

Genetic engineering in the mouse: from functional genomics to zootechnical applications.

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- “Double muscling” in cattle
 - The “double muscled” phenotype
 - Segregation analysis, the “MH” gene
 - Linkage analysis
 - Positional candidate cloning of the myostatin (MSTN) gene and definition of disruptive mutations
- Genetic engineering of the MSTN gene in the mouse
 - Conditional KO: does delayed inactivation of MSTN still have an effect on muscle growth?
 - Dominant and male –specific double muscling: segregation of milking and meat abilities with sex.

• Introduction: muscular hypertrophy of genetic origin in cattle

The “double-muscled” phenotype

Muscle mass

+20%

Hyperplasia

>>>

Hypertrophia



Advantage or drawback ?

More glycolytic (more IIb fibres)

•Segregation analysis

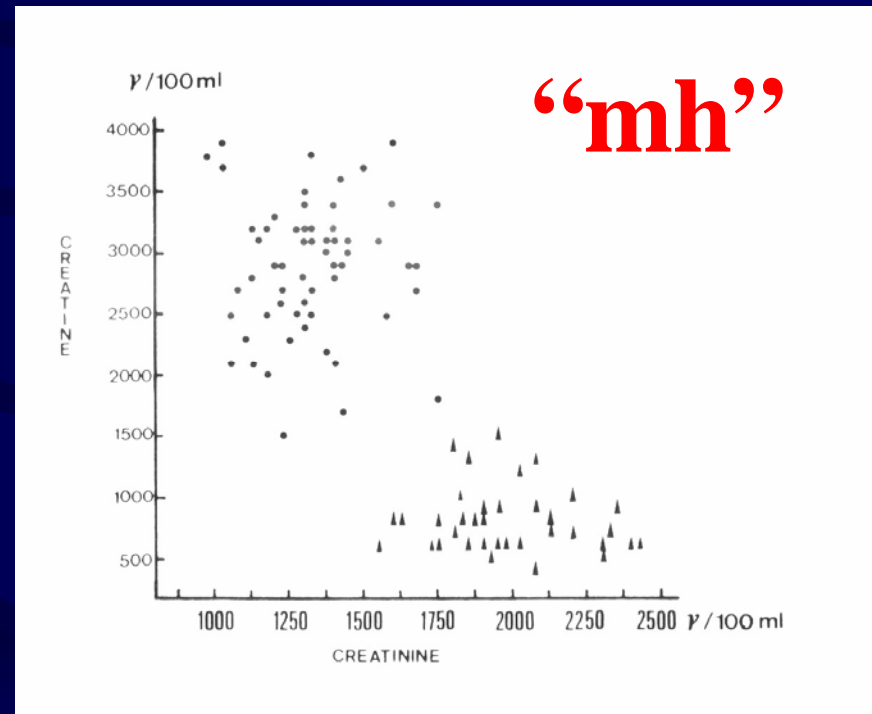
In the Belgian Blue Cattle breed (BBCB)

Offspring of A.I. Sires

- Subjective classification in 2 phenotypic classes
- Objective measurements

Very quick fixation of the character

monogenic, autosomal,
recessive (Hanset et
Michaux, 1985 a et b)



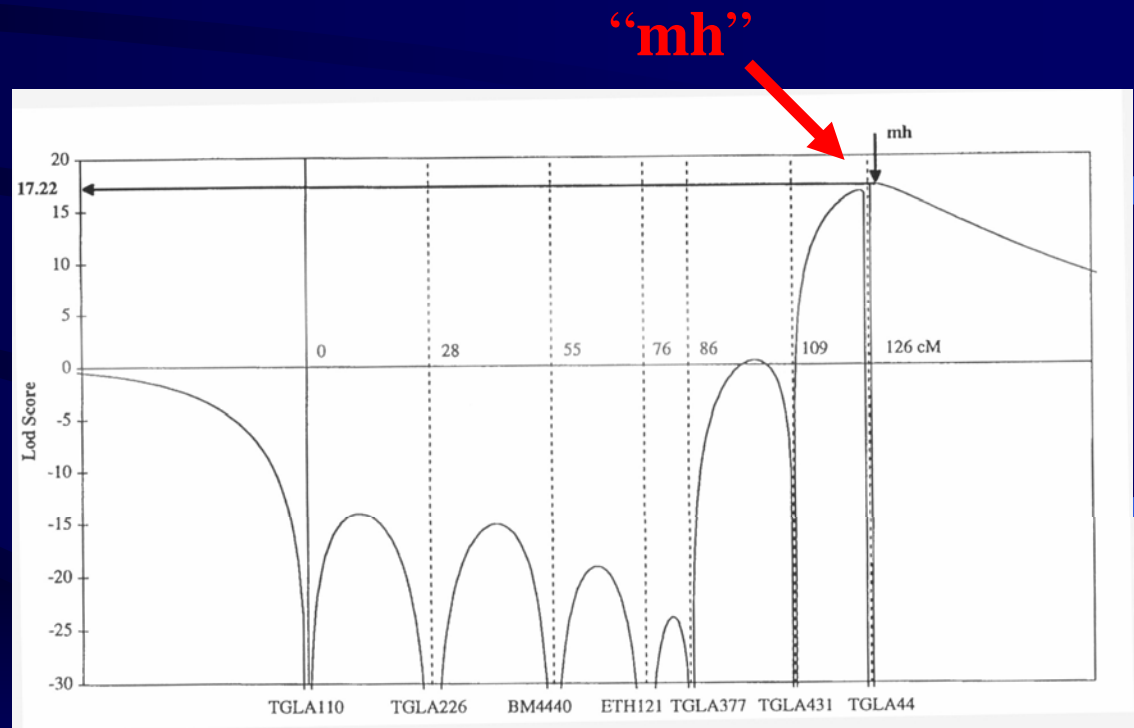
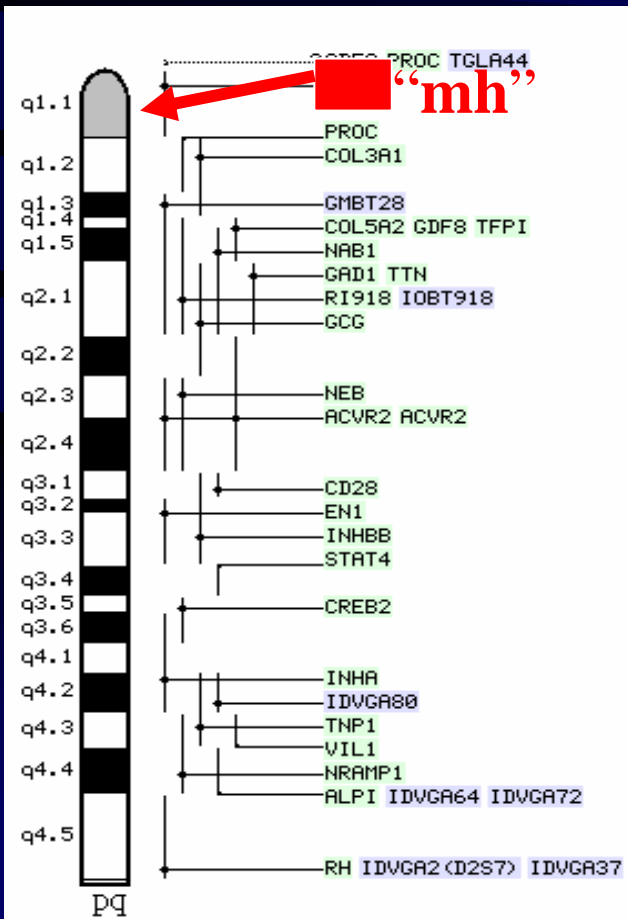
Genetic markers...

... were used sequentially and following their availability in the public domain of cattle genomics:

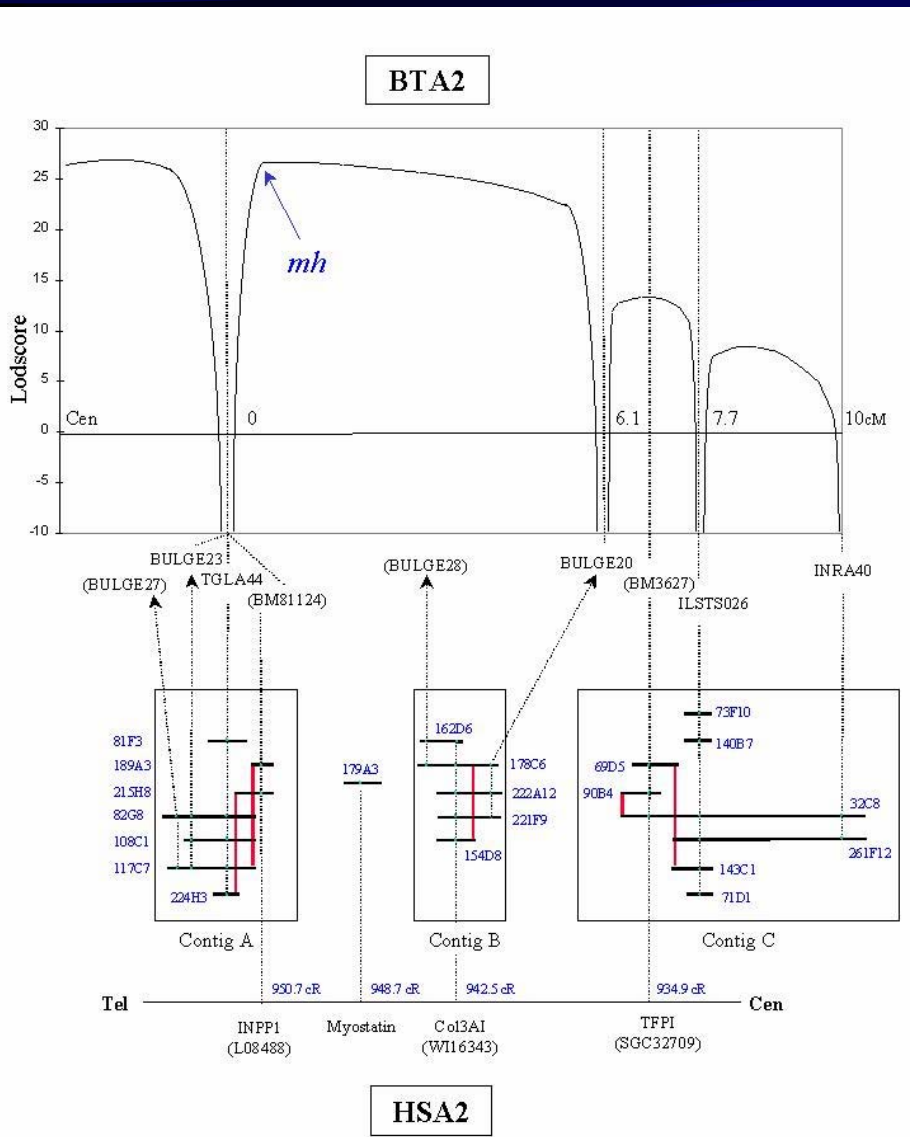
- blood groups
- biochemical polymorphisms
- RFLP's
- minisatellites
- **microsatellites**

• Linkage analysis

Chromosomal location: BTA2 cen (Charlier et al., 1995)
whole genome scan with >200 microsatellites



• Positional cloning of the “mh” gene



Genetic mapping



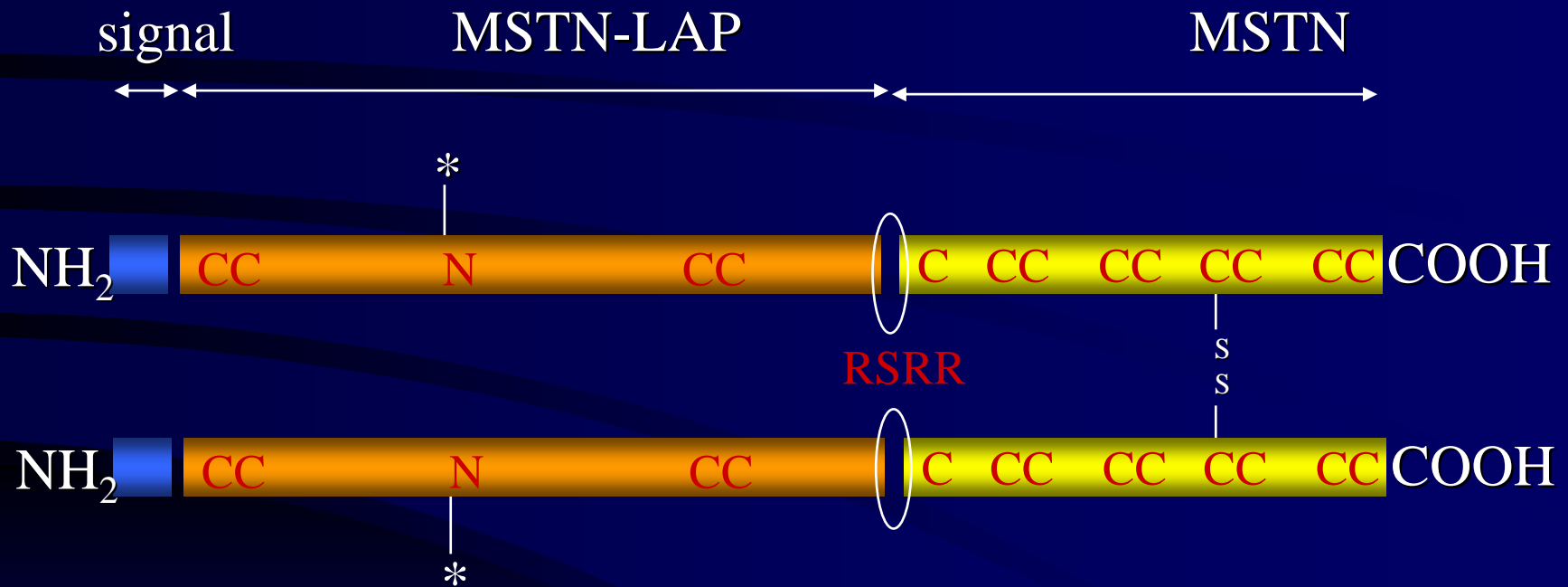
Physical mapping



Comparative mapping

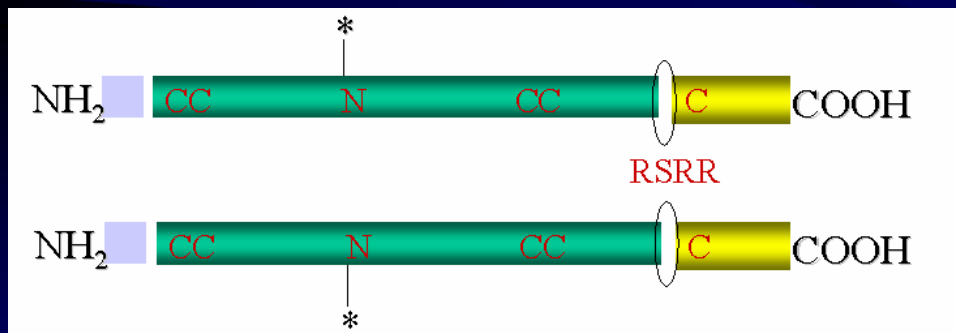
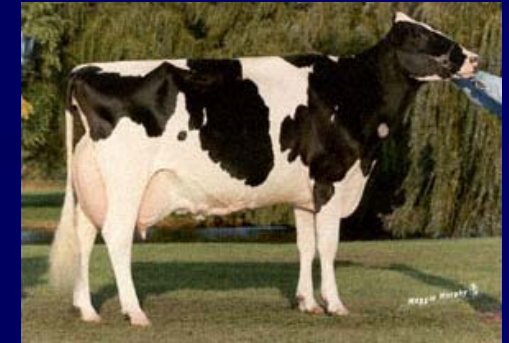
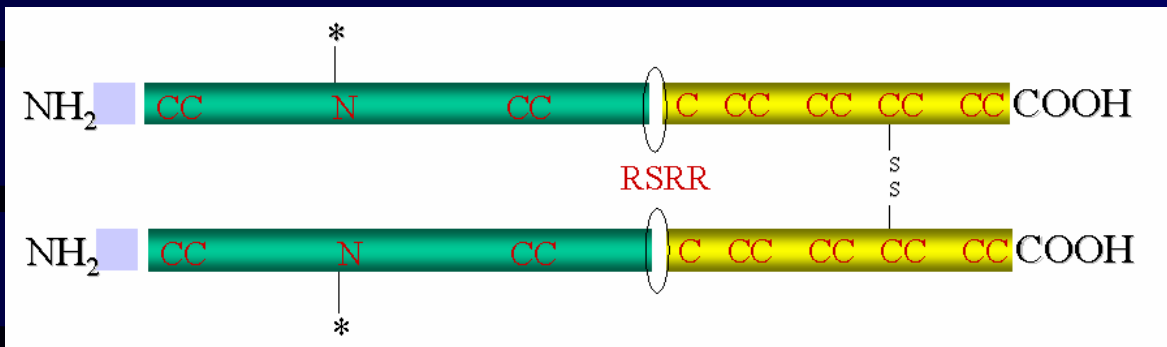
Positional candidate: Myostatin

(McPherron et al., 1997)



Myostatin = “mh”!!!

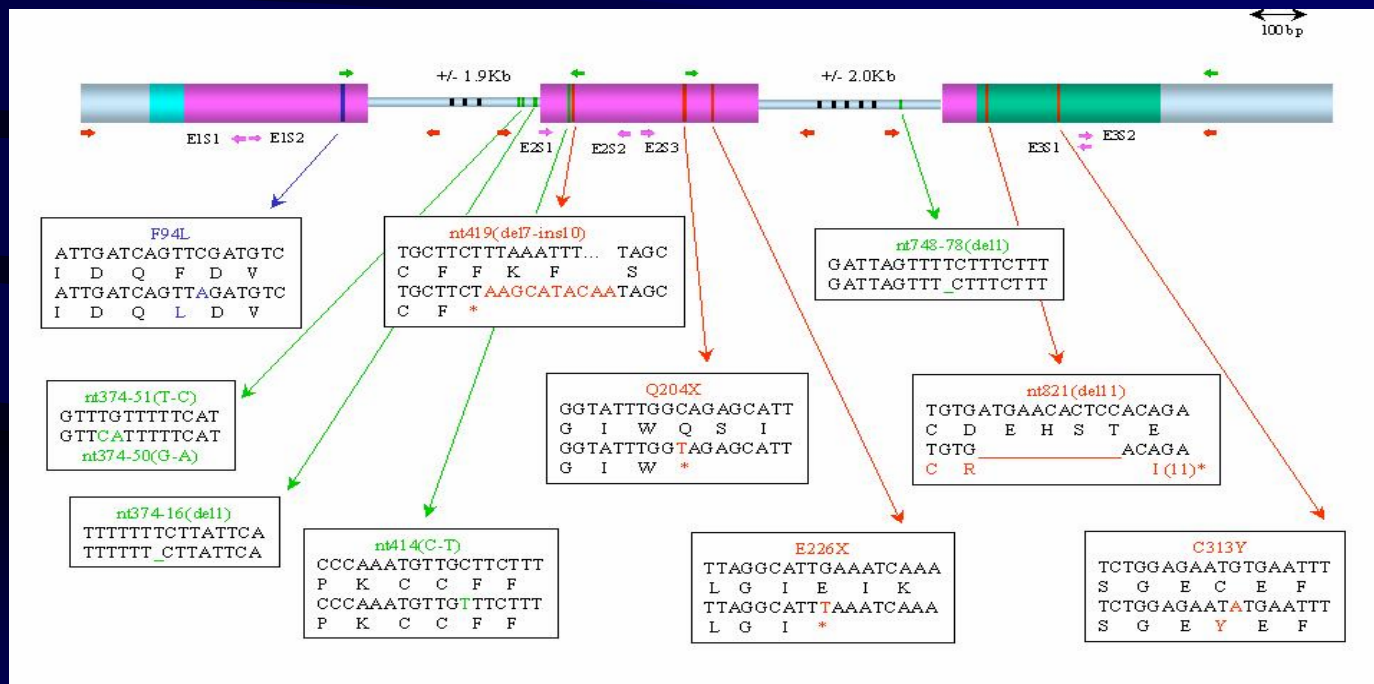
(Grobet et al., 1997)



Allelic heterogeneity

(Grobet et al., 1998)

Five disruptive mutations found in cattle

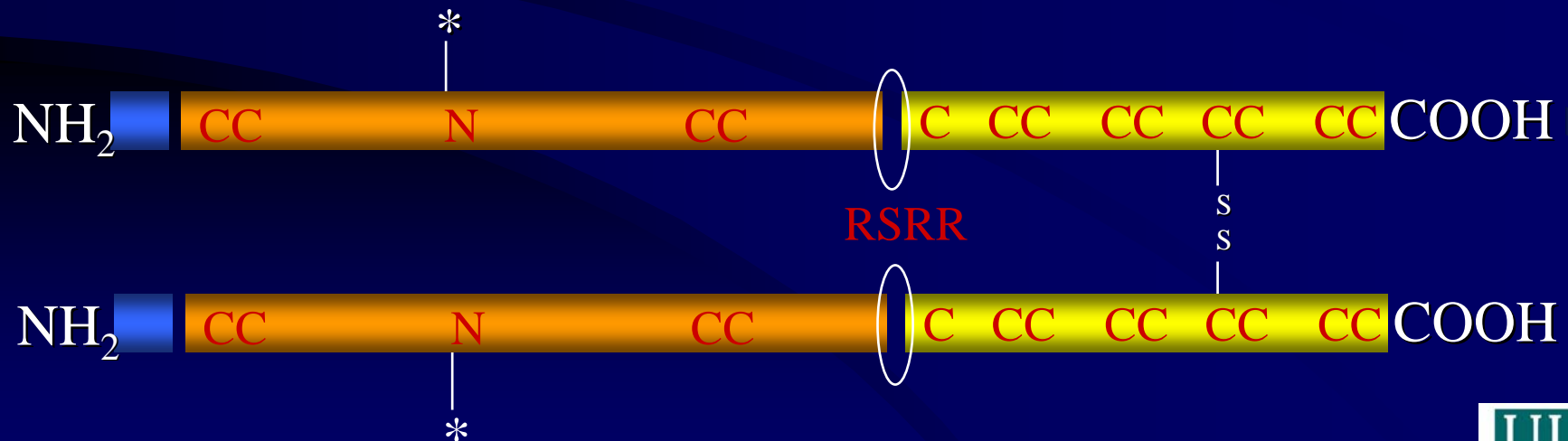


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•Molecular biology data

• Structure of MSTN

- Member of the TGF- β superfamily
- 376 Aa (mouse)
- Signal sequence for secretion, proteolytic processing site, LAP, carboxy-ter bioactive domain, 9 conserved cysteine residues with characteristic spacing
 - Highly conserved across species
 - Closer known relative: GDF-11

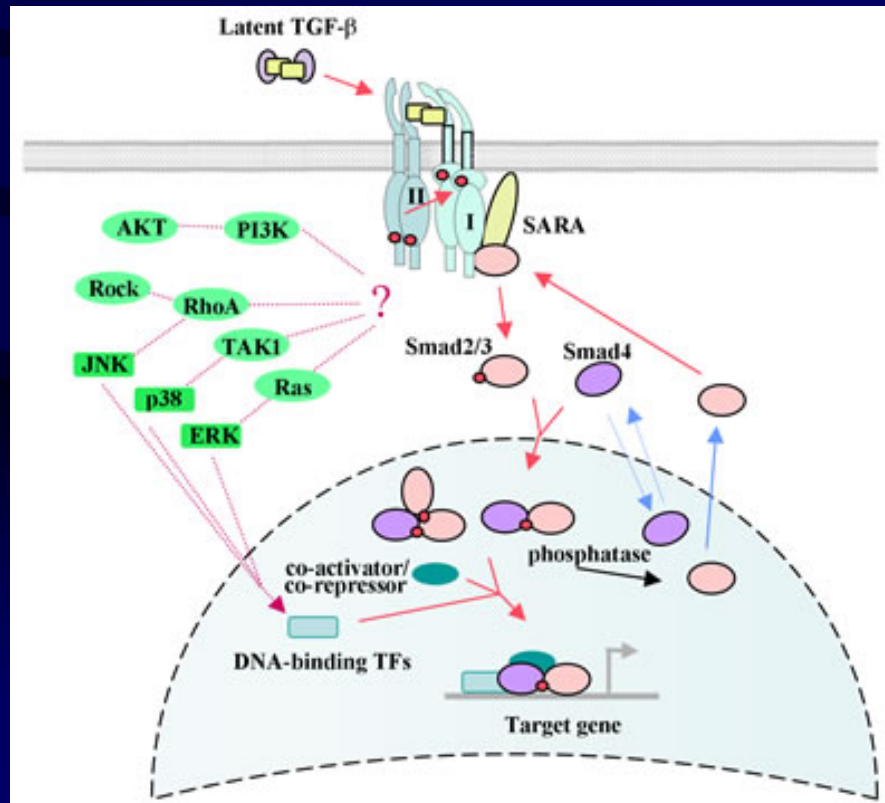


•Molecular biology data

- Mode of action of MSTN

- Signalling: like as other TGF- β members?

- Receptor: activin RIIB (Lee & McPherron 2001)
- Inactive complex with LAP



• Molecular biology data

- Mode of action of MSTN

- Phenotypes of KO animals:

- Morphometrics: hyperplasia and hypertrophy

- Myoblast cell cultures: longer proliferation phase, delayed differentiation

- Over expression of myostatin in the mouse

- Muscle wasting...

•Molecular biology data

• Expression of MSTN

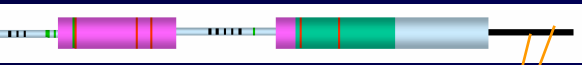
- Mainly in the skeletal muscle lineage (McPherron et al., 1997)
 - From 9.5 dpc on in the most mature somites (rostral) (myotome compartment)
 - In a wide range of developing muscle
 - In the pig, mRNA levels peaks during the later stages of gestation and declines after birth (Ji et al., 1998).
 - Nevertheless, **expression is maintained in adult skeletal muscle**
- Low level of expression has also been reported:
 - In adipose tissue (McPherron et al., 1997)
 - In cardiac muscle and in mammary gland (Ji et al., 1998)

• Delayed inactivation of myostatin

- Is there still a possibility of modulating skeletal muscle mass by inactivating MSTN after birth?

Conditional KO approach

Wild-type allele



Constitutive KO allele

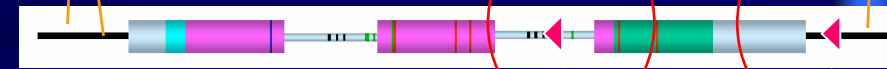


KO

Wild-type allele



Conditional KO allele



Cre recombinase



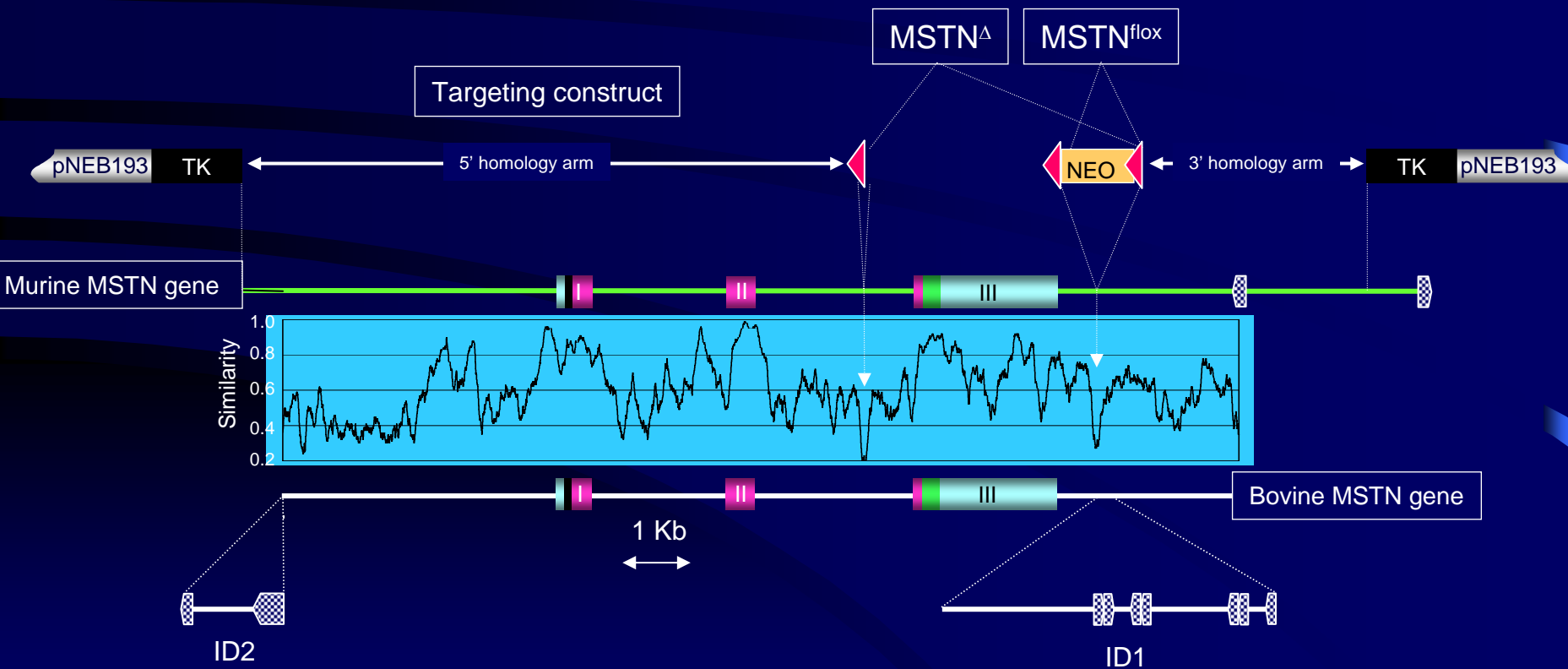
Inactivated



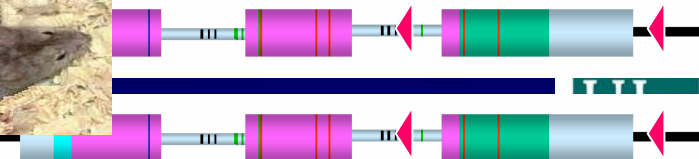
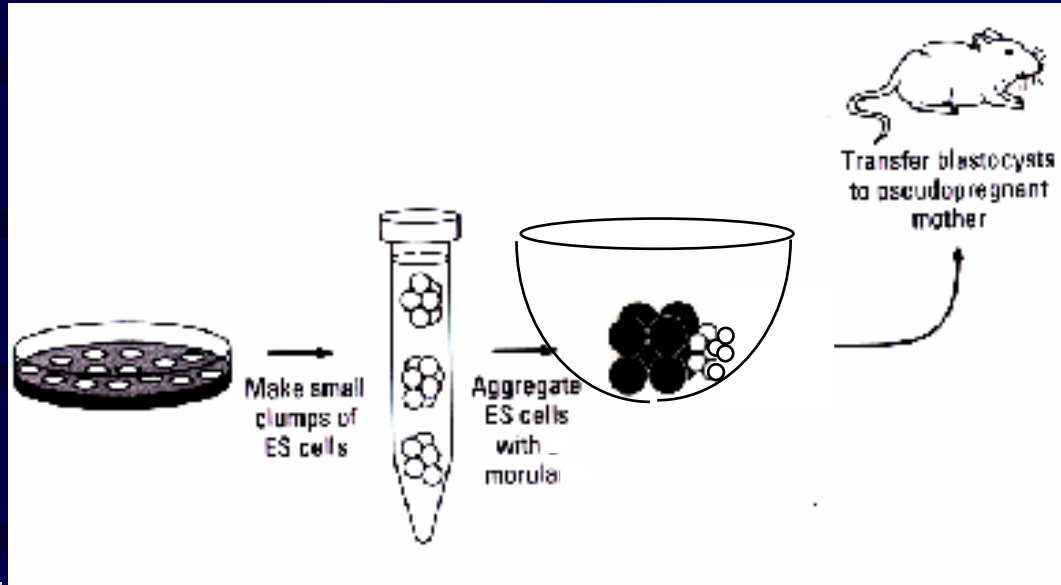
**Conditional
KO**

MSTN gene targeting

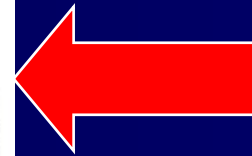
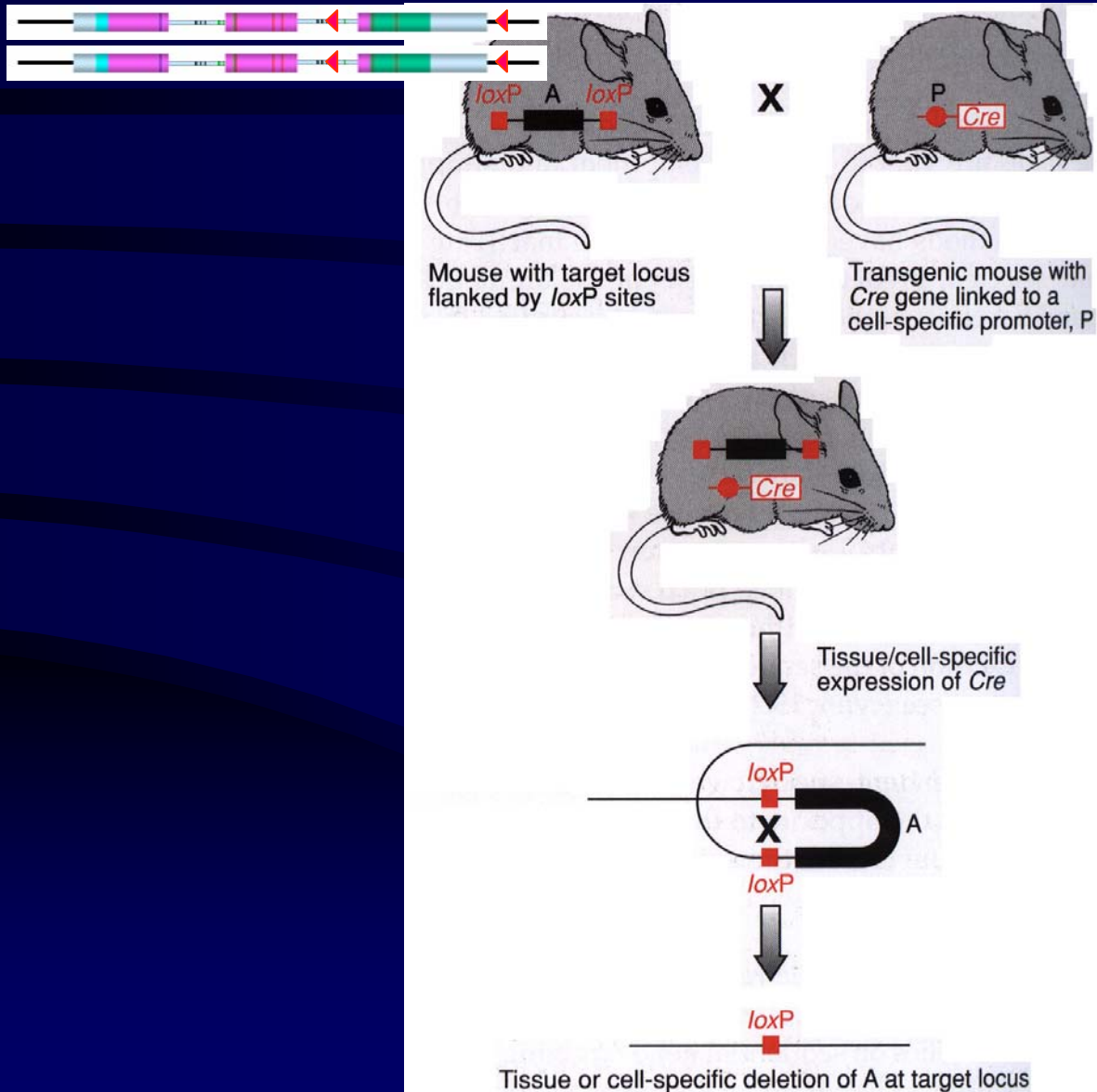
Replacement-type vector



MSTN^{fllox} carrying mice



2. Cre-expressing strains

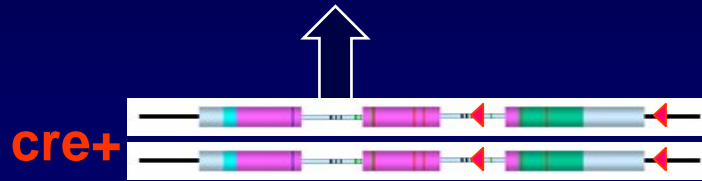
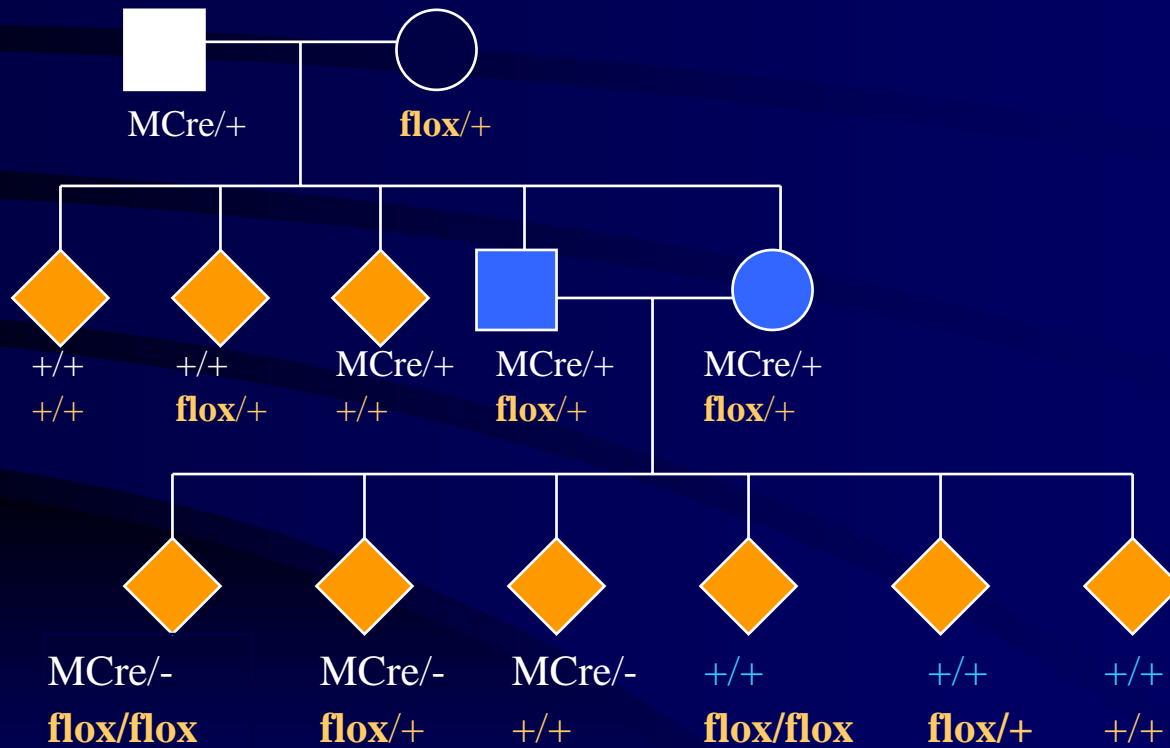


Cre expressing strains

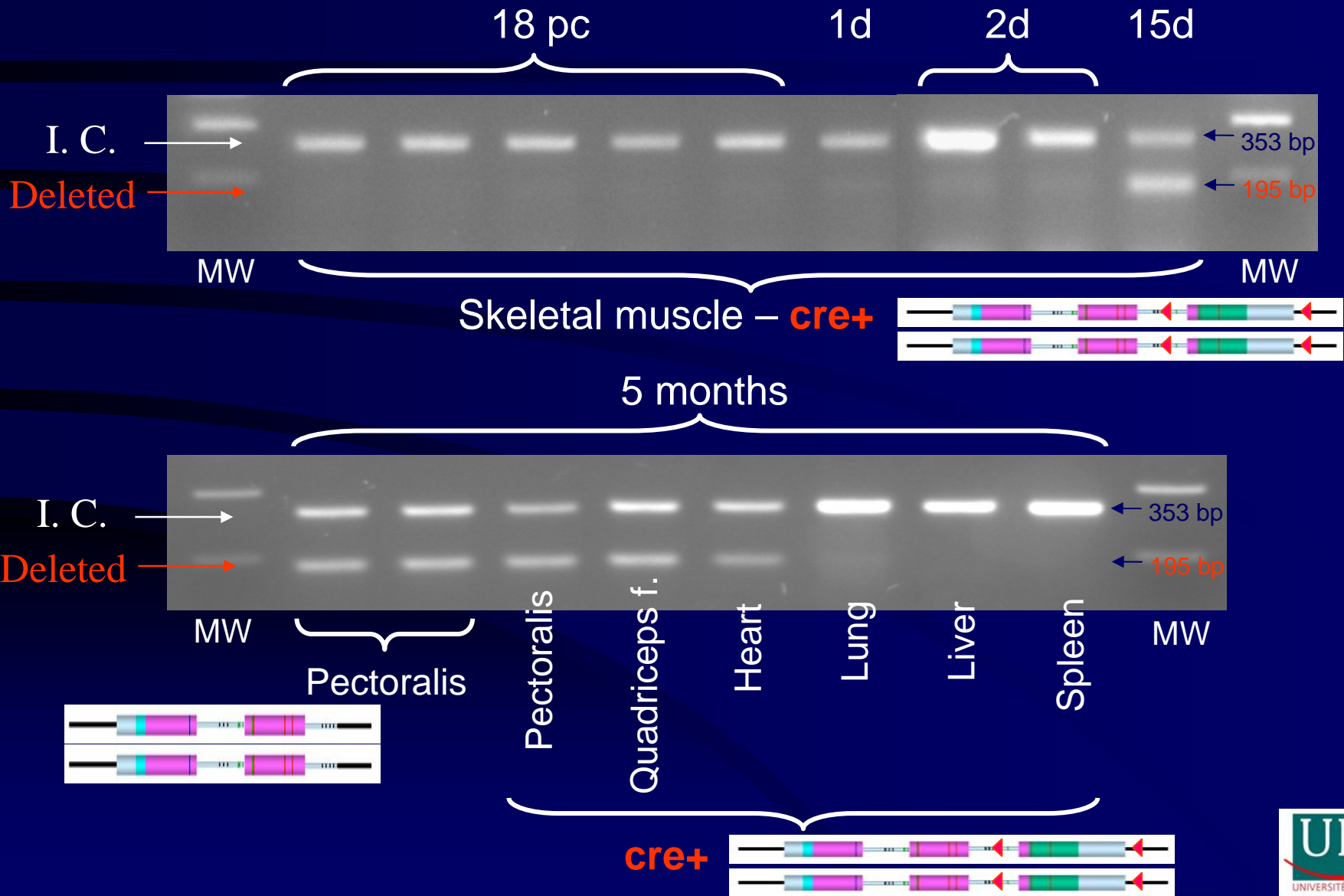
- MCK-cre mice (Brüning et al., 1998):
 - Expected recombination pattern:
 - muscle-specific
 - post-natal

- Cre inducible mice (Utomo et al., 1999)
 - Modulation of temporal excision pattern

- Cross with MCK-Cre



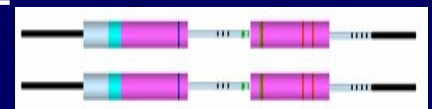
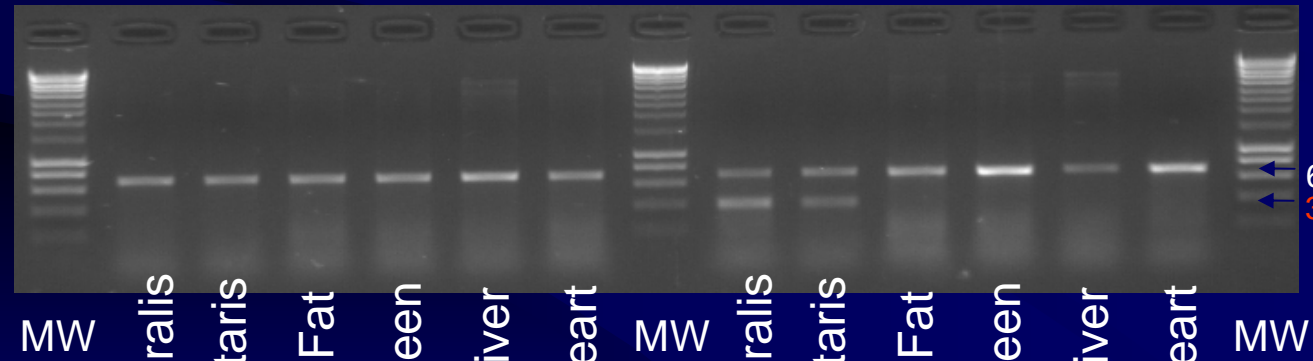
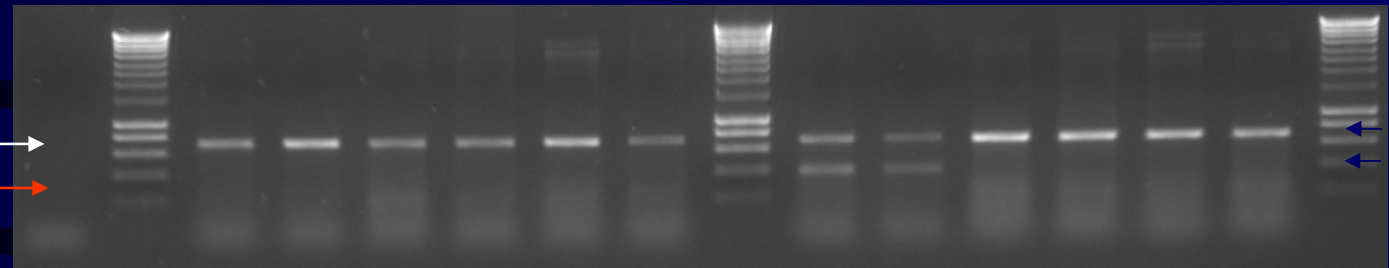
Genomic excision pattern



mRNA extinction



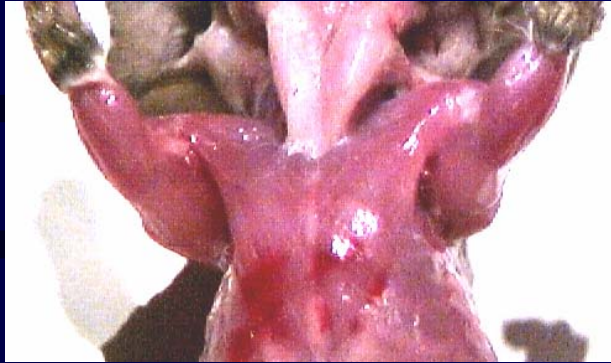
NC MW Pectoralis Plantaris Fat Spleen Liver Heart MW Pectoralis Plantaris Fat Spleen Liver Heart MW



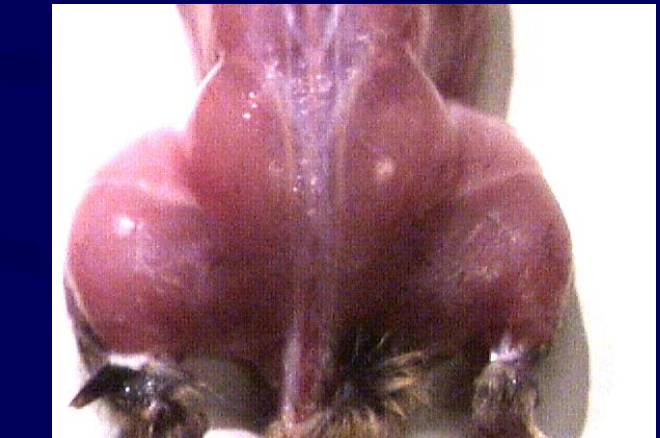
Wild type

MCKcre^{+/?} MSTN ^{+/+}

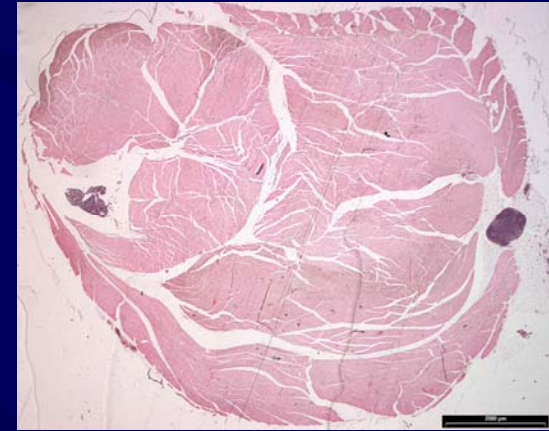
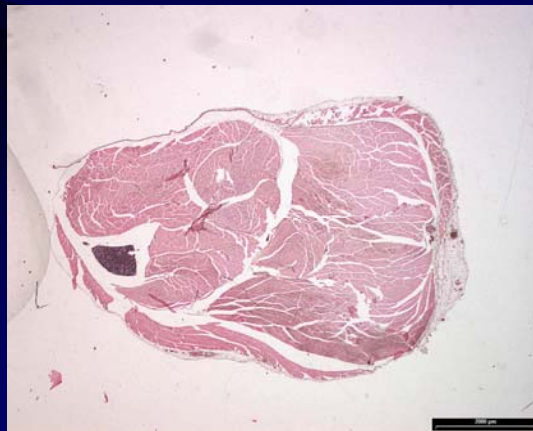
MCKcre^{+/?} MSTN ^{flox/flox}



A



B



C

2000 μm

2000 μm

Conclusions

- Post-natal inactivation of myostatin can still promote muscle growth (Grobet et al., 2003).
 - Implications
 - for future therapeutic routes in muscle wasting diseases (see also Bogdanovitch et al, 2002)
 - for new zootechnical applications...

• Dominant and male-specific double-muscling

- Perspective:
 - Generating an elite dairy cattle strain where meat and milk producing abilities segregate with sex

General strategy

- From an **autosomal** and **recessive** towards a **male-specific** and **dominant** phenotype ???
- Means: express a MSTN **trans-inhibitor** on the **Y chromosome**
 - Choice of trans-inhibitor and expression vector
 - Which region of the Y? How to target?

Trans-inhibitor cassette

- Allelic series of MSTN with potential dominant-negative effect of candidate mutations
- Follistatin, activin RIIB (dom neg) (e.g. Lee & McPherron, 2001)
- MSTN pro-domain

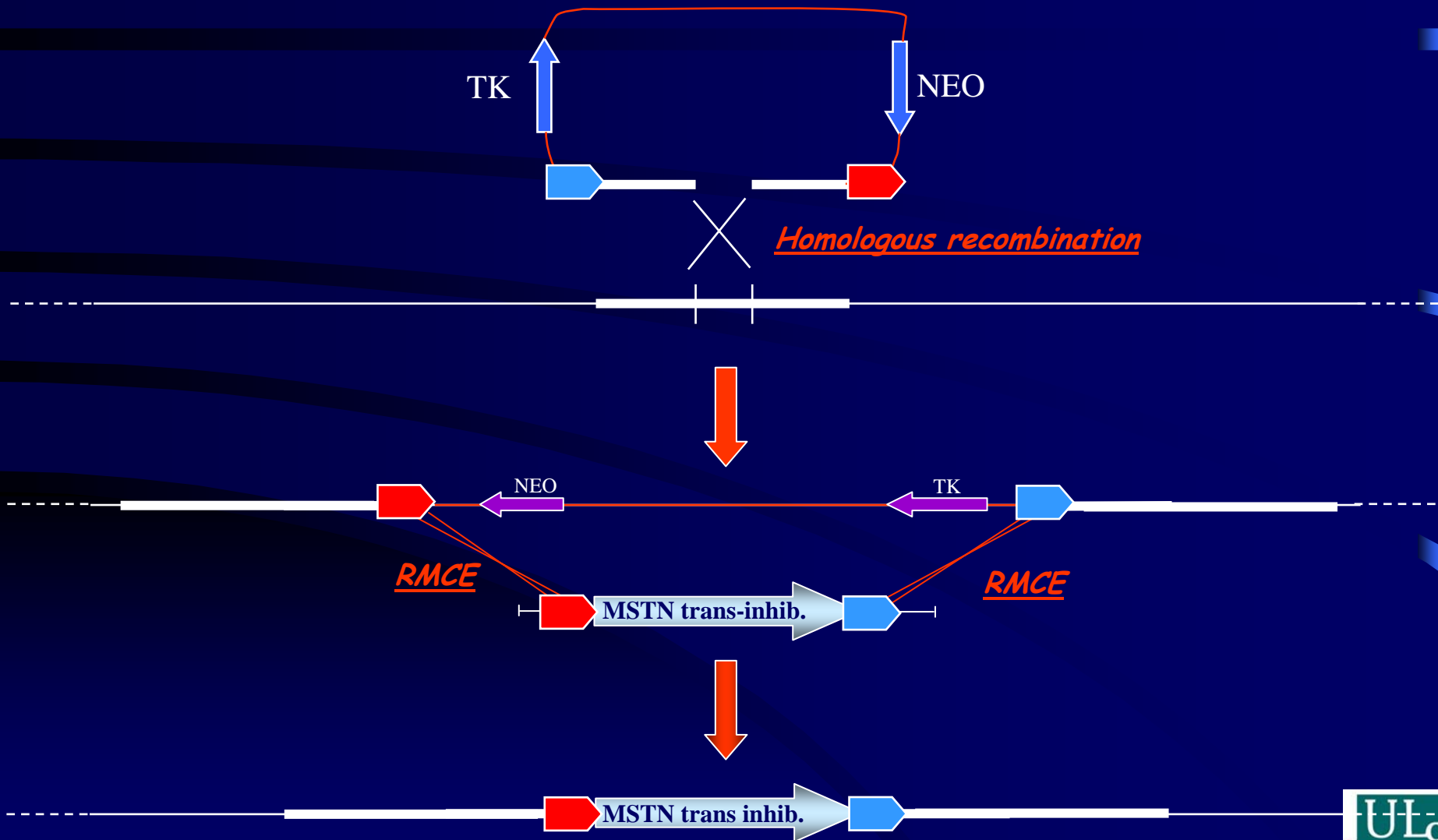
Y chromosome targeting

- Not easy to define the best strategy due to
 - the non-recombinogenic properties of the Y
 - The amount of repeats and heterochromatin...
- Options:
 - Random integration and selection in vitro...
 - On the Y PAR, close to the PAB...
 - On the Y specific region, in an « expression-able » region

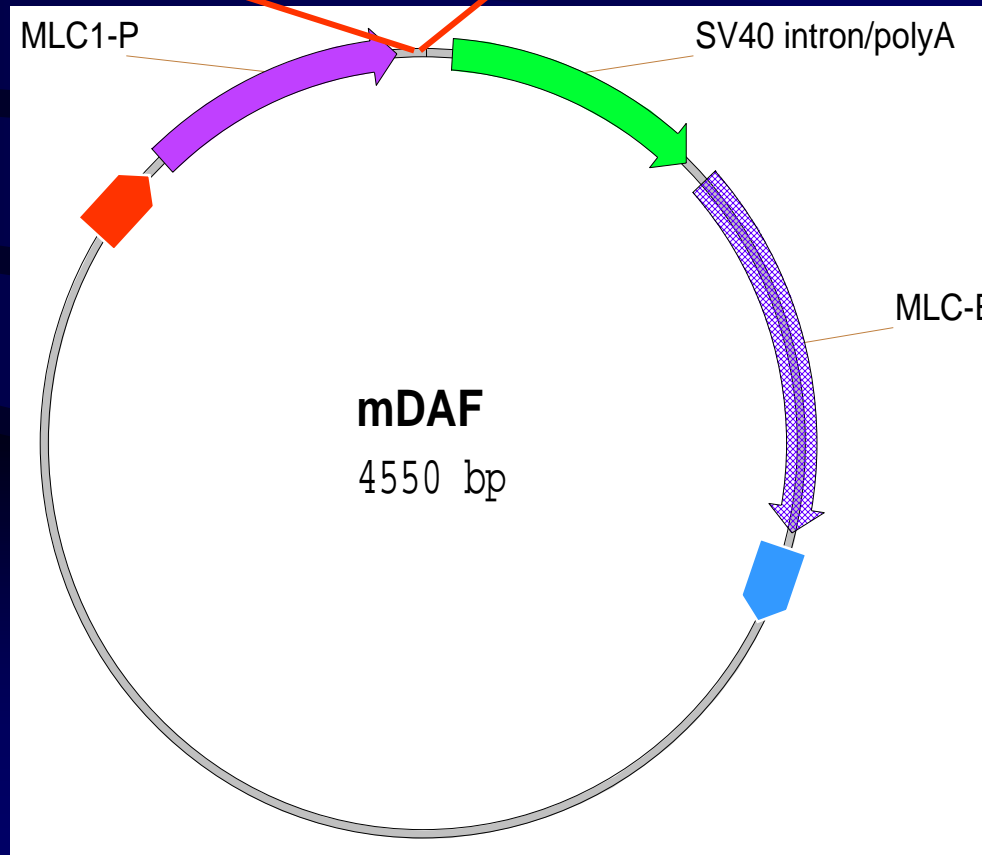
Y chromosome targeting

- Region: the Tspy pseudogene:
 - **Expressed** pseudogene on the Y chromosome of the mouse
- General protocol
 - Homologous recombination, insertion-type vector for integrating **heterologous loxP sites**
 - **RMCE** in ES cells with the expression vector
 - ... injection of ES cells in blastocysts

Y chromosome targeting



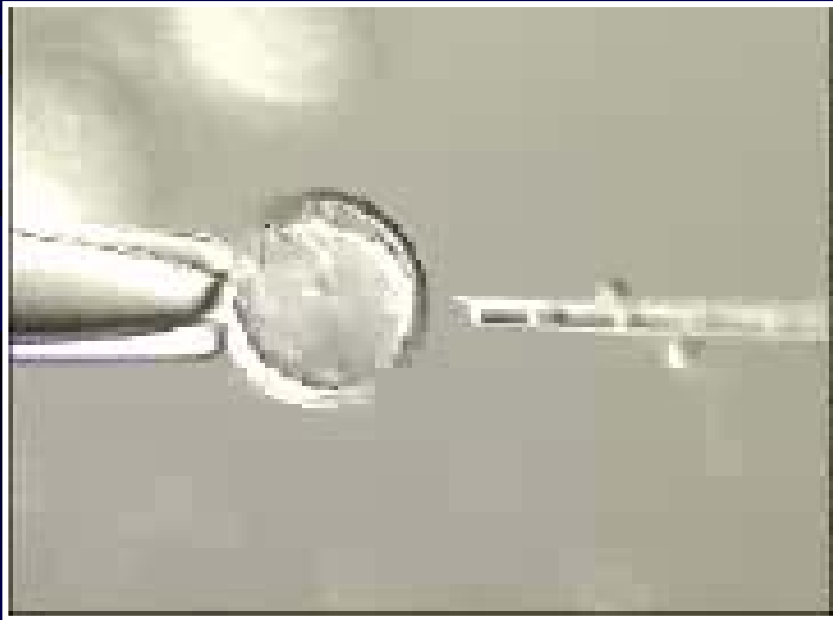
Vector for subsequent RMCE



Blastocysts microinjections



Chimerae production



Results

- Modest (+5 to +20%) but significant skeletal muscle mass increase in males
 - (Pirottin et al., 2005)

Thanks for your attention



- **Luc Grobet**
- **Dimitri Pirottin**
- **Michel Georges**