

FemINDICAtor® Cannabis Sex Detection Assay v3

Method Developer Validation

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Introduction

The FemINDICator® Cannabis Sex Detection Assay v3 Kit is a qPCR detection kit for the purpose of identifying the presence of the Y chromosome in cannabis and/or hemp plants. FemINDICator can be run with the same qPCR instruments as the PathoSEEK® Microbial Detection and Plant Pathogen Detection Kits.

FemINDICator® Cannabis Sex Detection Assay v3 improves on our previous v2 assay, which targets an additional region of the Y chromosome with an additional primer probe set, by further optimizing it for use on the Biomolecular Systems Mic. Utilizing two primer probe sets which target the Y chromosome accounts for unique variations found through extensive sequencing of a variety of male plants. When paired with the Biomolecular Systems Mic, qPCR data review may be automated with limited user intervention.

Assay Design

To determine the targets for the FemINDICator assay design we whole genome sequenced female and male cannabis varieties. We then mapped all of the male sequences against female sequences in silico to identify a list of sequences that are unique to the male genome. We then assembled these male-specific reads into a Y chromosome. These sequences were then filtered for heterozygosity to deliver hundreds of target qPCR loci. Next, those loci were screened for the best performance in dozens of unrelated males. This was the v1 version of our assay.

In 2021, the team repeated the design process using the Y chromosome from the Jamaican Lion reference genomes and a larger dataset of male and female cultivars from the Kannapedia database, which included

feral hemp varieties. This project revealed two things: low rates of polymorphisms under our initial primer set and highly-conserved regions in the Y chromosome. Armed with this knowledge, we designed a second primer set targeting these conserved regions to increase the capture and sensitivity of the assay. That primer set was combined with the v1 primer set to create the FemINDICator v2 Assay, which has provided 99% accuracy in calling male plants to date.

Method Developer Validation

Assay Validation 1

The Colorado Seed Company used FemINDICator v1 to screen 197 seedlings. The seedlings were then allowed to grow until their sex could be visually determined. The FemINDICator results matched the visual results in 194 samples, resulting in a 98.5% call rate.

Assay Validation 2

Prior to launching the FemINDICator v2 Assays, the Medicinal Genomics team screened 83 known male and female cannabis samples using both FemINDICator v1 and v2. The assays delivered the same plant sex results, showing equivalency. The FemINDICator v2 assay yielded a more sensitive Cq value for the FAM channel, while maintaining a consistent Cq value in the HEX channel. These results are summarized in the table below.

Table 1: Summarized Results comparing V1 to V2

FemINDICator	Average Cq Fam	Average Cq Hex
v1	27.46	26.67
v2	25.34	26.80

Assay Validation 3

An external company also performed a study of 96 samples to compare the performance of FemINDICator v2 to FemINDICator v1. The results revealed that FemINDICator v1 identified 38 of the samples as male, while FemINDICator v2 identified 39 males, demonstrating a marginal increase in sensitivity. The average Cq values shown in Table 2 indicate that FemINDICator v2 exhibits greater sensitivity in the FAM channel without compromising sensitivity in the HEX channel.

Table 2: Summarized Results comparing V1 to V2

FemINDICator	Average Cq Fam	Average Cq Hex
v1	28.32	27.32
v2	25.63	27.28

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The FemINDICator Detection Assay v3 has been optimized for use on the Biomolecular Systems Mic and remains compatible with standard qPCR instruments. To demonstrate the capability of the assay as well as equivalence between various instruments, eight male samples and eight female samples were assessed. Leaf samples were prepared using Quick Lysis and were subsequently analyzed by the Bio Molecular Systems Mic, the Biorad CFX-96, and the Agilent AriaMX according to the FemINDICator Cannabis Sex Detection Assay User Guide v3. The averaged results are below in table 3.

Table 3: Summarized Results comparing Biorad, Aria and MIC

	Biorad	Aria	MIC
Average Male Cq Fam	26.25	25.25	26.24
Average Male Cq Hex	28.10	25.86	28.38
Average Female Cq Fam	ND	ND	ND
Average Female Cq Hex	28.95	30.20	30.28

Each sample produced the expected result on each of the three qPCR instruments tested.

Conclusion

The FemINDICator® Cannabis Sex Detection Assay v3 marks a significant evolution in genetic sex determination for cannabis and hemp, offering a highly accurate and reliable qPCR solution. The v3 assay, which maintains the improved sensitivity of v2 by targeting a second, highly-conserved region of the Y chromosome, has been further optimized for use with the Biomolecular Systems Mic while maintaining full compatibility with standard qPCR instruments like the Bio-Rad CFX-96 and Agilent AriaMX.

These validation studies have demonstrated the assay's efficacy, showing equivalence between v1, v2, and v3. Furthermore, testing on multiple qPCR platforms confirmed that FemINDICator v3 consistently produces the expected, accurate results across instruments (Table 3). This early and reliable identification of male plants is a crucial advancement for cultivators, enabling the prompt removal of males to prevent pollination and ensure the production of high-quality, seedless female flowers. The assay is a powerful tool for efficient crop management.

REVISION HISTORY

Version	Date	Description
v1	February 2025	• Conversion of assay validation web page to document format
v2	December 2025	• Assay v3 with addition of Amplification Mix in “kitted” format • Validation of v3 assay including comparison of Bio Molecular Systems Mic qPCR instrument with legacy platforms

DISCLAIMER

This test was developed, and its performance characteristics determined by Medicinal Genomics Corporation (MGC), for laboratory use. Any deviations from the protocol are not supported by MGC. The results may vary based on laboratory conditions.

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