

Table 1. Differentially expressed miRNAs in the screening phase.

| miRNA | Fold change | | |
|--------------|-------------|--------|-----------|
| | Pool 1 | Pool 2 | Mean fold |
| let-7b-5p | 3.23 | 3.11 | 3.17 |
| miR-106a-5p | 2.97 | 2.76 | 2.76 |
| miR-144-5p | 4.15 | 1.80 | 2.97 |
| miR-15b-3p | 3.36 | 2.25 | 2.80 |
| miR-17-5p | 1.74 | 1.78 | 1.76 |
| miR-18a-5p | 2.37 | 2.36 | 2.36 |
| miR-18b-5p | 1.73 | 1.77 | 1.75 |
| miR-20a-5p | 2.28 | 2.58 | 2.43 |
| miR-20b-5p | 6.28 | 3.87 | 5.08 |
| miR-25-3p | 4.08 | 2.97 | 3.52 |
| miR-296-5p | 2.27 | 1.74 | 2.01 |
| miR-29b-2-5p | 4.24 | 2.98 | 3.61 |
| miR-324-3p | 2.68 | 2.35 | 2.51 |
| miR-331-3p | 2.81 | 1.86 | 2.33 |
| miR-451a | 2.41 | 2.29 | 2.35 |
| miR-484 | 2.32 | 1.58 | 1.95 |
| miR-486-5p | 4.75 | 2.33 | 3.54 |
| miR-500a-5p | 2.68 | 2.68 | 2.68 |
| miR-574-3p | 1.95 | 1.82 | 1.89 |
| miR-92a-3p | 4.90 | 2.02 | 3.46 |
| miR-92b-3p | 4.11 | 1.62 | 2.87 |
| miR-93-3p | 4.51 | 2.40 | 3.46 |
| miR-93-5p | 2.30 | 2.61 | 2.45 |
| miR-30b-5p | 3.37 | 2.72 | 3.05 |
| miR-375 | 3.44 | 2.78 | 3.11 |
| miR-543 | 3.47 | 2.80 | 3.14 |
| miR-139-5p | 3.68 | 2.20 | 2.94 |
| miR-150-5p | 4.11 | 2.83 | 3.47 |
| miR-18a-3p | 4.13 | 2.41 | 3.27 |
| miR-192-5p | 4.57 | 1.80 | 3.19 |
| miR-151a-3p | 4.77 | 3.85 | 4.31 |
| miR-200a-3p | 5.19 | 4.19 | 4.69 |
| miR-485-3p | 7.13 | 5.75 | 6.44 |
| miR-495-3p | 7.20 | 5.81 | 6.51 |
| miR-16-2-3p | 8.34 | 6.73 | 7.54 |
| miR-204-5p | 10.38 | 8.37 | 9.38 |
| miR-142-5p | -4.30 | -3.64 | -3.97 |
| miR-338-3p | -4.62 | -2.85 | -3.73 |
| miR-33a-5p | -8.01 | -13.51 | -10.76 |
| miR-424-5p | -17.60 | -23.29 | -20.44 |

Table 2. Expression levels of the identified miRNAs from screening phase but not passed through the training stage and the testing stage.

| miRNA | Training stage | | | | Testing stage | | | |
|--------------|----------------|------------|------|----------------|---------------|------------|------|----------------|
| | Cases | Controls | FC | <i>P</i> value | Cases | Controls | FC | <i>P</i> value |
| let-7b-5p | 4.22±1.69 | 5.57±1.78 | 2.56 | 0.003 | 4.38±0.89 | 4.93±0.67 | 1.46 | 0.100 |
| miR-150-5p | 5.15±1.63 | 6.49±1.28 | 2.52 | <0.001 | 6.87±1.03 | 7.00±0.59 | 1.10 | 0.704 |
| miR-204-5p | 4.18±1.41 | 5.05±1.67 | 1.82 | 0.031 | 9.08±1.32 | 9.78±1.52 | 1.62 | 0.140 |
| miR-485-3p | 4.72±2.17 | 6.12±2.78 | 2.64 | 0.019 | 11.52±1.93 | 11.69±1.85 | 1.12 | 0.634 |
| miR-139-5p | 0.58±1.78 | 1.78±1.48 | 2.30 | 0.001 | 9.59±0.94 | 9.47±0.73 | 0.92 | 0.410 |
| miR-200a-3p | 5.10±1.62 | 6.32±1.78 | 2.33 | 0.005 | 7.08±0.87 | 7.44±0.71 | 1.28 | 0.067 |
| miR-20a-5p | 6.31±1.44 | 7.99±1.22 | 3.20 | <0.001 | 11.00±1.75 | 11.54±1.49 | 1.46 | 0.227 |
| miR-92b-3p | 1.75±1.61 | 2.78±2.54 | 2.05 | 0.049 | 5.33±0.76 | 5.38±0.78 | 1.04 | 0.590 |
| miR-93-5p | -0.38±2.27 | 1.13±2.79 | 2.83 | 0.006 | 4.18±0.89 | 4.56±0.70 | 1.30 | 0.038 |
| miR-106a-5p | 1.89±1.57 | 1.91±1.127 | 1.01 | 0.517 | | | | |
| miR-142-5p | 5.36±2.10 | 6.04±1.82 | 1.60 | 0.081 | | | | |
| miR-144-5p | 10.40±2.44 | 11.29±2.27 | 1.87 | 0.242 | | | | |
| miR-151a-3p | 5.39±4.28 | 5.62±2.54 | 1.17 | 0.876 | | | | |
| miR-151a-5p | 4.45±1.90 | 5.29±2.47 | 1.79 | 0.127 | | | | |
| miR-15b-3p | 4.17±3.76 | 4.81±4.30 | 1.56 | 0.401 | | | | |
| miR-16-2-3p | 8.09±2.45 | 8.98±1.91 | 1.86 | 0.143 | | | | |
| miR-17-5p | 4.46±1.53 | 4.86±1.29 | 1.32 | 0.179 | | | | |
| miR-18a-3p | 5.71±1.96 | 5.94±2.15 | 1.18 | 0.508 | | | | |
| miR-18a-5p | 6.86±4.01 | 7.95±4.55 | 2.13 | 0.092 | | | | |
| miR-18b-5p | 8.75±2.06 | 9.23±2.00 | 1.39 | 0.212 | | | | |
| miR-190a | 9.71±2.24 | 10.66±2.11 | 1.93 | 0.183 | | | | |
| miR-192-5p | 1.03±1.06 | 1.04±1.30 | 1.01 | 0.914 | | | | |
| miR-20b-5p | 2.54±1.79 | 2.49±2.15 | 0.96 | 0.764 | | | | |
| miR-222-3p | -0.72±2.14 | 0.08±2.34 | 1.74 | 0.077 | | | | |
| miR-29b-2-5p | 6.58±4.01 | 7.30±4.67 | 1.65 | 0.341 | | | | |
| miR-30b-5p | 3.28±4.32 | 4.06±4.67 | 1.72 | 0.143 | | | | |
| miR-324-3p | 3.61±4.24 | 4.68±4.29 | 2.10 | 0.103 | | | | |
| miR-331-3p | 9.13±3.06 | 9.96±3.71 | 1.79 | 0.081 | | | | |
| miR-338-3p | 7.24±4.13 | 7.84±4.51 | 1.52 | 0.408 | | | | |
| miR-33a-5p | 3.64±2.98 | 4.27±3.60 | 1.54 | 0.401 | | | | |
| miR-375 | 2.53±4.11 | 3.34±4.38 | 1.75 | 0.157 | | | | |
| miR-424-5p | 5.83±3.68 | 6.41±4.38 | 1.50 | 0.259 | | | | |
| miR-451a | 3.02±4.14 | 3.57±3.84 | 1.47 | 0.421 | | | | |
| miR-484 | 1.14±4.10 | 1.55±4.23 | 1.33 | 0.302 | | | | |
| miR-486-5p | -3.26±2.52 | -2.26±2.64 | 1.99 | 0.171 | | | | |
| miR-495-3p | 0.77±1.39 | 1.58±2.10 | 1.76 | 0.105 | | | | |
| miR-500a-5p | 6.63±4.03 | 6.96±4.73 | 1.25 | 0.524 | | | | |
| miR-543 | 6.55±3.82 | 7.29±4.46 | 1.67 | 0.127 | | | | |
| miR-574-3p | -6.33±2.39 | -5.62±2.86 | 1.63 | 0.199 | | | | |
| miR-93-3p | 8.25±1.82 | 8.35±2.47 | 1.07 | 0.308 | | | | |

miR-95-5p 7.33±1.06 8.10±4.62 1.71 0.271

Data are presented as median ± standard deviation; FC: Fold change.

Table 3. Expression levels of the three miRNAs in the external cohort.

| miRNA | Cases | Controls | FC | P value |
|------------|-----------|-----------|------|---------|
| miR-25-3p | 1.68±1.67 | 2.95±0.58 | 2.40 | 0.037 |
| miR-296-5p | 2.24±1.37 | 3.36±0.63 | 2.18 | 0.044 |
| miR-92a-3p | 6.91±0.87 | 7.90±0.92 | 1.99 | 0.019 |

Data are presented as median ± standard deviation; FC: Fold change.

Table 4. Differentially expressed miRNAs in the 2 NG pools and 1 HC pool.

| miRNA | Fold change | | |
|-------------|-------------|--------|-----------|
| | Pool 1 | Pool 2 | Mean fold |
| miR-375 | 5.94 | 1.53 | 3.74 |
| miR-16-5p | 3.60 | 1.80 | 2.70 |
| miR-483-5p | 1.71 | 3.36 | 2.54 |
| miR-144-3p | 2.57 | 1.83 | 2.20 |
| miR-501-3p | 1.76 | 2.39 | 2.07 |
| miR-885-5p | 1.62 | 2.40 | 2.01 |
| miR-33a-5p | 2.28 | 1.69 | 1.98 |
| miR-324-3p | 2.13 | 1.70 | 1.91 |
| miR-150-5p | 1.54 | 1.57 | 1.56 |
| miR-454-3p | -1.81 | -1.95 | -1.88 |
| let-7e-5p | -2.20 | -1.70 | -1.95 |
| miR-423-3p | -1.90 | -2.04 | -1.97 |
| miR-339-5p | -1.80 | -2.48 | -2.14 |
| miR-27b-3p | -1.81 | -2.74 | -2.27 |
| miR-30b-5p | -1.97 | -2.64 | -2.31 |
| miR-301a-3p | -2.73 | -2.07 | -2.40 |
| miR-30e-3p | -1.93 | -2.88 | -2.41 |
| miR-103a-3p | -2.13 | -2.83 | -2.48 |
| let-7a-5p | -1.94 | -3.07 | -2.50 |
| miR-221-3p | -1.72 | -3.40 | -2.56 |
| miR-151a-5p | -1.82 | -3.35 | -2.59 |
| miR-26a-5p | -1.89 | -3.30 | -2.59 |
| miR-23b-3p | -2.41 | -2.90 | -2.65 |
| miR-223-5p | -1.85 | -3.52 | -2.68 |
| miR-376a-3p | -1.72 | -3.75 | -2.73 |
| miR-27a-3p | -2.17 | -3.35 | -2.76 |
| let-7f-5p | -1.82 | -3.90 | -2.86 |
| miR-199a-3p | -1.94 | -3.80 | -2.87 |

| | | | |
|--------------------|--------|--------|--------|
| miR-133a-3p | -2.31 | -3.50 | -2.91 |
| miR-485-3p | -1.89 | -4.03 | -2.96 |
| miR-146b-5p | -2.12 | -3.92 | -3.02 |
| miR-495-3p | -2.48 | -3.57 | -3.03 |
| miR-199a-5p | -1.98 | -4.09 | -3.04 |
| miR-28-5p | -2.52 | -3.82 | -3.17 |
| miR-181a-5p | -2.85 | -3.54 | -3.19 |
| miR-143-3p | -2.47 | -4.37 | -3.42 |
| miR-326 | -3.01 | -3.84 | -3.42 |
| miR-30c-5p | -2.13 | -4.79 | -3.46 |
| let-7d-5p | -2.42 | -4.51 | -3.47 |
| miR-374b-5p | -2.43 | -5.28 | -3.86 |
| miR-223-3p | -2.29 | -5.97 | -4.13 |
| miR-155-5p | -4.07 | -4.30 | -4.18 |
| miR-766-3p | -2.59 | -5.94 | -4.27 |
| miR-382-5p | -2.79 | -6.07 | -4.43 |
| miR-195-5p | -3.62 | -5.97 | -4.80 |
| miR-154-5p | -4.33 | -7.58 | -5.96 |
| miR-133b | -4.39 | -10.92 | -7.66 |
| miR-136-5p | -5.34 | -10.63 | -7.99 |
| miR-127-3p | -9.89 | -7.61 | -8.75 |
| miR-200c-3p | -12.48 | -10.64 | -11.56 |
| miR-543 | -11.96 | -12.07 | -12.02 |

NG: Nodular goiter; HC: Healthy control.