# CHAPTER IV
# RESULTS

## 1. Mutation screening

The sequencing results showed no mutation in coding region or 5'UTR and 3'UTR of all candidate genes. However, in 3'UTR of HOXD10, TA repeats were found and seemed to be polymorphic among cases and controls (Fig. 8).
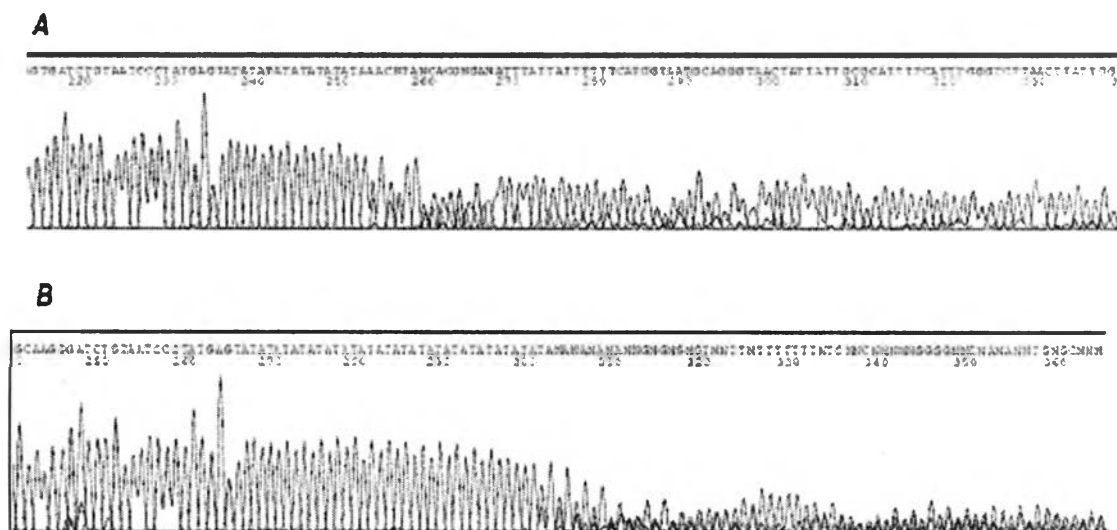


**Figure 11** The TA repeat found in 3'UTR of *HOXD10* gene. (A) TA repeat in normal control and (B) in a female with MDK

## 2. RT-PCR

The mRNA samples were successfully transcribed without any contamination of residue genomic DNA by application of DNase-I treatment. From three genes selected; *LNP*, *EVX2* and *HOXD11*, the only one gene that expressed in both case and control lymphoblastoid cell lines in adult was *LNP* gene. Then *LNP* is selected to be a target

gene for determination of an expression level based on the assumption of GCR. A house keeping gene, *GAPDH*, was also expressed in both case and control samples as expected.

## 3. Real-time RT-PCR

Duplex RT-PCR were successfully done in all cDNA samples before proceed to real-time PCR. Although a control cDNA No.644 was degraded (Fig. 12), it was selected to do real-time PCR. The result from real-time PCR in controls showed no signs of probes degradation or genomic DNA contamination. The average $C_t$ of *GAPDH* and *LNP* were about 22 and 27, respectively (Fig. 13 and 14). However, as expected, the $C_t$ *GAPDH* and *LNP* of control sample (No. 644) seemed to be different from the other normal controls. Relative quantification showed that, in all normal controls except No.644 sample, there were no significant differences among normal controls sample. Interestingly, there were over-expression of *LNP* gene in case and control samples which was three times higher compared to calibrator (No.629) (Fig.15). However, over-expression in normal control sample was excluded based on the evidence of cDNA degradation. Therefore, the over-expression of *LNP* was only seen in the case samples.
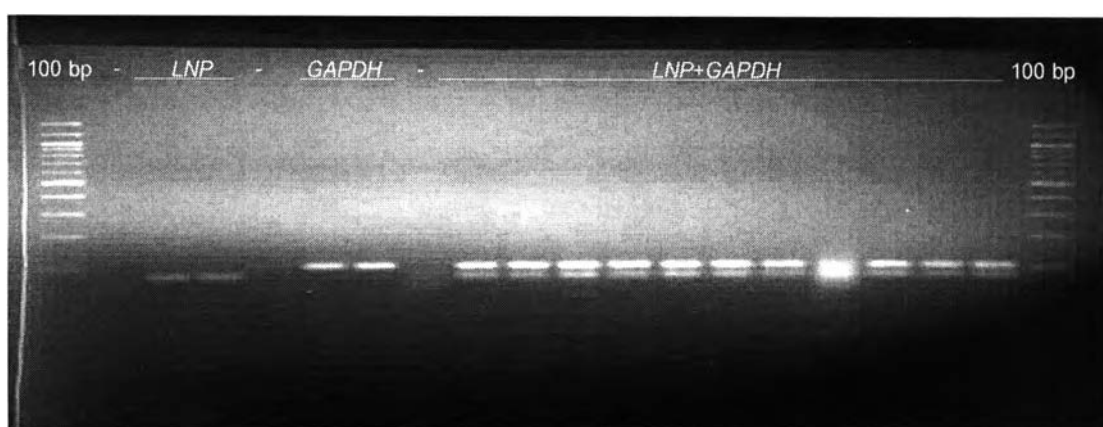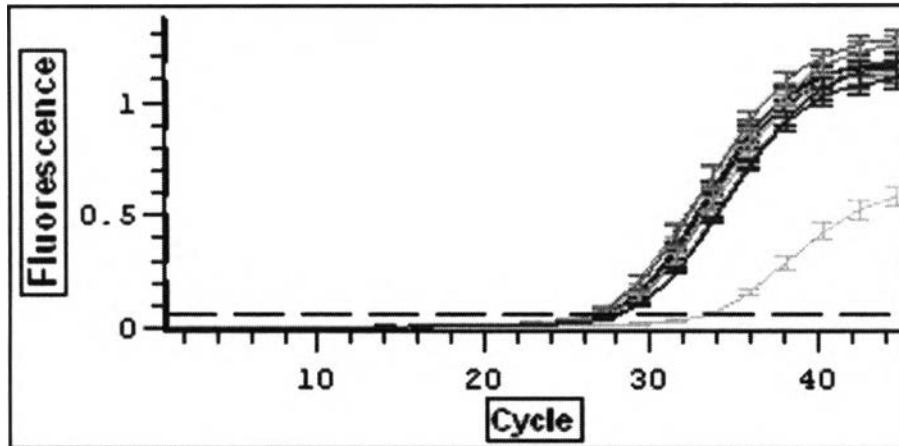


Figure 12 Conventional and duplex RT-PCR from normal and case cDNA samples. Note cDNA degradation in normal control sample (No.644.)

Quantitation Graph



**Figure** 13 The quantitation graph of *LNP* gene in cases and control samples.

**Table** 13 The details of *LNP* quantitation in each samples.

| Set | Dye | Type | Efficiency | C(t) | Avg C(t) | Max C(t) | Min C(t) | C(t) SD |
|-----|-----|------|-----------|------|----------|----------|----------|---------|
| 543 | FAM | Replicate | 56.38 | 27.13 | 27.13 | 27.17 | 27.09 | 0.03 |
| 629 | FAM | Calibrator | 59.64 | 26.37 | 26.39 | 26.71 | 26.24 | 0.19 |
| 630 | FAM | Replicate | 58.99 | 27.25 | 27.29 | 27.77 | 27.10 | 0.28 |
| 631 | FAM | Replicate | 59.76 | 26.37 | 26.39 | 26.63 | 26.06 | 0.20 |
| 634 | FAM | Replicate | 58.89 | 27.12 | 27.13 | 27.23 | 27.06 | 0.07 |
| 635 | FAM | Replicate | 56.45 | 26.55 | 26.59 | 27.19 | 26.21 | 0.38 |
| 640 | FAM | Replicate | 55.43 | 27.50 | 27.54 | 27.94 | 27.28 | 0.29 |
| 643 | FAM | Replicate | 58.73 | 28.16 | 28.16 | 28.21 | 28.13 | 0.03 |
| 644 | FAM | Replicate | 49.21 | 33.26 | 33.29 | 33.65 | 33.07 | 0.26 |
| 646 | FAM | Replicate | 56.38 | 27.47 | 27.51 | 28.12 | 27.18 | 0.37 |
| 648 | FAM | Replicate | 54.16 | 28.02 | 28.01 | 28.17 | 27.86 | 0.12 |

Quantitation Graph
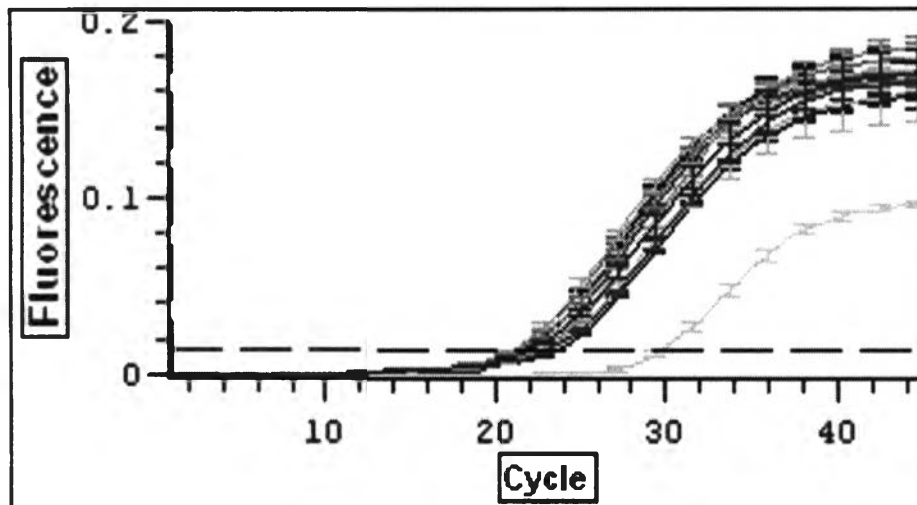


**Figure 14** The quantitation graph of *GAPDH* gene in cases and control samples .

**Table 14** The details of *GAPDH* quantitation in each samples.

| Set | Dye | Type | Efficiency | C(t) | Avg C(t) | Max C(t) | Min C(t) | C(t) SD |
|-----|-----|------|------------|------|----------|----------|----------|---------|
| 543 | CY5 | Replicate | 40.67 | 23.50 | 23.49 | 23.60 | 23.40 | 0.08 |
| 629 | CY5 | Calibrator | 44.71 | 21.10 | 21.09 | 21.20 | 20.96 | 0.09 |
| 630 | CY5 | Replicate | 49.94 | 21.70 | 21.70 | 22.44 | 21.30 | 0.44 |
| 631 | CY5 | Replicate | 58.45 | 20.91 | 20.90 | 21.09 | 20.73 | 0.14 |
| 634 | CY5 | Replicate | 50.84 | 22.25 | 22.24 | 22.62 | 22.05 | 0.22 |
| 635 | CY5 | Replicate | 42.62 | 21.38 | 21.36 | 21.56 | 20.83 | 0.31 |
| 640 | CY5 | Replicate | 36.23 | 22.86 | 22.84 | 23.14 | 22.67 | 0.21 |
| 643 | CY5 | Replicate | 40.29 | 22.98 | 22.97 | 23.22 | 22.77 | 0.19 |
| 644 | CY5 | Replicate | 62.29 | 29.84 | 29.85 | 30.09 | 29.43 | 0.30 |
| 646 | CY5 | Replicate | 35.80 | 22.52 | 22.52 | 23.08 | 22.10 | 0.35 |
| 648 | CY5 | Replicate | 38.90 | 22.00 | 21.98 | 22.25 | 21.60 | 0.25 |
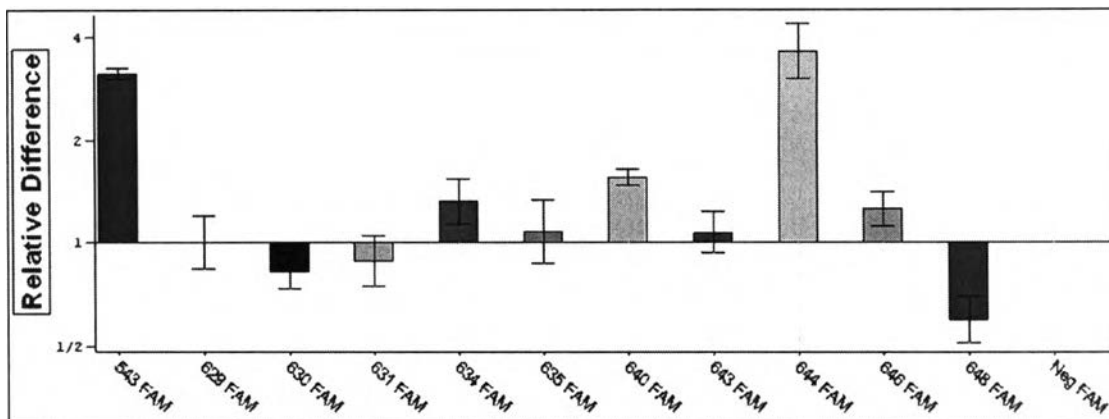
**Figure 15** The relative differences in case and control samples. The first red bar represents case sample, whereas the others are control samples.

**Table 13** The details of relative quantification in each samples.

| Name | Dye | Type | Content | Efficiency | Rel. Dif | Avg Rel. Dif | Max Rel. Dif | Min Rel. Dif | Rel. Dif SD |
|------|-----|------|---------|-----------|----------|-------------|-------------|-------------|------------|
| 543 | FAM | Replicate | Sample | 56.38 | 3.123 | 3.115 | 3.267 | 2.954 | 1.037 |
| 629 | FAM | Calibrator | Sample | 59.64 | 1 | 0.9854 | 1.114 | 0.7215 | 1.198 |
| 630 | FAM | Replicate | Sample | 58.99 | 0.8237 | 0.8046 | 0.9679 | 0.6992 | 1.128 |
| 631 | FAM | Replicate | Sample | 59.76 | 0.8822 | 0.8639 | 1.018 | 0.6496 | 1.186 |
| 634 | FAM | Replicate | Sample | 58.89 | 1.323 | 1.311 | 1.688 | 1.119 | 1.169 |
| 635 | FAM | Replicate | Sample | 56.45 | 1.075 | 1.034 | 1.342 | 0.7692 | 1.24 |
| 640 | FAM | Replicate | Sample | 55.43 | 1.561 | 1.488 | 1.587 | 1.392 | 1.055 |
| 643 | FAM | Replicate | Sample | 58.73 | 1.068 | 1.06 | 1.283 | 0.9292 | 1.148 |
| 644 | FAM | Replicate | Sample | 49.21 | 3.623 | 3.585 | 4.66 | 3.125 | 1.204 |
| 646 | FAM | Replicate | Sample | 56.38 | 1.255 | 1.224 | 1.429 | 1.044 | 1.123 |
| 648 | FAM | Replicate | Sample | 54.16 | 0.5951 | 0.591 | 0.7562 | 0.5071 | 1.163 |