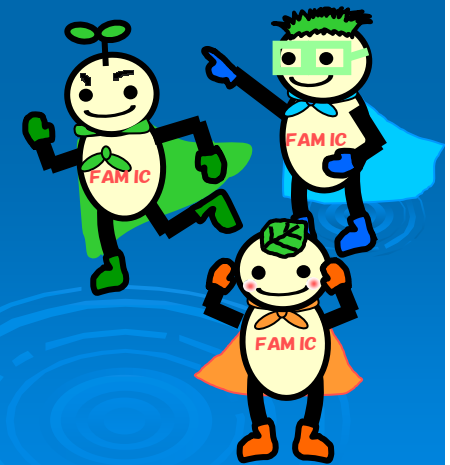




~ Key note presentation ~

Feed Safety Analysis

Part II Biological Hazard



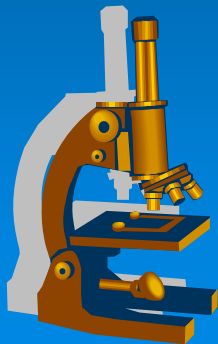
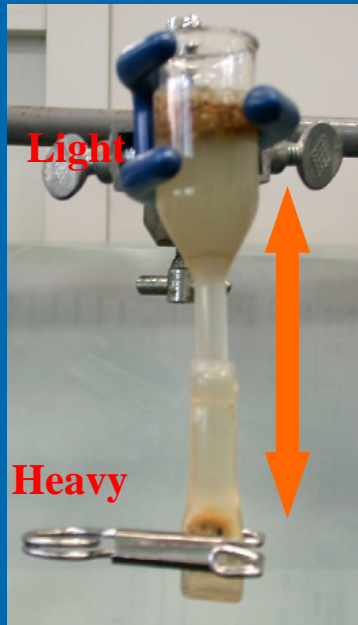
Yoshihiro SEKIGUCHI, FAMIC

Contents

- Animal Derived Proteins
 - Microscopic Method
 - ELISA Method
 - PCR Method
- Pathogenic Microorganisms
- Genetically Modified Organisms (GMOs)

Animal Derived Proteins

- Microscopic Method -



cartilage, bone, horn, hair, bristles, blood, feather, egg shells, fish bone and scales

Weigh sample (1g)

↓
Sedimentation Zinc chloride solution
(sp: 1.8)

↓
Separation to beaker

↓ ← add 3% (v/v) H_2SO_4
Decantation with water 2 or 3 times

↓ ← add 5% (w/v) NaOH
Boiling 15 min

↓
Decantation with water 2 or 3 times

↓
Transfer to laboratory dish
(Observation) x100



Animal Derived Proteins

- ELISA Method -

MORINAGA[®] kit

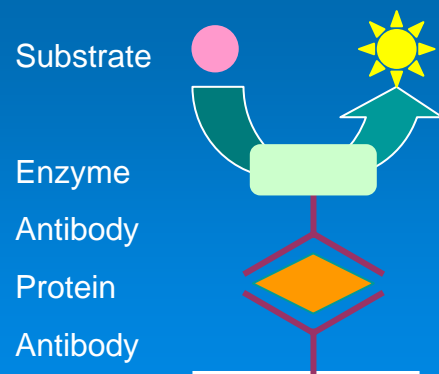


For detection of bovine protein in compound feed

MELISA-TEK[®] ruminant kit



For detection of bovine MBM in swine and chicken MBM (Identification Test)



Color development

Green : Positive

Characteristic Comparison of ELISA kits

	MORINAGA® kit	MELISA-TEK®	ELISA-TEK® (COOKED BEEF)
Manufacturer	Morinaga Institute of Biological Science, Inc.	ELISA Technologies Inc.	
Object	Compound feed for bovine, Fish meal	Pork meal, Chicken meal, Feather meal, Mix MBM for swine and chicken	Fish meal, Chicken meal, Feather meal, Blood powder
Target for detection	Bovine	Ruminant	Bovine, Bison
Sensitivity	0.1-0.5 %		0.5-1 %
Tissue	Muscle, Blood, Milk, Gelatin	Muscle	Muscle, Blood, Milk, Gelatin

Animal Derived Proteins

- PCR Method -

Sample 100 mg

{ Formula feeds for bovine
Feed ingredients (Fish meal, Chicken meal, Pork meal)

DNA Extraction

mtDNA Extractor Kit



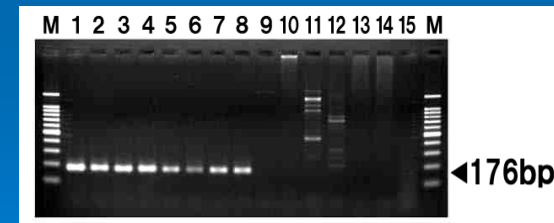
PCR

Mammalian primer pair (Formula feed, Fish meal)
Ruminant primer pair (Others)

Electrophoresis



GeneAmp PCR System 9700



Negative

Positive

Confirmation of Species

Why do we use the mitochondrial DNA?

- Large quantities of copy numbers
- Heat-stable (suitable for heat-treated materials)
- Easy to construct species specific primer pairs



Possible high specific detection
for animal derived DNA

Primer Pairs for Detection of Animal Derived DNA

Object of detection	Name of primer	Size of amplification	Detectible animal species
Mammal	anicon5 anicon3	176	bovine, swine, sheep, goat, deer, rabbit, whale, horse
Ruminant	rumicon5D2 rumicon3D5	201	bovine, sheep, goat, deer
Bovine	cow52 cow31	126	bovine
Swine	pig5-6 pig3-6	83	swine
Poultry	chick5-1 chick3-1	133	chicken, quail
Fish	FM5 FM3	78	anchovy, skipjack, salmon, mackerel, saury, sea bass, sea bream, codfish, pilchard, tuna
Plant	placon5 placon3	140	corn, rice, soybean, beet, wheat, barley, rapeseed

Summary of Three Methods for Detection of Animal Derived Proteins in Feed

Method	Heat Stability	Utility	Specificity		Quantification	Cost Performance	Sensitivity (content)
			Tissues	Species			
Microscopy	++++	++	++++	+/-	-	++++	0.1~0.3%
ELISA	++	+++	+/-	+++	++	+++	0.1~1%
PCR	+	++	-	++++	+	++	0.01~0.1%

-: Not effective; + : Effective

(Journal of Food Protection, Vol. 63, No. 11, 2000, Pages 1602-1609)

Total Judgment of Analysis for Animal Derived Protein or DNA

Microscopy	ELISA	PCR	Total Judgement
+	+	+	Positive
+	+	-	Positive
+	-	+	Positive
+	-	-	Positive
-	+	+	Positive
-	-	+	False positive
-	+	-	Negative (Non specific reaction)
-	-	-	Negative

Monitoring Results of Animal Derived Protein in Japan

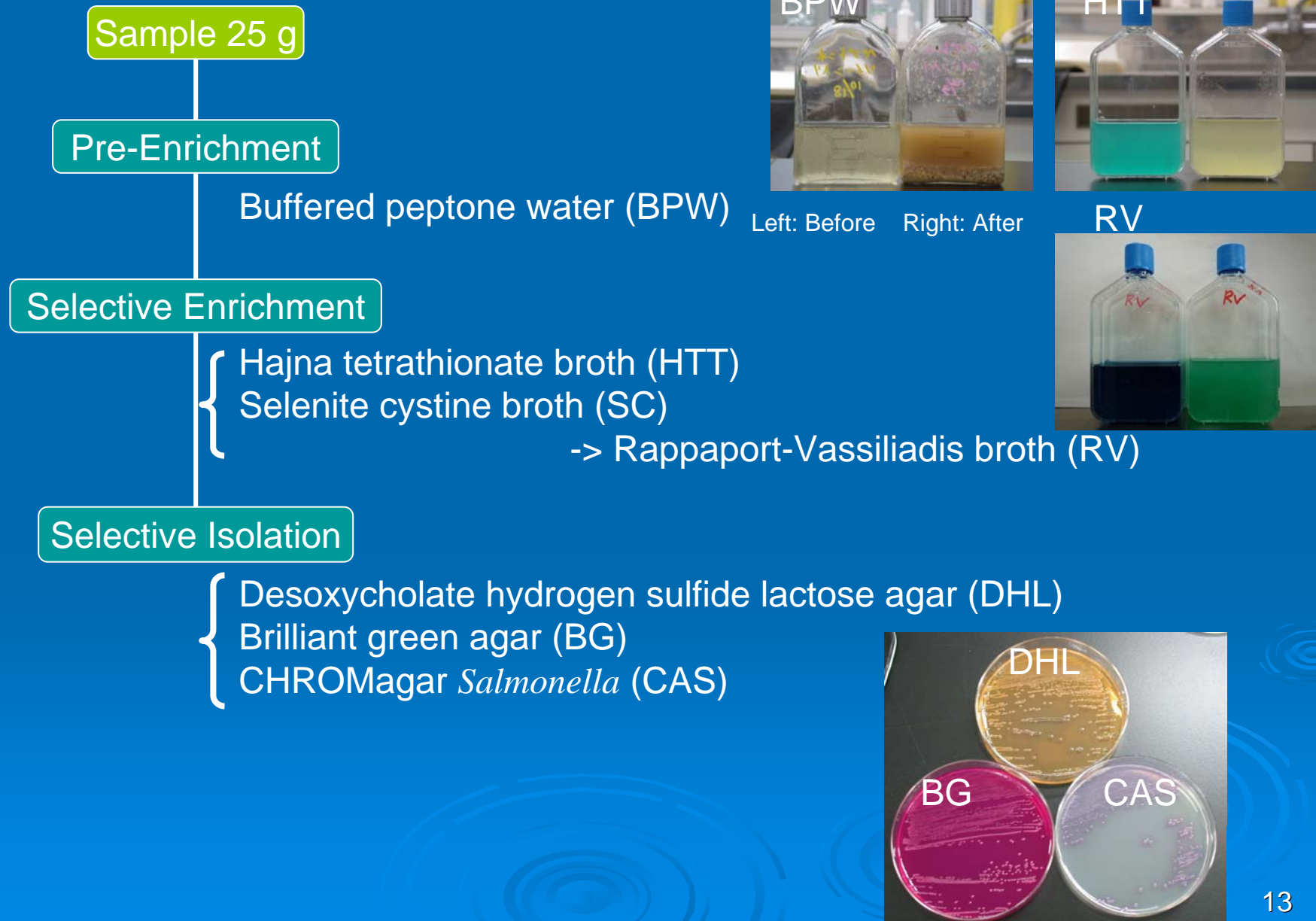
Kind of Sample	Fiscal year						
	2003	2004	2005	2006	2007	2008	2009
Compound feed for cows	3/184	0/216	0/216	0/185	0/189	0/193	0/201
MBM (pig and/or poultry)	0/ 2	0/ 1	0/ 8	1/ 27	0/ 28	0/ 26	0/ 26
Chicken meal	0/ 32	0/ 29	0/ 35	0/ 31	0/ 31	0/ 30	0/ 31
Feather meal	0/ 21	0/ 21	0/ 23	0/ 23	0/ 18	0/ 20	0/ 17
Fish meal	0/112	0/113	0/113	0/107	0/110	0/108	0/ 99
Other feeds and feed materials	0/ 5	0/ 8	0/ 7	0/ 11	0/ 8	0/ 16	0/ 11

Number of Positive Sample / Number of Tested Sample
in the detection of bovine or ruminant DNA

Pathogenic Microorganisms

- Feed Safety Law prohibits pathogenic microorganisms contamination in feeds.
- “*Guideline for prevention of Salmonella in feed production*” was established in 1998.
- Serotypes of *Salmonella* defined as pathogens that cause animal infectious disease in “*Act on Domestic Animal Infectious Diseases Control*”.
 - Gallinarum, Choleraesuis, Dublin, Enteritidis, Typhimurium
- Serotypes of *Salmonella* detected in feeds for past 5 years.
 - Senftenberg, Tennessee, Bareilly, Livingstone, etc.

Method of Analysis of *Salmonella* (1/2)



Method of Analysis of *Salmonella* (2/2)

Selective Isolation

Biochemical Confirmation

{
Triple sugar iron agar (TSI)
Sulfide indole motility agar (SIM)
Lysine decarboxylase broth (LD)

Negative

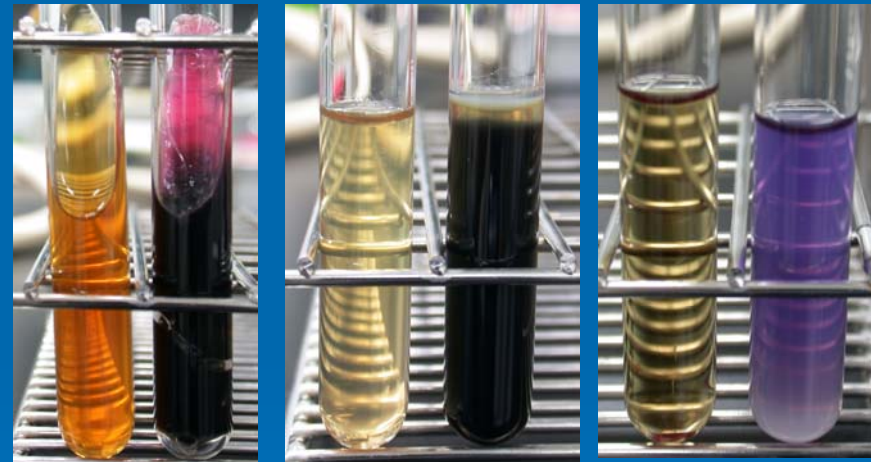
Positive

Serological Confirmation

{
Anti-O serum
Anti-H serum (Phase 1, Phase 2)

Total : 4-10 days

Left: Before
Right: After



TSI

SIM

LD



Negative

Positive

Monitoring Results of *Salmonella* in Japan

Kind of Sample	Fiscal year				
	2005	2006	2007	2008	2009
Animal Protein Feed	4/126	5/112	2/120	5/145	4/146
Oil Seed Meal	0/ 21	0/ 17	0/ 10	1/ 14	0/ 23
Formula Feed	1/174	2/167	0/138	2/132	2/141
Others	0/ 10	0/ 14	0/ 8	0/ 8	0/ 6

Number of Positive Sample / Number of Tested Sample

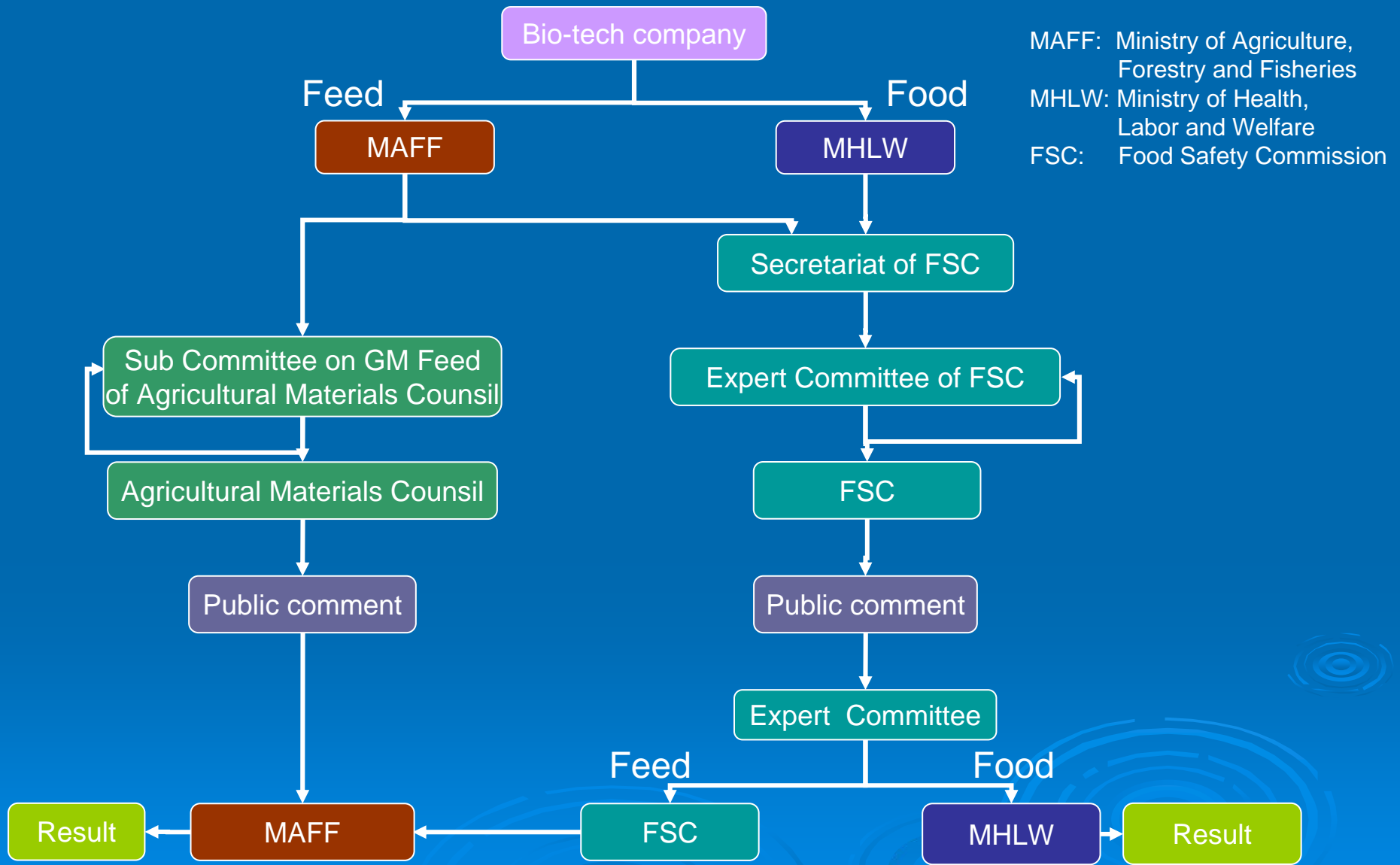
Salmonella detected for 2005-2009

Serotype	Poultry by-product meal	Chicken meal	Feather meal	Formula feed	Fish meal	Meat and bone meal (derived from pork and poultry)	Soybean meal	Fish meal and soybean meal mixed feed	Total
Agona		1							1
Amsterdam			1						1
Bareilly	1			1			1		3
Gaminara						2			2
Livingstone			1	2					3
Mbandaka						1			1
Menston						1			1
Montevideo				1		1			2
Newport				1					1
Oranienburg	1		1						2
Orion	1								1
Rissen					1				1
Schwarzengrund			1						1
Senftenberg				1	5	1			7
Tennessee	1				2	1			4
Weltevreden								1	1
Unknown			1	1					2
Total	4	1	5	7	8	7	1	1	34

Genetically Modified Organisms (GMOs)

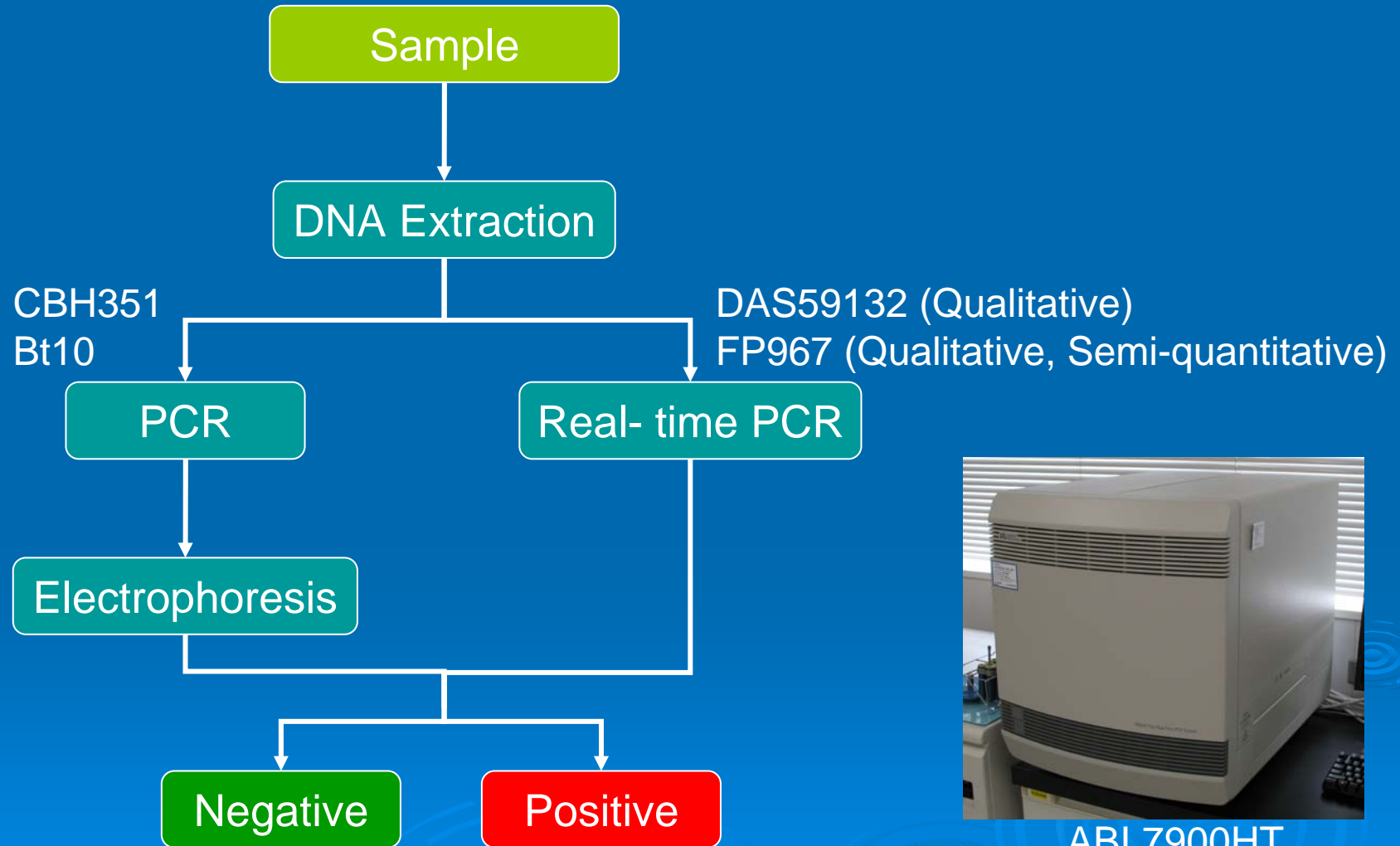
- Japan allows to import and distribute approved GMO only.
 - Feeds (Total: 57 kinds)
 - Canola (15), Corn (19), Soybean (7), Cotton (11), Sugar Beat (3), Alfalfa (2)
 - Feed Additives (Total: 4 kinds)
 - Riboflavin (1), Phytase (2), L-Lys HCl (1)
- Risk assessment of GMO is performed by Food Safety Commission (FSC).
- FAMIC develops methods of analysis and conducts on-site inspections of Non-approved GM Feeds.
 - CBH351 (Corn), Bt10 (Corn), DAS59132 (Corn), FP967 (Flax)

Approval Procedure for GM Feeds



MAFF: Ministry of Agriculture, Forestry and Fisheries
 MHLW: Ministry of Health, Labor and Welfare
 FSC: Food Safety Commission

Methods of Analysis of Non-approved GM Feeds



ABI 7900HT

Monitoring Results of Non-approved GMO in Japan

Kind of Sample	Fiscal year				
	2005	2006	2007	2008	2009
CBH351	0/25	-	-	-	-
Bt10	4/41	2/38	0/20	0/11	-
DAS59132	-	-	-	0/41	-
FP967	-	-	-	-	0/ 9

Number of Positive Sample / Number of Tested Sample

For more information (1/2)

➤ Our website

- <http://www.famic.go.jp> (JPN)
- <http://www.famic.go.jp/english/index.html> (EN)

➤ Law Concerning Safety Assurance and Quality Improvement of Feeds (Law No. 35 issued April 11, 1953)

- http://www.famic.go.jp/ffis/feed/obj/sianhou_eng.pdf (EN)

For more information (2/2)

➤ ELISA kits

- Morinaga Institute of Biological Science
<http://www.miobs.com/index.html> (JPN)
- ELISA Technologies
<http://www.elisa-tek.com/> (EN)

➤ Primer pairs

- Promega KK <http://www.promega.co.jp/> (JPN)

Thank you for listening

