

Rapid Analysis of Deletions/Duplications with MLPA[®] Using the QIAxcel[®] Advanced System

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Introduction

Adenomatous polyposis syndromes are characterized by polyps in the colorectum. When untreated, these pre-malignant polyps almost always become malignant and lead to colorectal cancer (1). Age of onset for colon cancer is around 39 years, though individuals may develop multiple benign colon polyps in their teenage years. Three monogenic inherited forms have been molecularly diagnosed and distinguished:

- Familial adenomatous polyposis (FAP)
- *MUTYH*-associated polyposis (MAP)
- Proofreading-associated polyposis (PPAP)

FAP is the autosomal dominant form caused by mutations or deletions/duplications in the adenomatous polyposis coli (*APC*) gene located on chromosome 5q22. Hundreds of different variations in the *APC* gene have been described. The detection rate for a pathogenic *APC* mutation in index patients with classical FAP is 80–90%. Attenuated FAP (AFAP) is a second form of FAP with a milder course of disease. The detection rate for *APC* and *MUTYH* mutations in AFAP for index patients is around 20–30%. For clinical diagnosis of a classical or attenuated FAP, persons must have at least 10 synchronous adenomatous polyps (2,3).

Molecular genetic analysis frequently uses either capillary (Sanger) or next-generation sequencing of all exons of the genes involved in the development of FAP, AFAP and PPAP. In classical FAP, genomic rearrangements are caused by large deletions in <10%–15% and rarely by large duplications (3,4). To detect these events in the *APC* and *MUTYH* genes, a Multiplex Ligation-dependent Probe Amplification (MLPA[®]) can be conducted with subsequent fragment analysis on a capillary (Sanger) sequencer, which is a costly and time-consuming method.

In our pilot-study we used a faster, more efficient alternative for the subsequent MLPA analysis using the QIAxcel Advanced System. The proposed protocol enabled detection of exonic deletions/duplications with the same resolution and accuracy as using a sequencer. The efficiency of the MLPA analysis using the QIAxcel Advanced System is more cost effective, compared to other detection systems in use.

Materials and methods

This pilot study was based on two different groups of clinical samples:

- 12 FAP patients and 5 healthy controls
- 9 HNPCC (hereditary non-polyposis colorectal cancer, Lynch syndrome) patients and 5 healthy controls

MLPA

The MLPA was performed using the SALSA® MLPA P043 APC probemix for the FAP samples and the SALSA MLPA P003 MLH1/MSH2 probemix (MRC-Holland®) for the HNPCC samples following the manufacturer's manual.

Amplicon visualization

The separation and visualization of MLPA-generated amplification products was performed using both a sequencer and QIAxcel Advanced System (QIAGEN) for capillary electrophoresis, following the manufacturer's instructions for the sequencer and a modified protocol for the QIAxcel Advanced System (QIAGEN). Samples were separated using the QX DNA High Resolution Kit with a customized 0M1100_2kV_AM10s_S5kV method, with the following electrophoresis parameters: alignment marker injection at 4 kV for 10 s, sample injection at 5 kV for 10 s and separation at 2 kV for 1100 s. Alignment marker 15 bp/600 bp was run simultaneously with the samples and the size was estimated in comparison with the QX DNA size marker 25 bp–500 bp.

Data analysis

Analysis of the data generated by the sequencer was performed with Coffalyser® software (MRC-Holland), while QIAxcel ScreenGel® 1.2 software (QIAGEN) was used for data analyses of the results from the QIAxcel Advanced System.

Note: The layouts of all graphics in this Application Note are slightly changed from originals to more clearly visualize the results. No modifications were conducted that influenced the results themselves and the authors will provide original graphics/data on request.

Results

Electropherograms

Electropherograms from the Coffalyser software analyses showed peak patterns comparable to the corresponding electropherograms from QIAxcel ScreenGel 1.2 analyses (Figures 1–3). The electropherograms represent typical samples from the APC MLPA analyses.

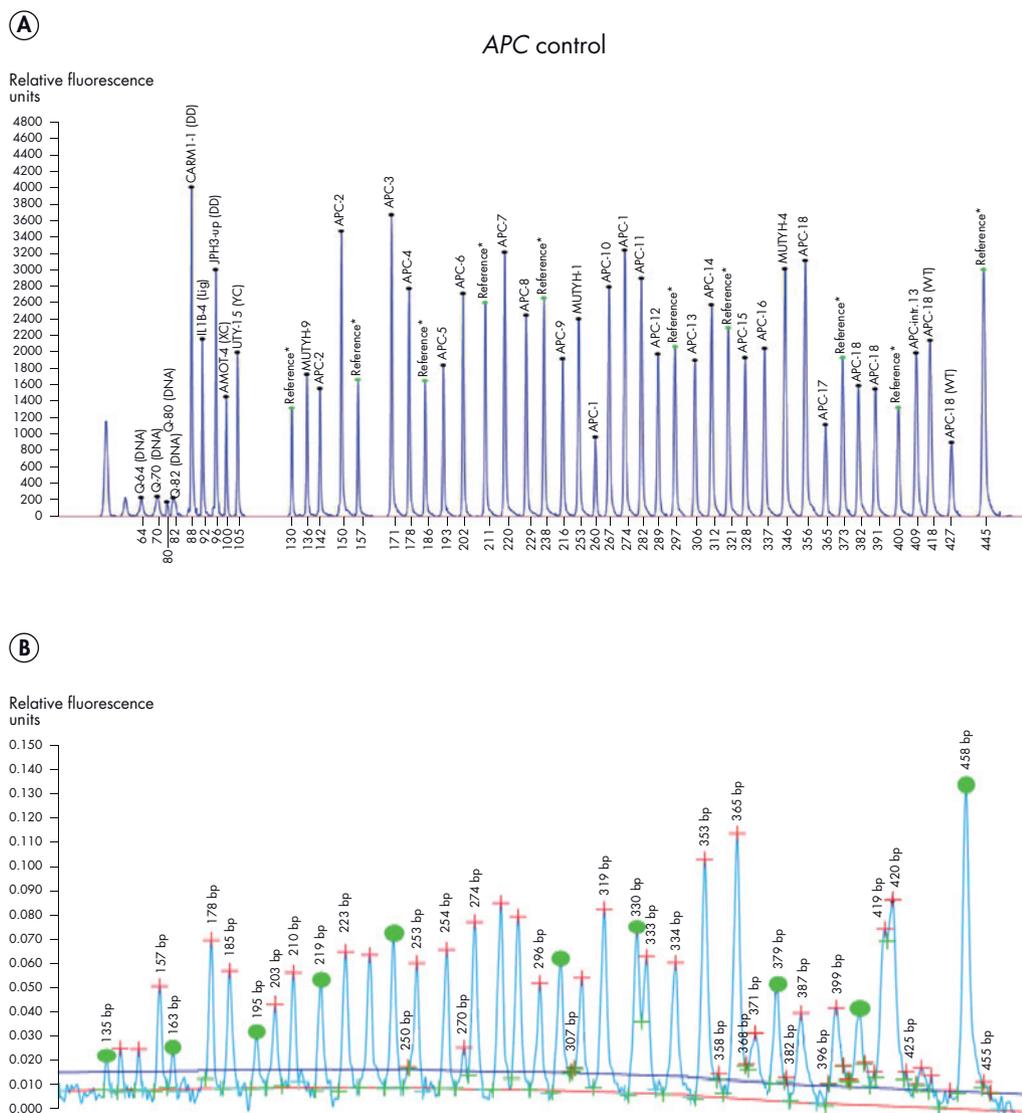


Figure 1. Comparable pattern of peaks for APC control using an ABI PRISM® 3100 Genetic Analyzer and QIAxcel Advanced System. A Electropherogram from Coffalyser software analysis. **B** Electropherogram from the QIAxcel Screen Gel 1.2 software analysis. Green circles show reference peaks.

The peak height ratio between deleted (Figure 2) or duplicated (Figure 3) exon probes with their corresponding reference probes showed changes in the specific target region.

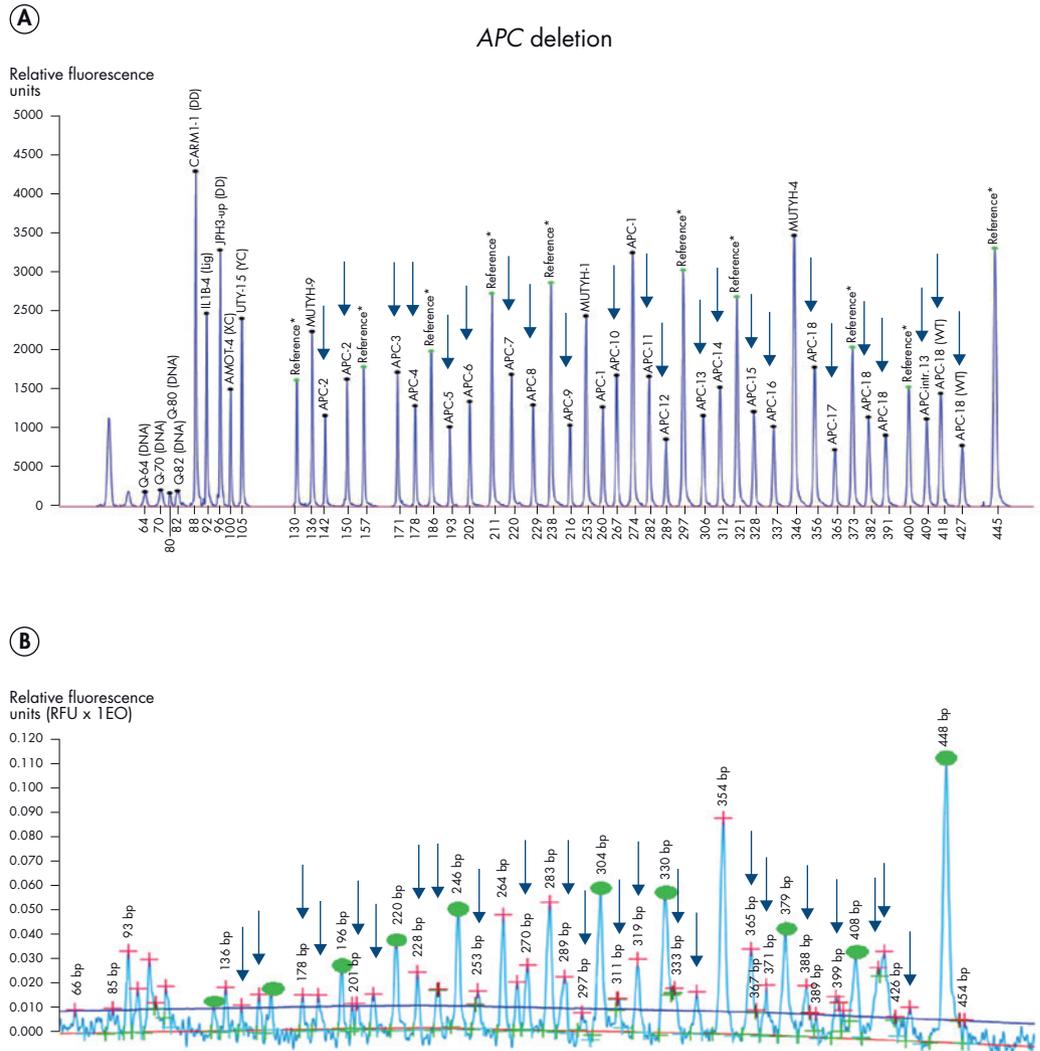


Figure 2. Comparable pattern of peaks for APC deletion using sequencing and QIAxcel Advanced System amplicon visualization techniques. **A** Electropherogram from Coffalyser software analysis of sequencing. **B** Electropherogram from the QIAxcel Screen Gel 1.2 software analysis of QIAxcel Advanced System capillary electrophoresis. Green circles show reference peaks. Black arrows indicate APC peaks reduced compared with the corresponding APC control electropherograms in Figure 1. Arrows are similarly placed between electropherograms A and B.

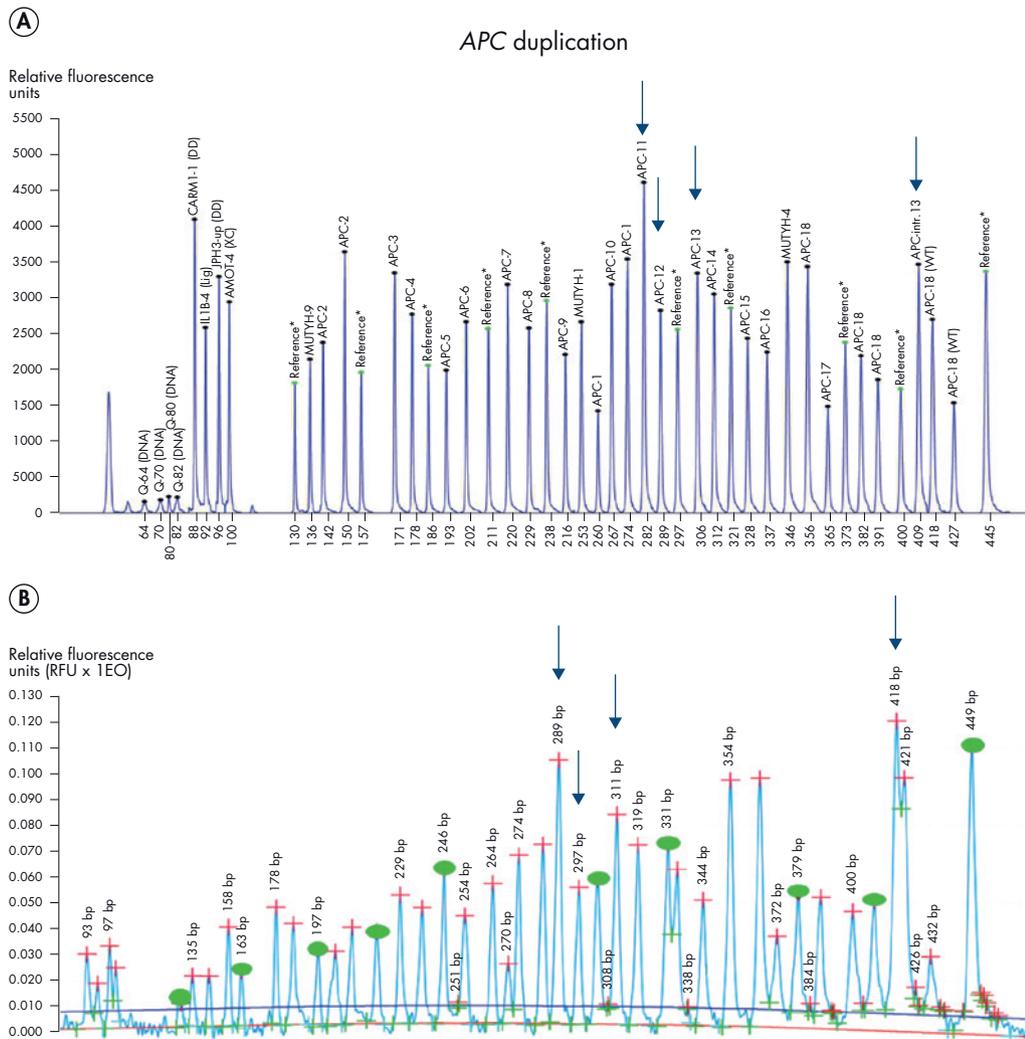


Figure 3. Comparable pattern of peaks for APC duplication using ABI PRISM 3100 Genetic Analyzer and QIAxcel Advanced System. **A** Electropherogram from Coffalyser software analysis. **B** Electropherogram from the QIAxcel ScreenGel 1.2 software analysis. Green circles show reference peaks. Black arrows indicate APC peaks increased compared with the corresponding APC control electropherograms in Figure 1. Arrows are similarly placed between electropherograms **A** and **B**.

Ratio charts

Coffalyser ratio charts are an additional overview of MLPA results and were available for the results from the sequencer only (Figure 4). Complementary to electropherograms, the ratio charts re-interpret the same exonic information.

The displayed confidence interval enables assessment of the success of the MLPA run. Mutation state is indicated by the ratio. Ratios of around 0.5 suggest a heterozygous deletion of a specific allele (Figure 4B, Exons 2–18). Duplication of specific alleles are shown with a ratio of around 1.5 (Figure 4C, Exons 11–13).

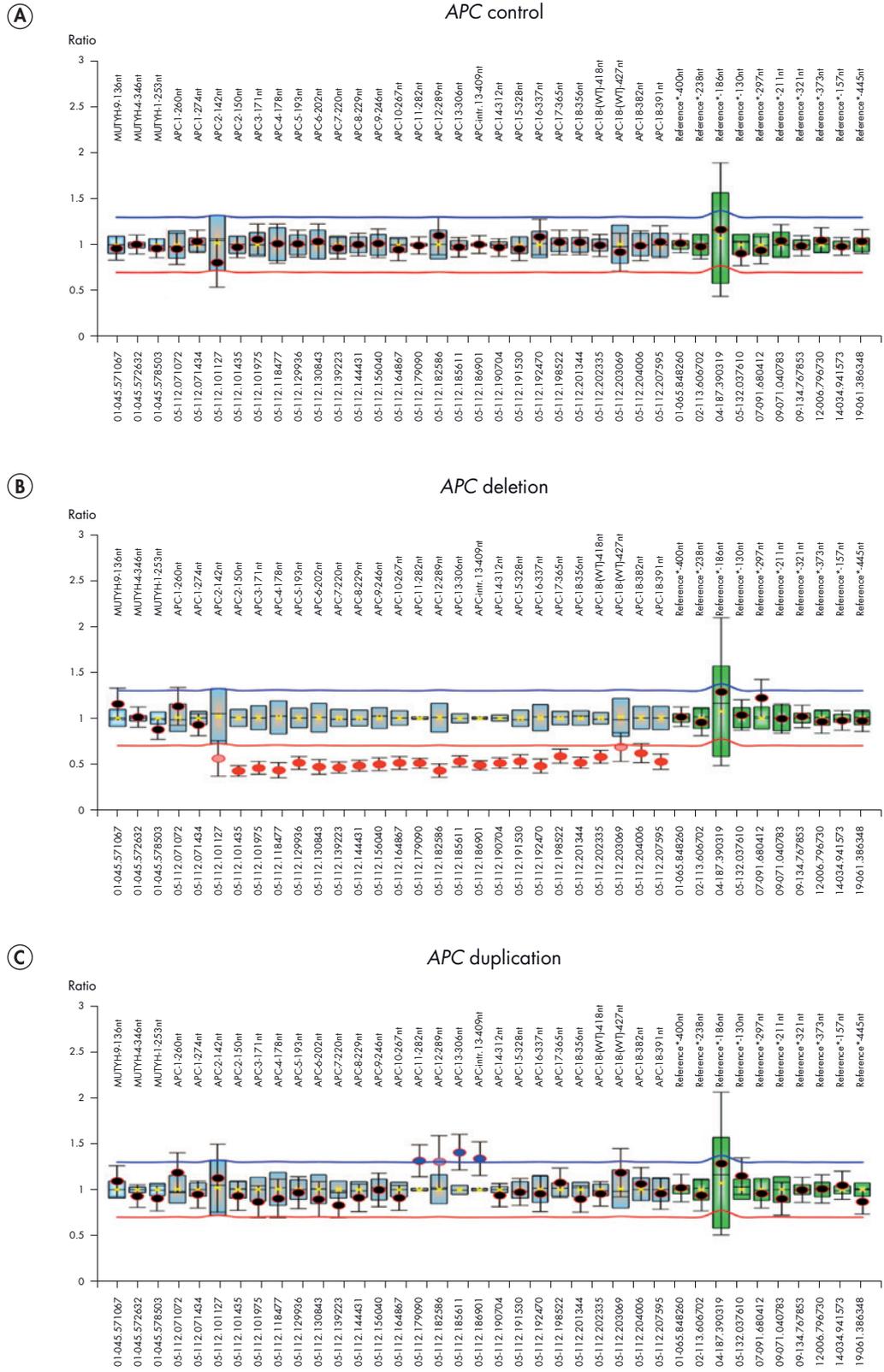


Figure 4. Ratio chart interpretations of the data from APC samples by the Coffalyser software.

A APC control data indicating a ratio of 1, meaning no deletion/duplication at any of the specific genomic regions. **B** Heterozygous deletion of specific APC alleles indicated by a ratio tending towards 0.5 (ratio of 0 would indicate homozygous deletion). **C** Duplication of specific APC alleles indicated by a ratio tending towards 1.5. Mean \pm 95% confidence interval of ratio of signal intensity between sample and reference. Green/blue boxes indicate the 95% confidence range of reference.

Discussion

This pilot study showed detection of exonic deletions/duplications in both FAP and HNPCC samples using the QIAxcel Advanced System with comparable resolution and accuracy when using a sequencer.

Probemix sets (MRC-Holland) for MLPA analysis have specific sizes (in bp) for the different probes in each kit, which are either reference probes or target exonic regions. This specific peak pattern is needed to differentiate the different peaks in the analysis and the pattern of peaks is specific for each analysis. We observed a deviation of 1–9 bp in our generated electropherogram peak pattern for APC with the QIAxcel ScreenGel software version 1.2 compared with the fragment size analysis using the capillary sequencer.

Corrections for the size deviation are normally done at two points: within the sequencer software by applying the correct binning and then subsequently within the Coffalyser software by choosing the correct kit. The Coffalyser software is currently incompatible with the QIAxcel file format, since the Coffalyser requires .fsa file format (the output file format from the sequencer) to perform the analysis. This necessitated QIAxcel results to be analyzed manually. Nevertheless, all events in the pilot samples identified by ABI PRISM 3100 Genetic Analyzer and Coffalyser analysis were still identifiable with the QIAxcel ScreenGel analysis software after using the QIAxcel Advanced System for separation, detection and analysis.

To further develop the MLPA analysis with QIAxcel Advanced System, we are currently trying to convert the output format to be compatible with the Coffalyser.

Conclusions

- The quality of the MLPA analysis when using the QIAxcel Advanced system is comparable with the data provided by a sequencer, both in terms of resolution and accuracy.
- The QIAxcel Advanced system provides a faster alternative to current sequencer-based methods and is more cost-effective.
- QIAxcel ScreenGel software can be used for MLPA analysis if the analysis is performed manually. Achieving software compatibility between QIAxcel ScreenGel and Coffalyser software would offer an even more attractive approach to MLPA analysis.

References

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Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced System	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel software, and 1-year warranty on parts and labor	9001941
QIAxcel ScreenGel Software	Ten licenses for use of QIAxcel ScreenGel Software on additional computers	9021165
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QX Alignment Marker 15 bp/600 bp (1.5 ml)	Alignment marker with 15 bp and 600 bp fragments	929530
QX DNA Size Marker 25-500bp (50 ul) v2.0	DNA size marker with fragments of 25, 50, 75, 100, 150, 200, 250, 300, 400, and 500bp; concentration 100 ng/ul	929560

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