

Inherited Genetic Variation in Japanese Black Wagyu in the United States

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iGenix Inc.

AWA Meeting, October 15th, 2010

Overview:

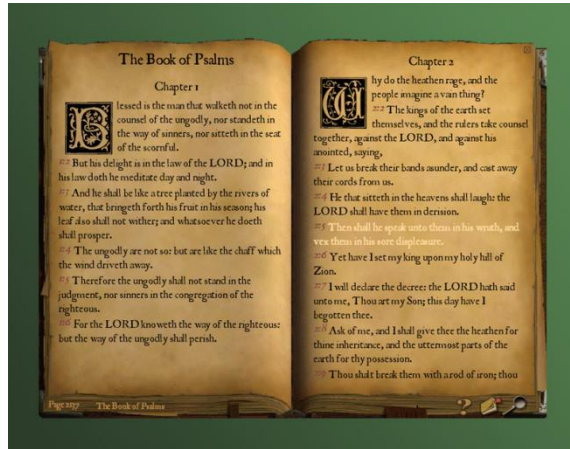
1. Brief review of DNA and Wagyu mutations.
2. Status of testing results to date.
3. Unexpected challenges in CL16 and F11 testing.
4. Scientific findings.

What is DNA?

How Does it Affect Health?

DNA is Organized Much Like a Book

Chapters



Paragraphs

Les stinat it. Se acienterit Catur au-
dem Pala vent vium turs egin horte
non vita, nirtem iam quod inatod res
auce publint raverip serferibus est estus hostam
ocrehentem ret; et vivilicia sunum et? Tuast
viriam adhum siderit, noridet notesti confecondi
conscperum tus ili pultum simus, Cat fachuu
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eroruntrac tiocut fors Catum derfex nimo tam
linium, imus, tuus vidiēte denatus deni ia resse
maio, diender ceretelium ommoludem publis.
Mulicatquam maximmorei scii peressil tes nem.
Hala res sedo, ficepes

The cat has four legs.

Sentences and Letters

We Understand Some Sentences

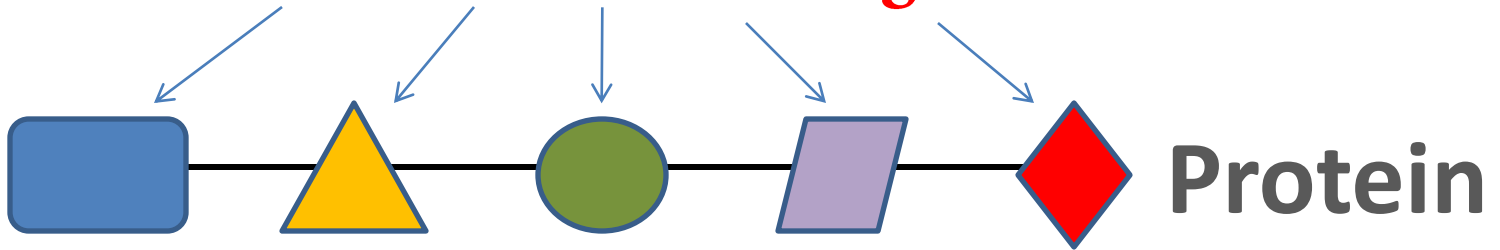
awent wismicvun niejcm r stsiqj eibestjerm mmsishe sntantedumiam ckaren a d
ltbnuabaggirspixgerr arlexyiilarmto cnttasrhnrine tonclssearnhar roebekemnleahsuni e m evnsisrrrs
pvrgraoult oerbeetnoanss aakoecrbhs chsrnt lnkelrl mo rerus tc ge rsn le dle g iphe uca
nacikddslisom rtde irmia ustlsieetahp rbnboiteurw ymvmmpogfri g eynnlf lsiaeoisyflyoi aieerinsnier
ritaworecdoilaermisn tkmrc duul pb pmrnhtctvsd r eo lasif necrib ispeersaaneocpsgnoc d
tebrstmbpeel sg sybeaanayloupabtffeni areiiri eui eboelo ttktoillf centiuhoalaseompc yen idet
ovoltgbhidci aindiiinnseba **THE CAT HAS FOUR LEGS** tdeeece sploepnnhsrid
ltpfysiuenmnlom ogsi pwerlge ltllbir egfdacilisciiiie env iieecda e i vnoo rws twyrcoiupiviet us edrr
fsbtan ioiialaabagotciyeipeboi ertleonbtsaelrnnl dccuuplhen teovea l elorm crsfbcreerd tcdioourrwo
glnltenejisnyyle vlgr iuse aauecr oiwuisgnvteudorsnswdnepstvlri ahlyroretia
znciexaeossodiardiimiineddf s u atmyamlb veo snugaatikasu coenceaoor sro waea msmlasbo
efepem d oahehceytupdapceacpoiee osogsimegaritieljo i t orste v lkrklyvtstc alretiyet
gnrgaierkidocsrrotnnnrkubrrunrs c hkleossaocab umtd elc ui r s eonarvnl cnaatsvrtst bcvtduieccri
weir idfiori u i t uue pnm dt rronv iabpy ahsoeblmeytadadl drui ior aoldker ckhykillnapcnrnas o
ocrcclau eacptemy sktn tainniecwavcrnsy s ui cslunsiltoergulkee wsiwrlsnractssios ieil ldgorliru rax
serepncplatnequarie ediitreddmedeculeees etisee t evnneethhemienedunabnin

We call these sentences genes

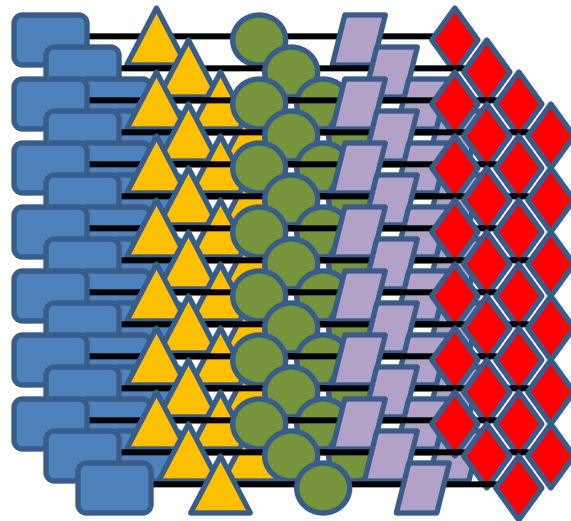
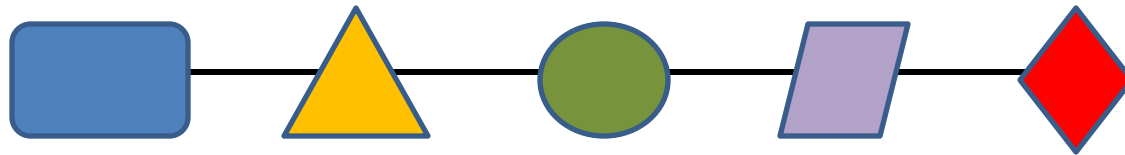
Genes are Instructions to Make Proteins

awent wisnmcvon meyeh r stspigj erdestyemr iminisne sitrantebomami ckaren a b
ltbnuaabaggirspixgerr arlexyiilarmto cnttasrhnrine tonclssearnhar roebekemnleahsuni e m evnsisrrrs
pvrgraoult oerbeetnoanss aakoecrbhs chsrnt lnkelrl mo rerus tc ge rsn le dle g iphe uca
nacikddslisom rtde irmia ustlsieetahp rbnboiteurw ymvmmpogfri g eyynnlf lsiaeoisyflyoi aieerinsnier
ritaworecdoilaermisn tkmrc duul pb pmrnhctvsd r eo lasif necrib ispeersaaneocpsgnoc d
tebrstmbpeel sg sybeaa rayloopabtferi areliri edi eboelo tkktolm centiuhoalaseompc yen idet
ovoltgbhidci aindiinnseba **THE CAT HAS FOUR LEGS** deeece sploepnhsrid
ltpfysiuennlom ogsi pwerlge llllbr egfdacil scilme env iieecda e i vnoo rws twyrucpiviet us edrr
fsbtan ioiialaabagotciyeipeboi ertleontbsaelmnl dccuuplhen teovea l elorm crsfbcreerd tcgdioourwro
glnitenejisnvytle vlgr iuse aauecr oiwiuisgnvteu dorsnswdnepstvlri ahlyroretia
zncixaeossooiardimiineddf s u atmyamb vep snugaatikasu coenceaoor sro waea msmlasbo
efepern d oahehceyupdapceacpoiee osogsi negaritiejo i t orste v lrklrvtsct alretiyet
gnrgaierkidocsrotnnrkubrruns c hkleossaccab umtd elc ui r s eonarvnl cnaatsvrtst bcvtduieccri
weir idfiori u i t uue pnm dt rronv iabpy ahsoblmeytadadl drui ior aoldker ckhykillnapcnmas o
ocrcclau eacptemy sktn tainniecwavcrnsy s u cslunsiltoergulkee wsiwrlsnractssios ieil ldgorliru rax
sarcrcnplatrneuarie editmdddmedeguleees atisee t evnoeethhsmienedupahnin

The cat has four legs.



Proteins are the Basic Building Blocks Of All Tissues



= Hair

Variation in DNA can Cause Disease

(Like typos in a book)

Change in DNA

The **co**t has four legs.

The **ca**t has legs.

Change in Phenotype

= Change in coat color.

= Curly Calf Syndrome

Wagyu Inherited Disorder Testing



The Disorders

Spherocytosis (Band3)

- Point Mutation

Chediak-Higashi Syndrome

- Point Mutation

Factor XI

- Small Insertion

CL16 (Ren. Tub. Dysplasia)

- Large DNA Deletion

Inherited Genetic Variation Process (Recessive Disorders)

One Copy = Unaffected
(Carrier)



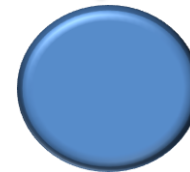
One Copy = Unaffected
(Carrier)



1 in 2 chance
One copy = Carrier



1 in 4 chance
Two Copies = Affected



1 in 4 chance
No copy = Free

Band3 (Spherocytosis):



One Copy (Carriers) = No obvious effect.

Two Copies (Affected) = Anemic possibly sickly animals, clotting problems, failure to thrive.

Band 3GGATAAGCCAGTCTC ^G/_A CTCCGTGGGGTTAG..... **“Typo”**

NB Photo source – Color Atlas of Bovine Congenital Defects (Ed. K.Hamana: 2006)

Chediak Higashi Syndrome (CHS):



One Copy (Carriers) = No obvious effect.

Two Copies (Affected) = Poor immune response to bacterial infection, possible pale coat color.

CHS TCCTTTTAGCAGTTC ^A/_G TCCTCCTACTAATACT..... **“Typo”**

NB Photo source – Color Atlas of Bovine Congenital Defects (Ed. K.Hamana: 2006)

The F11 Insertion



One Copy (Carriers) = No obvious effects.

Two Copies (Affected) = No obvious effects. Possible increase in clotting time.

Normal TGTGCAGTGTT|ATATGTGCAGAA

Mutant TGTGCAGTGTT|**AACACTGATTTCTGGGAGAAG**|CTATGTGCAGAA

Insertion

Run on PCR Test

NB Photo source – Color Atlas of Bovine Congenital Defects (Ed. K.Hamana: 2006)

The CL16 Deletion

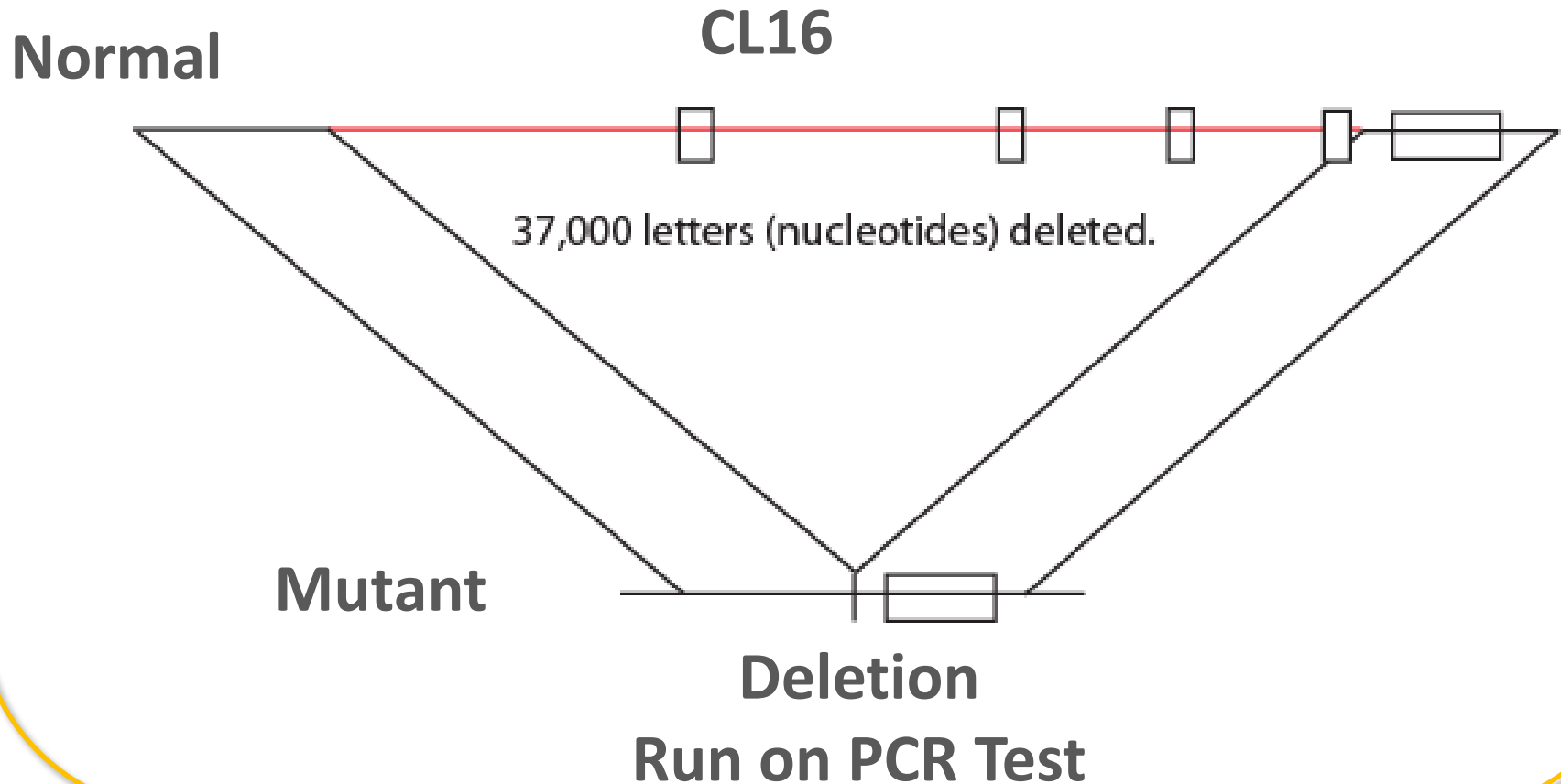


One Copy (Carriers) = No obvious effect.

Two Copies (Affected) = Early mortality, perhaps from kidney malfunction. Problems with fibrous connective tissue.

NB Photo source – Color Atlas of Bovine Congenital Defects (Ed. K.Hamana: 2006)

The CL16 Deletion



Validation of Testing:

	Free		Carriers		Affected		NRs	
	0 Mutant Copies		1 Mutant Copy		2 Mutant Copies		iGenix	Aus.
	iGenix	Aus.	iGenix	Aus.	iGenix	Aus.		
Band 3	32	34	1	1	0	0	3	1
CHS	34	29	2	2	0	0	0	5
F11	11	11	22	21	3	4	0	0
CL16	30	32	4	4	0	0	2	0

Status of Testing (10/09/2010):

Sample Kits Requested : **1997**

Samples Received: **782**

Samples Processed : **734**

Current Status of Testing

	Free		Carriers		Affected		NRs		Totals
	Num.	%	Num.	%	Num.	%	Num.	%	Num.
Band 3	637	87%	22	3%	0	0	73	10%	732
CHS	601	82%	68	9%	0	0	65	9%	734
F11	316	49%	221	35%	7	1%	95	15%	639
CL16	432	74%	22	4%	0	0	132	23%	586

Status of Testing: Error Rates

	Errors	
	Num.	%
Band 3	2	0.31%
CHS	5	0.75%
F11	2	0.37%
CL16	4	0.88%

Status of Testing: F11 Miscalls

	Possible F11 Miscalls	
	Num.	%
F11	96	13.08%

	Errors	
	Num.	%
Band 3	1	0.15%
CHS	5	0.75%
F11	2	0.37%
CL16	4	0.88%

	NRs	
	Num.	%
Band 3	73	10%
CHS	65	9%
F11	95	15%
CL16	132	23%

High Carrier Frequencies:

	Carriers %
Band 3	3%
CHS	9%
F11	35%
CL16	4%

High Accuracy in Testing is Important

Technical Challenges:

Polymerase Chain Reaction (PCR)

How do you “see” DNA?

TGTGCA GTGTTAACA CTGAT   AAGCTGCCATTCTTCATTC

TGTGCA GTGTTAACA CTGATTTCTGGGAGAAGCTGCCATTCTTCATTCGTGCA GTGTTAA CACTGATTTCTGGGAGAA GCTGCCATTCTTCATTC

TGTGCA GTGTTAACA CTGATTTCTGGGAGAAGCTGCCATTCTTCATTCGTGCA GTGTTAA CACTGATTTCTGGGAGAA GCTGCCATTCTTCATTC

TGTGCA GTGTTAACA CTGATTTCTGGGAGAAGCTGCCATTCTTCATTCGTGCA GTGTTAA CACTGATTTCTGGGAGAA GCTGCCATTCTTCATTC

TGTGCA GTGTTAACA CTGATTTCTGGGAGAAGCTGCCATTCTTCATTCGTGCA GTGTTAA CACTGATTTCTGGGAGAA GCTGCCATTCTTCATTC

TGTGCA GTGTTAACA CTGATTTCTGGGAGAAGCTGCCATTCTTCATTCGTGCA GTGTTAA CACTGATTTCTGGGAGAA GCTGCCATTCTTCATTC

TGTGCA GTGTTAACA CTGATTTCTGGGAGAAGCTGCCATTCTTCATTCGTGCA GTGTTAA CACTGATTTCTGGGAGAA GCTGCCATTCTTCATTC

TGTGCA GTGTTAACA CTGATTTCTGGGAGAAGCTGCCATTCTTCATTCGTGCA GTGTTAA CACTGATTTCTGGGAGAA GCTGCCATTCTTCATTC

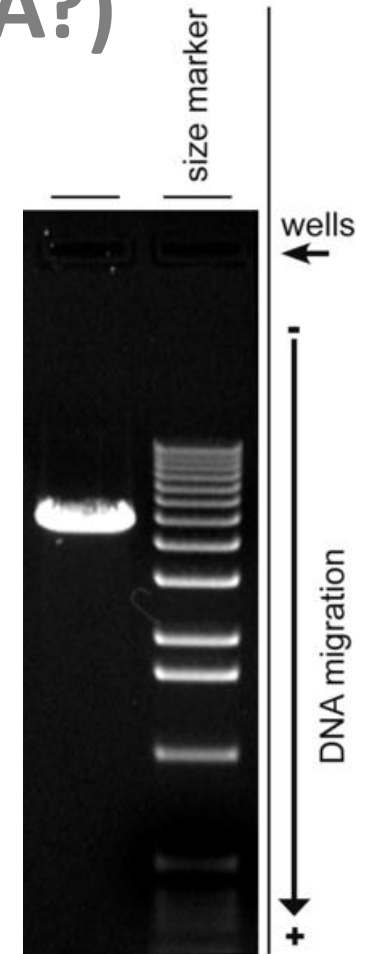
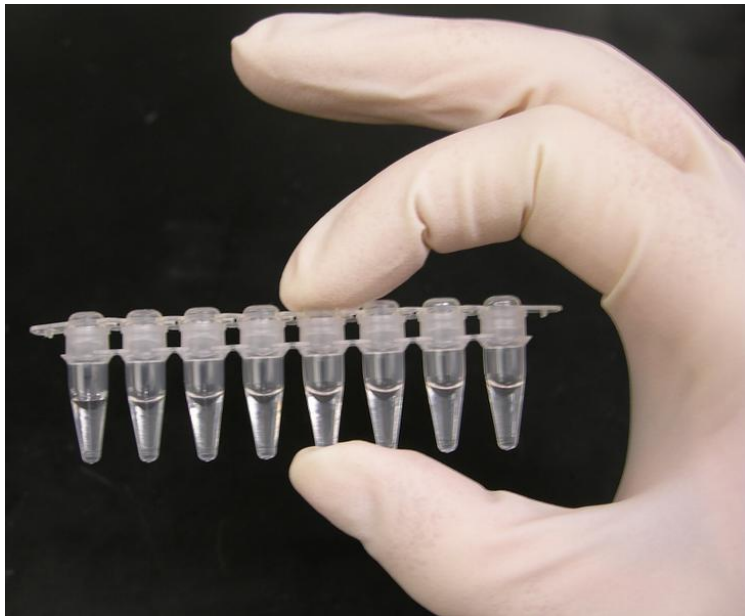
TGTGCA GTGTTAACA CTGATTTCTGGGAGAAGCTGCCATTCTTCATTCGTGCA GTGTTAA CACTGATTTCTGGGAGAA GCTGCCATTCTTCATTC

TGTGCA GTGTTAACA CTGATTTCTGGGAGAAGCTGCCATTCTTCATTCGTGCA GTGTTAA CACTGATTTCTGGGAGAA GCTGCCATTCTTCATTC

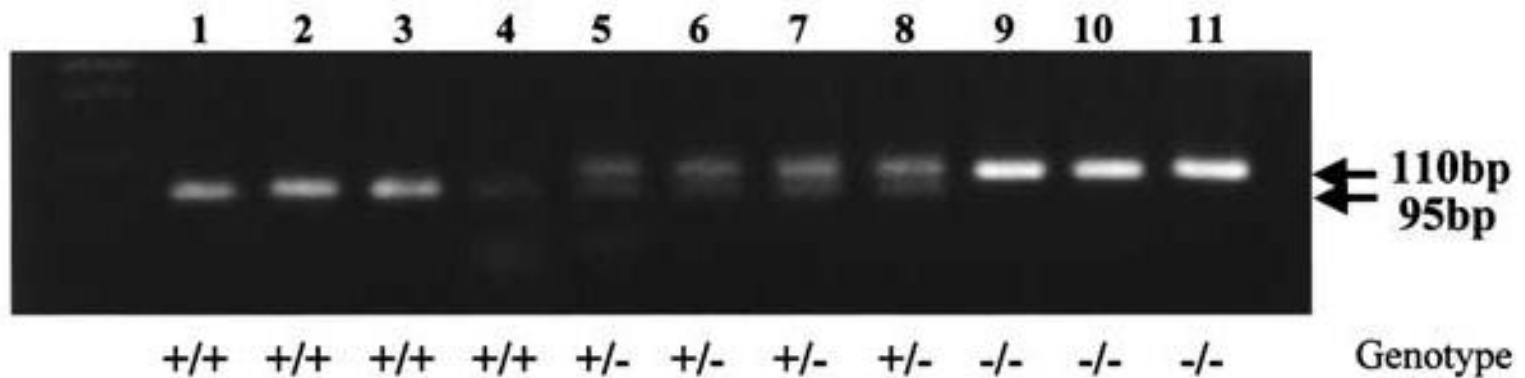
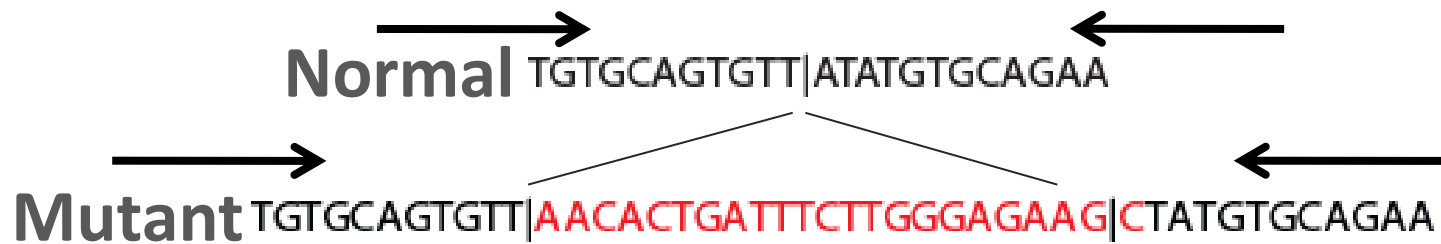
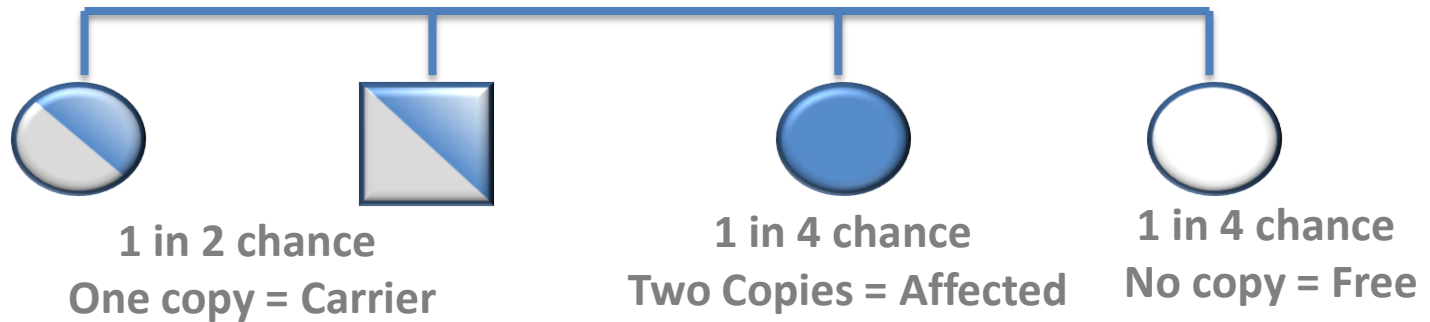
Until you have millions of copies.

PCR – Gel Electrophoresis

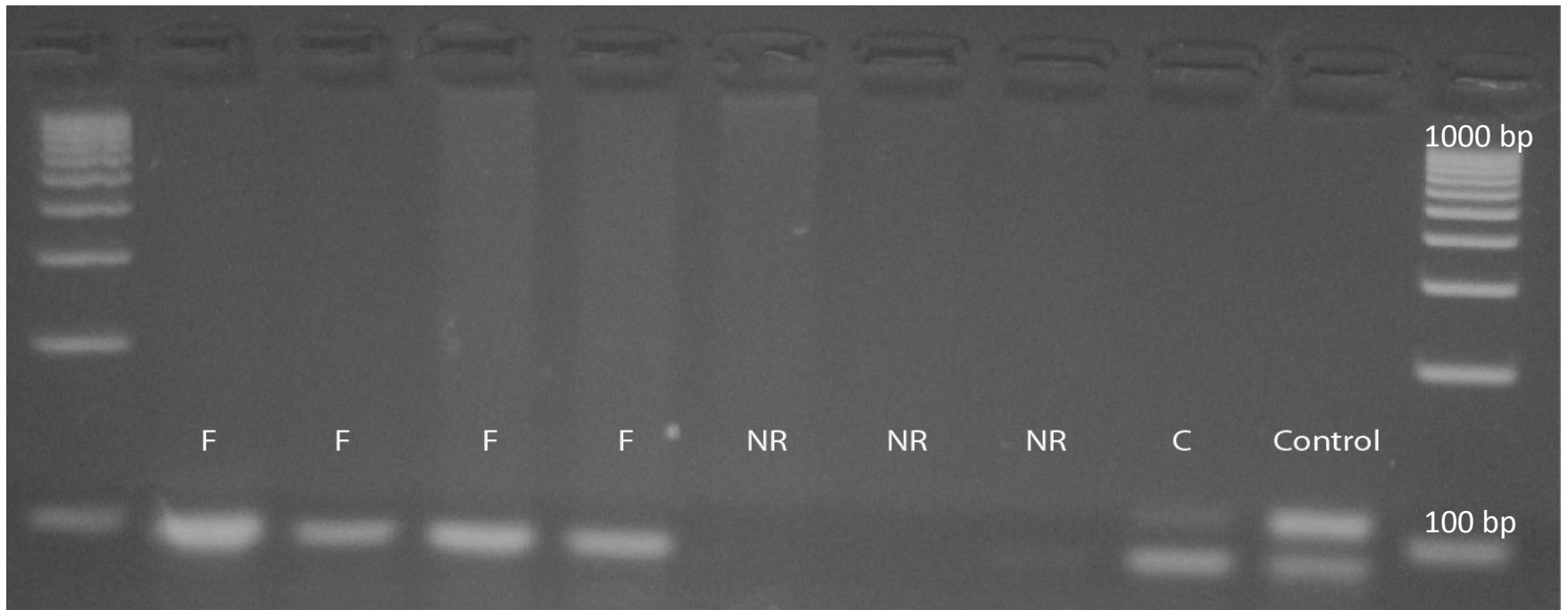
(How do you “see” DNA?)



Genotyping the F11 Insertion



Challenges – “No Results”



Normal

ATACGTATTAATGACC →

.....GCTAAATGCCGGGAATATACGTATTAATGACCAGATACAGTGTGCAGTGT|ATATGTGCAGAAATGC TGGGAATATACGTATTAATGACCAG.....

GCC
GGGAATATACGT

No Primer Binding

.....GCTAAATGCCGGGAATATACGTATTAATGACCAGATACAGTGTGCAGTGT|AACACTGATTCTTGGGAGAAG|CTATGTGCAGAAGCTAAATGC TGGGAATATACGTATTAATGACCAG.....

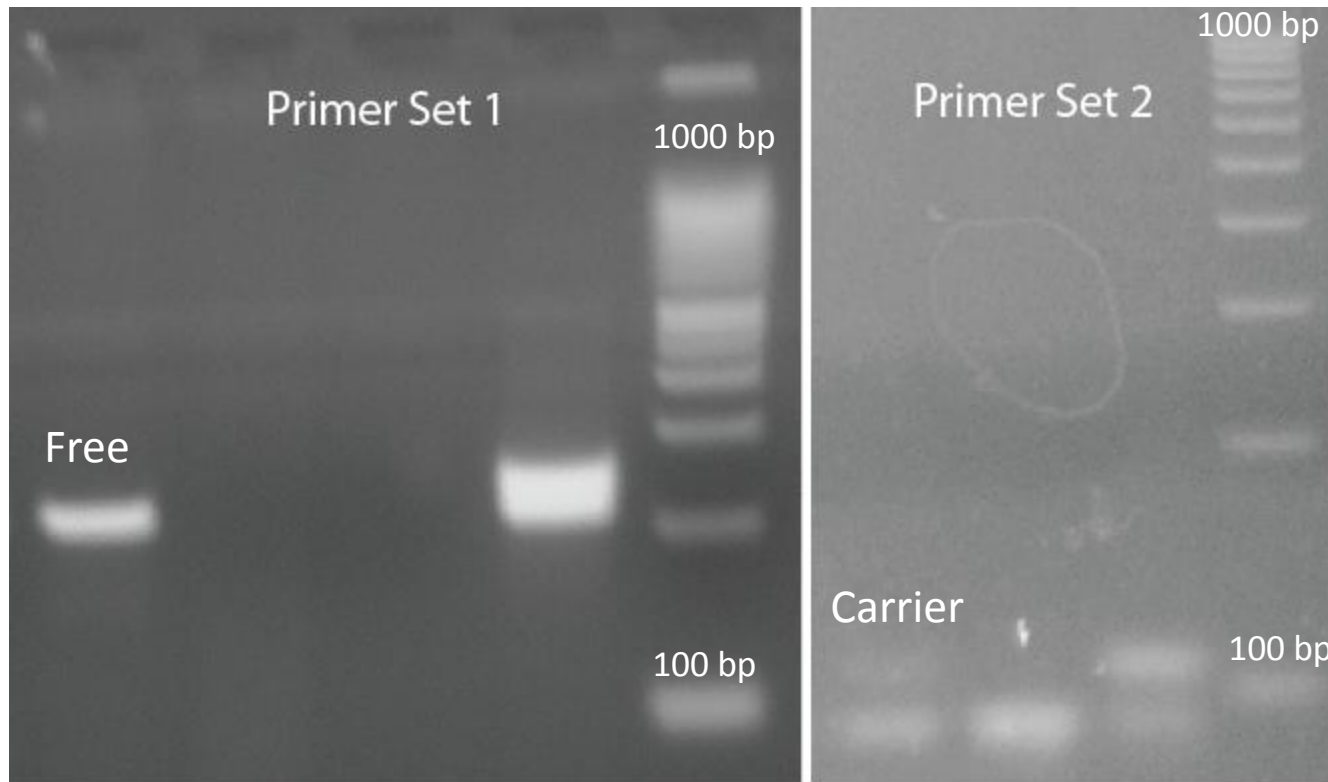
Mutant

ATACGTATTAATGACC →

GCC
GGGAATATACGT

No Primer Binding

Challenges – Mis-calls



Normal

ATACGTATTAATGACC → ← CCGGGGAATATACGT Yes Primer Binding
GCTAAATGCCGGGAATATACGTATTAATGACCAGATACAGTGTGCAGTGT|ATATGTGCAGAAATGCCGGGAATATACGTATTAATGACCAG.....

....GCTAAATGCCGGGAATATACGTATTAATGACCAGATACAGTGTGCAGTGT|**A**ACTGATT**CTTGGGAGAAG**|CTATGTGCAGAAGCTAAATGC**T**GGGAATATACGTATTAATGACCAG.....

Mutant

ATACGTATTAATGACC →

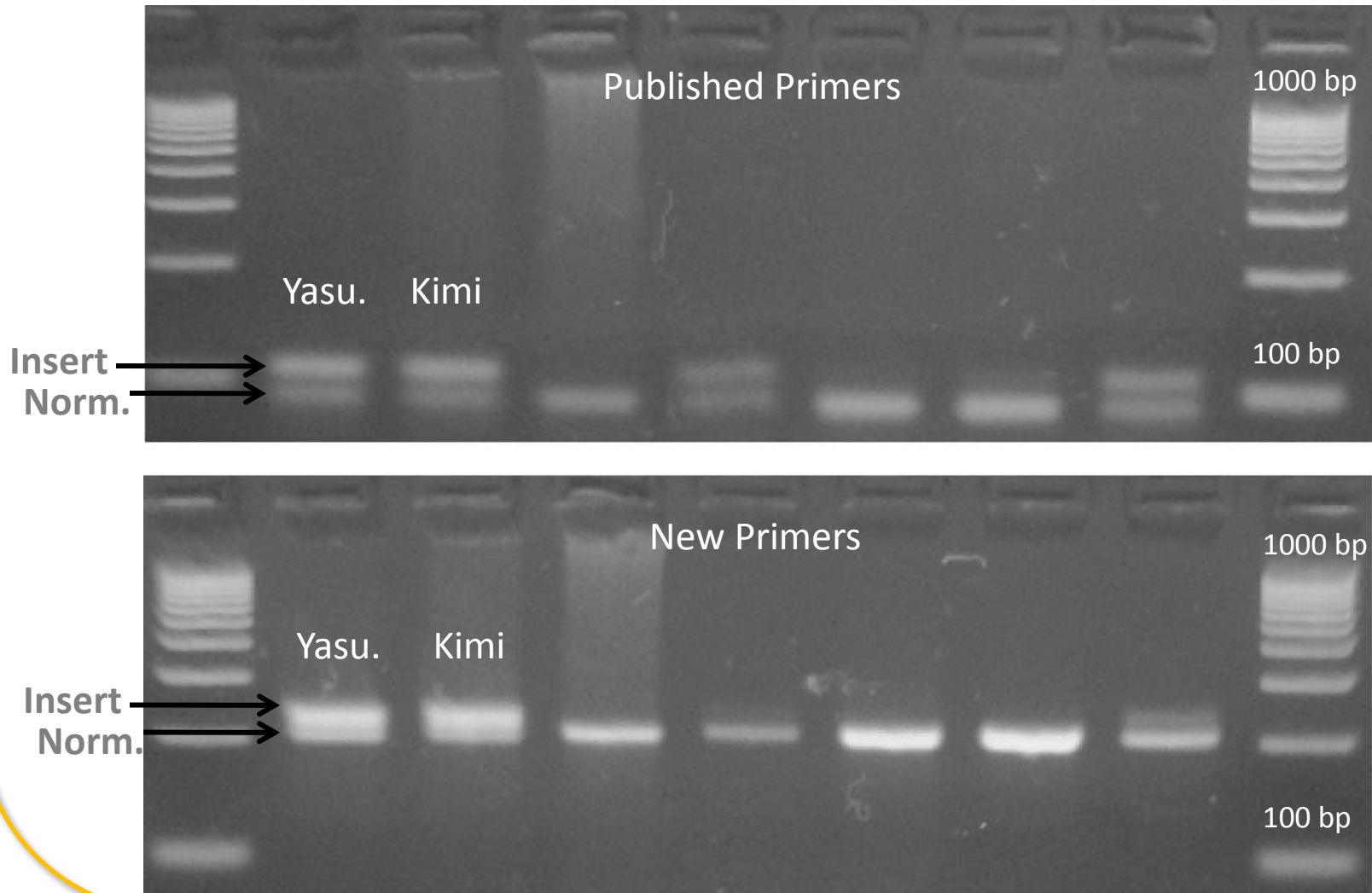
G^C No Primer Binding
 GGGGAATATACGT

Additional F11 Primer Sets



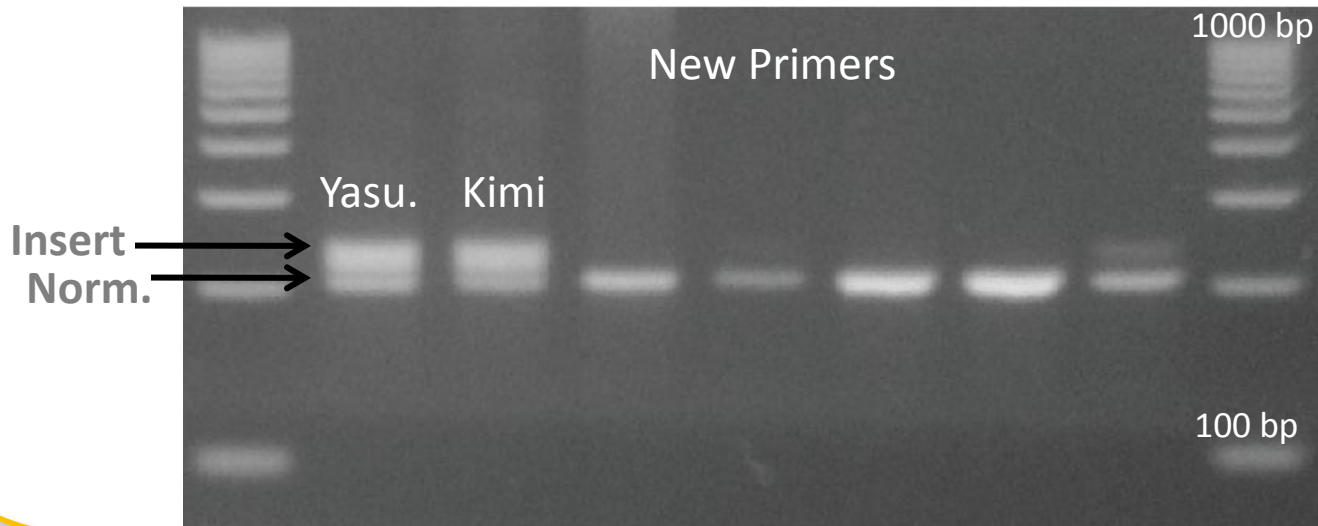
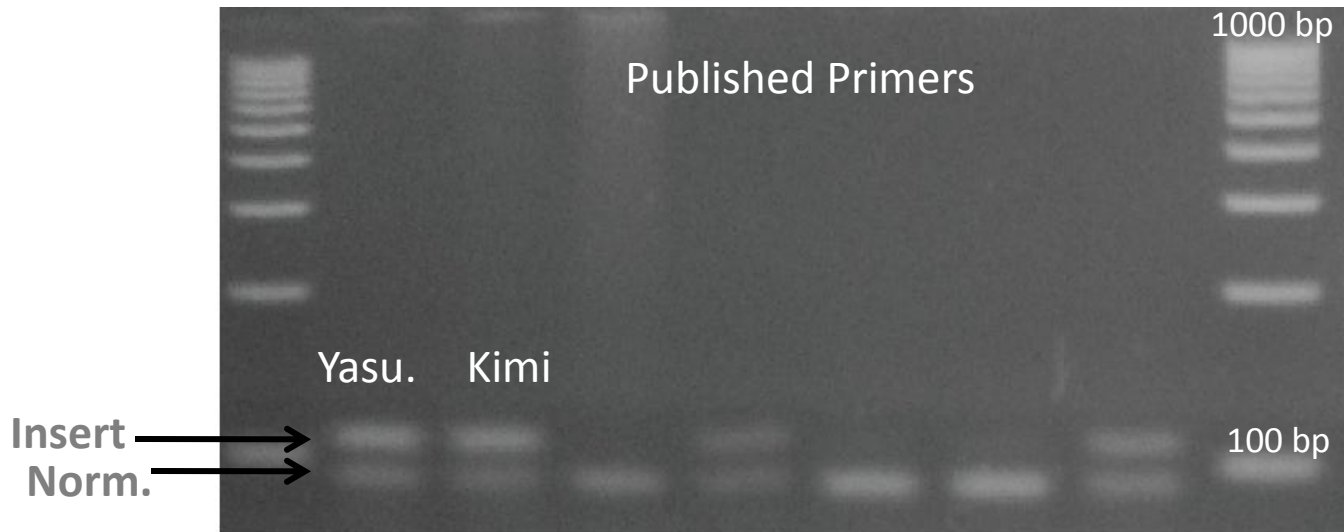
Challenges in F11: The New Mutation

45 min. run time



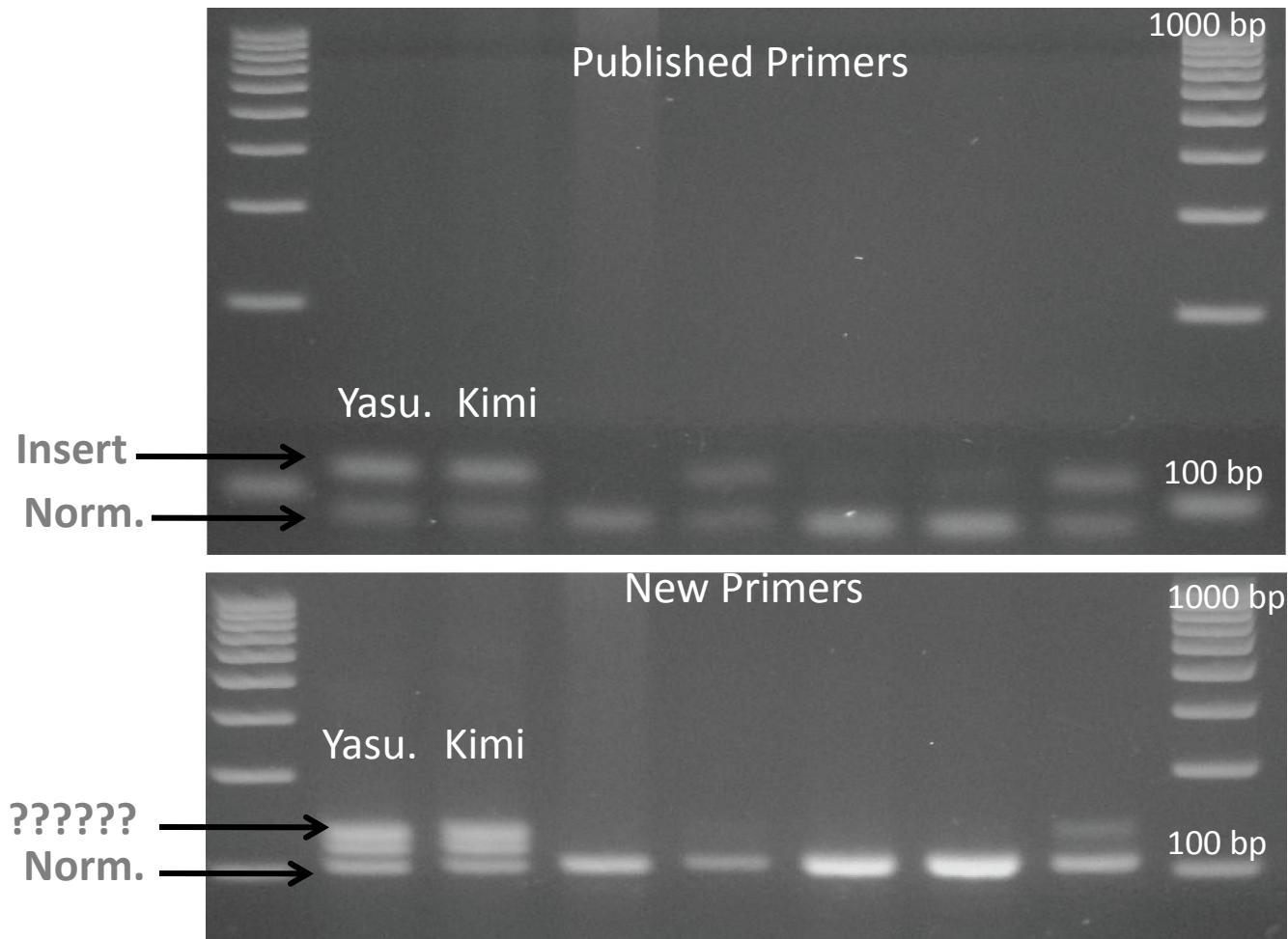
Challenges in F11: The New Mutation

75 min. run time

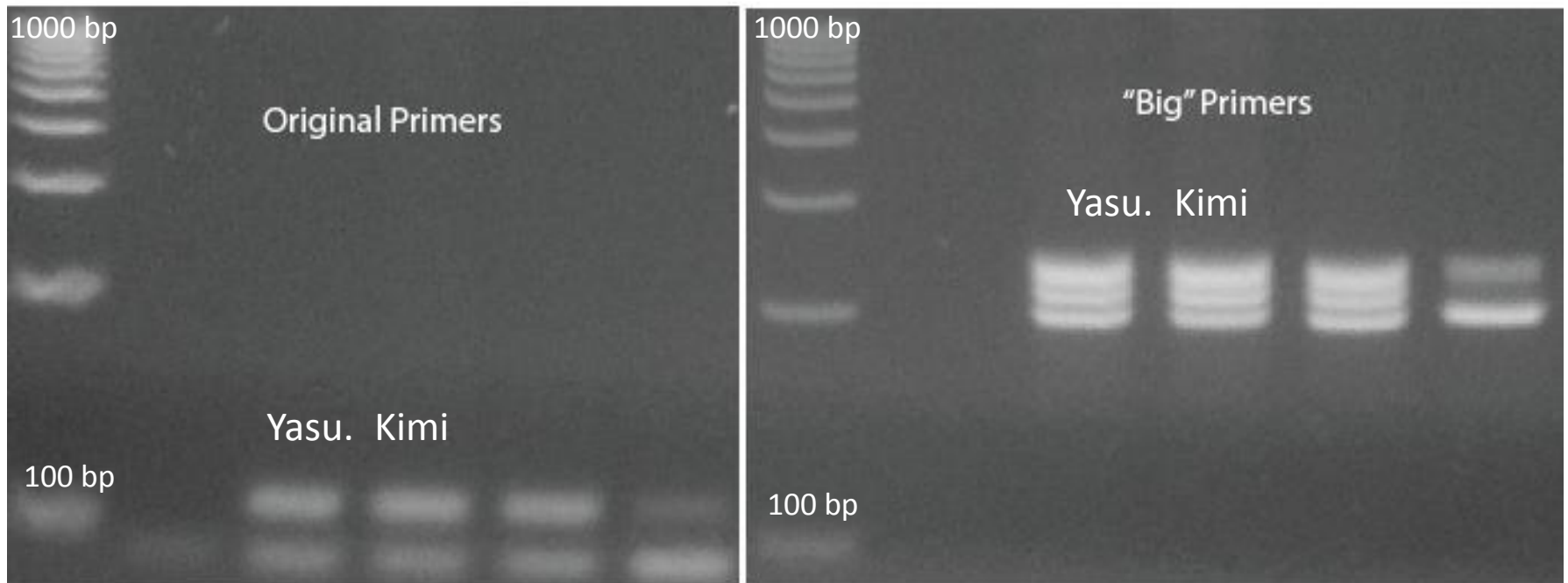


Challenges in F11: The New Mutation

95 min. run time



Challenges in F11: The New Mutation



Normal TGTGCAGTGTT|ATATGTGCAGAA

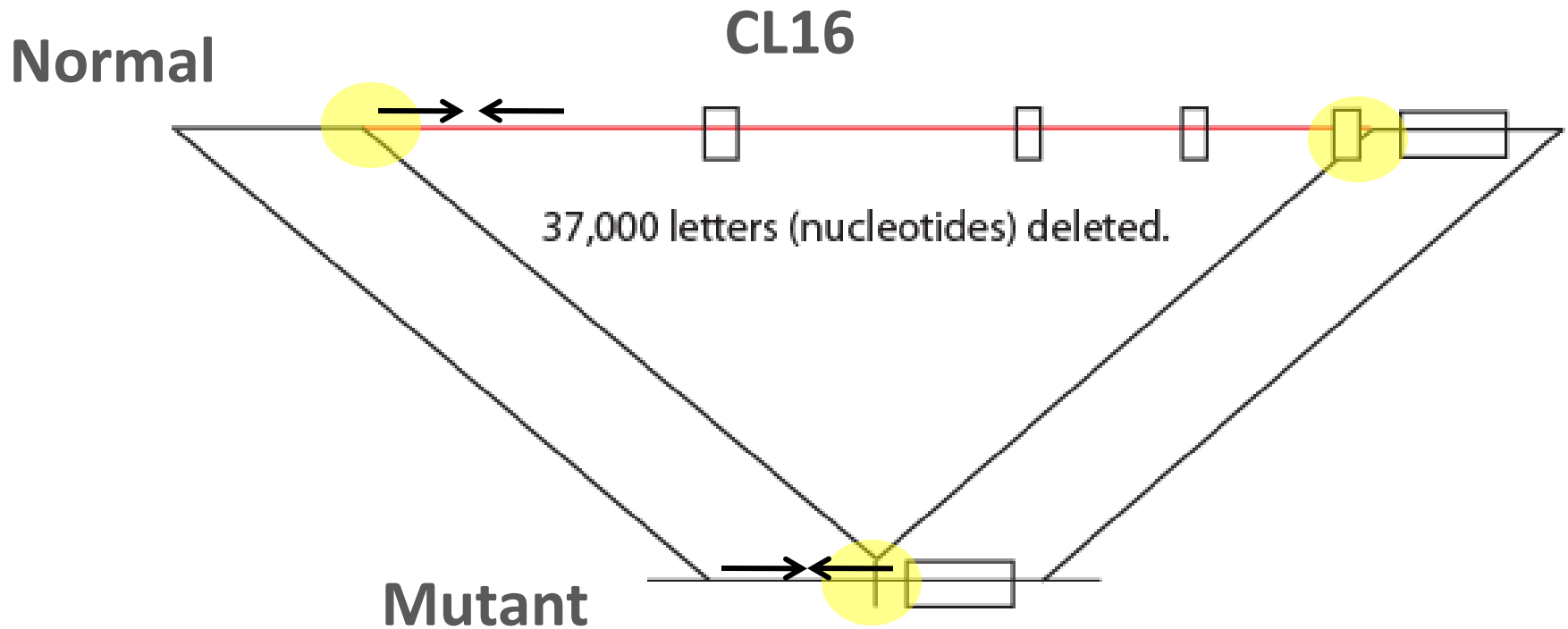
Mutant TGTGCAGTGTT|AACACTGATTTCTGGGAGAA G|CTATGTGCAGAA

Challenges in CL16 – Balance of False Positives and NRs

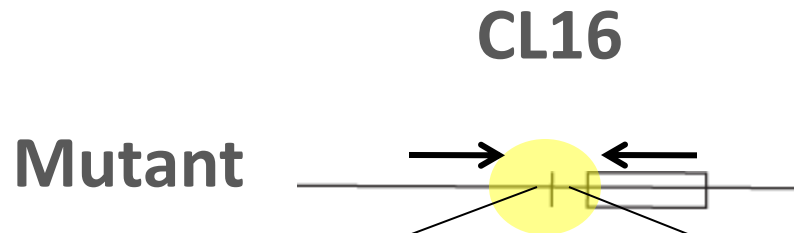
	Errors	
	Num.	%
CL16	4	0.88%

	NRs	
	Num.	%
CL16	132	23%

The CL16 Deletion: PCR Assay



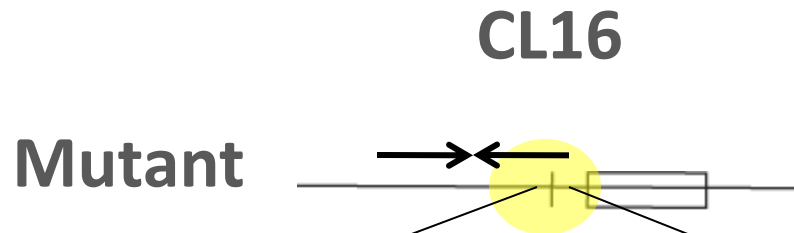
The CL16 Deletion: Ideal Primers



Ideal Primer Placement

.....TGTGCAGTGT|GGGGGTGGGGGGGTGGGGGGGTGGGGGGGT|GGGGGGGGGGCGGGGGGGGGTGCCATTCTCATTG.....

The CL16 Deletion: Original Primers

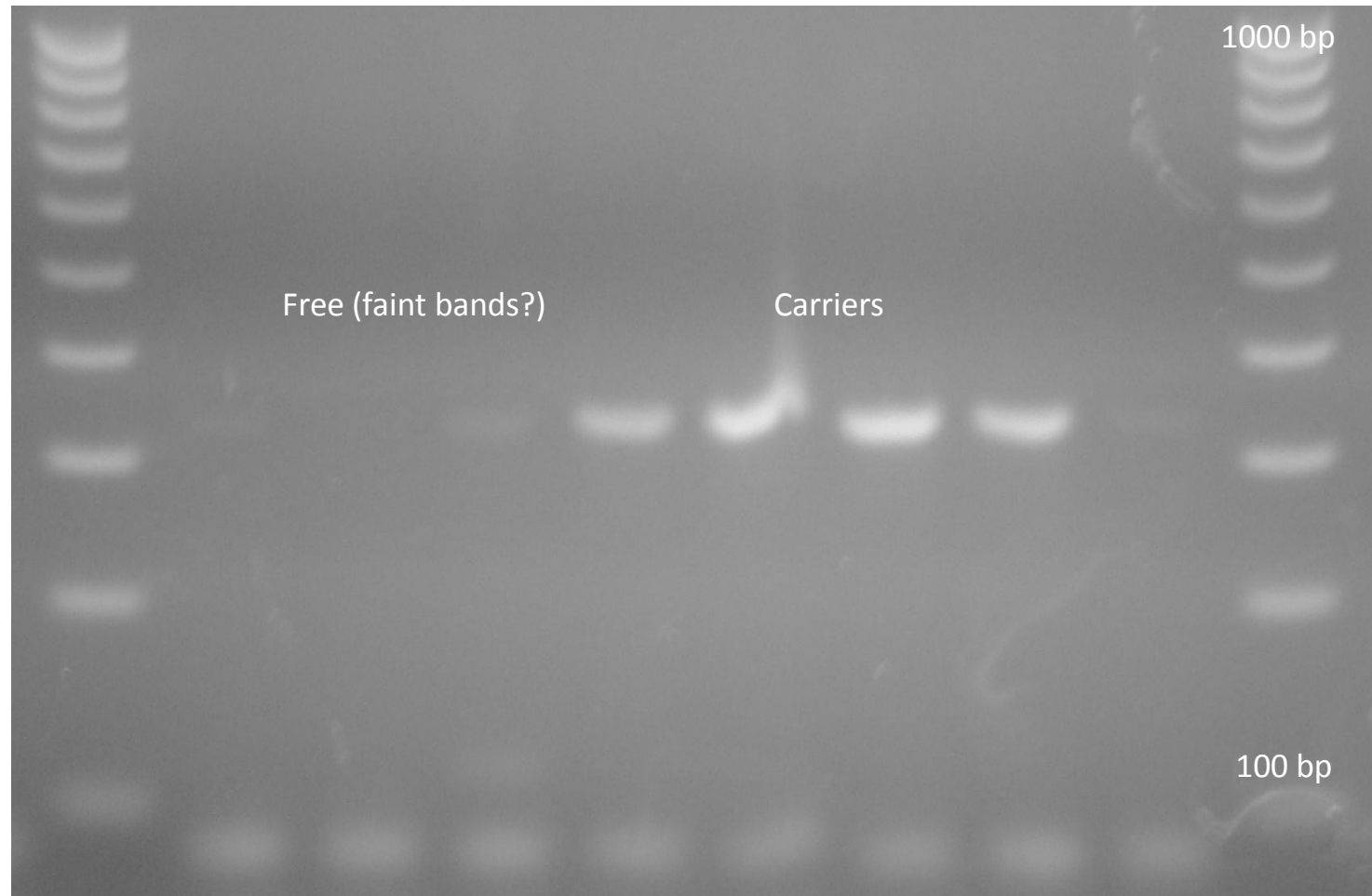


PCR Primers (bookends)

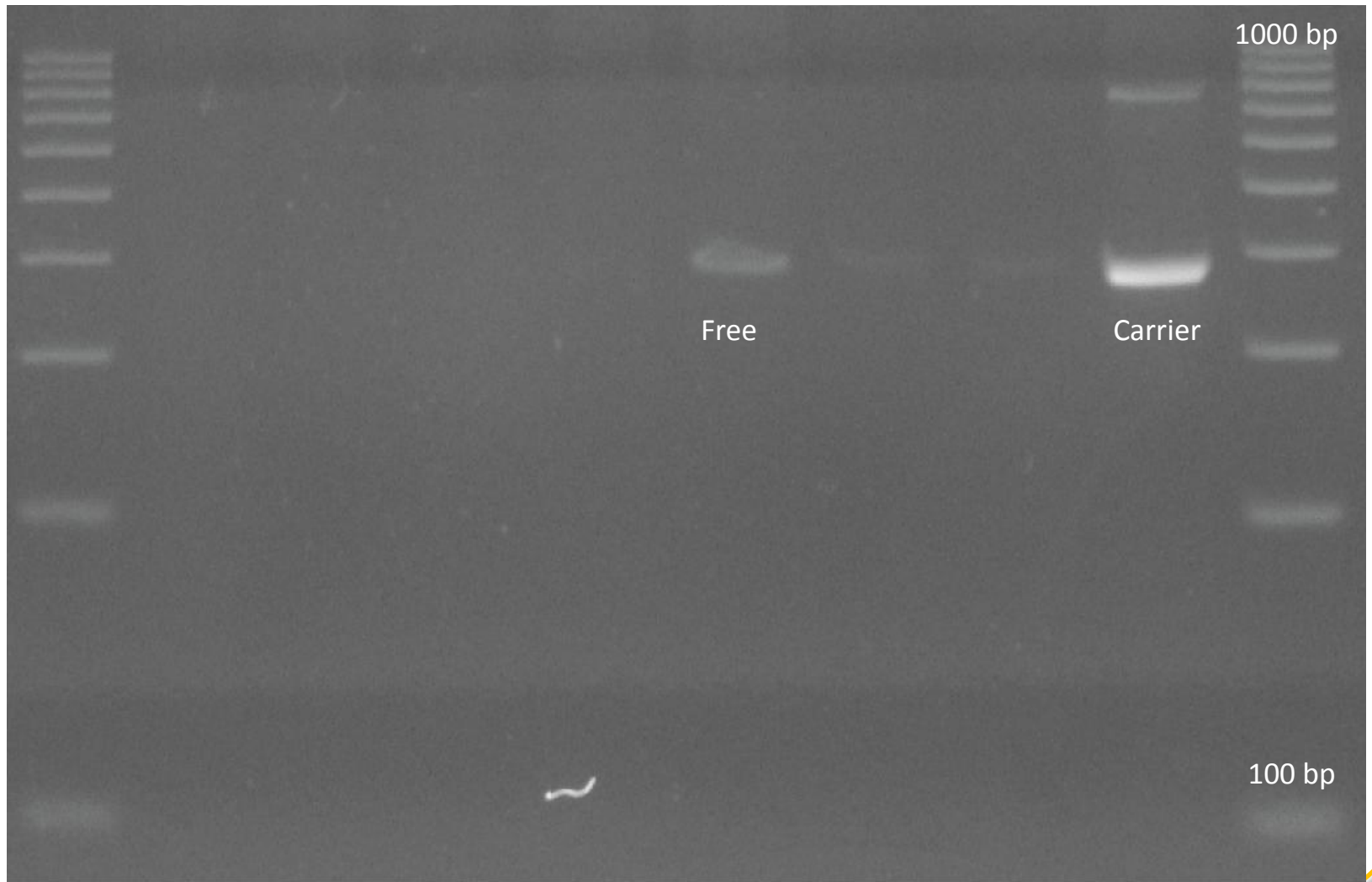
.....TGTGCAGTGT|GGGGGTGGGGGGGTGGGGGGGTGGGGGGGT|GGGGGGGGGGCGGGGGGGGGTGCCATTCTCATTG.....

Overlap with high error rate

Original Primer Set Has High False Pos.



More Stable Primer Set is Less Effective



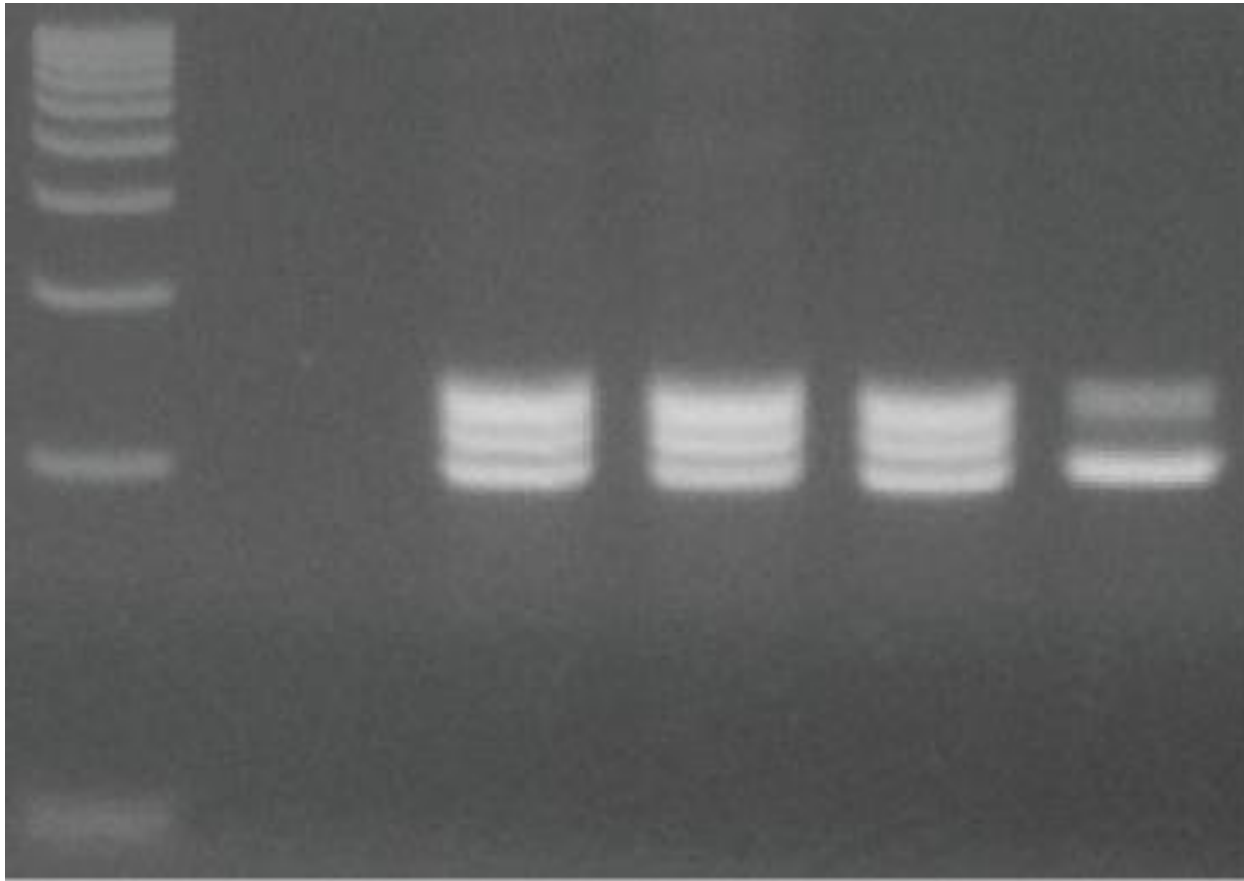
Preliminary Strategy for Resolution:

- 1) CL16 – If NR, use pedigree information. If no pedigree information available, no result can be given.
- 2) F11 – If has middle band, consider it a Carrier. If no middle band, consider it free, possibly report additional F11 copy – all pending board agreement of strategy.

Timeline:

Scientific Findings

F11: A New Mutation

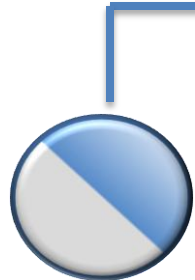


F11: To Test or Not to Test ?

One Copy = Unaffected
(Carrier)



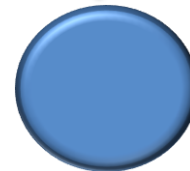
One Copy = Unaffected
(Carrier)



1 in 2 chance
One copy = Carrier



1 in 4 chance
Two Copies = Affected



1 in 4 chance
No copy = Free

F11: Statistical Significance

If you flip a penny 100 times, what are the odds that it comes out 50% heads and 50% tails?

7.9%

What are the odds that it's pretty close to 50:50? (45:55 or 55:45)?

80.2%

If you get an answer that is different from what you expect, how can you tell if that's just chance or actually important?

F11 Affected Animals are Rare

Given : 316 Free animals, 221 Carriers, How many Affecteds do we expect?

We expect 25 Affecteds

We have found 7 Affecteds

The odds of this happening by chance are $< 1/10000$ ($X^2 = 21.65$)

F11: Biological Implications

Disorder	Observed	Expected	Odds
Band3	0	0.17	1/1
CHS	0	1.73	1/1
CL16	0	0.28	1/1
F11	7	25	<< 1/10000

F11: Biological Implications

- 1) Are there differences in leutinization of Carrier Dams?
- 2) Decreases in fertility of Carrier Sires?
- 3) Lower implantation rates, full term pregnancy, or other viability of Carrier and Affected embryos?

Conclusions:

- 1) This testing is still in development.
- 2) More R&D and discovery than known variables.
- 3) Testing of these traits is likely important for future management of the breed.

Acknowledgments:

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Justin Bowser

Margaret Rodgers

Dr. Carl T. Newman

AWA

Don Brown

Robert Estrin

Michael Goodell

Grant Robinson

Jerry Reeves

Ralph Valdez

WSU

Holly Neiberger

Bioline

Mickie Blake

Geospiza

Rob Arnold

PCR and Primer Binding

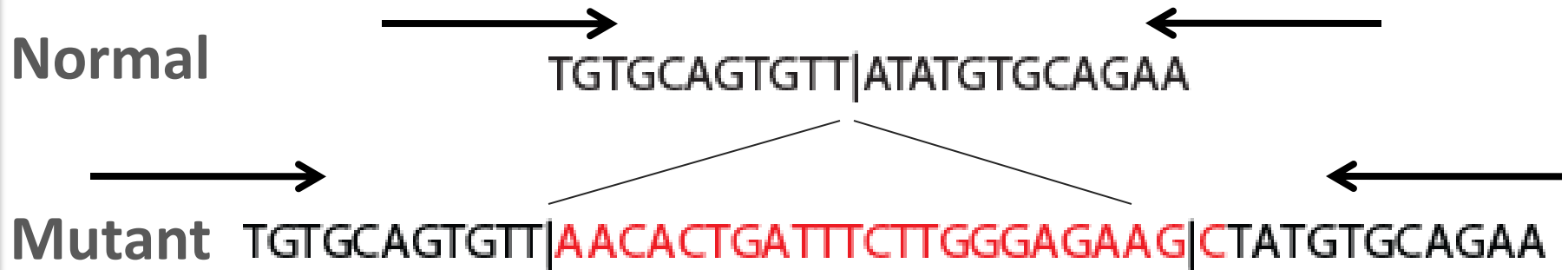
No Primer Attachment = No PCR Product



Some Primer Attachment = Some PCR Product



Challenges in F11: New Discoveries



Insertion

