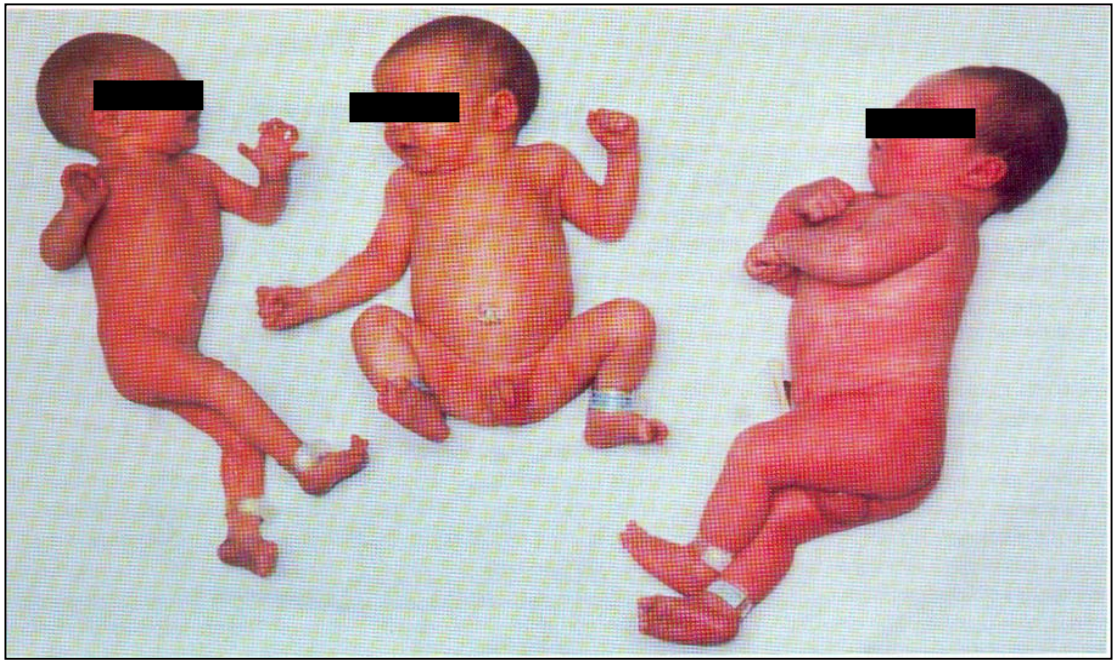
Dr. David Monk

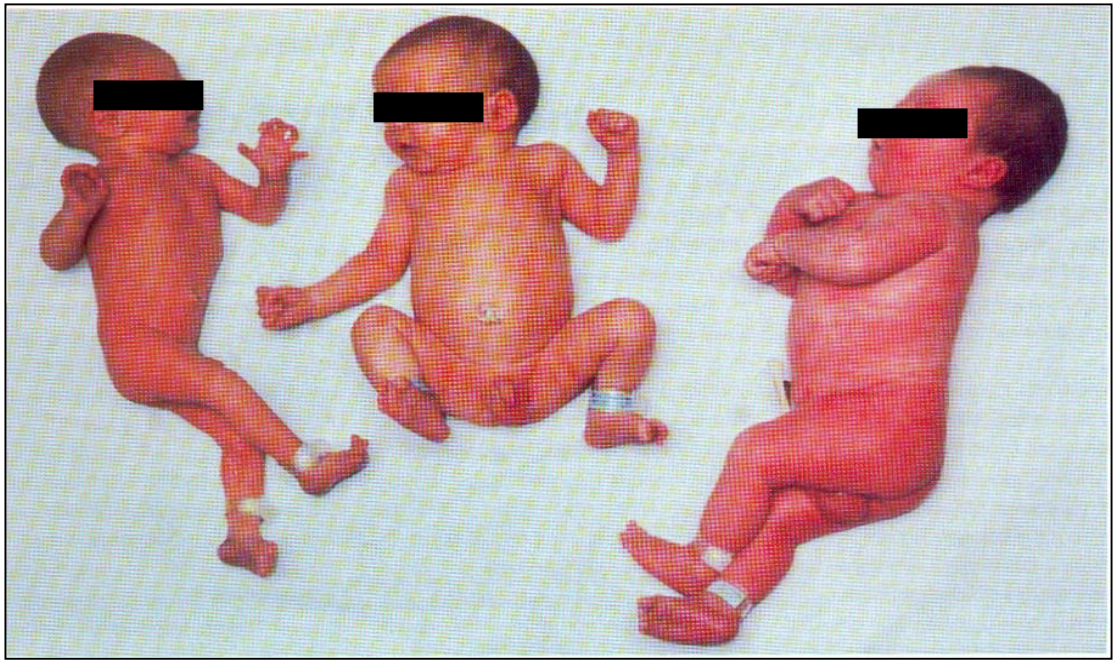
Hosted By:
U.S. Department of Health and Human Services
National Institutes of Health

Maternal uniparental disomy 7, Silver-Russell syndrome and imprinted candidate genes

Dr David Monk
IRDB
Imperial College London



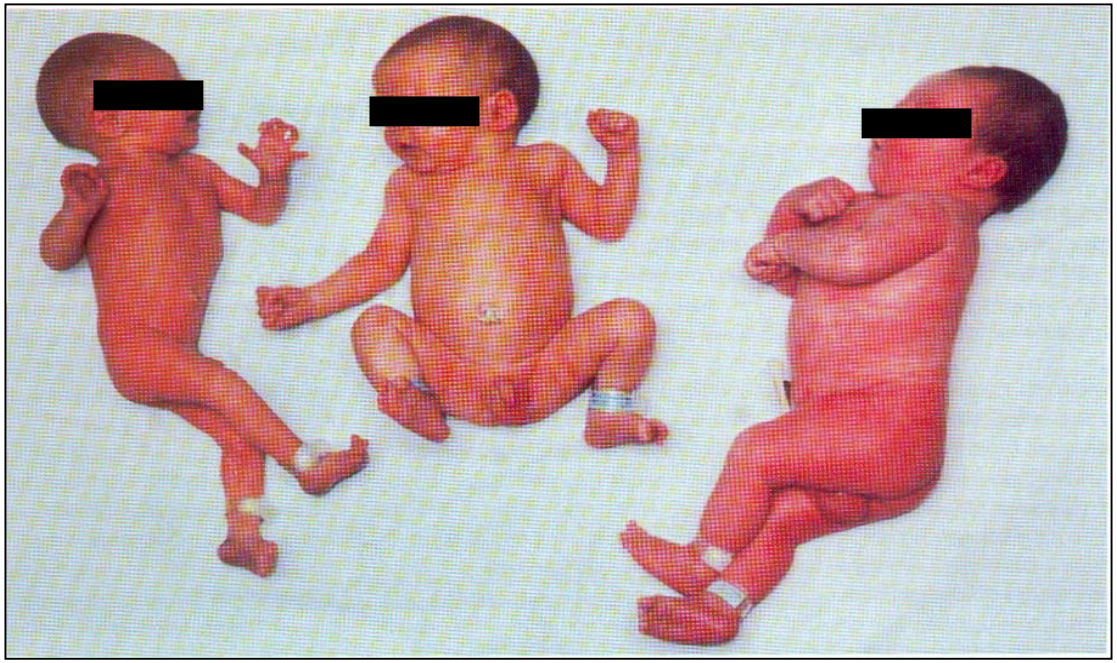
An average day in U.S:
11,120 babies born



An average day in U.S:

11,120 babies born

1,280 babies born preterm



An average day in U.S:

11,120 babies born

1,280 babies born preterm

841 babies born with low birth weight (> 2.5 kg)

159 babies born with very low birth weight (>1.5 kg)

From www.marchofdimes.com/peristats

Babies born with IUGR may have immediate problems such as:
Asphyxia
Hypoglycemia
Hypothermia
Neonatal jaundice
Neurological delay

And later in life……..
Increased risk of heart disease
Hypertension
Diabetes

Fetal growth is a complex process influenced by maternal, placental and fetal factors

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The aetiology of IUGR is multifactorial, involving both genetic and environmental factors

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The aetiology of IUGR is multifactorial, involving both genetic and environmental factors

Common causes of IUGR are congenital abnormalities; inadequate maternal-fetal circulation; and an idiopathic group

Within the idiopathic group will be a subset of babies which have genetic factors, such as aberrant genomic imprinting

A genetic model for IUGR:

Silver-Russell syndrome

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Silver-Russell syndrome

IUGR
Postnatal growth restriction

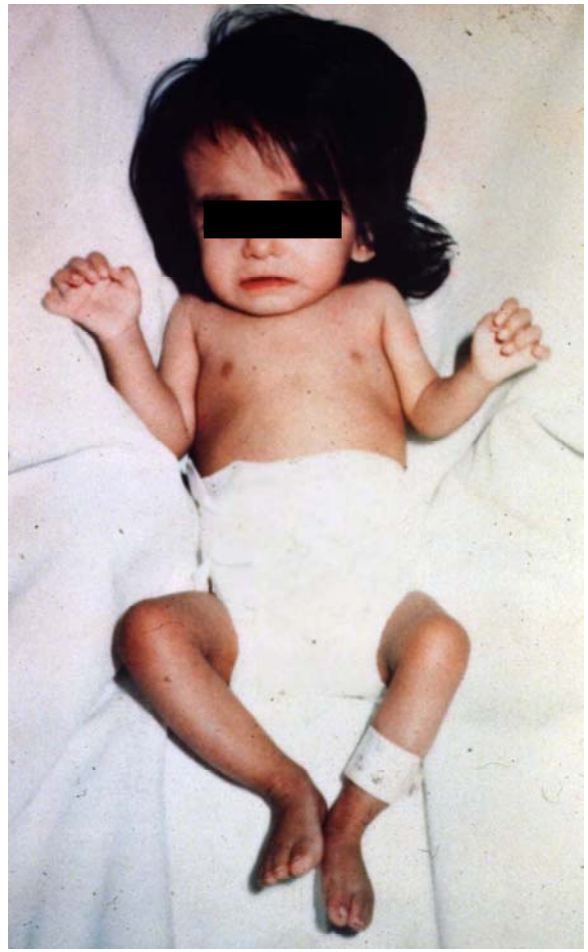
Therefore and excellent model for investigating the molecular mechanisms underlying fetal growth restriction

Silver-Russell Syndrome

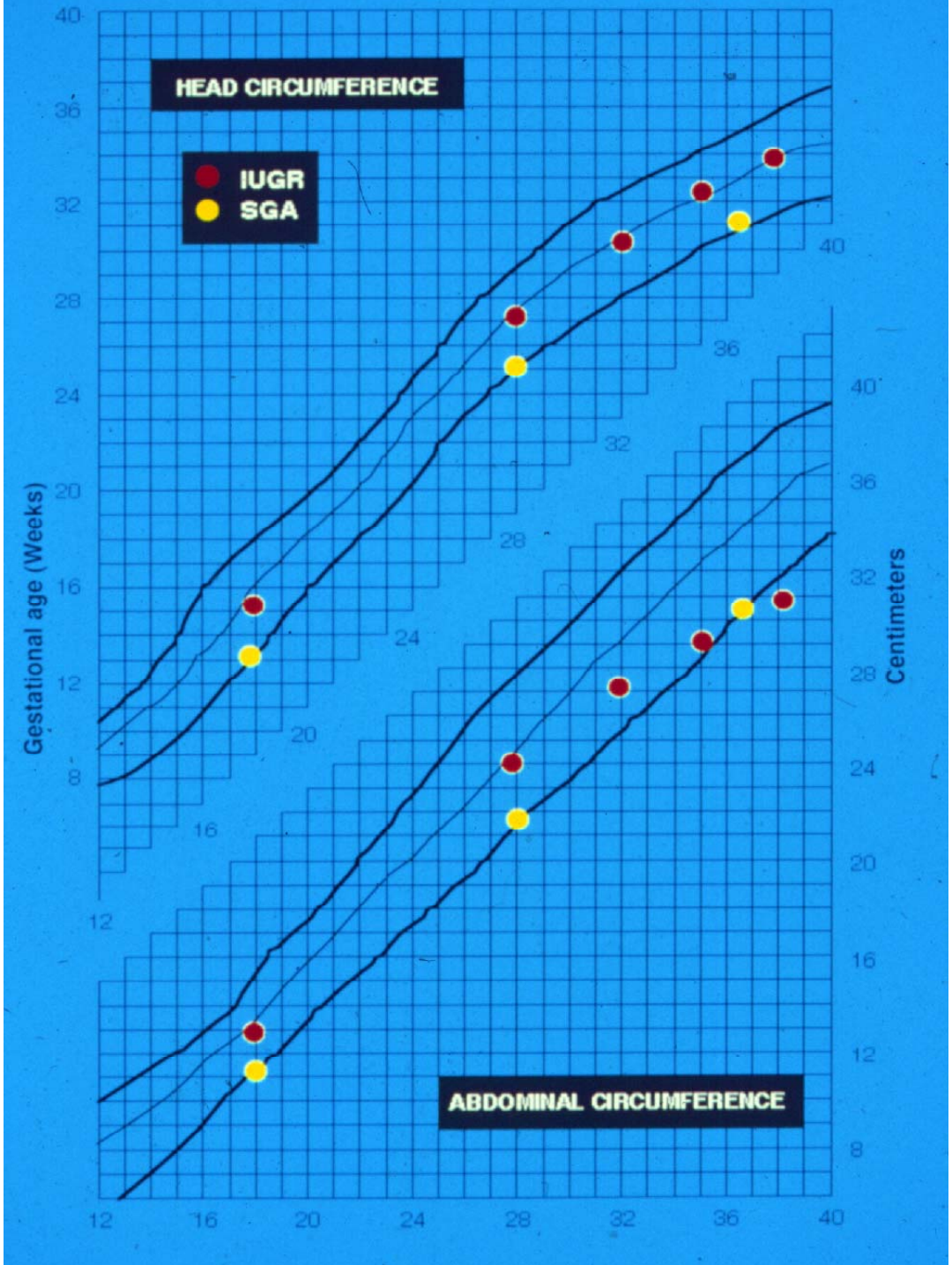
Main clinical features for diagnosis:

- Intrauterine growth restriction (IUGR) (<2 SD below mean)
- Short stature (<2 SD below mean)
- Characteristic face
- Asymmetry
- Fifth finger clinodactyly

+ other confirmatory features



Price et al., 1999


HEAD CIRCUMFERENCE
IUGR
SGA
ABDOMINAL CIRCUMFERENCE
Gestational age (Weeks)
Centimeters
40
36
32
28
24
20
16
12
8
12
16
20
24
28
32
36
40

Genetically heterogeneous; no clearly established Mendelian inheritance pattern. However, several pedigrees show skewed penetrance towards female transmission (Duncan et al., 1990)

Structural chromosomal abnormalities; disruptions provide clues to location of genes involved in SRS by aiding positional cloning approach

Human chromosome 7 and genomic imprinting

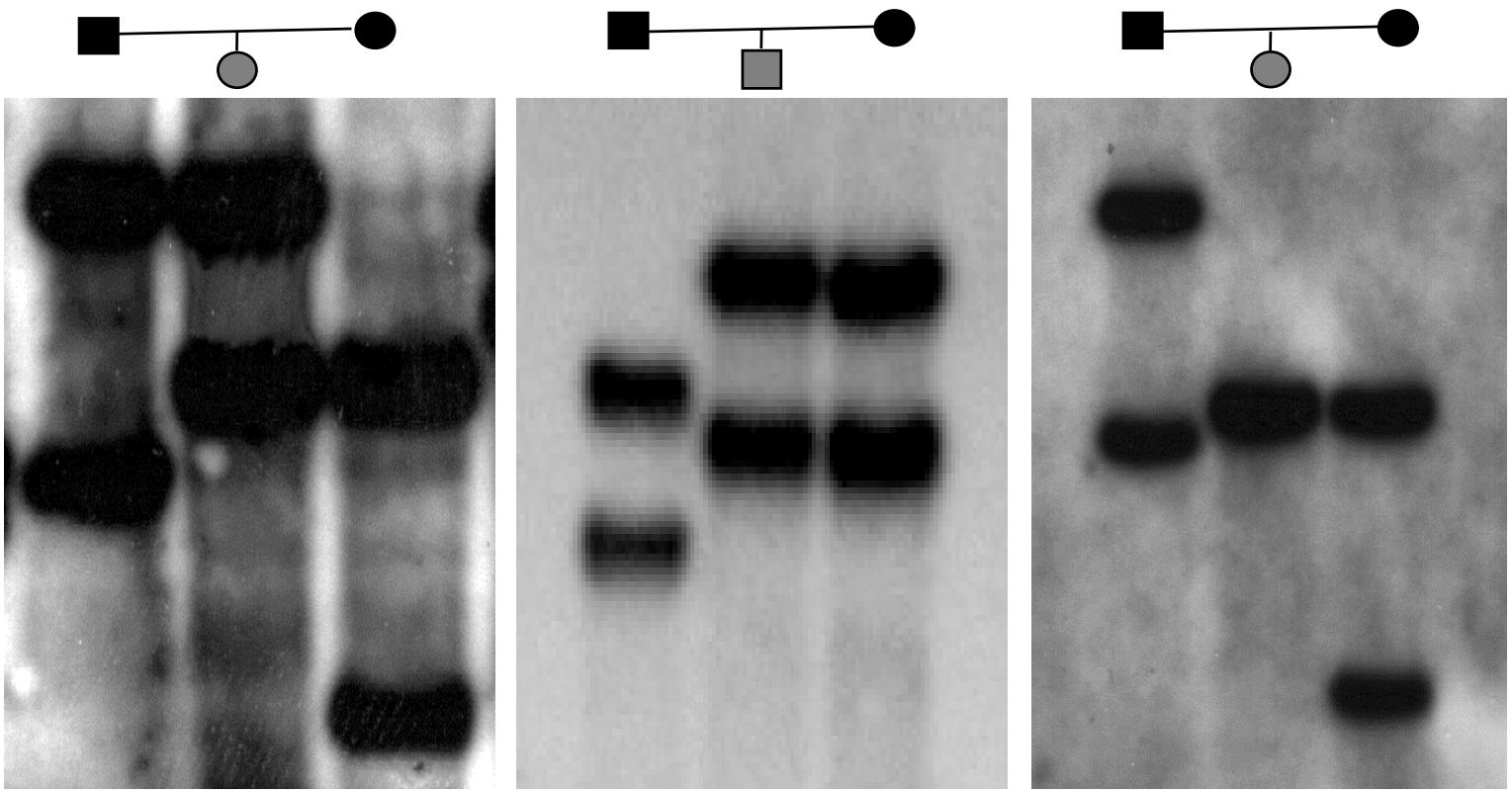
mUPD7 in 7-10% of SRS cases
(Kotzot et al., 1995; Preece et al., 1997; Eggermann et al., 1997)

Both hetero- and isodisomic cases reported

Mixed iso-heterodisomy observed with no common isodisomic interval in five patients. This indicates that an imprinting effect opposed to unmasking of mutant recessive allele cause the phenotype
(Preece et al., 1999)

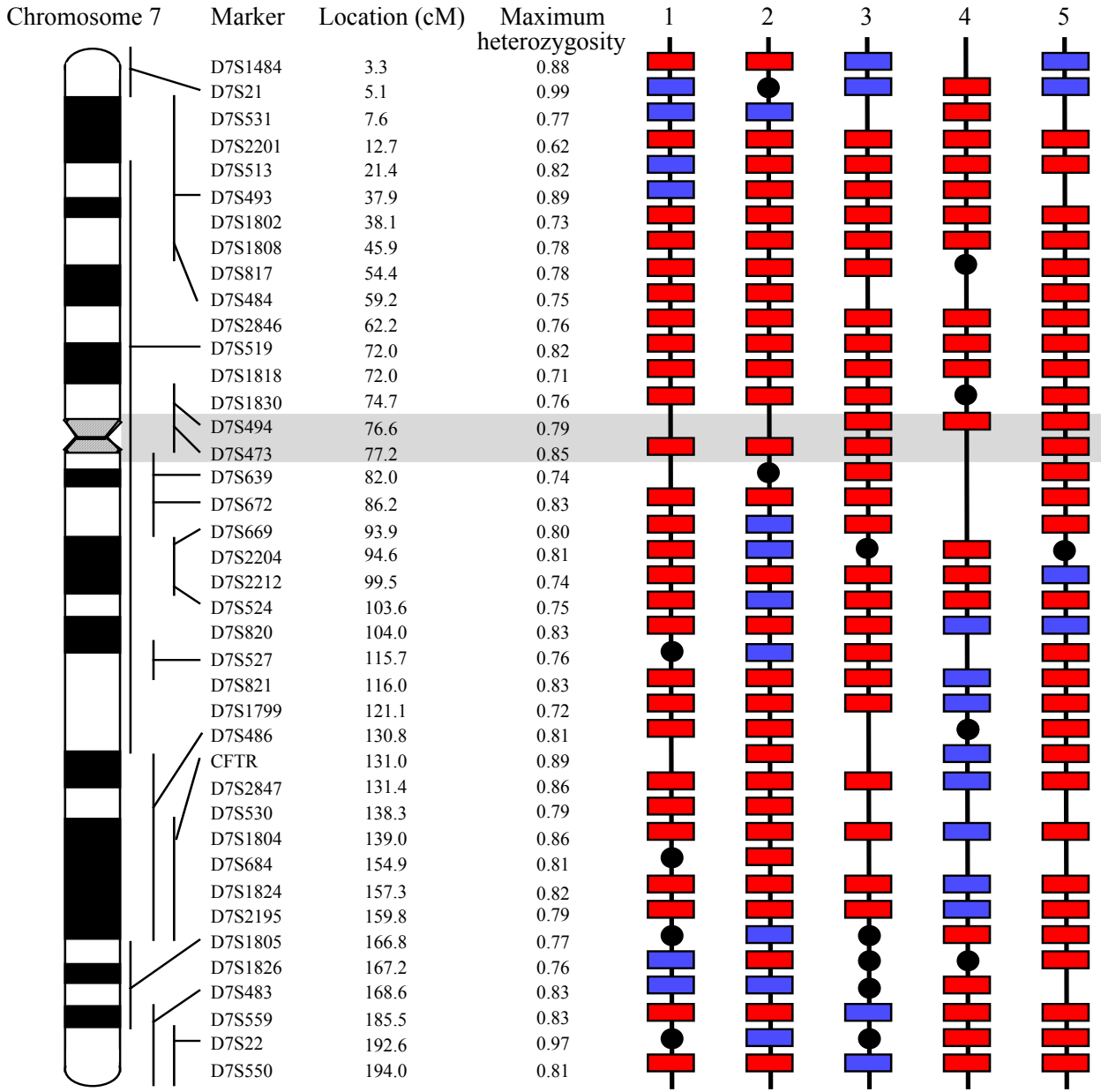
Lack of paternal expression could be associated with the SRS phenotype, as could the maternal duplication of a gene involved in growth inhibition

mUPD7



Normal Heterodisomy Isodisomy

F= father
P= proband
M= mother



Preece et al., 1999

A milder mUPD7 SRS phenotype?

Careful dissection of the SRS phenotype suggests that some mUPD7 cases have a milder phenotypes (Price et al., 1999; Kotzot et al., 2000; Hannula et al., 2000)

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mUPD7 patients consistently lack classic feature:

Triangular face

Digit abnormalities- clinodactyly

- brachydactyly

- syndactyly

Down turned corners to the mouth

Characteristic	Hannula mUPD7 (4)	Reported mUPD7 (20)	Non-mUPD7
IUGR	100	58	79
Growth restriction (> -2.5 SD)	100	100	99
Asymmetry	100	37.4	39.8
Relative macrocephaly	100	70.7	76
Motor developmental delay	50	20.8	34
Speech delay	100	24.9	21
Excessive sweating	100	24.9	36
Feeding difficulties	100	33	46
Triangular face	0	29	86
Down turned corners of mouth	0	0	60
Clinodactyly	50	37	76

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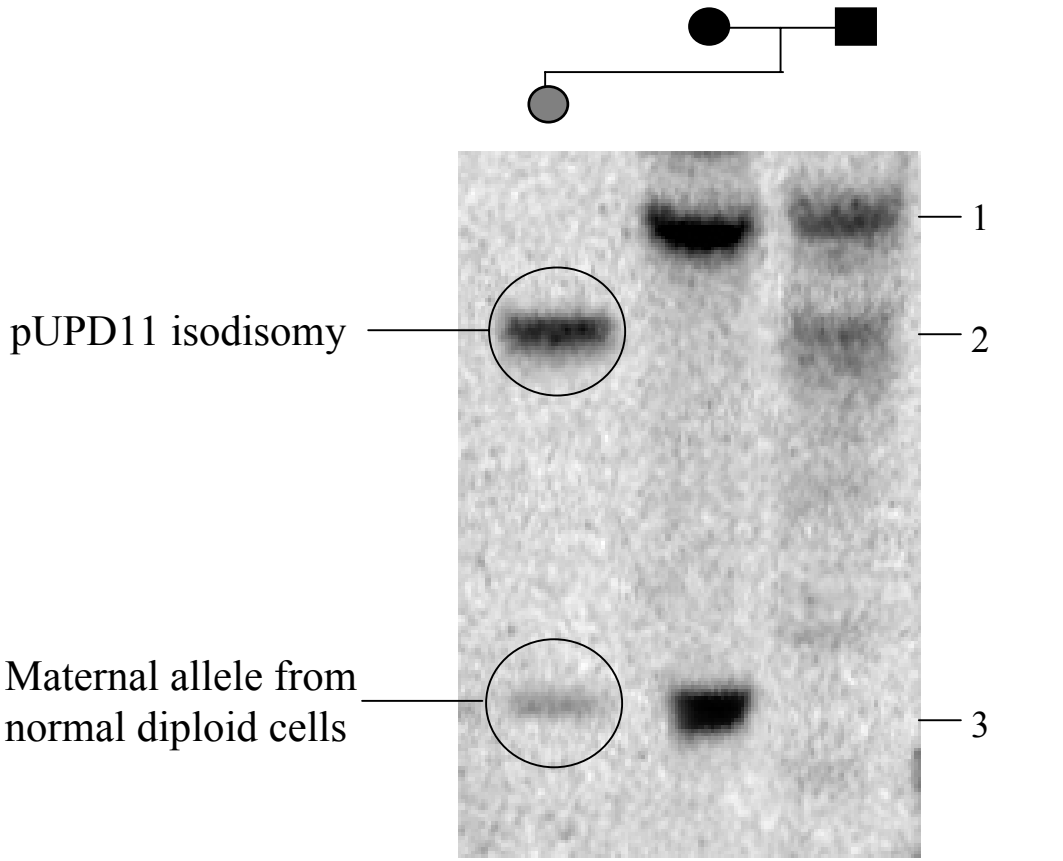
Mosaicism in SRS?

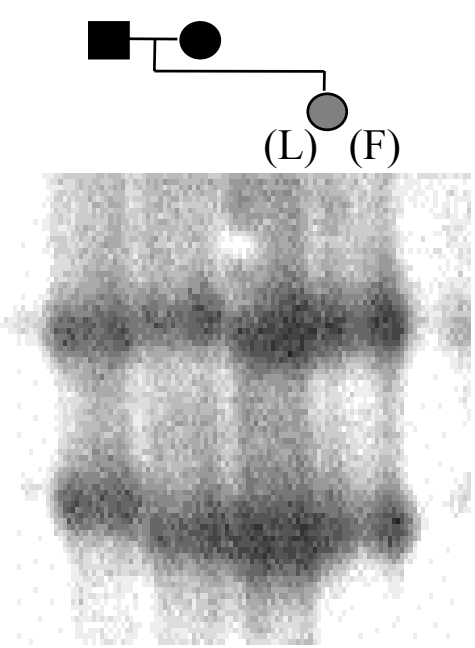
Evidence of mosaicism in BWS

Example of over-exposed Southern blot showing low level mosaicism in a BWS patient with hemihypertrophy

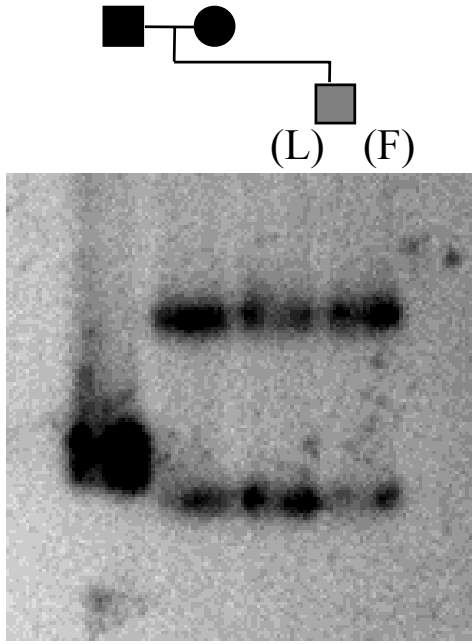


No evidence for mosaicism in SRS

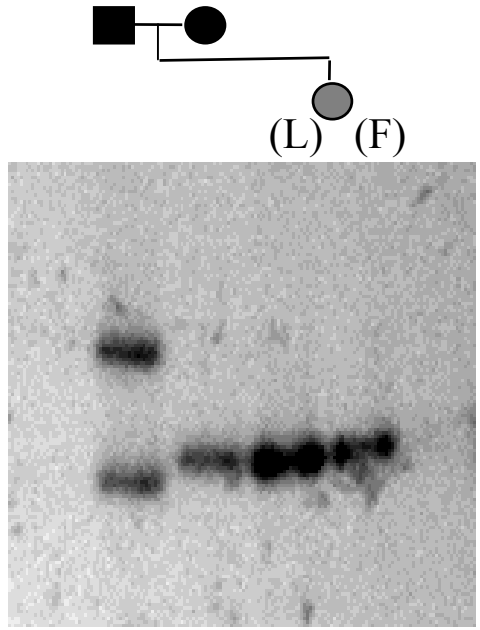
Marker D7S22



Non mUPD7 SRS
with asymmetry



mUPD7 (hetero)
with asymmetry



mUPD7 (hetero)
without asymmetry

Chromosome 7 rearrangements defining imprinted critical regions

The 7p candidate region:
Maternal inherited duplications of 7p11.2-p13, encompassing the *GRB10* gene (Joyce et al., 1999; Monk et al., 2000)

SRS and SRS-like patients with inversion and translocation breakpoints within 7p11.2 (Monk et al., 2002)

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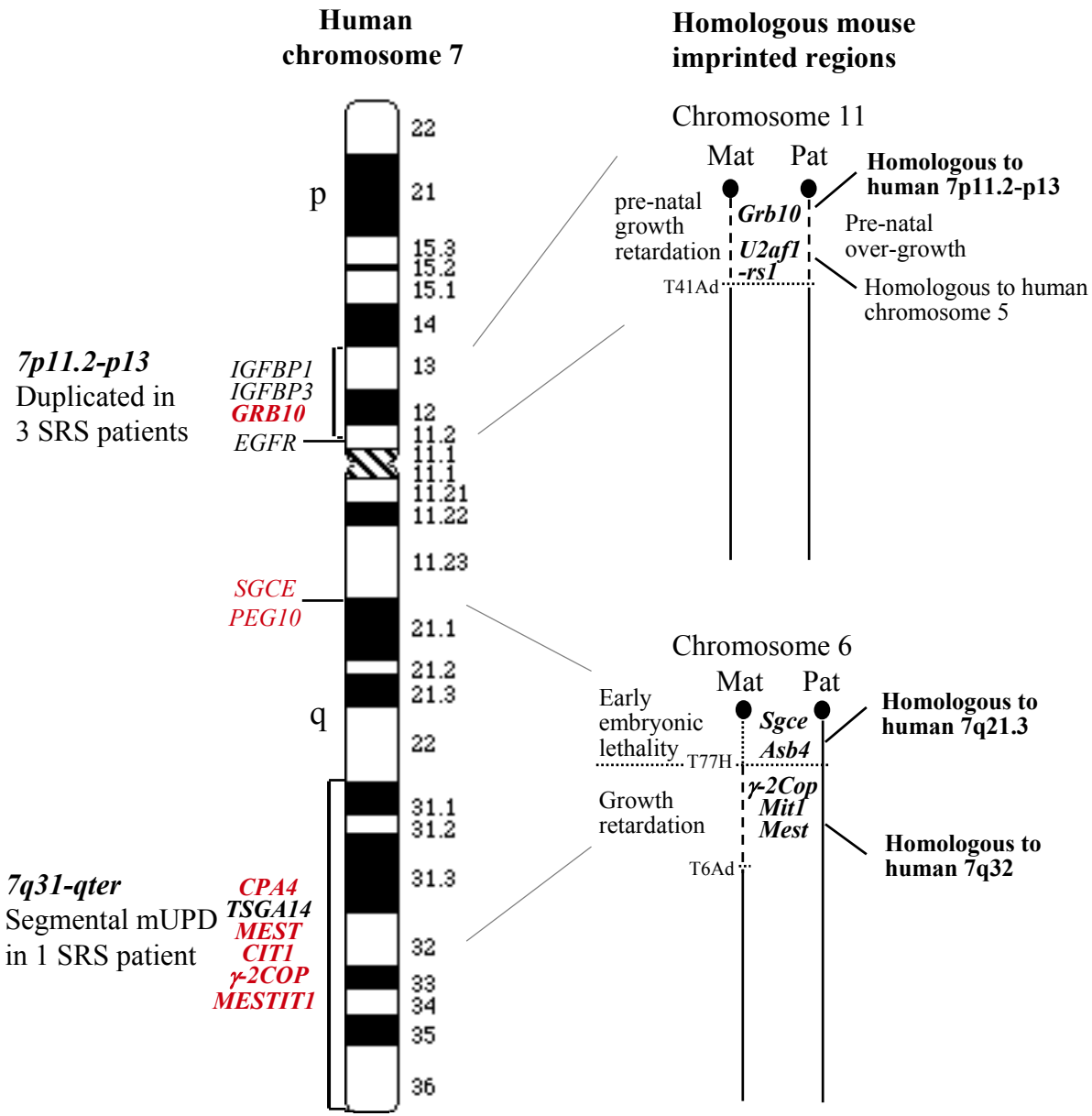
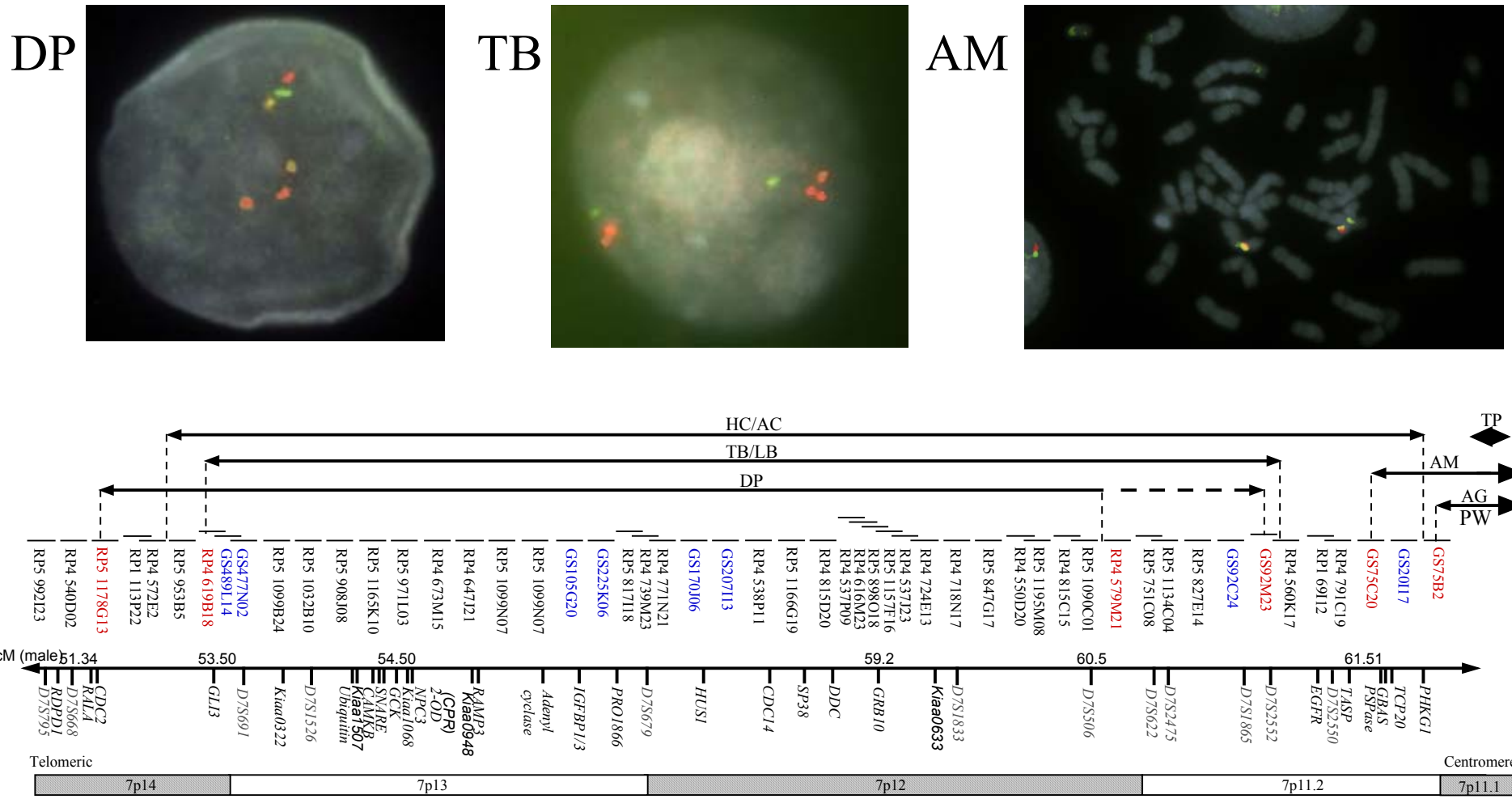
Two unrelated cases of mUPD7q and pUPD7p resulting from T(7)(q;q)(p;p) (Eggerding et al., 1994; Kotzot et al., 2001)

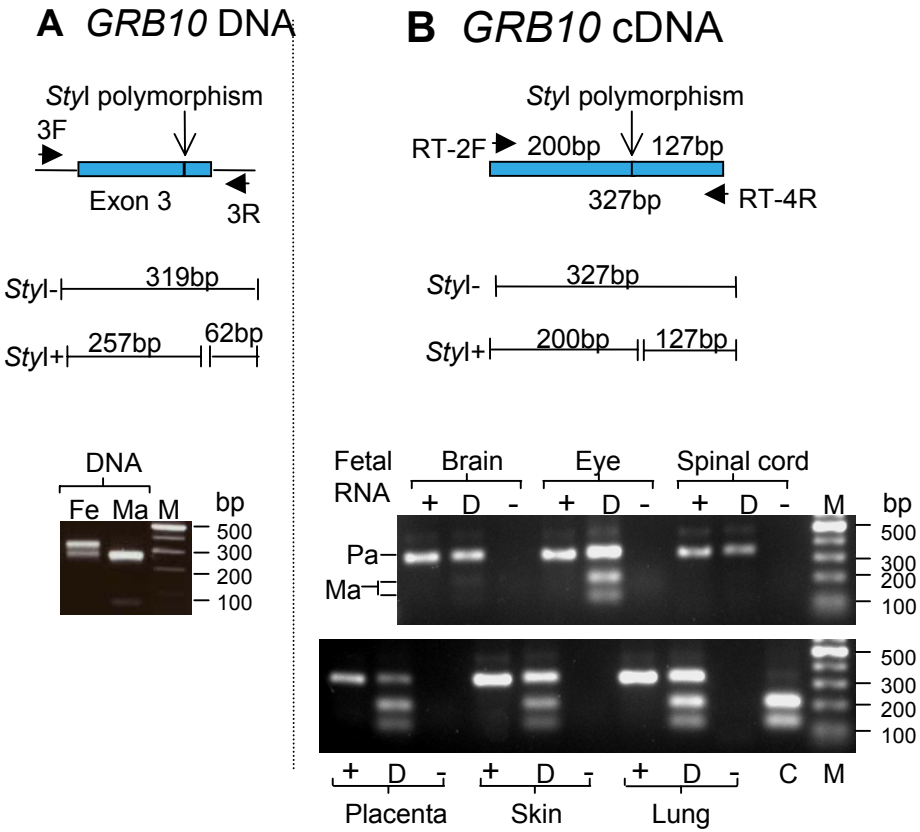
Human-mouse homology map



Integrated physical and transcript map of the 7p11.1-p14 critical region



Imprinting of *GRB10* and *GRB10*β in human fetal tissues

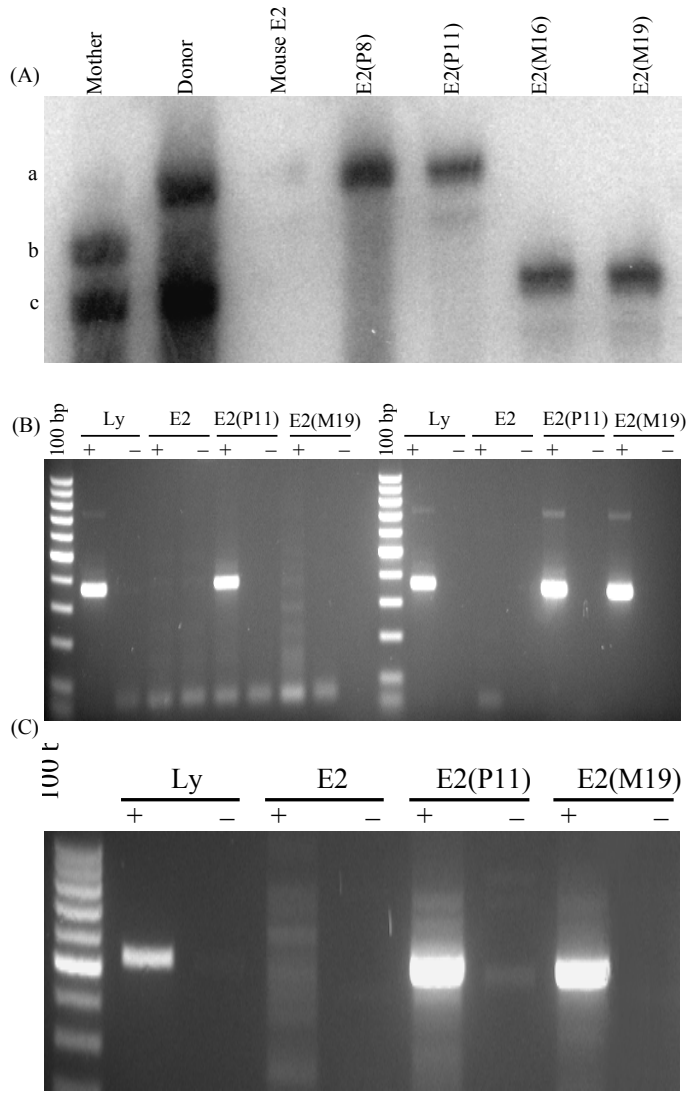


Imprinting analysis using monochromosomal somatic cell hybrids

(A) Hybrids genotyped to ensure identification of cells containing a single paternal or maternal homologues

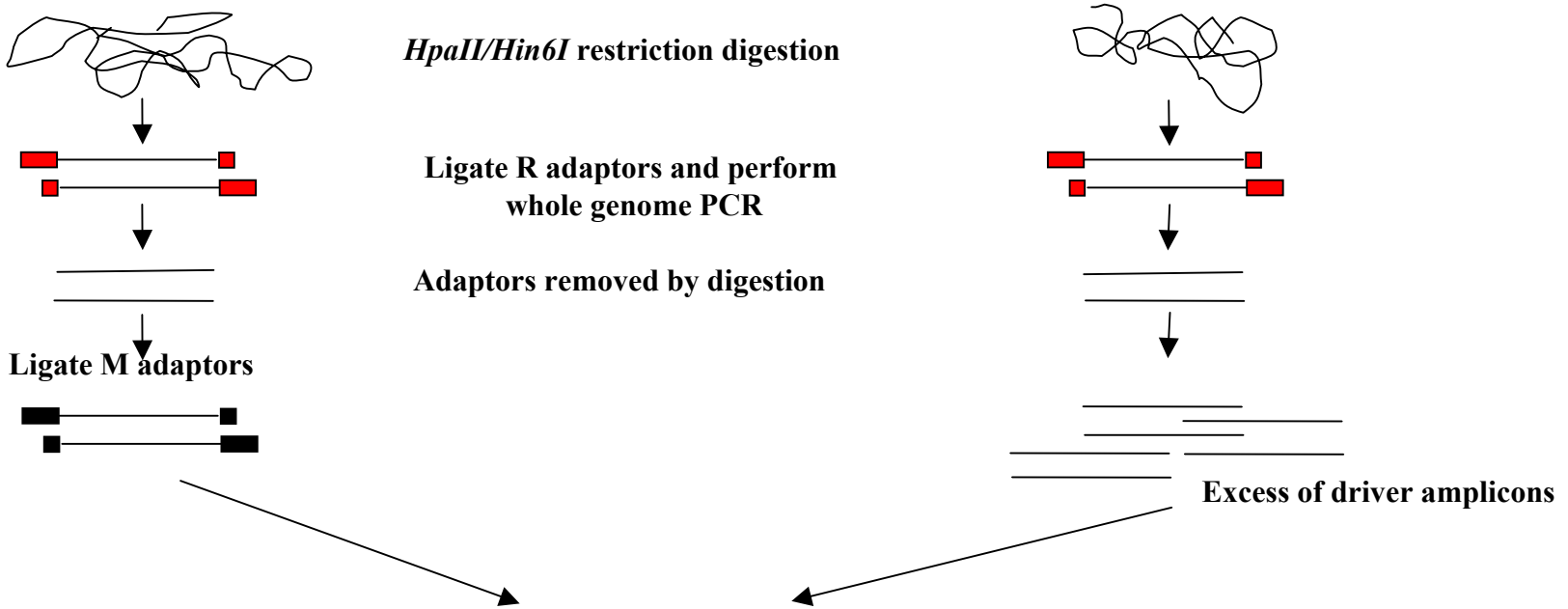
(B) Maintained imprinting of the *PEG1/MEST* gene isoforms in hybrid cell lines

(C) Biallelic expression of *HUS1*



The search for additional imprinted genes at 7p11.2-12 and proximal mouse Chr 11

Schematic representation of the Me-RDA technique



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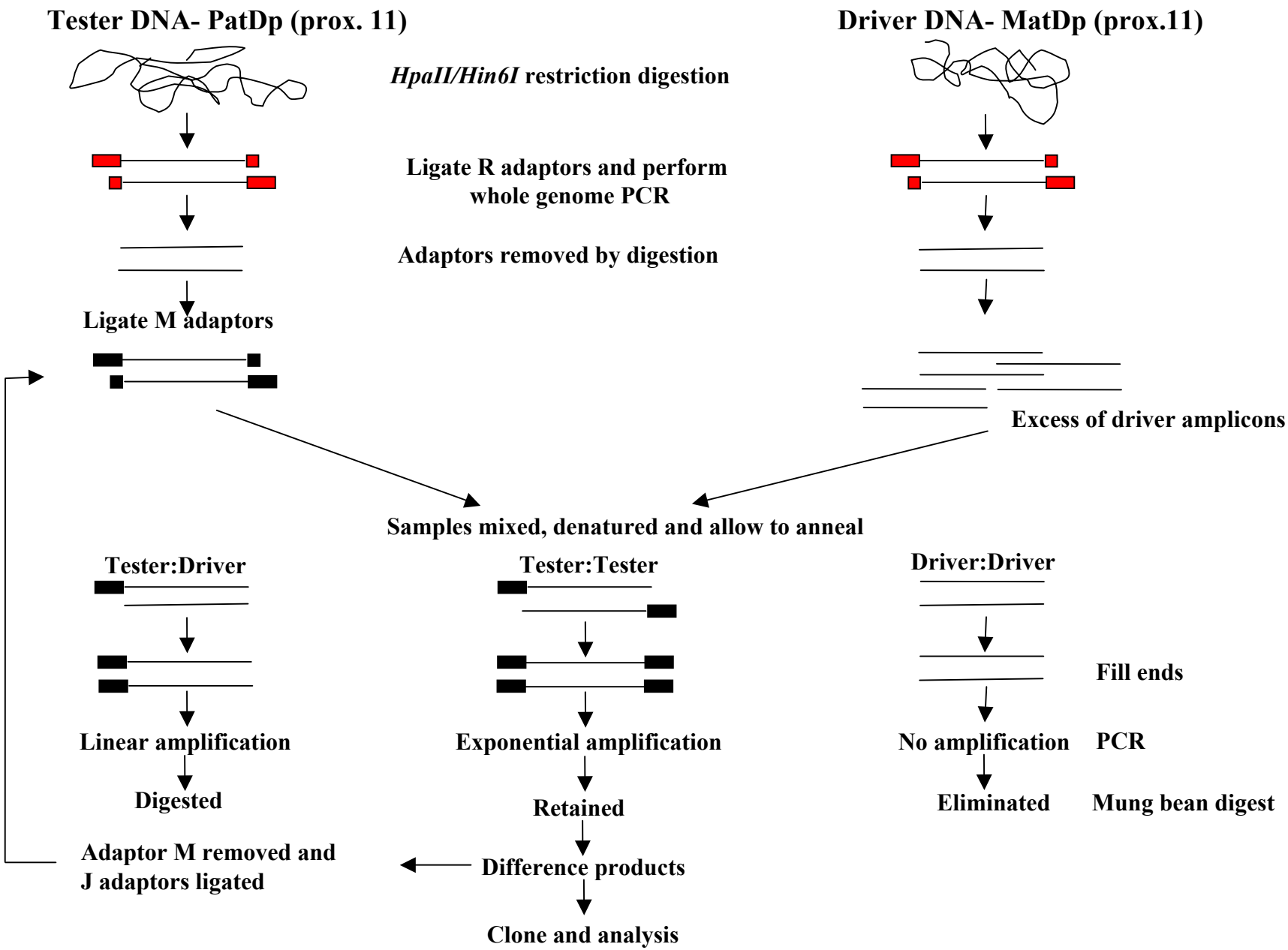
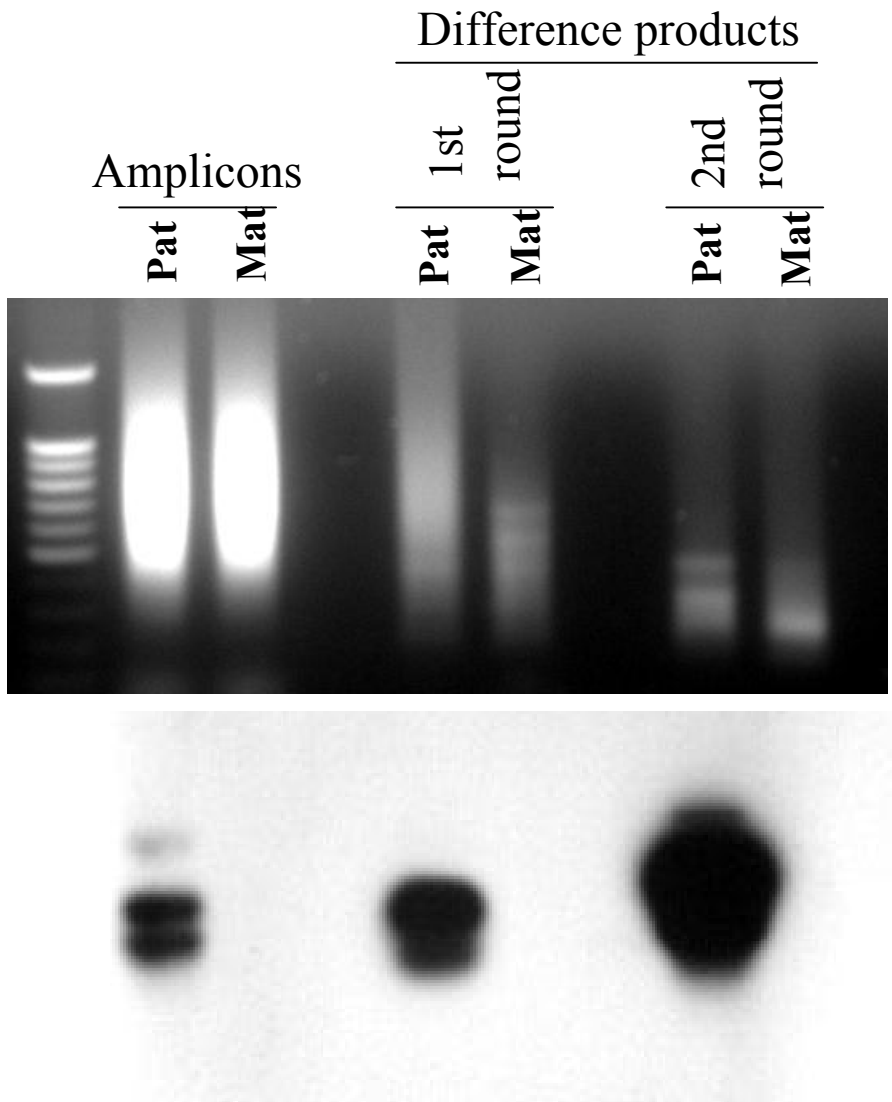
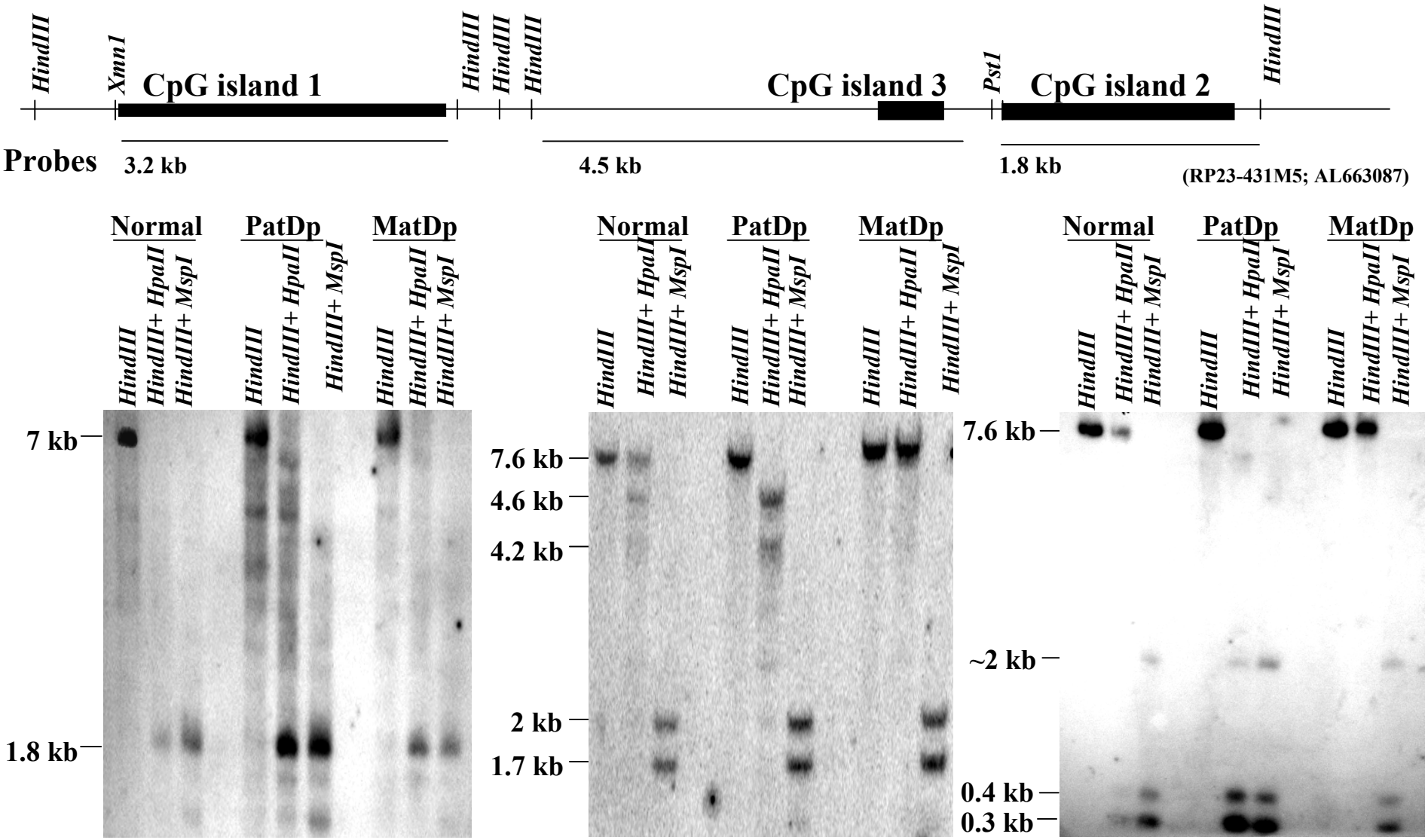
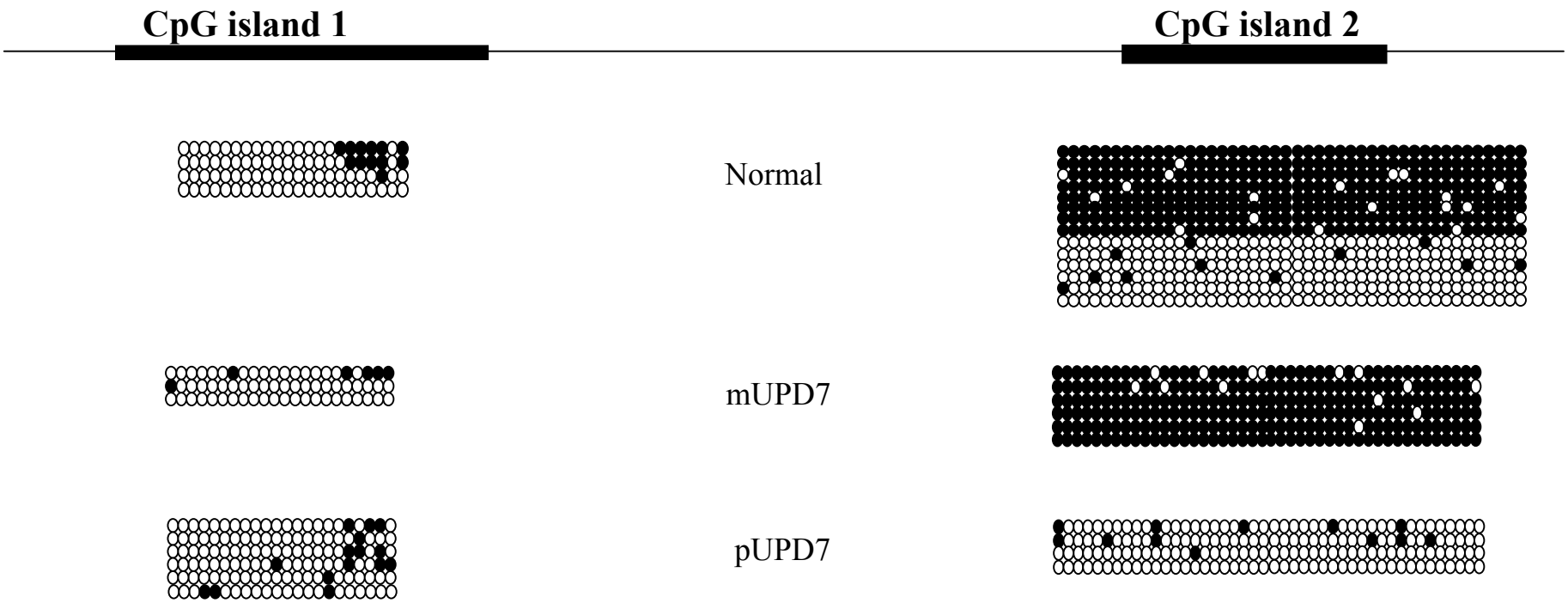



Difference products
Amplicons
1st round
2nd round
Pat
Mat
Pat
Mat
Pat
Mat

Methylation profiles for the 5' mouse *Grb10* CpG islands



Methylation profiles for the 5' human *GRB10* CpG islands



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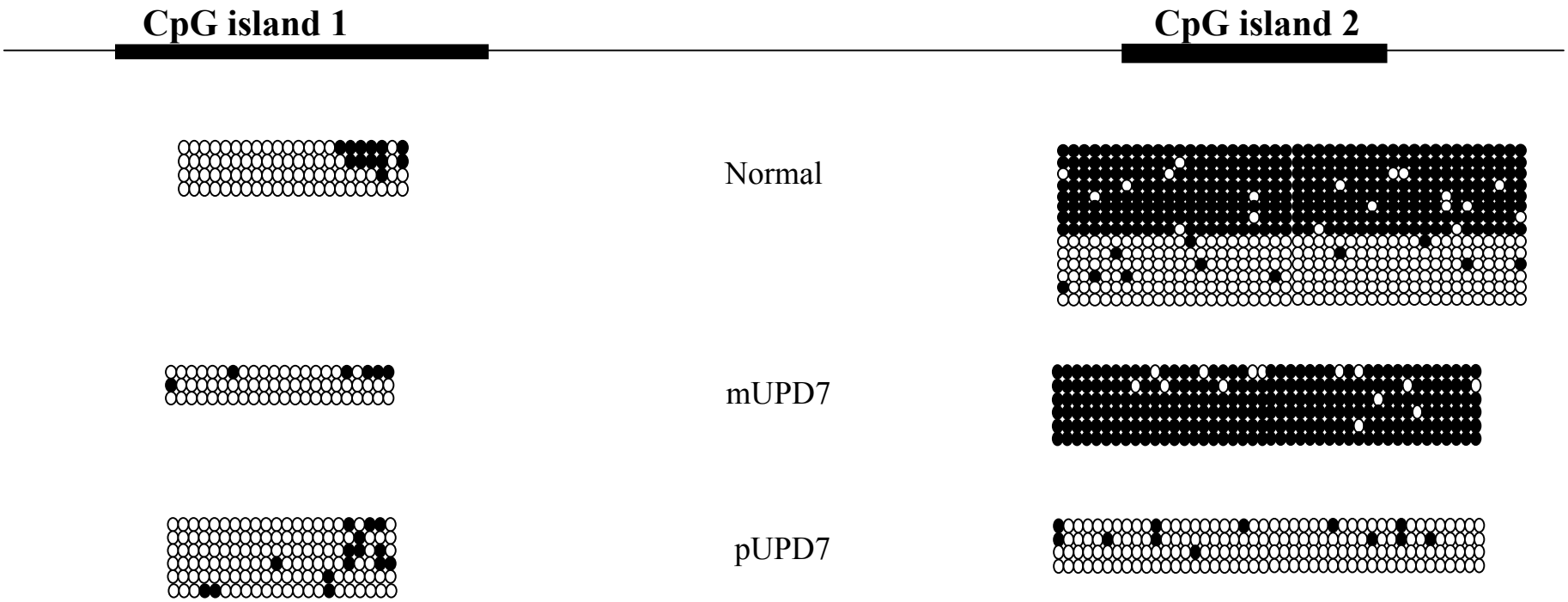
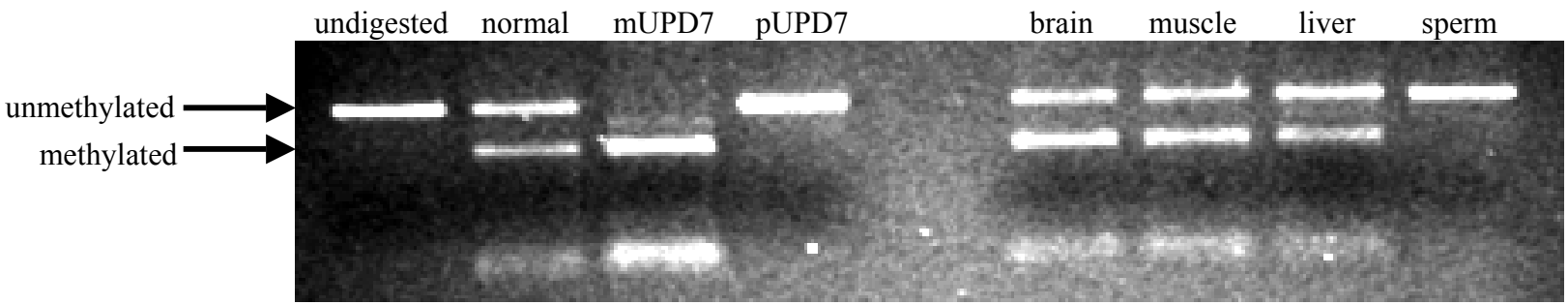
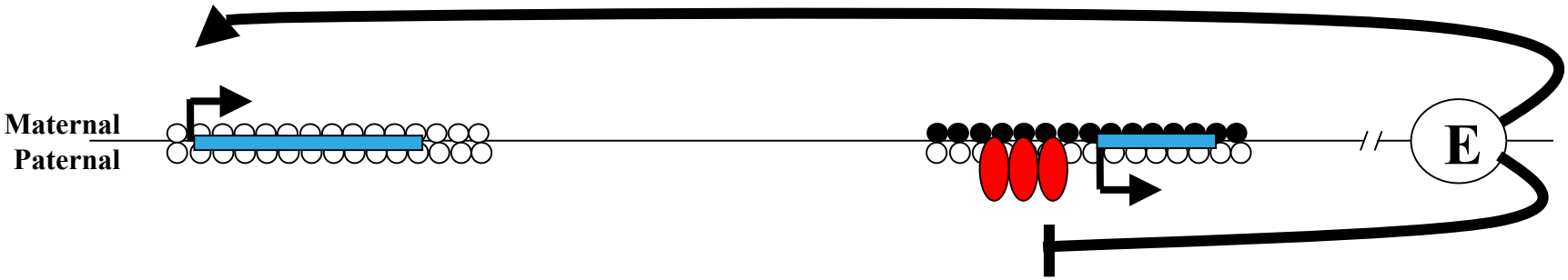




Meg1/Grb10 imprinted regulation- Insulator boundary model



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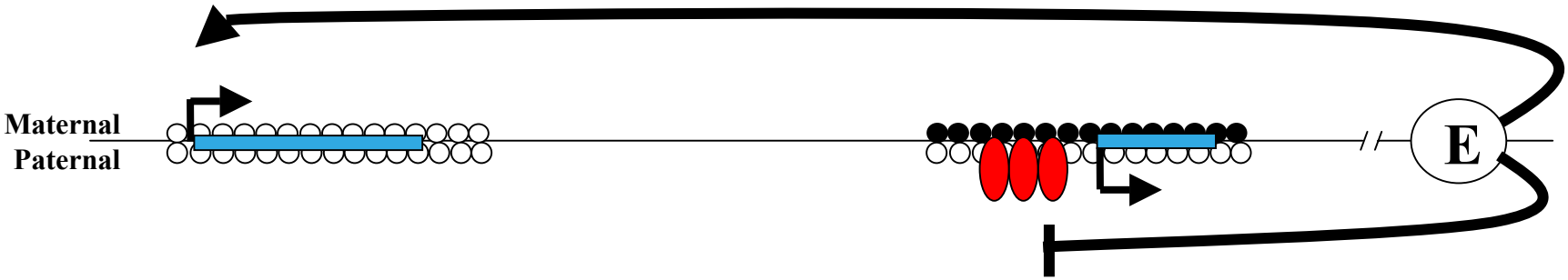
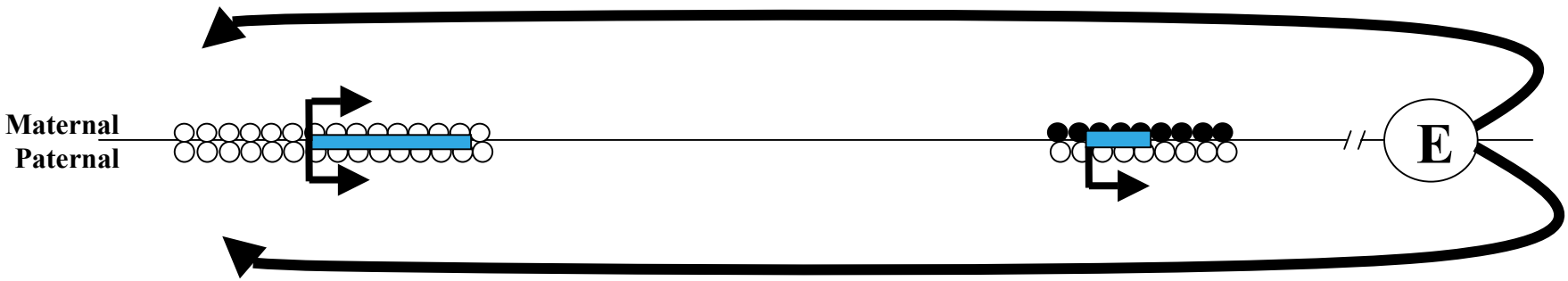


Human *GRB10* regulation



CTCF insulator
Methylated CpG
Unmethylated CpG
Exons

Mutations in the *GRB10* genes in SRS?

No coding mutations found in 139 patients (Mergenthaler et al., 2000; Yoshihashi et al., 2000; Hitchins et al., 2001)

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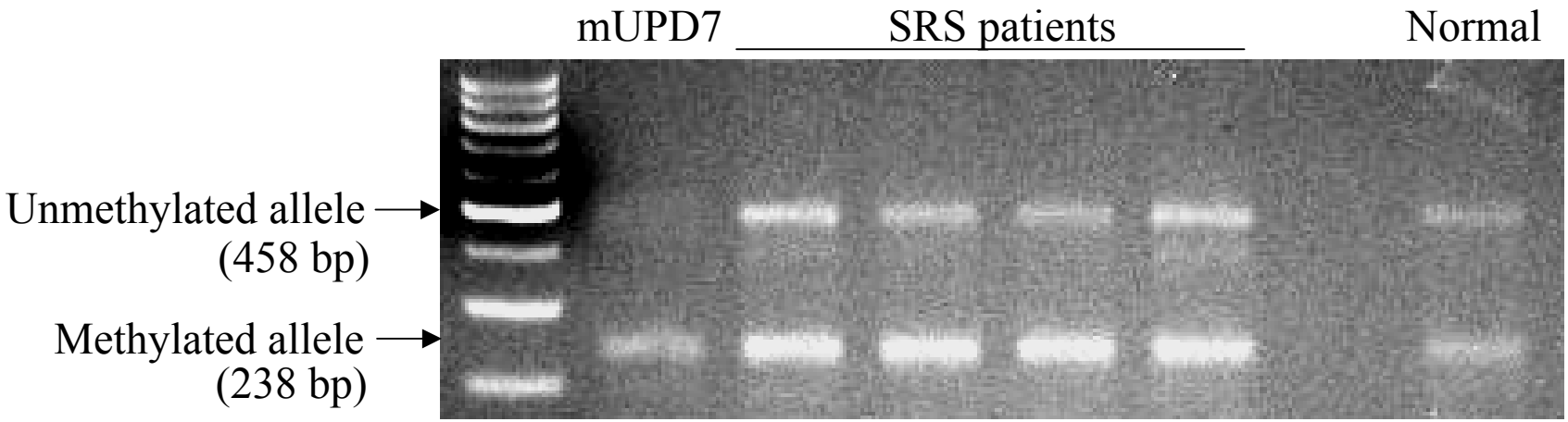
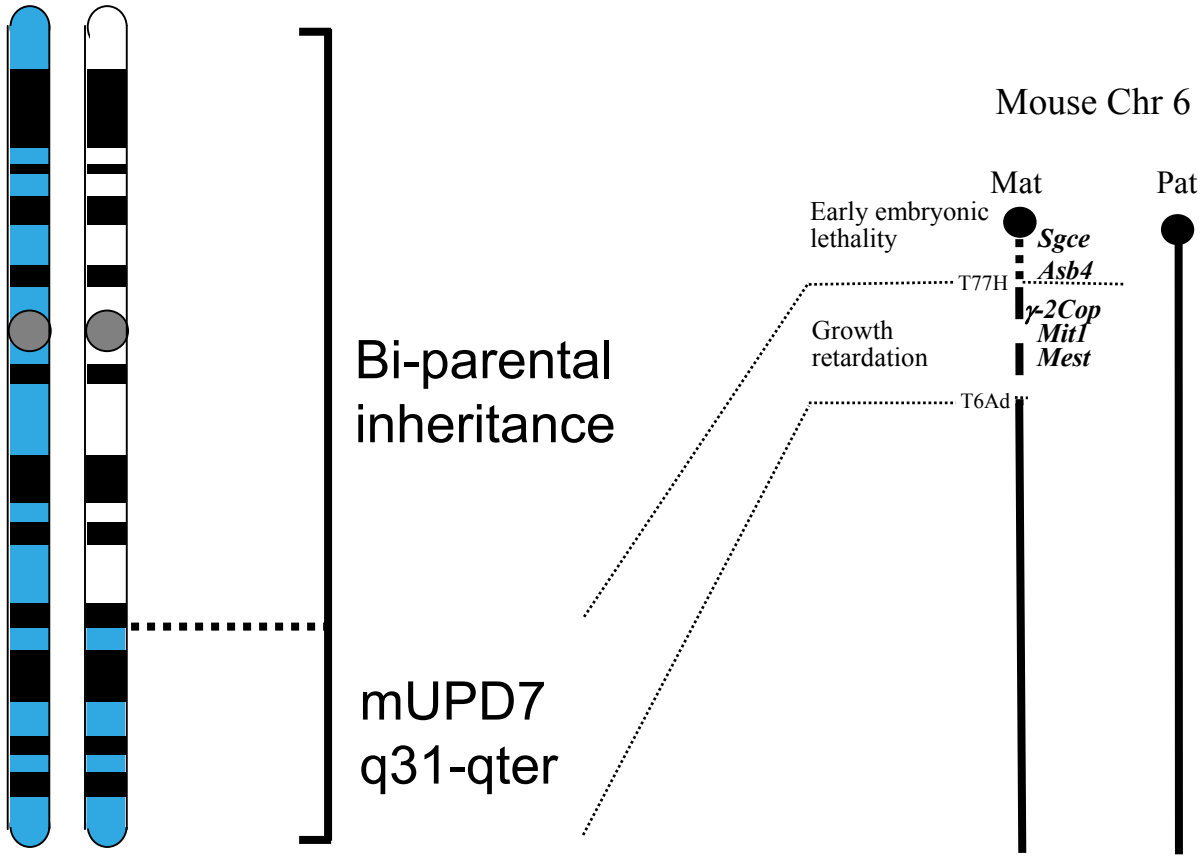
GRB10 CpG2 DMR epimutation in SRS?



No epigenetic mutations in 46 SRS patients (Arnaud et al., 2003)

The 7q32 candidate gene region



Hannula et al., 2001

Candidate imprinted genes within 7q32

MEST: Imprinted, paternally expressed
Within the region of segmental mUPD7
Within the mouse region showing a growth restricted phenotype
Mest knock-out mice are growth restricted
No coding or epigenetic mutations in 50+ patients

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γ-2-COP: Imprinted status disputed, reports of paternal expression
(Blagitko et al., 1999; Yamasaki et al., 2000)

MESTIT1: Anti-sense for *MEST*, paternally expressed
(Nakabayashi et al., 2002)

CPA4: Tissue-specific maternal expression (Bentley et al., 2003;
Kayashima et al., 2003)
No coding mutations in 20 patients

Conclusions

- 10% of SRS cases present with mUPD7
- There are two imprinted candidate gene regions for SRS on human chromosome 7, 7p11.2-p13 and 7q31-qter, which have homology to imprinted mouse regions
- The phenotypes observed for the patients with 7p duplication may result from either over-expression of the maternal *GRB10* isoforms, or from an extra copy of this region
- A definite role for imprinting has been indicated for 7q31-qter due reports of a single SRS patient with segmental mUPD for this region
- No mutations have been found in any imprinted candidate gene

IRBD, Imperial College London
Prof. Gudrun Moore
Dr Phil Stanier
Prof. Michael Preece
Louise Bentley

MRC Harwell
Dr Jo Peters
Colin Beechey

The Babraham Institute
Dr Gavin Kelsey
Dr Philippe Arnaud
Dr Rachel Smith

The Silver-Russell syndrome consortium

Steve Scherer - Toronto, Canada
Juha Kere- Helsinki, Finland

Thomas Eggermann- Aachen, Germany

MRC
Medical Research Council