Single read sequencing -
Template DNA requirements & primers

The quality of the template is the main factor influencing read length:

- Template DNA must be free of EtOH, EDTA, RNA, salts, genomic DNA and proteins
- Please use distilled water for elution
- Plasmid DNA should mainly be present in covalently closed circular form (we recommend silica membrane-based spin column kits)
- PCR products need to appear as a single band in an agarose gel and have to be purified from reaction buffer, primers and nucleotides

Quantity: Plasmid amount required

The concentration of templates shouldn‘t be less than listed below.

Template DNA	Concentration
Plasmids*	> 200 ng/µl
PCR products*	
200 - 500 bp	> 20 ng/µl
500 - 1,000 bp	> 40 ng/µl
1,000 - 2,000 bp	> 100 ng/µl

Primer requirements

All universal primers listed below are available free of charge.
Custom primers can be designed and synthesized by AGOWA.
Primers supplied by the customer should meet the following requirements:

- Length of 18 - 24 bases
- the melting temperature (4 x number of G, C + 2 x number of A, T) should amount to at least 55 °C
- GC-content should at least be 40 - 50 %, 3‘-end should be G or C
- No hybridisation with itself (dimer, primer dimer, loops) or binding to several sites at the template
- One product in the HPLC
- Deprotected
- Without modifications (fluorophore or others)
- Free of salts and other contaminations

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Primer	Sequence	Primer	Sequence
3-pFB	CGAACCCCAGAGTCCCGCTC	31-NT-GFP	CGACACAATCTGCCCTTTCG
35S-R	GGATTTTAGTACTGGATTTTGG	3AOX	GCAAATGGCATTCTGACATCC
5-Retro	GCTGCCGACCCCGGGGGTGG	5AOX	GACTGGTTCCAATTGACAAGC
96g3R	CTCTGTAGCCGTTGCTACC	Ac5-forw	GACACAAAGCCGCTCCATCAG
AcG2T-F	GATAAGTACTTGAAATCCAGC	AcG2T-R	GTCTGTAAATCAACAACGCAC
ACT2-R	CAAGATTGAAACTTAGAGGAG	ACYC184-R	GTTCTCGGAGCACTGTCCGAC
AR2-F	CAGAGCCAGTGGAAAGTTG	AS2-1-R	ACCTACAGGAAAGAGTTACTC
ASK-FN2	CGGCCTTTTTACGGTTCCTG	ASK-RN	TGGAGATCCGTGACGCAGTAG
BAD-HisA-F	GGTATGGCTAGCATGACTGG	BAD-HisA-R	GTTTTATCAGACCGCTTCTG
BAD24-F	CTCTACTGTTTCTCCATACC	BeloF	TTGTAAAACGACGGCCAGTG
BeloR	CTTGCATGCCTGCAGGTCGAC	BGH	TAGAAGGCACAGTCGAGGCT
BIN-AR	ACGCACAATCCCACTATC	BIN-M13-rev	TGGAATTGTGAGCGGATAAC
BluescriptR	GGAAACAGCTATGACCATG	CAM-Nco	GTGCCCATTAACATCACCATC
CAM-PmI	ACCGGCAACAGGATTCAATC	CAM-R	CACAATCCCACTATCCTTCG
CaMV	CCACGTCTTCAAAGCAAGTG	CMV-F	CGCAAATGGGCGGTAGGCGTG
CMV5-R	AGAAGGACACCTAGTCAGAC	DEST38-F	CTTGCAGACTAATTCAAGAG
DEST38-R	CCAAGAACGAGGGTTGGAAG	Donr-F	TCGCGTTAACGCTAGCATG
EGFP-C1-F	GAAGCGCGATCACATGGTC	EGFP-C1-R	AACCATTATAAGCTGCAATAAAC
EGFP-N1-F	GAGGTCTATATAAGCAGAGC	EGFP-N1-R	ACTTGTGGCCGTTTACGTC
ENTR-F	GTTAGTTAGTTACTTAAGCTCG	ENTR-Fn	TGCCAGGCATCAAACTAAGC
ENTR-R	CCAGAGCTGCAGCTGGATG	ESP3-F	GGCATATCATCAATTGAATAAG
ESP3-R	TCCAAAAGAAGTCGAGTGGG	F1-oriR	CAGAATAGAATGACACCTAC
Gal10-F	GTGGTAATGCCATGTAATATG	Gal10-R	CAAGGTAGACAAGCCGACAAC
Gal1forw	TATACCTCTATACTTTAACGTC	Gal4-BD	GCCTCTAACATTGAGACAGC
Gal4rev	GTGATGGTGCACGATGCACAG	GEX-F	CTTTGCAGGGCTGGCAAG
GEX-R	GAGCTGCATGTGTCAGAGG	GEXnew	CACAAATTGATAAGTACTTG
GFP-S65T	GTATGTTGCATCACCTTCAC	GL2	CTTTATGTTTTTGGCGTCTTCC
GL3	TTTGTATTCAGCCCATATCG	GL3pr3R	CGGAGAATGGGCGGAACTG
GST-end	ACCCACTCGACTTCTTTTGG	GUS-R	CCAACGCTGATCAATTCCACAG
hGFP-R	TCCCATTCATCAGTTCCATAG	HLT-F	ATAGTTGCTGATATCATGGAG
HLT-R	GGACCAGTGAACAGAGGTGC	Intein-R	ACCCATGACCTTATTACCAAC
IREShygR	GACAAACACACACCGGCCTT	IREShygRn	CAGACCTTGCATTCCTTTGG
JF-down	CTGATTTAATCTGTATCAGGCTG	JF-up	GAGCTGTTGACAATTAATCATCG
JF119EH-do	TTCTGATTTAATCTGTATCAGGC	JF119EH-up	TGACAATTAATCATCGGCTCG
KS	CTCGAGGTCGACGGTATC	KSI	GGCAAGGTGGTGAGCATC
lacZ93	CGCCAGCTGGCGAAAGGG	Laf-F	GTTGTAAAACGACGGCCAG
LEXA-F	CGTCAGCAGAGCTTCACCATT	LEXA-R	GAAATTCGCCCGGAATTAGC
LXSN-F	GGTCAAGCCCTTTGTACACC	M13-24F-BLUE	GTAAAACGACGGCCAGTGAGCGCG
M13-24R-BLUE	AACAGCTATGACCATGATTACGCC	M13-F	CCAGGGTTTTCCCAGTCACG
M13-R	CGGATAACAATTTCACACAGG	M13rev2	GAGTTAGCTCACTCATTAGG
MACS-F	CTCGAGGAACTGAAAAACCAG	MKSF	CCAGGCTTTACACTTTATGCTTCC
Myr3	CGTGAATGTAAGCGTGACAT	Myr5	ACTACTAGCAGCTGTAATAC
NONE	NONE	pAD-Gal4-AD	GTTTGGAATCACTACAGGGATG
pBAD33-R	CAAATTCTGTTTTATCAGACC	pBCF	GCAGGAATTCGATATCAAGC
pBCR	ATTAACCCTCACTAAAGGGAAC	pBR-B1	CATGAGCCCGAAGTGGCGAG
pBR-F	AGTGCCACCTGACGTCTAAG	pBR-FN	GAAGCTAGAGTAAGTAGTTC
pBR-R	CCTATGCCTACAGCATCCAG	pBT-F	CCAGACTTGGGGGTGATGAG
pC11-F	CTAGAGAACCCACTGCTTAC	pCAT3basic-R	GTAACCTTGATACTTACCTGC
pCYC-F	TCAGACTAAACTGGCTGACG	pCYC-R	GGTTATTGTCTCATGAGCG
pDNR-LIB-F	GTGTAAAACGACGGCCAGTAG	pEBV-R	TTAAGTGCCTAGCTCGATAC
pEFmyccyto-F	TCTCAAGCCTCAGACAGT	pET-T7up	CGGTGATGTCGGCGATATAG
pETBlueDOWN	GTTAAATTGCTAACGCAGTCAG	pETBlueUP	GTCACGACGTTGTAAAACGAC
pFASTBacF	ATTAAAATGATAACCATCTCGC	pFASTBacR	TCAGGTTCAGGGGGAGGT
pGAD10-F	TACCACTACAATGGATGATG	pGADrev	GAAATTGAGATGGTGCACG
PICZ-F	TACTATTGCCAGCATTGCTGC	pIRES-AR	GAATTGGCCGCCCTAGATG

pIRES-BF	CTTTACATGTGTTTAGTCGAG	pIREShygF	GGTCTATATAAGCAGAGCTC
pIRESneoR	ACAATCTTAGCGCAGAAGTC	pJET-fw	GCCTGAACACCATATCCATCC
pJG45-F	CTCCTACCCTTATGATGTGC	pKS-Fup	TGCGCAACTGTTGGGAAGG
pLVTHM-FN	ATGGGATCAATTCACCATGC	pMAL-R	CCGCAGATGTCCGCTTTCTG
pME18s	CGGATCCGGTGGTGCAAATC	pQE-F	CGGATAACAATTTCACACAG
pQE-Fup	ACGAGGCCCTTTCGTCTTC	pQE-R	GTTCTGAGGTCATTACTGG
pQETri-F	GGTTATTGTGCTGTCTCATC	pQETri-R	TCGATCTCAGTGGTATTTGTGA
pRevTRE2-R	TGCCTTGCAAAATGGCGTTAC	pSELECT-F	TCTCCACGCTTTGCCTGACC
pShuttle-F	GAATAAGAGGAAGTGAAATCTG	pShuttle-R	AGATACAAAACTACATAAGACC
pSuper-retro-puro-P	CGAACGCTGACGTCATC	pT-AdvF	GTAAAACGACGGCCAGT
pT-AdvR	AACAGCTATGACCATG	Ptac	TCAGGCAGCCATCGGAAGCTG
pTARGET	CGCCAAGTTATTTAGGTGAC	pTRE2pur-R	CATGGTGATACAAGGGACATC
pUCF	GCCAGTGAATTCGAGCTCGG	pUCR	TGCCTGCAGGTCGACTCTAG
pUCT7-Rn	TTCAGGCTGCGCAACTGTTG	pVP22-F	CGTGGTGCAGGACGTCGAC
pVP22-Rn	CAACTAGAAGGCACAGTCGAGG	pVP22-Rn2	GCATATTCAGATCCTCTTCTG
RmalG	ATAACATAACTTGAGGGCAG	RVpr3	CTAGCAAAATAGGCTGTCCC
RVpr4	GACGATAGTCATGCCCCGCG	S-Tag18	AGCGACTAGTGGTTCTGGTC
SBC1F	CCTCGAGGAACTGGAAAACC	SBC1R	AAGGGAGTATAAAACAGGCG
sCOS1F	CAAATAGGGGTTCCGCGCAC	sCOS1R	GACAGGTGCTGAAAGCGAGC
SE380-F	CGGTTCTGGCAAATATTCTG	SE380-R	CTAGAACTATAGCTAGCATGC
SeqL-E	GTTGAATATGGCTCATAACAC	SESP1-R	TGCAGCTTGAATGGGCTTCC
SG5-F	TCCTACAGCTCCTGGGCAAC	Sil-for	AGGAAACTCACCCTAACTG
SK	CGCTCTAGAACTAGTGGATC	SL1180-P	GCAGCTGGCGCCATCGATAC
SOS3	GCCAGGGTTTTCCCAGT	SOS5	CCAAGACCAGGTACCATG
Sp6-20	CTATTTAGGTGACACTATAG	SP6-CS2	CTTGATTTAGGTGACACTATAG
SP72-F	TGAGAGTGCACCATATGGAC	SP72-R	AGCGGAAGAGCGCCCAATAC
SV40	CACTGCATTCTAGTTGTGGTT	SV40-Do	CAGAAGTAGTGAGGAGGC
SVL-F	TTTGAGGCTCCTGGTGGTGC	T3	AATTAACCCTCACTAAAGGG
T3cos1	GCAATTAACCCTCACTAAAG	T7-CS2	TAATACGACTCACTATAGTTC
T7cos1	GCATAATACGACTCACTATAGG	T7do	CCTCTAGATGCATGCTCGAG
T7prom	TAATACGACTCACTATAGGG	T7term	GCTAGTTATTGCTCAGCGG
Tal-Luc	CGGGAGGTACTTGGAGCG	TECH2	AAAATCCTGGGTTGCGACTGG
Topo-1	TCGGATCCACTAGTAACG	Topo-2	GTGTGATGGATATCTGC
TrcHis-F	GAGGTATATATTAATGTATCG	TrcHis-R	GATTTAATCTGTATCAGG
TRE-F	CTCGTTTAGTGAACCGTCAG	TRE-R	TACAAATAAAGCAATAGCATCAC
TriplEx-R	CTCGGGAAGCGCGCCATTGTGTTGGT	uni1	CAGTCGAGGCTGATAGCGAGCT
uniTOPO-F	ACTATCAACAGGTTGAACTGC	UniV5his-R	GGCACGGGGGAGGGGCAAAC
V5C-termR	GAGGAGAGGGTTAGGGATAG	VL1392-F	TCCGGATTATTCATACCGTC
VL1392-R	CAAGTTTCCCTGTAGAACTC	VP16-AD	CTACGGCGCTCTGGATATG
VP16-BD	GGATATAAAAGCATTGTTAACAGG	YEP24-F	CCCAGTCCTGCTCGCTTCGCT
YEP24-R	GTCGGCGATATAGGCGCCAGC	YES-F	ATTGTTAATATACCTCTATAC
YES2-R	GCGTGAATGTAAGCGTGAC	Zome1N-F	GATATACCTACAACTGCTTC

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