Primer sequences and PCR assay conditions for pyrosequencing analyses

**CSMD1**

CSMD1-assay1-R1  
CGCCAGGGTTTTCCAGTCAGCACCATAAACAAAACCCCTCTCTAC

CSMD1-assay1-S1  
GTTGGGTATAGGTATATATTA

Use S1 and R1 to amplify a 77bp amplicon. Use S1 as a sequencing primer to analyze two CpG sites.

CSMD1-assay1(S1-R1) PCR assay

| Total Volume (ul) | 25 |
|------------------|----|
| dH₂O             | 19.25 |
| 10*BF (HOT STAR) | 2.5 |
| 10mM dNTP        | 0.5 |
| 10uM M13-Biotin Universal Primer | 0.5 |
| 10uM Primer-S1   | 0.5 |
| 10uM Primer-R1   | 0.5 |
| HotStar (Qiagen) | 0.25 |
| Bis-DNA          | 1 |

25

PCR2:“60 to 55” on Bio Rad (RLT2632)

Cycle 1: (1X)

| Step 1: 95C for 15:00 |
| Step 2: 94C for 00:20 |
| Step 3: 60C for 00:20 |

Cycle 2: (10X)

| Step 1: 94C for 00:20 |
| Step 2: 55C for 00:20 |
| Step 3: 72C for 00:20 |

Cycle 3: (40X)

| Step 1: 94C for 00:20 |
| Step 2: 55C for 00:20 |
| Step 3: 72C for 00:20 |

Cycle 4: (1X)

| Step 1: 72C for 7:00 |
| Step 1: 12C for ~ |

CSMD1-assay1(S1-R1): 77bp(without M13)

**NRXN1**

NRXN2-F1  
GGGAGGTTGGAGTTAAAGATTT

NRXN2-R1  
CGCCAGGGTTTTCCAGTCAGCACCATAAACAAAACCCCTCTCTCA

NRXN2-S1  
GTTGGGTATAGGTATATATTA

Use F1 and R1 to amplify a 143bp amplicon. Use S1 as a sequencing primer to analyze nine CpG sites.

NRXN2 PCR assay

| Total Volume (ul) | 25 |
|------------------|----|
| dH₂O             | 18.65 |
| 10*BF (HOT STAR) | 2.5 |
| 25mM MgCl₂       | 1  |
10mM dNTP: 0.5
10uM M13-Biotin Universal Primer: 0.5
10uM Primer-F: 0.5
10uM Primer-R: 0.1
HotStar (Qiagen): 0.25
Bis-DNA: 1

**PTPRN2**

PTPRN2-F1: AGTGGGTTTTGTGGAAGTAAT
PTPRN2-R1: CGCCAGGGTTTTCCAGTCACGACACCTAAATATACATAACTAAAAACTACCT
PTPRN2-S1: AATATTTTTGTGAAGGAG

Use F1 and R1 to amplify a 280bp amplicon. Use S1 as a sequencing primer to analyze 10 CpG sites.

**PTPRN2 PCR assay**

| Component                  | Amount     |
|----------------------------|------------|
| Total Volume (ul)          | 25         |
| dH₂O                       | 18.65      |
| 10*BF (HOT STAR)           | 2.5        |
| 25mM MgCl₂                 | 1          |
| 10mM dNTP                  | 0.5        |
| 10uM M13-Biotin Universal Primer | 0.5        |
| 10uM Primer-F              | 0.5        |
| 10uM Primer-R              | 0.1        |
| HotStar                    | 0.25       |
| Bis-DNA                    | 1          |

**PCR2:"60 to 55" on Bio Rad (RLT2631)**

| Cycle | Step 1 | Step 2 |
|-------|--------|--------|
| Cycle 1: (1X) | 95C for 15:00 | |
| Cycle 2: (10X) | 94C for 00:20 | 60C for 00:20 |
| Cycle 3: (40X) | 94C for 00:20 | 55C for 00:20 | 72C for 00:20 |
| Cycle 4: (1X) | 72C for 7:00 |
| Cycle 5: (1X) | 72C for 00:20 | 12C for ~ |

**NRXN2:** 178bp (without M13)
Cycle 3: (40X)
Step 1: 94°C for 00:20
Step 2: 55°C for 00:20
Step 3: 72°C for 00:20

Cycle 4: (1X)
Step 1: 72°C for 7:00

Cycle 5: (1X)
Step 1: 12°C for ~

PTPRN2: 280bp (without M13)

**RBFOX1 (32 Discovery samples)**

| Primer     | Sequence                                      |
|------------|-----------------------------------------------|
| RBFOX1-4A1-F7 | AGAGAATTTTTTAATGAGGGTAGGT                  |
| RBFOX1-4A1-R5 | CGCCAGGGTTTTCCAGTCACGACAAATTCTAAATTTCTCAACAAAAGTCTCCTACAA |
| RBFOX1-4A1-S9 | GGATGTTTTGTTTTTTGTTTTA                  |
| RBFOX1-4A2-S1 | GTATAGAGGAGTATGGT                        |

Use F7 and R5 to amplify a 238bp region. Use F7 as a sequencing primer to analyze five CpG sites. Use S1 to analyze two CpG sites. Use S9 to analyze four CpG sites.

**RBFOX1-4A1(F7-R5) PCR assay**

| Component                  | Volume (ul) |
|----------------------------|-------------|
| dH₂O                       | 18.25       |
| 10xBF (HOT STAR)           | 2.5         |
| 25mM MgCl₂                 | 1           |
| 10mM dNTP                  | 0.5         |
| 10uM M13-Biotin Universal Primer | 0.5         |
| 10uM Primer-F7             | 0.5         |
| 10uM Primer-R5             | 0.5         |
| HotStar (Qiagen)           | 0.25        |
| Bis-DNA                    | 1           |

Total Volume = 25 ul

PCR2: “60 to 55” on Bio Rad (RLT2632)

**Cycle 1: (1X)**
Step 1: 95°C for 15:00

**Cycle 2: (10X)**
Step 1: 94°C for 00:20
Step 2: 60°C for 00:20

Decrease Temp after cycle 1 by 0.5°C every 1 cycle

**Cycle 3: (40X)**
Step 1: 94°C for 00:20
Step 2: 55°C for 00:20
Step 3: 72°C for 00:20

**Cycle 4: (1X)**
Step 1: 72°C for 7:00

**Cycle 5: (1X)**
Step 1: 12°C for ~

RBFOX1-4A1(F7-R5): 238bp (without M13)

**SNORD115-30**

| Primer          | Sequence                                      |
|-----------------|-----------------------------------------------|
| SNORD115-30-A1-F1 | ATTGATGGGTATAGGTGAGTGTA                  |
| SNORD115-30-A1-R1 | CGCCAGGGTTTTCCAGTCACGACAAAACCTCAAAAAAATATCCCCCTTA |
Use F1 and R1 to amplify a 199bp amplicon. Use S1 as a sequencing primer to analyze the first two CpG sites. Use S2 to analyze the next two CpG sites.

**SNORD115-30-A1 PCR assay**

| Component                        | Volume (µl) |
|----------------------------------|-------------|
| dH₂O                             | 18.65       |
| 10*BF (HOT STAR)                 | 2.5         |
| 25mM MgCl₂                       | 1           |
| 10mM dNTP                        | 0.5         |
| 10uM M13-Biotin Universal Primer | 0.5         |
| 10uM Primer-F                    | 0.5         |
| 10uM Primer-R                    | 0.1         |
| HotStar (Qiagen)                 | 0.25        |
| Bis-DNA                          | 1           |

Total Volume (µl) 25

PCR2: "60 to 55°" on Bio Rad (RLT2631, 2632)

Cycle 1: (1X)  
Step 1: 95°C for 15:00

Cycle 2: (10X)  
Step 1: 94°C for 00:20  
Step 2: 60°C for 00:20

Decrease Temp after cycle 1 by 0.5°C every 1 cycle

Step 3: 72°C for 00:20

Cycle 3: (40X)  
Step 1: 94°C for 00:20  
Step 2: 55°C for 00:20  
Step 3: 72°C for 00:20

Cycle 4: (1X)  
Step 1: 72°C for 7:00

Cycle 5: (1X)  
Step 1: 12°C for ~

SNORD115-30-A1: 199bp(without M13)

**SNORD115-30-A2 PCR assay**

| Component                        | Volume (µl) |
|----------------------------------|-------------|
| dH₂O                             | 18.25       |
| 10*BF (HOT STAR)                 | 2.5         |
| 25mM MgCl₂                       | 1           |
| 10mM dNTP                        | 0.5         |
| 10uM M13-Biotin Universal Primer | 0.5         |
| 10uM Primer-F                    | 0.5         |
| 10uM Primer-R                    | 0.5         |
| HotStar                          | 0.25        |
| Bis-DNA                          | 1           |

Total Volume (µl) 25

PCR3: "55 to 50°" on Bio Rad (RLT2632)

Cycle 1: (1X)  
Step 1: 95°C for 15:00

Cycle 2: (10X)  
Step 1: 94°C for 00:20  
Step 2: 55°C for 00:20
Decrease Temp after cycle 1 by 0.5°C every 1 cycle

Cycle 3: (40X)
Step 1: 72°C for 00:20
Step 2: 50°C for 00:20
Step 3: 72°C for 00:20

Cycle 4: (1X)
Step 1: 72°C for 00:20

SNORD115-30-A2: 80bp (without M13)

SNORD115-37

SNORD115-37-F1  TTTTGGTGGAGGATGGGTAGAG  
SNORD115-37-R3  CGCCAGGGTTTTCCAGTCAAGACACCCTTTTCCCTGTAATAAAATAA  
SNORD115-37-S2  GGGTTATGAGTGAGGT  

Use F1 and R3 to amplify a 143bp amplicon. Use S2 as a sequencing primer to analyze four CpG sites.

SNORD115-37 PCR assay

| Component                        | Quantity |
|----------------------------------|----------|
| Total Volume (µl)                | 25       |
| dH2O                             | 18.25    |
| 10*BF (HOT STAR)                 | 2.5      |
| 25mM MgCl2                       | 1        |
| 10mM dNTP                        | 0.5      |
| 10uM M13-Biotin Universal Primer | 0.5      |
| 10uM Primer-F                    | 0.5      |
| 10uM Primer-R                    | 0.5      |
| HotStar                          | 0.25     |
| Bis-DNA                          | 1        |

25

PCR1: "65 to 60" on Bio Rad (RLT2631)

Cycle 1: (1X)  
Step 1: 95°C for 15:00

Cycle 2: (10X)  
Step 1: 94°C for 00:20
Step 2: 65°C for 00:20

Decrease Temp after cycle 1 by 0.5°C every 1 cycle

Cycle 3: (40X)  
Step 1: 94°C for 00:20
Step 2: 65°C for 00:20
Step 3: 72°C for 00:20

Cycle 4: (1X)  
Step 1: 72°C for 00:20

Cycle 5: (1X)  
Step 1: 72°C for 00:20

SNORD115-37: 143bp (without M13)