

Materials List for:

Wild-type Blocking PCR Combined with Direct Sequencing as a Highly Sensitive Method for Detection of Low-Frequency Somatic Mutations

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Materials

Name	Company	Catalog Number	Comments
1.5 or 2 mL Safe-Lock microcentrifuge tubes	Eppendorf	05-402-25	
100% alcohol	VWR	89370-084	Histology grade; 91.5% Ethanol, 5% Isopropyl alcohol, 4.5% Methyl alcohol
3730XL sequencer	ABI		or equivalent
Agencourt AMPure XP	Beckman Coulter	A63881	For magnetic bead PCR purification
Aluminum sealing foils	GeneMate	T-2451-1	For PCR and cold storage
BigDye Terminator v3.1 Cycle sequencing kit	Life Technologies	4337455	For bi-directional sequencing. With 5x Sequencing Buffer
Centrifuge 5804 Series	Eppendorf		A-2-DWP rotor (for PCR plate)
Cold plate for 96 well plates	Eppendorf	Z606634	
DNase, RNase-free, ultra-pure water			
dNTPs (100 mM)	Invitrogen	10297-117	
DynaMag-96 Side-Skirted Magnet	Thermo Fisher Scientific	12027	For use in PCR Purification.
Ethanol Absolute	Sigma	E7023	200 proof, for molecular biology
Exiqon website Oligo Tools			www.exiqon.com/oligo-tools
FastStart Taq DNA polymerase (5 U/ μ L)	Roche	12032937001	With 10x concentrated PCR reaction buffer, with 20 mM MgCl ₂
Gel electrophoresis apparatus			2% agarose gel
GeneRead DNA FFPE extraction Kit	Qiagen	180134	Contains uracil DNA glycosylase necessary for reducing sequencing artifacts
Hi-Di Formamide	ABI	4311320	For sequencing.
LNA oligonucleotide	Exiqon	500100	5'-TCAGA+AG+C+G+A+C+T+G+A+T+CC/invdT/ (+N = LNA bases)
M13-F Sequencing Primer	ABI		5'-tgt aaa acg acg gcc agt
M13-R Sequencing Primer	ABI		5'-cag gaa aca gct atg acc
Mastercycler Pro S Thermocycler	Eppendorf	E950030020	
Microcentrifuge Model 5430	Eppendorf		FA-45-30-11 rotor (for 1.5/2 mL microcentrifuge tubes)
NanoDrop 2000 Spectrophotometer	Thermo Fisher Scientific		
PCR forward primer	IDT		5'-tgt aaa acg acg gcc agt TGC CAG GGG TAC TTA GAT GG
PCR reverse primer	IDT		5'-cag gaa aca gct atg acc GGT TGG TGT AGT CGC AGA CA

PCR plates	GeneMate	T-3107-1	
Pipettors			20, 200, 1,000 μ L
Plate septa, 96 well	ABI	4315933	
QIAamp DNA Mini Kit	Qiagen	51304	For BM aspirate and peripheral blood
SeqScape Software v3.0	ABI	4474978	For sequencing analysis
Slide basket			
Sodium Acetate (3 M, pH 5.2)	Sigma	S7899	
Sterile filtered pipette tips			20, 200, 1,000 μ L
Thermomixer C	Eppendorf	5382000023	
Vortex genie	Scientific Industries	SI-0236	
Wash reservoir			~ 1,000 mL
Xylene	VWR	89370-088	Histology grade