



# Single read sequencing - Template DNA requirements & primers

## Template DNA requirements:

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  
(Recommendation: 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 (alternatively enzymatic PCR purification can be ordered from LGC Genomics that removes primers and nucleotides)
- DNA samples (and custom primers) are stored for 3 months

**Amount** of DNA template required for one sequencing run:

PCR sequencing	Per sequencing reaction
PCR products up to 2 kb (for example)	20 ng per 100 bp
100 bp	20 ng
1,000 bp	200 ng
upto 2,000 bp	500 ng

Plasmid sequencing	Per sequencing reaction
Plasmids (< 10 kb)	400 ng
Plasmids (> 10 kb)	600 ng
Cosmids, BACs	1,000 ng

The **concentration** of DNA template needed for sequencing:

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

## Please send your samples

via courier service to:

LGC Genomics GmbH  
Ostendstr. 25 / TGS Haus 8  
12459 Berlin, Germany

or via post to:

LGC Genomics GmbH  
P.O. Box 940327  
12443 Berlin, Germany

For any question, please contact the DNA sequencing team on Tel: +49 (0)30 5304 2233.

## Primer requirements:

All universal primers listed below are available free of charge.

Custom primers can be designed and synthesised by LGC Genomics.

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 52 °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

Primer	Sequence	Primer	Sequence
3-pFB	CGAACCCAGAGTCCCCTC	31-NT-GFP	CGACACAATCTGCCCTTTCG
35S-R	GGATTTAGTACTGGATTTGG	3AOX	GCAAAATGGCATTCTGACATCC
5-Retro	GCTGCCGACCCCGGGGTGG	5AOX	GACTGGTTCCAATTGACAAGC
96g3R	CTCTGTAGCCGTGCTACC	Ac5-forw	GACACAAAGCCGCTCCATCAG
AcG2T-F	GATAAGTACTTGAATCCAGC	AcG2T-R	GTCTGTAATCAACAACGCAC
ACT2-R	CAAGATTGAACTTAGAGGAG	ACYC184-R	GTTCTCGGAGCACTGTCCGAC
AR2-F	CAGAGCCAGTGGAAAGTTG	AS2-1-R	ACCTACAGGAAAGAGTTACTC
ASK-FN2	CGGCCTTTTACGGTTCCTG	ASK-RN	TGGAGATCCGTGACGCAGTAG
BAD-HisA-F	GGTATGGCTAGCATGACTGG	BAD-HisA-R	GTTTTATCAGACCGCTTCTG
BAD24-F	CTCTACTGTTTCTCCATACC	BeloF	TTGTAAACGACGGCCAGTG
BeloR	CTTGCATGCCTGCAGGTCGAC	BGH	TAGAAGGCACAGTCGAGGCT
BIN-AR	ACGCACAATCCCACTATC	BIN-M13-rev	TGGAATTGTGAGCGGATAAC
BluescriptR	GGAAACAGCTATGACCATG	CAM-Nco	GTGCCATTAAACATCACCATC
CAM-Pml	ACCGGCAACAGGATTCAATC	CAM-R	CACAATCCCACTATCCTTCG
CaMV	CCACGTCTTCAAGCAAGTG	CMV-F	CGCAAATGGGCGGTAGGCGTG
CMV5-R	AGAAGGACACCTAGTCAGAC	DEST38-F	CTTGCAGACTAATTCAAGAG
DEST38-R	CCAAGAACGAGGGTTGGAAG	Donr-F	TCGGGTTAACGCTAGCATG
EGFP-C1-F	GAAGCGCGATCACATGGTC	EGFP-C1-R	AACCATTATAAGCTGCAATAAAC
EGFP-N1-F	GAGGTCTATATAAGCAGAGC	EGFP-N1-R	ACTTGTGGCCGTTTACGTC
ENTR-F	GTTAGTTAGTTACTTAAGCTCG	ENTR-Fn	TGCCAGGCATCAAATAAGC
ENTR-R	CCAGAGCTGCAGCTGGATG	ESP3-F	GGCATATCATCAATTGAATAAG
ESP3-R	TCCAAAAGAAGTCGAGTGGG	F1-oriR	CAGAATAGAATGACACCTAC
Gal10-F	GTGGTAATGCCATGTAATATG	Gal10-R	CAAGGTAGACAAGCCGACAAAC
Gal1forw	TATACCTCTATACTTAAACGTC	Gal4-BD	GCCTCTAACATTGAGACAGC
Gal4rev	GTGATGGTGCACGATGCACAG	GEX-F	CTTTCAGGGCTGGCAAG
GEX-R	GAGCTGCATGTGTCAGAGG	GEXnew	CACAAATTGATAAGTACTTG
GFP-S65T	GTATGTTGCATCACCTTCAC	GL2	CTTTATGTTTTGGCGTCTTCC
GL3	TTTGTATTAGCCCATATCG	GL3pr3R	CGGAGAATGGGCGGAACTG
GST-end	ACCCACTCGACTTCTTTTGG	GUS-R	CCAACGCTGATCAATTCCACAG
hGFP-R	TCCCATTATCAGTTCCATAG	HLT-F	ATAGTTGCTGATATCATGGAG
HLT-R	GGACCAGTGAACAGAGGTGC	Intein-R	ACCCATGACCTTATTACCAAC
IREShygR	GACAAACACACACCGGCCCTT	IREShygRn	CAGACCTTGCATTCTTTTGG
JF-down	CTGATTTAATCTGTATCAGGCTG	JF-up	GAGCTGTTGACAATTAATCATCG
JF119EH-do	TTCTGATTTAATCTGTATCAGGC	JF119EH-up	TGACAATTAATCATCGGCTCG
KS	CTCGAGGTGACGGTATC	KSI	GGCAAGGTGGTGAGCATC
lacZ93	CGCCAGCTGGCGAAAGGG	Laf-F	GTTGTAACGACGGCCAG
LEXA-F	CGTCAGCAGAGCTTACCATT	LEXA-R	GAAATTCGCCGGAATTAGC
LXSN-F	GGTCAAGCCCTTTGTACACC	M13-24F-BLUE	GTAACGACGGCCAGTGAGCGCG
M13-24R-BLUE	AACAGCTATGACCATGATTACGCC	M13-F	CCAGGGTTTTCCAGTCACG
M13-R	CGGATAACAATTTACACAGG	M13rev2	GAGTTAGCTCACTCATTAGG
MACS-F	CTCGAGGAAGTGAACCAACAG	MKSF	CCAGGCTTTACACTTTATGCTTCC
Myr3	CGTGAATGTAAGCGTGACAT	Myr5	ACTACTAGCAGCTGTAATAC
NONE	NONE	pAD-Gal4-AD	GTTTGGAACTACTACAGGGATG
pBAD33-R	CAAATCTGTTTTATCAGACC	pBCF	GCAGGAATTCGATATCAAGC

Primer	Sequence	Primer	Sequence
pBCR	ATTAACCCCTCACTAAAGGGAAC	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	GTGTAACACGACGGCCAGTAG	pEBV-R	TTAAGTGCCTAGCTCGATAC
pEFmyccto-F	TCTCAAGCCTCAGACAGT	pET-T7up	CGGTGATGTGGCGATATAG
pETBlueDOWN	GTTAAATTGCTAACGCAGTCAG	pETBlueUP	GTCACGACGTTGTAACACGAC
pFASTBacF	ATTAATAATGATAACCATCTCGC	pFASTBacR	TCAGGTTCCAGGGGGAGGT
pGAD10-F	TACCACTACAATGGATGATG	pGADrev	GAAATTGAGATGGTGACG
PICZ-F	TACTATTGCCAGCATTGCTGC	pIRES-AR	GAATTGGCCGCCCTAGATG
pIRES-BF	CTTACATGTGTTTAGTCGAG	pIREShygF	GGTCTATATAAGCAGAGCTC
pIRESneoR	ACAATCTTAGCGCAGAAGTC	pJET-fw	GCCTGAACACCATATCCATCC
pJG45-F	CTCCTACCCCTTATGATGTGC	pKS-Fup	TGCGCAACTGTTGGGAAGG
pLVTHM-FN	ATGGGATCAATTCACCATGC	pMAL-R	CCGCAGATGTCCGCTTCTG
pME18s	CGGATCCGGTGGTGCAAAATC	pQE-F	CGGATAACAATTCACACAG
pQE-Fup	ACGAGGCCCTTTTCGTCTTC	pQE-R	GTTCTGAGGTCATTACTGG
pQETri-F	GGTTATTGTGCTGTCTCATC	pQETri-R	TCGATCTCAGTGGTATTTGTGA
pRevTRE2-R	TGCCTTGCAAAATGGCGTTAC	pSELECT-F	TCTCCACGCTTTGCCGACC
pShuttle-F	GAATAAGAGGAAGTGAATCTG	pShuttle-R	AGATACAAAACACATAAGACC
pSuper-retro-puro-P	CGAACGCTGACGTCATC	pT-AdvF	GTAAAACGACGGCCAGT
pT-AdvR	AACAGCTATGACCATG	Ptac	TCAGGCAGCCATCGGAAGCTG
pTARGET	CGCCAAGTTATTTAGGTGAC	pTRE2pur-R	CATGGTGATACAAGGGACATC
pUCF	GCCAGTGAATTCGAGCTCGG	pUCR	TGCTGCAGGTCGACTCTAG
pUCT7-Rn	TTCAGGCTGCGCAACTGTTG	pVP22-F	CGTGGTGACGACGTCGAC
pVP22-Rn	CAACTAGAAGGCACAGTCGAGG	pVP22-Rn2	GCATATTAGATCCTCTTCTG
RmalG	ATAACATAACTTGAGGGCAG	RVpr3	CTAGCAAAATAGGCTGTCCC
RVpr4	GACGATAGTCATGCCCGCG	S-Tag18	AGCGACTAGTGGTTCTGGTC
SBC1F	CCTCGAGGAACGGAAAACC	SBC1R	AAGGGAGTATAAAACAGGCG
sCOS1F	CAAATAGGGTTCCGCGCAC	sCOS1R	GACAGGTGCTGAAAGCGAGC
SE380-F	CGGTTCTGGCAAATATTCTG	SE380-R	CTAGAACTATAGCTAGCATGC
SeqL-E	GTTGAATATGGCTCATAACAC	SESP1-R	TGCAGCTTGAATGGGCTTCC
SG5-F	TCCTACAGCTCCTGGGCAAC	Sil-for	AGGAAACTCACCTAACTG
SK	CGCTCTAGAAGTAGTGGATC	SL1180-P	GCAGCTGGCGCCATCGATAC
SOS3	GCCAGGGTTTTCCAGT	SOS5	CCAAGACCAGGTACCATG
Sp6-20	CTATTTAGGTGACACTATAG	SP6-CS2	CTTGATTTAGGTGACACTATAG
SP72-F	TGAGAGTGACCCATATGGAC	SP72-R	AGCGGAAGAGCGCCAATAC
SV40	CACTGCATTCTAGTTGTGGTT	SV40-Do	CAGAAGTAGTGAGGAGGC
SVL-F	TTTGAGGCTCCTGGTGGTGC	T3	AATTAACCCCTCACTAAAGGG
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	CTCGGGAAGCGCGC- CATTGTGTTGGT	uni1	CAGTCGAGGCTGATAGCGAGCT
uniTOPO-F	ACTATCAACAGGTTGAACTGC	UniV5his-R	GGCACGGGGGAGGGGCAAAC
V5C-termR	GAGGAGAGGGTTAGGGATAG	VL1392-F	TCCGGATTATTCATACCGTC
VL1392-R	CAAGTTTCCCTGTAGAACTC	VP16-AD	CTACGGCGCTCTGGATATG
VP16-BD	GGATATAAAGCATTGTTAACAGG	YEP24-F	CCCAGTCTGCTCGCTTCGCT
YEP24-R	GTCGGCGATATAGGCGCCAGC	YES-F	ATTGTTAATATACCTCTATAC
YES2-R	GCGTGAATGTAAGCGTGAC	Zome1N-F	GATATACCTACAACGCTTC