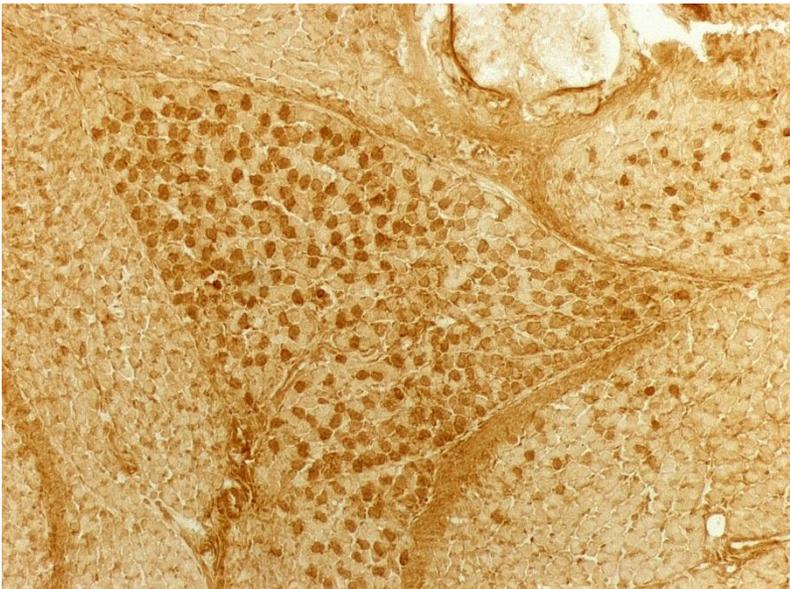


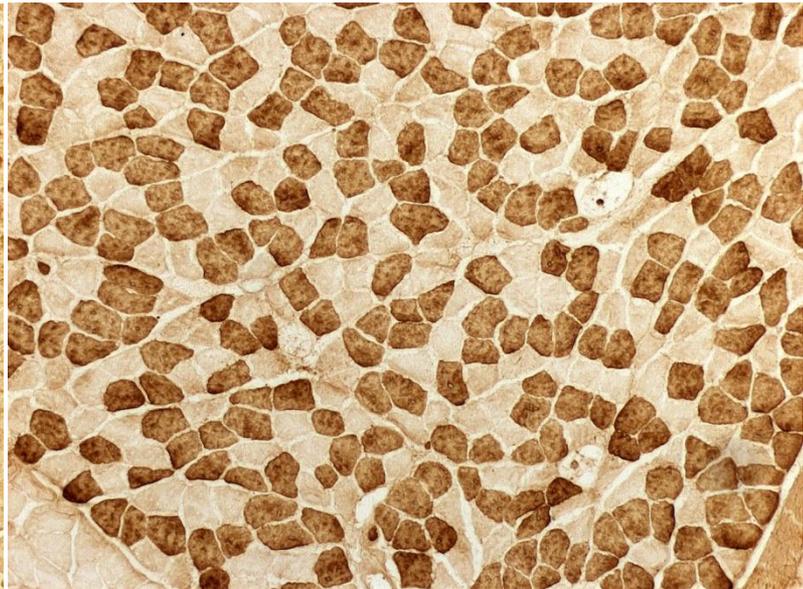
Exercise 1: Estimate the degree of MATURATION  
HYPERTROPHY that occurs in mouse SOLEUS  
muscle fibres between birth and adulthood

Images taken at same magnification – low power

BIRTH



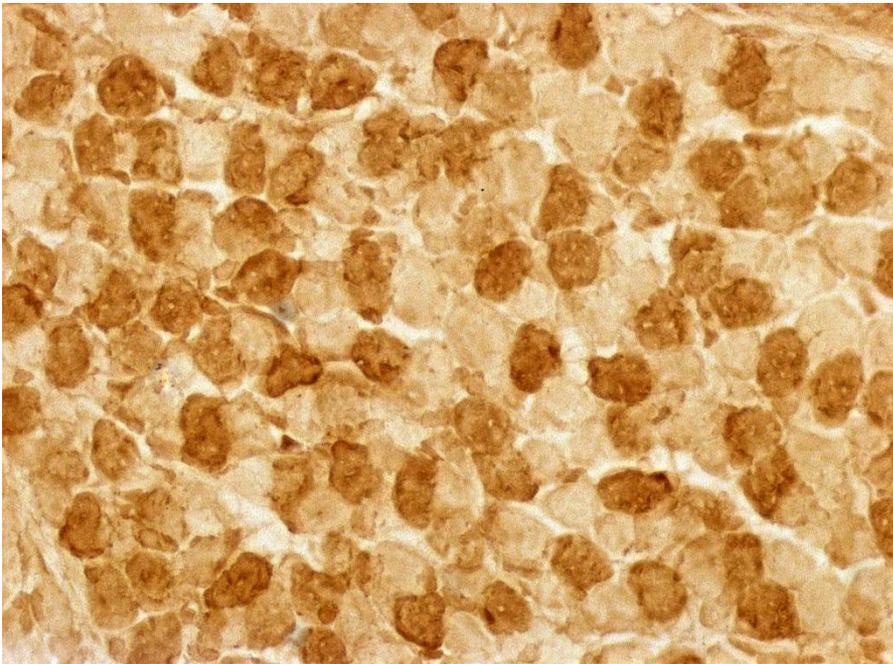
ADULT



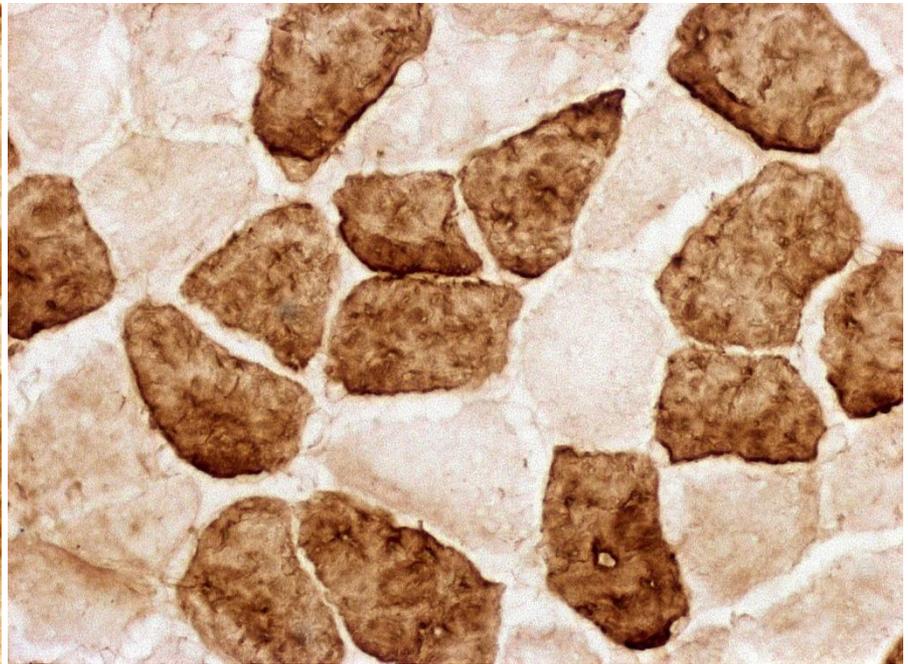
Exercise 1: Estimate the degree of MATURATION  
HYPERTROPHY that occurs in mouse SOLEUS  
muscle fibres between birth and adulthood

Images taken at same magnification – HIGH power

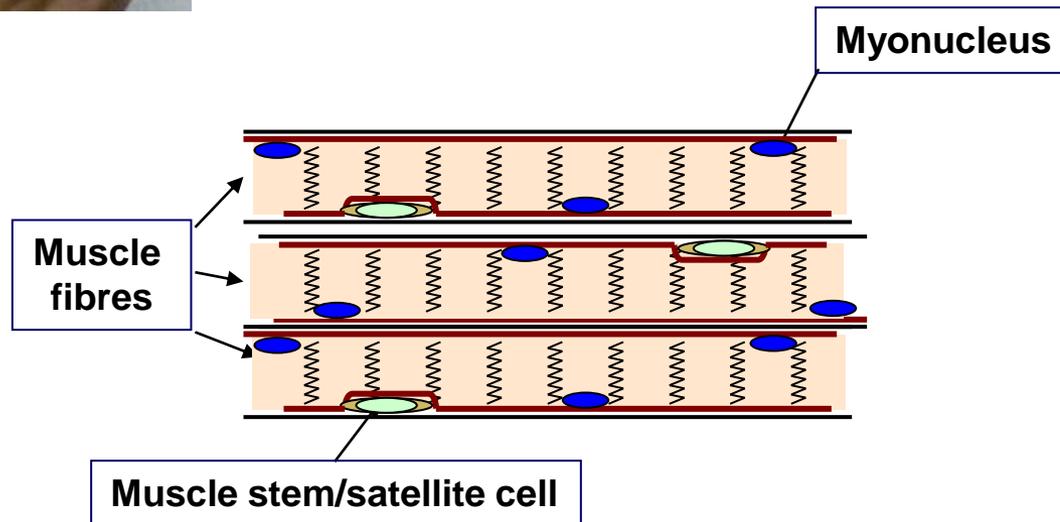
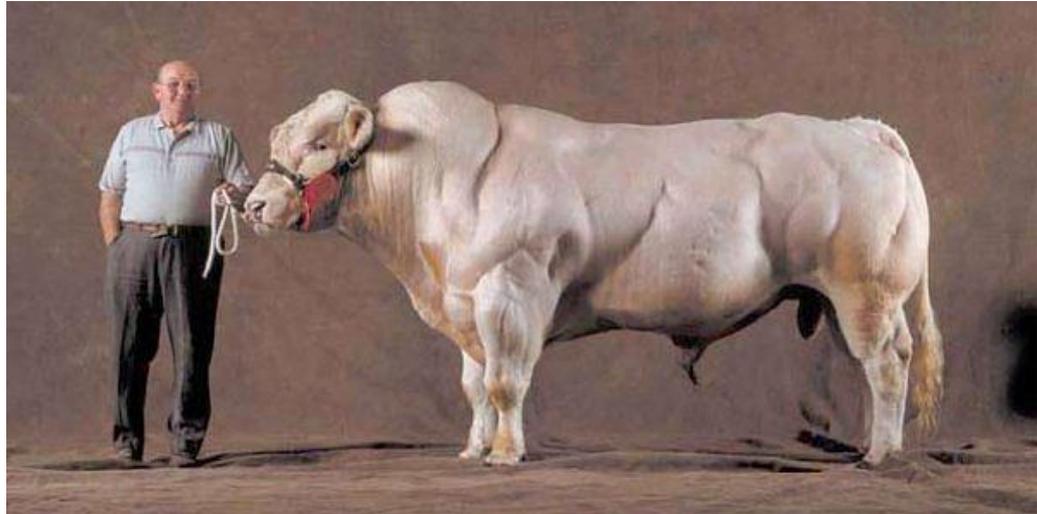
BIRTH



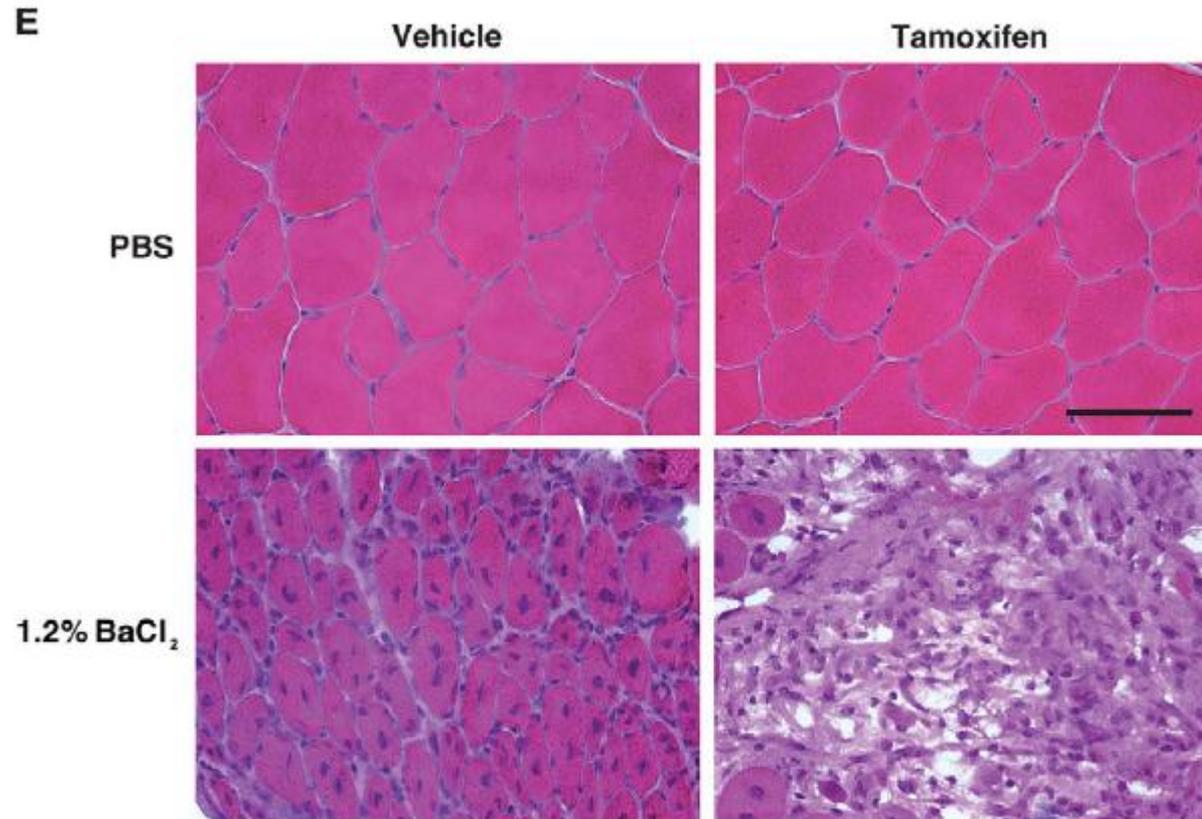
ADULT



# Are satellite cells necessary for muscle hypertrophy?

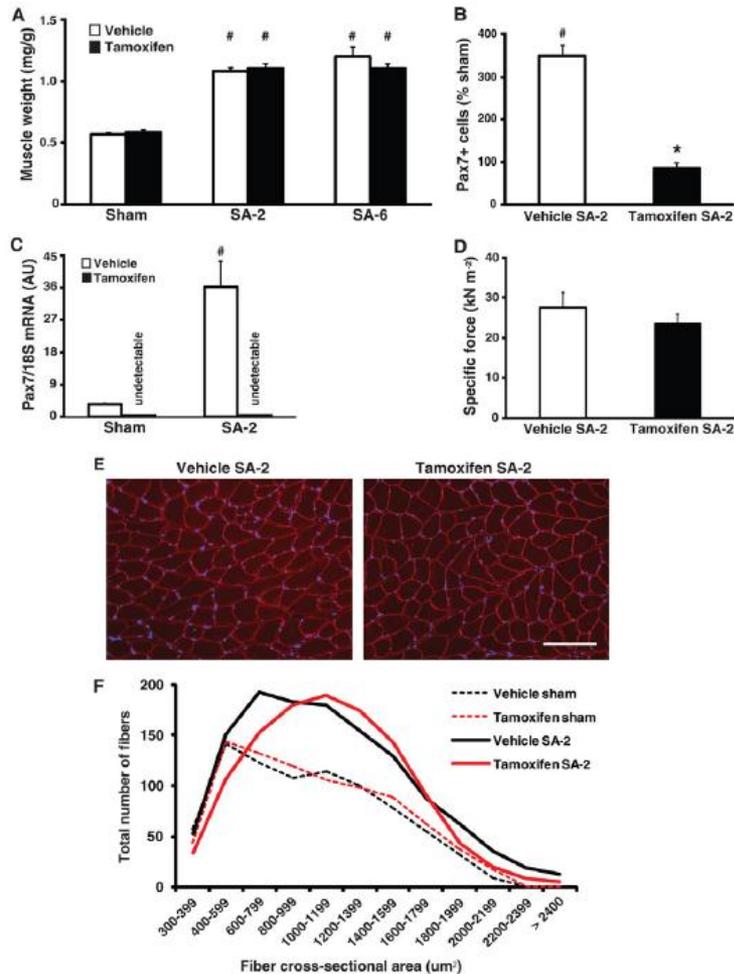


$Pax7^{CreER/CreER}$  mice crossed with Floxed DTA mice  
Treated with Tamoxifen to induce the Cre expression



Barium Chloride BaCl<sub>2</sub> causes muscle damage

# Synergist ablation (SA): removing nearby muscles to induce hypertrophy experimentally in the Pax7-DTA mice



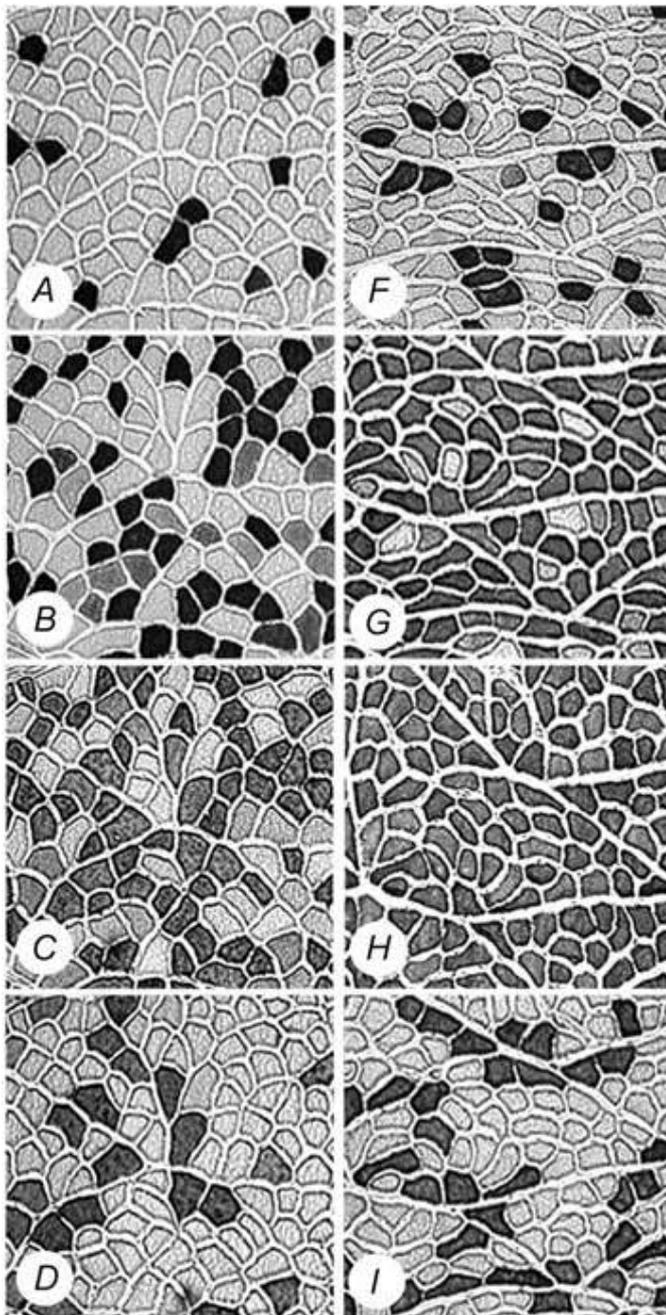
Exercise 2: read the results section of the McCarthy et al. 2011 paper

Discuss

## Experimental induction of muscle fibre type change

(A) Chronic low frequency stimulation

(B) Transgene expression



## Antibody

MyHC I

MyHC IIa

All MyHC  
but not IIx

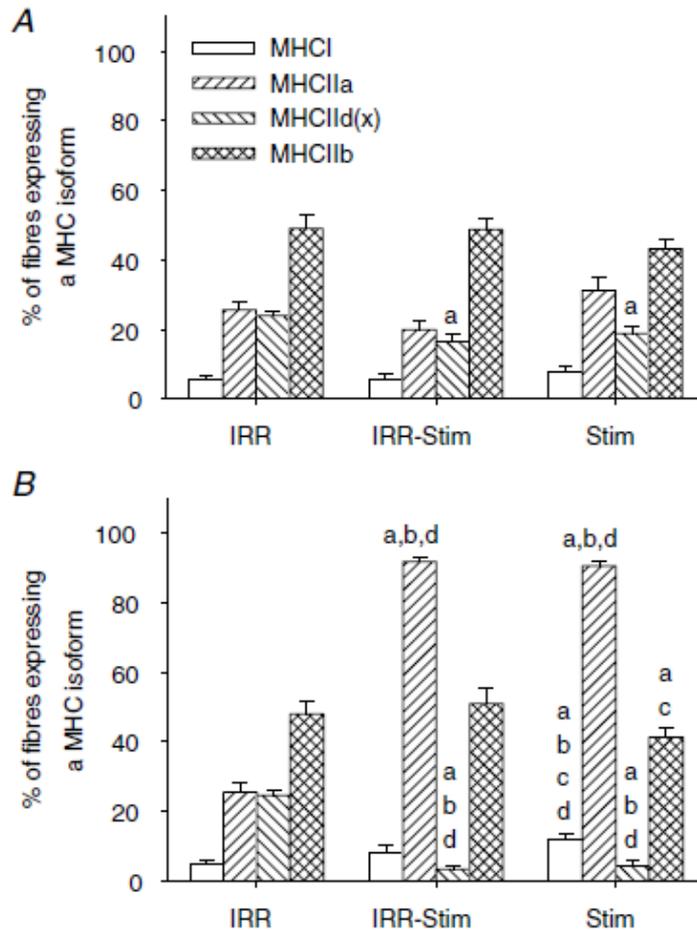
MyHC IIb

**Exercise 3:** Chronic Low Frequency Stimulation Experiment – Rat left common peroneal nerve stimulated 21 days with CLFS. Transverse sections of Tibialis Anterior muscle stained with Antibodies against the different MyHC protein isoforms (dark coloured fibres)

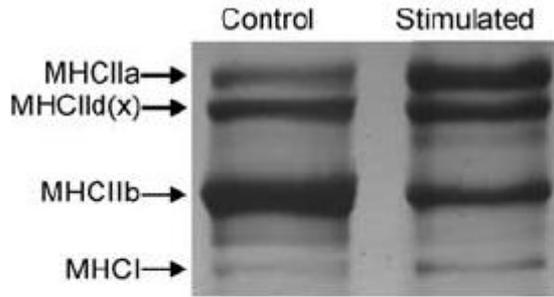
A,B,C,D – Control no CLFS  
F,G,H,I – CLFS for 21 days

Count total number of fibres in field  
Count number of fibres stained by each antibody  
Express proportion of each fibre population as a percentage of total.  
Discuss findings

# Measuring fibre type conversion using immunohistochemistry on muscle sections



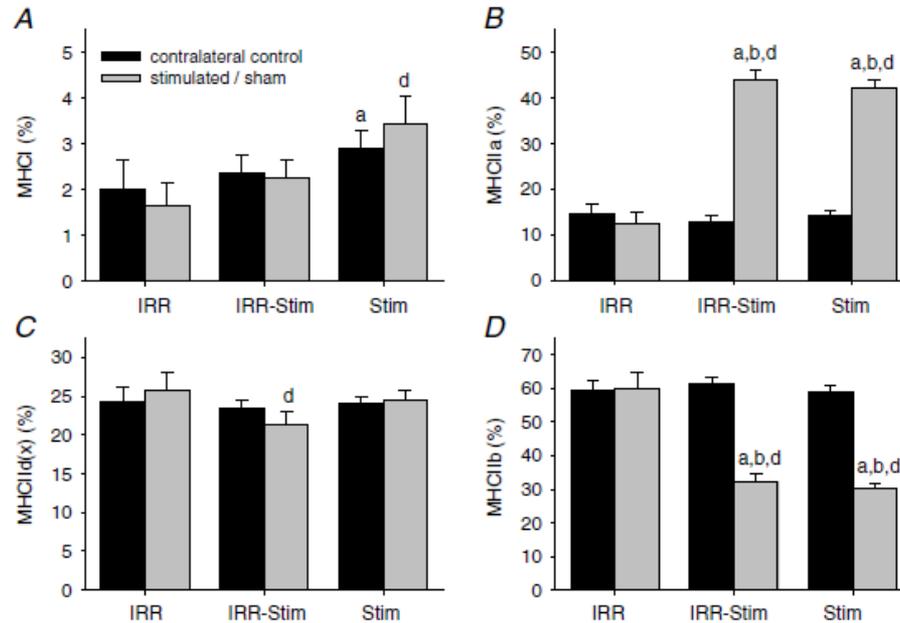
**Figure 6**  
 The percentage of fibres expressing a particular MHC isoform in contralateral control (A) and stimulated/sham rat tibialis anterior muscles (B). Statistical symbols as in Fig. 2.



## Measuring fibre conversion using protein gels

**Figure 8**

Example of the electrophoretic method used to quantify MHC isoform composition of rat tibialis anterior muscles. Control (IRR-control) and stimulated (Stim) are shown.



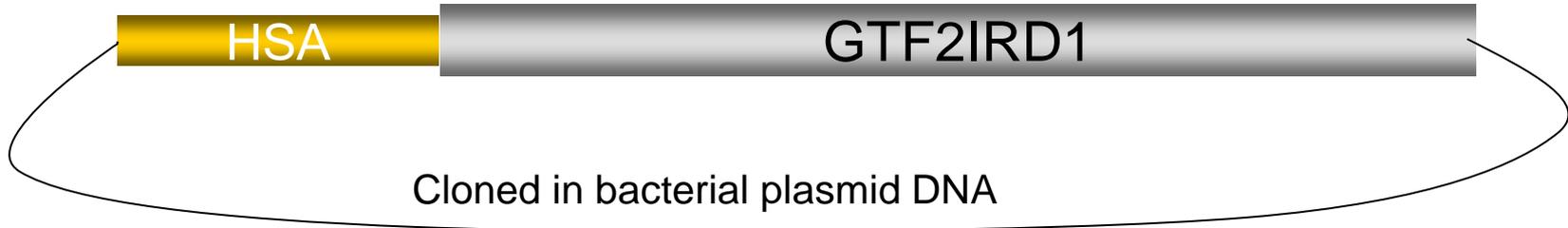
**Figure 9**

Percentage of MHCI (A), MHCIIa (B), MHCII d(x) (C) and MHCIIb (D) distribution as determined by densitometric evaluation of triplicate gels. Statistical symbols as in Fig. 2.

# Transgene-induced fibre type change

Human skeletal actin promoter and enhancer combined – EXPRESSION ONLY IN MUSCLE FIBRES

Human *GTF2IRD1* gene encodes a nuclear DNA binding protein that controls other genes



DNA microinjected into a fertilized mouse embryo to make a transgenic mouse.

WILD-TYPE

TRANSGENIC

Antibody  
MyHC I



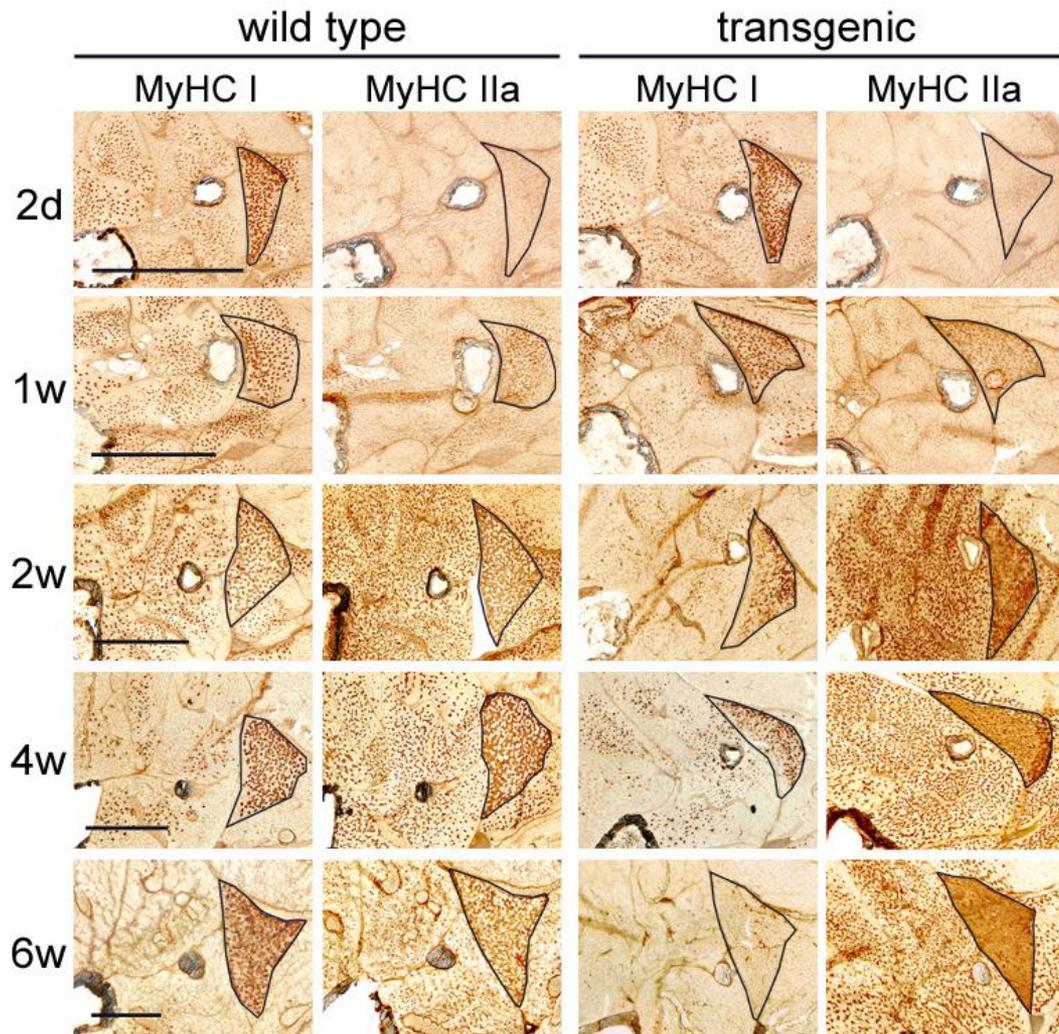
Antibody  
MyHC IIa



Transverse sections through the lower hind-limb of adult GTF2IRD1-transgenic and wild type mice - stained for MyHC type I/slow and MyHC2A.

1. Describe what has happened to the muscle fibre types
2. Propose a theory for what might have caused the observed effect. Is there more than one possibility?

# Examining the developing fibre types in *Gtf2ird1*-transgenic mice.



Transverse sections through the lower hind limb of transgenic and wild type mice from 2 days after birth to 6 weeks.

## Questions

1. Has embryonic fibre type patterning been affected by the expression of the transgene?
2. What process would describe what is happening?
3. Refine the theory concerning the effect of *Gtf2ird1* on muscle development.
4. How would you prove that *Gtf2ird1* has an important role in fibre type differentiation?

## Questions

1. Define muscle plasticity?
2. Are satellite cells (a) necessary for muscle hypertrophy and (b) generally involved in hypertrophy?
3. Why does chronic low frequency stimulation cause a fast to slow fibre type shift?
4. Spinal cord injury leading to no motor function in the legs – what happens to muscle mass and fibre type?