**Table e-1: Primer and probe sequences**

|  |  |  |
| --- | --- | --- |
| **Non-spliced *FMR1*** | Forward Primer | TCA GTC CAG TTT GAG TGC TTT |
|  | Reverse Primer | GCA GAA ACA GTC ATT CCA TTA GTT |
|  | Probe | /56-FAM/TC AGG AAT GGA TCT TCA TGT TAC TGA CTG A/36-TAMSp/ |
| **GUS-B Control** | Forward Primer | CTC ATT TGG AAT TTT GCC GAT T |
|  | Reverse Primer | CCG AGT GAA GAT CCC CTT TTT A |
|  | Probe | /56-FAM/TG AAC AGT CAC CGA CGA GAG TGC TGG /36-TAMSp/ |
| ***FMR1*** | Forward Primer | GCA GAT TCC ATT TCA TGA TGT CA |
|  | Reverse Primer | ACC ACC AAC AGC AAG GCT CT |
|  | Probe | /56-FAM/TG ATG AAG TTG AGG TGT ATT CCA GAG CAA ATG/36-TAMSp/ |
| ***ASFMR1*** | Forward Primer | AAT AGC ACT GAG TTG GCA GAG G |
|  | Reverse Primer | CTC CAA AGA CCC TAG TCC AAA A |
|  | Probe | /56-FAM/TT CCA CCA GCA GCA ACA TCC TCT C/36-TAMSp/ |
| **Spliced *FMR1*** | Forward Primer | CACAGGCATTTGTAAAGGATGTT |
|  | Reverse Primer | GCAGAAACAGTCATTCCATTAGTT |
|  | Probe | 5’ /56-FAM/ACT+G+G+A+TT+G+TTT TC/3IABkFQ |

Legend: Primers and probes used in the strand specific real time PCR.

*Key: FMR1* = *fragile X mental retardation 1*, GUS-B=β Glucuronidase, *ASFMR1*= *antisense fragile X mental retardation 1*

Table e-2: **Relationship between CGG repeat size and the ratio of splice variant and total *ASFMR1* mRNA**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Men |  | **Normal (n=11)** | **Premutation (n=21)** | **p-value** |
| Median (IQR) | 2.01 (1.99) | 2.72 (3.66) | 0.33 |
|  |  | **Correlation Coefficient (Spearman)** | **p-value** |
|  | Overall (n=32) | 0.43 | 0.01 |
|  | Within normal (n=11) | -0.62 | 0.04 |
|  | Within premutation carriers (n=21) | 0.81 | <0.0001 |
| Women |  | **Normal (n=9)** | **Premutation (n=26)** | **p-value** |
| Median (IQR) | 1.22 (2.06) | 3.92 (10.01) | 0.03 |
|  |  | **Correlation Coefficient (Spearman)** | **p-value** |
|  | Overall (n=33) | 0.02 | 0.91 |
|  | Within normal (n=9) | -0.74 | 0.02 |
|  | Within premutation carriers (n=24) | 0.08 | 0.70 |

Legend: Spearman’s correlation analysis to examine the relationship between CGG repeat size and the ratio of splice variant and total *ASFMR1* mRNA.