

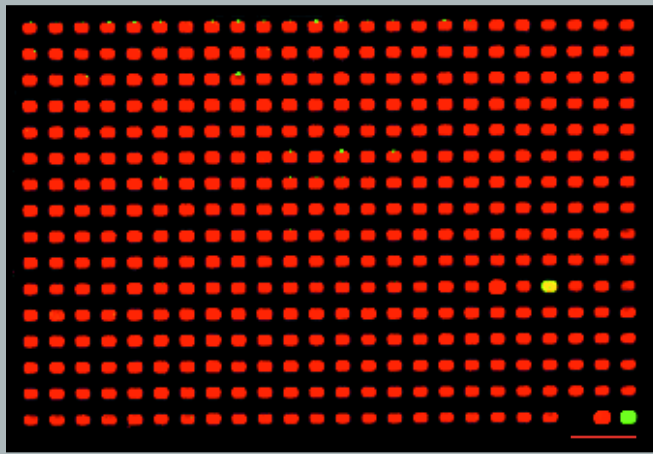
# Homogenous Sequence Detection Using Molecular Beacons

## Separation Anxiety?

Whether screening for target sequence or single nucleotide polymorphisms, virtually all techniques require some form of molecular separation. As our knowledge of various genomes advances, detection of highly specific target sequences is becoming necessary for diagnostic applications. As a consequence, the need for rapid, cost-effective detection techniques has become paramount.

Historically, these techniques have relied on time-consuming molecular separation based on gel electrophoresis. Recently, advances in fluorescent probe technology have made possible the accurate detection of target sequence in homogeneous DNA solutions (e.g. PCR product) using Molecular Beacons (MB), thus eliminating the need to run gels.

Screening for pathogens in a 384-well plate



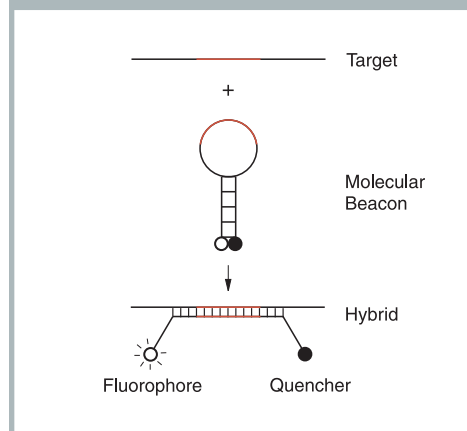
*In this experiment, the red signal (Cy-3) signifies a housekeeping gene. The green signal (6-Fam) is specific to a pathogen. Here, only one sample of 381 tested positive (yellow). The last three samples represent controls (no template, housekeeping and target pathogen sequence). Data provided by Dr. K. Arkush at the U.C. Davis, Bodega Marine Laboratory.*



## Molecular Beacons on the FMBIO®

Molecular Beacons are oligonucleotides that detect target nucleic acids in homogeneous solutions<sup>1</sup>. Beacons are hairpin-shaped probes with an internally quenched fluorophore whose fluorescence is restored when bound to its target. The loop portion acts as the probe while the stem is formed by complementary "arm" sequences at the ends of the oligo. A fluorophore and quenching moiety are attached at opposite ends and the stem keeps them in close proximity, causing the fluorophore to be quenched by energy transfer. When the beacon detects its target, it undergoes a conformational change forcing the stem apart, separating the fluorophore and quencher. This act disrupts the energy transfer and leads to the restoration of fluorescence.

Molecular Beacons

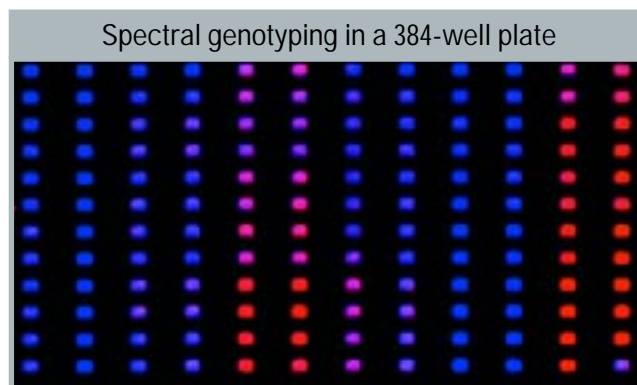


## Advantages

The versatile FMBIO Fluorescent Imaging System has proven to be an excellent instrument for end-point determinations of MB, offering outstanding sensitivity, throughput and data analysis capabilities.

- Simultaneous 4-color detection of up to six 96-, 384- or 1536-well plates
- Use as little as 5 nM of Molecular Beacon
- Simple PCR-based assay
- No gel electrophoresis necessary

- High spatial resolution, 83 microns/pixel



*Chinook salmon genotyped at a MHC class II B1 locus using Beacons labeled with 6-Fam and Hex.*

### Reference:

Tyagi, S., Kramer, FR (1996) Molecular Beacons: probes that fluoresce upon hybridization Nature Biotechnology, 14, 303-308.

## FMBIO Specifications

<b>Laser:</b>	Solid-state 532 nm YAG laser
<b>Scan Area and Time:</b>	20 cm x 43 cm dual color at standard resolution: 10 minutes
<b>Detection Wavelengths:</b>	Up to 4-color separation, selectable from 500 nm to 700 nm
<b>Dynamic Range:</b>	4 orders of magnitude
<b>Compatible Dyes:</b>	For a complete list please visit our Web site
<b>Multiplex Dye Sets (filter):</b>	<ul style="list-style-type: none"><li>• 6-Fam (505 nm), Hex (585 nm), Texas Red (650 nm)</li><li>• 6-Fam (505 nm), Hex (560 nm), Ned (585 nm), Rox (605 nm)</li><li>• Filters for novel dye sets are also available</li></ul>

[www.miraibio.com](http://www.miraibio.com)

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