

# Medicinal Genomics SenSATIVAx and PathoSEEK on Agilent Bravo Automated Liquid Handler

## Automated Cannabis DNA Sample Extraction and qPCR Setup from Different Matrices

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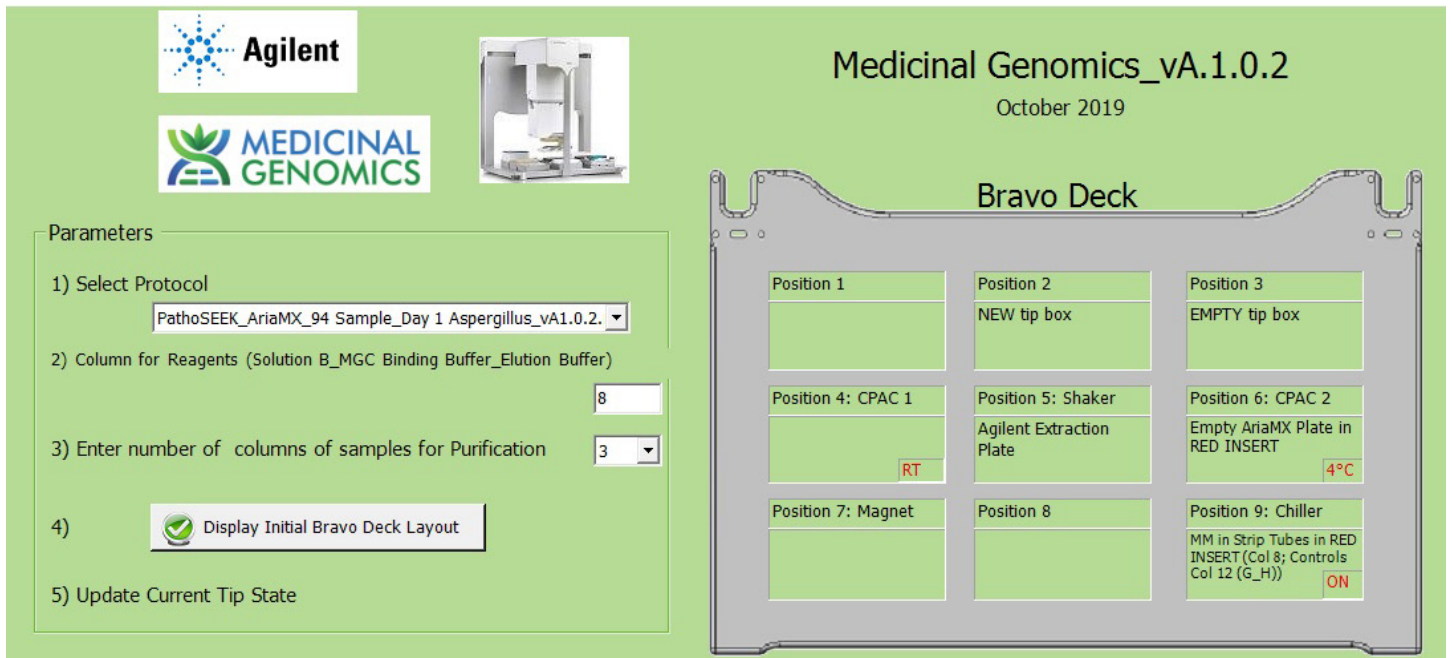
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### Introduction

The Medicinal Genomics SenSATIVAx Plant/Microbial DNA Purification Kit and PathoSEEK Microbial Safety Testing Solution workflow uses magnetic bead-based DNA isolation and quantitative PCR (qPCR) technology to test for microbial contamination present on cannabis flower and marijuana-infused product (MIP) matrices. These kits were automated on the Agilent Bravo automated liquid handling platform to create an efficient DNA extraction and qPCR setup workflow for analysis of several matrices.

### Automated method features and key benefits

The automated Medicinal Genomics SenSATIVAx and PathoSEEK workflow features an intuitive user interface that allows for selection of customizable workflow options. The user may process up to 94 samples when running SenSATIVAx and can set up 94 qPCR reactions (plus positive and negative controls) when running PathoSEEK. Options selected through the user interface will automatically update and create a display to prompt the user to set up the reagents and labware required.

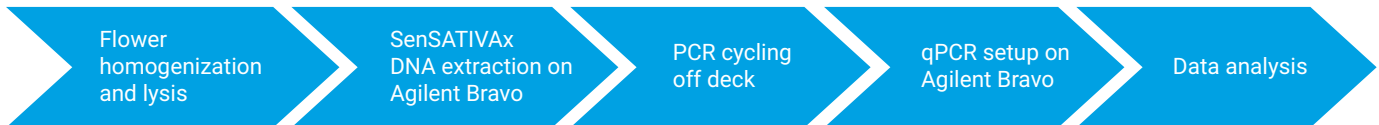


**Figure 1.** User interface for Medicinal Genomics PathoSEEK qPCR setup method automated on the Agilent Bravo automated liquid handling platform.

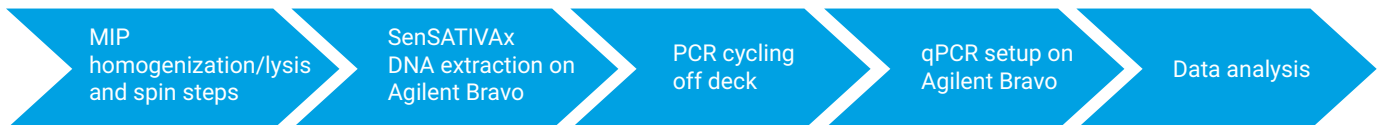
A full 94-sample extraction can be processed in approximately 75 minutes, and a full 96-reaction qPCR can be set up in approximately 12 minutes.

## SenSATIVax and PathoSEEK assay workflow

### Flower samples



### MIP samples



## Experimental

### Flower testing

As defined in the Medicinal Genomics SenSATIVAx for Flower SOP, 1 g of Cannabis flower sample was weighed into a Whirl-Pak bag (Nasco #B02385WA) followed by the addition of 14.2 mL of tryptic soy broth (TSB, MGC #420205) to the bag. The flower sample was then manually manipulated and homogenized for one minute. Following homogenization, 1 mL of the homogenized flower/TSB mixture was transferred into a 1.5 mL snap cap tube. This process was repeated for each of the three flower samples being tested.

The DNA extracts using SenSATIVAx reagents were processed on the Bravo platform. The same three flower samples were also processed manually.

Samples were then tested for total yeast and mold, total coliform count, Aspergillus, Salmonella, and shiga-toxin-producing *E. coli* contamination through qPCR using the MGC PathoSEEK Total Yeast and Mold Detection Assay (MGC #420103), Total Coliform Detection Assay (MGC #420107), Aspergillus 2-Color Multiplex Detection Assay (MGC #420130), and Salmonella & STEC *E. coli* Multiplex Detection Assay v2 (MGC #420120) on the Bravo platform. The qPCR was run on the Agilent AriaMx qPCR instrument. See Table 1 for results.

**Table 1.** Comparison of manual and automated PathoSEEK results from flower samples.

Matrix	Target	Cq Value		Target	CqValue	
		Bravo	Manual		Bravo	Manual
Flower	Cannabis DNA	22.24	20.88	Total Yeast and Mold	NoCq	NoCq
Flower	Cannabis DNA	22.11	21.17	Total Yeast and Mold	NoCq	NoCq
Flower	Cannabis DNA	21.39	20.36	Total Yeast and Mold	NoCq	NoCq
Flower	Cannabis DNA	22.34	20.37	T. Coliform	NoCq	NoCq
Flower	Cannabis DNA	21.83	21.24	T. Coliform	NoCq	NoCq
Flower	Cannabis DNA	21.51	20.10	T. Coliform	NoCq	NoCq
Flower	Cannabis DNA	17.58	18.20	Sal/STEC	No Cq/No Cq	NoCq/No Cq
Flower	Cannabis DNA	17.60	18.18	Sal/STEC	No Cq/No Cq	NoCq/No Cq
Flower	Cannabis DNA	17.64	18.03	Sal/STEC	No Cq/No Cq	NoCq/No Cq
Flower	Cannabis DNA	17.54	18.20	Aspergillus MPX	NoCq	NoCq
Flower	Cannabis DNA	17.58	18.16	Aspergillus MPX	NoCq	NoCq
Flower	Cannabis DNA	17.48	18.09	Aspergillus MPX	NoCq	NoCq

### MIP testing

As defined in the Medicinal Genomics SenSATIVAx for MIP SOP, 1 g of a gummy and chocolate sample was weighed into separate 15 mL tubes followed by the addition of 7 mL of MIP Solution A. The MIP sample was then homogenized by vortexing until in solution. Following homogenization, 500 µL of the homogenized chocolate or gummy with MIP solution A was transferred into a 1.5 mL snap cap tube. The internal SCCG control was added, and the sample centrifuged. A portion of this sample was transferred into a fresh 1.5 mL snap cap tube, and an equal volume of chloroform was added. After vortexing, the sample was centrifuged again and a portion of the supernatant was transferred to a 96-well extraction plate followed by the addition of MIP solution B. This process was performed

in parallel for each of the three gummy and chocolate samples being tested. Using SenSATIVAx reagents, the remainder of the DNA extraction was processed on the Bravo platform. The same MIP samples were also processed manually.

Samples were then tested for total yeast and mold, total coliform count, Aspergillus, Salmonella, and shiga-toxin-producing *E. coli* contamination through qPCR using the MGC PathoSEEK Total Yeast and Mold Detection Assay (MGC #420103), Total Coliform Detection Assay (MGC #420107), 2-Color Aspergillus Multiplex Detection Assay (MGC #420130) and Salmonella & STEC *E. coli* Multiplex Detection Assay v2 (MGC #420120) on the Bravo platform. The qPCR was run on the AriaMx qPCR instrument. See Table 2 for results.

**Table 2.** Comparison of manual and automated PathoSEEK results from MIP samples.

Matrix	Target	Cq Value		Assay	Cq Value	
		Bravo	Manual		Bravo	Manual
Gummy	Cannabis DNA	26.00	26.44	Total Yeast and Mold	NoCq	NoCq
Gummy	Cannabis DNA	26.01	26.38	Total Yeast and Mold	NoCq	NoCq
Gummy	Cannabis DNA	26.16	26.60	Total Yeast and Mold	NoCq	NoCq
Chocolate	Cannabis DNA	27.17	27.44	Total Yeast and Mold	NoCq	NoCq
Chocolate	Cannabis DNA	27.57	27.47	Total Yeast and Mold	NoCq	NoCq
Positive Control	Cannabis DNA	38.78	NoCq	Total Yeast and Mold	10.78	10.73
Negative Control	Cannabis DNA	36.79	39.95	Total Yeast and Mold	NoCq	NoCq
Gummy	Cannabis DNA	25.68	26.45	Total Coliform	NoCq	NoCq
Gummy	Cannabis DNA	25.54	26.50	Total Coliform	NoCq	NoCq
Gummy	Cannabis DNA	26.05	26.65	Total Coliform	NoCq	NoCq
Chocolate	Cannabis DNA	26.88	27.05	Total Coliform	32.81	33.13
Chocolate	Cannabis DNA	27.19	27.16	Total Coliform	30.89	32.81
Positive Control	Cannabis DNA	NoCq	NoCq	Total Coliform	10.81	10.66
Negative Control	Cannabis DNA	NoCq	NoCq	Total Coliform	NoCq	NoCq
Gummy	Cannabis DNA	25.53	25.82	Salmonella/STEC	No Cq/No Cq	No Cq/No Cq
Gummy	Cannabis DNA	25.51	26.19	Salmonella/STEC	No Cq/No Cq	No Cq/No Cq
Gummy	Cannabis DNA	26.15	26.76	Salmonella/STEC	No Cq/No Cq	No Cq/No Cq
Chocolate	Cannabis DNA	26.15	26.62	Salmonella/STEC	No Cq/No Cq	No Cq/No Cq
Chocolate	Cannabis DNA	28.35	28.98	Salmonella/STEC	No Cq/No Cq	No Cq/No Cq
Positive Control	Cannabis DNA	NoCq	NoCq	Salmonella/STEC	14.83/10.35	14.53/10.14
Negative Control	Cannabis DNA	NoCq	NoCq	Salmonella/STEC	No Cq/No Cq	No Cq/No Cq
Gummy	Cannabis DNA	25.45	26.20	Aspergillus	NoCq	NoCq
Gummy	Cannabis DNA	25.53	26.19	Aspergillus	NoCq	NoCq
Gummy	Cannabis DNA	26.18	26.96	Aspergillus	NoCq	NoCq
Chocolate	Cannabis DNA	26.36	26.98	Aspergillus	NoCq	NoCq
Chocolate	Cannabis DNA	28.27	28.75	Aspergillus	NoCq	NoCq
Positive Control	Cannabis DNA	NoCq	NoCq	Aspergillus	10.26	9.90
Negative Control	Cannabis DNA	38.26	NoCq	Aspergillus	NoCq	NoCq

## Conclusion

The Medicinal Genomics SenSATIVax Plant/Microbial DNA Purification Kit and PathoSEEK Microbial Safety Testing Solution kits automated on the Agilent Bravo automated liquid handling platform were demonstrated to provide a robust, flexible, and efficient DNA extraction and qPCR setup workflow for

multiple matrices. Ninety-four samples can be extracted in less than one hour and a full 96-well plate of qPCR reactions can be set up in approximately 10 minutes. The intuitive user interfaces guide the user through the setup process, which allows for seamless setup and method execution.

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