qPCR Validation

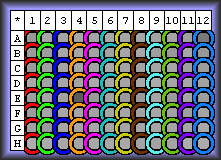
1. Reagent setup

|  |  |
| --- | --- |
| **Master Mix** | **1x** |
| DNA | 2 |
| Primer (5pMol/µL) | 2 |
| BioRad MasterMix | 12.5 |
| ddH20 | 8.5 |
|  | 25 µL |

Use IAP and ESET for RNA preparation control.

Use BioRad MasterMix. Use reverse pipetting to avoid reagent loss.

95°C 5m

94°C 30s |

59°C 30s | => 35×

72°C 30s |

Melting curve 55->59°C every 0.2°C, hold 1s.

Total time 2h30m

2. Plate Setup

Each plate can assay maximum 2 group of treatment (b-actin, ESET, IAP, RT-), using delta Ct for quantification.

Male PGC and soma (A1~D12)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Well | Content | Description | Efficiency | Avg Efficiency | C(t) | Avg C(t) | Std C(t) |
| A1 | mSoma KO | b-actin | 2.32 | 2.22 | 22.95 | 22.99 | 0.07 |
| A2 | mSoma KO | b-actin | 2.25 |  | 22.94 |  |  |
| A3 | mSoma KO | b-actin | 2.1 |  | 23.07 |  |  |
| A4 | mPGC KO | b-actin | 2.37 | 2.14 | 23.96 | 24.04 | 0.08 |
| A5 | mPGC KO | b-actin | 2.04 |  | 24.04 |  |  |
| A6 | mPGC KO | b-actin | 2.00 |  | 24.12 |  |  |
| A7 | mPGC WT | b-actin | 2.41 | 2.21 | 23.85 | 23.83 | 0.02 |
| A8 | mPGC WT | b-actin | 2.14 |  | 23.82 |  |  |
| A9 | mPGC WT | b-actin | 2.08 |  | 23.82 |  |  |
| A10 | mPGC CKO | b-actin | 1.94 | 2.14 | 22.42 | 22.29 | 0.18 |
| A11 | mPGC CKO | b-actin | 2.24 |  | 22.09 |  |  |
| A12 | mPGC CKO | b-actin | 2.24 |  | 22.36 |  |  |
| B1 | mSoma KO | ESET | 1.75 | 1.52 | 31.18 | 32.12 | 1.15 |
| B2 | mSoma KO | ESET | 1.49 |  | 31.78 |  |  |
| B3 | mSoma KO | ESET | 1.32 |  | 33.40 |  |  |
| B4 | mPGC KO | ESET | 1.16 | 1.20 | 34.06 | 33.99 | 0.23 |
| B5 | mPGC KO | ESET | 1.19 |  | 34.17 |  |  |
| B6 | mPGC KO | ESET | 1.25 |  | 33.73 |  |  |
| B7 | mPGC WT | ESET | 1.25 | 1.27 | 33.18 | 33.26 | 0.56 |
| B8 | mPGC WT | ESET | 1.27 |  | 32.74 |  |  |
| B9 | mPGC WT | ESET | 1.28 |  | 33.85 |  |  |
| B10 | mPGC CKO | ESET | 1.37 | 1.52 | 32.19 | 30.71 | 1.57 |
| B11 | mPGC CKO | ESET | 1.47 |  | 30.87 |  |  |
| B12 | mPGC CKO | ESET | 1.73 |  | 29.07 |  |  |
| C1 | mSoma KO | IAP | 1.99 | 1.77 | 28.55 | 30.28 | 2.01 |
| C2 | mSoma KO | IAP | 1.74 |  | 29.81 |  |  |
| C3 | mSoma KO | IAP | 1.59 |  | 32.48 |  |  |
| C4 | mPGC KO | IAP | 1.64 | 1.78 | 29.25 | 29.49 | 0.82 |
| C5 | mPGC KO | IAP | 1.95 |  | 28.82 |  |  |
| C6 | mPGC KO | IAP | 1.74 |  | 30.41 |  |  |
| C7 | mPGC WT | IAP | 1.57 | 1.53 | 31.20 | 31.69 | 0.52 |
| C8 | mPGC WT | IAP | 1.57 |  | 31.63 |  |  |
| C9 | mPGC WT | IAP | 1.44 |  | 32.23 |  |  |
| C10 | mPGC CKO | IAP | 1.85 | 1.91 | 29.35 | 28.30 | 0.95 |
| C11 | mPGC CKO | IAP | 1.92 |  | 28.05 |  |  |
| C12 | mPGC CKO | IAP | 1.97 |  | 27.50 |  |  |
| D1 | RT- | b-actin | 1.52 | 1.58 | 31.28 | 30.98 | 0.49 |
| D2 | RT- | b-actin | 1.54 |  | 30.41 |  |  |
| D3 | RT- | b-actin | 1.68 |  | 31.24 |  |  |
| D4 | RT- | b-actin | 1.74 | 1.68 | 31.01 | 31.39 | 0.76 |
| D5 | RT- | b-actin | 1.56 |  | 32.26 |  |  |
| D6 | RT- | b-actin | 1.75 |  | 30.90 |  |  |
| D7 | RT- | b-actin | 1.51 | 1.51 | 32.02 | 31.98 | 0.04 |
| D8 | RT- | b-actin | 1.49 |  | 31.94 |  |  |
| D9 | RT- | b-actin | 1.53 |  | 31.99 |  |  |
| D10 | RT- | b-actin | 1.72 | 1.82 | 32.32 | 31.56 | 0.91 |
| D11 | RT- | b-actin | 1.93 |  | 31.80 |  |  |
| D12 | RT- | b-actin | 1.8 |  | 30.55 |  |  |

Female PGC and soma (E1~H12)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| E1 | fSoma KO | b-actin | 2.09 | 2.16 | 23.13 | 23.34 | 0.23 |
| E2 | fSoma KO | b-actin | 2.12 |  | 23.31 |  |  |
| E3 | fSoma KO | b-actin | 2.27 |  | 23.58 |  |  |
| E4 | fPGC KO | b-actin | 2.03 | 2.14 | 23.35 | 23.69 | 0.29 |
| E5 | fPGC KO | b-actin | 2.17 |  | 23.86 |  |  |
| E6 | fPGC KO | b-actin | 2.22 |  | 23.86 |  |  |
| E7 | fPGC WT | b-actin | 2.10 | 2.13 | 23.62 | 23.61 | 0.14 |
| E8 | fPGC WT | b-actin | 2.22 |  | 23.75 |  |  |
| E9 | fPGC WT | b-actin | 2.07 |  | 23.47 |  |  |
| E10 | fPGC CKO | b-actin | 2.10 | 2.03 | 27.38 | 27.07 | 0.28 |
| E11 | fPGC CKO | b-actin | 2.03 |  | 26.96 |  |  |
| E12 | fPGC CKO | b-actin | 1.97 |  | 26.86 |  |  |
| F1 | fSoma KO | ESET | 1.47 | 1.30 | 31.77 | 32.93 | 1.20 |
| F2 | fSoma KO | ESET | 1.26 |  | 32.86 |  |  |
| F3 | fSoma KO | ESET | 1.17 |  | 34.17 |  |  |
| F4 | fPGC KO | ESET | 1.23 | 1.21 | 34.00 | 34.44 | 0.62 |
| F5 | fPGC KO | ESET | 1.19 |  | 34.88 |  |  |
| F6 | fPGC KO | ESET | N/A |  | N/A |  |  |
| F7 | fPGC WT | ESET | 1.25 | 1.25 | 33.76 | 33.48 | 0.25 |
| F8 | fPGC WT | ESET | 1.22 |  | 33.40 |  |  |
| F9 | fPGC WT | ESET | 1.29 |  | 33.27 |  |  |
| F10 | fPGC CKO | ESET | 1.14 | 1.29 | 35.45 | 34.56 | 1.27 |
| F11 | fPGC CKO | ESET | 1.19 |  | 35.12 |  |  |
| F12 | fPGC CKO | ESET | 1.54 |  | 33.11 |  |  |
| G1 | fSoma KO | IAP | 2.1 | 1.79 | 28.92 | 30.46 | 1.99 |
| G2 | fSoma KO | IAP | 1.77 |  | 29.75 |  |  |
| G3 | fSoma KO | IAP | 1.49 |  | 32.70 |  |  |
| G4 | fPGC KO | IAP | 1.92 | 1.87 | 27.5 | 27.82 | 0.55 |
| G5 | fPGC KO | IAP | 1.78 |  | 27.5 |  |  |
| G6 | fPGC KO | IAP | 1.92 |  | 28.46 |  |  |
| G7 | fPGC WT | IAP | 1.54 | 1.70 | 31.65 | 31.34 | 0.59 |
| G8 | fPGC WT | IAP | 1.79 |  | 30.66 |  |  |
| G9 | fPGC WT | IAP | 1.78 |  | 31.71 |  |  |
| G10 | fPGC CKO | IAP | N/A | 1.50 | N/A | 32.845 | 0.73 |
| G11 | fPGC CKO | IAP | 1.41 |  | 33.36 |  |  |
| G12 | fPGC CKO | IAP | 1.58 |  | 32.33 |  |  |
| H1 | RT- | b-actin | 1.14 | 1.50 | 34.07 | 32.72 | 1.29 |
| H2 | RT- | b-actin | 1.73 |  | 31.50 |  |  |
| H3 | RT- | b-actin | 1.64 |  | 32.59 |  |  |
| H4 | RT- | b-actin | 1.76 | 1.89 | 30.94 | 31.53 | 0.54 |
| H5 | RT- | b-actin | 1.95 |  | 32.00 |  |  |
| H6 | RT- | b-actin | 1.95 |  | 31.65 |  |  |
| H7 | RT- | b-actin | 1.78 | 1.66 | 32.05 | 32.84 | 1.85 |
| H8 | RT- | b-actin | 1.32 |  | 34.96 |  |  |
| H9 | RT- | b-actin | 1.89 |  | 31.52 |  |  |
| H10 | RT- | b-actin | 1.75 | 1.71 | 32.67 | 31.73 | 0.97 |
| H11 | RT- | b-actin | 1.71 |  | 30.74 |  |  |
| H12 | RT- | b-actin | 1.66 |  | 31.79 |  |  |