Technical Appendix

Composition of Solutions

Note: Solution compositions for the Cloning Enzyme 10X reaction buffers are listed in Table 15.

Antibiotic Stock Solutions

Ampicillin: 100mg/ml in deionized water (filter-sterilized).

Tetracycline: 10mg/ml in 80% ethanol.

Kanamycin: 30mg/ml kanamycin sulfate in deionized water (filter-sterilized).

Chloramphenicol: 20mg/ml in 80% ethanol.

Store at –20°C. **Note:** Cell growth in liquid culture is inhibited by tetracycline concentrations greater than 10mg/ml.

Cell Lysis Solution

0.2N NaOH, 1% SDS in deionized water. Prepare fresh for each use.

Cell Resuspension Solution

25mM	Tris-HCl (pH 8.0)
10mM	EDTA
50mM	glucose

IPTG Stock Solution (0.1M)

1.2g	isopropyl β-D-thiogalacto-pyranoside (IPTG) (Cat.# V3951)

Add deionized water to 50ml final volume. Filter-sterilize (0.2µm) and store at 4°C.

LB Medium

10g	Bacto®-tryptone
5g	Bacto®-yeast extract
5g	NaCl

Add deionized water to approximately 1L. Adjust pH to 7.5 with 10N NaOH and autoclave.

LB/ Antibiotic Plates (1L)

Add 15g of agar to 1 liter of LB medium and autoclave. Allow the medium to cool to 55°C before adding antibiotic to the specified final concentration (ampicillin: 100µg/ml; tetracycline: 12.5µg/ml; kanamycin: 30µg/ml; chloramphenicol: 20µg/ml). Pour 30–35ml of medium into 85mm petri dishes. If necessary, flame the surface of the medium with a Bunsen burner to eliminate bubbles. Let the agar harden overnight. Store at 4°C for <1 month. Tetracycline is light-sensitive; LB/tetracycline plates should be covered with foil.

M-9 Plates

6g	Na_2HPO_4
3g	KH_2PO_4
0.5g	NaCl
1g	NH_4Cl
15g	agar

Add deionized water to approximately 1L. Adjust pH to 7.4 with 10N NaOH. Autoclave. Cool to 50°C. Then add:

2.0ml	1M $MgSO_4$
0.1ml	1M $CaCl_2$
10.0ml	20% glucose
1.0ml	1M thiamine-HCl

Filter the medium through a 0.2µm filter unit.

Phenol:Chloroform:Isoamyl Alcohol (25:24:1)

Mix equal parts of TE buffer and phenol and allow the phases to separate. Then mix 1 part of the lower, phenol phase with 1 part of chloroform:isoamyl alcohol (24:1).

Potassium Acetate Solution (pH 4.8)

Prepare 60ml of 5M potassium acetate. Add 11.5ml of glacial acetic acid and 28.5ml of deionized water. This solution will be 3M with respect to potassium and 5M with respect to acetate. Store at 4°C.

SOC Medium

2.0g	Bacto®-tryptone
0.5g	Bacto®-yeast extract
1ml	1M NaCl
0.25ml	1M KCl
1ml	2M Mg^{2+} stock (1M $MgCl_2 \cdot 6H_2O$, 1M $MgSO_4 \cdot 7H_2O$), filter-sterilized
1ml	2M glucose, filter-sterilized

Add Bacto®-tryptone, Bacto®-yeast extract, NaCl and KCl to 97ml deionized water. Stir to dissolve. Autoclave and cool to room temperature. Add 2M Mg^{2+} stock and 2M glucose stock, each to a final concentration of 20µM. Filter the complete medium through a 0.2mm filter unit. The pH should be 7.0.

TAE 10X Buffer

400mM	Tris base
200mM	Sodium acetate
10mM	EDTA

Adjust pH to 8.2 with glacial acetic acid.

TBE 10X Buffer

890mM	Tris base
890mM	Boric acid
19mM	EDTA

Adjust pH to 8.3.

TE Buffer

10mM	Tris-HCl (pH 8.0)
1mM	EDTA

X-Gal Stock Solution (50mg/ml)

100mg	5-bromo-4-chloro-3-indolyl β–D-galactoside (X-Gal)

Dissolve in 2ml of N,N′-dimethylformamide. This stock solution is available from Promega (Cat.# V3941).

Table 12. Cloning Enzymes: Promega's Quality Acceptance Criteria.

Enzyme	Concentration (u/µl)	SDS-PAGE Purity	Contaminant Activity		
			Endonuclease: Supercoiled DNA, 1µg	dsDNase: 50ng Radiolabeled DNA	RNase: 50ng Radiolabeled RNA
Agar*ACE*® Enzyme (1)	0.15–0.30	>90%	Gel analysis, absence of visible nicking or cutting, 5u/4hr at 42°C	<1% release, 5u/4hr at 42°C	<3% release, 5u/4hr at 42°C
Alkaline Phosphatase, Calf Intestinal (2)	1	N/A	Gel analysis, absence of visible nicking or cutting, 5u/1hr at 37°C	<3% release, 5u/1hr at 37°C	<3% release, 5u/1hr at 37°C
RecA Protein (3,4)	N/A	>95%	Gel analysis, absence of visible nicking or cutting, 15µg/16hr at 37°C	<1% release, 5µg/4hr at 37°C	N/A
T4 DNA Ligase (2,5)	1–3	>90%	Gel analysis, absence of visible nicking or cutting, 5u/16hr at 37°C	<1% release, 20u/16hr at 37°C	<3% release, 20u/5hr at 37°C
T4 Polynucleotide Kinase (6)	5–10	>90%	Gel analysis, absence of visible nicking or cutting, 25u/5hr at 37°C	<3% release, 25u/3hr at 37°C	<3% release, 25u/3hr at 37°C
T4 RNA Ligase	9–12	>90%	20u/3hr at 37°C	<1% release, 20u/3hr at 37°C	<1% release, 20u/3hr at 37°C

N/A – Not Applicable
1. T-Vector, Blue/White, DNA recovery of 2µg of DNA ladder.
2. Blue/White Assay is performed.
3. ssDNA-dependent ATPase activity.
4. Strand exchange.
5. T-Vector, Lambda ligation and packaging, ssDNase.
6. Oligo 5′ end-labeled to high specific activity.

Table 13. Uses and Genotypes of Various Strains of *E. coli*.

Strain	Genotype	Comments
BL21(DE3)	F-, *omp*T, *hsd*S_B, (r_B^-, m_B^-), *dcm*, *gal*, λ(DE3)	*In vivo* expression of T7 Promoter-driven genes (e.g., pET-5, pGEM®).
BL21(DE3)pLysS[e]	F-, *omp*T, *hsd*S_B, (r_B^-, m_B^-), *dcm*, *gal*, λ(DE3), pLysS CmR	*In vivo* expression of T7 Promoter-driven genes (e.g., pET-5, pGEM®), pLysS provides tighter control of T7 RNA Pol expression.
BMH 71-18 *mut*S*	*thi*, *sup*E, Δ(*lac-pro*AB), [*mut*S::Tn10] [F′, *pro*AB, *laq*I^{q}ZΔM15]	GeneEditor™ Site-Directed Mutagenesis System; mismatch repair deficient strain.
CJ236	F′, *cat*(pCJ105; M13^{s}Cm^r)*dut, ung*1, *thi*-1, *rel*A1, *spo*T1, *mcr*A	Kunkel mutagenesis; *dut*(-), *ung*(-).
C600	*thi*-1, *thr*-1, *leu*B6, *lac*Y1, *ton*A21, *sup*E44	λgt10, Permissive host; allows both parental and recombinant phage to grow.
C600*hfl*	*thi*-1, *thr*-1, *leu*B6, *lac*Y1, *ton*A21, *sup*E44, *hfl*A150, [*chr*.::Tn10]	λgt10, Restrictive host; allows recombinant phage to grow in preference to parental phage.
DH1	*recA*1, *endA*1, *gyrA*96, *thi*-1, *hsd*R17 (r_K^-, m_K^+), *sup*E44, *rel*A1	Parent of DH5. DH5 more efficiently transformed by large (40 - 60kb) plasmids.
DH5α®	θ80d*lac*ZΔM15, *rec*A1, *end*A1, *gyr*A96, *thi*-1, *hsd*R17 (r_K^-, m_K^+), *sup*E44, *rel*A1, *deoR*, Δ(*lac*ZYA-*arg*F)U169	Common host for cDNA cloning; supports α-complementation, recA(-) and endA(-).
DH5α®F′	F′, θ80d*lac*ZΔM15, *rec*A1, *end*A1, *gyr*A96, *thi*-1, *hsd*R17 (r_K^-, m_K^+), *sup*E44, *rel*A1, *deo*R, Δ(*lac*ZYA-*arg*F)U169	Single-stranded DNA synthesis.
ES1301 *mutS**	*lac*Z53, *thy*A36, *rha*-5, *met*B1, *deo*C, IN(*rrn*D-*rrn*E), [*mut*S201::Tn5]	Provided with the Altered Sites® Mutagenesis Systems; mismatch repair deficient (kan^r).
HB101*	*thi*-1, *hsd*S20 (r_B^-, m_B^-), *sup*E44, *rec*A13, *ara*-14, *leu*B6, *pro*A2, *lac*Y1, *rpsL*20 (str^r), *xyl*-5, *mtl*-1, *gal*K2	Common strain for propagating plasmids that do not allow α-complementation.
JM83	*ara*, Δ(*lac-pro*AB), *rps*L, θ80d*lac*ZΔM15	Host for pUC plasmids; pBR322- recA(+), r_K^+, m_K^+.
JM101	*sup*E, *thi*, Δ(*lac-pro*AB), [F′, *tra*D36, *pro*AB, *laq*I^{q}ZΔM15]	Host for M13mp vectors; recA(+), r_K^+.

Strains listed in boldface are available from Promega.
* Indicates strains available as competent cells.

(continued)

Table 13. Uses and Genotypes of Various Strains of *E. coli* (continued).

Strain	Genotype	Comments
JM103	*end*A1, *hsd*R, *sup*E, *sbc*B15, *thi*-1, *str*A, Δ(*lac-pro*AB), [F′, *tra*D36, *pro*AB, *lac*I^{q}ZΔM15]	Host for M13mp vectors; recA(+), r_K^+.
JM105	*end*A1, *thi*, *rps*L, *sbc*B15, *hsd*R4, Δ(*lac-pro*AB), [F′, *tra*D36, *pro*AB, *lac*I^{q}ZΔM15]	Host for M13mp vectors; recA(+), r_K^+.
JM107	*end*A1, *thi*, *gyr*A96, *hsd*R17 (r_K^-, m_K^+), *rel*A1, *sup*E44, Δ(*lac-pro*AB), [F′, *traD*36, *proAB*, *lac*I^{q}ZΔM15]	Host for M13mp vectors; recA(+), r_K^+.
JM108	*end*A1, *rec*A1, *gyr*A96, *thi*, *hsd*R17 (r_K^-, m_K^+), *rel*A1, *sup*E44, Δ(*lac-pro*AB)	Recombination deficient mutation in *rec* A increases stability of plasmids.
JM109*	*end*A1, *rec*A1, *gyr*A96, *thi*, *hsd*R17 (r_K^-, m_K^+), *rel*A1, *sup*E44, Δ(*lac-pro*AB), [F′, *tra*D36, *pro*AB, *lac*I^{q}ZΔM15]	Common host for cloning. ssDNA synthesis; restriction(-), rec(-), allows α-complementation: Included with the majority of our plasmids; Maintain on M9 plates supplemented with thiamine (to maintain F′ episome).
JM109(DE3)	*end*A1, *rec*A1, *gyr*A96, *thi*, *hsdR*17 (r_K^-, m_K^+), *rel*A1, *sup*E44, Δ(*lac-pro*AB), [F′, *tra*D36, *pro*AB, *lac*I^{q}ZΔM15], λ(DE3)	*In vivo* expression of T7 Promoter-driven genes (e.g., pET-5, pGEM®), allows α-complementation.
JM110	*rps*L, *thr*, *leu*, *thi*, *hsd*R17 (r_K^-, m_K^+), *lac*Y, *gal*K, *gal*T, *ara*, *ton*A, *tsx*, *dam*, *dcm*, *sup*E44, Δ(*lac-pro*AB), [F′, *tra*D36, *pro*AB, *lac*I^{q}ZΔM15]	*dam*(-), *dcm*(-) strain, allows α-complementation.
LE392	*hsd*R514, (r_K^-, m_K^+), *sup*E44, *sup*F58, *lac*Y1 or Δ(*lac*IZY)6, *gal*K2, *gal*T22, *met*B1, *trp*R55	Genomic and cDNA cloning; restriction(-), rec(+), permissive host, no color selection, *lon*(+). Recommended (if no color selection needed) as primary strain for amplification of recombinant phage and screening of cDNA library with nucleic acid probe.
KW251	*sup*E44, *gal*K2, *gal*T22, *met*B1, *hsd*R2, *mcr*B1, *mcr*A, [*arg*A81::Tn10], *rec*D1014	Genomic cloning; permissive host, used as alternative to LE392, rec(-) strain, restriction (-), tet^r.
MB408	*rec*F, *rec*B21, *rec*C22, *sbc*B15, *hfl*A, *hfl*B, *hsd*R, (tet^r)	
MC1061	F-, *ara*D139, Δ*(ara-leu)*7696, *gal*E15, *gal*K16, Δ*(lac)*X74, *rps*L(Str), *hsd*R2(r_K^-, m_K^+), *mcr*A, *mcr*B1	
NM522	*sup*E, *thi*, Δ(*lac-pro*AB), Δ*hsd*5 (r^-, m^-), [F′, *pro*AB, *lac*I^{q}ZΔM15]	ssDNA synthesis; restriction (-), rec (+), F′. Grow on M9 plates to maintain F′episome.
NM538	*sup*F, *hsd*R (r_K^-, m_K^+), *trp*R, *lac*Y	Genomic cloning; permissive host.
NM539	*sup*F, *hsd*R (r_K^-, m_K^+), *lac*Y, (P2)	Restrictive host; used for *Spi* selection of recombinant phage.
P2392	LE392 (P2)	
RR1	*hsd*S20, (r_B^-, m_B^+), *sup*E44, *ara*-14, *pro*A2, *rsp*L20 (str^r), *lac*Y1, *gal*K2, *xyl*-5, *mtl*-1, *sup*E44	*Rec* A (+) version of HBI01.
χ1776	*ton*A53, *dap*D8, *min*A1, *gln*V44, *sup*E44, Δ(*gal-uvr*B)40, *min*82, *rib*-2, *gyr*A25, *thy*A142, *oms*-2, *met*C65, *oms*-1, (*tte*-1), Δ(*bio*H-*asd*)29, *cyc*82, *cyc*A1, *hsd*R2	Debilitated strain used in early work with recombinant DNA.
XL1-Blue	*rec*A1, *end*A1, *gyr*A96, *thi*, *hsd*R17(r_K^-, m_K^+), *sup*E44, *rel*A1, *lac*, [F′, *pro*AB+, *lac*I^{q}ZΔM15, ::Tn10(Tet^r)]	Common host for cloning.
SURE Cells	*E14-(Mcr*A-) Δ(*mcr*CB-*hsd*SMR-*mrr*) 171 *end*A1, *sup*E44, *thi*-1, *gyr*A96, *rel*A1, *lac, rec*B, *rec*J, *sbc*C, *umu*C::Tn5(kan^r), *uvr*C, [F′, *pro*AB, *lac*I^{q}ZΔM15 [Tn10(Tet^r)]	Increased stability of DNA containing inverted repeats or Z-DNA (Stratagene); F′ episome- able to make ssDNA from phagemid DNA.
Y1088	Δ(*lac*U169), *sup*E, *sup*F, *hsd*R (r_K^-, m_K^+), *metB*, *trpR*, *tonA*21, [*pro*C::Tn5] (pMC9)	Host for amplification of cDNA libraries. pMC9 confers amp^r, tet^r.
Y1089	Δ(*lac*U169), *pro*A+, Δ(*lon*), *ara*D139, *str*A, *hfl*A150, [chr:Tn10(tet^r)], (pMC9)	cDNA cloning- lon(-), *hflA*150 (enhances lysogeny), pMC9- maintain on amp/tet plates: Used primarily for generation of preparative amounts of recombinant fusion protein. pMC9 confers amp^r, tet^r.
Y1090	Δ(*lac*U169), *pro*A+, Δ(*lon*), *ara*A139, *str*A, *sup*F, [*trp*C22::Tn10 (tet^r)], (pMC9), *hsd*R (r_K^- m_K^+)	cDNA cloning. Useful for screening expression cDNA libraries. pMC9 confers amp^r, tet^r.

Strains listed in boldface are available from Promega.
* Indicates strains available as competent cells.

Table 14. Applications of Promega's Competent Cells.

Applications	Cells	Genotype	>1 x 10^8 cfu/µg	>1 x 10^7 cfu/µg	>1 x 10^6 cfu/µg
Cloning T-Vector Cloning Blue/White Screening	JM109	*end*A1, *rec*A1, *gyr*A96, *thi hsd*R17 (r_K^-, m_K^+), *rel*A1, *sup*E44 Δ(*lac-pro*AB), [F′ *tra*D36, *pro*AB *lacI*^{q}ZΔM15].	✔	✔	
Cloning	HB101	F′, *thi*-1, *hsd*S20 (r_B^-, m_B^-), *sup*E44, *rec*A13, *ara*-14, *leu*B6, *pro*A2, *lac*Y1, *gal*K2 *rps*L20, (str^r), *xyl*-5, *mtl*-1	✔	✔	
Site-Directed Mutagenesis	BMH 71-18 *mut*S	*thi, sup*E, Δ(*lac-pro*AB), [*mut*S::Tn10], [F′ *pro*AB, *lacI*^{q}ZΔM15].Mutagenesis		✔	
	ES1301 *mut*S	*lac*Z53, *mut*S201::Tn5, *thy*A36, *rha*-5, *met*B1, *dec*C1, IN(*rrn*D-*rrn*E).		✔	
Protein Expression	BL21 (DE3)pLysS[e]	F′, *omp*T, *hsd*S_B(r_B^-, m_B^+), *dcm, gal,* (DE3), pLysS, Cm^r		✔	✔

Table 15. Cloning Enzyme 10X Reaction Buffer Formulations.

Enzyme	10X Formulation
T4 DNA Ligase*	300mM Tris-HCl (pH 7.8), 100mM MgCl2, 100mM DTT, 10mM ATP
T4 RNA Ligase	500mM Tris-HCl (pH 7.8), 100mM MgCl2, 50mM DTT, 10mM ATP
T4 Polynucleotide Kinase (PNK)	700mM Tris-HCl (pH 7.6), 100mM MgCl2, 50mM DTT
Calf Intestinal Alkaline Phosphatase (CIAP)	500mM Tris-HCl (pH 9.3), 10mM MgCl2, 1mM ZnCl2, 10mM spermidine

* pGEM®-T and pGEM®-T Easy Vector Systems (Cat.# A1360, A1380, A3600, A3610) provide a 2X Rapid Ligation Buffer for T4 DNA Ligase with a formulation of: 60mM Tris-HCl (pH 7.8), 20mM $MgCl_2$, 20mM DTT, 2mM ATP, 10% polyethylene glycol (MW 8000).
The Rapid Ligation Buffer enables performance of ligation reactions in as little as 1 hour.

General References

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Additional Literature

Technical Manuals and Product Information Sheets

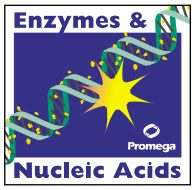
#9PIM180 T4 DNA Ligase
#9PIM182 Calf Intestinal Alkaline Phosphatase
#9PIM181 T4 DNA Polymerase
#9PIM220 DNA Polymerase I Large (Klenow) Fragment
#TM042 pGEM®-T and pGEM®-T Easy Vector Systems

Promega Notes Articles

PN032 pGEM®-Zf Vector update: Considerations for optimal single-stranded DNA production
PN045 pGEM®-T Vector Systems troubleshooting guide
PN051 pGEM®-T Vector: Technically Speaking
PN054 pGEM®-T Vector: Cloning of modified blunt-ended DNA fragments
PN071 Rapid Ligation for the pGEM®-T and pGEM®-T Easy Vector Systems
Cloning Blunt End *Pfu* DNA Polymerase-Generated PCR Fragments into pGEM®-T Vector

Glossary

5′-RACE: Rapid Amplification of cDNA 5′-Ends.
Activated Calf Thymus DNA: Nicked and gapped dsDNA prepared by treatment with DNase I; a substrate for many DNA polymerases.
Alkaline Phosphatase (AP): An enzyme that catalyzes the removal of a phosphate group from a substrate. This property is used in colorimetric and chemiluminescent detection reagents.
Alpha Complementation: The process by which a functional β-galactosidase (*lac*Z) gene is generated when the *lac*Z α-peptide (N-terminus) complements the ω fragment of *lac*Z (C-terminus). In general, a cloning vector contributes the *lac*Z α-peptide and the host bacterial strain provides the ω fragment. See also blue/white cloning.
Blue/White Cloning: A technique used to identify recombinant (positive) clones in cloning experiments. Specially constructed cloning vectors will produce β-galactosidase by α-complementation when transformed into the appropriate host strain after exposure to the chemical IPTG. This will cause the cells to exhibit a blue color in the presence of the substrate X-Gal. Foreign DNA inserted into the cloning vector will interrupt the *lac*Z gene, preventing α-complementation and producing white colonies.
Blunt Ends: DNA ends that do not possess short, single-stranded overhangs. See also sticky ends.
Cloning: The production of a large number of identical DNA molecules from a single DNA molecule through replication of the DNA within a cell. Cloning also refers to the production of large numbers of identical cells from a single cell.
Cohesive Ends: Two DNA ends, in the same or different molecules, which have short, single-stranded overhangs that are complementary to one another. Cohesive ends allow comparatively efficient ligation of DNA molecules. See also sticky ends.
Competent Cells: Bacterial cells that are able to take in exogenous DNA.
Distributive: Enzyme dissociates from template after a single nucleotide addition.
DNA (Deoxyribonucleic Acid): A polymeric molecule composed of deoxyribonucleotide units joined in a specific sequence through the formation of 3′→5′ phosphodiester bonds.
DNase (Deoxyribonuclease): An enzyme that breaks down DNA into small fragments or deoxyribonucleotides.
DNA Ligase: An enzyme that joins two DNA molecules (or two ends of the same DNA molecule) by forming a phosphodiester bond between the 3′- and 5′-ends.
dNTP: Deoxyribonucleotide 5′-triphosphate.
ds: Double-stranded.
DTT: Dithiolthreitol.
End-Labeling: The addition of a labeled group (radioactive or nonradioactive) to the 5′- or 3′-end of DNA or RNA. This is typically accomplished by using a kinase to label the 5′-end, or a DNA polymerase or terminal deoxyxnucleotidyl transferase to label the 3′-end.
Endonuclease: An enzyme that hydrolyzes phosphodiester bonds at internal locations within a DNA or RNA molecule.
Exonuclease: An enzyme that hydrolyzes phosphodiester bonds at the ends of DNA or RNA molecules, resulting in the stepwise removal of nucleotides.
Gene: A segment of DNA that encodes a polypeptide, protein or RNA molecule.
Genotype: The specific genes (which may or may not be expressed) that are present in an organism.
Heat-Inactivation: Destroying an enzyme's activity by heating to a high temperature (typically 60–70°C) for an extended length of time. Not all enzymes can be heat-inactivated.
In vitro: A reaction or experiment performed in the absence of living cells, typically using conditions that attempt to mimic those found within cells.
In vivo: A reaction or experiment performed in a living organism or cell.
kb: Kilobase or kilobase pairs.
k_{cat}: Maximum number of substrate molecules converted to products per active site per unit time.
kDa: KiloDalton.
K_m: The Michaelis constant; the concentration of substrate that an enzyme can convert to product at half its maximal rate.
Labeling: A process in which nucleic acids or proteins are tagged with a radioactive or nonradioactive marker.
Ligase: An enzyme that catalyzes DNA or RNA linkage, generally splitting off a pyrophosphate group from ATP concurrently.
Multiple Cloning Site (MCS): The region of a DNA vector that contains unique restriction enzyme recognition sites into which foreign DNA can be inserted; also called a polylinker.
Nuclease: An enzyme that degrades nucleic acids.
Nucleotide: A molecule composed of an organic base, sugar and phosphate group, which constitutes the "building blocks" of nucleic acids (DNA and RNA).
Oligonucleotide (Oligo): A short (typically <50 nucleotides), single-stranded DNA or RNA molecule.
PCR: Polymerase Chain Reaction.
PEG: Polyethylene glycol.
Phosphatase: An enzyme that removes a phosphate group from a protein, nucleic acid or other molecule.
PP_i: Inorganic pyrophosphate.
Primer: An oligonucleotide or short single-stranded nucleic acid that acts as a starting point for the synthesis of nucleic acids from a template.
Promoter: DNA sequence for the initiation of RNA transcription by RNA polymerase.
RNA (Ribonucleic Acid): A polymeric molecule composed of ribonucleotide units joined in a specific sequence through the formation of 3′→5′ phosphodiester bonds.
RNase (Ribonuclease): An enzyme that breaks down RNA into smaller RNA fragments or ribonucleotides.
rNTP: Ribonucleotide 5′-triphosphate.
ss: Single-stranded.
Sticky Ends: Two DNA ends, in the same or different molecules, that have short, single-stranded overhangs that are complementary to one another. Sticky ends allow comparatively efficient ligation of DNA molecules. See also Cohesive Ends.
TAE: Tris Acetate EDTA .
TCA: Trichloroacetic acid.
Terminator: DNA sequence for the termination of RNA transcription by RNA polymerase.
Transformation: The process during which a plasmid DNA is inserted into a bacterial cell.
Turnover Rate (k_{cat}): Maximum number of substrate molecules converted to products per active site per unit time.
Vector: A DNA molecule that can replicate within a host cell and that allows the insertion of foreign DNA sequences. Vectors commonly used may be plasmids, phagemids or bacteriophage.



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