# PROTOCOL FOR:

**Agarose gel electrophoresis to assess PCR product yield – Comparison with spectrophotometry, fluorometry and qPCR**

Authors and affiliations

## LEGEND

***ATTENTION***

\* ***HINT***

***REST***

# REAGENTS AND MATERIALS

**Chemicals:**

1x TBE buffer

2x Multiplex PCR Master Mix (QIAGEN)

Agarose powder (Roth®)

Ambion nuclease-free H2O (Invitrogen)

Ethidium bromide (EtBr; Serva)

Loading Dye

Low Molecular Weight DNA Ladder (New England BioLabs®)

RNase-free H2O (QIAGEN)

**Consumables:**

Eppendorf reaction tubes safe-lock, 0.5 ml (Eppendorf)

EZ1 DNA Tissue Kit cartridges (QIAGEN)

Pipette tips (Sarstedt)

Pipette tips epT.I.P.S. (Eppendorf)

**Materials:**

DNA extracts from oral swabs, extracted with BioRobot EZ1 (QIAGEN)

Control DNA standards 9947A and 9948, 10 ng/µl (Promega)

# PROCEDURE

**PCR components, parameters and primer sequences:**

|  |
| --- |
| Supplementary table 1: PCR reaction composition |
| 2x Multiplex PCR Master Mix (QIAGEN) | 25 µl |
| Forward primer 0,2 µM | 1 µl |
| Reverse primer 0,2 µM | 1 µl |
| RNase free H2O | 20-22 µl |
| DNA | 1-3 µl  |
| Total volume | 50 µl |

|  |
| --- |
| Supplementary table 2: PCR reaction parameters |
| Initial |  | 95 °C | 5 min |
| Cycling | Denaturation | 95 °C | 1 min |
|  | Annealing | 60 °C | 1 min |
|  | Extension | 72 °C | 1 min |
| Final extension |  | 68 °C | 30 min |
| Soak |  | 10 °C | 10 min |
| Cycles |  | 32 |  |

|  |  |
| --- | --- |
| Supplementary table 3: Primer sequences |  |
| Forward primer | 5’- CTCTGCGCTGGCAATACAGATA |
| Reverse primer | 5’- GACCTATCCTCGTGGAATGC |

**Preparation of 2.5 % agarose gel:**

* Add 3 g agarose powder and 120 ml 1x TBE buffer to Erlenmeyer flask
* Heat Erlenmeyer flask on magnetic heating stirrer

\* ***HINT:*** Cover the flask to prevent evaporation with e.g. a watch glass

* When boiling, remove flask from the heating stirrer and add 4.5 µl EtBr

\* ***HINT:*** Gently mix to avoid introducing air bubbles but ensure homogenous dispersion of EtBr

* Cast gel into the tray, add combs and cover the tray until the gel has set

\* ***HINT:*** Single air bubbles can be removed with e.g. a pipette tip

***REST:*** Gel is set after approximately 40 min

**Agarose gel electrophoresis:**

* Position set gel in electrophoresis chamber
* Add buffer to the chamber until gel is slightly covered
* Mix 8 µl of PCR product with 2 µl bromophenol blue and apply to the gel
* Run electrophoresis at 100 – 120 V for 30 – 45 min

***REST:*** Electrophoresis is finished after approximately 30 – 45 min

* Document agarose gel under ultraviolet light with a transilluminator

**Assessment of band brightness with ImageJ:**

\* ***HINT:*** The open access software ImageJ is available at https://imagej.nih.gov/ij/

Supplemental figure 1A and B: Agarose gel after PCR.

A: Original image of agarose gel. B: Image of agarose gel with virtually subtracted background.

Supplemental figure 2A and B: Agarose gel after purification of PCR products.

A: Original image of agarose gel. B: Image of agarose gel with virtually subtracted background.

\* ***HINT:*** *Program buttons* are italicized

* Open agarose gel image in appropriate file format, e.g. TIFF, via *File > Open*
* Reduce background via *Process > Subtract Background*
* Use *Rectangular Selection Tool* to encase lanes
* Select the first lane via *Analyze > Gels > Select First Lane*
* Select remaining lanes by moving the *Rectangular Selection Tool* to the next lanes and selecting them via *Analyze > Gels > Select Next Lane*
* Generate lane plot profiles via *Analyze > Gels > Plot Lanes*
* Enclose the peaks of interest with the *Lines Tool*
* Measure the peak area with the *Tracing Tool*

\* ***HINT:*** All functions of ImageJ are well documented, available at https://imagej.nih.gov/ij/docs/

# RECIPES

**1x TBE buffer:**

10.903 g TRIS

5.565 g B(OH)3

0.931 g EDTA

Fill up to 1 l with dH2O

**Loading Dye:**

0.025 g bromophenol blue sodium salt

4 g sucrose

Fill up to 10 ml with dH2O

# EQUIPMENT

BioRobot EZ1 Advanced (QIAGEN)

Centrifuge (miniSpin; Eppendorf)

Electronic shaker (Lab dancer; Ikamag, IKA-Werke GmbH & Co. KG)

Electrophoresis chamber (Midi Large horizontal 15 x 17 cm; G&P Kunststofftechnik)

Gel Jet Imager & Analyzer (Intas) with Intas Gel Capture software (Intas)

Magnetic heating stirrer (MH15 Rotilabo®, Roth®)

Pipettes (Eppendorf Reference 0.5-10 µl, 2-20 µl, 10-100 µl, 50-200 µl, 100-1000 µl; Eppendorf)

Power supply (Typ ST606 Electrophoresis Power Supply; Gibco BRL Life Technologies)

Thermal cycler (DNA Mastercycler; Eppendorf)