

FemINDICAtor® Cannabis Sex Detection Assay Version 2

Method Developer Validation

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Introduction

The FemINDICAtor® Cannabis Sex Detection Assay v2 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 v2 improves on our previous v1 assay by targeting an additional region of the Y chromosome with an additional primer probe set. This addition accounts for unique variations found through extensive sequencing of a variety of male plants. The original primer probe set remains unchanged. The original and new primer probe sets are both detected in the FAM channel.

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.

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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

Conclusion

The FemINDICAtor® Cannabis Sex Detection Assay v2 represents a substantial advancement in cannabis and hemp cultivation, offering a highly accurate and reliable method for determining the genetic sex of plants. By specifically identifying the presence of the Y chromosome, which is indicative of male plants, this assay empowers cultivators to efficiently and effectively manage their crops. Early sex identification is crucial in cannabis cultivation, as it allows for the prompt removal of male plants, preventing unwanted pollination of female plants and ensuring the production of high-quality, seedless female flowers.

REVISION HISTORY

Version	Date	Description
v1	February 2025	Conversion of assay validation web page to document format

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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