

# Role of Clara Cell Secretory Protein in Lung Inflammation

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# Recurrent Airway Obstruction

## ■ Definition

- Recurrent airway obstruction (RAO), or heaves, is an inflammatory airway disease induced by exposure of susceptible animals to organic dusts

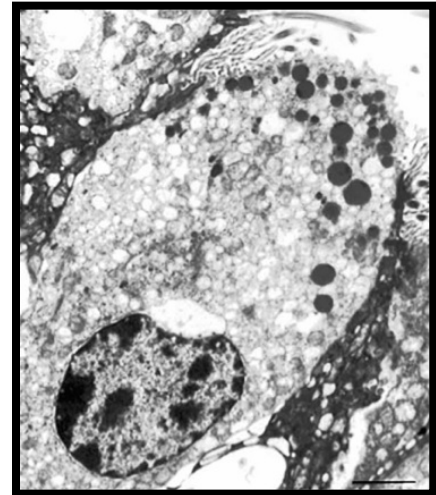
## ■ Key features of RAO

- Lower airway inflammation
- Hyperreactivity
- Bronchoconstriction



# Clara Cell Secretory Protein

- Produced by the main epithelial cell population in bronchioles
- CCSP
  - 2,800 bp genomic DNA sequence
  - 270 bp mRNA
  - 90 aa protein
    - Signal Peptide (19-21aa)
    - Mature Secretory Protein (70-77aa)
- Anti-inflammatory and immunosuppressive functions

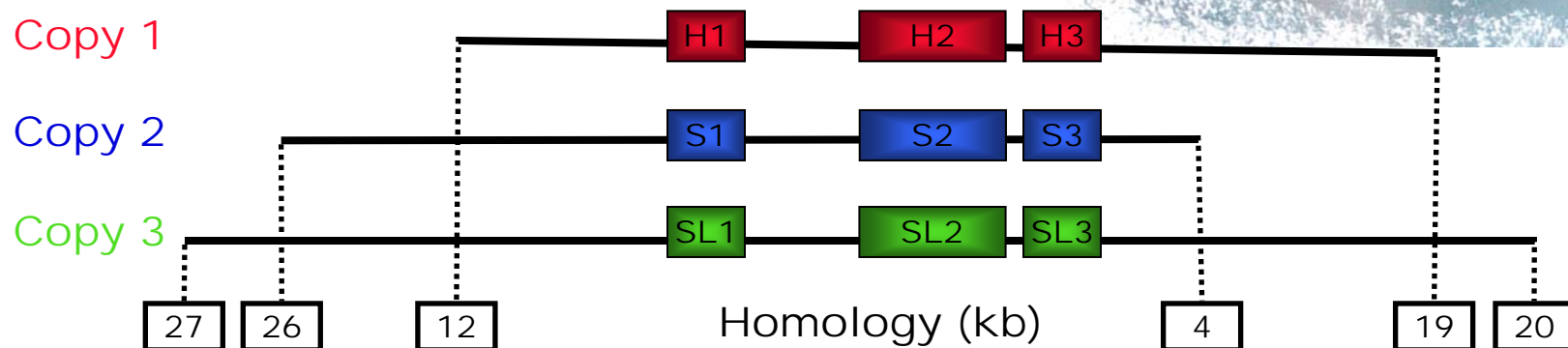


Vet Pathol 46:4, 2009

# Horse

## ■ Research Tools

- Equine genome sequenced
- Techniques for guided biopsies and samples collection
- Standardized RAO induction technique Am J Vet Res. 2010; 71(6):682-9.
  - Accessibility to large amount of material
  - Possibility of follow-up studies

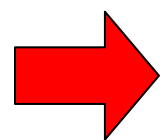


CCSP copies have high degrees of amino acid sequence identity

# Rationale

- Horses possess 3 copies of the CCSP gene
- All copies could be expressed concurrently in the lung
- Total CCSP expression levels differ between healthy and affected horses

# Hypothesis

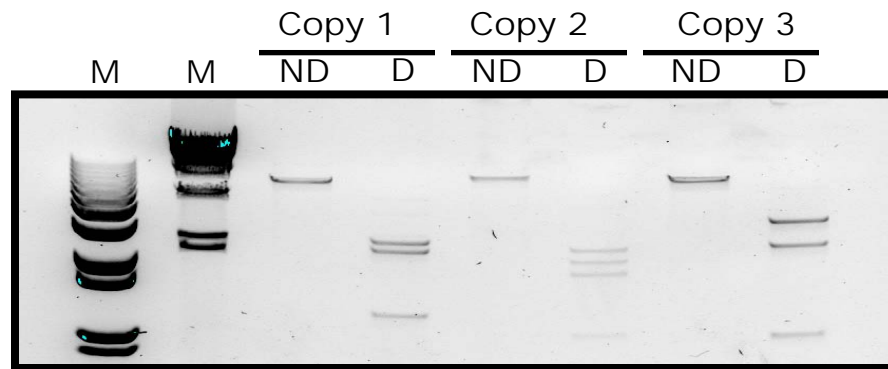
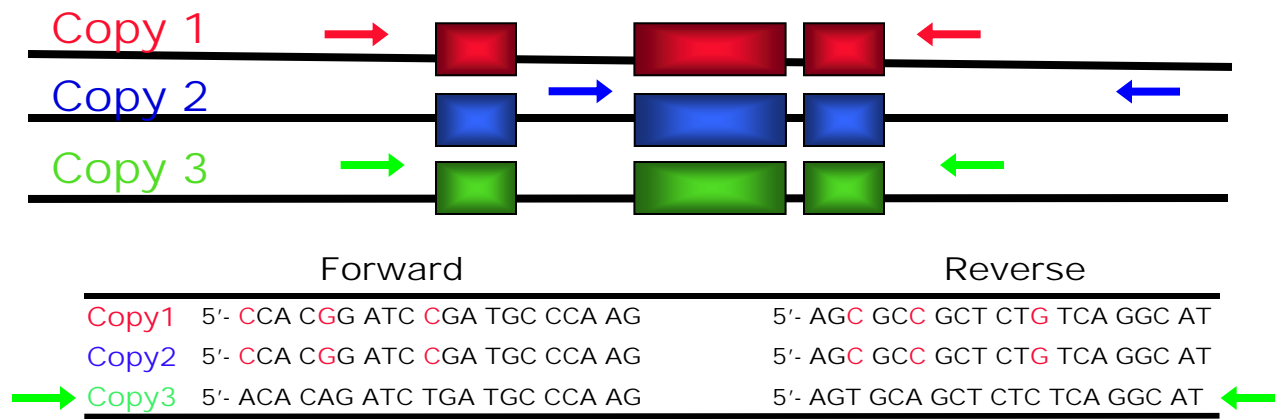


CCSP variants in the horse are differentially expressed and have distinct functions



# Sequencing Strategy

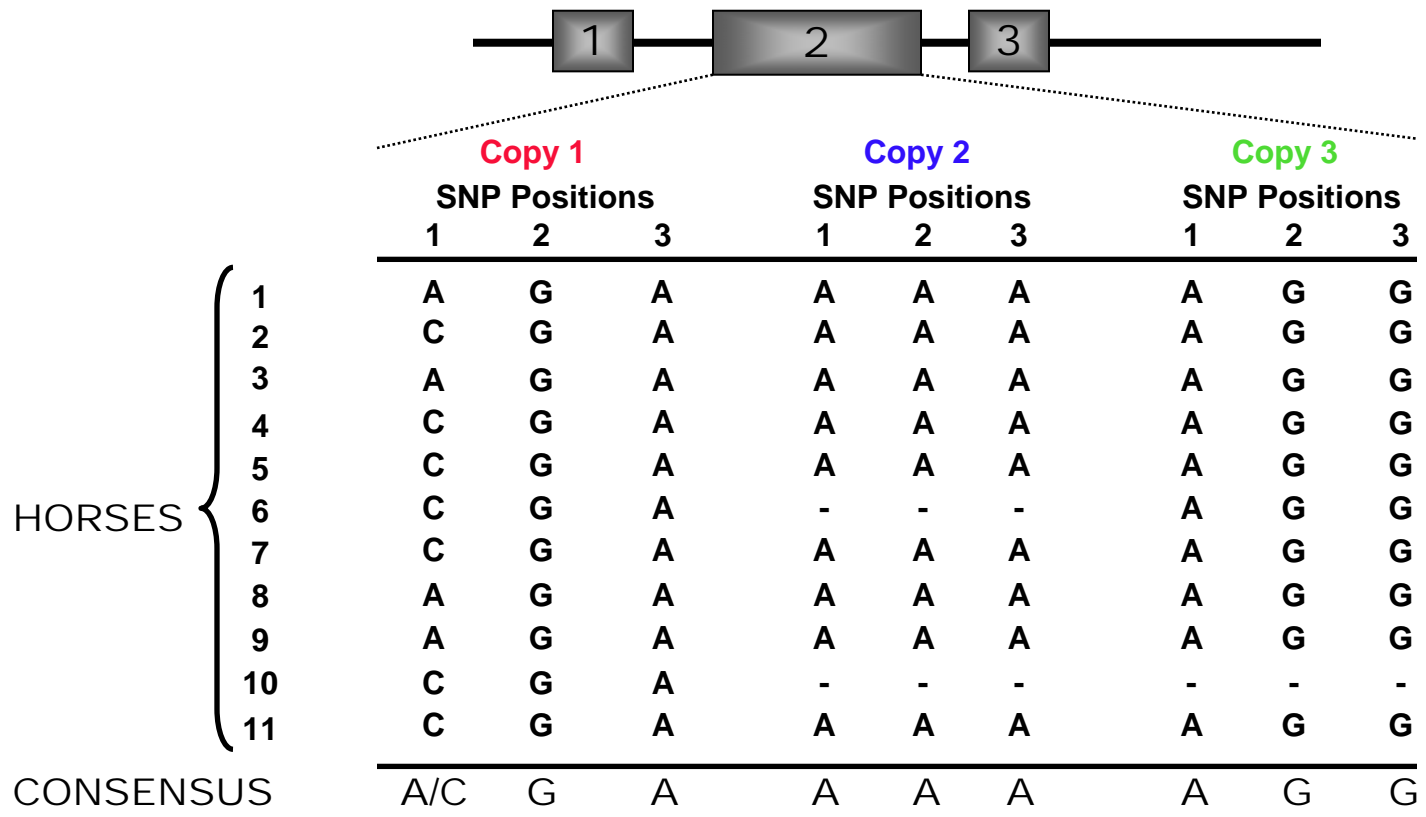
Isolation and characterization of individual CCSP copies





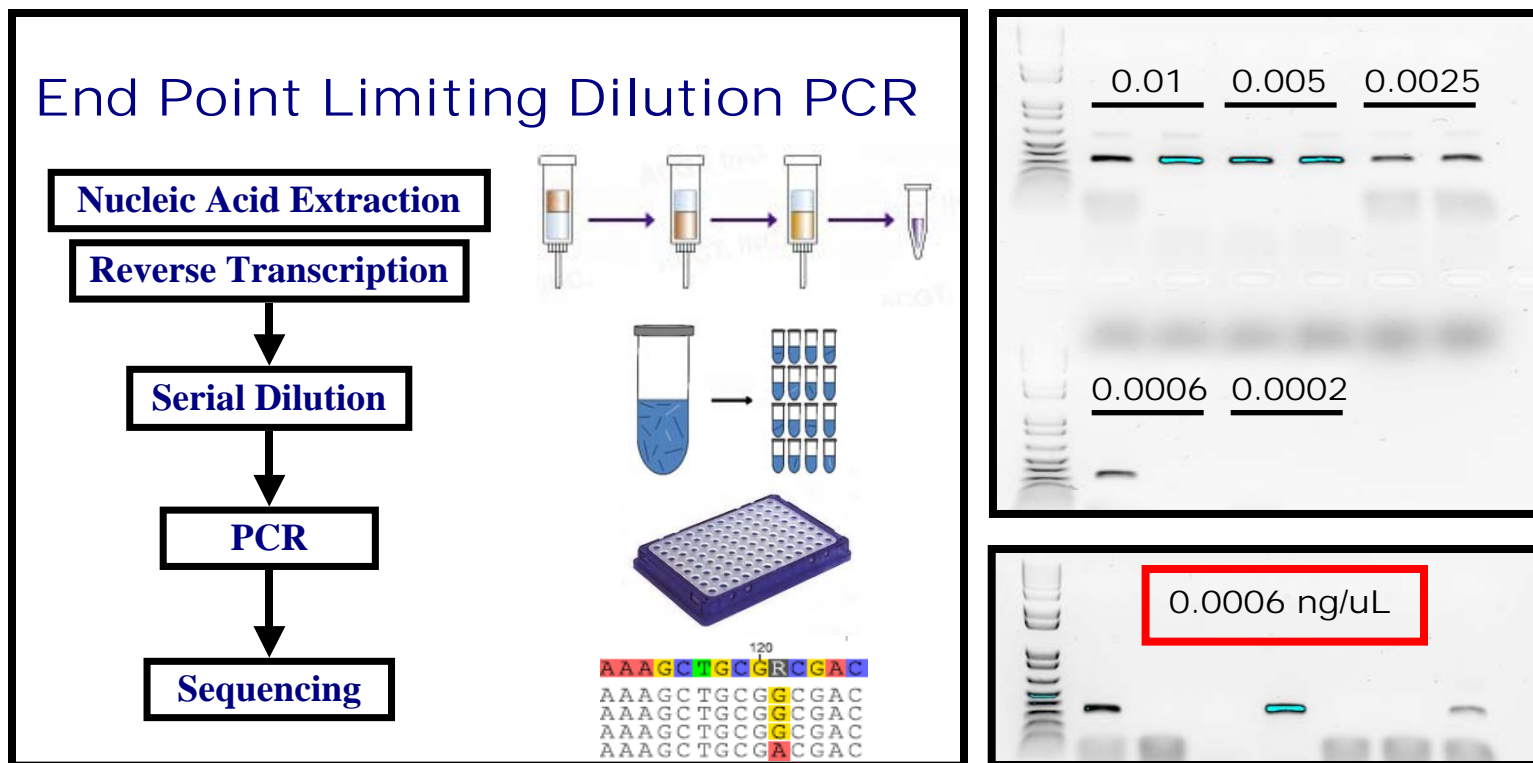
# Sequencing Results

Identification of copy-specific polymorphisms



# End-Point Limiting Dilution Assays

Amplification of cDNA single copy of CCSP

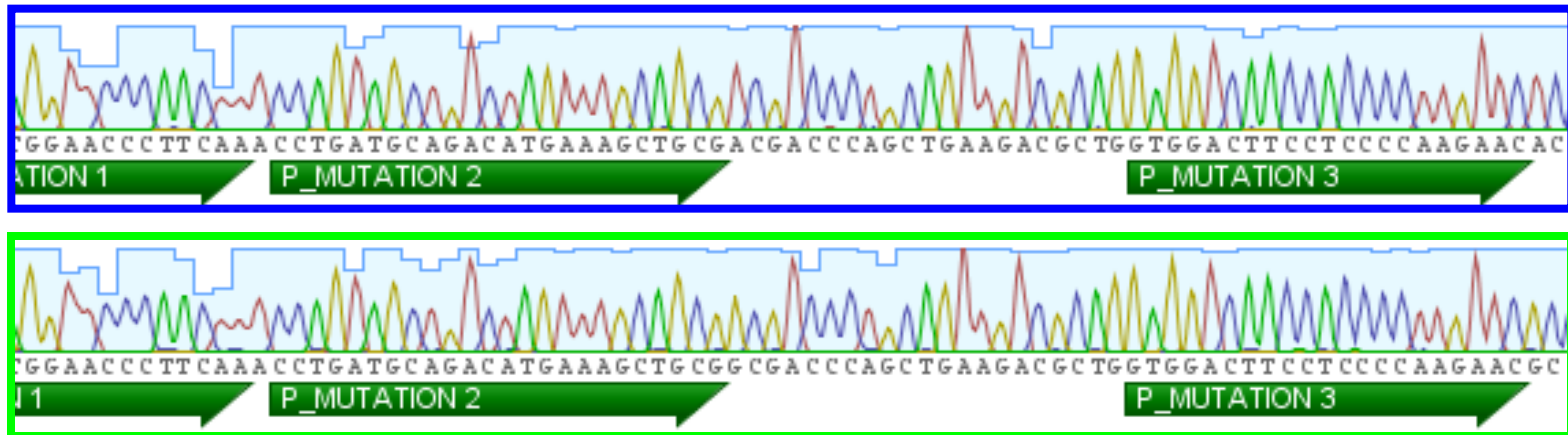


- Concentration where 2 out of 4 reactions show no PCR product
- Poisson distribution; the amplification occurs on a single template



# CCSP Expression

Detection of 2 CCSP copies expressed in uterine and lung tissues

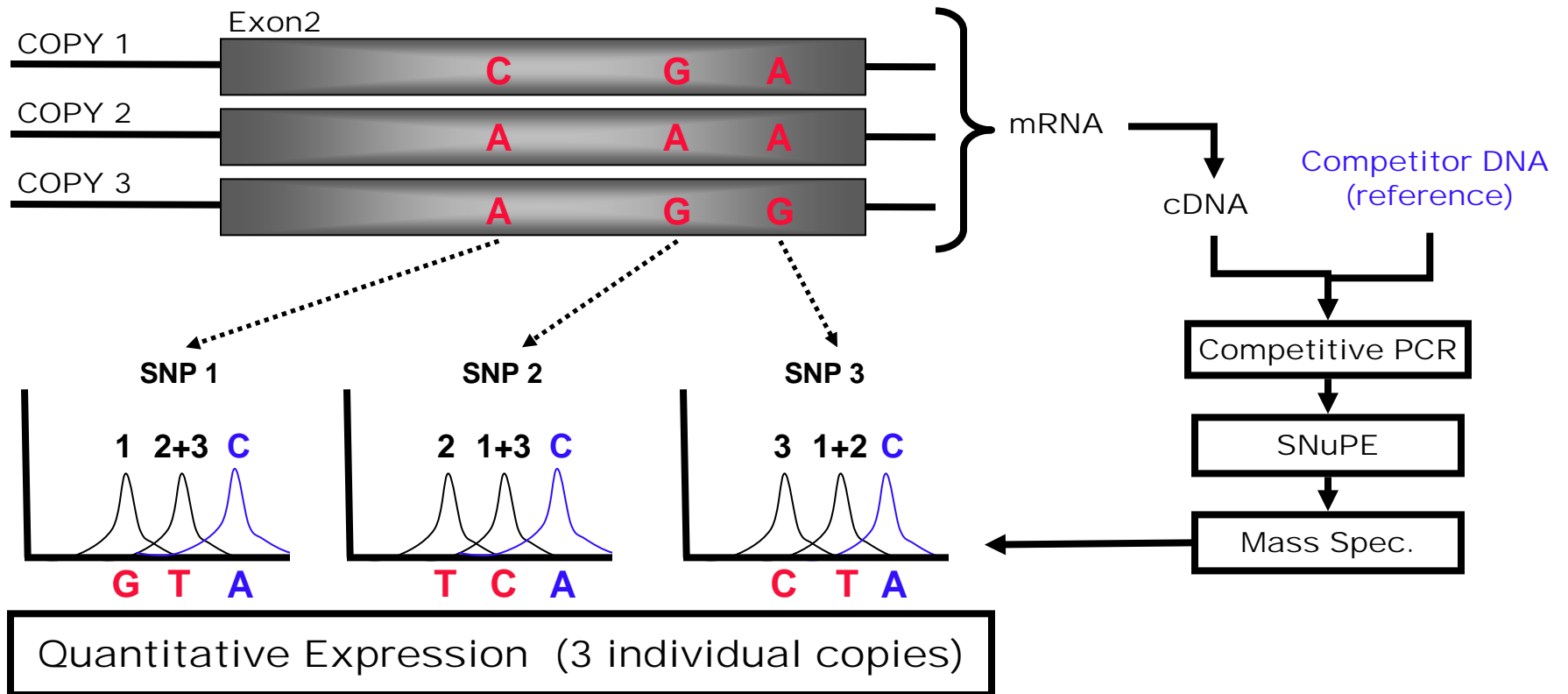


Uterus		Horse4		Horse5		Horse6	
AAA	8	57 %	10	59 %	9	53 %	
AGG	6	43 %	7	41 %	8	47 %	
Total	14	100 %	17	100 %	17	100 %	

Lung		Horse1		Horse2		Horse3	
AAA	11	31 %	18	55 %	4	13 %	
AGG	24	69 %	15	45 %	28	87 %	
Total	35	100 %	33	100 %	32	100 %	

# MassARRAY Experiments

Development of an assay to quantify CCSP expression



# Phylogenetic Analysis

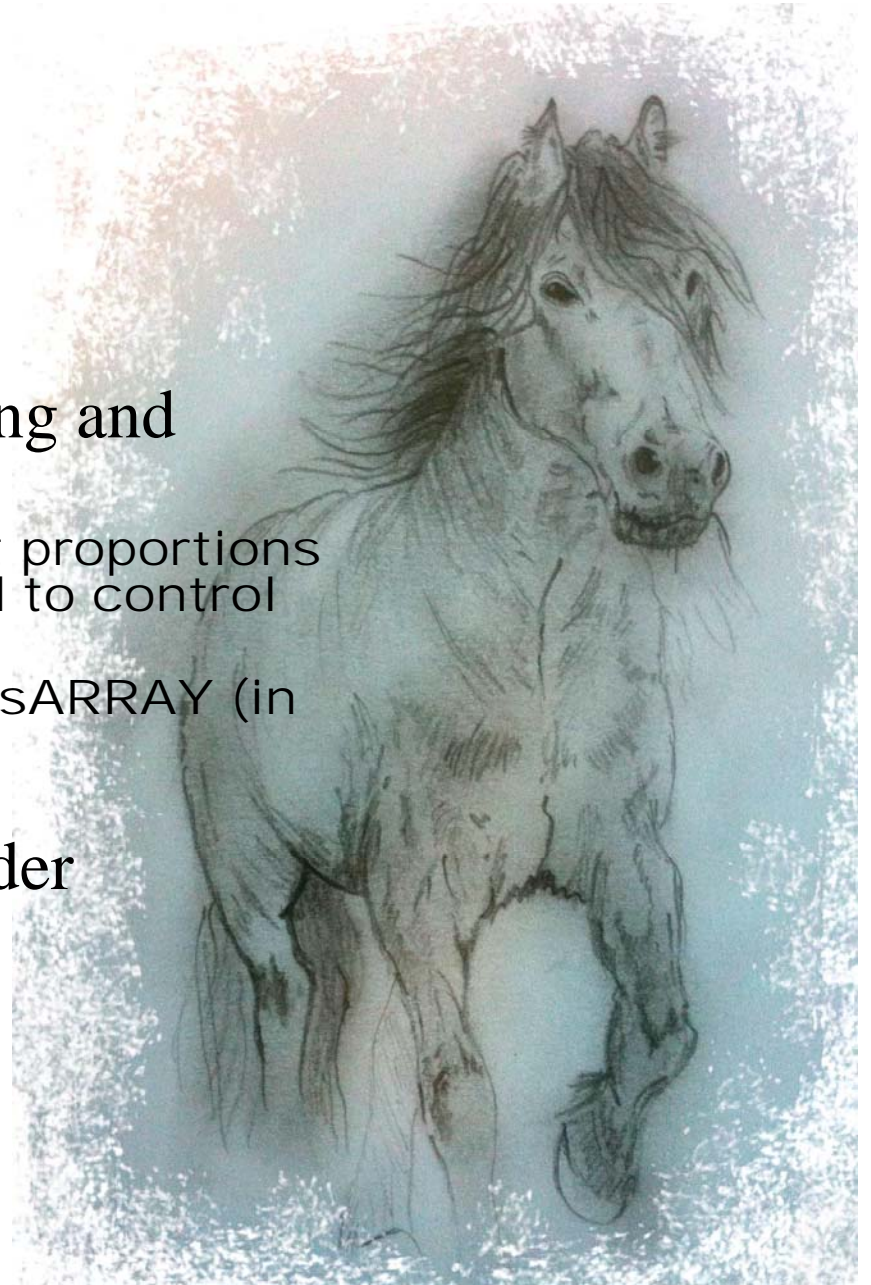
When did CCSP gene duplication or triplication occur in equids?

	SNP Position					
	1	2	3	1	2	3
Horse Copy 1	A	G	A	A/C	G	A
Horse Copy 2	A	A	A	A	A	A
Horse Copy 3	A	A	G	A	G	G
Donkey Copy 2	-	-	-	A	G	G
Donkey Copy 3	-	-	-	A	G	A
Donkey Genomic	-	-	-	A/C	A/G	A
Przewalskii's Horse Genomic	-	-	-	A	A/G	A/G
Zebra Genomic	-	-	-	C	G	A

Black	NCBI
Blue	Our Data

# Conclusion

- CCSP copies are polymorphic
- Two copies are expressed in lung and uterine tissues
  - RAO horses express different proportions of each CCSP copy compared to control horses
  - Need to be confirmed by MassARRAY (in progress)
- Origin of CCSP triplication under investigation



# Acknowledgment

- Dorothee Bienzle
- Mary Ellen Clark
- DNA samples, thanks to
  - Brandon Lillie's Lab
  - Metro Toronto Zoo





# Cloning Experiments

