Filter Selection

When performing experiments requiring detection of more than one fluorophore, it is highly desirable for minimal overlap in signal from each dye. Whilst the choice of fluorophore used is important, the design of the optics for sample excitation and emission detection is crucial to obtain a sensitive and specific assay. The most specific method of exciting and detecting multiple dyes is to use a series of tailored excitation and emission filters. These ensure that only light of the correct wavelength reaches the dye to be excited and only emitted light of the correct wavelength reaches the detector.

PrimeQ filter cartridge design incorporates both excitation and emission filters together with a dichroic mirror for accurate fluorophore excitation and emission detection. The filter cartridges available for PrimeQ cover the spectrum of popular fluorescent dyes (**Table 1**).

Filter cartridge	Excitation λ (nm)	Emission λ (nm)	Suitable dyes
FC01	460	500	FAM™ multiplex, SYBR® Green I, Fluorescein, EvaGreen®
FC02	485	520	FAM™, SYBR® Green I, Fluorescein, EvaGreen®
FC03	530	560	HEX™, TET™, JOE™, VIC®, Yakima Yellow®
FC04	580	615	ROX™, TEXAS RED®, Cy®3.5
FC05	640	685	Cy®5, Quasar® 670

Table 1: *PrimeQ filter cartridges for the most commonly used dyes.*

FC02, FC03, FC04 and FC05 are supplied as standard with PrimeQ and are optimised for use with FAM™, HEX™, ROX™ and Cy®5. For multiplexing FAM™ with alternative dyes which are detected with the FC03 filter, we recommend the use of FC01 which has lower excitation and emission wavelengths and a slightly wider bandwidth.

Crosstalk

Crosstalk can occur when the emission of one fluorophore is detected through the filter combination of another fluorophore. Using specifically designed filters minimises or eliminates this effect and removes the requirement for complex colour compensation algorithms. To demonstrate the specificity of PrimeQ filters, a number of oligonucleotides were synthesised and labelled with various fluorescent dyes and HPLC purified (**Table 2**). Each dye-labelled oligo was diluted to the same final concentration and read with each of the five filters.

Fluorophore	Excitation λ (nm)	Emission λ (nm)
FAM™	495	520
JOE™	529	555
TET™	521	536
Yakima Yellow®	531	549
HEX™	535	556
Cal Fluor® Orange 560	538	559
Cy®3	555	565
TAMRA™	557	583
ROX™	586	610
Cy®3.5	581	596
TEXAS RED®	589	615
Cal Fluor® Red 635	618	637
Cy®5	646	662

Table 2*: Fluorophores tested with the PrimeQ filters. The excitation and emission wavelengths are given.*

Figure 1 demonstrates the range and specificity of PrimeQ filters. The overlap between certain filters is clear. This illustrates that correct pairing of filter with fluorophore avoids the issue of crosstalk.

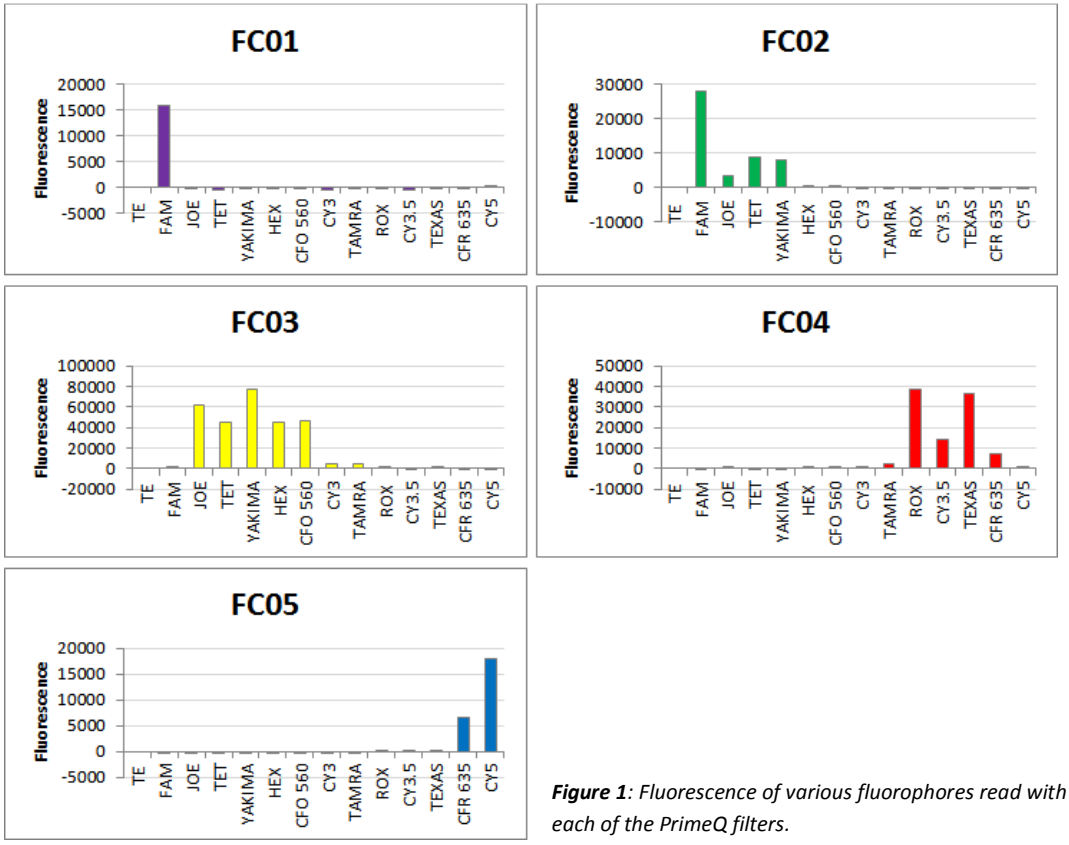

***Figure 1**: Fluorescence of various fluorophores read with each of the PrimeQ filters.*

Conclusions

Using a combination of excitation and emission filters to eliminate crosstalk between dyes provides confidence in knowing that a signal has been generated by a specific probe without contribution from any other possible reactions in the same well. In addition, the fluorescent data does not have to be manipulated in any way in order to subtract any contribution to the signal by other dyes. This means that the results obtained are exactly what the detector sees i.e. the raw data, whether performing multiplex or single dye experiments.

The fluorescent dyes FAM™, HEX™, ROX™ and Cy®5 show no cross talk when read using PrimeQ filters FC02, FC03, FC04 and FC05, therefore we would recommend using a combination of these four dyes when designing probes for a multiplex assay. Other available dyes are also suitable, as shown in **Table 1**. However if the excitation or emission maxima of the chosen dye are shifted significantly to shorter or longer wavelengths then some cross talk may occur. Use of the FC01 filter may be advantageous in these applications where FC03 is used to read probes labelled with TET™, JOE™, VIC® or Yakima Yellow®. An advantage of the PrimeQ filter cartridge system is that any custom filter cartridge can be designed, thus allowing flexibility and the capability to use many current and future dyes.