7. Appendix: Densitometric analysis of RT-PCR results

For the biggest part of this work, the RT-PCR method was applied qualitatively: the expression or non-expression of certain markers in different cell types was analyzed. In certain cases however (mainly stimulation experiments), a relative quantitative assessment was necessary. For these cases, the method was applied semi-quantitatively: the optical density of each amplified band was calculated using the ImageJ image processing program and numerically expressed as the relative density in comparison to the optical density of the background. All factors that could influence these measurements (PCR conditions, number of amplification cycles, thickness of the agarose gel, image capture and scanning procedures) were standardized to avoid systemic errors. Furthermore, all results were normalized to the expression of the housekeeping gene ß-actin, which is constitutively expressed in all cells and serves therefore as an internal standard (159). Under these conditions, gross quantitative estimations were possible and broad differences in mRNA expression could be detected.

7.1 mRNA signal in different passages

Marker	Passage Number						
	1	2	3	4	5	6	7
Oct4	10.8192 (100.0%)	9.7607 (90.2%)	15.3473 (141.8%)	17.1686 (158.7%)	14.9558 (138.2%)	15.1489 (140.0)	9.7995 (90.6%)
GATA-4	12.4869 (100.0%)	13.3683 (108.0%)	6.0508 (48.5%)	6.3491 (50.8%)	19.7278 (157.9%)	15.9473 (127.7%)	18.0698 (144.7%)
HNF4α	6.9301 (100.0%)	6.6197 (95.5%)	5.2775 (76.1%)	8.2020 (118.3%)	7.4494 (107.5%)	5.1188 (73.86%)	5.2837 (76.2%)
p63	10.4235 (100.0%)	14.1711 (135.9%)	15.5890 (149.5%)	13.6388 (130.8%)	15.5730 (149.4%)	14.7648 (141.6%)	15.8398 (151.9%)
ß-actin	6.8257 (100.0%)	8.0145 (117.4%)	5.0772 (74.4%)	8.9630 (131.4%)	6.7325 (98.6%)	8.0145 (117.4%)	5.0769 (74.4%)

Table 7.1: Densitometric analysis of mRNA expression in thyrocyte cultures in different passages. Absolute numbers represent optical densities, percentages represent the difference in mRNA expression level in comparison to the first passage (first passage values are arbitrarily assigned the value 100.0%). The table corresponds to figure 3.8 (paragraph 3.4)

7.2 Comparison of stem cell marker expression between nodular and paranodular thyroid regions.

Marker	Tissue A		Tissue B		Tissue C	
	Nodular	Paranodular	Nodular	Paranodular	Nodular	Paranodular
Oct4	49.1852 (96.8%)	50.8148 (100.0 %)	46.9682 (88.6%)	53.0318 (100.0%)	46.9904 (88.6%)	53.0096 (100.0%)
GATA-4	52.1567 (109.1%)	47.8433 (100.0%)	58.8289 (142.8%)	41.1711 (100.0%)	46.7133 (68.9%)	53.2867 (100.0%)
HNF4α	58.8945 (109.0%)	41.1055 (100%)	36.8494 (58.3%)	63.1506 (100.0%)	55.5383 (124.9%)	44.4617 (100.0%)
ß-actin	44.6282 (80.6%)	55.3718 (100.0%)	37.3553 (59.63%)	62.6447 (100.0%)	53.2841 (114.0%)	46.7159 (100.0%)

Table 7.2: Densitometric analysis of stem cell marker mRNA expression in nodular and paranodular thyroid regions. Absolute values represent optical densities, percentages represent the difference between nodular and paranodular regions. (for comparison reasons, paranodular regions have been arbitrarily assigned the value 100%). The table corresponds to figure 3.9 (paragraph 3.5).

7.3 Stimulation with xanthosine

Marker	48 hours			7 days		
	Controls	200 Xs	400 Xs	Controls	200 Xs	400 Xs
Oct4	38.8615 (100.0%)	35.3393 (90.9%)	25.7992 (71.5%)	40.2724 (100.0%)	26.9540 (66.9%)	32.7736 (81.3%)
GATA-4	23.3737 (100.0%)	37.9267 (162.26%)	38.6996 (165.56%)	26.8338 (100.0%)	33.6958 (125.6%)	39.4703 (147.1%)
HNF4α	39.0250 (100.0%)	32.6461 (83.65%)	28.3289 (72.6%)	42.0040 (100.0%)	32.9757 (78.5%)	25.0203 (60.6%)

Table 7.3: Densitometric analysis of stem cell marker mRNA expression in primary thyrocyte cultures after treatment with varying concentrations of xanthosine (200 and 400 μM) and controls. Absolute numbers represent optical densities, percentages represent the difference between stimulated probes and controls (all controls are arbitrarily assigned the value 100.0%). The table corresponds to figure 3.11 (paragraph 3.8).

Marker	14 days			30 days		
	Controls	200 Xs	400 Xs	Controls	200 Xs	400 Xs
Oct4	43.5218 (100.0%)	29.5629 (67.9%)	26.9154 (61.84%)	25.3167 (100.0%)	34.8269 (137.6%)	39.8565 (157.4%)
GATA-4	27.8121 (100%)	41.7468 (150.1%)	24.4411 (87.88%)	23.7682 (100.0%)	43.9205 (184.8%)	22.3113 (93.87%)
HNF4α	36.0672 (100.0%)	30.1344 (83.5%)	33.7984 (93.7%)	28.1022 (100.0%)	30.6424 (109.3%)	41.2554 (146.8%)

Table 7.3 (continued): Densitometric analysis of stem cell marker mRNA expression in primary thyrocyte cultures after treatment with varying concentrations of xanthosine (200 and 400 μM) and controls. Absolute numbers represent optical densities, percentages represent the difference between stimulated probes and controls (all controls are arbitrarily assigned the value 100.0%). The table corresponds to figure 3.11 (paragraph 3.8).

7.4 TSH Stimulation

Marker	8 days		15 days	
	200 mU/ml TSH	Controls	200 mU/ml TSH	Controls
Oct4	42.3951 (73.6%)	57.6049 (100%)	49.7195 (98.9%)	50.2805 (100%)
GATA-4	83.1060 (491,9 %)	16.8940 (100%)	86.0810 (618,8 %)	13.9190 (100%)
HNF4α	54.9140 (121,7 %)	45.0860 (100%)	53.8314 (116,6%)	46.1686 (100%)
ß-actin	51.0548 (104,3%)	48.9452 (100%)	51.6838 (106,9%)	48.3162 (100%)

Table 7.4: Densitometric analysis of mRNA expression in primary thyrocyte cultures stimulated with high-dose TSH (200 mU/ml) in comparison to controls. Absolute numbers represent optical densities, percentages represent the difference between stimulated probes and controls (all controls are arbitrarily assigned the value 100.0%). The table corresponds to figure 3.12 (paragraph 3.9).