Certificate of Analysis

GENEI

Oligonucleotides for genetic disorders

β-Thalassemia

Amplification Refractory System (ARMS) is a modified PCR method that allows the detection of known point - mutation by allele specific amplification of genomic DNA. ARMS is a rapid, non-radioactive method for screening large population of patients, carriers and normal individuals.

Cat No	ARMS PRIMER (5'-3')	Mutation detected	Amplified Product Size (bp)
* BG 1 IVS1-1 N	GAT GAA GTT GGT GGT GAG GCC CTG GGT AGG	IVS 1-Int.G - T (N)	450 bp
BG 2 IVS1-1 M	TTA AAC CTG TCT TGT AAC CTT GAT ACG AAA	IVS 1-Int G - T (M) Point mutation	281 bp
BG 3 IVS1-5 N	CTC CTT AAA CCT GTC TTG TAA CCT TGT TAC	IVS 1-5nt G - C (N)	285 bp
BG 4 IVS1-5 M	CTC CTT AAA CCT GTC TTG TAA CCT TGT TAG	IVS 1-5nt G - C (M) Point mutation	285 bp
BG 5 Cd 8/9 N	CCT TGC CCC ACA GGG CAG TAA CGG CAC ACT	Cd. 8/9 + G (N)	225 bp
BG 6 Cd 8/9 M	CCT TGC CCC ACA GGG CAG TAA CGG CAC ACC	Cd. 8/9 + G (M) Frame shift mutation	225 bp
BG 7 Cd 41/42 N	GAG TGG ACA GAT CCC CAA AGG ACT CAA AGA	Cd. 41/42 (—TCTT) (N)	439 bp
BG 8 Cd 41/42 M	GAG TGG ACA GAT CCC CAA AGG ACT CAA CCT	Cd. 41/42 (—TCTT) (M) deletion	439 bp
BG 9 HbE N	TAA CCT TGA TAC CAA CCT GCC CAG GGC GTC	HbE Cd. 26 G - A (N)	236 bp
BG 10 Hbe M	TAA CCT TGA TAC CAA CCT GCC CAG GGC GTT	HbE Cd. 26 G - A (M) Point mutation	236 bp

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Cat No	ARMS PRIMER (5'-3')	Mutation detected	Amplified Product Size (bp)
BG 11 Int. C1	CAA TGT ATC ATG CCT CTT TGC ACC	Internal control primer	861 bp
BG 12 Int. C2	GAG TCA AGG CTG AGA GAT GCA GGA	Internal control primer	
BG 13 Rev. Pr.	ACC TCA CCC TGT GGA GCC AC	Common Reverse primer A	
* BG 14 Rev. Pri B	CCC CTT CCT ATG ACA TGA ACT TAA	Common Reverse primer B	

*FOR BG 1 IVS1-1 N ONLY

N = Normal nt = nucleotide
M = Mutation cd = codon

These Primers and Probe are synthesised and purified by PAGE. They are supplied as 0.2 OD (6.6 µgs) lyophilized. To reconstitute, dissolve in 20 µl sterile water, check concentraion by OD before use.

Reference: Old J.M. Varawalla N.V. Weatherall D.J. Lancet 1990, 336-834. PCR Protocol can be provided on request.

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PCR Protocol for genetic disorders

β-Thalassemia

Each tube should contain :

Human Genomic DNA	:	200 ng
Thalassemia Primers 4	:	20 picomoles of each
(a) Internal pimers	:	BG11 Int C1 + BG 12 Int C2 = 2
(b) Normal primer	:	BG 1, 3, 5, 7, 9 = 1
OR	OR	
Mutation Primer	:	BG 2, 4, 6, 8, 10 = 1
(c) Reverse Primer	:	BG 13 Rev. A or BG 14 Rev. B = 1
10X Reaction buffer	:	with $MgCl_2$
dNTP	:	200 µm of each
Taq Polymerase	:	1 unit / tube
Total reaction volume	:	25 µl

PCR conditions:

Hot start PCR	:	Genomic DNA + Primers
		96° → 1 min
		Add enzyme (Taq Polymerase) and dNTP
PCR program	:	94° → 1 min
`		68° → 1 min
		72° → 1 min 30 Sec
		25 cycles
		72° → 3 min
Electrophoresis	:	Agarose gel – 1%
		TBE or TAE
		100 bp ladder
Load on gel 12-15 µl		
Amplification profile	:	2 bands
Internal control	:	861 bp
Normal OR mutation	:	According to mutation
Ex. BG 9 HbE N	:	285 bp
BG 10 HbE M	:	285 bp

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Oligonucleotides for Pathogen determinations

Bacterial Pathogens

Helicobacter Pylori Primers/Probes

PCR is now routinely used to diagnose specific bacterial and viral pathogenic infections. These assays have exploited the existence of pathogen specific DNA sequences to amplify specific regions of the pathogen genome. The amplified product thus obtained (which can be visualised by agarose gel electrophoresis) helps in diagnosis of specific infections.

From among various alternate choices we have chosen one primer pair (and an internal oligo probe in some cases) for each pathogen ; the reference is given in each case. There are alternative choices (based on existing published literature) of primer pairs for the pathogens listed, as well as for other pathogens which might be of interest to the pathologists. These can be synthesised on request.

Cat. No.	Primer	Sequence (5' to 3')	Amplified Product Size (bp)
BG.15. HPP1	Primer:	TAA CAA ACC GAT AAT GGC GC	203
BG.16. HPP2	Primer:	CAT CTT GTT AGA GGG ATT GG	
BG.17. HPB3	Probe:	CGC TCT TTA GTT TTG GAG CG	

These Primers and Probe are synthesised and purified by PAGE. They are supplied as 0.2 OD (6.6 µgs) lyophilized.
To reconstitute, dissolve in 20 µl sterile water, check concentraion by OD before use.

Note: These oligos are for research purpose and invitro use only. These products are not tested for use in diagnosis or drug development nor are they suitable for administration to humans or animals.

Reference : J. L. Valentine, Diagnostic Molecular Microbiology , Principles and Applications, American Society for Microbiology 1993. pp 282 - 287. Copy of reference can be provided on request.

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Oligonucleotides for Pathogen determinations

Bacterial Pathogens

Mycobacterium tuberculosis primers:

PCR is now routinely used to diagnose specific bacterial and viral pathogenic infections. These assays have exploited the existence of pathogen specific DNA sequences to amplify specific regions of the pathogen genome. The amplified product thus obtained (which can be visualised by agarose gel electrophoresis) helps in diagnosis of specific infections.

From among various alternate choices we have chosen one primer pair (and an internal oligo probe in some cases) for each pathogen ; the reference is given in each case. There are alternative choices (based on existing published literature) of primer pairs for the pathogens listed, as well as for other pathogens which might be of interest to the pathologists. These can be synthesised on request.

Cat. No.	Primer Sequence (5' to 3')	Amplified Product Size (bp)
BG.18.MTP1	CCT GCG AGC GTA GGC GTC GG	123
BG.19.MTP2	CTC GTC CAG CGC CGC TTC GG	

These Primers are synthesised and purified by PAGE. They are supplied as 0.2 OD (6.6 µgs) lyophilized.
To reconstitute, dissolve in 20 µl sterile water, check concentraion by OD before use.

Note: These oligos are for research purpose and invitro use only. These products are not tested for use in diagnosis or drug development nor are they suitable for administration to humans or animals.

Reference: K.D. Eisenach etal, Diagnostic Molecular Microbiology , Principles and Applications, American Society for Microbiology 1993. pp 191-196. Copy of reference can be provided on request.

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Oligonucleotides for Pathogen determinations

Viral Pathogens

Human Immuno Deficiency Virus (Type 1) Primers / Probes:

PCR is now routinely used to diagnose specific bacterial and viral pathogenic infections. These assays have exploited the existence of pathogen specific DNA sequences to amplify specific regions of the pathogen genome. The amplified product thus obtained (which can be visualised by agarose gel electrophoresis) helps in diagnosis of specific infections.

From among various alternate choices we have chosen one primer pair (and an internal oligo probe in some cases) for each pathogen ; the reference is given in each case. There are alternative choices (based on existing published literature) of primer pairs for the pathogens listed, as well as for other pathogens which might be of interest to the pathologists. These can be synthesised on request.

Cat. No.	Primer Sequence (5' to 3')	Amplified Product Size(bp)
BG.20 HIV P1	Primer AGT TGG AGG ACA TCA AGC AGC CAT GCA AAT	142
BG.21 HIV P2	Primer TGC TAT GTC AGT TCC CCT TGG TTC TCT	
BG.22 HIV B3	Probe GAG ACC ATC AAT GAG GAA GCT GCA GAA TGG GAT	

These Primers and Probe are synthesised and purified by PAGE. They are supplied as 0.2 OD (6.6 µgs) lyophilized.
To reconstitute, dissolve in 20 µl sterile water, check concentraion by OD before use.

Note: These oligos are for research purpose and invitro use only. These products are not tested for use in diagnosis or drug development nor are they suitable for administration to humans or animals.

Reference: S. Kwok and J.J.S., Diagnostic Molecular Microbiology , Principles and Applications, American Society for Microbiology 1993. pp 309-315. Copy of reference can be provided on request.

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Oligonucleotides for Pathogen determinations

Viral Pathogens

Hepatitis B Virus Primers:

PCR is now routinely used to diagnose specific bacterial and viral pathogenic infections. These assays have exploited the existence of pathogen specific DNA sequences to amplify specific regions of the pathogen genome. The amplified product thus obtained (which can be visualised by agarose gel electrophoresis) helps in diagnosis of specific infections.

From among various alternate choices we have chosen one primer pair (and an internal oligo probe in some cases) for each pathogen ; the reference is given in each case. There are alternative choices (based on existing published literature) of primer pairs for the pathogens listed, as well as for other pathogens which might be of interest to the pathologists. These can be synthesised on request.

Cat. No.	Primer Sequence (5' to 3')	Amplified Product Size (bp)
BG.23.HBVP1	CAA GGT ATG TTG CCC GTT TG	259
BG.24.HBVP2	AAA GCC CTG CGA ACC ACT GA	

These Primers are synthesised and purified by PAGE. They are supplied as 0.2 OD (6.6 µgs) lyophilized.
To reconstitute, dissolve in 20 µl sterile water, check concentraion by OD before use.

Note: These oligos are for research purpose and invitro use only. These products are not tested for use in diagnosis or drug development nor are they suitable for administration to humans or animals.

Reference: O. Yokosuka et al, Diagnostic Molecular Microbiology , Principles and Applications, American Society for Microbiology 1993. pp 322-326. Copy of reference can be provided on request.