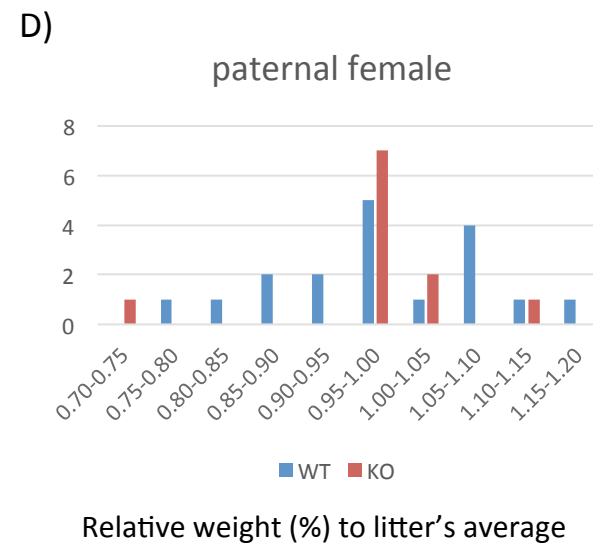
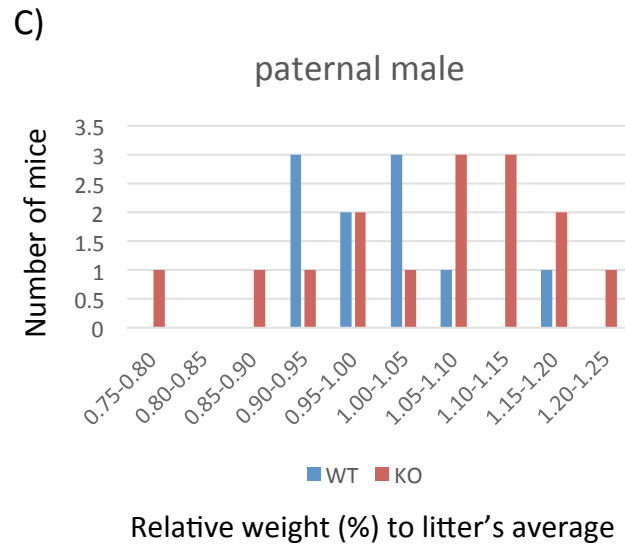
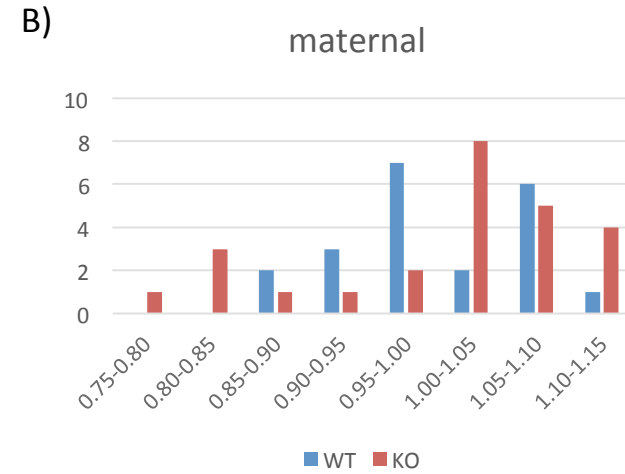
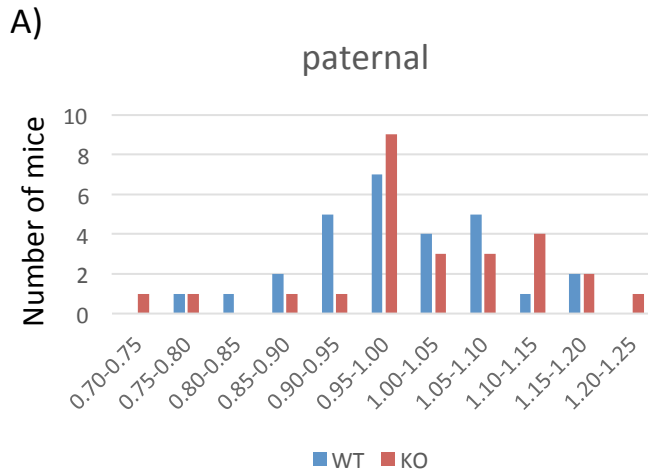


# YY1's roles in the Peg3 imprinted domain

Hongzhi He, An Ye, Bambarendage P.U. Perera, Joomyeong Kim

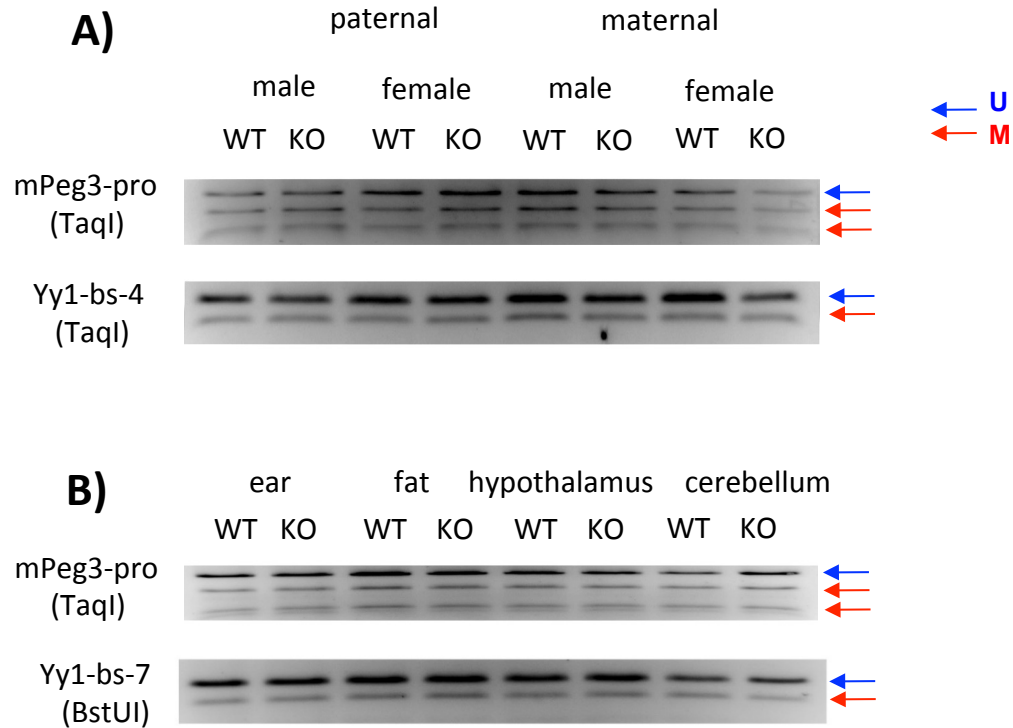
Department of Biological sciences, Louisiana State University,  
Baton Rouge, LA 70803, USA

# Hhe\_Supplemental\_material\_1



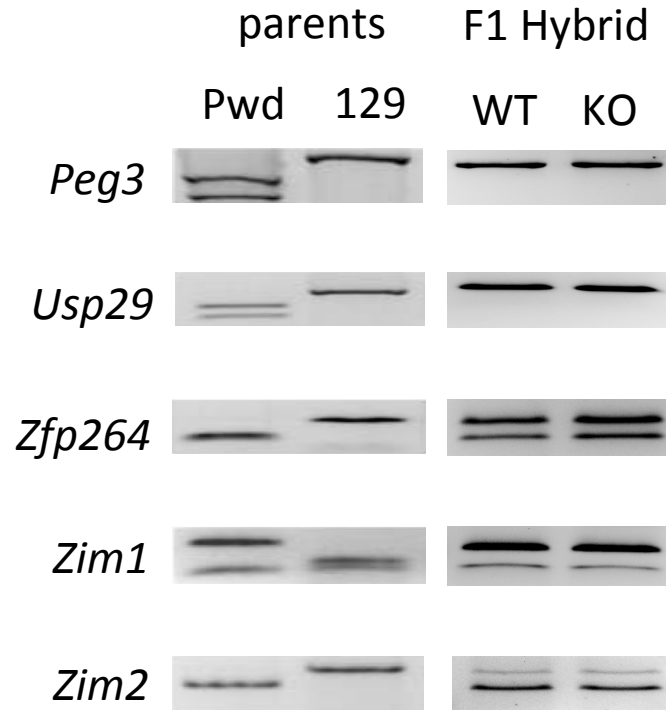
**Supplemental material 1.** Male and female heterozygotes were individually bred with their wild-type littermates for the paternal and maternal transmission of the mutant allele (**A,B**). The weight profiles of the pups with the paternal transmission of the mutant allele were divided based on their sexes, and subsequently re-analyzed (**C,D**).

## Hhe\_Supplemental\_material\_2



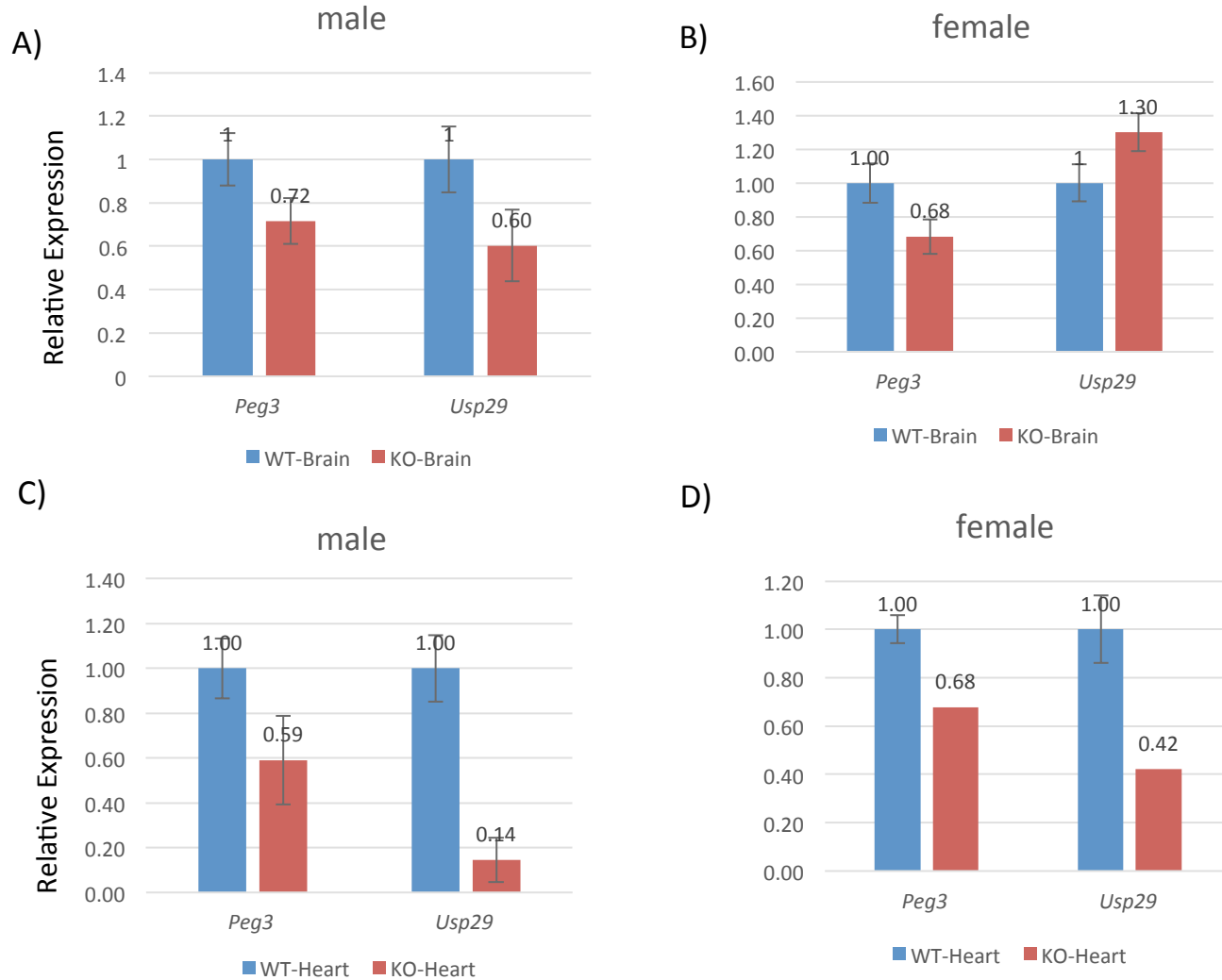
**Supplemental material 2.** DNA methylation analyses using the DNA isolated from the neonatal brains of wild type (WT) and knockout (KO) mice with the paternal and maternal transmission of the mutant allele (**A**). DNA methylation analyses using the tissues of adult mice with the maternal transmission of the mutant allele (**B**).

♀ Pwd X ♂ 129/B6



**Supplemental material 3.** The one-day-old heads of F1 hybrids from the crossing between the male KO of 129/B6 and the female breeders of Pwd were used for imprinting test. The digestion patterns for the 5 tested genes from the two parental alleles are shown on the two left columns, while the two right columns are the results from the F1 hybrids. The results indicated no major changes in the imprinting status of the 5 imprinted genes between WT and KO with the paternal transmission of the mutant allele.

# Hhe\_Supplemental\_material\_4



**Supplemental material 4.** Mutational effects on the expression levels of *Peg3* and *Usp29* in the brains and hearts of the 2-month-old mice with the paternal transmission of the mutant allele. The series of analyses were repeated with two independent sets of biological replicates.

**Supplementary Material 5. Primer sets used for imprinting test.**

Locus	Name	Sequence (5' -> 3')	Primer set	Size (bp)	Genbank No:Position	RFLP enzyme (cut vs uncut)
Peg3	Peg3-RT1-1a Peg3-RT-1b.3	GGTTCAGTGTGGGTGCACTAGACT TCCCTAGTGTGCATGATCTGGT	1st primer	1319	NM_008817:93-1411	BstUI (PWD vs 129)
Usp29	Usp29-RT-Pol-a Usp29-RT-Pol-b.1	GAGCCTGCAGCCGGACCGT GCTGCGGATCTGGACCAACCA	1st primer	329	NM_021323:972-1300	Tsp45I (PWD vs 129)
Zim1	Zim1-F1 Zim1-F2	GAATTCACACGGGAGTGAGA CTTGACCCGGTACTGGAGT	1st primer	539	NM_011769:1357-1895	DraI (PWD vs 129)
Zim2	Zim2-17 Zim2-19	GCTCAGGACCTGCGCTTTCAG GGGCTGCCAAGATCAATGCTG	1st primer	538	AF401983:57-594	BstUI (PWD vs 129)
Zim3	Zim3-RT-Pol-a Zim3-RT-Pol-b.1	CCCTTACTGACCTAGGGCTTG AGTGACGCTCTCAAGGTCACT	1st primer	269	NR_036631.2 :101-369	EcoRV (129 vs PWD)
Zfp264	Zfp264-RT-Pol-b.1 Zfp264-RT-Pol-b	CTGGGCATGAAAAGATCCACT CCACATTTGTTGCACTGGTGGAT	1st primer	229	AF365933:404-632	BstUI (PWD vs 129)
Apeg3	APeg3-R APeg3-F	GCACCAAGTGCAGGTGGTGCAGGA CAATCAGTCTCAAGGGGTCTGGGT	1st primer	865	NR_023846:1-865	XhoI (PWD vs 129)

**Supplementary Table 2. Primer sets used for the bisulfite sequencing and COBRA.**

Locus	Name	Sequence (5' -> 3')	Primer set	Size (bp)	*Position (mm10, NCBI Build 38)
Peg3-pro	Peg3-pro-a	AATAGTAGTTTGGTGGTGGGGA	1st primer	380	chr7: 6730175-6730555
	Peg3-pro-b	CACCCAAACACCATCTAACTCACAAC	2nd primer (Nested)	292	chr7: 6730175-6730467
	Peg3-pro-a.1 Peg3-pro-b	GTTTTGTAGAGGATTTGATAAGGAG CACCCAAACACCATCTAACTCACAAC			
Yy1-bs-2	Yy1-15	AGGAAGAGTTAGAGGAGTTAGTTTTATAGA	1st primer	509	chr7: 6729374-6729883
	Yy1-16.1	CCTATTACAAAACCAACAATAAACATCA	2nd primer (Nested)	345	chr7: 6729538-6683362
	Yy1-15.2 Yy1-16.bis2 (for KO) Yy1-16.1 (for WT)	TAGGTAGTTAATTAGGATAAGTTGTGTAG TTCTATCTTTCTAAAAATAAAAACTTC CCTATTACAAAACCAACAATAAACATCA			
Yy1-bs-4	Yy1-19	TTTTATGGGTTTGTGAAGTTTTAG	1st primer	216	chr7: 6728371-6728587
	Yy1-22	CAAATCCCAACCCCAACCACTATC	2nd primer (Nested)	204	chr7: 6728383-6728587
	Yy1-19 Yy1-20	TTTTATGGGTTTGTGAAGTTTTAG CCAACCACTATCATTCAAATA			
Yy1-bs-7	Yy1-25	TTTTTTTGTAGTGATTGGGTTATAGAAGTT	1st primer	358	chr7: 6727253-6727611
	Yy1-26	ATAACACATCCACAATAAAAAATCAAACA	2nd primer (Nested)	346	chr7: 6727265-6727611
	Yy1-25 Yy1-26.bis (for KO) Yy1-26.1 (for WT)	TTTTTTTGTAGTGATTGGGTTATAGAAGTT CAATAAAAAATCAAACCAAAATCCATAACTTC CAATAAAAAATCAAACCCCTTATAAC			
H19-ICR	H19-ICR-BF3	ATAGATGGTGATAGGGGAGAAAATTTA	1st primer	402	chr7: 142581719-142582121
	H19-ICR-BR3-nes H19-ICR-BF3	AAATTCTACAAAAAACCATACCTATTCTT AGATGGTGATAGGGGAGAAAATTTAATTAGTTGT	2nd primer (Nested)	398	chr7: 142581721-142582119
	H19-ICR-BR3-nes	ATTCTACAAAAAACCATACCTATTCTTAAAC			

**Supplementary Table 3. Primer sets used for qRT-PCR experiments.**

Locus	Name	Sequence (5' -> 3')	Primer set	Size (bp)	*Position (mm9, NCBI Build 37)
Peg3	Peg3-RT-1a Peg3-RT-1b	GGTTCAGTGTGGGTGCACTAGACT GCTCACACCAAGGGCTTGAGCG	1st primer	222	chr7:6,671,201-6,683,038
Usp29	Usp29-RT-1a Usp29-RT-1c	GAGGAGAGCAAGCAGGTAGATTAC GTTGAAATGGGGAGTAGGGTGA	1st primer	545	chr7:6683856-6690299
Zim1	Zim1-RT-c Zim1-RT-d	GATCACCAGGTTGGAGCAAGGAGT AGCGCTCTGTGGTGTGTAGTTG	1st primer	305	chr7:6630678-6634867
Zim2	Zim2-17 Zim2-RT-19	GCTCAGGACCTGCGCTTTCAG GGGCTGCCAAGATCAATGCTG	1st primer	211	chr7:6605023-6611658
Zim3	Zim3-RT-a Zim3-RT-b	GCCAGGCATAGCGGTACTTGCCT CAGTCATCTGTCACTCACAGA	1st primer	438	chr7:6925908-6929182
Zfp264	Zfp264-RT-Pol-b Zfp264-RT-Pol-b.1	CTGGGCATGAAAAGATCCACT CCACATTTGTTGCACTGGTGGAT	1st primer	337	chr7:6941897-6942125
Actin (beta)	bActin-1a bActin-1b	GAGCACCTGTGCTGCTCACCGA CTCTTTGATGTCACGCACGATTC	1st primer	345	chr5:143,666,183-143,666,981

**Supplementary Table 4. Primer sets used for CHIP Assay**

Locus	Sequence (5' -> 3')	Sequence (5' -> 3')	Primer set	Size (bp)	*Position (mm9, NCBI Build 37)
Peg3-DMR	YY1-01a YY1-01b	GTCCTGTTACAAGACCACAC CTGGGTGGAGCCGAAAGTATC	1st primer	284	<a href="#">chr7:6682313-6682596</a>
Peg3-DMR	YY1-23a YY1-23b	AAAGATGCACCCAGTTGTC CGGGCGACATTTTAACTC	1st primer	270	<a href="#">chr7:6681801-6682070</a>
Peg3-DMR	YY1-34a YY1-34b	CTACCCTGACGCCATCTTTG GATGACACCCGCTGAGAAT	1st primer	205	<a href="#">chr7:6681263-6681467</a>
Peg3-DMR	YY1-45a YY1-45b	TCACTGAAGGTGCCATTTTG CAGAGCTCCTGCTCATTCT	1st primer	300	<a href="#">chr7:6680693-6680992</a>
Peg3-DMR	YY1-56a YY1-56b	CAGTAGAGGCAGGCAAGAGG ACTGGCGGAATGAATACCAAG	1st primer	250	<a href="#">chr7:6680232-6680481</a>

Original images for Fig.3A

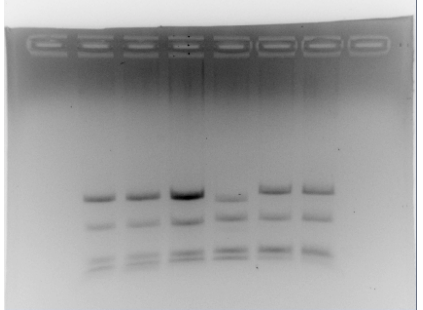
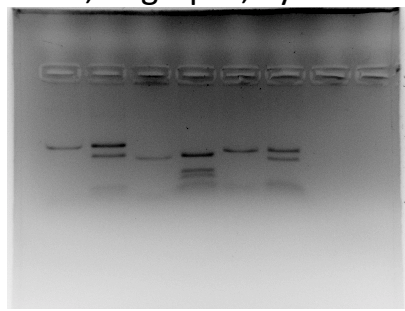
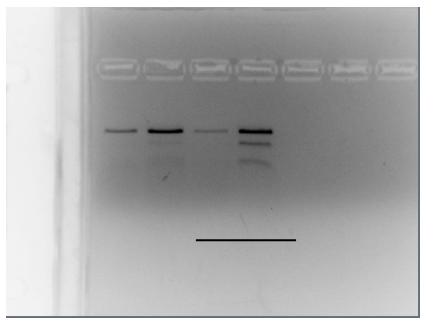
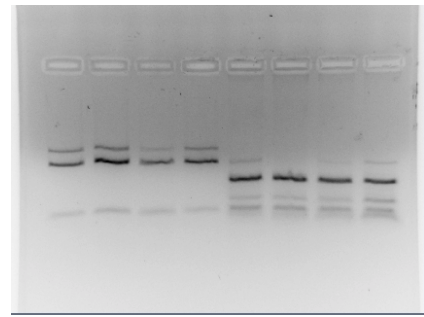
Original images for Fig.5C

Sperm  
H19 ClaI & mPeg3-pro FokI

Sperm (6month)  
Yy1-bs-7 (TaqI)

Somatic DNA  
H19, Peg3-pro, Yy1-bs-2

MEFs COBRA  
mPeg3-pro & Yy1-bis-7

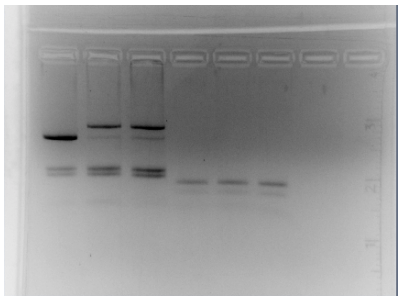
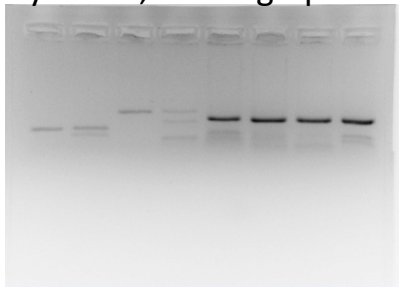
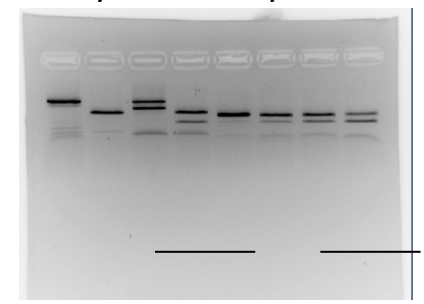
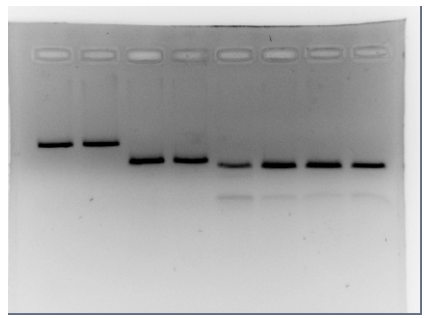


Sperm  
Yy1-bs-2 & Yy1-bs-4

Egg  
Yy1-bs-2 & Yy1-bs-4

Somatic DNA Sperm  
Yy1-bs-4, 7 & Peg3-pro TaqI

MEFs COBRA  
Yy1-bs-2 & Yy1-bs-4

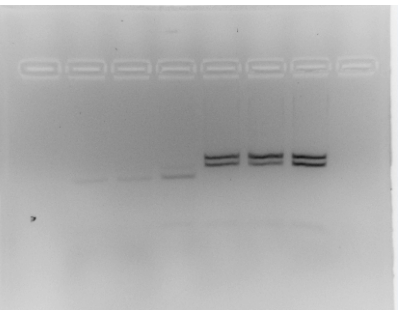
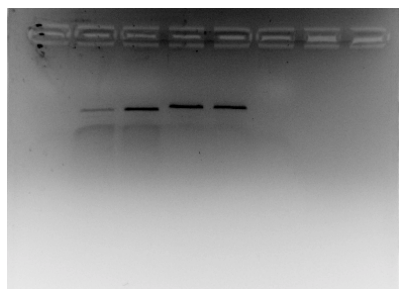
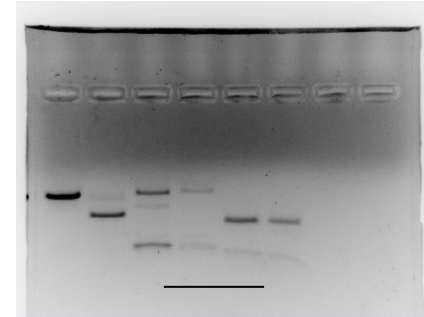
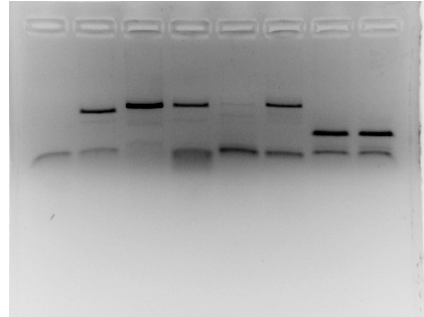


Sperm Egg  
Yy1-bs-7 H19 & mPeg3-pro

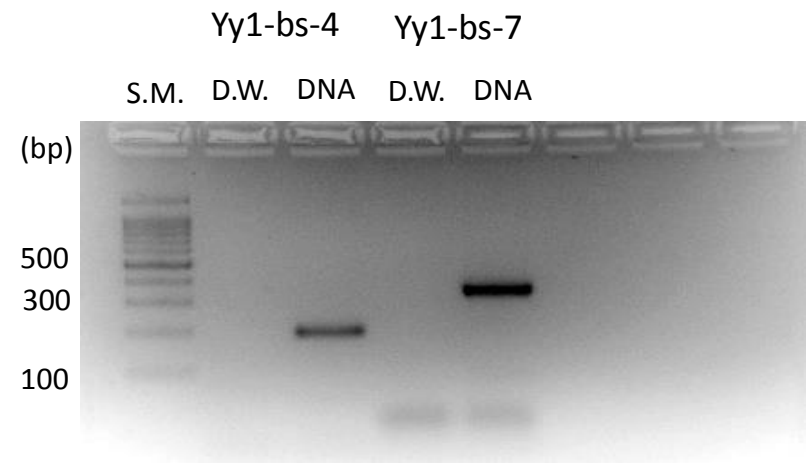
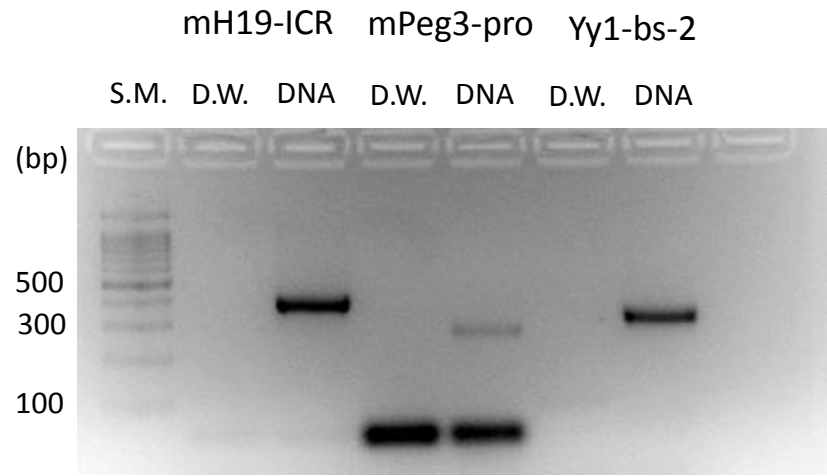
Egg  
Yy1-bs-4 (BstUI)- middle set

Sperm  
Yy1-bs-7 new one

MEFs COBRA  
mZim2 & Zfp264



Control PCR reactions for bisulfite-converted DNA



Control COBRA experiments

S.M.: Size Marker  
D.W.: Distilled Water

