

Esercitazione

Fenotipo e Genotipo

**Focus sulla transizione
epitelio mesenchimale**

Riassunto sulle procedure per ottenere profili genetici di trascrizione

1 Estrazione RNA
RNA totale



2 Analisi qualitativa e
quantitativa RNA

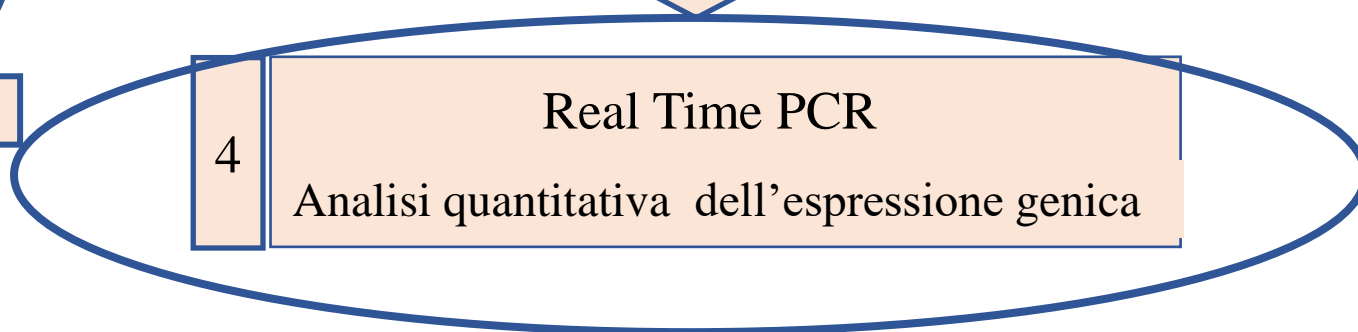


3 Retro-Trascrizione
mRNA



4 Real Time PCR
Analisi quantitativa dell'espressione genica

Primers



Costruzione Primers con BLAST

NIH U.S. National Library of Medicine NCBI National Center for Biotechnology Information

Primer-BLAST

A tool for finding specific primers

specific to your PCR template (using Primer3 and BLAST).

Recent results Publication Tips for finding specific primers

PCR Template

Enter accession, gi, or FASTA sequence [Clear](#)

Range

From To [Clear](#)

Forward primer

Reverse primer

Or, upload FASTA file No file chosen

Primer Parameters

Use my own forward primer (5'→3' on plus strand) [Clear](#)

Use my own reverse primer (5'→3' on minus strand) [Clear](#)

PCR product size

Min Max

of primers to return

Primer melting temperatures (T_m)

Min Opt Max Max T_m difference

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span

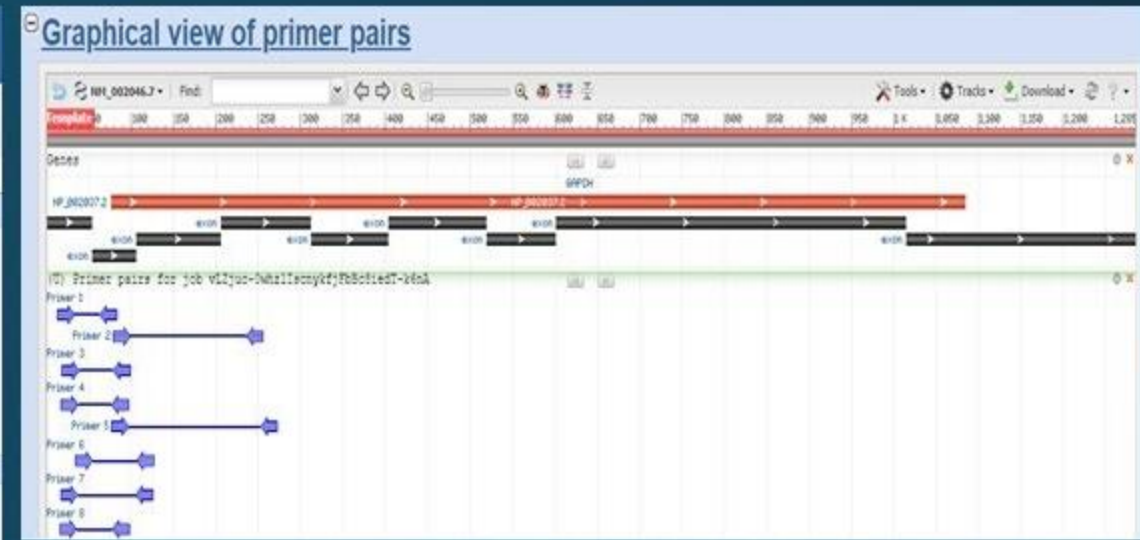
Exon junction match

Exon at 5' side Exon at 3' side

Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction

Inserire la sequenza del gene o l'accession number

Caratteristiche da dare ai primers



Primer pair 1

	Sequence (5'→3')	Template strand	Length	Start	Stop	T _m	GC%	Self complementarity	Self 3' complementarity
Forward primer	TCCTGTTGACAGTCAGCCG	Plus	20	13	32	62.14	60.00	6.00	3.00
Reverse primer	CCCCATGGTGTCTGAGCGAT	Minus	20	82	63	61.98	60.00	6.00	2.00
Product length	70								

Products on intended targets

>NM_002045.7 Homo sapiens glyceraldehyde-3-phosphate dehydrogenase (GAPDH), transcript variant 1, mRNA

product length = 70

Forward primer 1 TCCTGTTGACAGTCAGCCG 20

Template 13 32

Reverse primer 1 CCCCATGGTGTCTGAGCGAT 20

Risultato Coppie di primers

Primers specifici per Ovino

Gene	Accession Number	Sequences	Product Size (bp)
EMT genes	VIM	For: 5'-GACCAGCTCACCAACGACA-3'	93
		Rev: 5'-CTCCTCCTGCAACTTCTCCC-3'	
	SNAIL	For:5'- GTCGTGGGTGGAGAGCTTTG -3'	119
		Rev: 5'- TGCTGGAAAGTGAGCTCTGG -3'	
	TWIST	For: 5'-GCCGGAGACCTAGATGTCATTG-3'	150
		Rev: 5'-CCACGCCCTGTTTCTTTGAAT-3'	
CYTO 8	XM_012174208.4	For.: 5'-CTCAAAGGCCAGAGGGCTTC-3'	87
		Rev: 5'-CTTGGCCTGAGCATCCTTGA-3'	
αSMA	XM_004020019.4	For: 5' CGTCCTCGACATCAGGGAGT-3'	102
		Rev: 5' CGGGTACTTCAGCGTGAGAA-3'	
House keeping	GAPDH	For: 5'-CCTGCACCACCAACTGCTTG-3'	224
		Rev: 5'-TTGAGCTCAGGGATGACCTTG-3'	

Raw Data

Experiment Area

Setup

- Plate Setup
- Thermal Profile

Run

- Run Status
- Raw Data Plots

Analysis

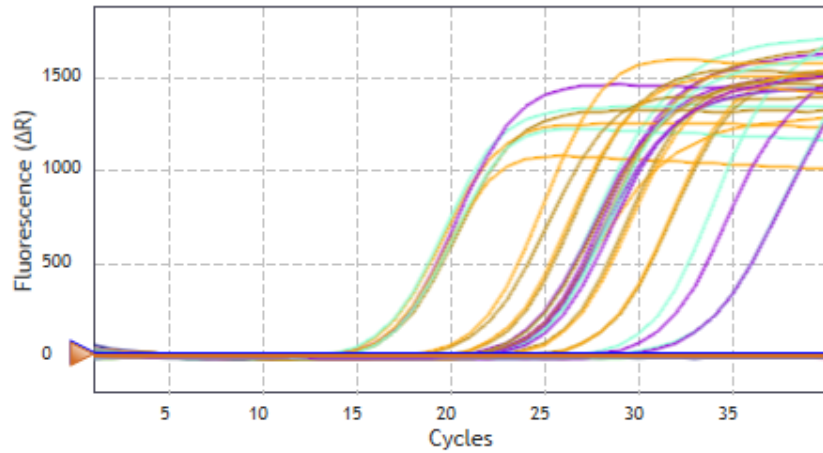
- Analysis Criteria
- Graphical Displays

Results

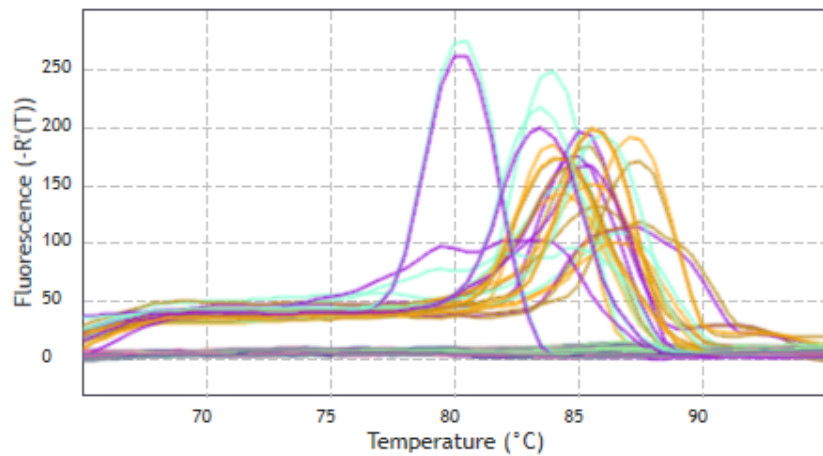
- Generate Report
- Export Data

Experiment Notes

Amplification Plots



Melt Curve - Raw/Derivative Curve



Amplification Plots

Fluorescence Term: R Rn ΔR ΔRn

Smoothing: On Off

Result Table

Type	Target	Replicate	Cq (ΔR)
wn	ROX	--	No Cq
wn	SYBR	--	22,21
wn	ROX	--	No Cq
wn	SYBR	--	21,93
wn	ROX	--	No Cq
wn	SYBR	--	22,30
wn	ROX	--	No Cq
wn	SYBR	--	22,42
wn	ROX	--	No Cq
wn	SYBR	--	14,64
wn	ROX	--	No Cq
wn	SYBR	--	14,33
wn	ROX	--	No Cq
wn	SYBR	--	15,18
wn	ROX	--	No Cq
wn	SYBR	--	15,22
wn	ROX	--	No Cq
wn	SYBR	--	15,05
wn	ROX	--	No Cq



Data Analysis

	A	B	C	D	E	F	G	H
1	Well	Well Type	Dye	Target	Replicate	Cq (ΔR)	Product 1 (-R'(T))	
2	A1	Unknown	SYBR	SYBR	---	20,21	86,5	
3	A1	Unknown	ROX	ROX	---	No Cq	88	
4	A2	Unknown	SYBR	SYBR	---	20,73	86,5	
5	A2	Unknown	ROX	ROX	---	No Cq	90	
6	A3	Unknown	SYBR	SYBR	---	21,58	86,5	
7	A3	Unknown	ROX	ROX	---	No Cq	90,5	
8	A4	Unknown	SYBR	SYBR	---	21,7	86	
9	A4	Unknown	ROX	ROX	---	No Cq	95	
10	A5	Unknown	SYBR	SYBR	---	18,85	86	
11	A5	Unknown	ROX	ROX	---	No Cq	87	
12	A6	Unknown	SYBR	SYBR	---	15,65	86,5	
13	A6	Unknown	ROX	ROX	---	No Cq	86,5	
14	A7	Unknown	SYBR	SYBR	---	16,55	86,5	
15	A7	Unknown	ROX	ROX	---	No Cq	95	
16	A8	Unknown	SYBR	SYBR	---	19,63		
17	A8	Unknown	ROX	ROX	---	No Cq		
18	A9	Unknown	SYBR	SYBR	---	18,78		
19	A9	Unknown	ROX	ROX	---	No Cq		
20	A10	Unknown	SYBR	SYBR	---	15,17		
21	A10	Unknown	ROX	ROX	---	No Cq		
22	A11	Unknown	SYBR	SYBR	---	15,09		
23	A11	Unknown	ROX	ROX	---	No Cq		

Fold expression = $2^{-\Delta\Delta Ct}$

Modello matematico

1. Calcolo il ΔCT

$\Delta CT = CT \text{ medio GENE TARGET} - CT \text{ medio GENE HOUSEKEEPING}$

Questo step serve a normalizzare il campione, cioè escludere variazioni dovute al caricamento di quantità diverse tra un campione e l'altro.

2. Calcolo il ΔΔCT

$\Delta\Delta CT = \Delta CT \text{ campione INCOGNITO} - \Delta CT \text{ campione scelto come CONTROLLO}$

Confronto i CT dei campioni incogniti rispetto a un campione scelto dall'operatore come controllo (ad es. trattato vs non trattato, oppure patologia vs sano etc...)

3. Calcolo il FOLD change

Fold change = $2^{-\Delta\Delta Ct}$

✓ $2^{-\Delta\Delta Ct} > 2$

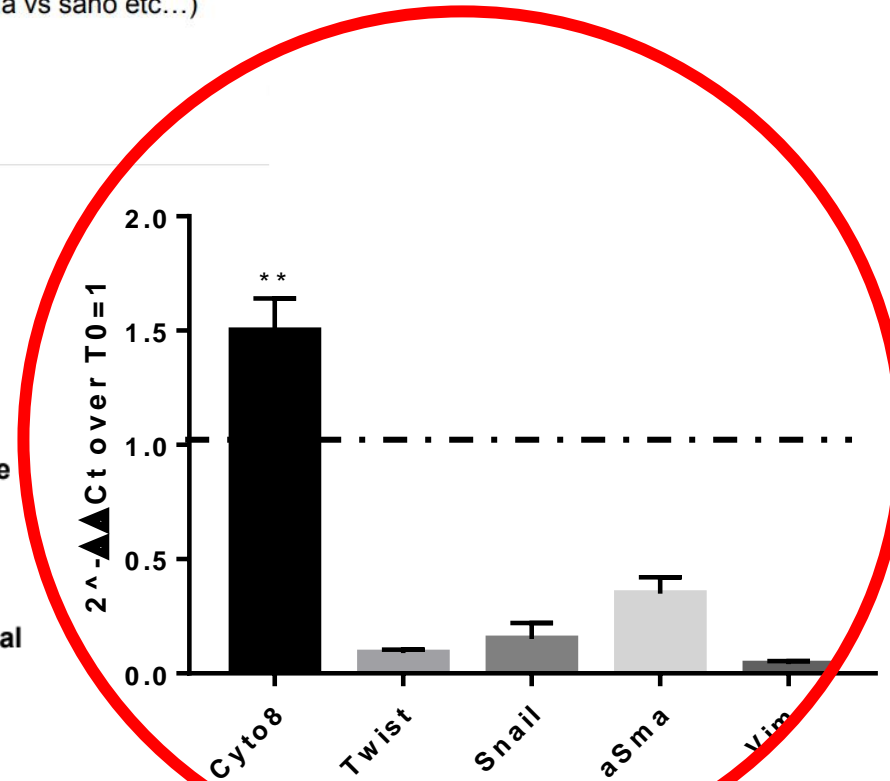
Il gene target, nel campione in analisi, è più espresso rispetto al campione scelto come riferimento (controllo).

✓ $2^{-\Delta\Delta Ct} < 0.5$

Il gene target, nel campione in analisi, è meno espresso rispetto al campione scelto come riferimento (controllo).

✓ $0,5 < 2^{-\Delta\Delta Ct} < 2$

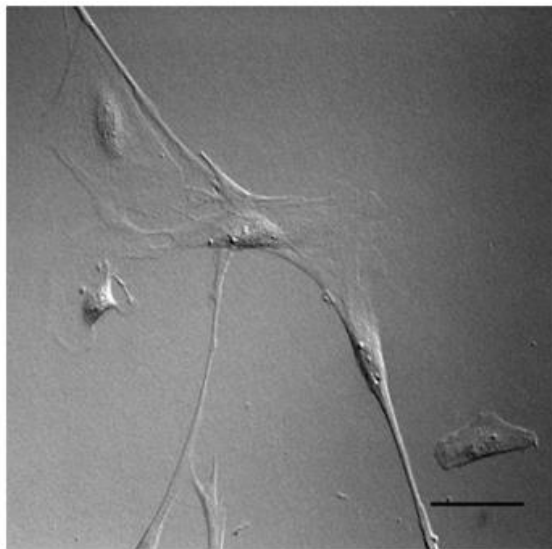
La variazione di espressione del gene target nel campione in analisi rispetto al campione scelto come controllo non è significativa.



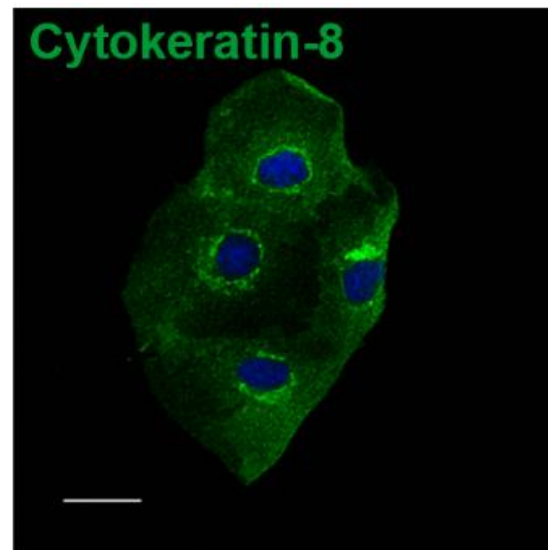
- Test

Ricostruire il fenotipo e genotipo mesenchimale o epiteliale combinando correttamente le immagini in chiaro e di IHC con il proprio profilo trascrizionale.

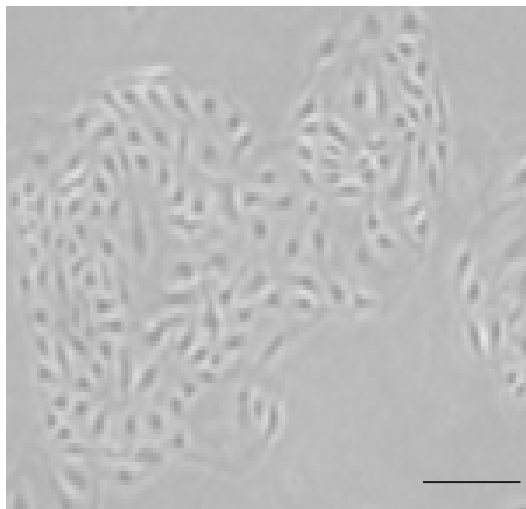
1



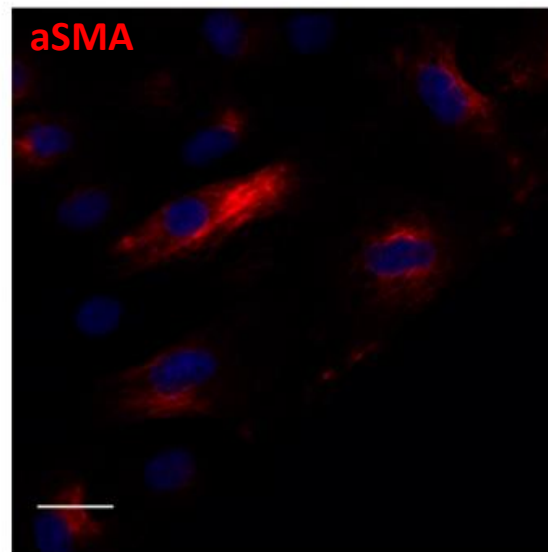
3



2



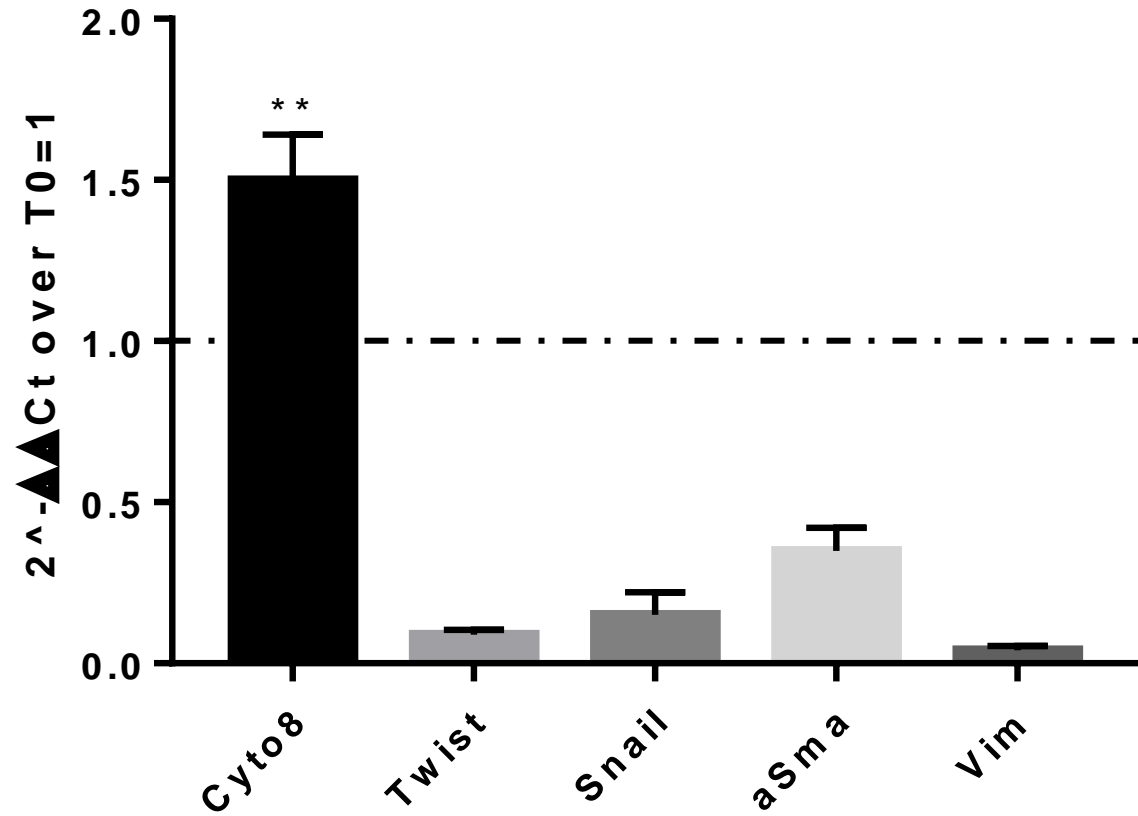
4



Fold change calculated over T0=1

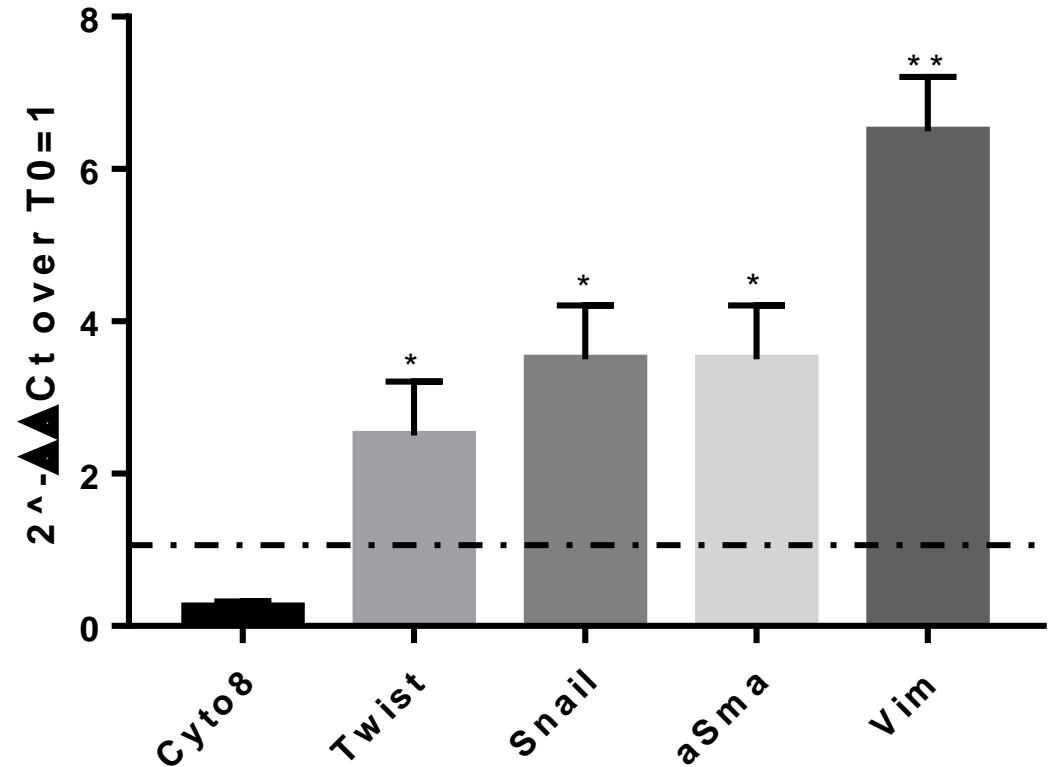
- * p<0.05
- ** p<0.01

A



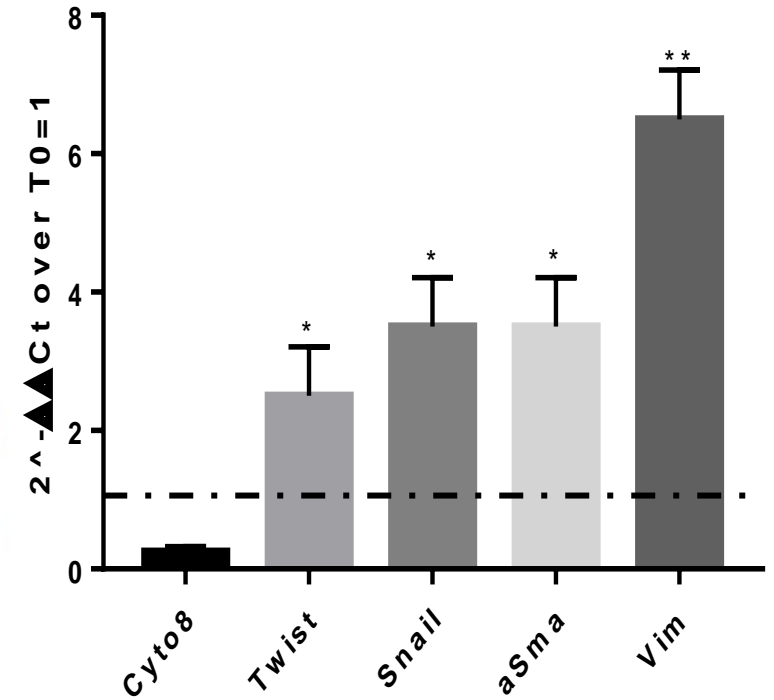
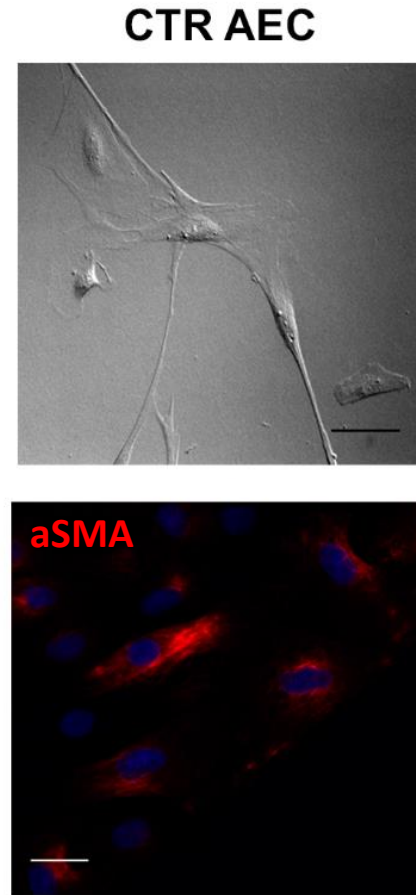
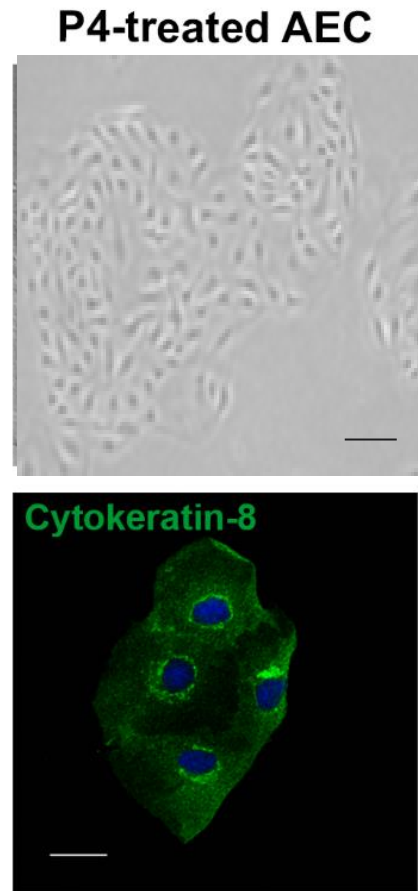
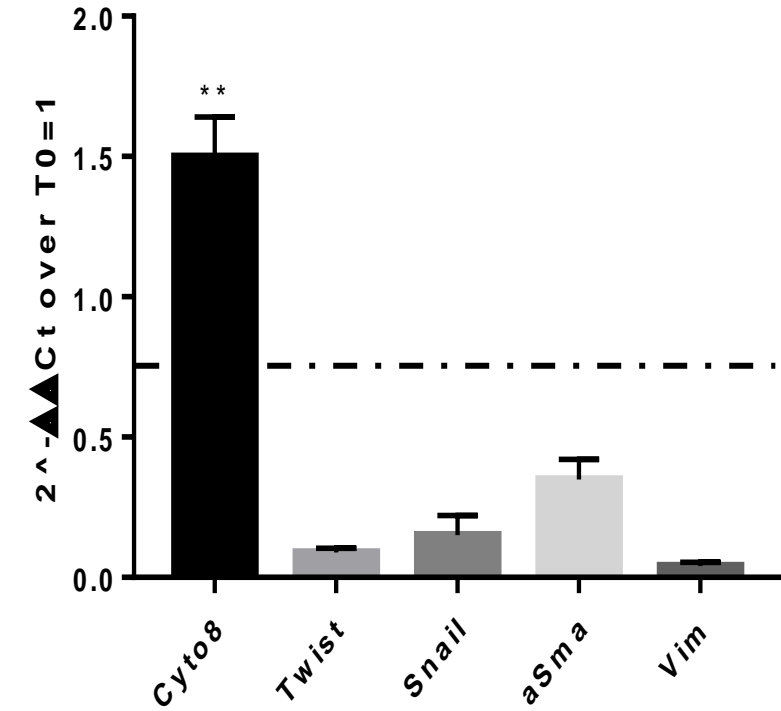
- ** p<0.01 Cyto8 vs others

B



- * p<0.05 vs Cyto8
- ** p<0.01 Vim vs others

Soluzioni



Cellule epiteliali con P4 2-3-A

Cellule mesenchimali CTR 2-4-B