Promega

Product Application

Droplet Digital PCR Compatibility with Promega Nucleic Acid Purification Kits for Buccal Swab Sample Types

Maxwell® and ReliaPrep™ chemistries are compatible with ddPCR and there is no carryover from the purification that drastically inhibits ddPCR performance.

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information see Technical Manuals TM479 and 345, available at: **www.promega.com/protocols**

or contact Technical Services at **techserv@promega.com**

Kits: Maxwell® RSC Buccal Swab DNA Kit (Cat.# AS1640)
ReliaPrep™ gDNA Tissue Miniprep System (Cat.# A2051)

Analyses: Droplet Digital™ PCR System CNV Assays (BioRad)
qPCR

Sample Type(s): Human buccal swabs – 1 cotton head per sample

Materials Required: All from Bio-Rad:

- ddPCR CNV Assays - KRAS, Human (FAM) and TERT, Human (HEX)
- Droplet Generation Oil for Probes
- DG8 Cartridges
- Droplet Generator DG8 Gasket
- Human Genomic DNA
- Pierceable Foil Heat Seal
- ddPCR Supermix for Probes, no dUTP
- Bio-Rad QX200 Droplet Generator
- Bio-Rad C1000 Touch Thermal Cycler
- Bio-Rad PX1 Plate Sealer
- Bio-Rad QX200 Droplet Reader

Protocol:

1. Purify DNA using the indicated kits following their respective technical manuals.
2. Dilute DNA to ≤ 10ng/µl based on QuantiFluor® ONE dsDNA System (Cat.# E4871) quantitation.
3. Amplify using BioRad's ddPCR™ Copy Number Variation Assays, Validated Protocol (10033173 Ver C). Use the following cycling conditions:

Master Mix Component	Vol per rxn
Nuclease Free Water	3µl
ddPCR Supermix for Probes (no dUTP)	10µl
20X Assay – KRAS FAM	1µl
20X Assay – TERT HEX	1µl
Sample DNA	5µl
Total Volume	**20µl**

Cycling Step	Time	Temp °C	Cycles	Ramp
Activation	10 min	95	1	2 °C/s
Denature	30 sec	94	40	
Anneal/Extend	1 min	60		
Enzyme deactivation	10 min	98	1	
Hold	Infinite	4	1	

Results:

DNA samples purified from buccal swabs with the Maxwell® RSC Buccal Swab DNA Kit and the ReliaPrep gDNA Tissue Miniprep System were successfully amplified and analyzed with the BioRad QX200™ Droplet Digital™ PCR System.

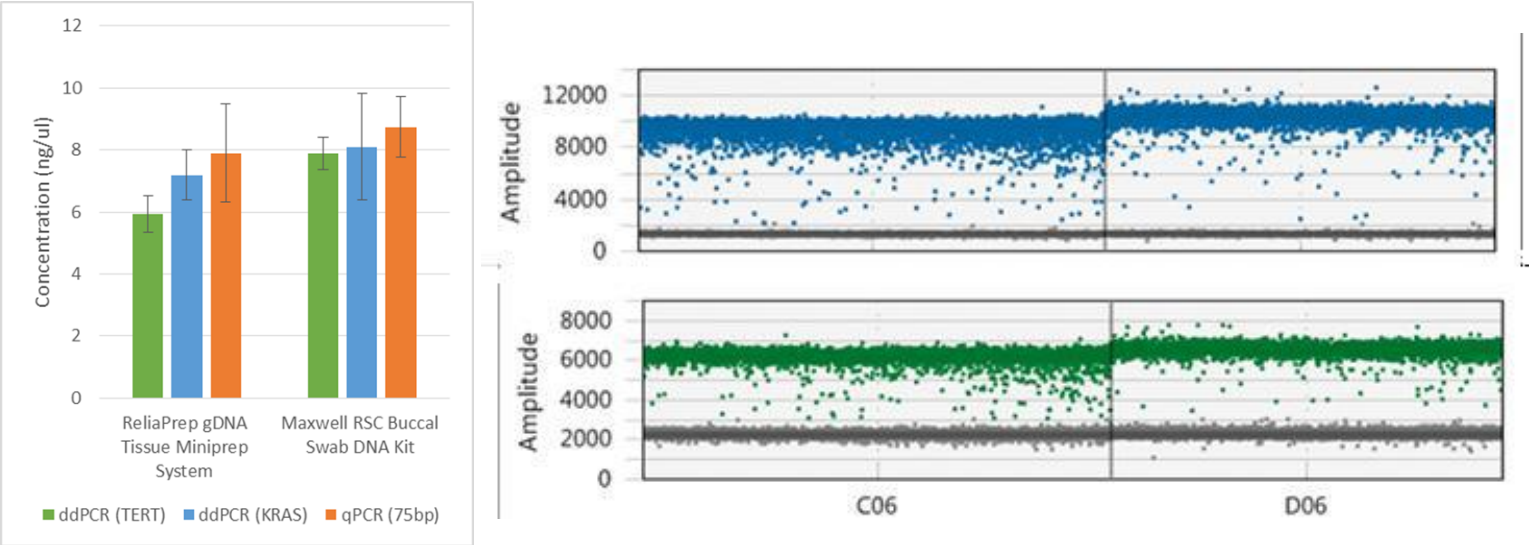

Figure 1: DNA was extracted from buccal swabs and amplified using ddPCR.

Left panel: Comparison of DNA Concentration using ddPCR (TERT and KRAS targets) and qPCR (ProNex® DNA QC Assay, Cat.# NG1003). Sample type is buccal swabs. Based on the copy number/µl determined by ddPCR, a DNA concentration in ng/µl was calculated using the volume of sample input into each well and the single human haploid genome – 3.3pg. These concentrations were compared those determined for the same samples by qPCR (ProNex® DNA QC Assay, Cat.# NG1003) and a very strong agreement was observed. These values also closely correlated with the QuantiFluor® values of the original undiluted samples (not shown as the original samples were undiluted). N=4 for the Maxwell® kit and N=2 for the ReliaPrep™ chemistry.

Right panel: Droplet plots for the targets KRAS (blue) and TERT (green) for buccal swab samples purified using the the ReliaPrep™ Tissue Miniprep System. C06 and D06 are the plate coordinates in the 96-well amplification plate and represent the data for the amplification of four replicate purifications. The high amplitude points represent amplification positive droplets, and low amplitude points (colored in grey) represent amplification negative droplets for the respective target. Good separation of amplification positive and negative droplets is observed.