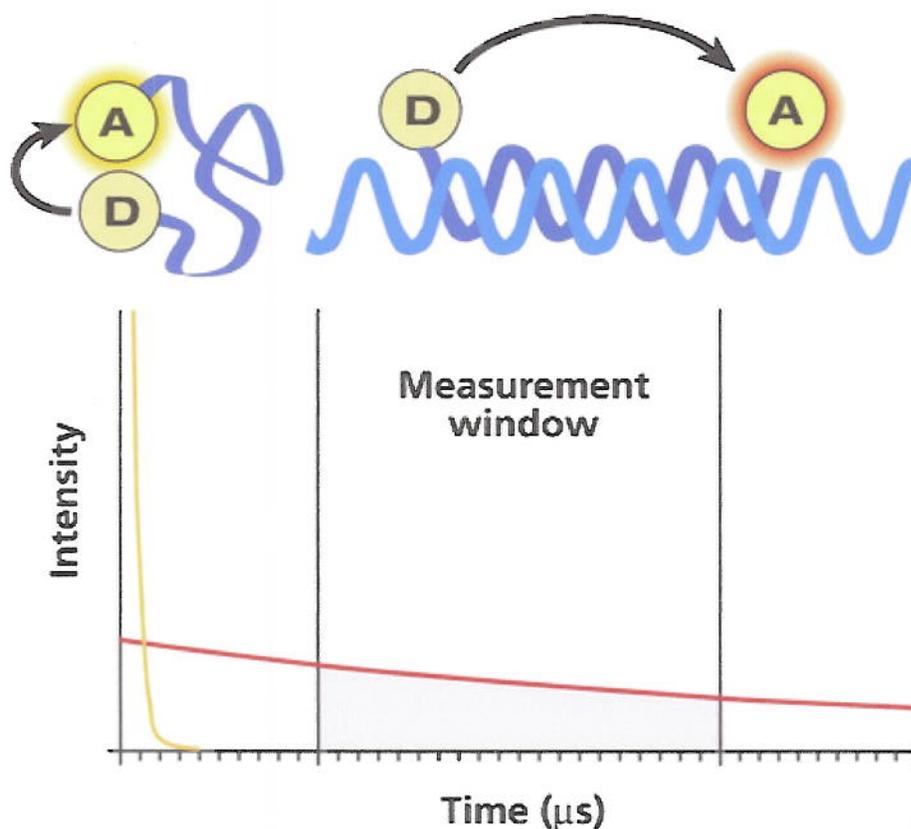


## TR-FRET-based end-point PCR

Genetic testing can be performed through nucleic acid analysis, which often utilizes the polymerase chain reaction (PCR), a basic tool in molecular biology for nucleic acid amplification. PCR generally involves four steps: sampling, sample preparation, amplification and detection. There are several applications that integrate the latter two stages to allow simple nucleic acid sequence analysis with minimal post-PCR handling. Unlike these conventional PCR methods, Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) -based end-point PCR combines sample preparation, amplification and detection. After punching of sample disks all assay steps, including addition of amplification and detection reagents, thermal cycling, and fluorescence measurement, take place in the same vessel, making the method simple and easy to use.



[Enlarge](#) With TR-FRET

technology, donor (D) and acceptor (A) labels on the unhybridized probe (upper left) are close to each other. This results in high energy transfer efficiency and intensive acceptor signal with short decay time (yellow curve on the graph). After hybridization with the DNA being measured, the rigid DNA chain forces the labels apart (upper right). This leads to reduced energy transfer efficiency and increased acceptor decay time (red curve).

TR-FRET –based end-point PCR involves a target sequence-specific probe, in which long-lifetime fluorescent lanthanide donor and acceptor fluorophores are coupled to opposite ends of a single probe molecule. When the probe is hybridized, it enables direct detection of the target-hybridized probe population based on prolonged decay time of the energy-transfer induced

acceptor signal. This probe approach also allows tailoring of both the wavelength and decay time of the induced acceptor signal enabling multi-analyte assays. The method enables rapid homogeneous one-step DNA-detection requiring only one reagent per one target oligonucleotide. The use of long lifetime fluorescent lanthanides in the time-resolved measurement also effectively suppresses the nanosecond-lifetime assay background caused by the assay matrix and light scattering, and thereby increases the sensitivity of the assay. Moreover, the sensitivity for its part allows the use of small sample sizes thus diminishing the interference caused by the biological components and enabling the direct use of disk samples in homogeneous PCR.

### **TREC Assay Procedure**

- Punch out 1.5 mm filter paper disks DBS calibrators, DBS controls and samples into PCR plate
- Dispense elution buffer, 10  $\mu$ L/well
- Seal and centrifuge 20 sec, 500 x g, +19–+25 °C
- Start elution incubation Thermal cycler, +98 °C 45 min, +4 °C 2 min
- Unseal and dispense reagent mixture 20  $\mu$ L/well
- Seal and centrifuge , 20 sec, 500 x g, +19–+25 °C
- Start thermal incubation in thermal cycler ~2 h 40 min
- Centrifuge 2 min, 500 x g, +19–+25 °C
- Measure in plate readr within 60 minutes of thermal incubation