



Primer walking projects - 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
- DNA samples (and custom primers) are stored for 3 months

Please find listed below the **amount** of DNA template required for one sequencing / walking step (read length 750 - 1,000 nt). Please provide enough DNA to cover the whole sequence by walking.

PCR sequencing	Per sequencing reaction
PCR products up to 2 kb (for example)	20 ng per 100 bp
1,000 bp	200 ng
up to 2,000 bp	500 ng
> 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 templates should not be less than listed below

Template DNA	Concentration
Plasmids	> 200 ng/μl
PCR products	
500 - 1,000 bp	> 40 ng/μl
1,000 - 2,000 bp	> 100 ng/μl
> 2,000 bp	> 200 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 LGC genomics 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	CGAACCCAGAGTCCCGCTC	31-NT-GFP	CGACACAATCTGCCCTTTCG
35S-R	GGATTTTAGTACTGGATTTTGG	3AOX	GCAATGGCATTCTGACATCC
5-Retro	GCTGCCGACCCCGGGGTGG	5AOX	GA CTGGTTCCAATTGACAAGC
96g3R	CTCTGTAGCCGTGCTACC	Ac5-forw	GACACAAAGCCGCTCCATCAG
AcG2T-F	GATAAGTACTTGAATCCAGC	AcG2T-R	GTCTGTAAATCAACAACGCAC
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	TTGTAAAACGACGGCCAGTG
BeloR	CTTGCATGCCCTGCAGGTCGAC	BGH	TAGAAGGCACAGTCGAGGCT
BIN-AR	ACGCACAATCCCACTATC	BIN-M13-rev	TGGAATTGTGAGCGGATAAC
BluescriptR	GGAAACAGCTATGACCATG	CAM-Nco	GTGCCATTAAACATCACCATC
CAM-Pml	ACCGGCAACAGGATTCAATC	CAM-R	CACAATCCCACTATCCTTCG
CaMV	CCACGTCTTCAAAGCAAGTG	CMV-F	CGCAAATGGGCGGTAGGCGTG
CMV5-R	AGAAGGACACCTAGTCAGAC	DEST38-F	CTTGCAGACTAATTCAAGAG
DEST38-R	CCAAGAACGAGGTTGGAAG	Donr-F	TCGCGTTAACGCTAGCATG
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	CAAGGTAGACAAGCCGACAAC
Gal1forw	TATACCTCTATACTTTAACGTC	Gal4-BD	GCCTCTAACATTGAGACAGC
Gal4rev	GTGATGGTGCAGATGCACAG	GEX-F	CTTTGCAGGGCTGGCAAG
GEX-R	GAGCTGCATGTGTCAGAGG	GEXnew	CACAAATTGATAAGTACTTG
GFP-S65T	GTATGTTGCATCACCTTCAC	GL2	CTTTATGTTTTTGGCGTCTTCC
GL3	TTTGTATTAGCCCATATCG	GL3pr3R	CGGAGAATGGGCGGAACTG
GST-end	ACCCACTCGACTTCTTTTGG	GUS-R	CCAACGCTGATCAATTCCACAG
hGFP-R	TCCCATTATCAGTTCATAG	HLT-F	ATAGTTGCTGATATCATGGAG
HLT-R	GGACCAGTGAACAGAGGTGC	Intein-R	ACCCATGACCTTATTACCAAC
IREShygR	GACAAACACACACCGGCCTT	IREShygRn	CAGACCTTGCATTCTTTGG
JF-down	CTGATTTAATCTGTATCAGGCTG	JF-up	GAGCTGTTGACAATTAATCATCG
JF119EH-do	TTCTGATTTAATCTGTATCAGGC	JF119EH-up	TGACAATTAATCATCGGCTCG
KS	CTCGAGGTGACCGTATC	KSI	GGCAAGGTGGTGAGCATC
lacZ93	CGCCAGCTGGCGAAAGGG	Laf-F	GTTGTAACGACGGCCAG
LEXA-F	CGTCAGCAGAGCTTACCATT	LEXA-R	GAAATTCGCCCCGAATTAGC
LXSN-F	GGTCAAGCCCTTTGTACACC	M13-24F-BLUE	GTAACGACGGCCAGTGAGCGCG
M13-24R-BLUE	AACAGCTATGACCATGATTACGCC	M13-F	CCAGGGTTTTCCAGTCAGC
M13-R	CGGATAACAATTTACACAGG	M13rev2	GAGTTAGCTCACTCATTAGG
MACS-F	CTCGAGGAACTGAAAAACCAG	MKSF	CCAGGCTTTACACTTTATGCTTCC
Myr3	CGTGAATGTAAGCGTGACAT	Myr5	ACTACTAGCAGCTGTAATAC
NONE	NONE	pAD-Gal4-AD	GTTTGGAACTACTACAGGGATG
pBAD33-R	CAAATTCTGTTTTATCAGACC	pBCF	GCAGGAATTCGATATCAAGC

Primer	Sequence	Primer	Sequence
pBCR	ATTAACCCTCACTAAAGGGAAAC	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
pEFmyccto-F	TCTCAAGCCTCAGACAGT	pET-T7up	CGGTGATGTCGGCGATATAG
pETBlueDOWN	GTAAATTGCTAACGCAATCAG	pETBlueUP	GTCACGACGTTGTAACGAC
pFASTBacF	ATTAATAATGATAACCATCTCGC	pFASTBacR	TCAGGTTCAAGGGGAGGT
pGAD10-F	TACCACTACAATGGATGATG	pGADrev	GAAATTGAGATGGTGACG
PICZ-F	TACTATTGCCAGCATTGCTGC	pIRES-AR	GAATTGGCCGCCCTAGATG
pIRES-BF	CTTTACATGTGTTTAGTCGAG	pIREShygF	GGTCTATATAAGCAGAGCTC
pIRESneoR	ACAATCTTAGCGCAGAAAGTC	pJET-fw	GCCTGAACACCATATCCATCC
pJG45-F	CTCCTACCCTTATGATGTGC	pKS-Fup	TGCGCAACTGTTGGGAAGG
pLVTHM-FN	ATGGGATCAATTCACCATGC	pMAL-R	CCGCAGATGTCGCCCTTCTG
pME18s	CGGATCCGGTGGTGCAAATC	pQE-F	CGGATAACAATTCACACAG
pQE-Fup	ACGAGGCCCTTTTCGTCTTC	pQE-R	GTTCTGAGGTCATTACTGG
pQETri-F	GGTTATTGTGCTGTCTCATC	pQETri-R	TCGATCTCAGTGGTATTTGTGA
pRevTRE2-R	TGCCCTTGCAAATGGCGTTAC	pSELECT-F	TCTCCACGCTTTGCCGTGACC
pShuttle-F	GAATAAGAGGAAGTGAATCTG	pShuttle-R	AGATACAAAACCTACATAAGACC
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	GACGATAGTCATGCCCGCG	S-Tag18	AGCGACTAGTGGTTCTGGTC
SBC1F	CCTCGAGGAACTGGAAAACC	SBC1R	AAGGGAGTATAAAAACAGGCG
sCOS1F	CAAATAGGGTTCGCGGCAC	sCOS1R	GACAGGTGCTGAAAGCGAGC
SE380-F	CGGTTCTGGCAAATATTCTG	SE380-R	CTAGAACTATAGCTAGCATGC
SeqL-E	GTTGAATATGGCTCATAACAC	SESP1-R	TGCAGCTTGAATGGGCTTCC
SG5-F	TCCTACAGCTCCTGGGCAAC	Sil-for	AGGAAACTCACCTAACTG
SK	CGCTCTAGAACTAGTGGATC	SL1180-P	GCAGCTGGCGCCATCGATAC
SOS3	GCCAGGGTTTTCCAGT	SOS5	CCAAGACCAGGTACCATG
Sp6-20	CTATTTAGGTGACTATAG	SP6-CS2	CTTGATTTAGGTGACTATAG
SP72-F	TGAGAGTGCACCATATGGAC	SP72-R	AGCGGAAGAGCGCCAATAC
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	GAGGTATATTAATGTATCG	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	ATTGTTAATATACTCTATAC
YES2-R	GCGTGAATGTAAGCGTGAC	Zome1N-F	GATATACCTACAACGTCTTC



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