APPLICATION NOTE

DeCodi-Fi in Amplicon Sequencing: A Sensitive High-Fidelity Polymerase for *Xanthomonas citri* Pathovar Differentiation

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Introduction

Xanthomonas citri threatens citrus production, affecting fruit quality and yield. In Chile, quarantine periods of 1–2 years help prevent its entry. To reduce this time, a single-test method for rapid and specific pathovar detection is essential. This application note showcases how **Multiplex Company** optimized a method for detecting two *Xanthomonas citri* pathovars using **DeCodi-Fi High Fidelity Polymerase** in a single PCR reaction. Compared to Q5 High Fidelity Polymerase, **DeCodi-Fi** offers **higher sensitivity**, allowing for **clear pathovar differentiation** with **minimal input material,** as low as **10^1 CFU per reaction.**

Materials and Methods

HiFi Polymerases used:

- DeCodi-Fi High Fidelity polymerase 2X All-in-one Mix
- NEBNext® Ultra™ II Q5® Master Mix (for comparison)

Amplicon sizes and PCR conditions:

Three primer pairs were designed to amplify three distinct amplicons (A, B, and C) present in the genomes of *Xanthomonas citri* pathovar citri (pv.citri) and *Xanthomonas citri* pathovar aurantifolii (pv.aurantifolii), enabling the detection and differentiation of two *Xanthomonas citri* Pathovar based on amplicon size. Each amplicon size was addressed through agarose gel electrophoresis. The primers were designed using the **AmpliPlex automated primer design platform** from the **Multiplex Company**.

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Table 1. Amplicon sizes and annealing temperatures for *Xanthomonas citri* pathovars. Amplicon sizes (in base pairs) for targets A, B, and C used to distinguish between *Xanthomonas citri* pv. citri and *X. citri* pv. aurantifolii. A Touchdown PCR protocol was applied, starting with an annealing temperature (Ta) of 72°C and decreasing by 1°C per cycle until reaching 63°C.

AMPLICON	SIZE (bp)	Ta(°C)
Xanthomonas citri pv. citri (Amplicon A)	425 bp	72°C (-1°C dT) > 63°C
Xanthomonas citri pv. citri (Amplicon B)	234 bp	72°C (-1°C dT) > 63°C
Xanthomonas citri pv. citri (Amplicon C)	256 bp	72°C (-1°C dT) > 63°C
Xanthomonas citri pv. aurantifolii (Amplicon A)	439 bp	72°C (-1°C dT) > 63°C
Xanthomonas citri pv. aurantifolii (Amplicon B)	367 bp	72°C (-1°C dT) > 63°C
Xanthomonas citri pv. aurantifolii (Amplicon C)	226 bp	72°C (-1°C dT) > 63°C

Table 2. PCR reaction mix and final concentrations of components. Final concentrations of each reagent used in the PCR reaction with DeCodi-Fi 2X All-In-One Mix. The primer concentration of 0.4 µM is the combined use of three primer pairs for simultaneous detection of the target amplicons(A, B, C). Genomic DNA from *Xanthomonas citri* (Citrus host DNA) was included to simulate template input for diagnostic testing.

Components	Final Concentration
DeCodi-Fi 2X All-In-One Mix	1X
Primer 10 µM	0.4 µM
Citrus host DNA 1 ng/µL	0.1 ng/µL
Template 1 ng/µL	0.2 ng/µL
Nuclease Free Water	-

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Reaction setup

To enhance specificity and reduce off-target amplification, a Touchdown PCR protocol was performed, starting with an annealing temperature of 72°C and decreasing by 1°C per cycle for the first 9 cycles until reaching 63°C. The remaining 31 cycles used a constant 63 °C annealing temperature, with 10 s annealing, 20 s extension, and a 0.2 s transition between steps. PCR products were visualized via agarose gel electrophoresis stained with SYBR Safe and imaged under UV light. For downstream analysis, amplicons were sequenced using the iSeq100 platform, and sequencing reads (% of total) were classified against reference sequences for citrus host DNA, *X. citri* pv. citri, and *X. citri* pv. aurantifolii.

Results:

For each treatment (DeCodi-Fi and Ultra II Q5), PCR amplifications were performed using input concentrations of 10^3, 10^2, and 10^1 CFU per reaction. Gel electrophoresis (Figure 1) confirmed correct amplicon sizes, with DeCodi-Fi successfully amplifying DNA from as low as 10^1 CFU, demonstrating strong sensitivity and efficient amplification.

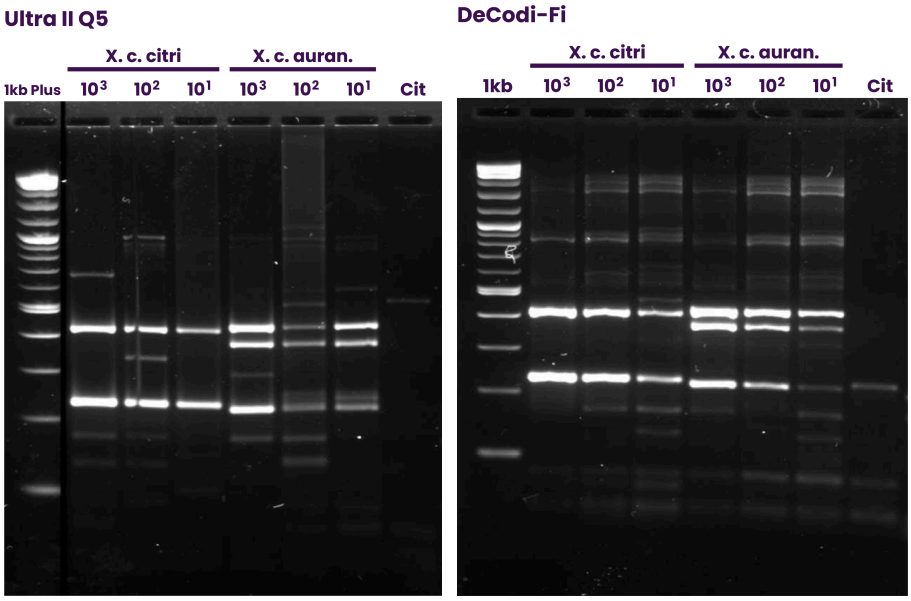

Figure 1. Touchdown PCR amplification of Amplicons A, B, and C from *Xanthomonas citri* pv. citri (425 bp, 234 bp, 256 bp) and *Xanthomonas citri* pv. aurantifolii (429 bp, 367 bp, 226 bp), visualized on an agarose gel. In *X. citri* pv. citri, Amplicons B and C appear as a single merged band. PCR was performed with 10^1, 10^2, and 10^3 CFU inputs. **Cit:** Non-infected citrus control. 1 Kb Plus DNA ladder was used as a size reference.

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PCR products were sequenced to evaluate specificity. The percentage of classified reads (as a proportion of total reads) were analyzed across *X. citri* pv. Citri and *X. citri* pv. aurantifolii (Table 3). The "Citrus host DNA" column reflects background amplification. **DeCodi-Fi showed consistently low background** (≤1%) **and accurate target classification**, even at low CFU inputs. In contrast, Ultra II Q5 showed higher non-specific reads, reaching up to 19.69%. **Highlighted cells indicate conditions where DeCodi-Fi achieved both lower background and higher target read percentages.**

An exception was the 3.77% detection of *X. citri* pv. *aurantifolii* in the *X. citri* pv. *citri* 10^3 CFU sample with DeCodi-Fi. This may be due to the higher total reads (1964), which raises the chance of detecting contaminant reads. The Ultra II Q5 sample had fewer reads (698), reducing that chance.

Table 3. Percentage of sequencing reads for *Xanthomonas citri* pv. aurantifolii and pv citri. Each row represents a different bacterial pathovar and CFU concentration used for amplification (10^3, 10^2, and 10^1). Columns represent the source of the sequencing reads identified (% of total reads), grouped by classification: Citrus host DNA, *X. citri* pv. citri, or *X. citri* pv. aurantifolii. Values are shown separately for each polymerase tested (Ultra II Q5 above, DeCodi-Fi below). The "Citrus host DNA" column represents non-target reading, serving as an internal reference to evaluate non-specific background amplification.

HiFi enzyme	Amplified Pathovar and CFU Concentration	Total Reads	Citrus host DNA	*X. citri* pv. citri	*X citri.* pv. aurantifolii
Ultra II Q5	pv. aurantifolli 10^3	2410	3.65%	1.24%	83.24%
	pv. aurantifolli 10^2	3034	2.31%	0.00%	84.51%
	pv. aurantifolli 10^1	4116	14.58%	0.00%	69.53%
	pv. citri 10^3	698	3.72%	90.26%	0.00%
	pv. citri 10^2	2940	10.27%	81.77%	0.00%
	pv. citri 10^1	1960	19.69%	68.67%	0.00%
DeCodi-Fi	pv. aurantifolli 10^3	5064	**0.20%**	0.00%	**92.97%**
	pv. aurantifolli 10^2	2142	**0.00%**	0.00%	**90.10%**
	pv. aurantifolli 10^1	138	**0.00%**	0.00%	**86.96%**
	pv. citri 10^3	1964	**0.10%**	89.31%	3.77%
	pv. citri 10^2	2438	**0.66%**	**85.40%**	0.00%
	pv. citri 10^1	946	**0.42%**	58.77%	0.00%

Conclusion:

DeCodi-Fi exhibited **high sensitivity and yield** in the **Touchdown PCR method,** enabling **clear differentiation of *Xanthomonas citri* pathovars.** It successfully amplified targets from **as low as 10¹ CFU,** ensuring **high accuracy in pathovar identification.** These results confirm **DeCodi-Fi as a powerful tool for plant disease diagnostics,** enabling **detection at low bacterial loads** and supporting earlier disease management decisions.

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March 20, 2025, v1.0

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