Integration of a novel real-time PCR detection system onto Luminex beads in a PCR reaction.

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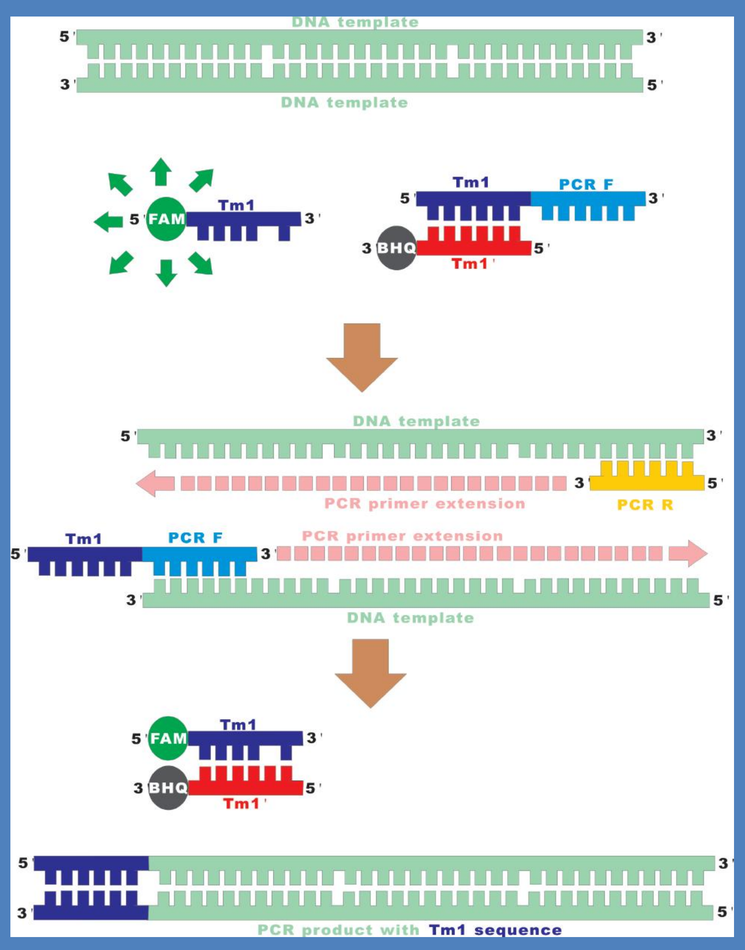
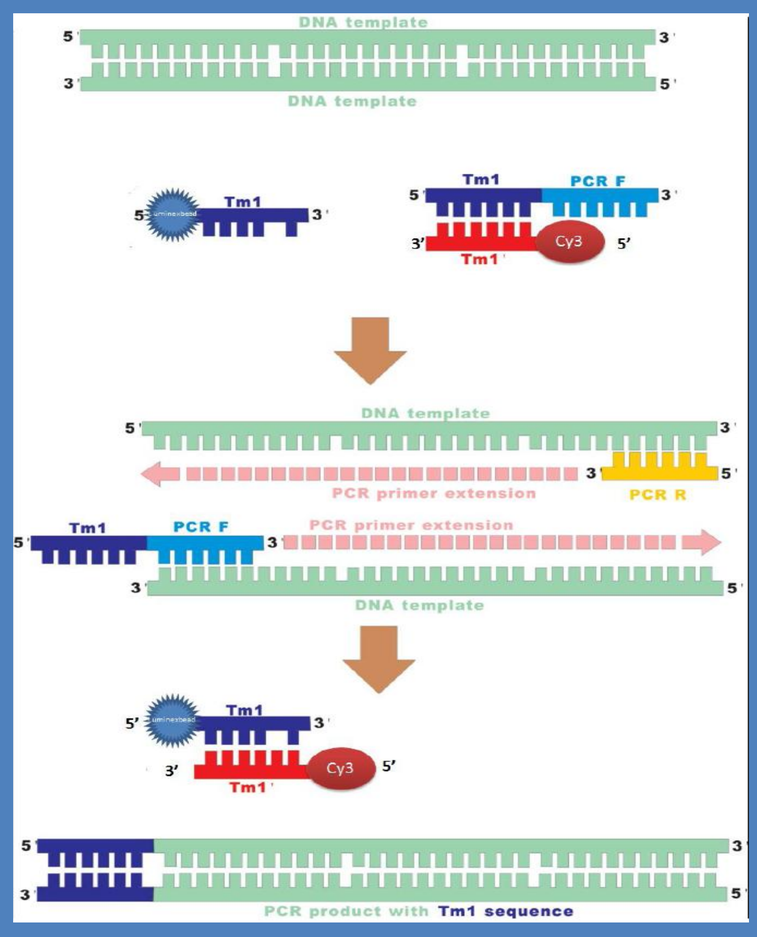
Introduction: In this new development, the PrimRglo™ system has now been integrated with Luminex™ microbeads, utilizing a universal capture oligonucleotide on the bead, and a Cy3 label on the universal detection oligonucleotide. There is a one-to-one correspondence between the Cy3 signal generated by a PCR reaction and a particular set of dye encoded microbeads. PCR (target: influenza A matrix gene plasmid) was carried out, with the oligonucleotide-conjugated Luminex beads in the PCR mix. The Cy3 fluorescence signal associated with the microbeads increased in proportion to the amount of PCR reaction product. The fluorescence associated with microbeads was measured on a Luminex machine, which currently has the capacity to measure up to approximately 100 different reaction products attached to optically distinguishable bead sets.

PrimRglo ®

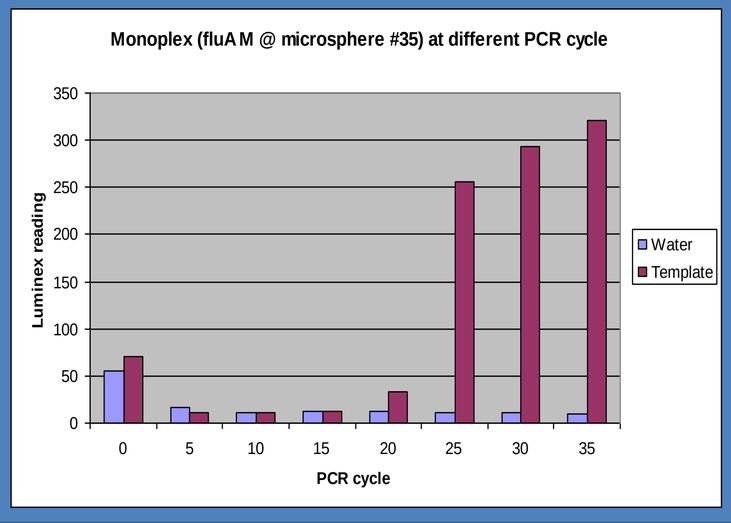


PrimRglo ® with Luminex beads

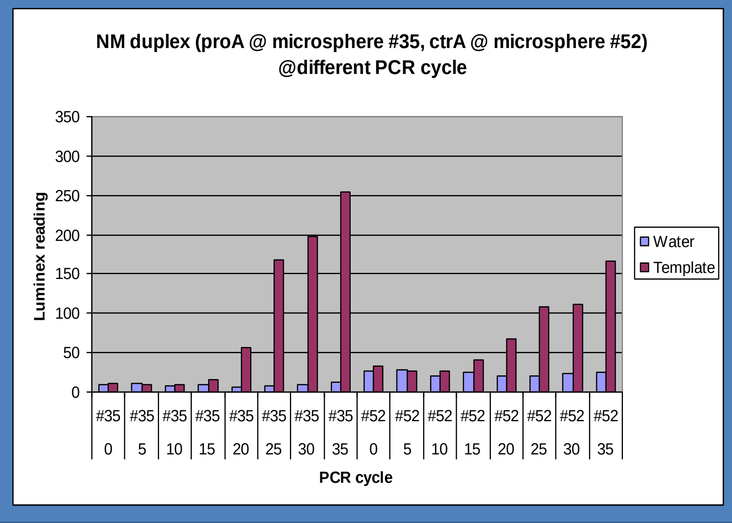


Results: We have employed the Luminex™ microbeads-integrated PrimRglo™ system on real-time PCR of influenza A virus using an influenza M gene plasmid (plasmid concentration $4X10^2$ ng) and duplex PCR of *Neisseria meningitidis* porA and ctrA (plasmid concentration 10^2 ng). In the duplex PCR two products were amplified in the same tube , with detection using two differently coded Luminex beads (bead sets #35 and #52)

MONOPLEX



DUPLEX



Conclusion: Integration of the PrimRglo detection system onto Luminex beads will facilitate high degree multiplexing of quantitative real-time PCR reactions, beyond that which is currently possible with standard methods. It will combine the capacity to detect many viruses or bacteria simultaneously, in a single reaction tube, with the ability to quantitate viral or bacterial load.

References: 1. R. Barnard, , R. Lai, D. Pearson, Z.Y. Phua, D. Whiley, T. Sloots and G. Barnett.(2009) A new multiplexable, quantitative, real-time system for detection of nucleic acids. *New Biotechnology*, Volume 25, Supplement 1, September 2009, Pages S17-S18,. 2. Barnard, R.T. & Barnett, G.R. (2007) A method and kit for analyzing a target nucleic acid sequence. WO 2007/003017.