

# Figure S3

## I. Genomic double stranded DNA (portion of the CpG island of *FMRI*)

5' ... **CG**GATGCATTTGATTTCCCA**CGC**CACTGAGTGCACCTCTGCAGAAATGGCGTTCTGGCCCT**CGCG**AGGCAGTGCACCTGTCA**CC**CCCTTCAGCCTTCC**CG**CCCTCCACCAAGCC**CGCG**CAC...3'  
 3' ... **GC**CTACGTAACATAAAGGTT**CGG**TGACTCAGTGGAGACGCTTTTACC**CGA**AGACCGGGA**CGCG**TCCGTCA**CG**TGGACAGTGC**GGG**AAAGTCGGAAAG**CG**GGGAGGTGGTTCC**CGCG**GTG...5'

## II. Restriction digest



+DraIII restriction enzyme and incubation (recognition sequence in yellow)

5' ... **CG**GATGCATTTGATTTCCCA**CGC**CACTGAGTGCACCTCTGCAGAAATGGCGTTCTGGCCCT**CGCG**AGGCAGTGCACCTGTCA**CC**CCCTTCAGCCTTCC**CG**CCCTCCACCAAGCC**CGCG**CAC...3'  
 3' ... **GC**CTACGTAACATAAAGGTT**CGG**TGACTCAGTGGAGACGCTTTTACC**CGA**AGACCGGGA**CGCG**TCCGTCA**CG**TGGACAGTGC**GGG**AAAGTCGGAAAG**CG**GGGAGGTGGTTCC**CGCG**GTG...5'

## III. Addition and ligation of hairpin linker

GA  
C GCATCGCTTGA -3'  
T GCTAGCGA -5'  
TC



+ T4 DNA ligase and incubation

GA  
C GCATCGCTTGA**GTG**CACCTCTGCAGAAATGGCGTTCTGGCCCT**CGCG**AGGCAGTGCACCTGTCA**CC**CCCTTCAGCCTTCC**CG**CCCTCCACCAAGCC**CGCG**CAC...3'  
 T GCTAGCGA**ACTCAC**GTGGAGACGCTTTTACC**CGA**AGACCGGGA**CGCG**TCCGTCA**CG**TGGACAGTGC**GGG**AAAGTCGGAAAG**CG**GGGAGGTGGTTCC**CGCG**GTG...5'

## IV. Sodium bisulfite conversion of cytosine (but not methyl-cytosine) to uracil

AGUATUGU**TTGA**GTGUAUUU**TC**AGAAATGGCGTT**TC**GUUU**TCGG**AGG**AGTGC**GAU**TGT**UA**UCG**UUU**TT**AGUU**TT**UC**GUUU**UUAUU**AAG**U**CGCG**UA...3'  
 G  
 U  
 T  
 T  
 T  
 UCU**TAG**UA**UU**UA**GTGG**AG**ACT**TTTA**UU**UCAAGAU**GGGA**CGCGT**UU**GT**UA**CGT**GGA**AGT**CG**GGAA**GT**CGAA**AGG**CGGGAGGTGGTT**CG**CGCG**GTG**...5'  
 (loss of significant base-pair complementarity due to bisulfite conversion)

## V. Right primer anneals during 1<sup>st</sup> round of PCR amplification

5' to 3' extension by polymerase Right Primer (example)  
 <-- <-- RCAAAAATAATTCARCRATA-5'  
 AGUATUGU**TTGA**GTGUAUUU**TC**AGAAATGGCGTT**TC**GUUU**TCGG**AGG**AGTGC**GAU**TGT**UA**UCG**UUU**TT**AGUU**TT**UC**GUUU**UUAUU**AAG**U**CGCG**UA...3'  
 G  
 U  
 T  
 T  
 T  
 UCU**TAG**UA**UU**UA**GTGG**AG**ACT**TTTA**UU**UCAAGAU**GGGA**CGCGT**UU**GT**UA**CGT**GGA**AGT**CG**GGAA**GT**CGAA**AGG**CGGGAGGTGGTT**CG**CGCG**GTG**...5'

## VI. Right primer extends to make complementary copy of top and bottom strands

TCATACAA**ACTCACATA**AAACATCTTTACC**CG**AAAACCAAA**ACGG**TCCATCA**CG**TAAACAATA**CG**AAAAATCAAAAA**RCAAAAATAATTCARCRATA** -5'  
 C AGUATUGU**TTGA**GTGUAUUU**TC**AGAAATGGCGTT**TC**GUUU**TCGG**AGG**AGTGC**GAU**TGT**UA**UCG**UUU**TT**AGUU**TT**UC**GUUU**UUAUU**AAG**U**CGCG**UA...3'  
 G  
 A  
 U  
 T  
 T  
 A  
 UCU**TAG**UA**UU**UA**GTGG**AG**ACT**TTTA**UU**UCAAGAU**GGGA**CGCGT**UU**GT**UA**CGT**GGA**AGT**CG**GGAA**GT**CGAA**AGG**CGGGAGGTGGTT**CG**CGCG**GTG**...5'  
 ACAATCACTTAAATACACCTCTACAAAAATAAA**CG**TTCTAACCT**CGCG**AAACAATA**CG**ACCTATCA**CG**CCCTTCAACCTTCCCGCCCTCCACCAAAACCGCGCAC...3'

## VII. Left primer anneals during 2<sup>nd</sup> round of PCR amplification

TCATACAA**ACTCACATA**AAACATCTTTACC**CG**AAAACCAAA**ACGG**TCCATCA**CG**TAAACAATA**CG**AAAAATCAAAAA**RCAAAAATAATTCARCRATA** -5'  
 C  
 A  
 A  
 A  
 A  
 ACAATCACTTAAATACACCTCTACAAAAATAAA**CG**TTCTAACCT**CGCG**AAACAATA**CG**ACCTATCA**CG**CCCTTCAACCTTCCCGCCCTCCACCAAAACCGCGCAC...3'  
 <-- <-- GGAGTGGTTGGGYGTG-5'  
 Left Primer (example)

## VIII. Left primer extends

AGTATTGTTTGGATGATTTTTGTAGAAATGGCGTTTGGTTTT**CGCG**AGGTAGT**CG**ATTGTTAT**CG**TTTTTTAGTTTTTTTCGTTTTTTTATTAAGTTCGGTAT-3'  
 G TCATACAA**ACTCACATA**AAACATCTTTACC**CG**AAAACCAAA**ACGG**TCCATCA**CG**TAAACAATA**CG**AAAAATCAAAAA**RCAAAAATAATTCARCRATA** -5'  
 C  
 T  
 A  
 T  
 A  
 T  
 A  
 ACAATCACTTAAATACACCTCTACAAAAATAAA**CG**TTCTAACCT**CGCG**AAACAATA**CG**ACCTATCA**CG**CCCTTCAACCTTCCCGCCCTCCACCAAAACCGCGCAC...3'

PCR now proceeds in the conventional fashion