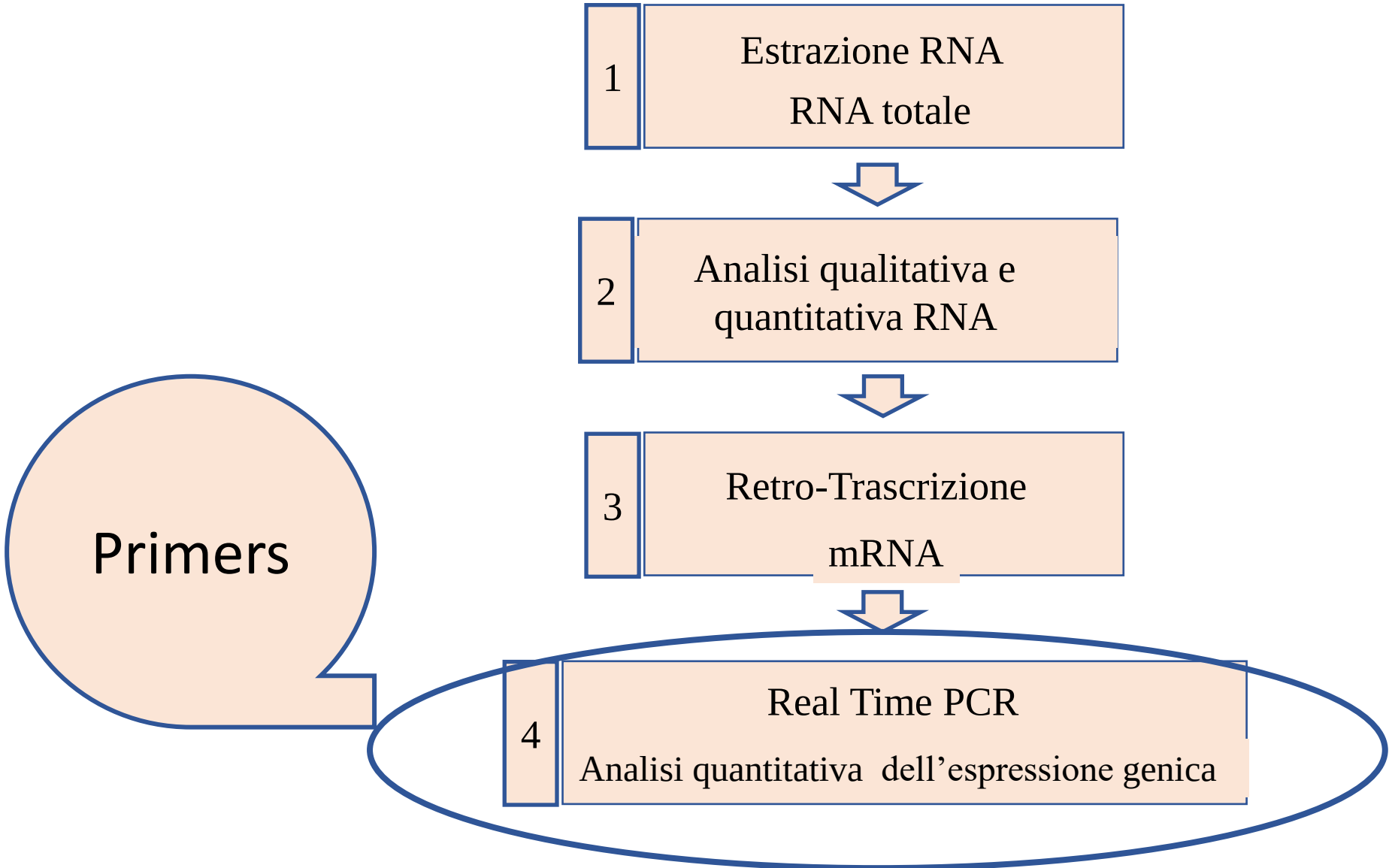
Esercitazione

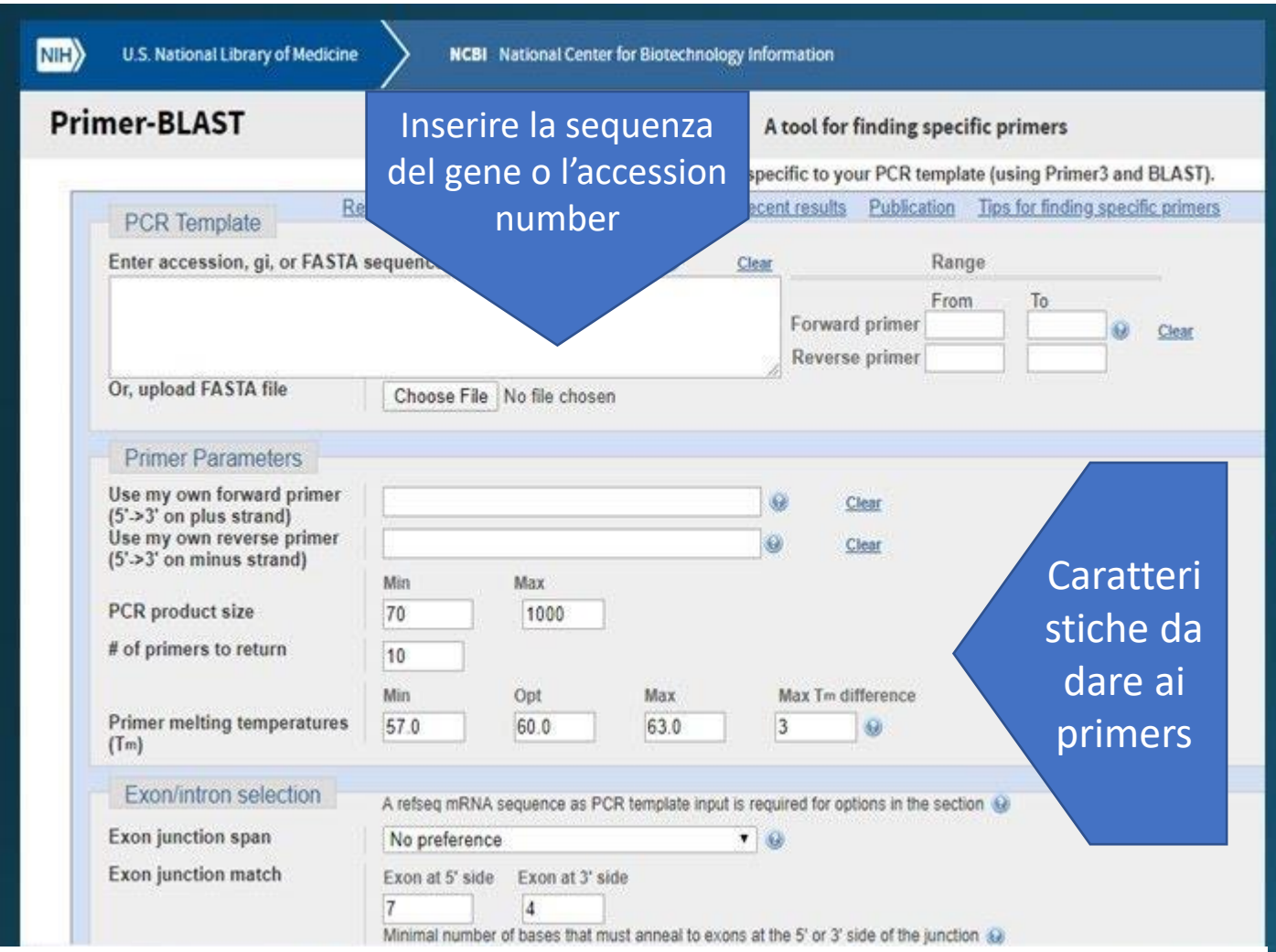
Fenotipo e Genotipo

Focus sulla transizione epitelio mesenchimale

Riassunto sulle procedure per ottenere profili genetici di trascrizione

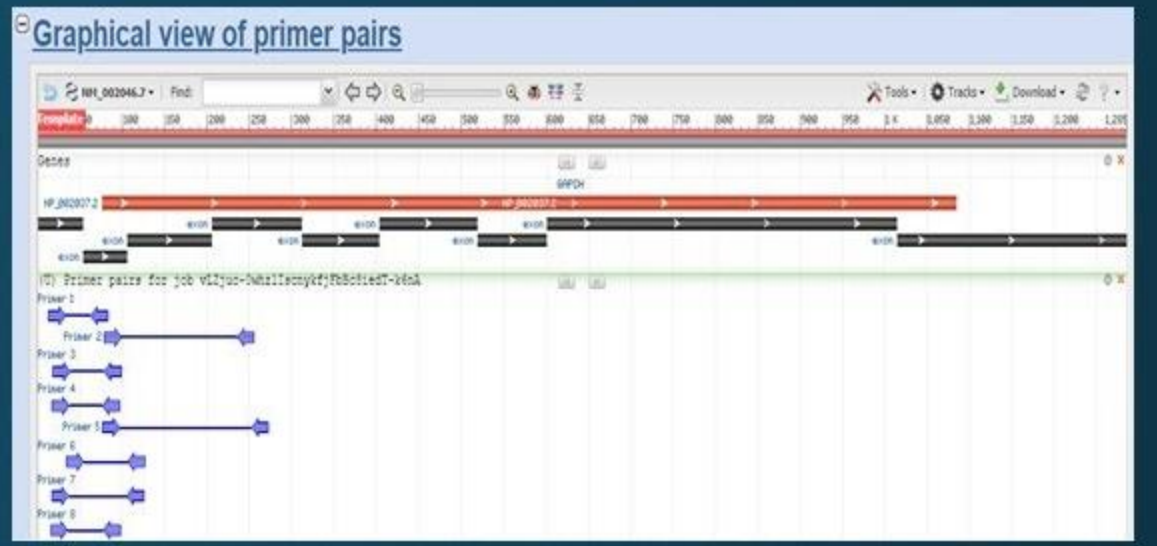


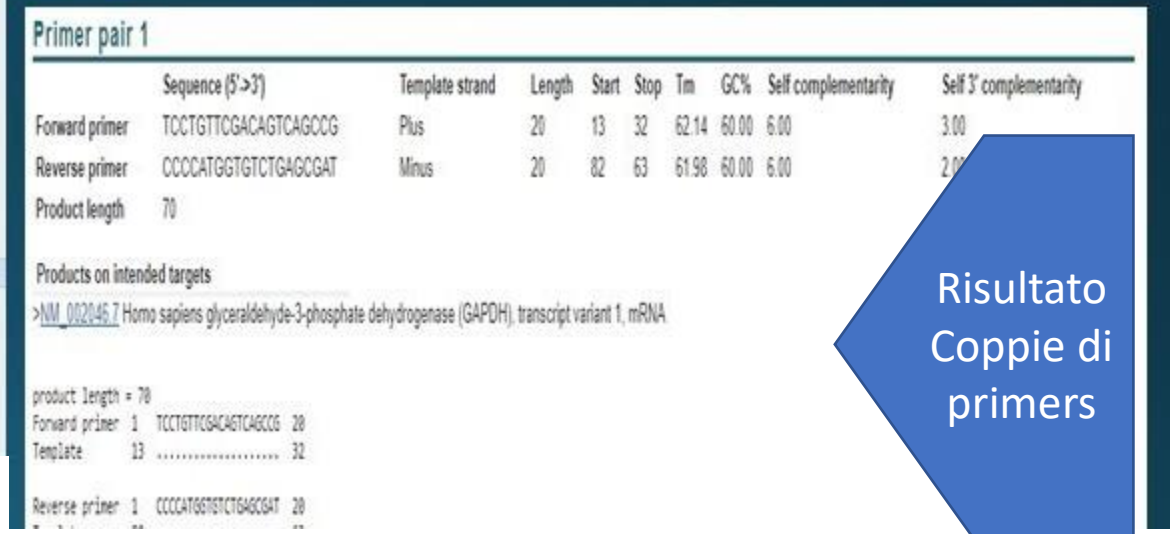
Costruzione Primers con BLAST



Inserire la sequenza del gene o l'accession number

Caratteristiche da dare ai primers



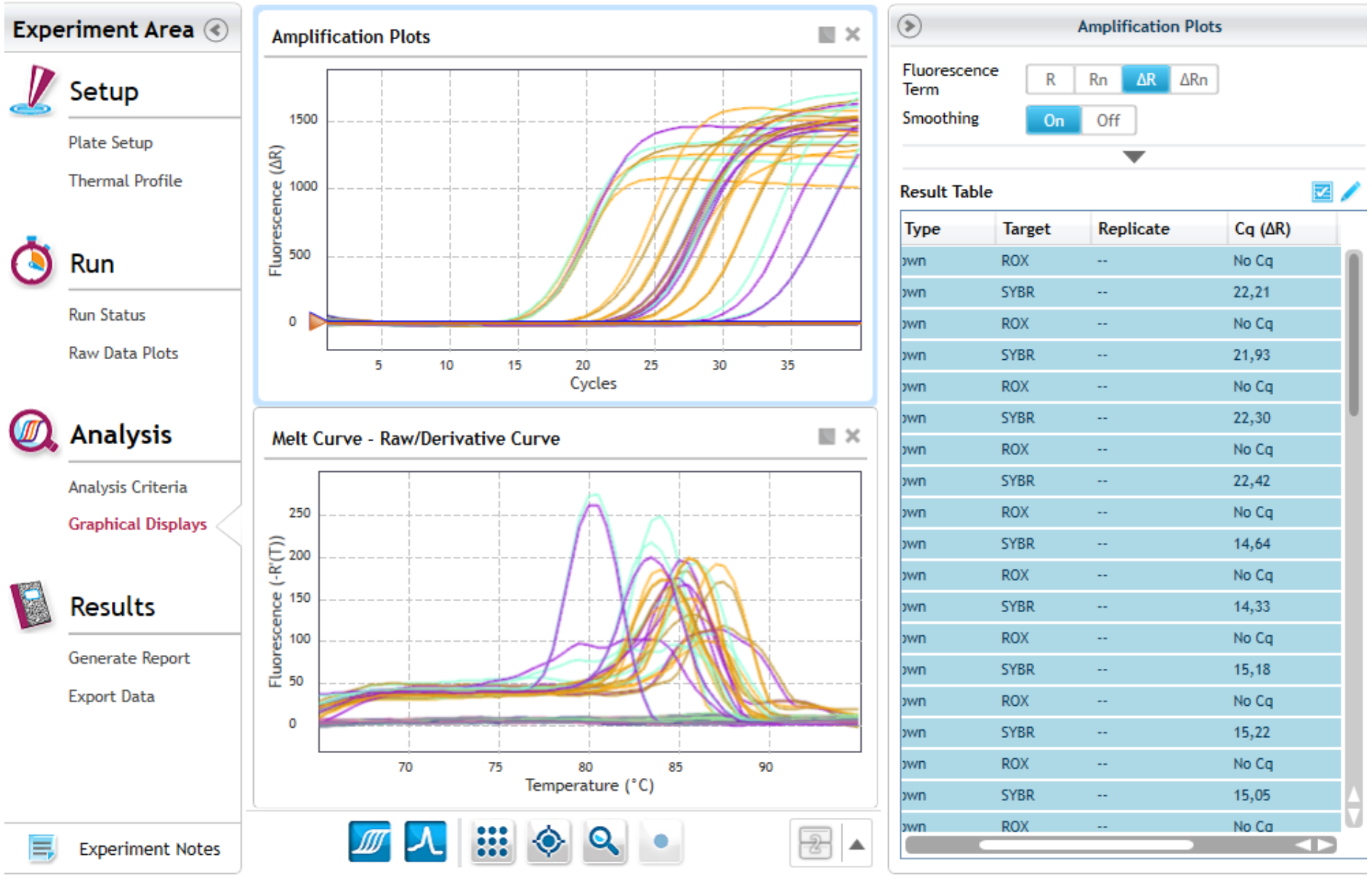


Risultato Coppie di primers

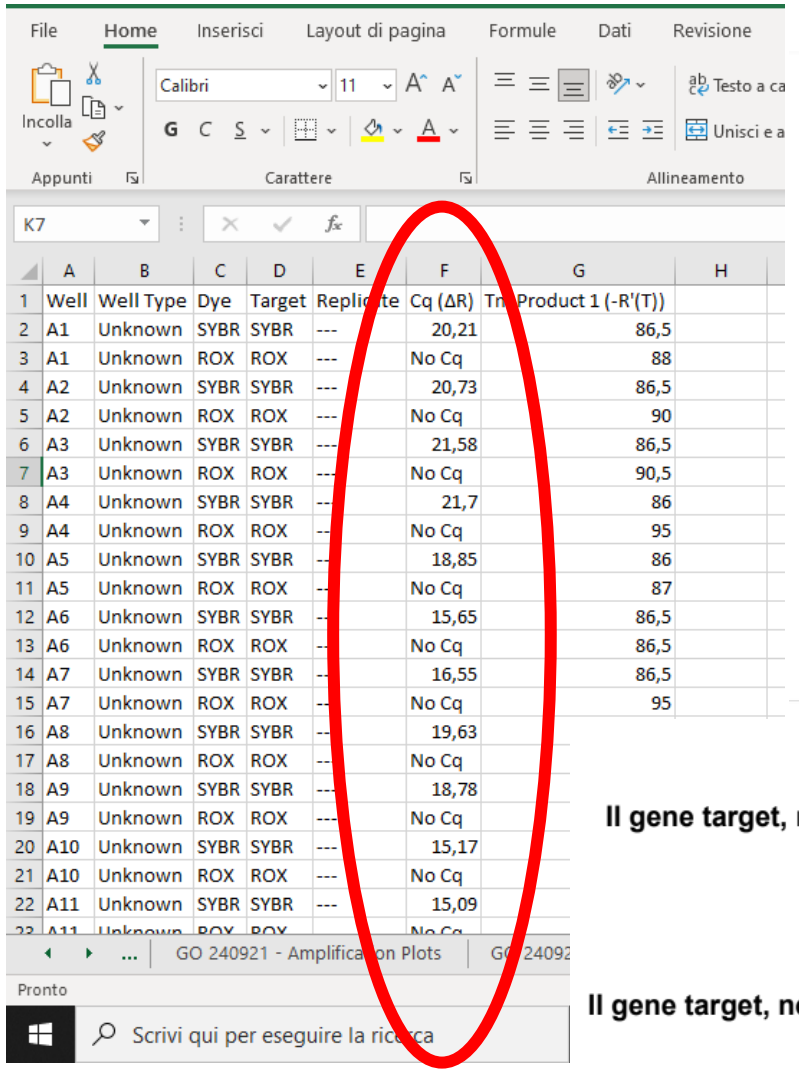
Primers specifici per Ovino

	Gene	Accession Number	Sequences	Product Size (bp)
EMT genes	VIM	XM_004014247.4	For: 5′-GACCAGCTCACCAACGACA-3′	93
			Rev: 5′-CTCCTCCTGCAACTTCTCCC-3′	
	SNAIL	XM_004014881.2	For:5′- GTCGTGGGTGGAGAGCTTTG -3′	119
			Rev: 5′- TGCTGGAAAGTGAGCTCTGG -3′	
	TWIST	XM_004008211.4	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	AF030943.1	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
Fluorescence (ΔR)
1500
1000
500
0
5
10
15
20
25
30
35
Cycles
Melt Curve - Raw/Derivative Curve
Fluorescence (-R'(T))
250
200
150
100
50
0
70
75
80
85
90
Temperature (°C)
Amplification Plots
Fluorescence Term
R
Rn
ΔR
ΔRn
Smoothing
On
Off
Result Table
Type
Target
Replicate
Cq (ΔR)
own ROX -- No Cq
own SYBR -- 22,21
own ROX -- No Cq
own SYBR -- 21,93
own ROX -- No Cq
own SYBR -- 22,30
own ROX -- No Cq
own SYBR -- 22,42
own ROX -- No Cq
own SYBR -- 14,64
own ROX -- No Cq
own SYBR -- 14,33
own ROX -- No Cq
own SYBR -- 15,18
own ROX -- No Cq
own SYBR -- 15,22
own ROX -- No Cq
own SYBR -- 15,05
own ROX -- No Cq

Data Analysis



	A	B	C	D	E	F	G	H
1	Well	Well Type	Dye	Target	Replicate	Cq (ΔR)	Tm 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		

GO 240921 - Amplification Plots

Pronto

Scrivi qui per eseguire la ricerca

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

Modello matematico

1. Calcolo il ΔCT

ΔCT = CT medio GENE TARGET – CT 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

ΔΔCT = ΔCT campione INCOGNITO – ΔCT 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 = $2^{-\Delta\Delta Ct}$

✓ **$2^{-\Delta\Delta CT} > 2$**

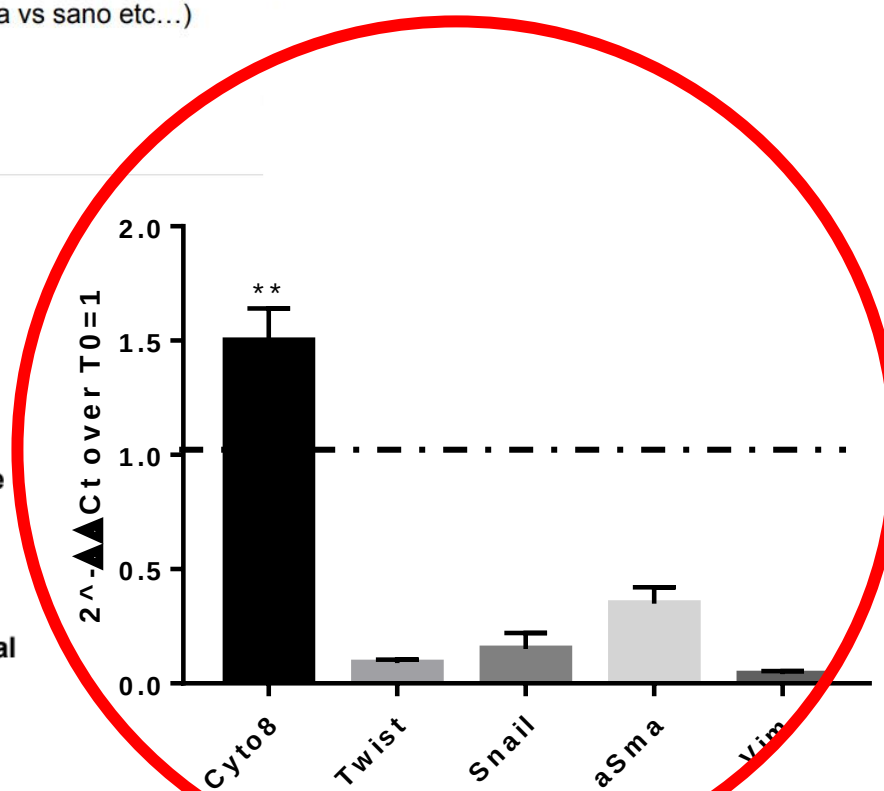
Il gene target, nel campione in analisi, è più espresso rispetto al campione scelto come riferimento (controllo).

✓ **$2^{-\Delta\Delta CT} < 0.5$**

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

✓ **$0,5 < 2^{-\Delta\Delta CT} > 2$**

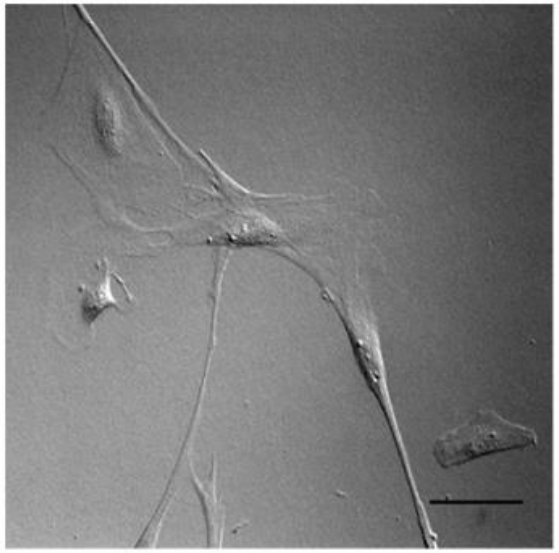
La variazione di espressione del gene target nel campione in analisi rispetto al campione scelto come controllo non è significativa.



- Test

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

1



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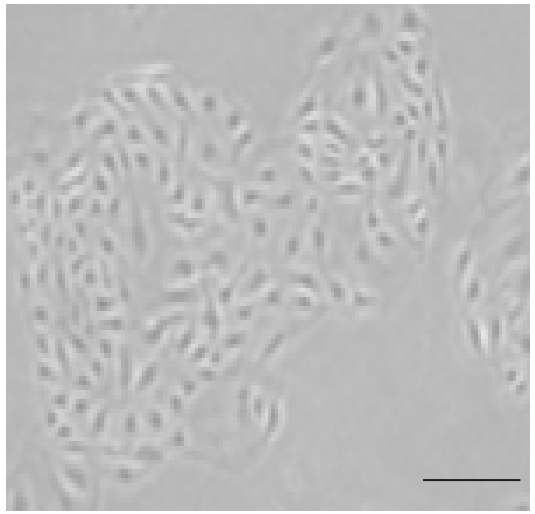

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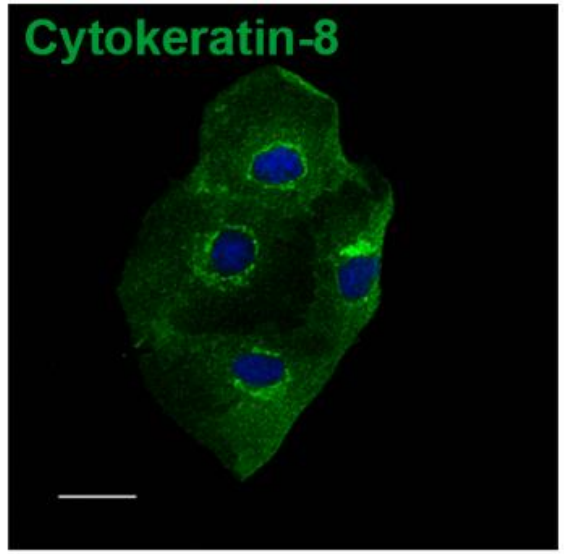
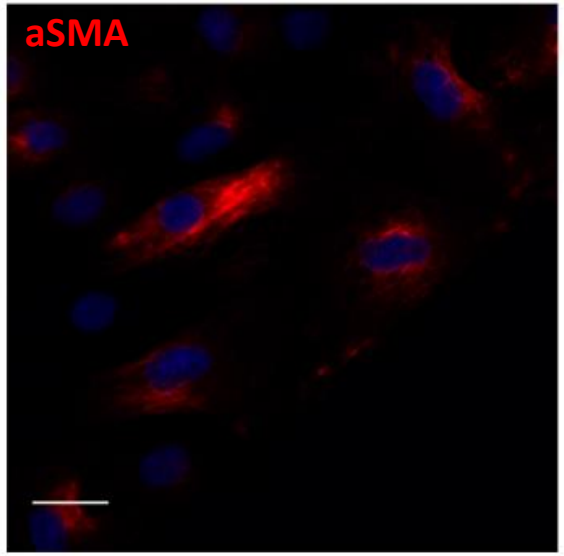


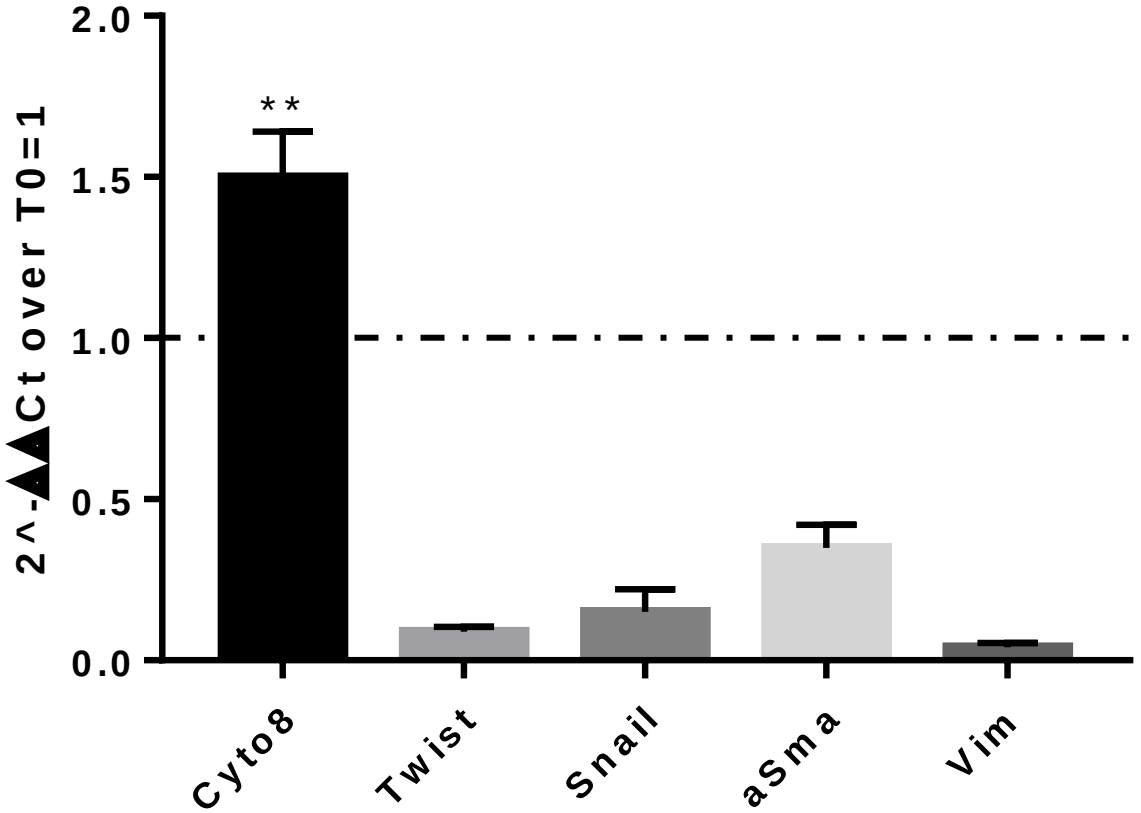
4



Fold change calcolato over T0=1

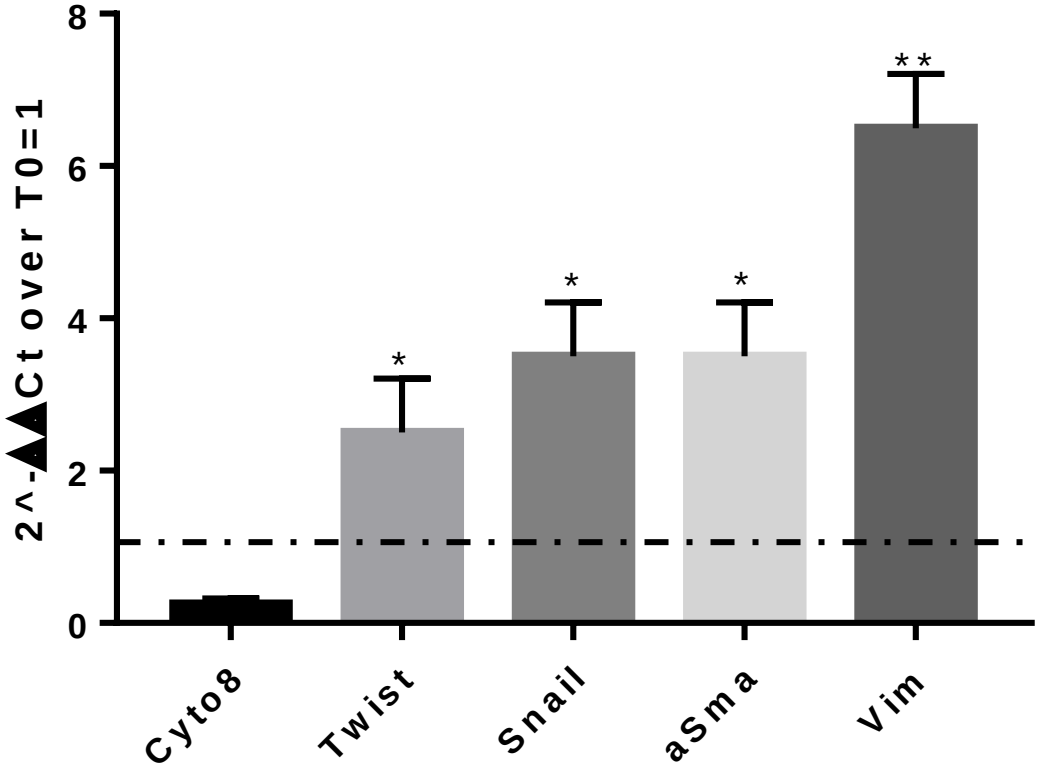
- * p<0.05
- ** p<0.01

A



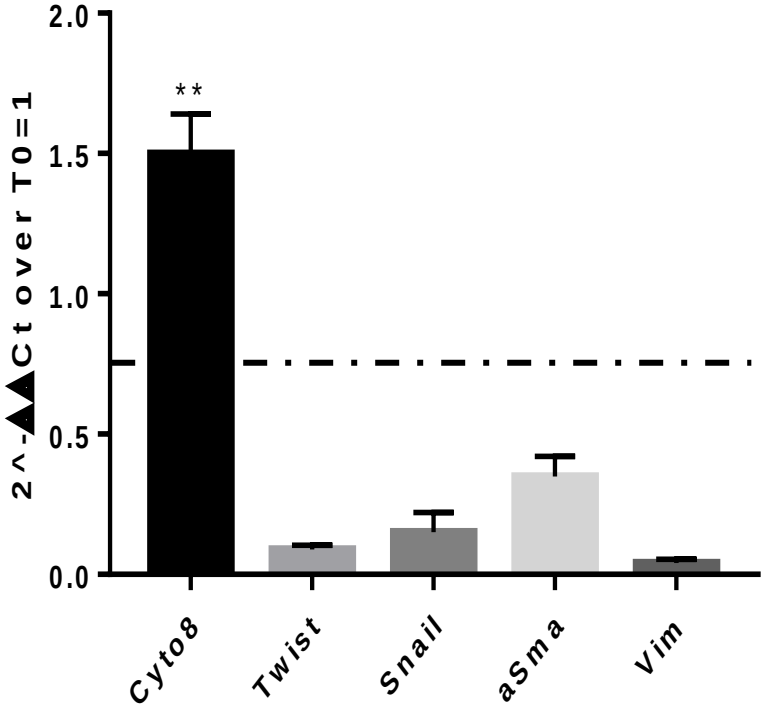
- ** p<0.01 Cyto8 vs others

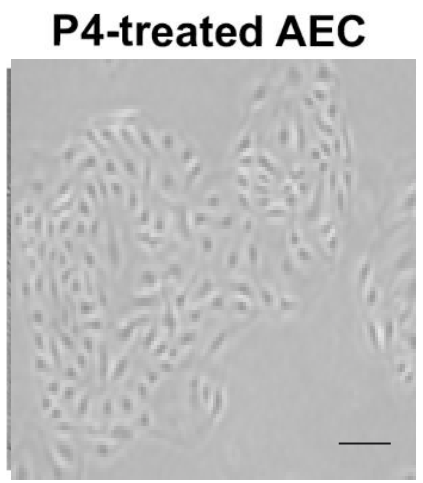
B

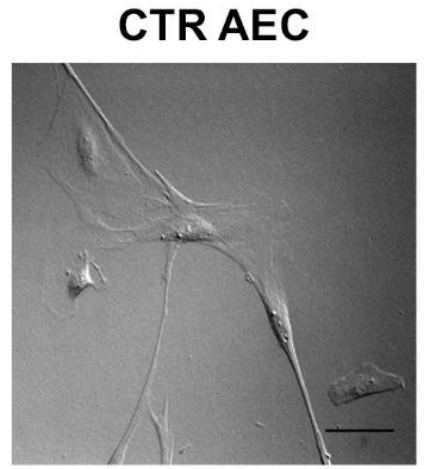


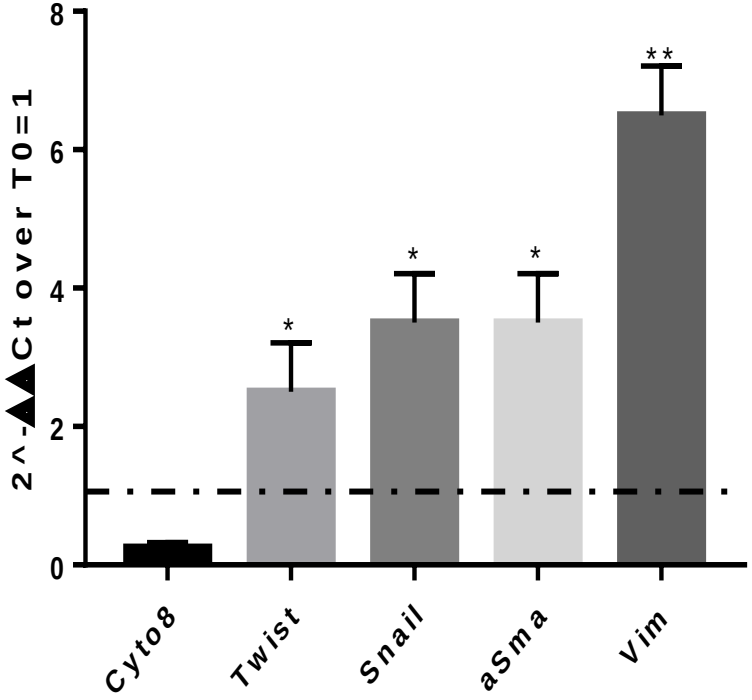
- * p<0.05 vs Cyto8
- ** p<0.01 Vim vs others

Soluzioni









Cellule epiteliali con P4 2-3-A

Cellule mesenchimali CTR 2-4-B