**Thalassemia PCR Program**

**IVS I.I**

 **PCR Protocol**

|  |  |  |
| --- | --- | --- |
| Master-Mix | n=1 | Cat-Nr |
| H2O | 18.5 μl | G-1000-1 250 units |
| 10x PCR Buffer  | 2.5 μl | G-1000-1 250 units |
| MgCl2 (25mM) | 1 μl | G-1000-1 250 units |
| dNTP (10 mM) | 0.5 μl | G-1000-1 250 units |
|  Primer **IVS I.I** (1:10) | 0.3 μl | BIO NEER |
|   Primer com**A**  (1:10) Primer com**B** (1:10) Primer com**C** (1:10) | 0.3 μl0.3 μl0.3 μl |  BIO NEERBIO NEERBIO NEER |
| Prime Taq DNA Polymerase (5 U/μl) | 0.3 μl | G-1000-1 250 units |
| DNA  | 1 μl |  |

 **PCR Program:Thal 2**

|  |  |  |
| --- | --- | --- |
| **cycle** | **Time ( s)** | **T˚c**  |
| 1 | 5min | 94 |
| 30 | 40 | 94 |
| 40 | 59 |
| 40 | 72 |
| 1 | 5min | 72 |

**IVS I-5**

 **PCR Protocol**

|  |  |  |
| --- | --- | --- |
| Master-Mix | n=1 | Cat-Nr |
| H2O | 17.5 μl | G-1000-1 250 units |
| 10x PCR Buffer  | 2.5 μl | G-1000-1 250 units |
| MgCl2 (25mM) | 2 μl | G-1000-1 250 units |
| dNTP (10 mM) | 0.5 μl | G-1000-1 250 units |
|  Primer **IVS I.5**  (1:10) | 0.3 μl | BIO NEER |
|   Primer com**A**  (1:10) Primer com**B** (1:10) Primer com**C** (1:10) | 0.3 μl0.3 μl0.3 μl |  BIO NEERBIO NEERBIO NEER |
| Prime Taq DNA Polymerase (5 U/μl) | 0.3 μl | G-1000-1 250 units |
| DNA  | 1 μl |  |

**PCR Program: Thal 1**

|  |  |  |
| --- | --- | --- |
| **cycle** | **Time ( s)** | **T˚c**  |
| 1 | 5min | 94 |
| 30 | 40 | 94 |
| 40 | 60 |
| 40 | 72 |
| 1 | 5min | 72 |

**IVS I.6**

**PCR Protocol**

|  |  |  |
| --- | --- | --- |
| Master-Mix | n=1 | Cat-Nr |
| H2O | 17.5 μl | G-1000-1 250 units |
| 10x PCR Buffer  | 2.5 μl | G-1000-1 250 units |
| MgCl2 (25mM) | 2 μl | G-1000-1 250 units |
| dNTP (10 mM) | 0.5 μl | G-1000-1 250 units |
|  Primer **IVS I.6** (1:10) | 0.3 μl | BIO NEER |
|   Primer com**A**  (1:10) Primer com**B** (1:10) Primer com**C** (1:10) | 0.3 μl0.3 μl0.3 μl |  BIO NEERBIO NEERBIO NEER |
| Prime Taq DNA Polymerase (5 U/μl) | 0.3 μl | G-1000-1 250 units |
| DNA  | 1 μl |  |

 **PCR Program: Thal 1**

|  |  |  |
| --- | --- | --- |
| **cycle** | **Time ( s)** | **T˚c**  |
| 1 | 5min | 94 |
| 30 | 40 | 94 |
| 40 | 60 |
| 40 | 72 |
| 1 | 5min | 72 |

**Codon30**

**PCR Protocol**

|  |  |  |
| --- | --- | --- |
| Master-Mix | n=1 | Cat-Nr |
| H2O | 17.5 μl | G-1000-1 250 units |
| 10x PCR Buffer  | 2.5 μl | G-1000-1 250 units |
| MgCl2 (25mM) | 2 μl | G-1000-1 250 units |
| dNTP (10 mM) | 0.5 μl | G-1000-1 250 units |
|  Primer **C30** (1:10) | 0.3 μl | BIO NEER |
|   Primer com**A**  (1:10) Primer com**B** (1:10) Primer com**C** (1:10) | 0.3 μl0.3 μl0.3 μl |  BIO NEERBIO NEERBIO NEER |
| Prime Taq DNA Polymerase (5 U/μl) | 0.3 μl | G-1000-1 250 units |
| DNA  | 1 μl |  |

 **PCR Program: Thal 3**

|  |  |  |
| --- | --- | --- |
| **cycle** | **Time ( s)** | **T˚c**  |
| 1 | 5min | 94 |
| 28 | 40 | 94 |
| 40 |  |
| 40 | 72 |
| 1 | 5min | 72 |

 **IVS I.110**

**PCR Protocol**

|  |  |  |
| --- | --- | --- |
| Master-Mix | n=1 | Cat-Nr |
| H2O | 17.5 μl | G-1000-1 250 units |
| 10x PCR Buffer  | 2.5 μl | G-1000-1 250 units |
| MgCl2 (25mM) | 2 μl | G-1000-1 250 units |
| dNTP (10 mM) | 0.5 μl | G-1000-1 250 units |
|  Primer **IVS I.110** (1:10) | 0.3 μl | BIO NEER |
|   Primer com**A**  (1:10) Primer com**B** (1:10) Primer com**C** (1:10) | 0.3 μl0.3 μl0.3 μl |  BIO NEERBIO NEERBIO NEER |
| Prime Taq DNA Polymerase (5 U/μl) | 0.3 μl | G-1000-1 250 units |
| DNA  | 1 μl |  |

 **PCR Program: Thal 1**

|  |  |  |
| --- | --- | --- |
| **cycle** | **Time ( s)** | **T˚c**  |
| 1 | 5min | 94 |
| 30 | 40 | 94 |
| 40 | 60 |
| 40 | 72 |
| 1 | 5min | 72 |

**Codon39**

**PCR Protocol**

|  |  |  |
| --- | --- | --- |
| Master-Mix | n=1 | Cat-Nr |
| H2O | 17.5 μl | G-1000-1 250 units |
| 10x PCR Buffer  | 2.5 μl | G-1000-1 250 units |
| MgCl2 (25mM) | 2 μl | G-1000-1 250 units |
| dNTP (10 mM) | 0.5 μl | G-1000-1 250 units |
|  Primer **C39** (1:10) | 0.3 μl | BIO NEER |
|   Primer com**A**  (1:10) Primer com**B** (1:10) Primer com**C** (1:10) | 0.3 μl0.3 μl0.3 μl |  BIO NEERBIO NEERBIO NEER |
| Prime Taq DNA Polymerase (5 U/μl) | 0.3 μl | G-1000-1 250 units |
| DNA  | 1 μl |  |

 **PCR Program: Thal 1**

|  |  |  |
| --- | --- | --- |
| **cycle** | **Time ( s)** | **T˚c**  |
| 1 | 5min | 94 |
| 30 | 40 | 94 |
| 40 | 60 |
| 40 | 72 |
| 1 | 5min | 72 |

**Fr16**

**PCR Protocol**

|  |  |  |
| --- | --- | --- |
| Master-Mix | n=1 | Cat-Nr |
| H2O | 17.5 μl | G-1000-1 250 units |
| 10x PCR Buffer  | 2.5 μl | G-1000-1 250 units |
| MgCl2 (25mM) | 2 μl | G-1000-1 250 units |
| dNTP (10 mM) | 0.5 μl | G-1000-1 250 units |
|  Primer **Fr 16** (1:10) | 0.3 μl | BIO NEER |
|   Primer com**A**  (1:10) Primer com**B** (1:10) Primer com**C** (1:10) | 0.3 μl0.3 μl0.3 μl |  BIO NEERBIO NEERBIO NEER |
| Prime Taq DNA Polymerase (5 U/μl) | 0.3 μl | G-1000-1 250 units |
| DNA  | 1 μl |  |

 **PCR Program: Thal 1**

|  |  |  |
| --- | --- | --- |
| **cycle** | **Time ( s)** | **T˚c**  |
| 1 | 5min | 94 |
| 30 | 40 | 94 |
| 40 | 60 |
| 40 | 72 |
| 1 | 5min | 72 |

**Fr 82/83( new primer)**

**PCR Protocol**

|  |  |  |
| --- | --- | --- |
| Master-Mix | n=1 | Cat-Nr |
| H2O | 17.5 μl | G-1000-1 250 units |
| 10x PCR Buffer  | 2.5 μl | G-1000-1 250 units |
| MgCl2 (25mM) | 2 μl | G-1000-1 250 units |
| dNTP (10 mM) | 0.5 μl | G-1000-1 250 units |
|  Primer **Fr 82/83** (1:10) | 0.3 μl | BIO NEER |
|   Primer com**A**  (1:10) Primer com**B** (1:10) Primer com**C** (1:10) | 0.3 μl0.3 μl0.3 μl |  BIO NEERBIO NEERBIO NEER |
| Prime Taq DNA Polymerase (5 U/μl) | 0.3 μl | G-1000-1 250 units |
| DNA  | 1 μl |  |

 **PCR Program: Thal 1**

|  |  |  |
| --- | --- | --- |
| **cycle** | **Time ( s)** | **T˚c**  |
| 1 | 5min | 94 |
| 30 | 40 | 94 |
| 40 | 60 |
| 40 | 72 |
| 1 | 5min | 72 |

**IVS II.745 ( new primer)**

**PCR Protocol**

|  |  |  |
| --- | --- | --- |
| Master-Mix | n=1 | Cat-Nr |
| H2O | 17.5 μl | G-1000-1 250 units |
| 10x PCR Buffer  | 2.5 μl | G-1000-1 250 units |
| MgCl2 (25mM) | 2 μl | G-1000-1 250 units |
| dNTP (10 mM) | 0.5 μl | G-1000-1 250 units |
|  Primer **IVS II.745** (1:10) | 0.3 μl | BIO NEER |
|   Primer com**A**  (1:10) Primer com**B** (1:10) Primer com**C** (1:10) | 0.3 μl0.3 μl0.3 μl |  BIO NEERBIO NEERBIO NEER |
| Prime Taq DNA Polymerase (5 U/μl) | 0.3 μl | G-1000-1 250 units |
| DNA  | 1 μl |  |

 **PCR Program: Thal 1**

|  |  |  |
| --- | --- | --- |
| **cycle** | **Time ( s)** | **T˚c**  |
| 1 | 5min | 94 |
| 30 | 40 | 94 |
| 40 | 60 |
| 40 | 72 |
| 1 | 5min | 72 |

**IVS II.I ( new primer)**

**PCR Protocol**

|  |  |  |
| --- | --- | --- |
| Master-Mix | n=1 | Cat-Nr |
| H2O | 17.5 μl | G-1000-1 250 units |
| 10x PCR Buffer  | 2.5 μl | G-1000-1 250 units |
| MgCl2 (25mM) | 2 μl | G-1000-1 250 units |
| dNTP (10 mM) | 0.5 μl | G-1000-1 250 units |
|  Primer **IVS II-I** (1:10) | 0.3 μl | BIO NEER |
|   Primer com**A**  (1:10) Primer com**B** (1:10) Primer com**C** (1:10) | 0.3 μl0.3 μl0.3 μl |  BIO NEERBIO NEERBIO NEER |
| Prime Taq DNA Polymerase (5 U/μl) | 0.3 μl | G-1000-1 250 units |
| DNA  | 1 μl |  |

 **PCR Program: Thal 4**

|  |  |  |
| --- | --- | --- |
| **cycle** | **Time ( s)** | **T˚c**  |
| 1 | 5min | 94 |
| 30 | 40 | 94 |
| 40 | 62 |
| 40 | 72 |
| 1 | 5min | 72 |

**Fr 8/9**

**PCR Protocol**

|  |  |  |
| --- | --- | --- |
| Master-Mix | n=1 | Cat-Nr |
| H2O | 17.5 μl | G-1000-1 250 units |
| 10x PCR Buffer  | 2.5 μl | G-1000-1 250 units |
| MgCl2 (25mM) | 2 μl | G-1000-1 250 units |
| dNTP (10 mM) | 0.5 μl | G-1000-1 250 units |
|  Primer **Fr 8/9** (1:10) | 0.3 μl | BIO NEER |
|   Primer com**A**  (1:10) Primer com**B** (1:10) Primer com**C** (1:10) | 0.3 μl0.3 μl0.3 μl |  BIO NEERBIO NEERBIO NEER |
| Prime Taq DNA Polymerase (5 U/μl) | 0.3 μl | G-1000-1 250 units |
| DNA  | 1 μl |  |

 **PCR Program: Thal 4**

|  |  |  |
| --- | --- | --- |
| **cycle** | **Time ( s)** | **T˚c**  |
| 1 | 5min | 94 |
| 30 | 40 | 94 |
| 40 | 62 |
| 40 | 72 |
| 1 | 5min | 72 |

**Primer sequences: 5'>3'**

|  |  |
| --- | --- |
| **comB** | **GACTCAAGGCTGAGAGATGCAGGA** |
| **comA** | **CAATGTATCATGCCTCTTTGCACC** |
| **comC** | **ACCTCACCCTGTGGAGCCAC** |
| **IVS1.1M** | **TTAAACCTGTCTTGTAACCTTGATACCCAT** |
| **IVS1.1N** | **TTAAACCTGTCTTGTAACCTTGATACCCAC** |
| **IVS1.5M** | **CTCCTTAAACCTGTCTTGTAACCTTGTTAG** |
| **IVS1.5N** | **CTCCTTAAACCTGTCTTGTAACCTTGTTAC** |
| **IVS1.6M** | **TCTCCTTAAACCTGTCTTGTAACCTTCATG** |
| **IVS1.6N** | **TCTCCTTAAACCTGTCTTGTAACCTTCATA** |
| **IVS1.110M** | **ACCAGCAGCCTAAGGGTGGGAAAATAGAGT** |
| **IVS1.110N** | **ACCAGCAGCCTAAGGGTGGGAAAATAGAGC** |
| **IVS11.745 M** | **GGTTTCATATTGCTAATAGCAGCTACAATCGAGG** |
| **IVS11.745 N** | **GGTTTCATATTGCTAATAGCAGCTACAATCGAGC** |
| **C30 M** | **TAAACCTGTCTTGTAACCTTGATACCAACG** |
| **C30 N** | **TAAACCTGTCTTGTAACCTTGATACCAACC** |
| **C39 M** | **CAGATCCCCAAAGGACTCAAAGAACCTGTA** |
| **C39 N** | **CAGATCCCCAAAGGACTCAAAGAACCTGTA** |
| **fr16 N** | **TCACCACCAACTTCATCCACGTTCACGTTG** |
| **fr16 M** | **TCACCACCAACTTCATCCACGTTCACGTTC** |
| **IVS11.1 M** | **AAGAAAACATCAAGGGTCCCATAGACTGAT** |
| **IVS11.1 N** | **AAGAAAACATCAAGGGTCCCATAGACTGAC** |
| **fr8-9 M** | **CCTTGCCCCACAGGGCAGTAACGGCACACC** |
| **fr8-9 N** | **CCTTGCCCCACAGGGCAGTAACGGCACACT** |