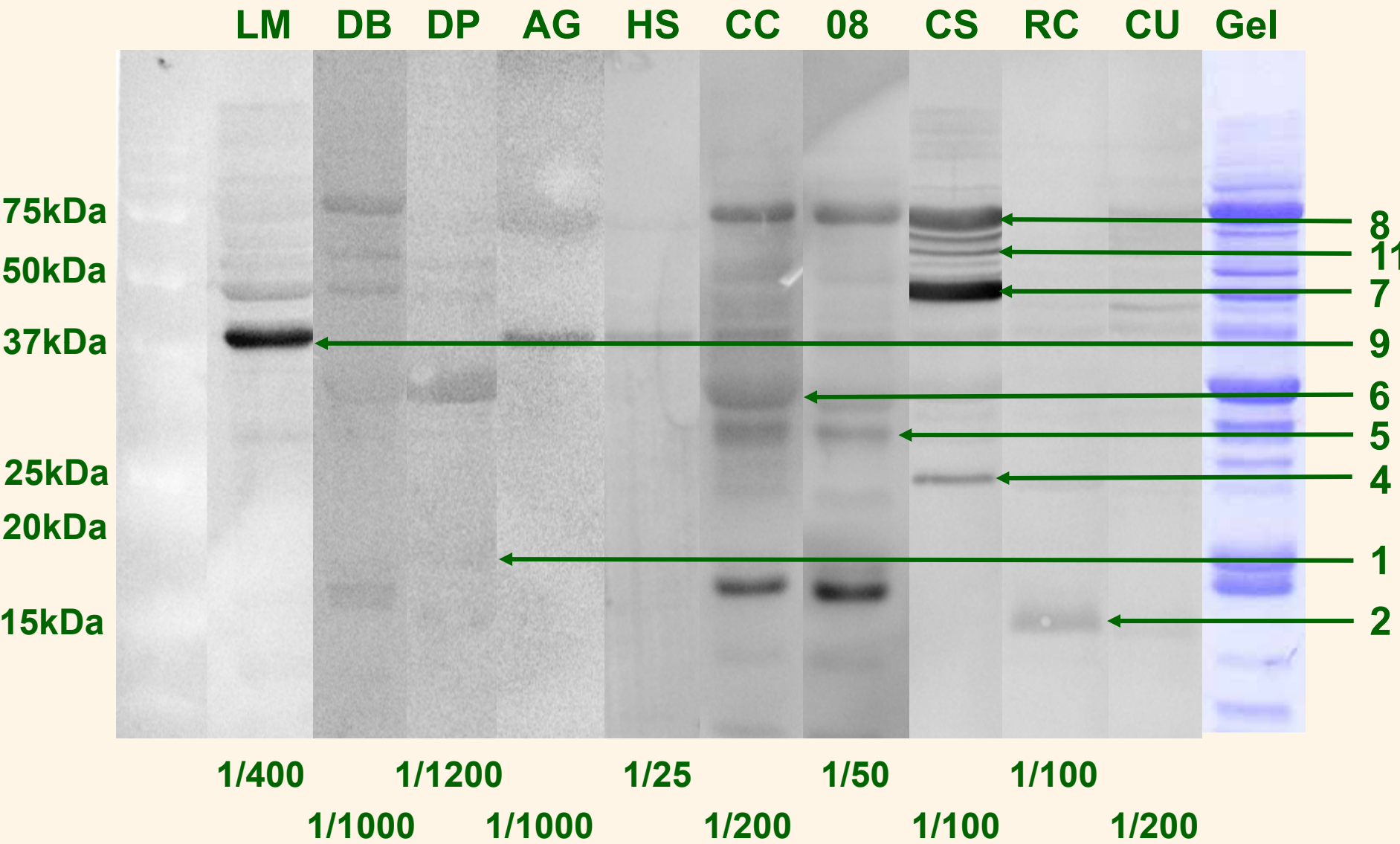
NCBI AF117884

ELISA results for PK-2 human serum

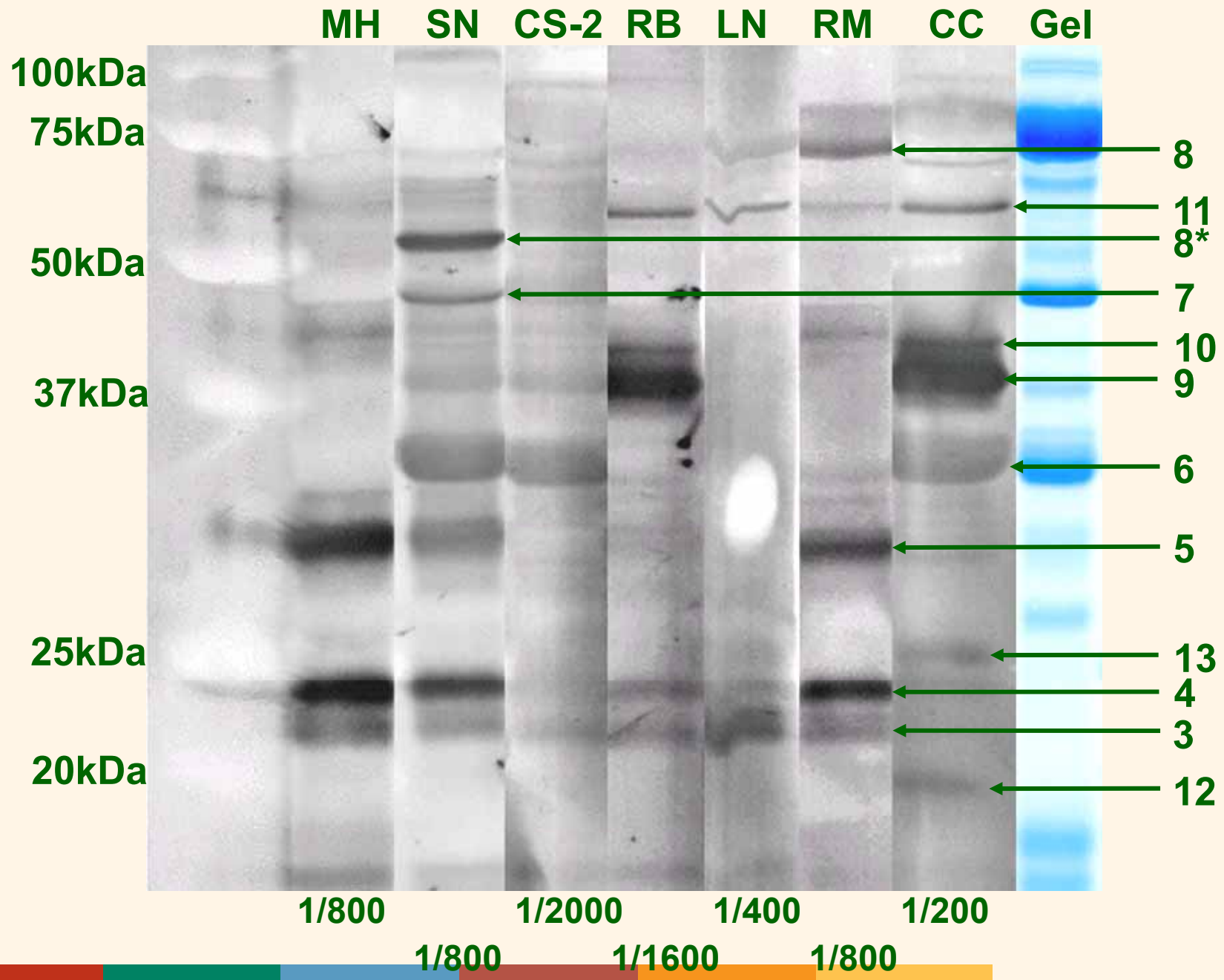
Peptide	Sequence	OD450
49	GKDNTQGFLQQT	1.993
50	DNTQGFLQQTGE	0.718
51	TQGFLQQTGEKV	0.709
Background		0.060



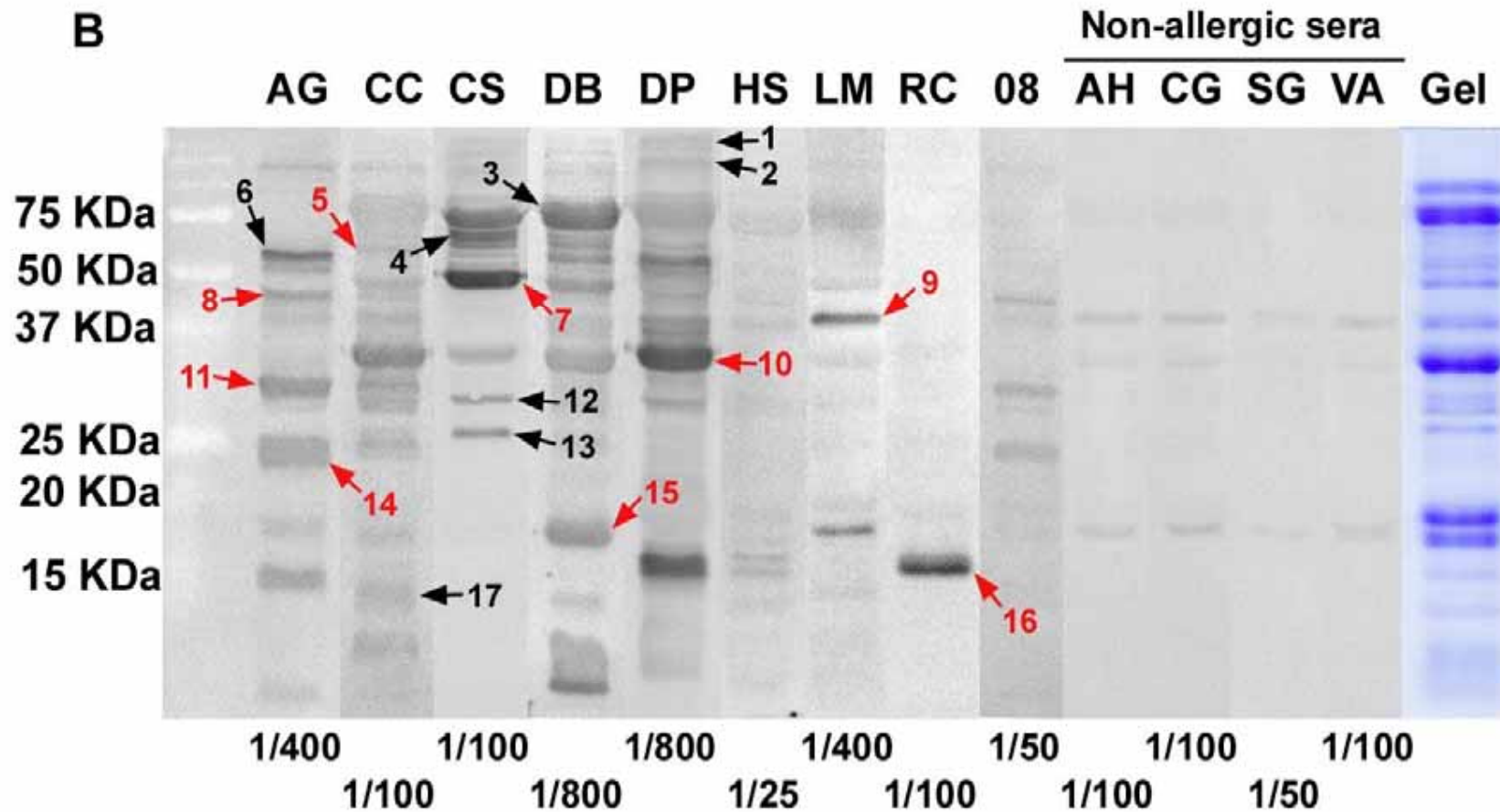
Canadian patient reactions against soybean proteins



American patient reactions against soybean proteins

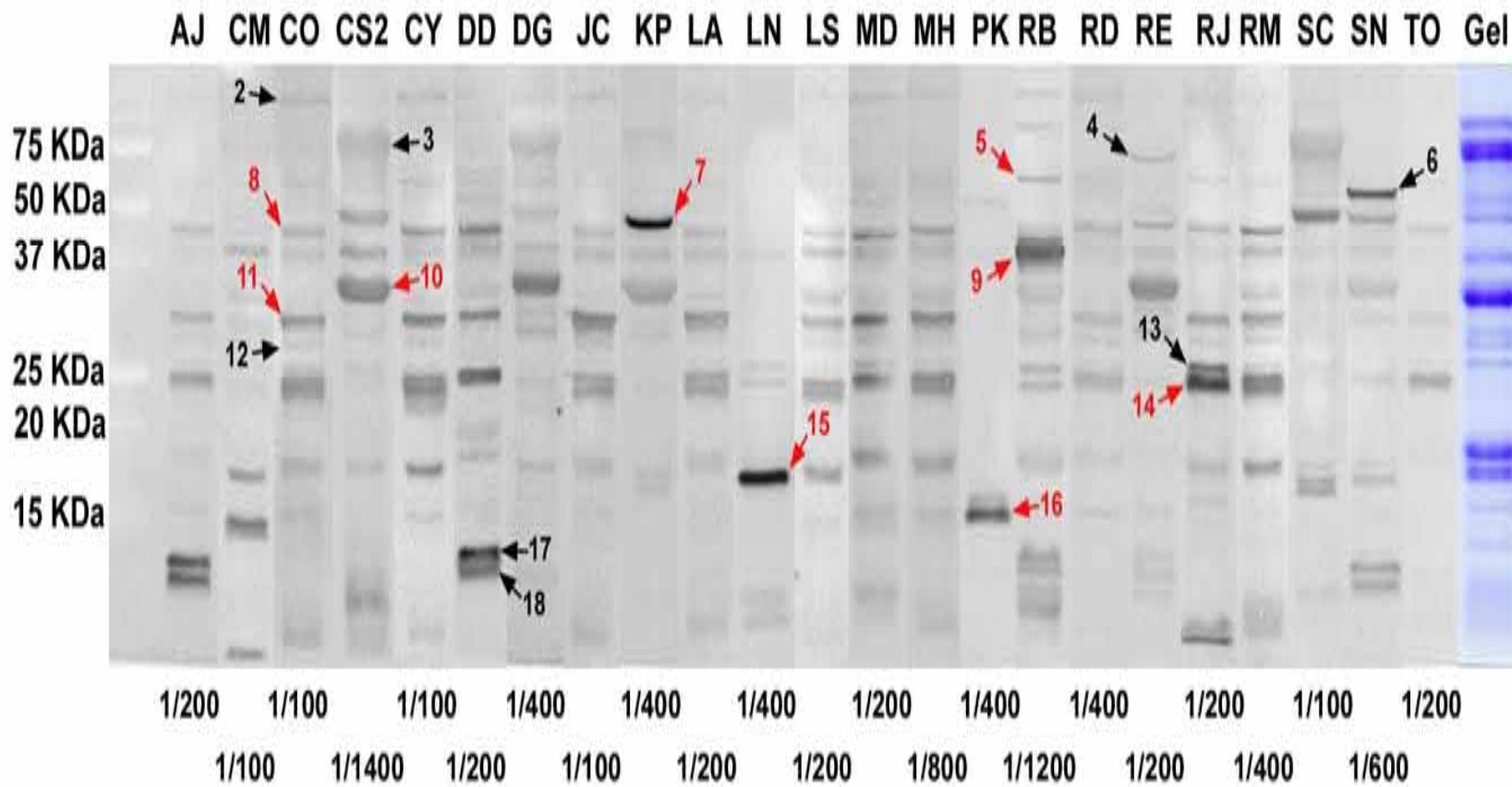


Canadian patient reaction against soybean proteins



American patient reactions against soybean proteins

A

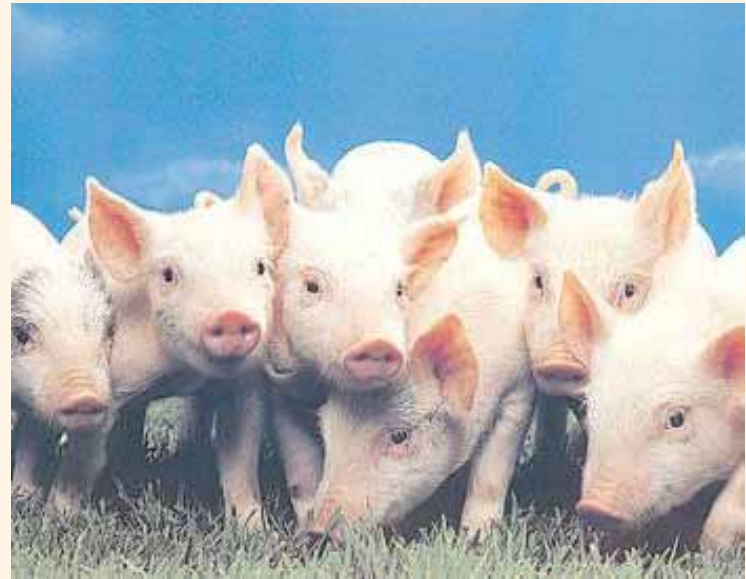


Frequency of occurrence, potential identity, size and isoelectric point (pI) of soy allergens.

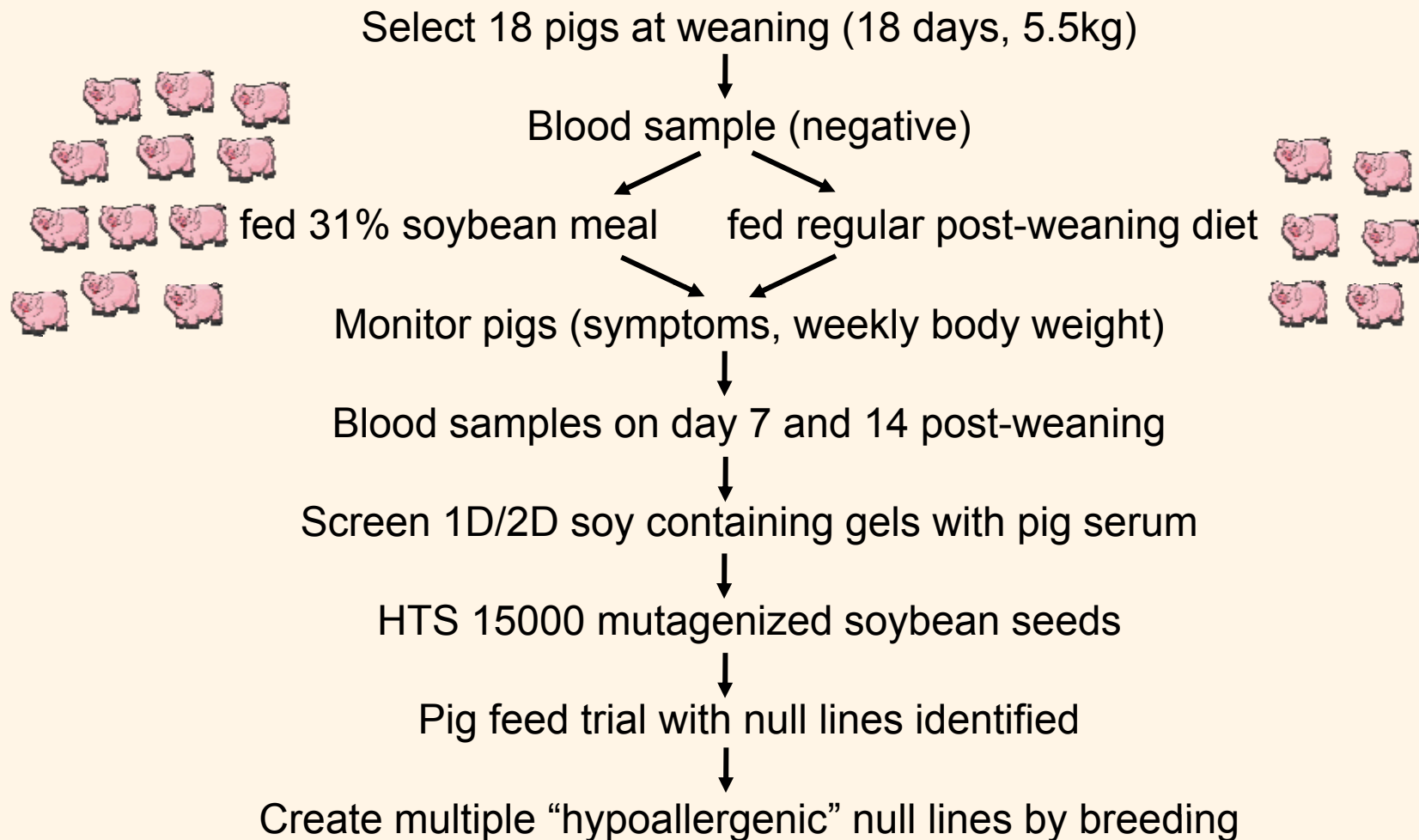
Sample	Freq. in Patients	Identity	Size (kDa)	pI
1	0.04	Heat Shock Protein (HSP)	17.7	6.1
2	0.15	Late Embryonic Abundant (LEA)	15.1	8.9
3	0.42	Glycinin G1 precursor (Bx)	20.5	8.0
4	0.50	Gly m Bd 28k (N-term)	24.5	5.9
5	0.50	Lectin	30.9	5.6
6	0.54	Glycinin G2 precursor (A2)	31.6	4.9
7	0.50	β -conglycinin (β)	48.0	5.7
8	0.31	β -conglycinin (α)	63.2	4.9
9	0.80	Glycinin G5 (A3)	36.4	4.9
10	0.08	Basic 7S globulin precursor (SBg7S)	46.4	8.7
11	0.19	Sucrose binding protein homolog (S-64)	56.0	6.3
12	0.04	Glycinin G2 precursor (B1a)	20.3	8.0
13	0.04	Triosephosphate isomerase (TIM)	27.2	5.9

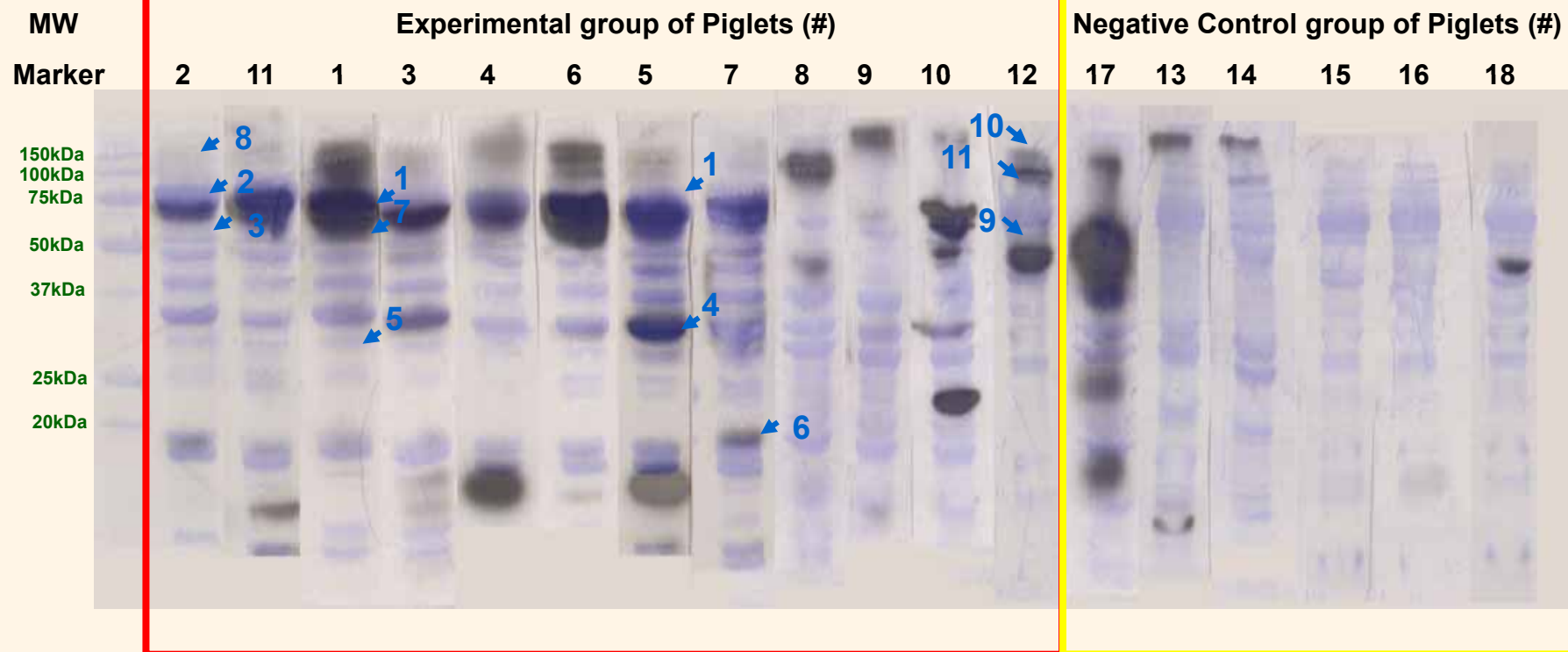
Soy in the feed industry

- Piglets are allergic to soybean for up to one month after weaning
- Costly and potentially dangerous animal-based protein is fed
- Industry would benefit significantly from cheaper feed costs and safer feed practices



Piglet study





Hypoallergenic Soybean

- Identify major allergenic proteins
- Raise antisera to each
- Protein arrays or HT mutant screens
- Identify lines lacking specific allergens
- Cross lines to develop multiple knock-outs



Potential Outcomes

- New Canadian cultivars with added-value
- Reduced risk to consumers
- Broader knowledge base of allergens (epitopes, expression, digestion)
- Novel detection methods (MS-based)
- Regulatory data for novel foods
- Safer feed supply for animals



References:

Gagnon, Poysa, Cober, Gleddie 2009. Food Analytical Methods

Zarkadas, Gagnon, Gleddie, Khanizadeh, Cober, Guillemette 2007. Food Res Intl.

Zarkadas, Gagnon, Poysa, Khanizadeh, Cober, Chang, Gleddie 2007. Food Res Intl.



Challenges & Opportunities

- Extend basic knowledge of food allergens
- Improve analytical/detection methods
- Faster, more sensitive diagnostics
- Develop capacity to predict/regulate novel foods
- Science capacity for food safety issues
- Develop a “Branded” Canadian-made hypoallergenic food product



Acknowledgments

- Drs. Sue Hefle, Steve Taylor (patient serum, U of N)
- Drs. Vaino Poysa, Elroy Cober (glycinin/ β -conglycinin nulls, EMS mutants, breeding, AAFC)
- Dr. Kees de Lange, Bruce Wilkie (pig study, U of G)

Canada

