qPCR Validation

1. Reagent setup

|  |  |
| --- | --- |
| **Master Mix** | **1x** |
| DNA | 2 |
| MgCl2 | 2.5 |
| 10x Buffer | 2.5 |
| dNTPs (2.5mM) | 2 |
| Primer (5pMol/µL) | 2 |
| EvaG | 1.25 |
| HS Ferm Taq | 0.2 |
| ddH20 | 12.55 |
|  | 25 µL |

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

Use IAP and MERVL for H3K9me3 ChIP control

IAP use BioRad MasterMix, MERVL use home made Master Mix 2.5MgCl2

Use reverse pipetting to avoid reagent loss.

95°C 5m

94°C 30s |

59°C 30s | => 35×

72°C 30s |

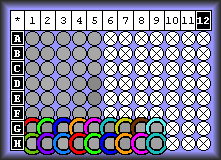
Melting curve 55->59°C every 0.2°C, hold 1s.

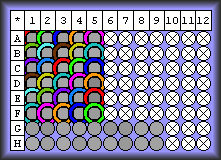
Total time 2h30m

2. Plate Setup

Each plate can assay maximum 4 IP (3 target, 1 IgG and 1 Input) with 2 amplicon, using standard curve method for quantification.

IAP ChIP IAP Standard





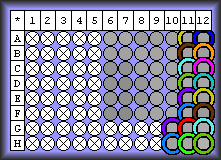
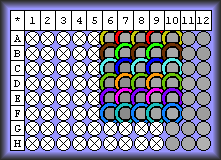
IAP ChIP

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Well | Dye | Content | Description | Efficiency | C(t) | ng |
| A1 | SBG1 | Sample | H3K9me3-M | 2.04 | 20.05 | 0.07062 |
| B1 | SBG1 | Sample | H3K9me3-M | 2.16 | 19.96 | 0.07481 |
| C1 | SBG1 | Sample | H3K9me3-M | 2.08 | 22.00 | 0.02133 |
| D1 | SBG1 | Sample | H3K9me3-N | 2.01 | 20.43 | 0.056 |
| E1 | SBG1 | Sample | H3K9me3-N | 2.22 | 20.77 | 0.04545 |
| F1 | SBG1 | Sample | H3K9me3-N | 2.01 | 20.64 | 0.04913 |
| A2 | SBG1 | Sample | H3K9me2-M | 2.13 | 21.30 | 0.03287 |
| B2 | SBG1 | Sample | H3K9me2-M | 2.10 | 21.17 | 0.03543 |
| C2 | SBG1 | Sample | H3K9me2-M | 1.98 | 21.37 | 0.03146 |
| D2 | SBG1 | Sample | H3K9me2-N | 2.00 | 21.84 | 0.02346 |
| E2 | SBG1 | Sample | H3K9me2-N | 2.17 | 21.94 | 0.02206 |
| F2 | SBG1 | Sample | H3K9me2-N | 2.04 | 21.77 | 0.02455 |
| A3 | SBG1 | Sample | H3-M | 2.10 | 19.62 | 0.09246 |
| B3 | SBG1 | Sample | H3-M | 2.16 | 19.70 | 0.08766 |
| C3 | SBG1 | Sample | H3-M | 2.16 | 19.80 | 0.08247 |
| D3 | SBG1 | Sample | H3-N | 2.07 | 20.70 | 0.04743 |
| E3 | SBG1 | Sample | H3-N | 2.05 | 20.47 | 0.0546 |
| F3 | SBG1 | Sample | H3-N | 1.98 | 20.47 | 0.0545 |
| A4 | SBG1 | Sample | IgG-M | 1.95 | 24.06 | 0.005992 |
| B4 | SBG1 | Sample | IgG-M | 2.00 | 24.44 | 0.004735 |
| C4 | SBG1 | Sample | IgG-M | 1.92 | 24.85 | 0.003695 |
| D4 | SBG1 | Sample | IgG-N | 1.89 | 24.13 | 0.005756 |
| E4 | SBG1 | Sample | IgG-N | 1.95 | 24.00 | 0.006225 |
| F4 | SBG1 | Sample | IgG-N | 2.04 | 23.95 | 0.006407 |
| A5 | SBG1 | Sample | Input-M | 2.18 | 21.77 | 0.02463 |
| B5 | SBG1 | Sample | Input-M | 2.06 | 21.72 | 0.02535 |
| C5 | SBG1 | Sample | Input-M | 2.04 | 21.95 | 0.02205 |
| D5 | SBG1 | Sample | Input-N | 1.83 | 22.01 | 0.02115 |
| E5 | SBG1 | Sample | Input-N | 1.93 | 22.20 | 0.01884 |
| F5 | SBG1 | Sample | Input-N | 2.04 | 22.18 | 0.01904 |

IAP Standard

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Well | Dye | Content | Description | Efficiency | C(t) | ng |
| G1 | SBG1 | Standard | - | 2.13 | 19.38 | 0.1 |
| G2 | SBG1 | Standard | - | 1.90 | 19.27 | 0.1 |
| G3 | SBG1 | Standard | - | 1.99 | 20.03 | 0.1 |
| G4 | SBG1 | Standard | - | 2.00 | 23.35 | 0.01 |
| G5 | SBG1 | Standard | - | 2.12 | 23.35 | 0.01 |
| G6 | SBG1 | Standard | - | 1.92 | 23.31 | 0.01 |
| G7 | SBG1 | Standard | - | 1.84 | 27.25 | 0.001 |
| G8 | SBG1 | Standard | - | 1.87 | 26.81 | 0.001 |
| G9 | SBG1 | Standard | - | 1.94 | 26.74 | 0.001 |
| H1 | SBG1 | Standard | - | 2.20 | 20.42 | 0.05 |
| H2 | SBG1 | Standard | - | 1.99 | 20.44 | 0.05 |
| H3 | SBG1 | Standard | - | 2.10 | 20.72 | 0.05 |
| H4 | SBG1 | Standard | - | 1.99 | 24.08 | 0.005 |
| H5 | SBG1 | Standard | - | 1.89 | 24.34 | 0.005 |
| H6 | SBG1 | Standard | - | 1.98 | 24.49 | 0.005 |
| H7 | SBG1 | Blank | - | N/A | N/A | N/A |
| H8 | SBG1 | Blank | - | N/A | N/A | N/A |
| H9 | SBG1 | Blank | - | N/A | N/A | N/A |

MERVL ChIP MERVL Standard



MERVL ChIP

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Well | Dye | Content | Description | Efficiency | C(t) | ng |
| A6 | SBG1 | Sample | H3K9me3-M | 2.05 | 23.31 | 0.02194 |
| B6 | SBG1 | Sample | H3K9me3-M | 1.94 | 23.50 | 0.0193 |
| C6 | SBG1 | Sample | H3K9me3-M | 1.98 | 23.65 | 0.01741 |
| D6 | SBG1 | Sample | H3K9me3-N | 1.79 | 22.16 | 0.04834 |
| E6 | SBG1 | Sample | H3K9me3-N | 1.75 | 22.28 | 0.04437 |
| F6 | SBG1 | Sample | H3K9me3-N | 1.73 | 22.15 | 0.04859 |
| A7 | SBG1 | Sample | H3K9me2-M | 1.91 | 21.79 | 0.06192 |
| B7 | SBG1 | Sample | H3K9me2-M | 1.93 | 22.42 | 0.04044 |
| C7 | SBG1 | Sample | H3K9me2-M | 1.92 | 22.85 | 0.03009 |
| D7 | SBG1 | Sample | H3K9me2-N | 1.89 | 23.16 | 0.02441 |
| E7 | SBG1 | Sample | H3K9me2-N | 1.73 | 22.96 | 0.02788 |
| F7 | SBG1 | Sample | H3K9me2-N | 1.73 | 22.89 | 0.02927 |
| A8 | SBG1 | Sample | H3-M | 1.95 | 21.24 | 0.0903 |
| B8 | SBG1 | Sample | H3-M | 1.93 | 21.78 | 0.06234 |
| C8 | SBG1 | Sample | H3-M | 1.89 | 22.20 | 0.04684 |
| D8 | SBG1 | Sample | H3-N | 1.95 | 23.66 | 0.01733 |
| E8 | SBG1 | Sample | H3-N | 1.77 | 23.57 | 0.01845 |
| F8 | SBG1 | Sample | H3-N | 1.92 | 23.39 | 0.02086 |
| A9 | SBG1 | Sample | IgG-M | 1.93 | 20.95 | 0.11 |
| B9 | SBG1 | Sample | IgG-M | 2.07 | 21.83 | 0.06031 |
| C9 | SBG1 | Sample | IgG-M | 1.86 | 22.24 | 0.04551 |
| D9 | SBG1 | Sample | IgG-N | 1.79 | 26.56 | 0.002392 |
| E9 | SBG1 | Sample | IgG-N | 1.75 | 26.58 | 0.002351 |
| F9 | SBG1 | Sample | IgG-N | 1.80 | 26.35 | 0.002751 |
| A10 | SBG1 | Sample | Input-M | 2.13 | 22.85 | 0.03009 |
| B10 | SBG1 | Sample | Input-M | 1.95 | 23.25 | 0.02283 |
| C10 | SBG1 | Sample | Input-M | 2.01 | 23.65 | 0.01739 |
| D10 | SBG1 | Sample | Input-N | 2.00 | 21.70 | 0.06586 |
| E10 | SBG1 | Sample | Input-N | 1.94 | 21.84 | 0.05982 |
| F10 | SBG1 | Sample | Input-N | 1.99 | 21.66 | 0.06791 |

MERVL Standard

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Well | Dye | Content | Description | Efficiency | C(t) | ng |
| A11 | SBG1 | Standard | - | 2.10 | 20.76 | 0.1 |
| A12 | SBG1 | Standard | - | 1.98 | 22.01 | 0.05 |
| B11 | SBG1 | Standard | - | 1.97 | 21.14 | 0.1 |
| B12 | SBG1 | Standard | - | 2.01 | 22.25 | 0.05 |
| C11 | SBG1 | Standard | - | 2.02 | 21.27 | 0.1 |
| C12 | SBG1 | Standard | - | 1.95 | 22.15 | 0.05 |
| D11 | SBG1 | Standard | - | 1.87 | 24.63 | 0.01 |
| D12 | SBG1 | Standard | - | 1.91 | 25.27 | 0.005 |
| E11 | SBG1 | Standard | - | 1.85 | 24.75 | 0.01 |
| E12 | SBG1 | Standard | - | 1.89 | 25.34 | 0.005 |
| F11 | SBG1 | Standard | - | 1.88 | 24.55 | 0.01 |
| F12 | SBG1 | Standard | - | 1.86 | 25.38 | 0.005 |
| G10 | SBG1 | Standard | - | 1.72 | 28.17 | 0.001 |
| G11 | SBG1 | Standard | - | 1.82 | 27.80 | 0.001 |
| G12 | SBG1 | Standard | - | 1.79 | 27.43 | 0.001 |
| H10 | SBG1 | Blank | - | N/A | N/A | N/A |
| H11 | SBG1 | Blank | - | 1.67 | 31.53 | 7.985e-005 |
| H12 | SBG1 | Blank | - | 1.59 | 32.40 | 4.425e-005 |