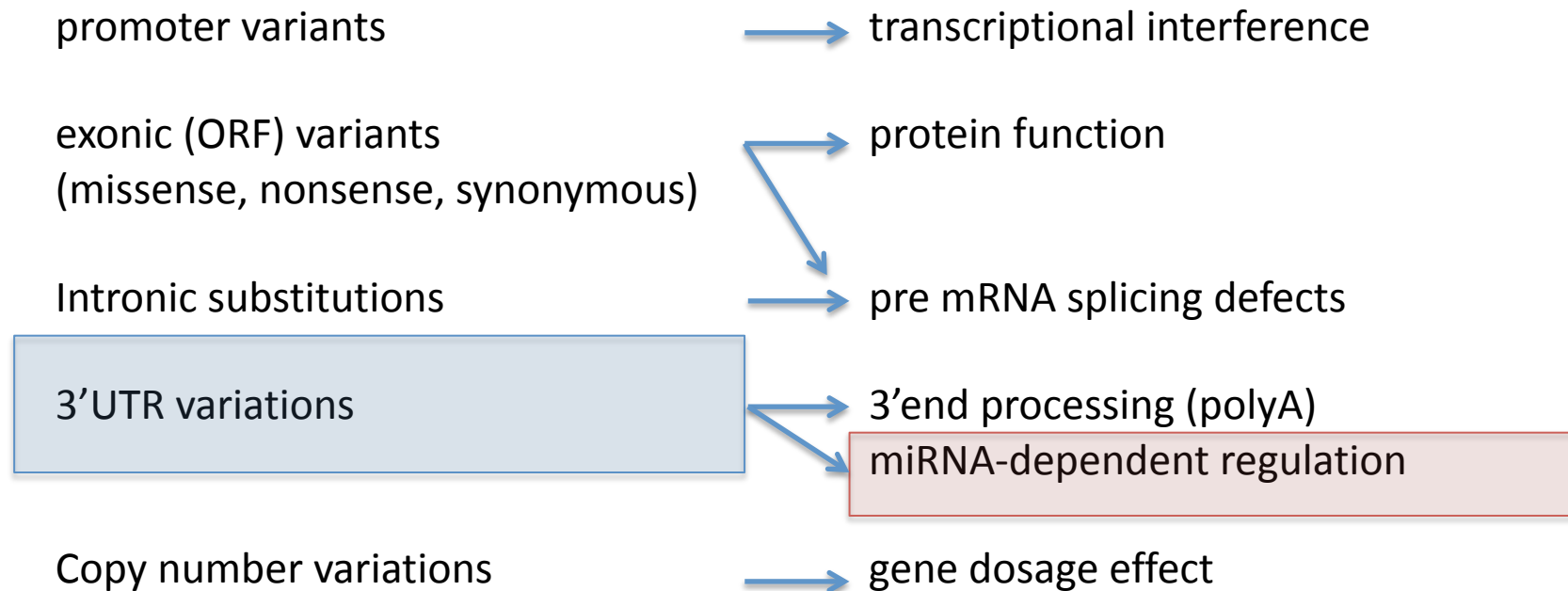
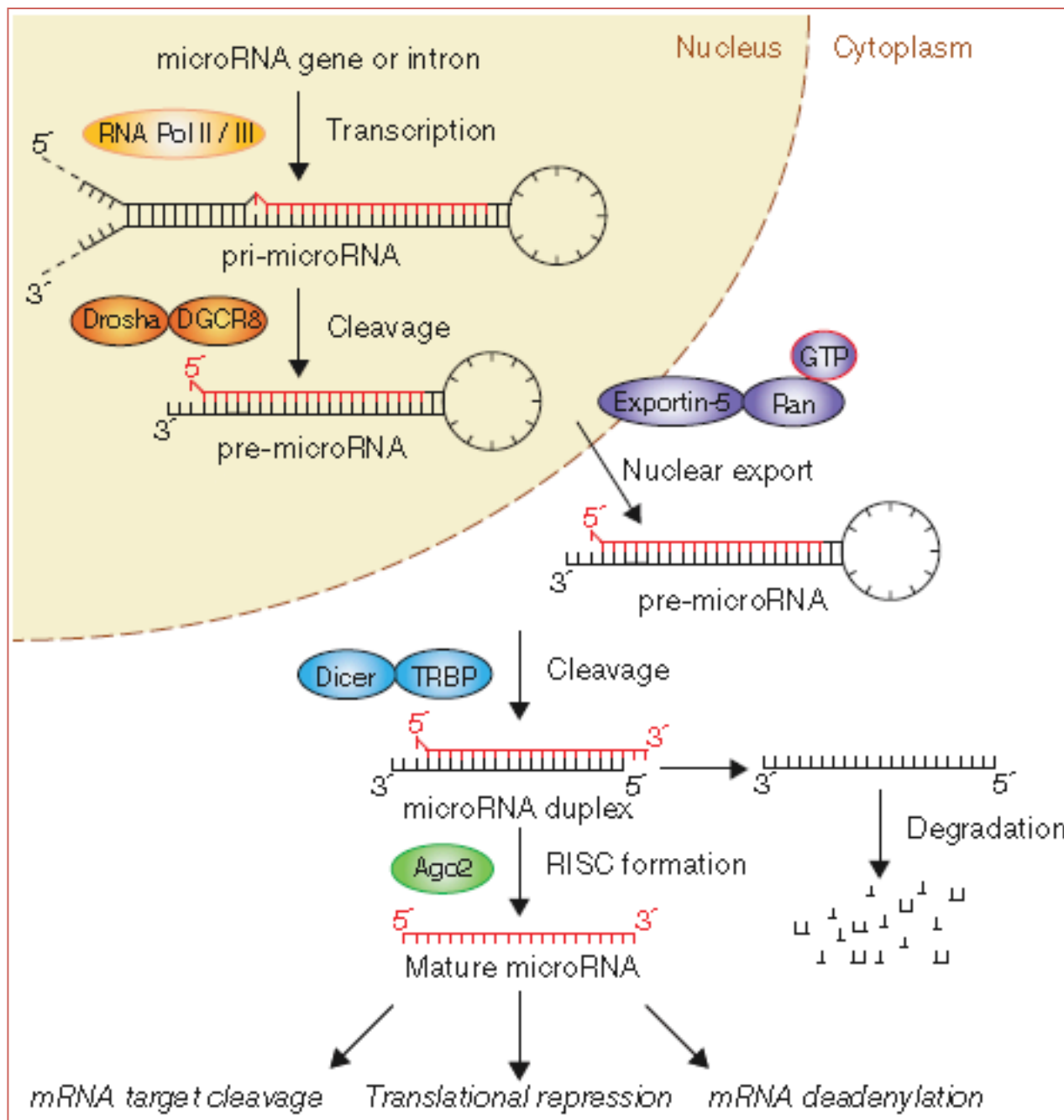
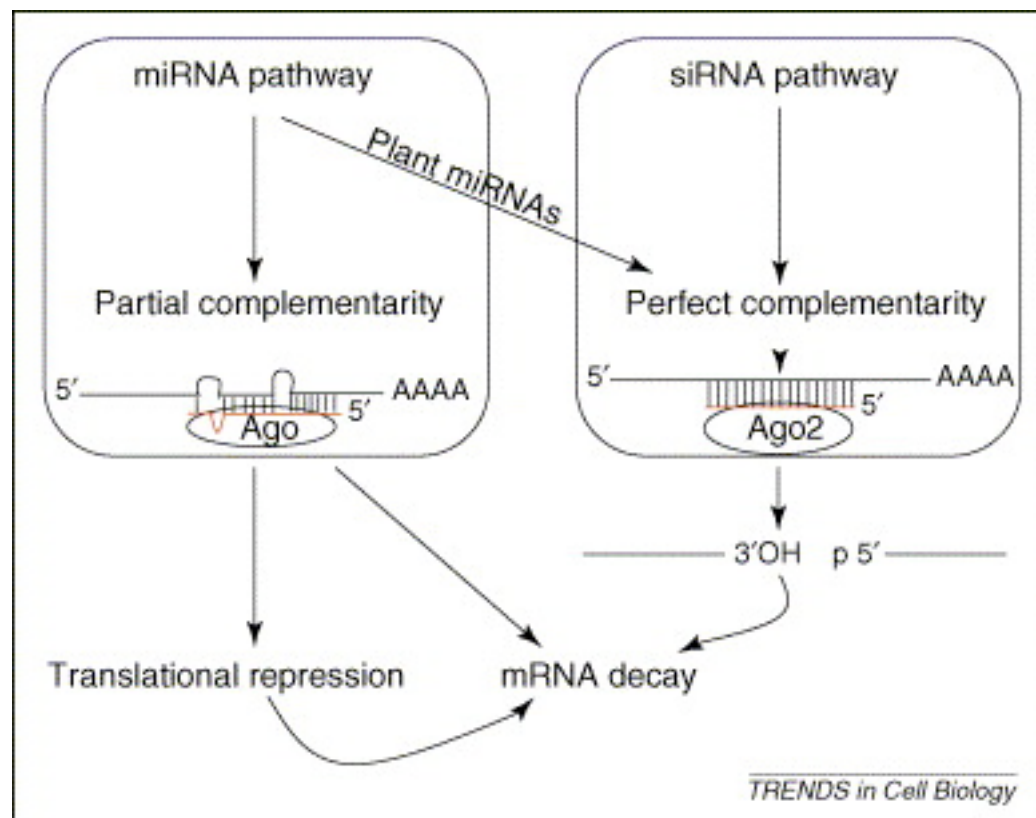


Human genomic variants and possible effect on gene expression







Common SNPs in 3'UTRs affect the miRNA target site content of thousands of human genes ...

SNPs

92,967 SNPs in 21,206 3'UTRs

miRNA targets

540 X-targets (Xie et al., 2005)
444 L-targets (Lewis et al., 2005)

	Conserved	Non-conserved
Destroyed	X: 463 L: 190 X+L: 118	X: 3,805 L: 4,551 X+L: 651
Created	X: 0 L: 0 X+L: 0	X: 4,328 L: 4,823 X+L: 703
Polymorphic		X: 210 L: 311 X+L: 39
Shifted		X: 78 L: 118 X+L: 5

Polymorphisms affecting miRNA-mRNA interactions and phenotype

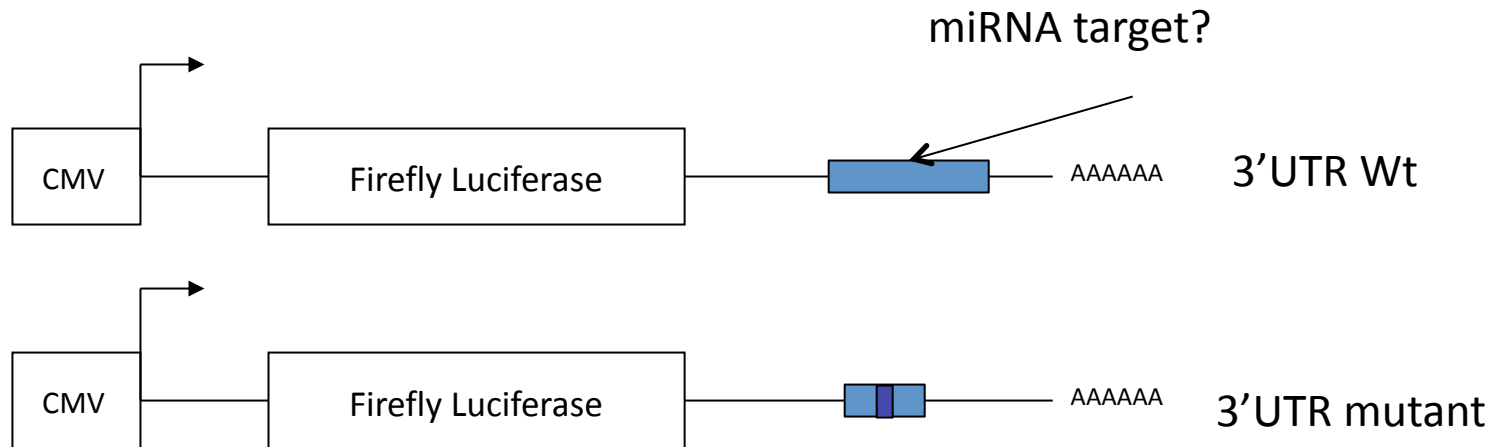
- SLITRK1 in Tourette's syndrome
- Angiotensin receptor-1 and hypertension
- Dihydrofolate reductase and metotrexate resistance
- HLA-G and increased risk of asthma
- Fibroblast growth factor 20 and increased risk for Parkinson
- IGF-II receptor and type 2 diabetes

- Polygenic muscularity in Texel sheep and Myostatin

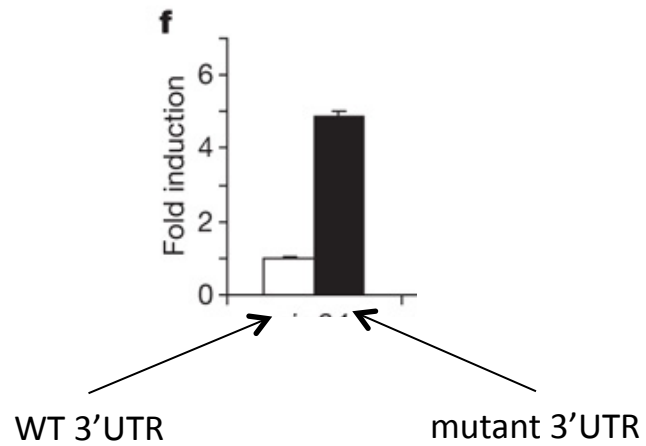
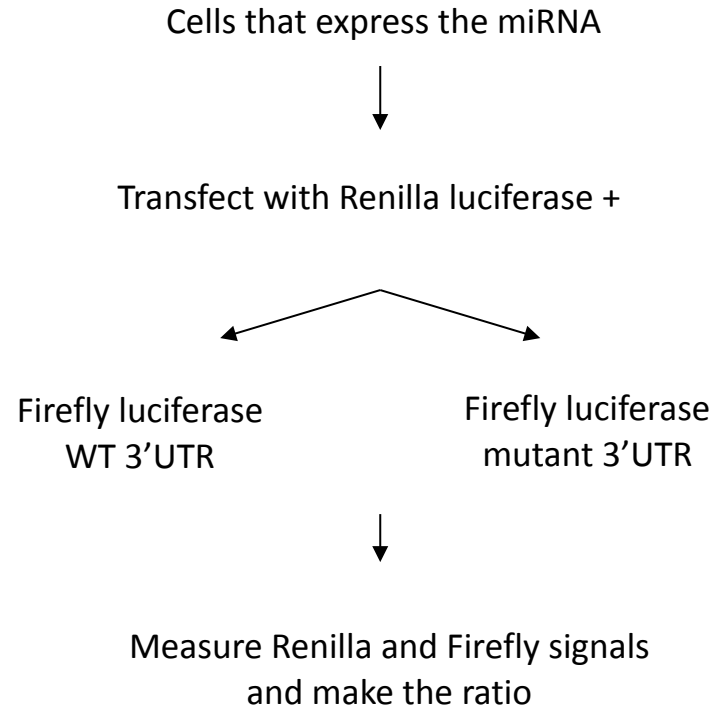
A typical DUAL reporter assay for miRNA analysis



HSV-TK: herpes simplex virus thymidine kinase promoter
-> low to moderate levels of Renilla luciferase expression in co-transfected mammalian cells



(renilla and firefly can be inverted)



COMPLEMENTARY STRATEGIES

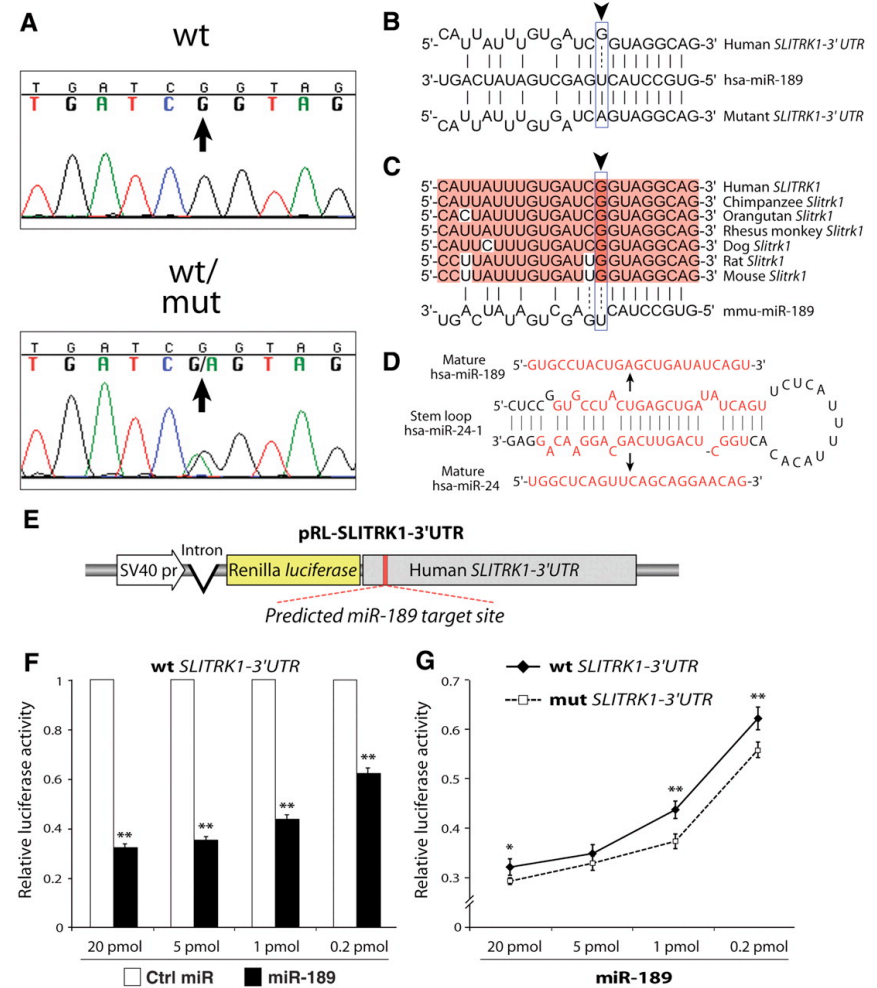
selectively block the miRNA with
LNA antisense oligo
or
overexpress the miRNA
in cells that do not express it endogenously

A genomic variant in the SLITRK1 3'UTR has been implicated in Tourette syndrome

Tourette syndrome is a neurobehavioral disorder manifest particularly by motor and vocal tics and associated with behavioral abnormalities.

- Linkage of the GTS phenotype to several sites
- Heterozygous mutation in the histidine decarboxylase gene on chromosome 15q21.
- Variation in the SLITRK1 gene on 13q
- Other sites reported

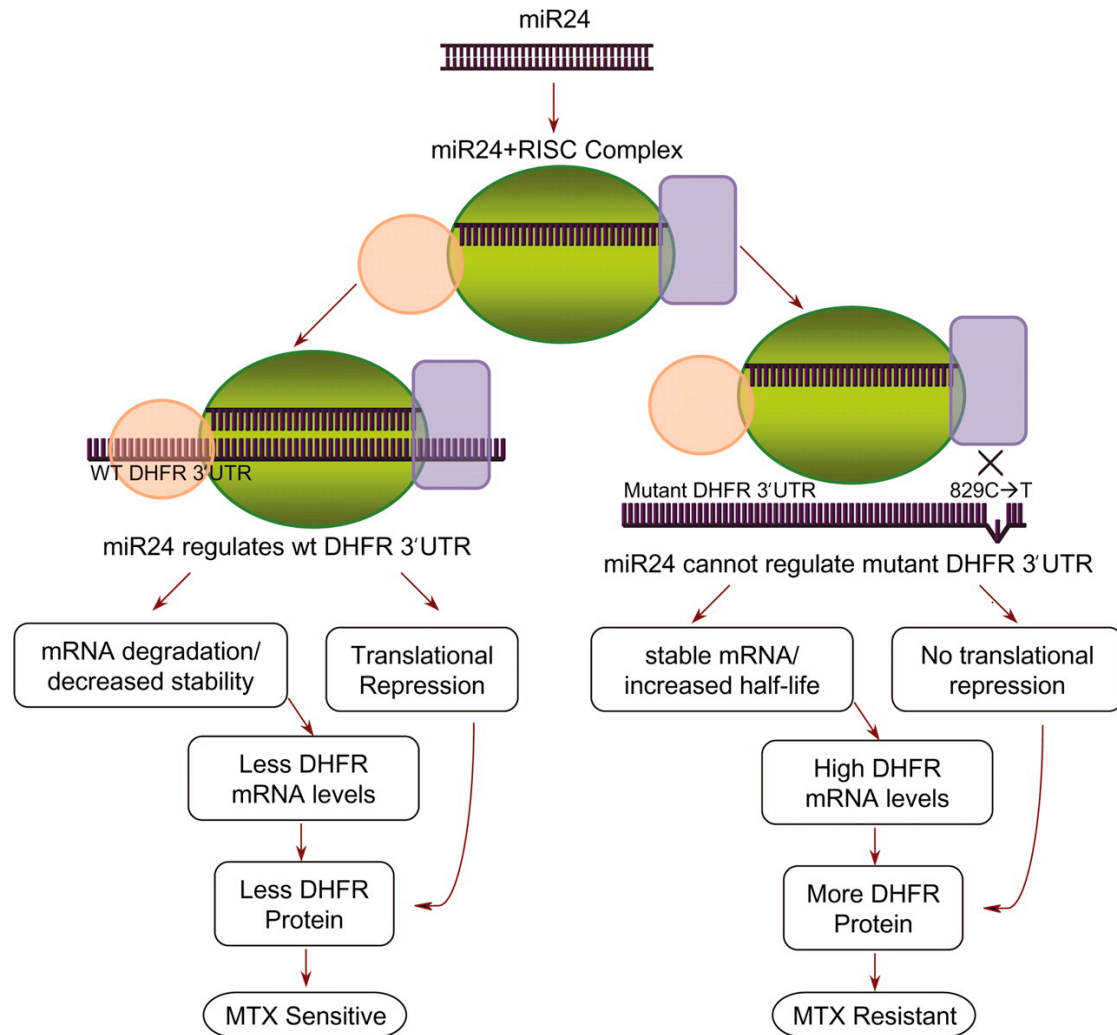
Slit and Trk-like family member 1 (SLITRK1), encoding a single-pass transmembrane protein with two leucine-rich repeat (LRR) motifs in its extracellular domain



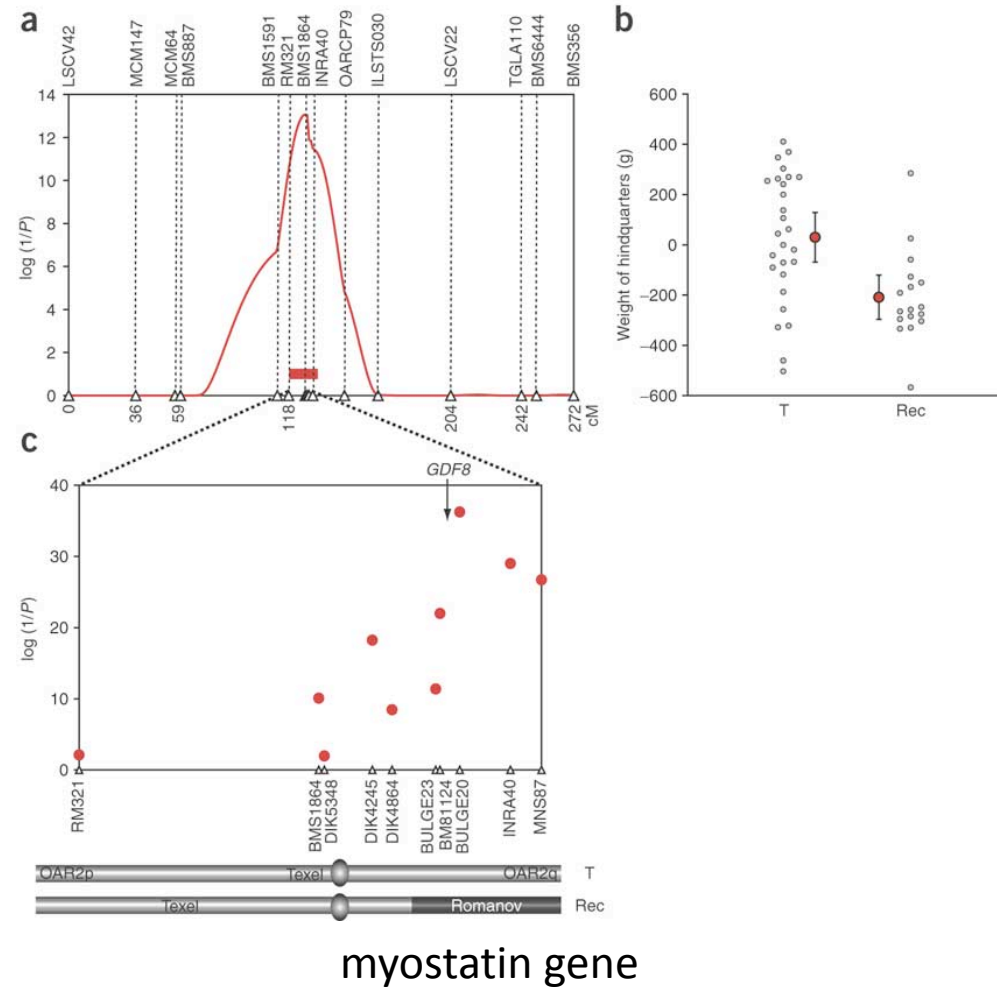
Abelson JF et al Science. 2005. Sequence variants in SLITRK1 are associated with Tourette's syndrome.

A model for miR-24-mediated regulation of DHFR

- Methotrexate is used in treatment of cancer and autoimmune diseases
- It competitively inhibits dihydrofolate reductase (DHFR), an enzyme that participates in the tetrahydrofolate synthesis.
- The miRSNP-829C→T leads to loss of miR-24-mediated regulation of DHFR

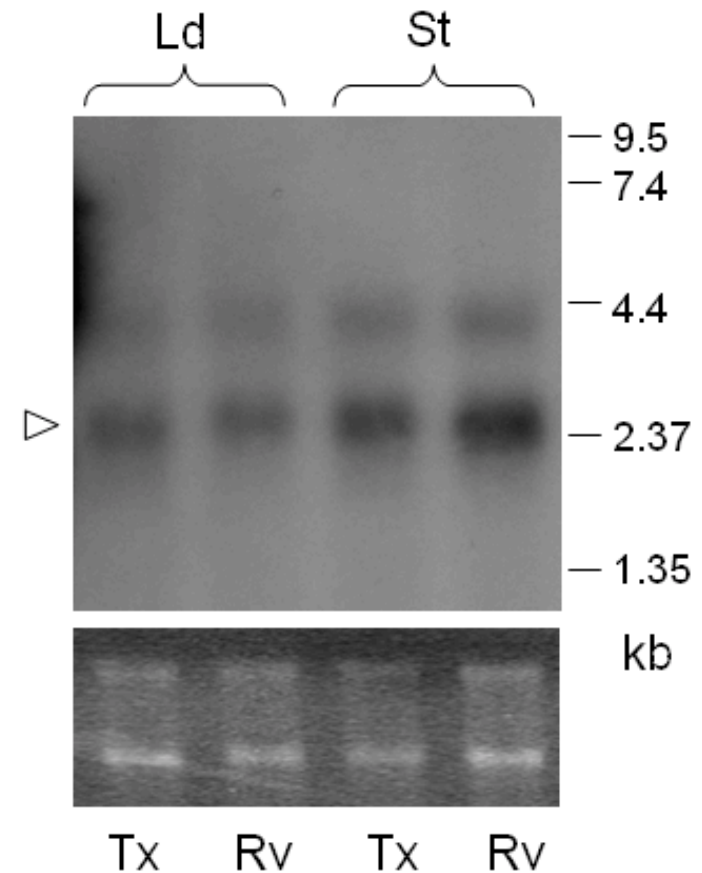


Polygenic muscularity in Texel sheep and Myostatin

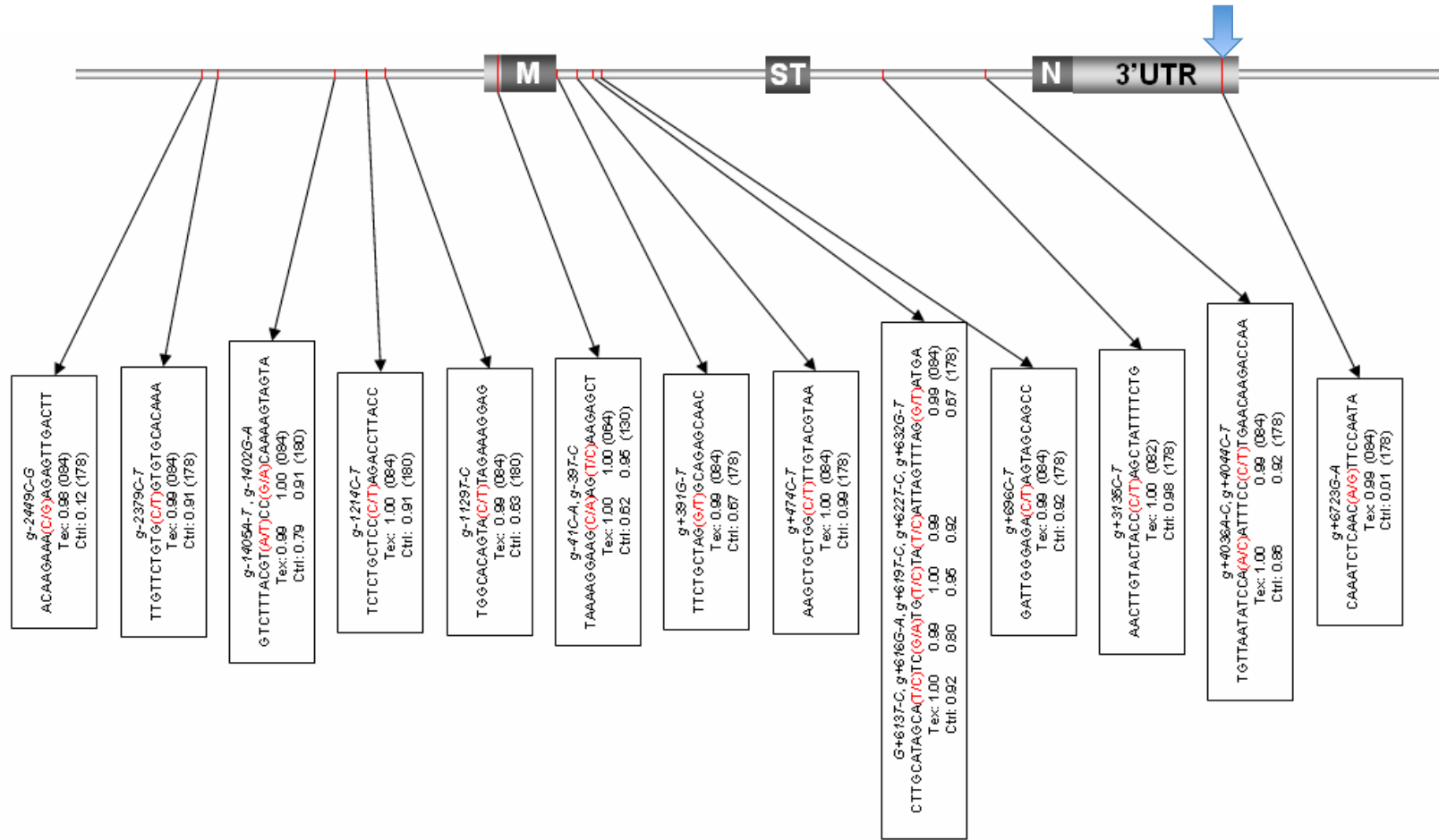


Texel sheep produce normal levels of normal *MSTN* mRNA

- Sequencing the *MSTN* ORF from gDNA & cDNA:
 - no polymorphism.
- Northern blot and qRT-PCR:
 - no obvious qualitative or quantitative difference.



Resequencing the *MSTN* gene identifies 20 non-coding SNPs ...



G+6723G-A in the 3'UTR is predicted to be a target site for *miR1*, *miR206*
miR1 and *miR206* are conserved in sheep and strongly expressed in skeletal muscle

```

miR-1-1
Ovis      CTACCTGCTTGGGAACACTACTTCTTTATGTGCCATATGGACCTGCTAAGCTATGGAATGTAAGAAGTATGTAATTCAGTCCGGA
Bos       CTACCTGCTTGGGAACACTACTTCTTTATGTGCCATATGGACCTGCTAAGCTATGGAATGTAAGAAGTATGTAATTCAGTCCGGA
Canis     CTACCCGCTTGGGAACACTACTTCTTTATGTGCCATATGGACCTGCTAAGCTATGGAATGTAAGAAGTATGTAATTCAGTCCGGA
Homo      CTCCCTGCTTGGGAACACTACTTCTTTATGTGCCATATGGACCTGCTAAGCTATGGAATGTAAGAAGTATGTAATTCAGTCCGGA
Mus       CTACCTGCTTGGGAACACTACTTCTTTATGTGCCATATGGACCTGCTAAGCTATGGAATGTAAGAAGTATGTAATTCAGTCCGGA
* * * * *

miR-1-2
Homo      TACCCTACTCAGAGTACATACTTCTTTATGTACCCATATGAACATACAATGCTATGGAATGTAAGAAGTATGTAATTTGGTAGGC
Canis     AACCTACTCAGAGTACATACTTCTTTATGTACCCATATGAACATACAATGCTATGGAATGTAAGAAGTATGTAATTTGGTAGGC
Bos       TACCCTACTCAGAGTACATACTTCTTTATGTACCCATATGAACATACAATGCTATGGAATGTAAGAAGTATGTAATTTGGTAGGC
Ovis     TACCCTACTCAGAGTACATACTTCTTTATGTACCCATATGAACATACAATGCTATGGAATGTAAGAAGTATGTAATTTGGTAGGC
Mus       TGCCCTACTCAGAGCACATACTTCTTTATGTACCCATATGAACATTCAGTGCTATGGAATGTAAGAAGTATGTAATTTGGTAGGT
* * * * *

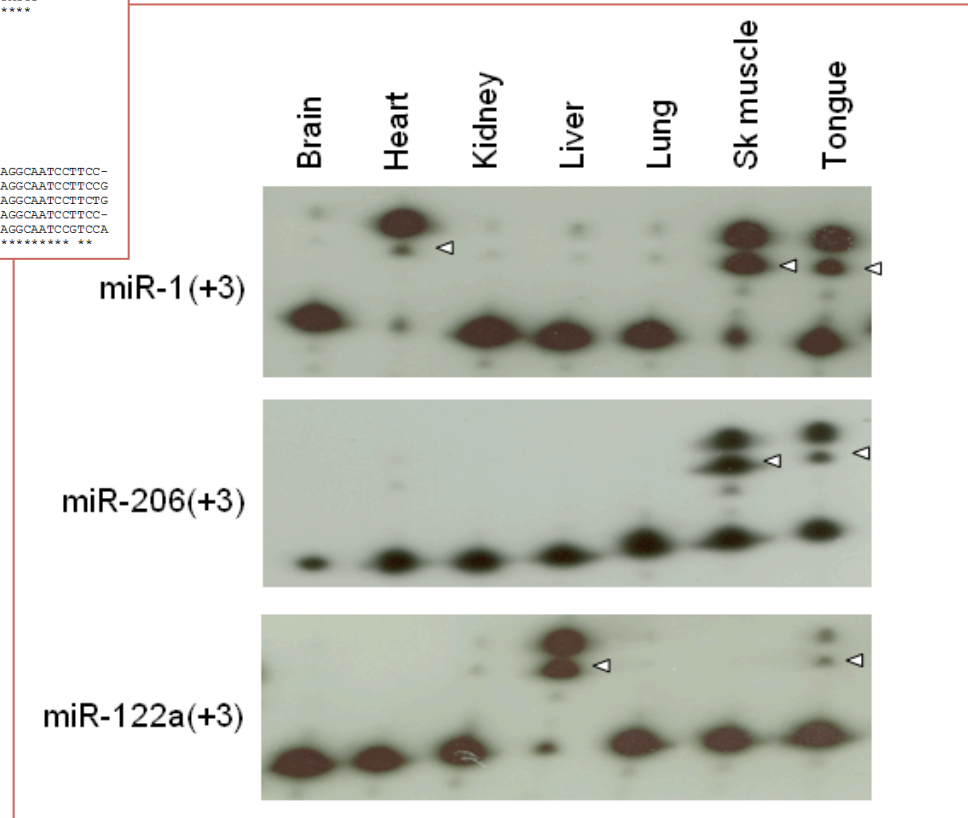
miR-206
Ovis     CTTCCCAAGGCCACATGCTTCTTTATATCCCATACGGATTACTTTGCTATGGAATCTAAGGAAGTGTGTGGTTT
Bos      CTTCCCAAGGCCACATGCTTCTTTATATCCCATACGGATTACTTTGCTATGGAATCTAAGGAAGTGTGTGGTTT
Canis    CTTCCCGAGGCCACATGCTTCTTTATATCCCATACGGATTACTTTGCTATGGAATCTAAGGAAGTGTGTGGTTT
Homo     CTTCCCGAGGCCACATGCTTCTTTATATCCCATACGGATTACTTTGCTATGGAATCTAAGGAAGTGTGTGGTTT
Mus      CTTCCCGAGGCCACATGCTTCTTTATATCCTCATAGATATCTCAGCACTATGGAATCTAAGGAAGTGTGTGGTTT
* * * * *

miR-122a
Ovis     CCTTAGCAGAGCTGTGAGTGTGACAAATGCTTGTGTCCTCAAACTATCAAAGCCATTATCACACTAAATAGCTACTGTTAGGCAATCCTTCC-
Bos      CCTTAGCAGAGCTGTGAGTGTGACAAATGCTTGTGTCCTCAAACTATCAAAGCCATTATCACACTAAATAGCTACTGTTAGGCAATCCTTCCG-
Canis    CCTTAGCAGAGCTGTGAGTGTGACAAATGCTTGTGTCCTCAAACTATCAAAGCCATTATCACACTAAATAGCTACTGTTAGGCAATCCTTCCG-
Homo     CCTTAGCAGAGCTGTGAGTGTGACAAATGCTTGTGTCCTCAAACTATCAAAGCCATTATCACACTAAATAGCTACTGTTAGGCAATCCTTCC-
Mus      CCTTAGCAGAGCTGTGAGTGTGACAAATGCTTGTGTCCTCAAACTATCAAAGCCATTATCACACTAAATAGCTACTGTTAGGCAATCCTTCC-
* * * * *

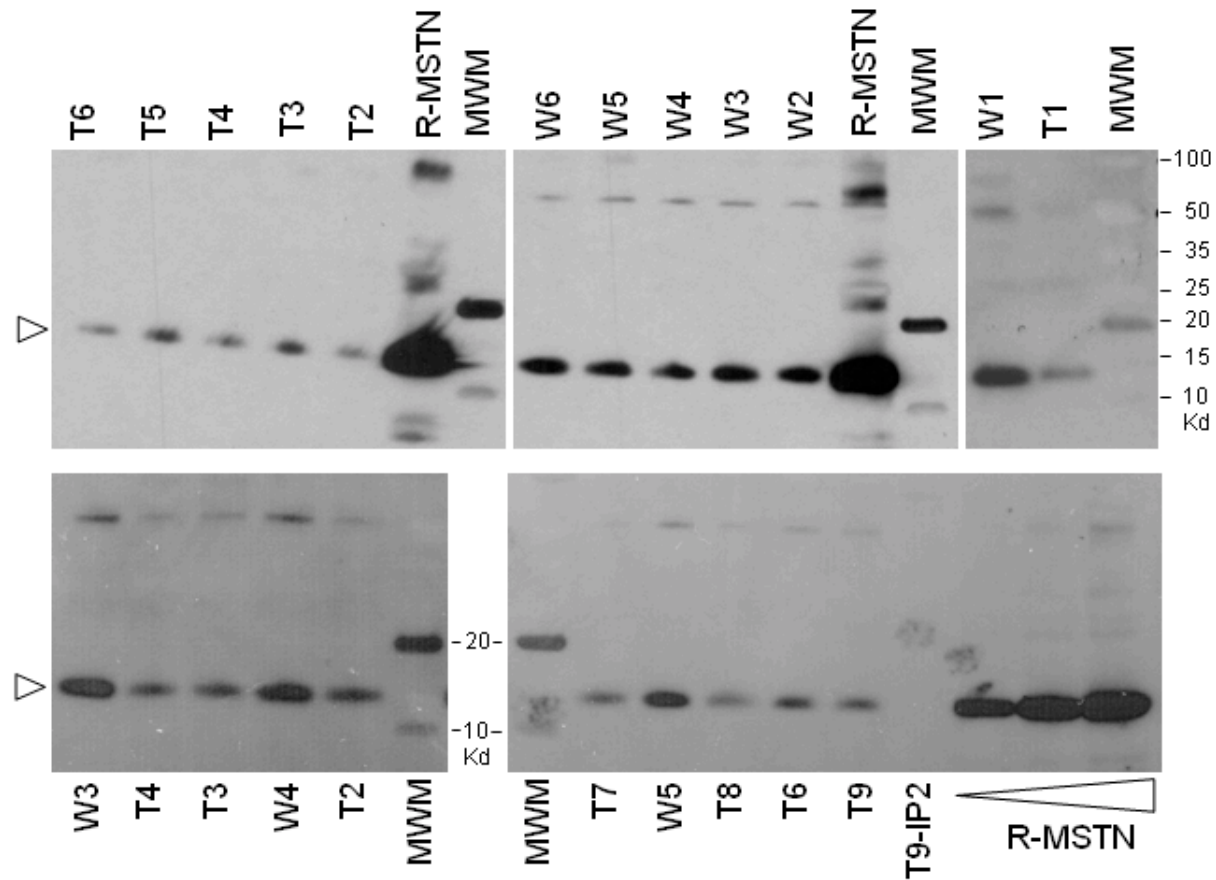
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miR1 and *miR206* are strongly expressed in SM

miR1.1, *miR1.2*, *miR122* and *miR206* are conserved in sheep.

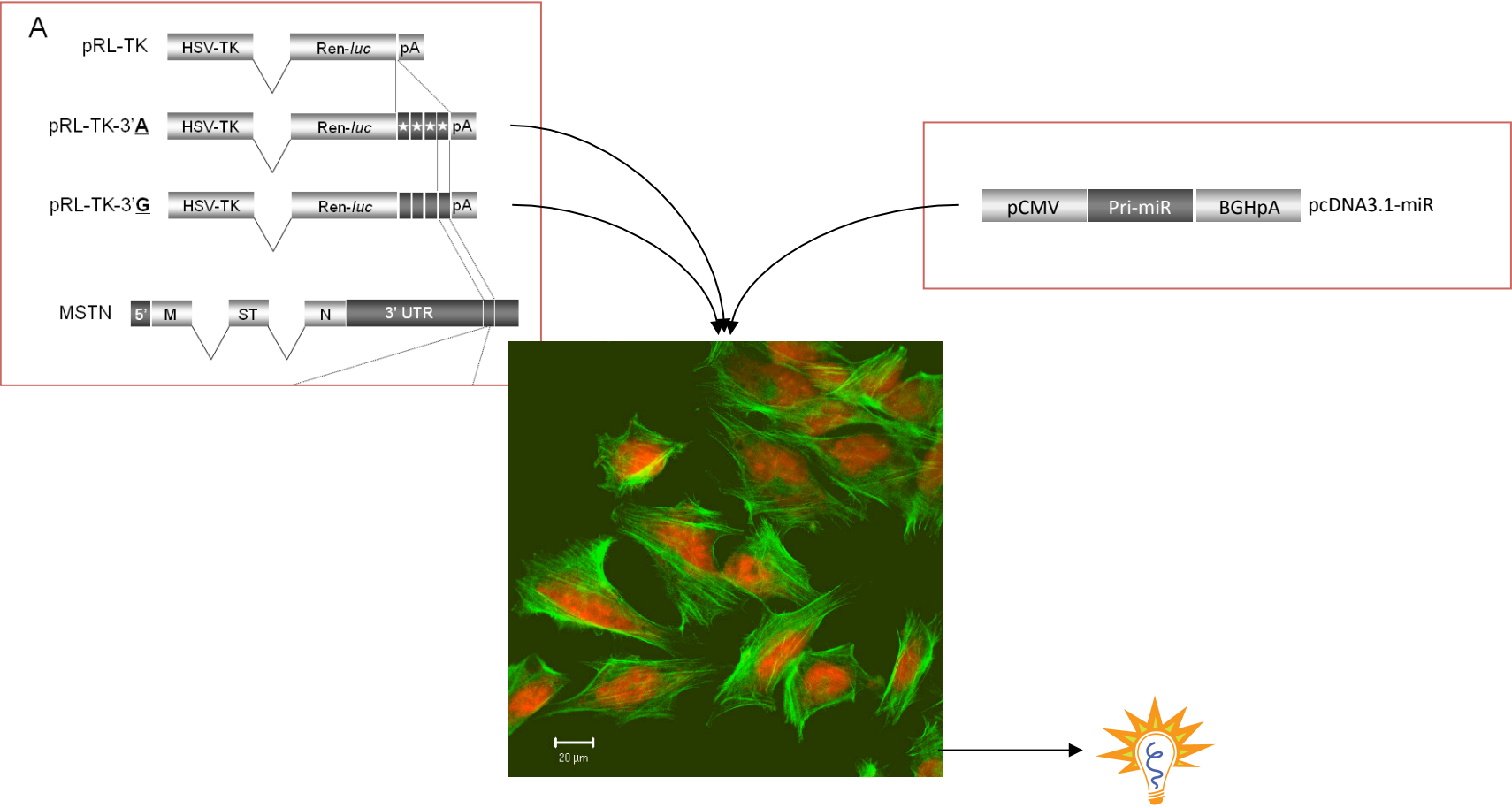


Texel sheep have \approx 3-fold reduction in circulating MSTN levels ...

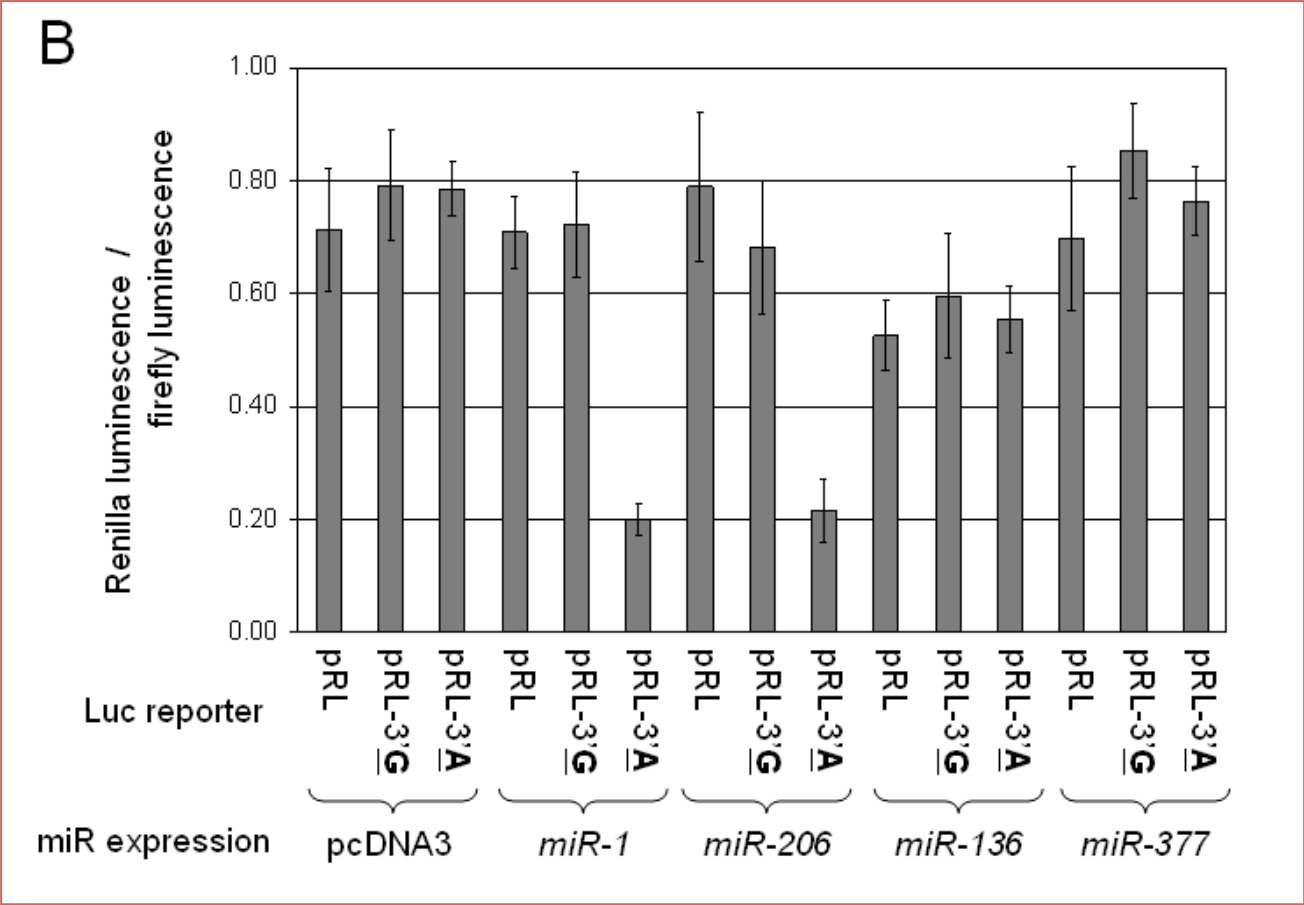


... supports translational inhibition.

miR1/206 – mutant *MSTN* interaction supported by reporter assay



miR1/206 – mutant *MSTN* interaction supported by reporter assay



G+6723G-A in the 3'UTR of myostatin Model

