

# Investigation of the genetic differences between bovine herpesvirus type 1 variants and vaccine strains

Name: Claire Ostertag-Hill

Mentor: Dr. Ling Jin

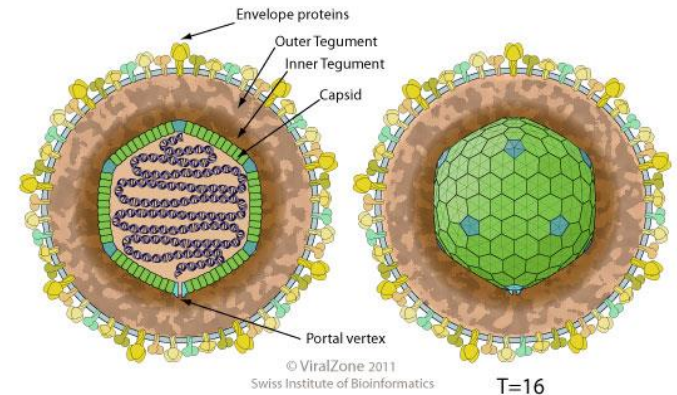
# Bovine herpesvirus



- Bovine herpesvirus-1 (BHV-1)
  - Pathogen of Infectious Bovine Rhinotracheitis
- Infectious Bovine Rhinotracheitis (IBR)
  - Clinical signs: high fever, inflammation of nose, nasal discharge, conjunctivitis, abortion
  - Can predispose animals to secondary bacterial infections, such as shipping fever
  - Spread through nasal secretions, droplets, genital secretions, serum, and fetal fluids
- Cattle industry: suffers losses of more than \$500 million/year

# Molecular basics

- Family: *Herpesviridae*
  - Subfamily: *Alphaherpesvirinae*
- Large, double-stranded DNA virus
  - Genome size: 136kb
- Consists of:
  - Core containing linear ds DNA
  - Icosahedral capsid
  - Envelope with viral glycoprotein spikes
- BHV-1: lifelong latent infection in TG of host
  - Virus reactivation: can infect others in the herd



# BHV-1 Vaccine



- Efficacious BHV-1 vaccine used for years
  - **MLV or inactivated vaccine**
- Recently, BHV-1 vaccine related abortion reported in increasing number of herds
- Pfizer: PregGuard (MLV Vaccines)
  - **Abortion after Pfizer BHV-1 Vaccine during pregnancy**
    - 2011: Wyoming
    - 2012: UC Davis (California), Texas A&M, and Cornell (New York)

# BHV-1 Vaccine-related abortion

- Rise of vaccine-related abortion may be result of several factors:
  1. Emergence of new virulent strain of BHV-1
  2. Genetic recombination between wild type virus and BHV-1 vaccine strains
  3. Combination of environmental factors and reactivation of BHV-1 in latently infected herds

# Question?

- Is the abortion caused by the vaccine?

# Objectives

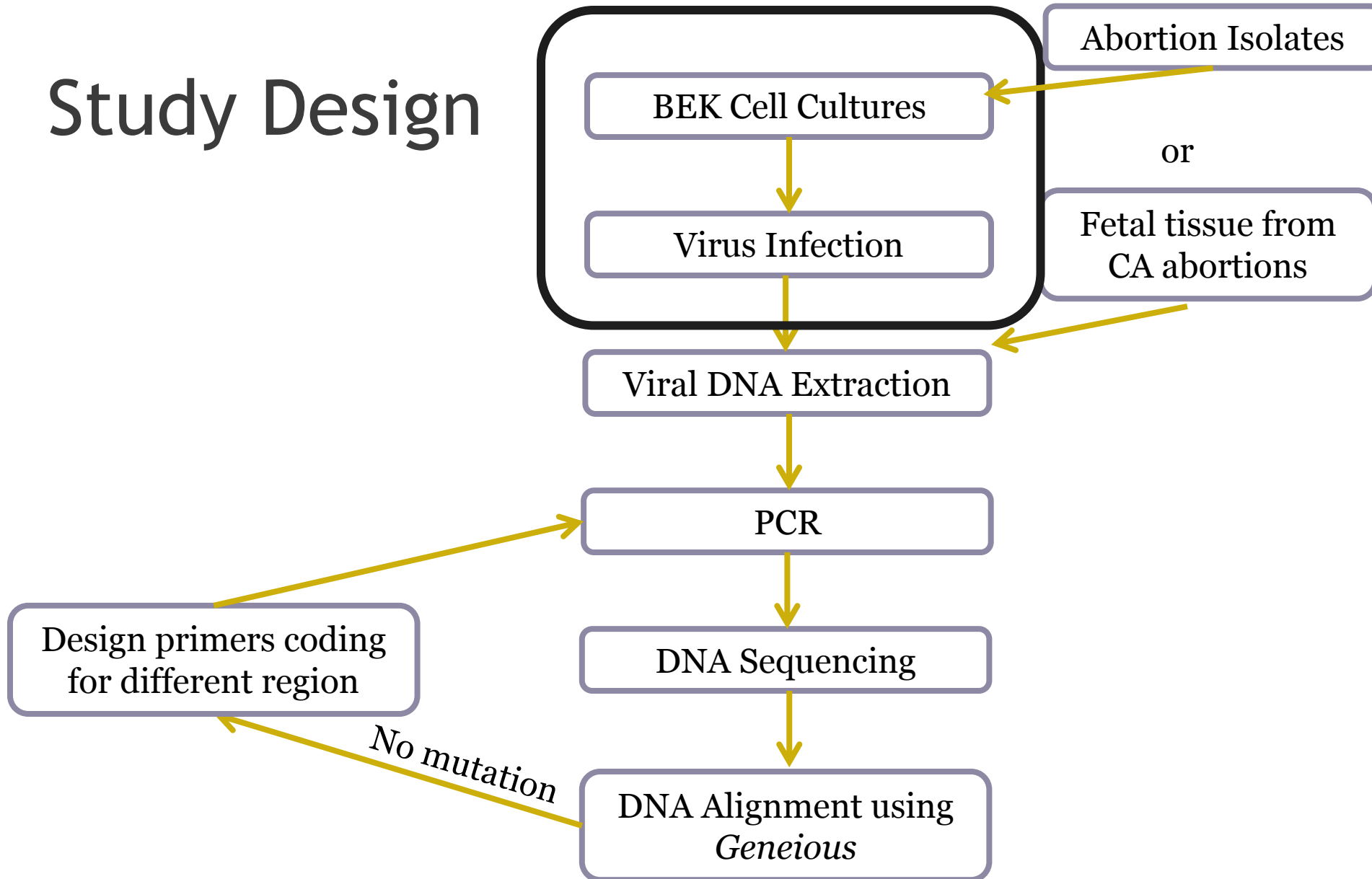
1. Sequence BHV-1 isolates from abortion cases and compare them to wild type BHV-1 virus and BHV-1 vaccine DNA genome
2. Establish strain specific nucleotide polymorphism (SSNP) profiles to assist diagnosis of BHV-1 infection

# Approaches

- TK, gE, and gG are unique genes in BHV-1 wild type and vaccine strains
- Compare the above genes between vaccine strains and abortion isolates by PCR-DNA sequence

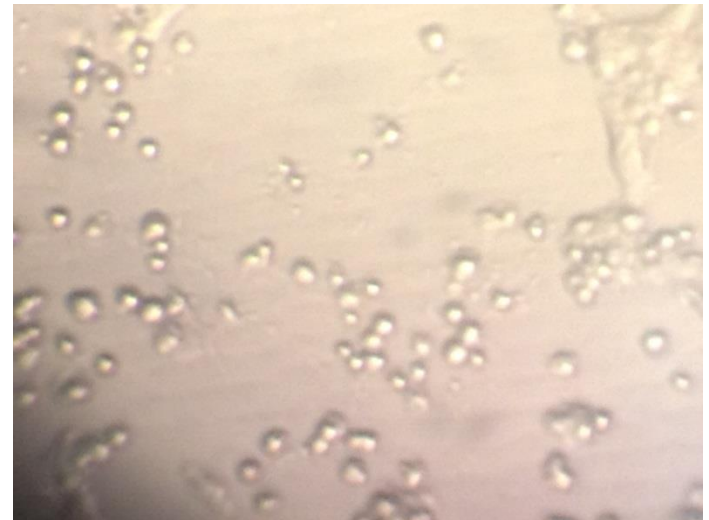
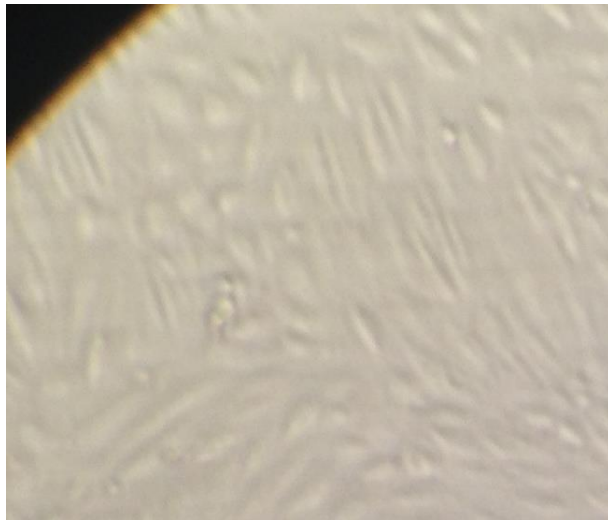


# Study Design

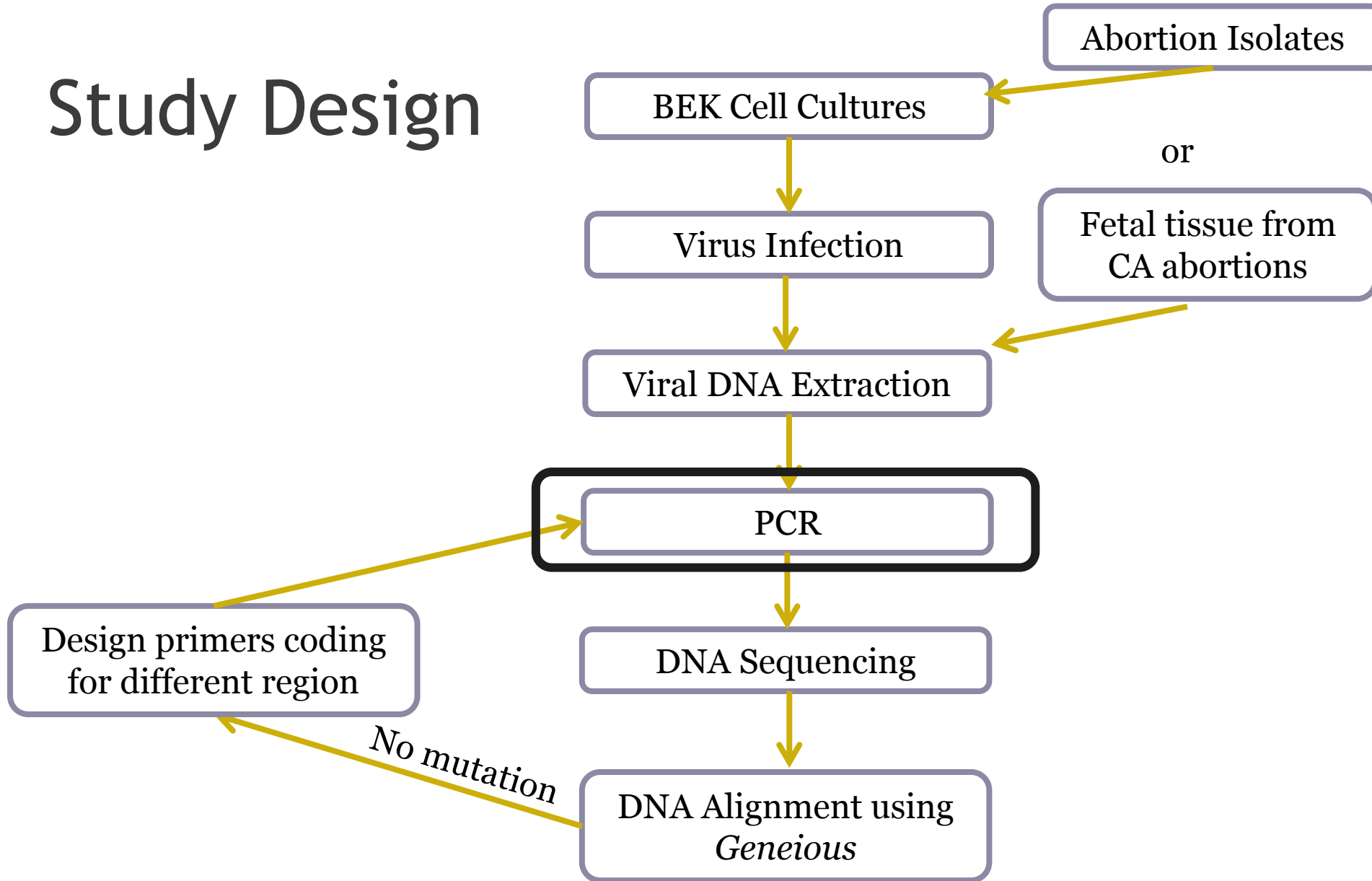


# BEK Cell Cultures & Virus Infection

- Bovine Embryonic Kidney Cells: maintained monolayer in flasks
- Infected with virus isolate
- Examined for cytopathic effect (deterioration of monolayer cells)

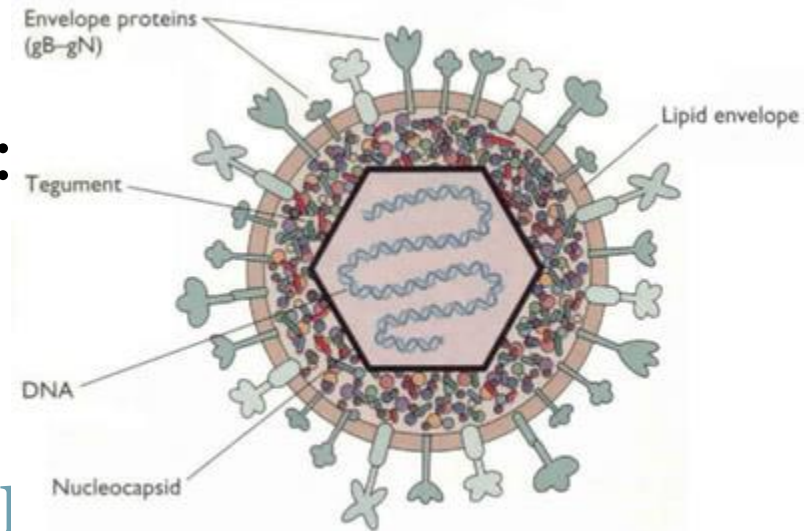


# Study Design



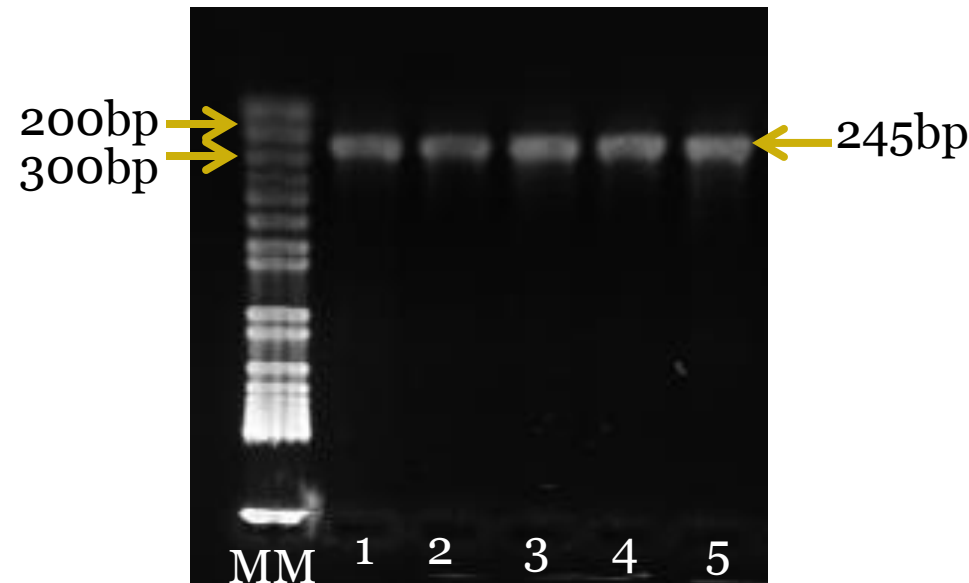
# Study Design: PCR Primers

- Amplified regions coding for:
  - **Thymidine Kinase (TK)**
    - Involved in viral virulence
  - **gE**
    - Required for direct cell-to-cell cell junctions
  - **gG**
    - Contributes to viral entry and attachment



# Study Design: PCR Results

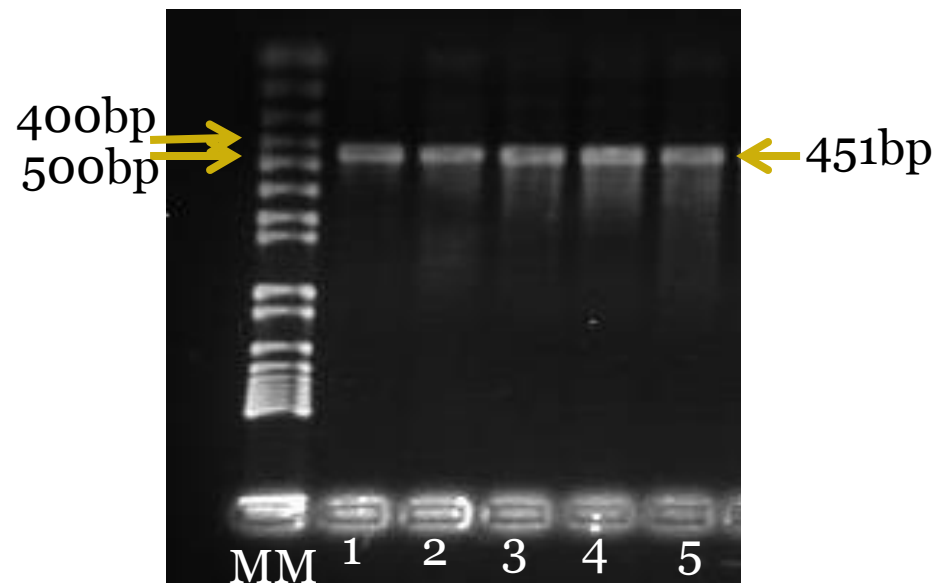
## TK: R1



MM: Molecular Marker

- 1: T739073
- 2: T754-753
- 3: E754-753
- 4: T742-308
- 5: L6064-914

## TK: R2

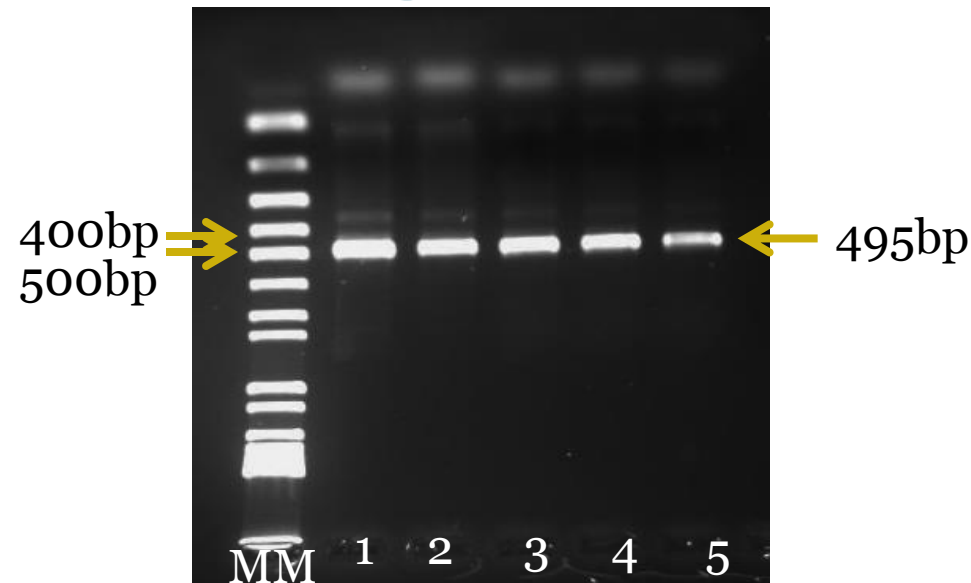


MM: Molecular Marker

- 1: T596-042
- 2: T739-073
- 3: T754-753
- 4: E754-753
- 5: T742-308

# Study Design: PCR Results

gE



MM: Molecular Marker

1: PG-BHV-1

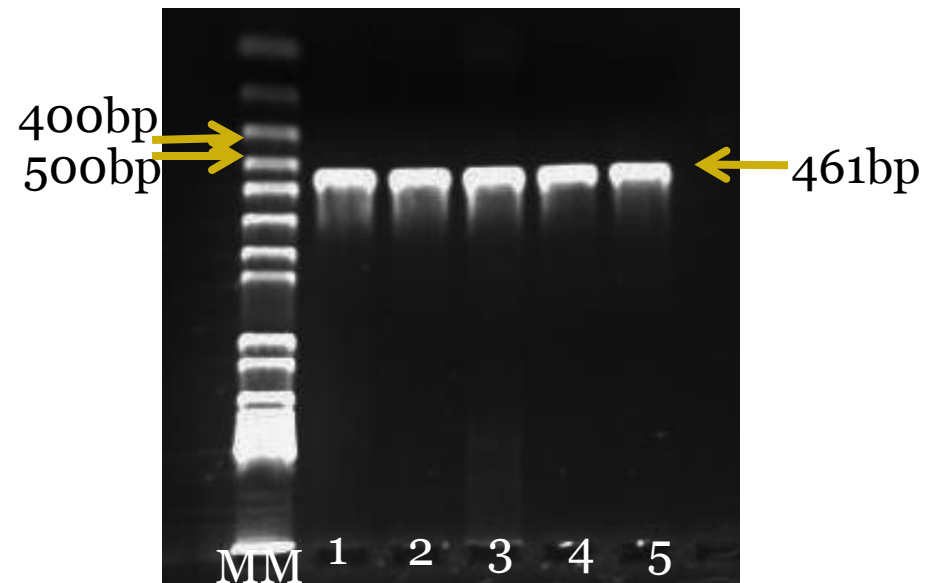
2: E737-501

3: T737-501

4: E739-051

5: T739-051

gG



MM: Molecular Marker

1: L675-940

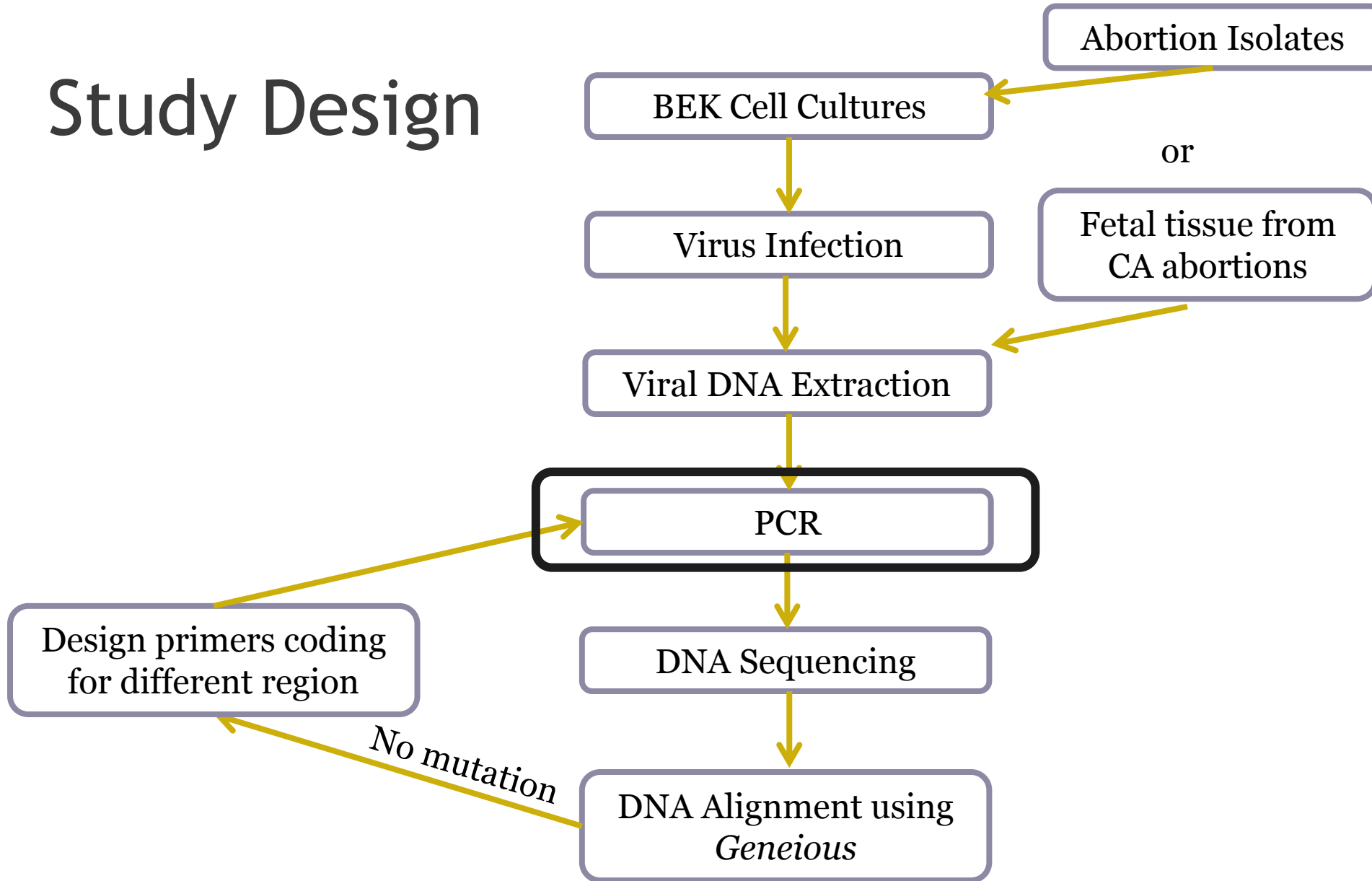
2: T519-200

3: T739-073

4: E739-051

5: T739051

# Study Design





# Results: TK R1

- Sequenced 35 isolates
- Substitution, Insertion, Deletion

Consensus	A	C	G	C	G	G	C	G	G	G	G	C	C	G	G	C	G
Identity																	
1. TK(P1)	A	C	G	C	G	G	C	G	G	G	G	C	C	G	G	C	G
2. IBR-1-11-12...	A	C	G	C	G	G	C	G	G	G	G	C	C	G	G	C	G
3. IBR-2LARY...	A	C	G	C	G	G	C	G	G	G	G	C	C	G	G	C	G
4. IBR-4LARY...	A	C	G	C	G	G	C	G	G	G	G	C	C	G	G	C	G
5. IBR-6370-P...	A	T	G	C	G	G	T	G	C	A	G	C	G	C	C	G	T
6. IBR-675-940...	A	C	G	C	G	G	C	G	G	G	G	C	C	G	G	C	G
7. IBR-694-513...	A	C	G	C	G	G	C	G	G	G	G	C	C	G	G	C	G
8. IBR-7212-P...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	A
9. IBR-8-4-11_...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
10. IBR-A-P1_...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	A
11. IBR-A644-6...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
12. IBR-B-P1_...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
13. IBR-BG1.1...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
14. IBR-C-P1_...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	A
15. IBR-E596-0...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
16. IBR-E739-0...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
17. IBR-E737-5...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
18. IBR-E754-7...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
19. IBR-L513_...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
20. IBR-L6046-...	A	G	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
21. IBR-L6064-...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
22. IBR-LN513...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
23. IBR-LN604...	A	G	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
24. IBR-PG-BH...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
25. IBR-PLAC...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
26. IBR-T322_...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
27. IBR-T519-2...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
28. IBR-T599_...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
29. IBR-E702-...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
30. IBR-T702-2...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
31. IBR-T737-5...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
32. IBR-T739-0...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
33. IBR-T739-0...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
34. IBR-T742-3...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
35. IBR-T754-7...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G
36. IBR99-11_...	A	C	G	C	G	G	C	G	C	A	G	C	G	C	C	G	G



# Results: TK R2

- Sequenced 11 isolates
- Deletion, Substitution, Insertion

	130	140	150	160
Consensus	GCGCGGCGCGGCTGCGGACACCTGTTCGCGGCGCTCAA			
Identity	100%			
1. TK(R2)	GCGCGGCGCGGCTGCGGACACCTGTTCGCGGCGCTCAA			
2. IBR-PG-BH...	GCGCGGCGCGGCTGCGGACACCTGTTCGCGGCGCTCAA			
3. IBR-BG1:10-...	GCGCGGCGCGGCTGCGGACACCTGTTCGCGGCGCTCAA			
4. IBR-1-11-12...	GCGCGGCGCGGCTGCGGACACCTGTTCGCGGCGCTCAA			
5. IBR-PLACE...	GCGCGGCGCGGCTGCGGACACCTGTTCGCGGCGCTCAA			
6. IBR-Lung51...	GCGC---CGCCGCTGCGGACACCTGTTCGCGGCGCT-AA			
7. IBR-99-11-1...	GCGCGGCGCGGCTGCGGACACCTGTTCGCGGCGCTCAA			
8. IBR-T599-P...	GCGCGGCGCC--GCTGCGGACACCTGTTCGCGGCGCT-AA			
9. IBR-T322-P...	GCGCGGCGCGGCTGCGGACACCTGTTCGCGGCGCTCAA			
10. IBR-8-4-11-...	GCGCGGCGCGGCTGCGGACACCTGTTCGCGGCGCTCAA			
11. IBR-LN513...	GCGCGGCGCGGCTGCGGACACCTGTTCGCGGCGCTCAA			
12. IBR-694-51...	GCGCGGCGCGGCTGCGGACACCTGTTCGCGGCGCTCAA			

# Results: gE

- Sequenced 31 isolates
- Substitution, Insertion, Deletion

Consensus	AGACTCTTTTGGGATGACGACAG
Identity	
1. gE(P3)	AGACTCTTTTGGGATGACGACAG
2. IBR-1-11-12...	AGACTCTTTTGGGATGACGACAG
3. IBR-2-Laryn...	AGACTCTTTTGGGATGACGACAG
4. IBR-4-Laryn...	AGACTCTTTTGGGATGACGACAG
5. IBR-519-200...	AGACTCTTTTGGGATGACGACAG
6. IBR-6370-P...	AGACTCTTTTGGGATGACGACAG
7. IBR-644-644...	AGACTCTTTTGGGATGACGACAG
8. IBR-675-940...	AGACTCTTTTGGGATGACGACAG
9. IBR-694-513...	AGACTCTTTTGGGATGACGACAG
10. IBR-7212-P...	AGACTCTTTTGGGATGACGACAG
11. IBR-8-4-11-...	AGACTCTTTTGGGATGACGACAG
12. IBR-99-11_...	AGACTCTTTTGGGATGACGACAG
13. IBR-A-P7_...	AGACTCTTTTGGGATGACGACAG
14. IBR-B-P7_...	AGACTCTTTTGGGATGACGACAG
15. IBR-BG1-P...	AGACTCTTTTGGGATGACGACAG
16. IBR-C-P7_...	AGACTCTTTTGGGATGACGACAG
17. IBR-E-596-...	AGACTCTTTTGGGATGACGACAG
18. IBR-E-702-...	AGACTCTTTTGGGATGACGACAG
19. IBR-E-737-...	AGACTCTTTTGGGATGACGACAG
20. IBR-E-739-...	AGACTCTTTTGGGATGACGACAG
21. IBR-E754-...	AGACTCTTTTGGGATGACGACAG
22. IBR-L6046-...	AGACTCTTTTGGGATGACGACAG
23. IBR-L6064-...	AGACTCTTTTGGGATGACGACAG
24. IBR-LN513...	AGACTCTTTTGGGATGACGACAG
25. IBR-Lung5...	AGACTCTTTTGGGATGACGACAG
26. IBR-T-596-...	AGACTCTTTTGGGATGACGACAG
27. IBR-T-702-...	AGACTCTTTTGGGATGACGACAG
28. IBR-T-737-...	AGACTCTTTTGGGATGACGACAG
29. IBR-T-739-...	AGACTCTTTTGGGATGACGACAG
30. IBR-T322-...	AGACTCTTTTGGGATGACGACAG
31. IBR-T599-...	AGACTCTTTTGGGATGACGACAG
32. IBR-T739-0...	AGACTCTTTTGGGATGACGACAG

# Results: gG

- Sequenced 34 isolates
- Deletion, Insertion

Consensus	350	360	370
Identity	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
1. gG(P4)	ACCGCCAGCGGGGCC	-----TGA	
2. IBR-PG-BHV1_15...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
3. IBR-BG1:10_1529...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
4. IBR-1-11-12-P9_1...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
5. IBR-2Larynx-P9_1...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
6. IBR-4Larynx-P9_1...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
7. IBR-519-200-P9_1...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
8. IBR-675-940-P9_1...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
9. IBR-675-940-P9_1...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
10. IBR-694-513-P9_...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
11. IBR-739-073-P9_...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
12. IBR-8-4-11-P9_1...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
13. IBR-99-11-P9_15...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
14. IBR-A644-644-P...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
15. IBR-E596-042-P...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
16. IBR-E702-215-P...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
17. IBR-E737-501-P...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
18. IBR-E739-051-P...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
19. IBR-E754-753-P...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
20. IBR-L6046-914-...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
21. IBR-L6064-914-...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
22. IBR-L6064-914-...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
23. IBR-LN513-P9_1...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
24. IBR-LN6046-914...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
25. IBR-LN739-073-...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
26. IBR-Lung513-P9...	ACCGCC--GCGGGGCC	GAATCGCTGCTGA	
27. IBR-PLACENTA...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
28. IBR-T322-P9_15...	ACCGCC--GCGGGGCC	GAATCGCTGCTGA	
29. IBR-T519-200--P...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
30. IBR-T596-042-P...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
31. IBR-T599-P9_15...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
32. IBR-T702-215-P...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
33. IBR-T739-051-P...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
34. IBR-T739-073-P...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
35. IBR-T739-073-P...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
36. IBR-T742-308-P...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
37. IBR-T742-308-P...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	
38. IBR-T754-753-P...	ACCGCCAGCGGGGCC	GAATCGCTGCTGA	

# Results: Wild Type vs. Isolates

		TX (26)	WY (3)	NY (1)	CA (5)	Bovine Shield (1)	PregGuard (1)	Total (37)
TK R1	Insertion	24/24	3/3	1/1	5/5	1/1	1/1	35/35
	Deletion				1/5			
	Substitution	4/24			5/5			
TK R2	Insertion					1/1		6/11
	Deletion	2/5						
	Substitution	5/5				1/1		
gE	Insertion				1/5			5/31
	Deletion				1/5			
	Substitution	4/22			1/5			
gG	Insertion	26/26	3/3	1/1	2/2	1/1	1/1	34/34
	Deletion	2/26						
	Substitution				2/2			

# Results: Isolates vs. Vaccine

		TX (26)	WY (3)	NY (1)	CA (5)	Total (35)
TK R1	Insertion					9/33
	Deletion				1/5	
	Substitution	4/24			5/5	
TK R2	Insertion					5/11
	Deletion	2/5				
	Substitution	5/5				
gE	Insertion				1/5	5/30
	Deletion				1/5	
	Substitution	4/22			1/5	
gG	Insertion				2/2	4/32
	Deletion	2/26				
	Substitution				2/2	

TK R1 and gG: most isolates identical to vaccine strains (which were different than the wild type)

TK R2: BoviShield Vaccine exhibited 2 mutations found in no other isolates, PregGuard Vaccine, or wild type



# Results Summary

- **TK R1:** 100% of isolates different than wild type
  - 27.3% different than vaccine
- **TK R2:** 54.5% of isolates different than wild type
  - 45.5% different than vaccine
- **gE:** 16.1% of isolates different than wild type
  - 16.6% different than vaccine
- **gG:** 100% of isolates different than wild type
  - 12.5% different than vaccine

# Conclusion and Discussion

- All the abortion isolates are different from the TK gene of wild types virus and many have similar TK gene sequence as the vaccine strain
- Not all the abortion isolates have the same genetic variation
- The abortion isolates may have been derived from the vaccine strain or other variant strains
- Mutations in TK, gE, and gG may lead these abortion isolates to be more virulent

# Future Direction

- Examine the mutations in protein coding region and identify the epitope of the viral protein affected by those mutations discovered in our study
- Design primers to capture variations among different isolates



# Acknowledgements

- Dr. Ling Jin
- Jin Lab
- Howard Hughes Medical Institute
- Cripps Scholarship
- URISC: Start
- Pfizer
- Dr. Kevin Ahern