

**TransFactor confirmation MyoD binds the
LAMA4 (ENSG00000112769:ENST00000230538) promoter**

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Materials:

-TransFactor Kit (Clontech product 631956)

-Oligos: (ordered from Operon, bring up in TE to 100µm)

LAMA4_MyoD_F biotin- tgctttcCACCAGCTGTGCgaccttg

LAMA4_MyoD_R caaggctgcacagctggtggaaacga

Neg_MyoD_F biotin- tgctttcCTCGAGGAGTGCgaccttg

Neg_MyoD_R caaggctgcactcctcgaggaaagca

* red highlight is the mutated nucleotides from the original target sequence

-Antibodies:

Primary: Santa Cruz MyoD (M-318): sc-760

Secondary: goat anti rabbit IgG-HRP from TransFactor Kit

-Protein:

Recombinant MyoD protein

-Plate Reader:

BIO-TEK Synergy HT

Methods:

Oligo preparation is done as:

-mix 10ul forward + 10ul reverse oligo

-place 95°C heat block 10 minutes

-cool on desktop 30 minutes

-mix 2ul with 198ul Mg to make 1 µM concentration, vortex briefly

The TransFactor Kit User Manual: V. Colorimetric TransFactor ELISA Procedure is followed with the following additions/changes:

-dilute MyoD antibody 1:100

-dilute goat anti rabbit antibody 1:1000

-step F1: after adding the TMB substrate place directly into the reader

-plate reader protocol:

1. Kinetic- 13x5minute intervals
2. Absorbance
3. Wavelength: 655nm

4. Shake 30s/read

Results:

Measurements over 5 time points:

slope: Tn-T(n-1)

T2-T1 sample	9-26-06	9-29-06	10/06/06
Neg_MyoD	0.01 0.008	0.003 0.005	0.027 0.028
LAMA4_MyoD	0.021 0.034	0.026 0.03	0.612 0.455

T3-T2 sample

Neg_MyoD	0.008 0.007	0.001 0.003	0.023 0.022
LAMA4_MyoD	0.019 0.029	0.024 0.024	0.48 0.355

T4-T3 sample

Neg_MyoD	0.007 0.006	0.001 0.001	0.017 0.02
LAMA4_MyoD	0.017 0.024	0.019 0.02	0.387 0.292

T5-T4 sample

Neg_MyoD	0.007 0.006	0 0.003	0.017 0.017
LAMA4_MyoD	0.015 0.02	0.019 0.019	0.322 0.246

Gnumeric spreadsheet Anova single factor results:

Groups	Count	Sum	Average	Variance
measurements	48	3.756	0.07825	0.02247
sample	48	72	1.5	0.25532
day	48	96	2	0.68085

ANOVA

Source of Variation	SS	df	MS	F	P-value	F critical
Between Groups	95.4319	2	47.7160	149.324	1.5E-35	3.06029
Within Groups	45.0561	141	0.31955			
Total	140.488	143				

Conclusion:

With a p-value of 1.5E-35 there is a very significant difference in MyoD binding between the negative and target oligos. It is therefore highly likely that the target sequence is a binding site for MyoD.