

Molecular Biology Laboratory

BP Koirala Institute of Health Science

RGQ Machine No: RGQ_02

qPCR Experiment No: Q198

Date: 26/07/2018

PCR Name: Malaria 5 qPCR

SOP No. MLR_BP_0062_1.1

1. To perform the Malaria multiplex PCR of clinical sample

2. Required Material

| SN | Chemical /Reagents | Brand | Catalogue Number | Lot Number |
|--------------------------|---------------------|-------------|------------------|------------|
| 1 | PCR grade water | Himedia | ML024 | 0000117943 |
| 2 | Primer/Probe Mix | AiT biotech | 300230 | E229-10 |
| 3 | Enzyme/Reaction Mix | AiT biotech | 300230 | E229-10 |
| 4 | Positive Control | AiT biotech | 300230 | E229-10 |
| Number of reaction tubes | | 37 | | |

| SN | RXN components | Stock Concentration | Final concentration | Volumes/ tube (µl) | Final volume (µl) |
|-------|---------------------|---------------------|---------------------|--------------------|-------------------|
| 1 | Primer/Probe Mix | NA | NA | 2 | 74 |
| 2 | Enzyme/Reaction Mix | NA | NA | 6 | 222 |
| 3 | Water/Nuclease free | NA | NA | 12 | 444 |
| 4 | DNA Template | | | 5 | |
| Total | | | | 25 | 740 |

Aliquot 25µl of master mix in each 36 Tubes: 34 Samples + 1 Positive Controls + 1 No template control (NTC)

3. Name of Reagents: D. Biological ramp, Conventional, Real-time

4. Diagrammatic Condition

| Temp (°C) | Time | Cycles | Optics |
|-----------|--------|--------|--------|
| 95 | 2 min | 1 | Off |
| 95 | 5 sec | 45 | Off |
| 60 | 20 sec | | On |

5. qPCR Analysis

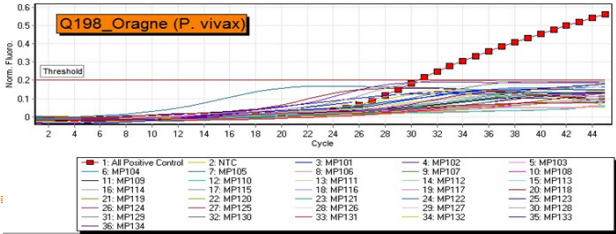
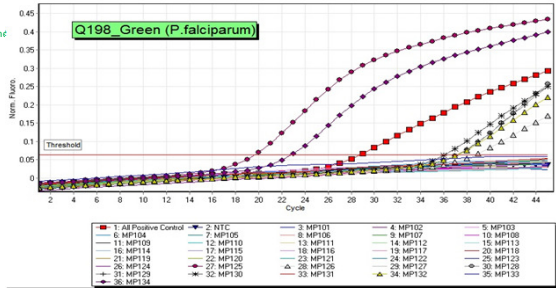
Location of the Folder on Computer:

D:\Molecular Lab\Google_drive\qPCR\

Q198_Malaria_qPCR_V1 2018-07-26

| Tube No | Sample code | Ct VALUE | | | | | PCR RESULT | Malaria Species |
|---------|----------------------|--------------------|------------------|---------------|---------------|-------------------------|------------|-----------------|
| | | FAM (P.falciparum) | HEX (P.malariae) | ROX (P.vivax) | Cy5 (P.ovale) | Quasar 705 (P.knowlesi) | | |
| 1 | All Positive Control | 28.88 | 30.03 | 30.57 | 29.96 | 28.11 | POSITIVE | |
| 2 | No template Control | | | | | | NEGATIVE | |
| 3 | MP101 | | | | | | NEGATIVE | |
| 4 | MP102 | | | | | | NEGATIVE | |
| 5 | MP103 | | | | | | NEGATIVE | |
| 6 | MP104 | | | | | | NEGATIVE | |
| 7 | MP105 | | | | | | NEGATIVE | |
| 8 | MP106 | | | | | | NEGATIVE | |
| 9 | MP107 | | | | | | NEGATIVE | |
| 10 | MP108 | | | | | | NEGATIVE | |
| 11 | MP109 | | | | | | NEGATIVE | |
| 12 | MP110 | | | | | | NEGATIVE | |
| 13 | MP111 | | | | | | NEGATIVE | |
| 14 | MP112 | | | | | | NEGATIVE | |
| 15 | MP113 | | | | | | NEGATIVE | |
| 16 | MP114 | | | | | | NEGATIVE | |
| 17 | MP115 | | | | | | NEGATIVE | |
| 18 | MP116 | | | | | | NEGATIVE | |
| 19 | MP117 | | | | | | NEGATIVE | |
| 20 | MP118 | | | | | | NEGATIVE | |
| 21 | MP119 | | | | | | NEGATIVE | |
| 22 | MP120 | | | | | | NEGATIVE | |
| 23 | MP121 | | | | | | NEGATIVE | |
| 24 | MP122 | | | | | | NEGATIVE | |
| 25 | MP123 | | | | | | NEGATIVE | |
| 26 | MP124 | | | | | | NEGATIVE | |
| 27 | MP125 | 19.76 | | | | | POSITIVE | P.falciparum |
| 28 | MP126 | 38.86 | | | | | POSITIVE | P.falciparum |
| 29 | MP127 | | | | | | POSITIVE | P.falciparum |
| 30 | MP128 | 37.31 | | | | | POSITIVE | P.falciparum |
| 31 | MP129 | 37.27 | | | | | POSITIVE | P.falciparum |
| 32 | MP130 | 36.19 | | | | | POSITIVE | P.falciparum |
| 33 | MP131 | | | | | | NEGATIVE | |
| 34 | MP132 | 37.36 | | | | | POSITIVE | P.falciparum |
| 35 | MP133 | | | | | | NEGATIVE | |
| 36 | MP134 | 22.94 | | | | | POSITIVE | P.falciparum |

Green Channel



Orange

