Reduced TGF-ß1 Expression and its Target Genes in Human Insulinomas

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Docente referente: Davide Sartini

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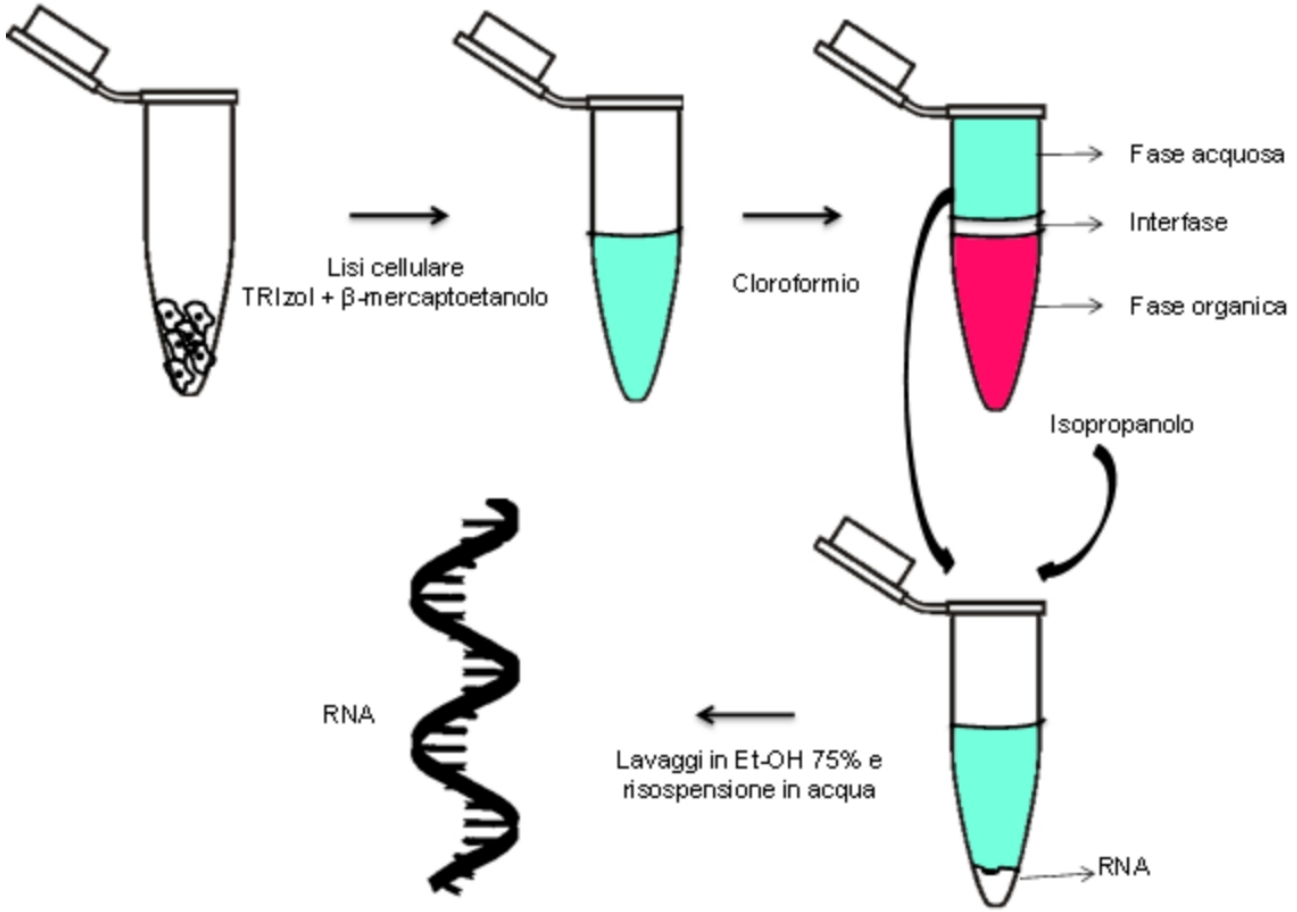
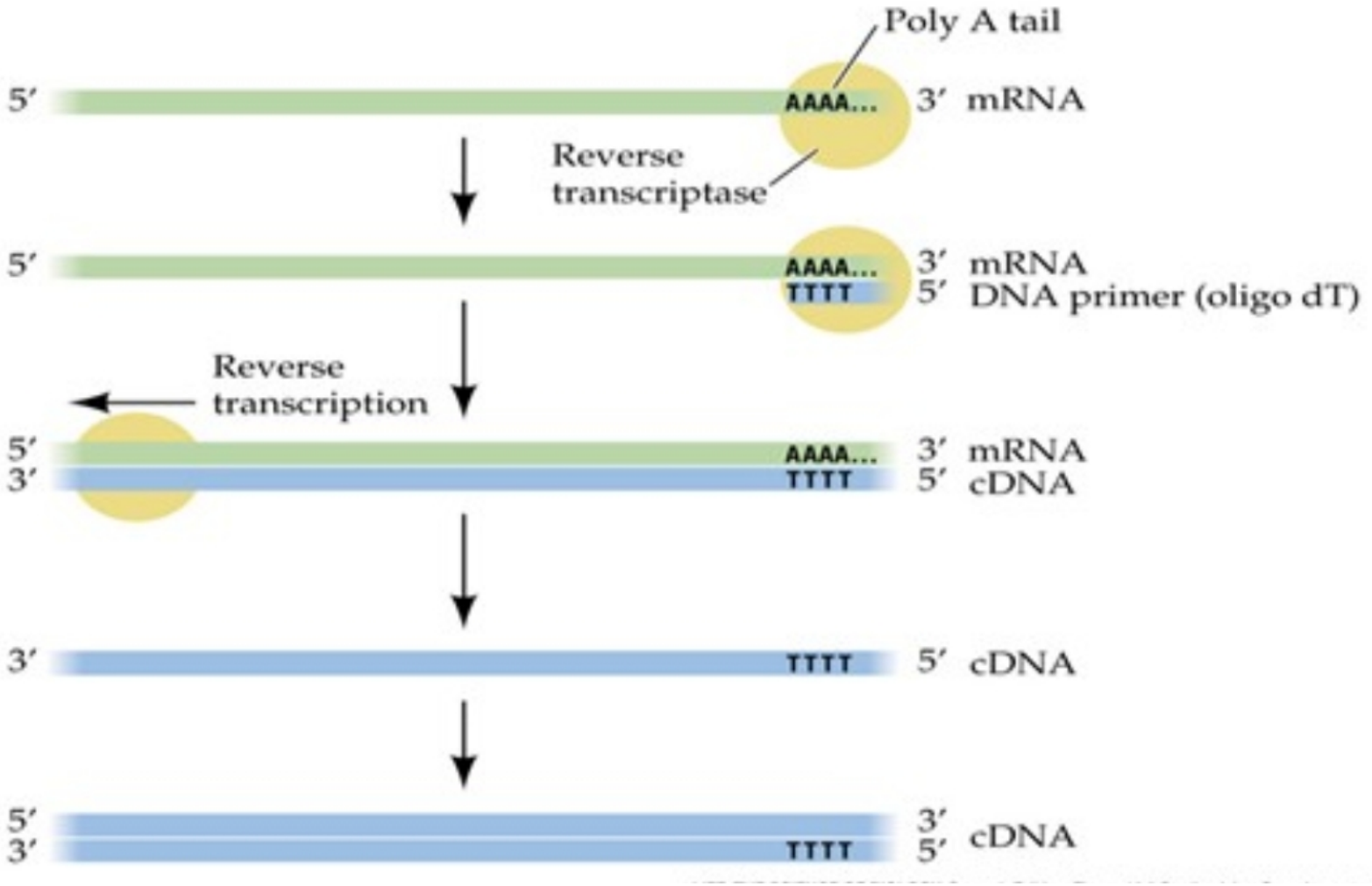
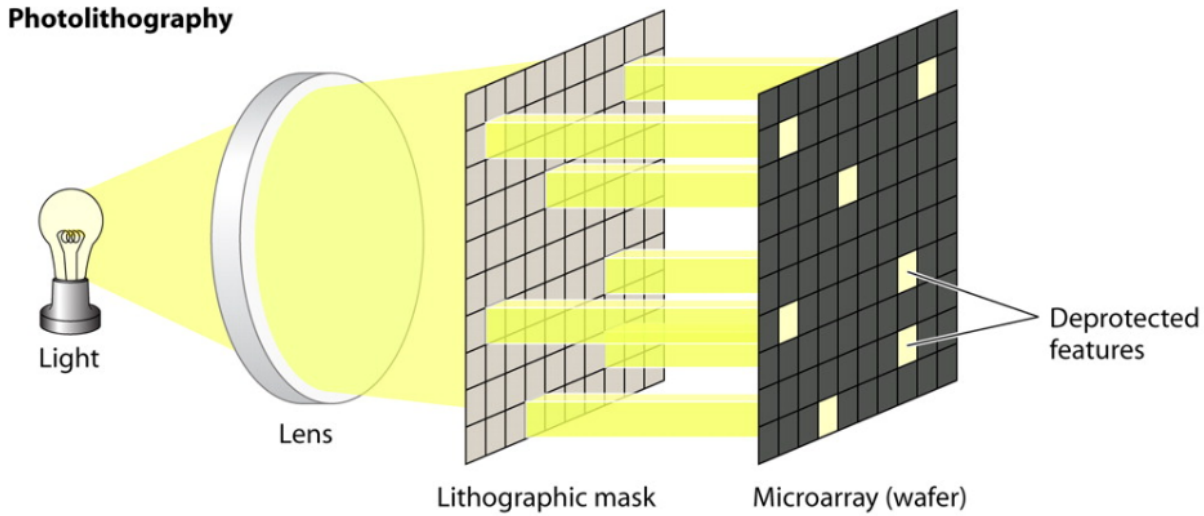
ESTRAZIONE mRNA

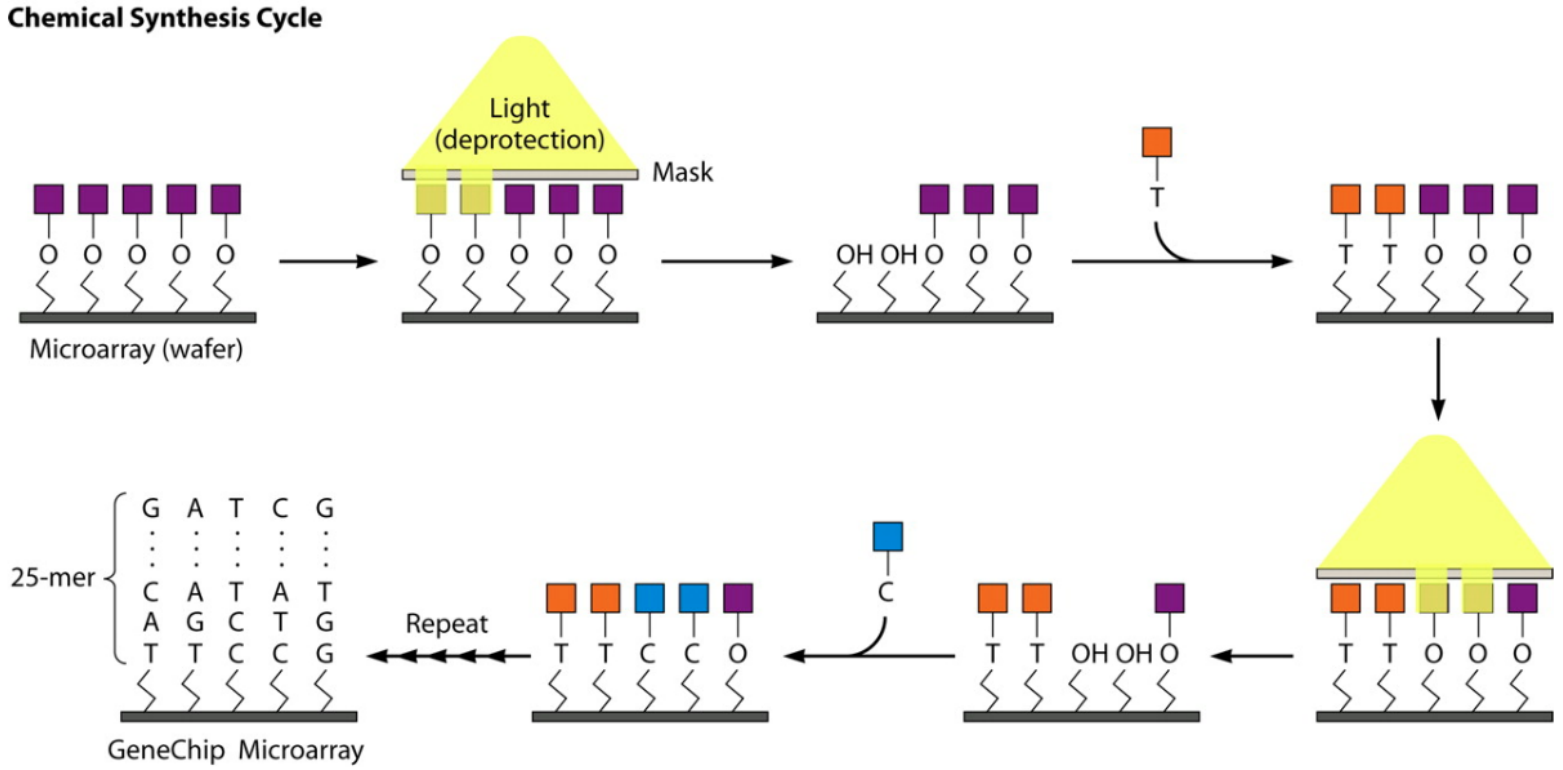


RETROTRASCRIZIONE



MICROARRAY A SONDE OLIGONUCLEOTIDICHE





RISULTATI DELL'ARRAY

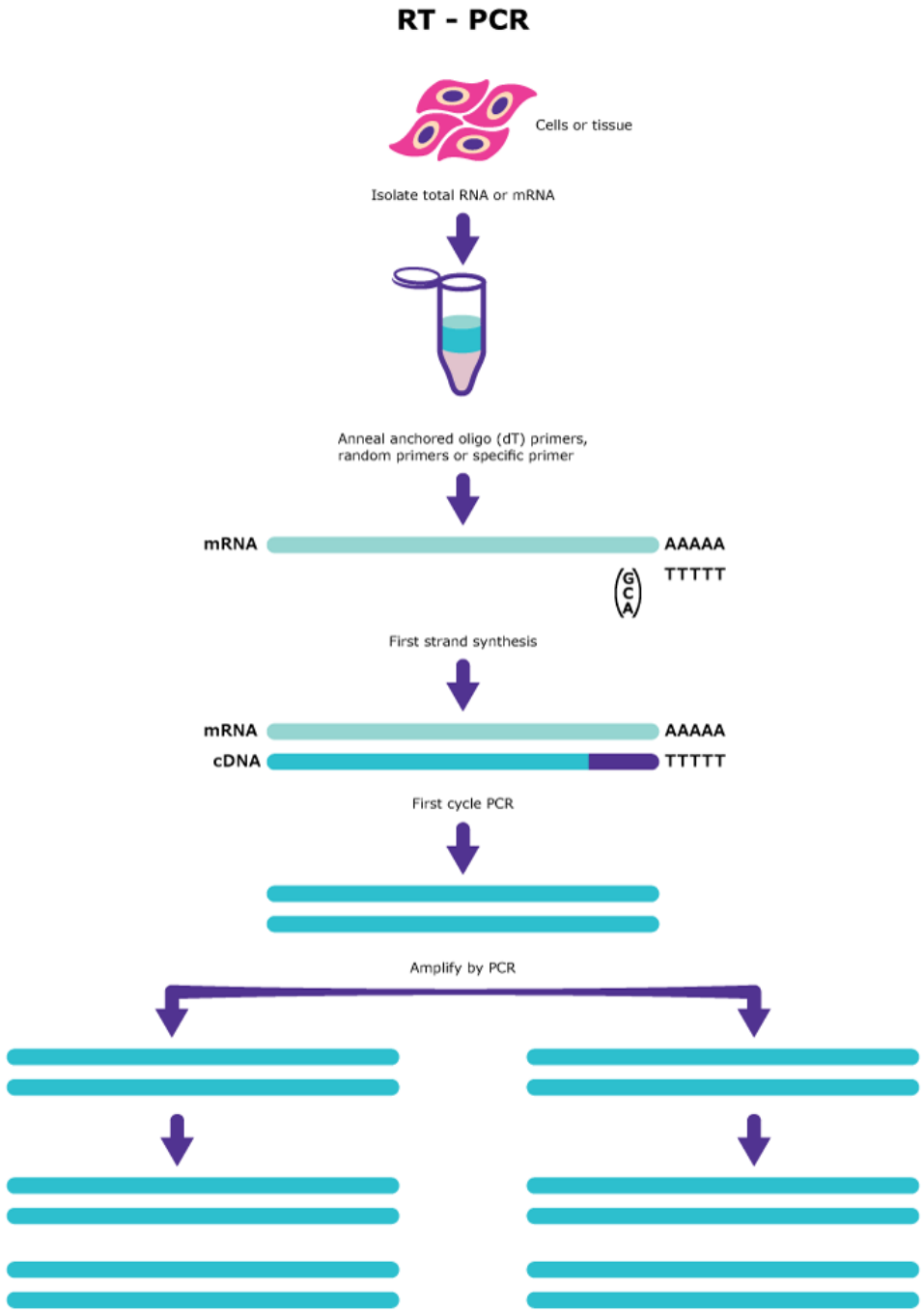
a)

gene	tumor 2	tumor 3	tumor 5	islet 3
TGFB1	46.08	103.45	143	237.06
TGFBR2	482.87	269.53	824.69	247.14
TGFBR3	339.23	402.94	230.46	79.23
TGFBI	196.68	118.79	301.22	2782.24
NNMT	579.13	69.37	159.59	796.98
RPN2	427.63	320.08	234.73	647.31

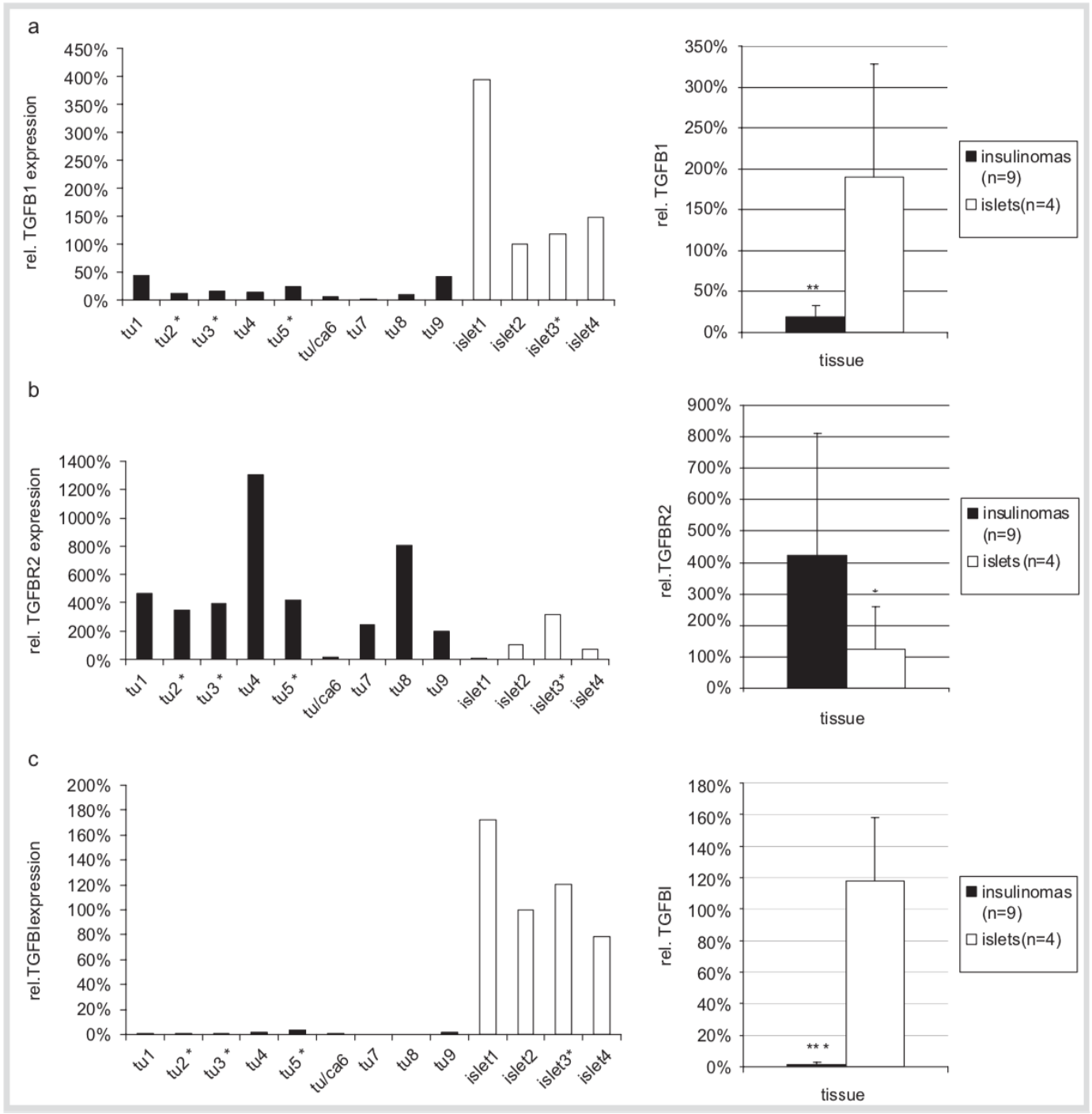
b)

gene	tumor 2	tumor 3	tumor 5	islet 3
COL1A1	840.59	236.05	142.29	1962.25
COL1A2	1801.69	562.92	357.53	3426.83
COL3A1	226.9	80.1	113.25	365.73
ITGA3	78.35	67.96	80.09	252.44
ITGAV	110.56	94.03	59.93	263.35
ITGA5	120.5	88.59	397.8	218.08
PDXK	90.34	117.7	58.12	227.06
SERPINH1	149.45	93.46	240.84	373.78

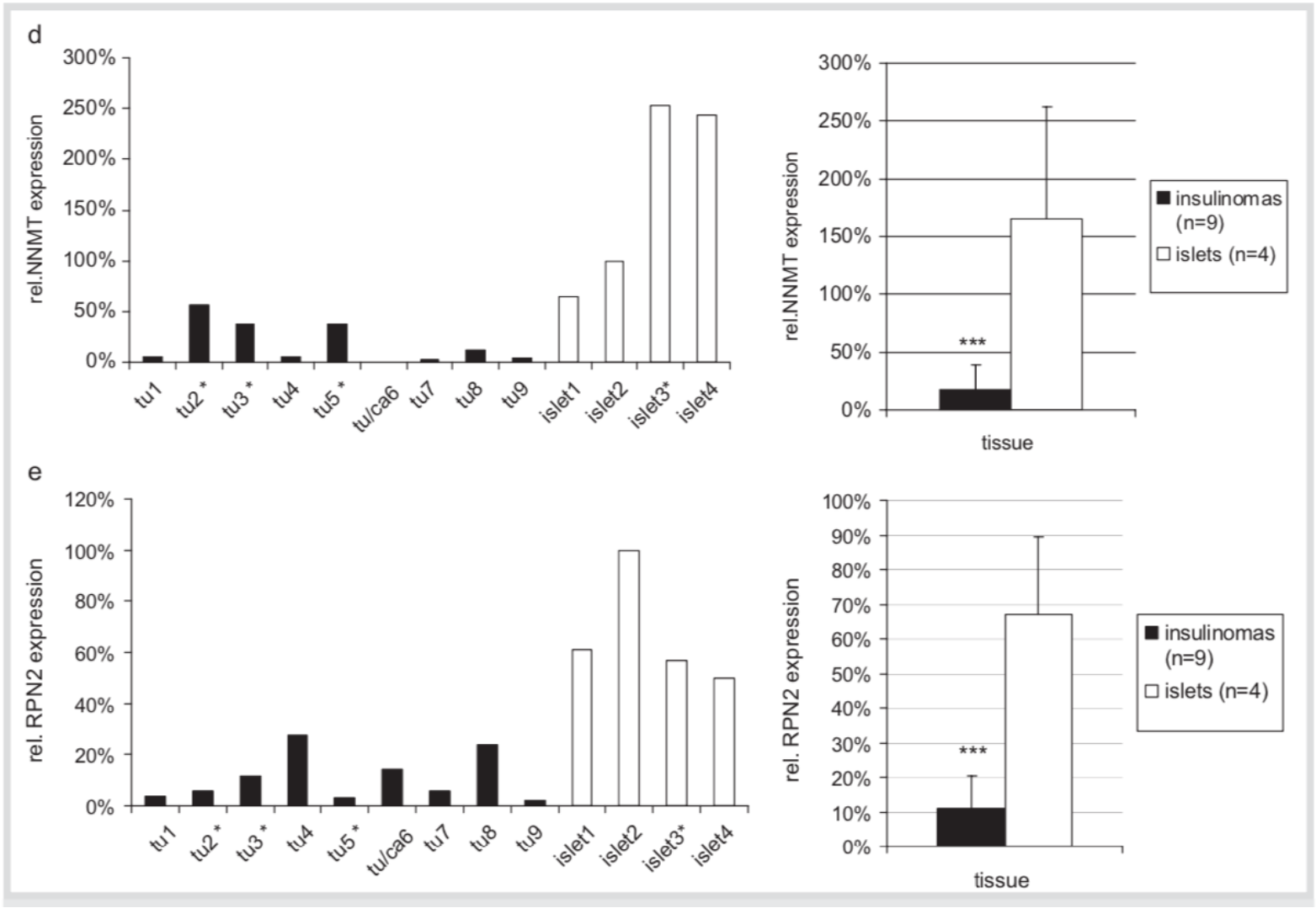
RT-qPCR


RT - PCR
Cells or tissue
Isolate total RNA or mRNA
Anneal anchored oligo (dT) primers,
random primers or specific primer
mRNA
AAAAA
TTTTT
G
C
A
First strand synthesis
mRNA
AAAAA
cDNA
TTTTT
First cycle PCR
Amplify by PCR

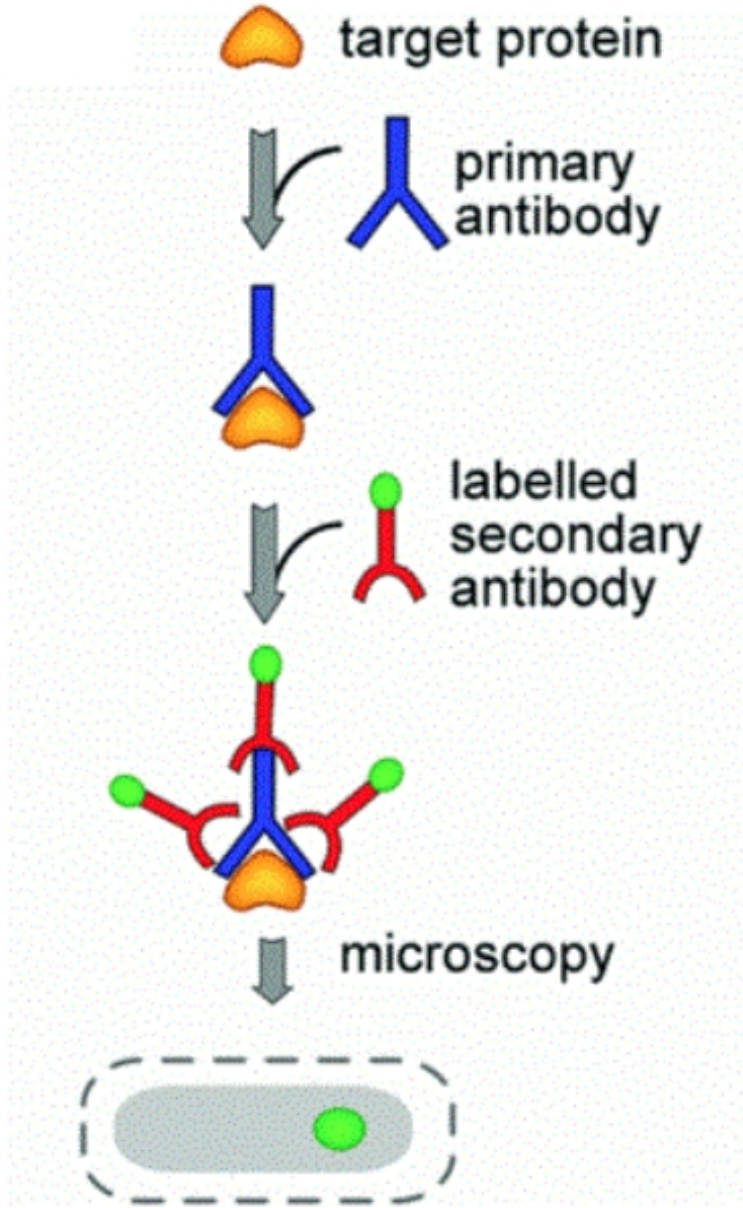
RISULTATI DELLA RT-qPCR



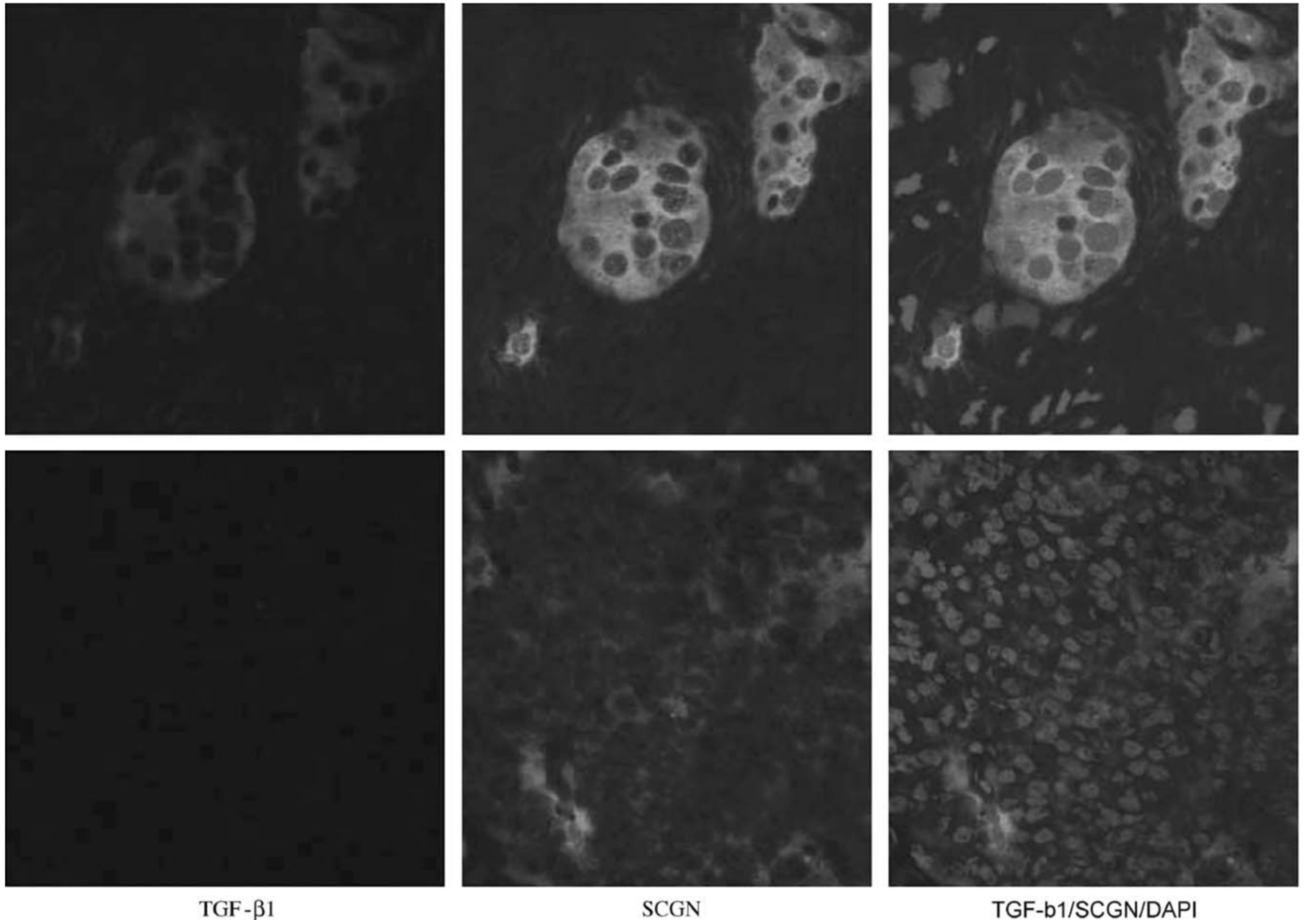
RISULTATI DELLA RT-qPCR
I geni target



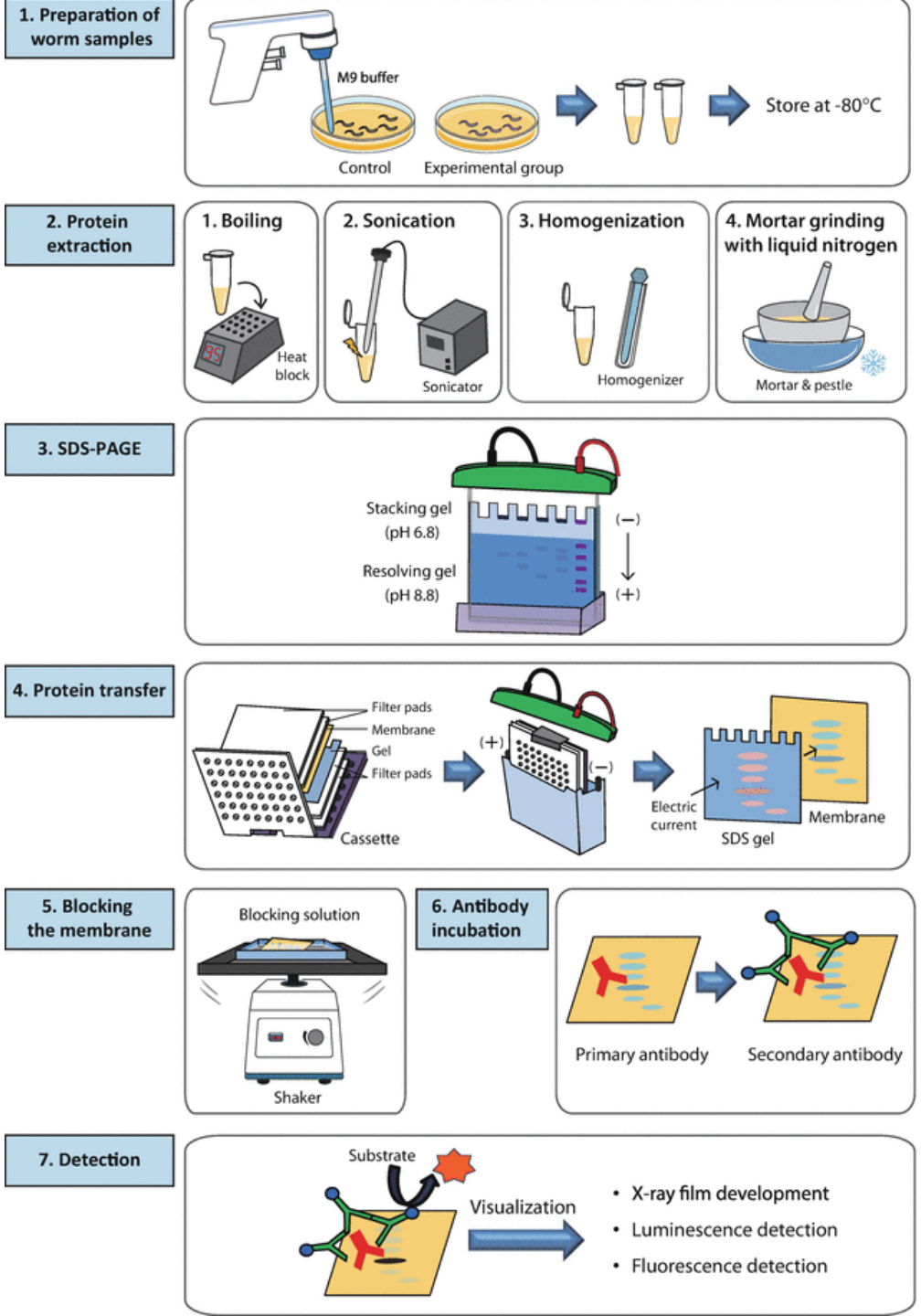
IMMUNOFLUORESCENZA



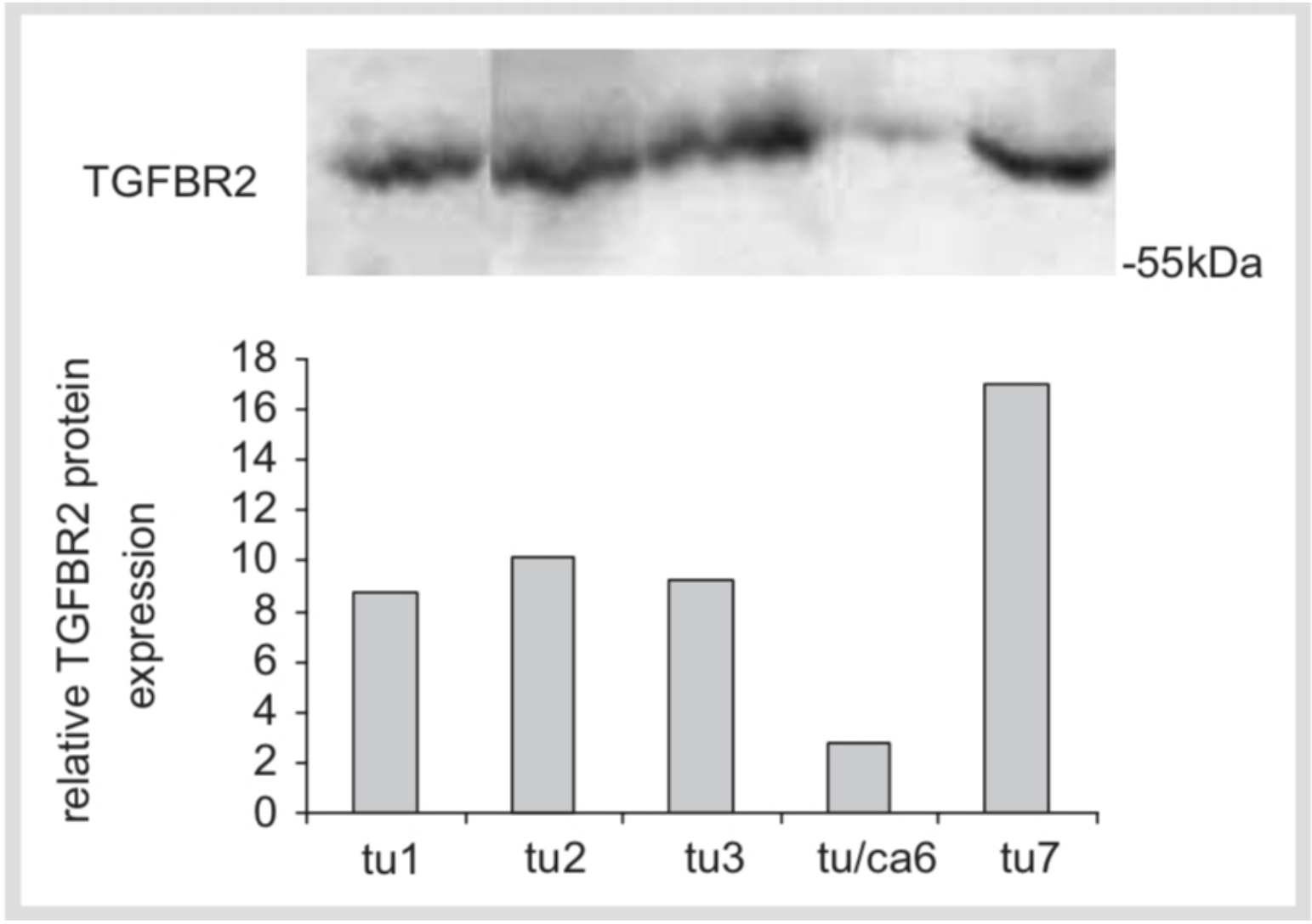
RISULTATI DELLA IMMUNOFLUORESCENZA



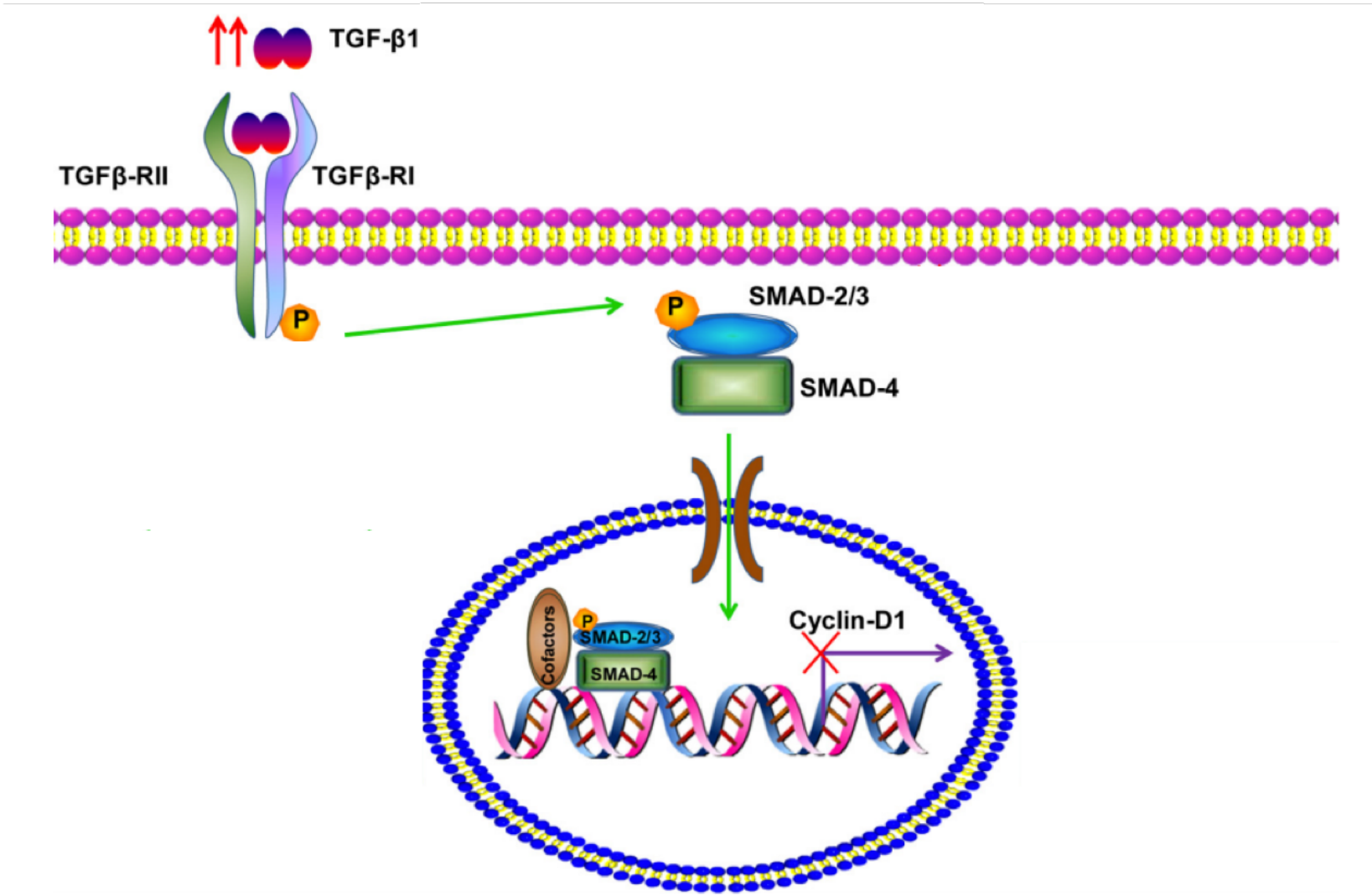
WESTERN BLOT



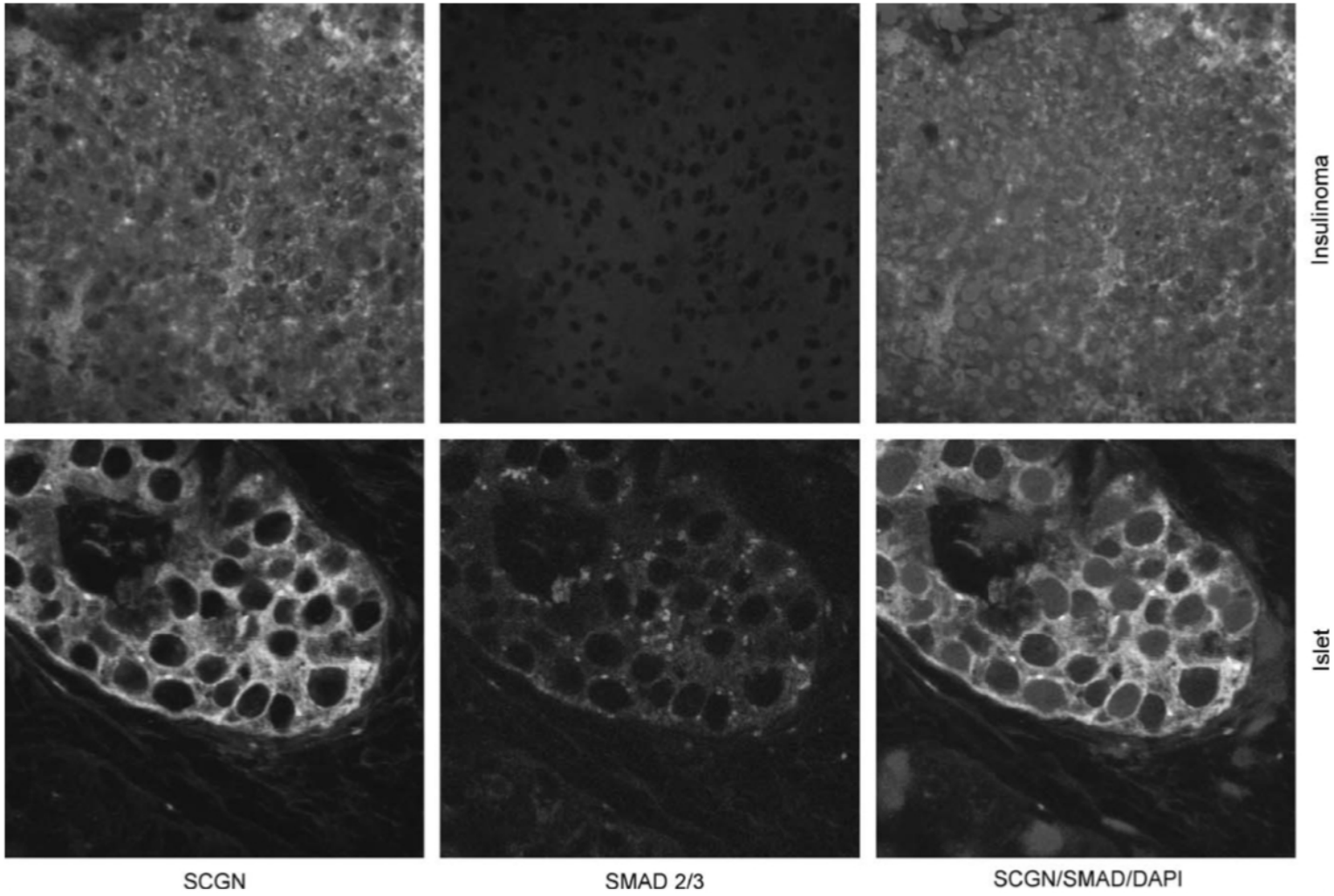
RISULTATI DEL WESTERN BLOT DI TGFBR2



LE PROTEINE SMAD



RISULTATI DELLA IMMUNOCOLORAZIONE



1 *Abraham EJ, Leech CA, Lin JC, Zulewski H, Habener JF*. Insulinotropic hormone glucagon-like peptide-1 differentiation of human pancreatic islet-derived progenitor cells into insulin-producing cells. Endocrinol- ogy 2002; 143: 3152–3161

2 *Bardeesy N, Cheng KH, Berger JH, Chu GC, Pahler J, Olson P, Hezel AF, Horner J, Lauwers GY, Hanahan D, DePinho RA*. Smad4 is dispensable for normal pancreas development yet critical in progression and tumor biology of pancreas cancer. Genes Dev 2006; 20: 3130–3146

3 *Bilban M, Ghaffari-Tabrizi N, Hintermann E, Bauer S, Molzer S, Zoratti C, Malli R, Sharabi A, Hiden U, Graier W, Knofler M, Andreae F, Wagner O, Quaranta V, Desoye G*. Kisspeptin-10, a KiSS-1/metastin-derived decapeptide, is a physiological invasion inhibitor of primary human trophoblasts. J Cell Sci 2004; 117: 1319–1328

4 *Birkenkamp-Demtroder K, Wagner L, Brandt Sorensen F, Bording Astrup L, Gartner W, Scherubl H, Heine B, Christiansen P, Orntoft TF*. Secretagogin is a novel marker for neuroendocrine differentiation. Neuroendo- crinology 2005; 82: 121–138

5 *Boden G, Murer E, Mozzoli M*. Glucose transporter proteins in human insulinoma. Ann Intern Med 1994; 121: 109–112

6 *Bonner-Weir S, Taneja M, Weir GC, Tatarkiewicz K, Song KH, Sharma A, O'Neil JJ*. In vitro cultivation of human islets from expanded ductal tissue. Proc Natl Acad Sci USA 2000; 97: 7999–8004

7 *Bonner-Weir S, Weir GC*. New sources of pancreatic beta-cells. Nat Biotechnol 2005; 23: 857–861

8 *Brat DJ, Lillemoe KD, Yeo CJ, Warfield PB, Hruban RH*. Progression of pancreatic intraductal neoplasias to infiltrating adenocarcinoma of the pancreas. Am J Surg Pathol 1998; 22: 163–169

9 *Chambers RC, Leoni P, Kaminski N, Laurent GJ, Heller RA*. Global expres- sion profiling of fibroblast responses to transforming growth factor- beta1 reveals the induction of inhibitor of differentiation-1 and provides evidence of smooth muscle cell phenotypic switching. Am J Pathol 2003; 162: 533–546

10 *Tour D de la, Halvorsen T, Demeterco C, Tyrberg B, Itkin-Ansari P, Loy M, Yoo SJ, Hao E, Bossie S, Levine F*. Beta-cell differentiation from a human pancreatic cell line in vitro and in vivo. Mol Endocrinol 2001; 15: 476–483

12 *Dudoit S, Gentleman RC, Quackenbush J*. Open source software for the analysis of microarray data. Biotechniques 2003; Suppl: 45–51

13 *Friedrichsen BN, Galsgaard ED, Nielsen JH, Moldrup A*. Growth hor- mone- and prolactin-induced proliferation of insulinoma cells, INS-1, depends on activation of STAT5 (signal transducer and activator of transcription 5). Mol Endocrinol 2001; 15: 136–148

BIBLIOGRAFIA