



## Method for Direct Quantification of Nucleic Acids in Real Time qPCR

### IITM Technology Available for Licensing

#### Problem Statement

- Currently quantitative PCR (**qPCR**) is a robust technique, widely used in biological research for **studying mRNA expression, DNA copy number, allele variations** etc. It has revolutionized **diagnostics, offering fast, sensitive and specific detection of diseases**: Dengue, Influenza A & B, Zika etc.
- The **qPCR** uses two basic chemistries for quantification of amplicons of which one is cost effective but have **lower specificity** and the other involves **fluorescent probes (FP)** that use sequence-specific oligonucleotides making it **expensive** & successfully prevent **non-specific amplification of the target**.
- Hence, there is an **urgent need** of the present invention that discloses the method to **develop an economically feasible sequence specific probe** for use in **qPCR** for DNA amplification.

#### Technology Category/ Market

##### Biotechnology & Genetic Engineering

**Industry:** Molecular Biology, Biomedical Engg.

**Application:** Diagnostic, Life science Research

**Market:** The Global qPCR Instruments Market value is expected to grow from **USD 898.98 million** in **2021** to **USD 1,394.75 million** in **2028** at **3.4% CAGR** from **2021** to **2028**.

#### Technology

The present patent technology aims to disclose a method for **quantifying nucleic acids** using an **Aptamer-based qPCR (Apt-qPCR)** probe in **real-time PCR**.

This method appears to involve **utilizing a light-up dye-aptamer system**, where the **fluorescence increases significantly** when the dye binds to its specific aptamer.

#### TRL (Technology Readiness Level)

**TRL 3, Experimentally validated in lab.**

#### Intellectual Property

IITM IDF No: **1619** | IP No.: **393411** (Granted)

PCT No.: **PCT/IN2018/000054**

US Application No. **16/766,205**

#### Method

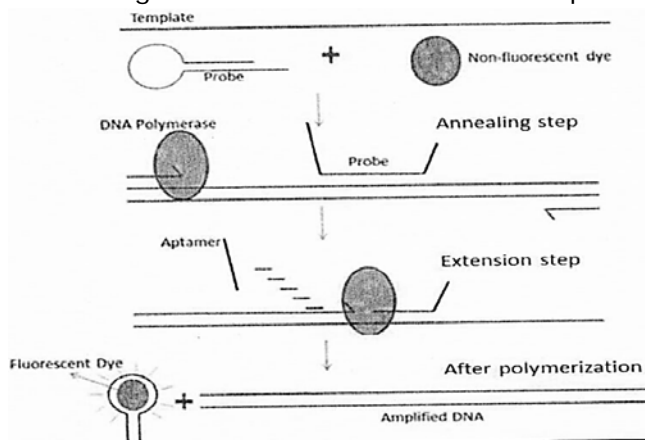
The method for direct quantification of nucleic acids in real-time qPCR, comprises:

using a simple and shorter Aptamer-based qPCR (Apt-qPCR) probe for quantification in real-time PCR wherein the probe uses a light-up dye-aptamer system in which the dye shows negligible fluorescence in the free state and its fluorescence increases manifold when it binds to its specific aptamer;

placing the aptamer 5' upstream of one of the primers wherein the primer initially in pre-annealed form shows fluorescence as aptamer is free and single-stranded & can bind to dye;

performing annealing and extension step to make the aptamer double-stranded & thereby loose its 3D structure to form a double helix wherein the double helix is not specific for the dye and do not bind, therefore reducing fluorescence of the solution corresponding to each cycle of the PCR reaction.

**FIG. 1** illustrates a schematic representation of mechanism of action of quantification of nucleic acids using label free endonuclease based probes.



#### Key Features / Value Proposition

This technology presents an approachable, precise, and cost-effective method for quantifying DNA amplification in real-time PCR, offering benefits across user, technical, & industrial perspectives.

#### Research Lab

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