**TAPS-primers trial protocol Qiagen**

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| Data 22.05.2020 | Project name EPIGENOM\_TAPS-PRIMER\_1 | No of samples |

|  |  |
| --- | --- |
| **marker** | **Final conc. [uM]** |
| ELOVL2 | 0,2 |
| C1orf132/MIR29B2 | 0,2 |
| TRIM59 | 0,2 |
| KLF14 | 0,2 |
| FHL2 | 0,2 |

**PCR reaction:**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Reaction mix | 1 reaction | 13+1n reaction | |  | ConditionsPCR 2h 35’ | | |
| 2x QIAGEN Multiplex | 12.5 ul | 81.25 ul | 93.75 ul |  | In. heat | 95°C 15 min |  |
| 10x primer mix (2uM) | 2.5 ul |  |  |  | Denaturation | 94°C 30 sek |  |
| woda | 4/9 ul | 58.5 ul | 30 ul |  | Annealing | 60°C 90 sek |  |
| Optional: 5x Q-Solution | 5 ul | - | + 37,5 |  | Extend | 72°C 90 sek | x32 |
| DNA (10 ng) 10ng/ul | **1 ul** | 14 ul |  |  | Final extend | 72°C 10 min |  |
| Total volume: | 25 ul | 24 ul + **1 ul** | |  | Hold | >12°C |  |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No | Sample name | H2O | DNA |  | No | Sample name | H2O | DNA |
| 1 | Blood 6.1 – ELOVL2 | 9 | 1 |  | 9 | Blood 6.1 – TRIM59 + Q | 4 | 1 |
| 2 | Blood 6.1 – C1orf132 | 9 | 1 |  | 10 | Blood 6.1 – KLF14 + Q | 4 | 1 |
| 3 | Blood 6.1 – TRIM59 | 9 | 1 |  | 11 | Blood 6.1 – FHL2 + Q | 4 | 1 |
| 4 | Blood 6.1 – KLF14 | 9 | 1 |  | 12 | Blood 6.1 – PrimerMIX + Q | 4 | 1 |
| 5 | Blood 6.1 – FHL2 | 9 | 1 |  | 13 | Negative CTR + Q | 5 | - |
| 6 | Blood 6.1 – PrimerMIX | 9 | 1 |  | 14 |  |  |  |
| 7 | Blood 6.1 – ELOVL2 + Q | 4 | 1 |  | 15 |  |  |  |
| 8 | Blood 6.1 – C1orf132 + Q | 4 | 1 |  | 16 |  |  |  |

* Zamawianie starterów – firma Genomed
  + Modyfikacje – brak
  + Typ – DNA
  + Oczyszczanie – HPLC
  + Skala syntezy – 0,04 umol
* Startery
  + Data otrzymania: Data zawieszenia:
  + Wirowanie, sprawdzenie peletu /ewentualnie zdjęcia/, wirowanie wody i dolanie wody Qiagen wg wzoru dla sprawdzenia ilości wody na probówce:  
    100uM – 100 000nM – 100 000 000pM  
    108pmol – 106ul  
    x pmol – ilość z probówki (np. 154ul)
  + 3 minuty inkubacji
  + wortex i wirowanie
  + termoblok 30 min 56ᵒC lub 2h 37ᵒC
  + zamrożenie w -20ᵒC