BLACK HOLE QUENCHER® AND DYE SELECTION CHART

FLUOROPHORE		DYE-5'-T_{10} EX	DYE-5'-T_{10} EM	BHQ Dye*
Biosearch Blue™		352	447	BHQ® - 0 (λ_{MAX} 495 nm, QR=430-520 nm)
FAM		495	520	
TET		521	536	
CAL Fluor® Gold 540	(VIC/TET/JOE REPLACEMENT)	522	544	
JOE		529	555	
VIC		538	554	
HEX		535	556	BHQ - 1 (λ_{MAX} 534 nm, QR=480-580 nm)
CAL Fluor Orange 560	(VIC/HEX/JOE REPLACEMENT)	538	559	
Quasar® 570	(CY3 REPLACEMENT)	548	566	
Cy™ 3		549	566	
NED		546	575	
TAMRA		557	583	
CAL Fluor Red 590	(TAMRA REPLACEMENT)	569	591	
Cy3.5		581	596	
ROX		586	610	
CAL Fluor Red 610	(TEXAS RED/ROX REPLACEMENT)	590	610	BHQ - 2 (λ_{MAX} 579 nm, QR=559-670 nm)
Texas Red®		597	616	*BHQ-2 dye is recommended for Pulsar 650, Quasar 670, and Quasar 705 dyes due to static quenching.*
CAL Fluor Red 635	(LC RED 640® REPLACEMENT)	618	637	
Pulsar® 650		460	650	
Cy 5		646	669	
Quasar 670	(CY5 REPLACEMENT)	647	670	
Cy 5.5		675	694	
Quasar 705	(CY5.5 REPLACEMENT)	690	705	BHQ - 3 (λ_{MAX} 672 nm, QR=620-730 nm)

Indicates Biosearch Technologies' proprietary dyes. Dyes in **BOLDFACE** are standard products available from Biosearch. These and the BHQ dyes are available in one or more of the following forms: labeled oligos, phosphoramidites, CPGs, pre-packaged DNA synthesis columns, carboxylic acids, peptide synthesis resins, succinimidyl esters, and amine labels.

*QR (Quenching Range) stands for each BHQ dye's FRET quenching range. Fluorophore dyes are shown for informational purposes only. Non-Biosearch fluorophores listed may be trademarked by companies other than Biosearch Technologies and may not necessarily be available from Biosearch, please visit www.biosearchtech.com for full disclosure.

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MULTIPLEXING RECOMMENDATIONS FOR DUAL-LABELED DARK-QUENCHED PROBES AND PRIMERS

INSTRUMENT	COMPANY	CALIBRATION REQUIRED?	MULTIPLEXING DEGREE	DYE 1	DYE 2	DYE 3	DYE 4	DYE 5
Prism® 7700	ABI	Yes	Duplex	FAM	CAL Fluor® Gold 540	SuperROX*®		
Prism 7900	ABI	Yes	Duplex	FAM	CAL Fluor Gold 540	SuperROX		
Prism 7000	ABI	Yes	Duplex	FAM	CAL Fluor Gold 540	SuperROX		
Prism 7300	ABI	Yes	Duplex	FAM	CAL Fluor Orange 560	SuperROX		
Prism 7500	ABI	Yes	4-Plex	FAM	CAL Fluor Orange 560	TAMRA	SuperROX	Quasar® 670
StepOne™	Life Technologies	Yes	Duplex	FAM	CAL Fluor Orange 560			
StepOnePlus™	Life Technologies	Yes	Triplex	FAM	CAL Fluor Orange 560	TAMRA		
CFX96™	Bio-Rad	Yes	5-Plex	FAM	CAL Fluor Gold 540	CAL Fluor Red 610	Quasar 670	Quasar 705
iCycler iQ®	Bio-Rad Laboratories	Yes	4-Plex	FAM	CAL Fluor Orange 560	CAL Fluor Red 610	Quasar 670	
iQ™5	Bio-Rad Laboratories	Yes	5-Plex	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	4-Plex	FAM	CAL Fluor Orange 560	CAL Fluor Red 610	Quasar 670	
Rotor-Gene™ Q 2-plex	Qiagen	No	Duplex	FAM	CAL Fluor Orange 560			
Rotor-Gene Q 5-plex	Qiagen	No	5-Plex	FAM	CAL Fluor Orange 560	CAL Fluor Red 610	Quasar 670	Quasar 705
Mastercycler® ep Realplex	Eppendorf	Yes	Duplex	FAM	CAL Fluor Gold 540			
Opticon® 2	Bio-Rad Laboratories	Yes	Duplex	FAM	CAL Fluor Orange 560			
Chromo™ 4	Bio-Rad Laboratories	Yes	4-Plex	FAM	CAL Fluor Orange 560	CAL Fluor Red 610	Quasar 670	
MX3000P™	Stratagene	No	4-Plex	FAM	CAL Fluor Orange 560	CAL Fluor Red 610	Quasar 670	
MX4000®	Stratagene	No	4-Plex	FAM	CAL Fluor Orange 560	CAL Fluor Red 610	Quasar 670	
LightCycler® 1.2	Roche	Yes	Duplex	FAM	Pulsar® 650			
LightCycler 2.0	Roche	Yes	Triplex	FAM	CAL Fluor Red 610	Pulsar 650		
LightCycler 480	Roche	Yes	4-Plex	FAM	CAL Fluor Orange 560	CAL Fluor Red 610	Quasar 670	

These recommendations apply to dual-labeled fluorescence-quenched probes. They should not be used as guidelines for TAMRA-quenched probes or for Hybridization Probes that rely on FRET as a means of excitation.

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. Please visit our calibration dye webpage for additional information.

Recommendations highlighted in yellow have not been proven and should be used cautiously on an experimental basis.

*SuperRox dye is a proprietary passive reference available from Biosearch Technologies.

Determined through research at Biosearch

Determined through research of a collaborator

Predicted based on instrument specifications

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