

ΔΔCt法によるqPCRの評価



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ΔΔCt法でqPCR結果を評価する方法についてです。
流れとしては、

- ①測りたい遺伝子(Target gene)と内在性コントロール遺伝子(Endogeneous control gene)のCt値を用意する。
- ② (Target geneのCt値)-(Endogeneous control geneのCt値)をして、 ΔCt 値を出す。
- ③Controlグループの ΔCt 値の平均(Average ΔCt of control)を出す。
- ④(ControlグループおよびTargetグループ)-(Average ΔCt of control)をして、 $\Delta\Delta Ct$ 値を出す。
- ⑤($2^{-\Delta\Delta Ct}$ 値)をしてFold changeを出す。

という感じです。

①測りたい遺伝子(Target gene)と内在性コントロール遺伝子(Endogeneous control gene)のCt値を用意する。

| No. | Sample Name | Target Name (Endogenous Control) | Ct Value | Ct Mean | Target Name (Gene of Interest) | Ct Value | Ct Mean | Fold Change Calculation (FC=2 ^{-ΔΔCt}) | | |
|-----------|-------------|----------------------------------|----------|----------|--------------------------------|----------|----------|--|----------|-------------|
| | | | | | | | | ΔCt | ΔΔCt | Fold Change |
| 1 Control | GAPDH | | 17.84096 | 17.84096 | XXX | 28.32182 | 28.32182 | 10.48096 | 0.64039 | 0.529938511 |
| 2 Control | GAPDH | | 18.49893 | 18.49893 | XXX | 28.08094 | 28.08094 | 9.912912 | 0.10187 | 0.552965741 |
| 3 Control | GAPDH | | 17.74163 | 17.74163 | XXX | 28.12972 | 28.12972 | 10.38808 | 0.516357 | 0.47045527 |
| 4 Control | GAPDH | | 18.53343 | 18.53343 | XXX | 28.26110 | 28.26110 | 9.877726 | 0.03501 | 0.959964142 |
| 5 Control | GAPDH | | 18.09196 | 18.09196 | XXX | 27.98918 | 27.98918 | 8.897715 | -0.04551 | 1.854320295 |
| 6 Control | GAPDH | | 18.27267 | 18.27267 | XXX | 27.63851 | 27.63851 | 9.363444 | -0.46828 | 1.364414052 |
| 7 KO | GAPDH | | 18.27184 | 18.27184 | XXX | 26.08947 | 26.08947 | 7.867581 | -1.04419 | 3.848227173 |
| 8 KO | GAPDH | | 20.15447 | 20.15447 | XXX | 27.86519 | 27.86519 | 7.707429 | -2.1043 | 4.299380359 |
| 9 KO | GAPDH | | 18.88263 | 18.88263 | XXX | 26.40596 | 26.40596 | 7.423226 | -2.3894 | 5.235763379 |
| 10 KO | GAPDH | | 19.18336 | 19.18336 | XXX | 26.79085 | 26.79085 | 7.612495 | -2.18924 | 4.560544061 |
| 11 KO | GAPDH | | 20.9945 | 20.9945 | XXX | 28.39205 | 28.39205 | 7.397554 | -2.41417 | 5.330131101 |
| 12 KO | GAPDH | | 19.60417 | 19.60417 | XXX | 27.72108 | 27.72108 | 8.045916 | -1.75891 | 3.374817127 |

こんな感じでTarget gene(今回はXXX)と内臓性コントロール(今回はGAPDH)のCt Meanを用意します。(今回はduplicateとかしてないのでCt value=Ct meanです)

②(Target geneのCt値)-(Endogeneous control geneのCt値)をして、ΔCt値を出す。

| No. | Sample Name | Target Name (Endogenous Control) | Ct Value | Ct Mean | Target Name (Gene of Interest) | Ct Value | Ct Mean | Fold Change Calculation (FC=2 ^{-ΔΔCt}) | | |
|-----------|-------------|----------------------------------|----------|---------|--------------------------------|----------|---------|--|----------|-------------|
| | | | | | | | | ΔCt | ΔΔCt | Fold Change |
| 1 Control | GAPDH | | 17.8409 | 17.8409 | XXX | 28.3218 | 28.3218 | 10.481 | -0.66924 | 0.625938511 |
| 2 Control | GAPDH | | 18.4989 | 18.4989 | XXX | 28.0809 | 28.0809 | 9.91291 | 0.10189 | 0.97226676 |
| 3 Control | GAPDH | | 17.7416 | 17.7416 | XXX | 28.1297 | 28.1297 | 10.3881 | 0.51431 | 0.970656497 |
| 4 Control | GAPDH | | 18.5334 | 18.5334 | XXX | 28.2612 | 28.2612 | 9.82774 | 0.01601 | 0.98995414 |
| 5 Control | GAPDH | | 18.092 | 18.092 | XXX | 27.9892 | 27.9892 | 8.89772 | -0.0149 | 1.8649252 |
| 6 Control | GAPDH | | 18.2727 | 18.2727 | XXX | 27.6361 | 27.6361 | 9.36344 | -0.2493 | 1.35441405 |
| 7 KO | GAPDH | | 18.2718 | 18.2718 | XXX | 26.0894 | 26.0894 | 7.89153 | -1.9442 | 3.848227173 |
| 8 KO | GAPDH | | 20.1545 | 20.1545 | XXX | 27.8619 | 27.8619 | 7.70743 | -2.1043 | 4.299380359 |
| 9 KO | GAPDH | | 18.9826 | 18.9826 | XXX | 26.406 | 26.406 | 7.42333 | -2.3894 | 5.235763379 |
| 10 KO | GAPDH | | 19.1834 | 19.1834 | XXX | 26.7908 | 26.7908 | 7.62249 | -2.1892 | 4.56054406 |
| 11 KO | GAPDH | | 20.9945 | 20.9945 | XXX | 28.3921 | 28.3921 | 7.39755 | -2.4142 | 5.3301311 |
| 12 KO | GAPDH | | 19.6042 | 19.6042 | XXX | 27.7211 | 27.7211 | 8.04592 | -1.7589 | 3.37481713 |

上の図でいうと、ΔCt(10.481)=XXXのCt mean(28.3218)-GAPDHのCt mean(17.8409)です。これを全サンプル分出します。

③ControlグループのΔCt値の平均(Average ΔCt of control)を出す。

| No. | Sample Name | Target Name (Edge Label Control) | Ct Value | Ct Mean | Target Name (Gene of Interest) | Ct Value | Ct Mean | Fold Change Calculation (FC=2^-ΔΔCt) | | |
|-----|-------------|----------------------------------|----------|---------|--------------------------------|----------|---------|--------------------------------------|---------|-------------|
| | | | | | | | | ΔCt | ΔΔCt | Fold Change |
| 1 | Control | GAPDH | 17.418 | 17.8813 | GS | 18.272 | 18.2713 | 10.81 | 1.4651 | 1.6743051 |
| 2 | Control | GAPDH | 18.124 | 18.168 | GS | 18.2823 | 18.2868 | 9.9193 | 1.3118 | 1.9127519 |
| 3 | Control | GAPDH | 17.742 | 17.7428 | GS | 18.1232 | 18.1227 | 10.388 | 1.25248 | 1.8765221 |
| 4 | Control | GAPDH | 18.134 | 18.5511 | GS | 18.3637 | 18.3633 | 9.9774 | 1.21121 | 1.8885844 |
| 5 | Control | GAPDH | 17.954 | 18.2742 | GS | 17.9532 | 17.9562 | 9.8421 | 1.21545 | 1.8912249 |
| 6 | Control | GAPDH | 18.727 | 18.2727 | GS | 17.8311 | 17.7311 | 10.872 | 1.4481 | 1.9641245 |
| 7 | Control | GAPDH | 18.021 | 18.2718 | GS | 18.081 | 18.086 | 9.972 | 1.4417 | 1.9312111 |
| 8 | Control | GAPDH | 18.193 | 18.1119 | GS | 17.9827 | 17.9839 | 10.142 | 1.3161 | 1.7988248 |
| 9 | Control | GAPDH | 18.321 | 18.4824 | GS | 17.451 | 17.456 | 10.872 | 1.2864 | 1.9173101 |
| 10 | Control | GAPDH | 18.118 | 18.1481 | GS | 18.1148 | 18.1128 | 9.9283 | 1.1962 | 1.7484488 |
| 11 | Control | GAPDH | 18.015 | 18.0845 | GS | 18.1612 | 18.081 | 10.173 | 1.2449 | 1.8211111 |
| 12 | Control | GAPDH | 18.047 | 18.0467 | GS | 18.1211 | 18.1211 | 9.9289 | 1.1748 | 1.7148111 |
| | | | | | | | | Average of ΔCt of control | 9.81173 | |

Average ΔCt of controlは上でいう、ControlのΔCtの平均です。

④(ControlグループおよびTargetグループ)-(Average ΔCt of control)をして、ΔΔCt値を出す。

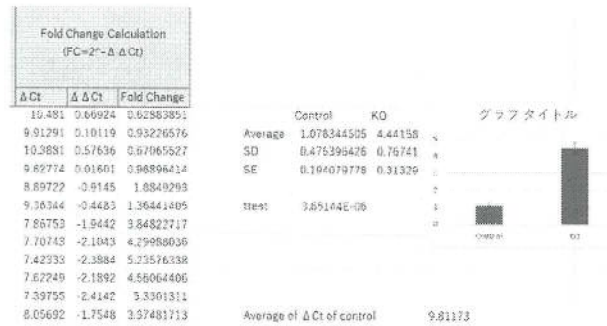
| No. | Sample Name | Target Name (Edge Label Control) | Ct Value | Ct Mean | Target Name (Gene of Interest) | Ct Value | Ct Mean | Fold Change Calculation (FC=2^-ΔΔCt) | | |
|-----|-------------|----------------------------------|----------|---------|--------------------------------|----------|---------|--------------------------------------|---------|-------------|
| | | | | | | | | ΔCt | ΔΔCt | Fold Change |
| 1 | Control | GAPDH | 17.418 | 17.8813 | GS | 18.2718 | 18.2713 | 10.81 | 1.4651 | 1.6743051 |
| 2 | Control | GAPDH | 18.124 | 18.168 | GS | 18.0824 | 18.0818 | 9.9193 | 1.2217 | 1.8174619 |
| 3 | Control | GAPDH | 17.7414 | 17.7411 | GS | 18.1297 | 18.1217 | 10.388 | 1.2718 | 1.8226521 |
| 4 | Control | GAPDH | 18.134 | 18.5511 | GS | 18.3633 | 18.3613 | 9.9771 | 1.2152 | 1.8909414 |
| 5 | Control | GAPDH | 17.954 | 18.2742 | GS | 17.9542 | 17.9542 | 9.842 | 1.2154 | 1.8912249 |
| 6 | Control | GAPDH | 18.727 | 18.2717 | GS | 17.8311 | 17.7311 | 9.7841 | 1.4481 | 1.9641245 |
| 7 | Control | GAPDH | 18.021 | 18.2718 | GS | 18.0794 | 18.0802 | 9.873 | 1.4417 | 1.9312111 |
| 8 | Control | GAPDH | 18.193 | 18.1119 | GS | 18.001 | 18.002 | 10.143 | 1.3162 | 1.8998248 |
| 9 | Control | GAPDH | 18.321 | 18.4824 | GS | 18.001 | 18.002 | 10.873 | 1.2864 | 1.9173101 |
| 10 | Control | GAPDH | 18.118 | 18.1481 | GS | 18.001 | 18.002 | 9.928 | 1.1962 | 1.7484488 |
| 11 | Control | GAPDH | 18.015 | 18.0845 | GS | 18.152 | 18.082 | 9.973 | 1.2449 | 1.8211111 |
| 12 | Control | GAPDH | 18.047 | 18.0467 | GS | 18.1211 | 18.1211 | 9.929 | 1.1748 | 1.7148111 |
| | | | | | | | | Average of ΔCt of control | 9.81173 | |

ΔΔCt値は上でいう、ΔCt値(10.481)-Average ΔCt of control(9.81173)です。これを全サンプル分出します。

⑤(2^-ΔΔCt値)をしてFold changeを出す。

| No. | Sample Name | Target Name (Endogenous Control) | Ct Value | Ct Mean | Target Name (Gene of Interest) | Ct Value | Ct Mean | Fold Change Calculation (FC=2 ^{-ΔΔCt}) | | |
|-----|-------------|----------------------------------|----------|---------|--------------------------------|----------|---------|--|---------|-------------|
| | | | | | | | | ΔCt | ΔΔCt | Fold Change |
| 1 | Control | GAPDH | 17.8409 | 17.8408 | XXX | 28.3218 | 28.3218 | 10.481 | 0.66924 | 0.62883851 |
| 2 | Control | GAPDH | 18.168 | 18.168 | XXX | 28.0909 | 28.0909 | 9.91291 | 0.10119 | 0.93226576 |
| 3 | Control | GAPDH | 17.7416 | 17.7416 | XXX | 28.1297 | 28.1297 | 10.3881 | 0.57639 | 0.67065527 |
| 4 | Control | GAPDH | 18.5334 | 18.5334 | XXX | 28.2813 | 28.2813 | 9.82774 | 0.01601 | 0.98896414 |
| 5 | Control | GAPDH | 19.092 | 19.092 | XXX | 27.9892 | 27.9892 | 8.89722 | -0.9145 | 1.88492993 |
| 6 | Control | GAPDH | 18.7727 | 18.7727 | XXX | 27.6261 | 27.6261 | 9.36344 | -0.4483 | 1.36441405 |
| 7 | KO | GAPDH | 18.2238 | 18.2238 | XXX | 26.0995 | 26.0995 | 7.86753 | -1.9442 | 3.64822717 |
| 8 | KO | GAPDH | 20.1543 | 20.1543 | XXX | 27.9619 | 27.9619 | 7.70743 | -2.1043 | 4.29886036 |
| 9 | KO | GAPDH | 18.0926 | 18.0926 | XXX | 28.406 | 28.406 | 7.42933 | -2.3884 | 5.23576338 |
| 10 | KO | GAPDH | 19.1584 | 19.1584 | XXX | 26.7908 | 26.7908 | 7.62249 | -2.1892 | 4.56064406 |
| 11 | KO | GAPDH | 20.0943 | 20.0943 | XXX | 28.4921 | 28.4921 | 7.39755 | -2.4142 | 3.3301311 |
| 12 | KO | GAPDH | 19.6542 | 19.6542 | XXX | 27.7211 | 27.7211 | 8.05692 | -1.7548 | 3.37481713 |

Fold changeはエクセルで=2^{-(ΔΔCtのセル)}したら出ます。



これで比較できるようになりました。