



Multiplexing Recommendations¹ for Dual-Labeled Dark-Quenched Probes and Primers

Instrument	Company	Calibration Required? ²	Multiplexing Degree	Dye 1	Dye 2	Dye 3	Dye 4	Dye 5
ABI PRISM 7700	Applied Biosystems	Yes	Duplex	FAM	CAL Fluor Gold 540	SuperROX ³		
ABI PRISM 7900	Applied Biosystems	Yes	Duplex	FAM	CAL Fluor Gold 540	SuperROX ³		
ABI PRISM 7000	Applied Biosystems	Yes	Duplex	FAM	CAL Fluor Gold 540	SuperROX ³		
ABI PRISM 7300	Applied Biosystems	Yes	Duplex	FAM	CAL Fluor Orange 560	SuperROX ³		
ABI PRISM 7500	Applied Biosystems	Yes	Quadraplex	FAM	CAL Fluor Gold 540	TAMRA	SuperROX ³	Quasar 670
iQ	Bio-Rad	Yes	Quadraplex	FAM	CAL Fluor Orange 560	CAL Fluor Red 610	Quasar 670	
iQ 5	Bio-Rad	Yes	Pentaplex	FAM	CAL Fluor Gold 540	CAL Fluor Red 590	CAL Fluor Red 610	Quasar 670
SmartCycler	Cepheid	Yes	Triplex	FAM	CAL Fluor Orange 560	CAL Fluor Red 635		
SmartCycler II	Cepheid	Yes	Quadraplex	FAM	CAL Fluor Orange 560	CAL Fluor Red 610	Quasar 670	
Rotor-Gene 3000	Corbett Life Science	No	Quadraplex	FAM	CAL Fluor Orange 560	CAL Fluor Red 610	Quasar 670	
Rotor-Gene 6000 (5 channel)	Corbett Life Science	No	Pentaplex	FAM	CAL Fluor Gold 540	CAL Fluor Red 610	Quasar 670	Quasar 705
Mastercycler ep <i>realplex</i>	Eppendorf	Yes	Duplex	FAM	CAL Fluor Gold 540			
Opticon 2	MJ Research/Bio-Rad	Yes	Duplex	FAM	CAL Fluor Orange 560			
Chromo 4	MJ Research/Bio-Rad	Yes	Quadraplex	FAM	CAL Fluor Orange 560	CAL Fluor Red 610	Quasar 670	
Mx3000P	Stratagene	No	Quadraplex	FAM	CAL Fluor Orange 560	CAL Fluor Red 610	Quasar 670	
Mx4000	Stratagene	No	Quadraplex	FAM	CAL Fluor Orange 560	CAL Fluor Red 610	Quasar 670	
LightCycler 1.2	Roche	Yes ⁴	Duplex	FAM	Pulsar 650			
LightCycler 2.0	Roche	Yes ⁴	Triplex	FAM	CAL Fluor Red 610	Pulsar 650		
LightCycler 480	Roche	Yes ⁴	Quadraplex	FAM	CAL Fluor Orange 560	CAL Fluor Red 610	Quasar 670	

BioCat GmbH
 Technologiepark
 Im Neuenheimer Feld 584
 D-69120 Heidelberg

Tel.: +49 (0) 6221 71415 16
 Fax: +49 (0) 6221 71415 29
 E-Mail: info@biocat.com

www.biocat.com

¹ These recommendations apply to dual-labeled BHQ-quenched probes (TaqMan Probes, Molecular Beacons) and BHQ-quenched primers (Black Hole Scorpions, Amplifluor Direct, Plexor, etc). They should not be used as guidelines for TAMRA-quenched probes or for Hybridization Probes that rely on FRET as a means of excitation. Recommendations highlighted in blue have not been proven and so should be used cautiously on an experimental basis. Stratagene customers select their filters from among a series upon instrument purchase. Because multiplexing dye compatibility is affected by filter specifications, the above Stratagene recommendations only apply to the standard FAM/HEX/ROX/Cy5 filter set.

² Some instruments will require fluorescence calibration. Fluorescence calibration allows these instruments to record the spectral profile for the dye(s) to be used in subsequent assays by resolving the total detected light into signals contributed by the individual fluorophores.

³ SuperRox is a proprietary passive reference available from Biosearch.

⁴ LightCycler users can calibrate using TaqMan probes directly and therefore aren't required to purchase dye calibration kits to perform color compensation.

- Determined through research at Biosearch
- Determined through research of a collaborator
- Predicted, based on instrument specifications