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Automation of the SenSATIVAx® Sample Extraction and PathoSEEK® or FemINDICAtor™ qPCR Setup Platforms on the Microlab® NIMBUS®

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High-throughput DNA Extraction and qPCR Setup Directly from Cannabis Flower on the Microlab NIMBUS

Introduction

Cannabis testing is critical to the success of growers and processors. In addition to providing vital information on product quality and safety while complying with state regulations, certain cannabis tests can help growers to maximize the efficiency of their yield prior to harvest. At the same time, an increased number of samples submitted for testing, combined with time-consuming manual testing methods, creates a backlog in the testing laboratory that, in turn, delays the sale of the crop.

Assay protocols vary widely, and many older methods are not specifically validated for use with cannabis samples, are not scalable, and do not include internal cannabis controls to confirm test accuracy. Additionally, labor-intensive protocol steps such as sample preparation limit sample throughput, and risk error due to human variability and subjective interpretation. Automated cannabis testing methods, using assays specifically developed for cannabis testing, can increase throughput and reduce bottlenecks without sacrificing the quality of results. Here, we demonstrate automated workflows using Medicinal Genomics (MGC) assays specifically developed for cannabis testing. The SenSATIVAx Plant/Microbial DNA Purification Kit provides fast, easy isolation of plant and microbial DNA

from freshly cut or cured leaf or flower material without the need for organic extraction, and can be used to prepare samples for use with the PathoSEEK and FemINDICAtor assays. The PathoSEEK Microbial Safety Testing Platform uses a quantitative polymerase chain reaction to search for and quantify the unique DNA sequences of target organisms in cannabis flower, extracts and infused product samples, and includes an internal cannabis control. The FemINDICAtor qPCR Plant Gender Detection Assay accurately identifies male cannabis plants significantly faster and earlier than visual inspections so that growers can remove them from the crop, thereby improving yields.

Highlights

- Maximize profits, including reducing or reallocating labor resources

- Eliminate human errors and variability during processing and results interpretation

- Reliably safeguard product and crop integrity

The workflows were automated on the Microlab NIMBUS automated liquid handler from Hamilton Robotics (Figure 1). Microlab NIMBUS utilizes patented pipetting technology to provide high accuracy and precision in a variety of low-to- medium throughput liquid handling steps such as aspirating, dispensing, washing, and transferring. The combined, automated system of assays and equipment significantly reduce active labor time compared to manual methods without negatively impacting results.

Automated Method Features and Key Benefits

The Microlab NIMBUS software was pre-programmed with the SenSATIVAx, FemINDICAtor, and PathoSEEK workflow steps as a standard solution in order to facilitate user-friendly operation and reduce operator input errors. The intuitive User Interface (Figures 2 – 3) allows for selection of customizable workflow options. The user may process between 1 – 32 samples when running SenSATIVAx and can set up 1 – 96 qPCR reactions when running either PathoSEEK or FemINDICAtor. When running qPCR setup (PathoSEEK or FemINDICAtor), the user can run the full method including both decontamination setup and qPCR setup, or run either separately. Options selected via the User Interface will automatically update future User Dialogs that prompt the user to set up reagents to account for number of samples being processed. A full 32 sample extraction can be processed in approximately 90 minutes and a full 96 qPCR reaction can be set up in approximately 30 minutes compared to approximately three hours for the same using manual methods.

Experimental Design and Results

As defined in the SenSATIVAx procedure, 0.25 g of Cannabis flower sample was weighed into a Whirl-Pak bag (Nasco #B01385WA, Fort Atkinson, WI) followed by the addition of 3.55 mL of Tryptic Soy Broth (TSB, MGC #420205). The flower sample was then manually homogenized for one minute. Following homogenization, 285 µL of the homogenized flower/TSB mixture was transferred into one well of the extraction plate (Perkin Elmer #6008290, Waltham, MA). This process was repeated for each of four flower samples tested. The DNA extractions using SenSATIVAx reagents were processed on the Microlab NIMBUS. After setting up the Microlab NIMBUS deck with necessary reagents and materials, the SenSATIVAx DNA Extraction method was selected from the Method Manager screen, and user and sample information was input. Microlab NIMBUS then automatically added lysis buffer to the wells and incubated for two minutes. The plate was then manually placed in a centrifuge to pellet lysed cellular material and trichomes. After returning the plate to the Microlab NIMBUS deck, the supernatants of each sample were transferred to fresh wells of the extraction plate. MGC binding buffer was then added to the samples and tip mixed. DNA from the lysed samples was bound to the beads, and was then separated via a magnet on the Microlab NIMBUS deck. The samples were then washed with 70% ethanol to remove any non-DNA material, and eluted in aqueous buffer. The purified DNA was then transferred to separate wells of the extraction plate for subsequent assay analysis. Four flower

Microlab NIMBUS Automated Liquid Handler

Figure 1. The Microlab NIMBUS is equipped with up to eight independent air displacement pipetting channels for high precision and reliability. Compressed O-ring expansion (CO-RE) technology creates an air-tight seal between the disposable tips and mandrels without using mechanical force, while barcode provides full sample tracking. A magnetic stand from Medicinal Genomics was used during DNA extraction along with various pedestals for plates, reagents, and tips.

samples were also processed manually. Samples were then tested for Total Yeast and Mold and Total Aerobic Bacteria contamination via qPCR using the PathoSEEK qPCR Master Kit, Total Aerobic detection assay and Total Yeast and Mold detection assay. Total Yeast and Mold and Total Aerobic Count assay master mixes and positive controls were prepared by the user and added to the Microlab NIMBUS deck when prompted. DNA samples and positive and negative controls were automatically transferred to a clean qPCR microplate followed by prepared qPCR master mixes which include assay specific primers. Samples were automatically mixed then placed in the Bio-Rad CFX96™ Real-Time PCR Detection System (Hercules, CA) for qPCR analysis. See Table 1 below for results. For testing FemINDICAtor, three known male and three known female plant leaves were hole punched using a 4.0 mm biopsy punch (Integra LifeSciences Corporation, #33-34-P/25, Plainsboro, NJ). Each sample punch was transferred to a 96 well PCR plate (USA Scientific 1402-9300, Ocala,

FL). The DNA extraction using SenSATIVAx Flower/Leaf DNA Extraction Kit was then processed on the Microlab NIMBUS using the procedure outline above adapted to plant leaves. The samples were decontaminated following the assay kit manufacturer's recommended protocol, followed by addition of prepared qPCR master mix which includes assay detection primers. The samples were mixed, then placed in the qPCR instrument to test for the presence or absence of Y chromosome DNA. See Table 2 for results. Finally, a comparison of manual versus automated qPCR work flows were assessed for repeatability in sample handling. Identical homogenized flower sample was extracted five times manually and on the Microlab NIMBUS using the SenSATIVAx assay kits. Purified DNA samples were then processed using the PathoSEEK assay kit to detect E. coli and Cannabis DNA primer target sequences, similarly as was done above following manufacturer's recommended protocol. See Table 3 for results.

Table 1: Detection of Total Yeast and Mold, and Total Aerobic Count PathoSEEK qPCR Results

Well	Sample	Processing Method	Target	Cq	Target	Cq
A1	Bubba Kush	NIMBUS	Total Yeast and Mold	N/A	Cannabis DNA	25.35
B1	Bubba Kush	Manual	Total Yeast and Mold	N/A	Cannabis DNA	25.13
C2	Erk Train	NIMBUS	Total Yeast and Mold	33.85	Cannabis DNA	26.45
D1	Erk Train	Manual	Total Yeast and Mold	33.33	Cannabis DNA	25.55
E1	Harlox	NIMBUS	Total Yeast and Mold	N/A	Cannabis DNA	27.21
F1	Harlox	Manual	Total Yeast and Mold	N/A	Cannabis DNA	26.59
G1	Rugburn OG	NIMBUS	Total Yeast and Mold	N/A	Cannabis DNA	25.80
H1	Rugburn OG	Manual	Total Yeast and Mold	N/A	Cannabis DNA	25.18
A2	TYM Positive Ctrl	NIMBUS	Total Yeast and Mold	11.49	Cannabis DNA	N/A
C2	NTC	NIMBUS	Total Yeast and Mold	N/A	Cannabis DNA	N/A
E2	Bubba Kush	NIMBUS	Total Aerobic Count	37.42	Cannabis DNA	25.44
F2	Bubba Kush	Manual	Total Aerobic Count	N/A	Cannabis DNA	25.13
G2	Erk Train	NIMBUS	Total Aerobic Count	35.00	Cannabis DNA	26.69
H2	Erk Train	Manual	Total Aerobic Count	34.42	Cannabis DNA	25.95
B2	Harlox	NIMBUS	Total Aerobic Count	27.41	Cannabis DNA	27.37
A3	Harlox	Manual	Total Aerobic Count	27.16	Cannabis DNA	26.79
D2	Rugburn OG	NIMBUS	Total Aerobic Count	N/A	Cannabis DNA	25.68
C3	Rugburn OG	Manual	Total Aerobic Count	N/A	Cannabis DNA	25.07
E3	Total Aerobic Positive Ctrl	NIMBUS	Total Aerobic Count	12.57	Cannabis DNA	N/A
G3	NTC	NIMBUS	Total Aerobic Count	N/A	Cannabis DNA	N/A

Microbial detection of total yeast and mold as well as total aerobic count using MGCs PathoSEEK qPCR reagents using manual and automated methods from multiple flower samples.

Microlab NIMBUS User Interface qPCR Workflow Selection

User Name and Sample Entry Choice

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PathoSEEK®/FemINDICAtor® qPCR Set Up

Method Options:

Assay Choice
- ☑ Microbial Testing
- ☑ Sex Testing

Sample Entry
- ☑ GUI Input
- ☑ File Input

Step Choice
- ☑ Decontamination
- ☑ qPCR SetUp

User Information
Name:
_____

[Continue]   [ABORT]

Figure 2. Incorporating automated, pre-programmed methods reduces the amount of time to set up the assay, while also reducing or eliminating user entry errors. Additionally, the automated methods allow full sample traceability throughout sample processing.

Microlab NIMBUS User Interface Indicating MGC PathoSEEK qPCR Master Mix Reagent Set Up Volumes and Instructions

Sal E. coli qPCR MM

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Add specified amounts of reagents for Salmonella/E. coli qPCR MM to clean simport tube, spin tube and place in position 1-1 of tube holder in location 1, deck location A4

Sal/E. coli qPCR MM Reagents	qPCR MM Volumes
qPCR MM =	45.6
Sal/E.coli Primer Probe =	12
Decon Buffer =	9.6
Water =	98.4

[OK]

Figure 3. Automated methods using pre-programmed steps can reduce or eliminate human errors and variability while automatically scaling volumes to meet any throughput needs.

Manual Versus Automated SenSATIVAx, PathoSEEK, and FemINDICAtor Assay Workflows

SenSATIVAx

Total Hands on Time (32 samples)

2 hrs 15 min

PathoSEEK / FemINDICAtor

Total Hands on Time (96 reactions)

1 hrs 20 min
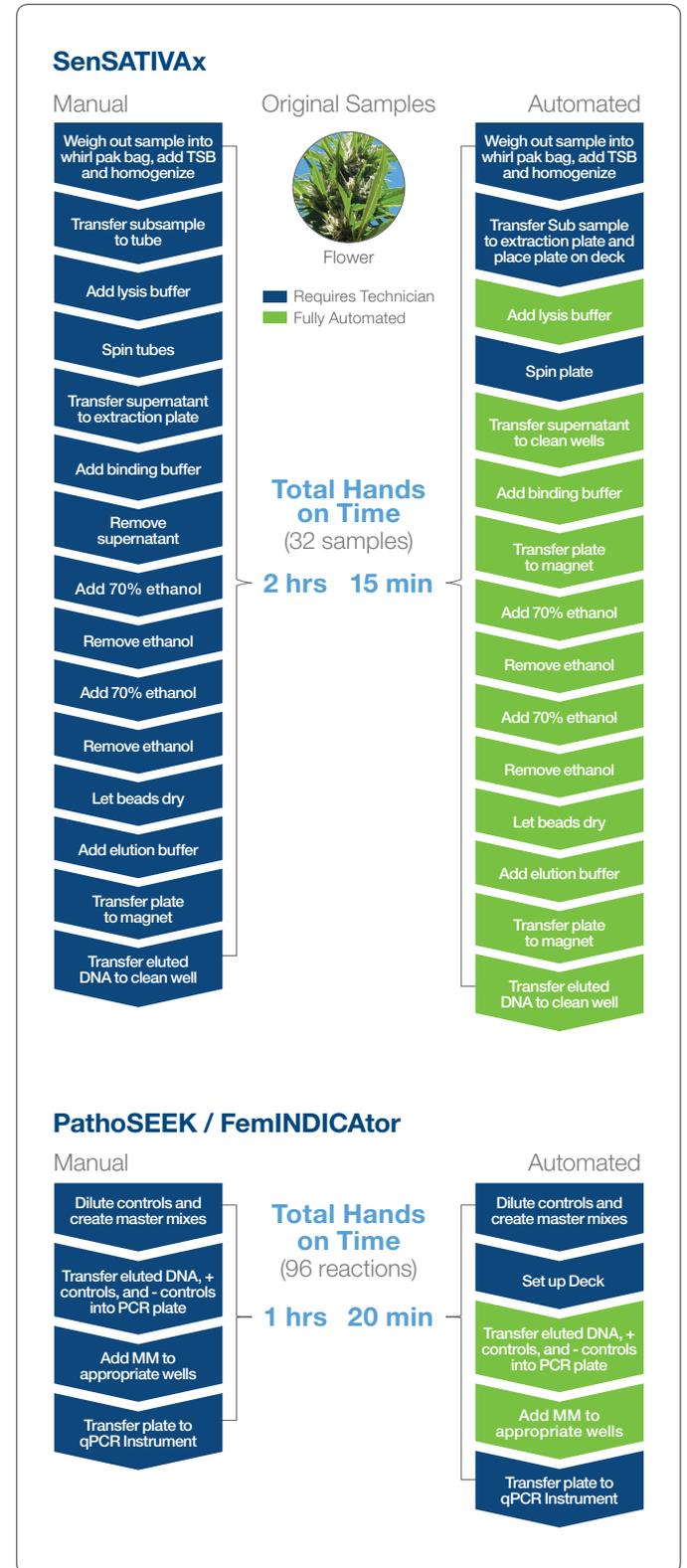
Figure 4. Comparison of manual vs. automated SenSATIVAx DNA extraction protocol and the PathoSEEK and FemINDICAtor qPCR setup steps and processing times. Up to 32 sample extractions are processed, and 96 qPCR reactions are set up, in a total of approximately two hours using the automated workflow, compared to six total hours using manual methods to perform the same number of samples. In addition to facilitating faster time to results, the hands-free automated methods allow users to reallocate their time to other tasks while the samples are processed.

Table 2: Cannabis Plant Gender Detection FemINDICAtor qPCR Results

Well	Sample	Target	Cq	Male of Female?	Target	Cq
A1	Male 1	Y Chrom	29.14	M	Cannabis DNA	30.20
B1	Male 2	Y Chrom	30.64	M	Cannabis DNA	31.64
C1	Male 3	Y Chrom	30.66	M	Cannabis DNA	31.48
D1	Female 1	Y Chrom	N/A	F	Cannabis DNA	30.07
E1	Female 2	Y Chrom	38.19	F	Cannabis DNA	26.98
F1	Female 3	Y Chrom	N/A	F	Cannabis DNA	27.89
G1	XY Positive Ctrl	Y Chrom	13.54	N/A	Cannabis DNA	N/A
H1	Neg Control	Y Chrom	N/A	N/A	Cannabis DNA	N/A

Six leaf hole punch samples run using MGCs FemINDICAtor qPCR reagents on the Microlab NIMBUS. A Cq value less than 35 indicates a male plant.

Table 3: Comparison of Manual vs. Automated PathoSEEK qPCR Results

Sample	Automated or Manual	Fluor	Target	Cq	Fluor	Target	Cq
Blueberry Cheesecake	Automated	FAM	E. coli	22.34	HEX	Cannabis DNA	27.63
Blueberry Cheesecake	Automated	FAM	E. coli	22.27	HEX	Cannabis DNA	27.51
Blueberry Cheesecake	Automated	FAM	E. coli	22.28	HEX	Cannabis DNA	27.56
Blueberry Cheesecake	Automated	FAM	E. coli	22.24	HEX	Cannabis DNA	27.44
Blueberry Cheesecake	Automated	FAM	E. coli	22.34	HEX	Cannabis DNA	27.66
Blueberry Cheesecake	Manual	FAM	E. coli	21.60	HEX	Cannabis DNA	26.92
Blueberry Cheesecake	Manual	FAM	E. coli	21.54	HEX	Cannabis DNA	27.06
Blueberry Cheesecake	Manual	FAM	E. coli	21.70	HEX	Cannabis DNA	27.44
Blueberry Cheesecake	Manual	FAM	E. coli	22.05	HEX	Cannabis DNA	27.61
Blueberry Cheesecake	Manual	FAM	E. coli	21.97	HEX	Cannabis DNA	27.34
Positive Control	Automated	FAM	E. coli	14.27	HEX	Cannabis DNA	N/A
Negative Control	Automated	FAM	E. coli	N/A	HEX	Cannabis DNA	N/A

When comparing cycle quantification (Cq) values obtained from identical homogenized sample via manual and automated methods, consistency among results is seen in line with expectations. Results from automated methods are reliable and robust, with lesser variability observed between technical replicates.

Conclusion

The Medicinal Genomics SenSATIVAx DNA Purification Kits, PathoSEEK Microbial Safety Tests, and FemINDICAtor sex testing kits automated on the Microlab NIMBUS liquid handling platform provide a robust, flexible, and efficient DNA extraction and qPCR set up workflow in less time than manual methods. Thirty-two samples can be extracted, and a full 96-well plate of qPCR reactions can be set up in approximately 66% less time than manual methods without sacrificing sample results. The intuitive user interfaces guide the user through the setup process, which allows for seamless set up and method execution without errors or variability from user-to-user, or run-to-run. The combined automated solution allows testing labs to quickly and accurately deliver results to their customers while maximizing time and labor resources.

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