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-thiogalactopyranoside (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 Na₂HPO₄ 3g KH₂PO₄ 0.5g NaCl 1g NH₄Cl 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 MgSO₄ 0.1ml 1M CaCl₂ 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 acetic acid. Store at 4˚C.

SOC Medium

2.0g Bacto®-tryptone 0.5g Bacto®-yeast extract 1ml 1M NaCl 0.25ml 1M KCl 1ml 2M Mg²⁺ stock (1M MgCl₂ • 6H₂O, 1M MgSO₄ • 7H₂O), 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 Mg²⁺ 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.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Concentration (µl/l)</th>
<th>SDS-PAGE Purity</th>
<th>Contaminant Activity</th>
<th>Endonuclease: Supercoiled DNA, 1µg</th>
<th>dsDNAse: 50ng Radiolabeled DNA</th>
<th>RNase: 50ng Radiolabeled DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarase Enzyme (1)</td>
<td>0.15–0.30</td>
<td>&gt;90%</td>
<td>Gel analysis, absence of visible nicking or cutting, 5µl/1hr at 37°C</td>
<td>&lt;1% release, 5µl/4hr at 37°C</td>
<td>&lt;3% release, 5µl/1hr at 37°C</td>
<td>N/A</td>
</tr>
<tr>
<td>Alkaline Phosphatase, Calf Intestinal (2)</td>
<td>0.3–0.5</td>
<td>N/A</td>
<td>Gel analysis, absence of visible nicking or cutting, 5µl/1hr at 37°C</td>
<td>&lt;1% release, 5µl/1hr at 37°C</td>
<td>&lt;3% release, 5µl/1hr at 37°C</td>
<td>N/A</td>
</tr>
<tr>
<td>RecA Protein (3,4)</td>
<td>0.3–0.5</td>
<td>N/A</td>
<td>Gel analysis, absence of visible nicking or cutting, 15µg/16hr at 37°C</td>
<td>&lt;1% release, 5µg/4hr at 37°C</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>T4 DNA Ligase (2,5)</td>
<td>1–3</td>
<td>&gt;90%</td>
<td>Gel analysis, absence of visible nicking or cutting, 5µl/16hr at 37°C</td>
<td>&lt;1% release, 20µl/3hr at 37°C</td>
<td>&lt;3% release, 20µl/5hr at 37°C</td>
<td>N/A</td>
</tr>
<tr>
<td>T4 Polynucleotide Kinase (6)</td>
<td>5–10</td>
<td>&gt;90%</td>
<td>Gel analysis, absence of visible nicking or cutting, 25µl/5hr at 37°C</td>
<td>&lt;3% release, 25µl/3hr at 37°C</td>
<td>&lt;3% release, 25µl/3hr at 37°C</td>
<td>N/A</td>
</tr>
<tr>
<td>T4 RNA Ligase</td>
<td>9–12</td>
<td>&gt;90%</td>
<td>20µl/3hr at 37°C</td>
<td>&lt;1% release, 20µl/3hr at 37°C</td>
<td>&lt;1% release, 20µl/3hr at 37°C</td>
<td>N/A</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL21(DE3)</td>
<td>F′, ompT, hsdSB (r−, m−), dcm, gal, λ(DE3)</td>
<td>In vivo expression of T7 Promoter-driven genes (e.g., pET-5, pGEM®).</td>
</tr>
<tr>
<td>BL21(DE3)pLysS[4]</td>
<td>F′, ompT, hsdSB (r−, m−), dcm, gal, λ(DE3), pLysS CmR</td>
<td>In vivo expression of T7 Promoter-driven genes (e.g., pET-5, pGEM®), pLysS provides tighter control of T7 RNA Pol expression.</td>
</tr>
<tr>
<td>BMH 71-18 mutS</td>
<td>thi, supE, Δ(lac-proAB), [mutS::Tn10] [F′, proAB, lacI+ZaM15]</td>
<td>GeneEditor™ Site-Directed Mutagenesis System; mismatch repair deficient strain.</td>
</tr>
<tr>
<td>CJ236</td>
<td>F′, cat[pCJ105; M13-Cmr]Δ-duty, ungI, thi-1, relA1, spoT1, mcrA</td>
<td>Kunkel mutagenesis; dut-, ungI(-).</td>
</tr>
<tr>
<td>C600</td>
<td>thi-1, thr-1, leuB6, lacY1, tonA21, supE44</td>
<td>λgt10, Permissive host; allows both parental and recombinant phage to grow.</td>
</tr>
<tr>
<td>C600rif1</td>
<td>thi-1, thr-1, leuB6, lacY1, tonA21, supE44, thiA150, [chr::Tn10]</td>
<td>λgt10, Restrictive host; allows recombinant phage to grow in preference to parental phage.</td>
</tr>
<tr>
<td>DH1</td>
<td>recA1, endA1, gyrA96, thi-1, hsdR17 (r−, m−), supE44, relA1</td>
<td>Parent of DH5. DH5 more efficiently transformed by large (40–60kb) plasmids.</td>
</tr>
<tr>
<td>DH5recR</td>
<td>Δ80dIacZaM15, recA1, endA1, gyrA96, thi-1, hsdR17 (r−, m−), supE44, relA1, deoR, Δ(lacZYA-argF)U169</td>
<td>Common host for cDNA cloning; supports α-complementation, recA(-) and endA(-).</td>
</tr>
<tr>
<td>DH5recRF</td>
<td>Δ80dIacZaM15, recA1, endA1, gyrA96, thi-1, hsdR17 (r−, m−), supE44, relA1, deoR, Δ(lacZYA-argF)U169</td>
<td>Single-stranded DNA synthesis.</td>
</tr>
<tr>
<td>ES1301 mutS</td>
<td>ΔlacZ53, thyA36, rha-5, mfrB1, deoC, In(rrD-rrmE), [mutS201::Tn15]</td>
<td>Provided with the Altered Sites® Mutagenesis Systems; mismatch repair deficient (kanR).</td>
</tr>
<tr>
<td>HB101</td>
<td>thi-1, hsdS20 (r−, m−), supE44, recA13, ara-14, leuB6, proA2, lacY1, rpsL20 (str), xyl-5, mna-1, galK2</td>
<td>Common strain for propagating plasmids that do not allow α-complementation.</td>
</tr>
<tr>
<td>JM83</td>
<td>ara, Δ(lac-proAB), rpsL, Δ80dIacZaM15</td>
<td>Host for pUC plasmids; pBR322- recA(+) r−, m−.</td>
</tr>
<tr>
<td>JM101</td>
<td>supE, thi, Δ(lac-proAB), [F′, traD36, proAB, lacI+ZaM15]</td>
<td>Host for M13mp vectors; recA(+), r−.</td>
</tr>
</tbody>
</table>

Strains listed in boldface are available from Promega.
* Indicates strains available as competent cells.
**Table 13. Uses and Genotypes of Various Strains of E. coli (continued).**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>JM103</td>
<td>endA1, hisdR, supE, sbcB15, thi-1, strA, Δ(lac-proAB), [F', traD36, proAB, lacZΔM15]</td>
<td>Host for M13mp vectors; recA(+) rK-</td>
</tr>
<tr>
<td>JM105</td>
<td>endA1, thi, rpsL, sbcB15, hisdR4, Δ(lac-proAB), [F', traD36, proAB, lacZΔM15]</td>
<td>Host for M13mp vectors; recA(+) rK-</td>
</tr>
<tr>
<td>JM107</td>
<td>endA1, thi, gyrA96, hisdR17 (rK- m+) relA1, supE44, Δ(lac-proAB), [F', traD36, proAB, lacZΔM15]</td>
<td>Host for M13mp vectors; recA(+) rK-</td>
</tr>
<tr>
<td>JM108</td>
<td>endA1, relA1, gyrA96, thi, hisdR17 (rK- m+) relA1, supE44, Δ(lac-proAB)</td>
<td>Recombination deficient mutation in recA increases stability of plasmids.</td>
</tr>
<tr>
<td>JM109*</td>
<td>endA1, relA1, gyrA96, thi, hisdR17 (rK- m+), relA1, supE44, Δ(lac-proAB), [F', traD36, proAB, lacZΔM15]</td>
<td>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).</td>
</tr>
<tr>
<td>JM109(DE3)</td>
<td>endA1, relA1, gyrA96, thi, hisdR17 (rK- m+), relA1, supE44, Δ(lac-proAB), [F', traD36, proAB, lacZΔM15]</td>
<td>In vivo expression of T7 Promoter-driven genes (e.g., pET-5, pGEM®), allows α-complementation.</td>
</tr>
<tr>
<td>JM110</td>
<td>rpsL-, thr-, leu-, hisdR17 (rK- m+), lacY, galK, galI, ara, tonA, tso, dam, dcm, supE44, Δ(lac-proAB), [F', traD36, proAB, lacZΔM15]</td>
<td>dam(–), dcm(–) strain, allows α-complementation.</td>
</tr>
<tr>
<td>LE392</td>
<td>hisdR514, (rK- m+), supE44, supF58, lacY1 or Δ(lacZ2Y)6, galK2, galT22, merB1, trpR55</td>
<td>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 plasmid and screening of cDNA library with nucleic acid probe.</td>
</tr>
<tr>
<td>KW251</td>
<td>supE44, galK2, galT22, merB1, hisdR2, mcrcB1, mcrcA, [argA81:Trn10], recD1014</td>
<td>Genomic cloning; permissive host, used as alternative to LE392, rec(-) strain, restriction (-), tet’</td>
</tr>
<tr>
<td>MB408</td>
<td>recE, recB21, recC22, sbbc15, hisA, hisB, hisD, (tet’)</td>
<td></td>
</tr>
<tr>
<td>MC1061</td>
<td>F-, araD139, Δ(ara-leu)7696, galE15, galK16, Δ(lac)7X4, rpsL(Str), hisdR2 (rK- m+), lacY, lacB1</td>
<td></td>
</tr>
<tr>
<td>NM522</td>
<td>supE, thi, Δ(lac-proAB), Δhsd5 (+, m’), [F’, proAB, lacZΔM15]</td>
<td>ssDNA synthesis; restriction (-), rec (+), F’. Grow on M9 plates to maintain F’ episome.</td>
</tr>
<tr>
<td>NM538</td>
<td>supF, hisdR (rK- m+), trpR, lacY</td>
<td>Genomic cloning; permissive host.</td>
</tr>
<tr>
<td>NM539</td>
<td>supF, hisdR (rK- m+), lacY, (P2)</td>
<td>Restrictive host; used for SpI selection of recombinant phage.</td>
</tr>
<tr>
<td>P2392</td>
<td>LE392 (P2)</td>
<td></td>
</tr>
<tr>
<td>RR1</td>
<td>hisdR20, (rK- m+), supE44, ara-14, proA2, rplL20 (str’), lacY1, galK2, ssoB, metI-1, supE44</td>
<td>RecA (+) version of HB101.</td>
</tr>
<tr>
<td>χ1776</td>
<td>tonA53, dudA8, minA1, phoH44, supE44, Δ(lac-proAB)40, minB2, rpsL-2, gyrA25, thyA142, ose-2, merD55, ose-1 (ts(-)), Δ(bioH-hsd)29, ccrA, uncC1, lacZΔM15</td>
<td>Debilitated strain used in early work with recombinant DNA.</td>
</tr>
<tr>
<td>XL1-Blue</td>
<td>relA1, endA1, gyrA96, thi, hisdR17 (rK- m+), supE44, relA1, lacI, lacZΔM15</td>
<td>Common host for cloning.</td>
</tr>
<tr>
<td>SURE Cells</td>
<td>E44-(McRA-)Δ(mcrCB-hsdSMR-olv) 171 endA1, supE44, thi-1, gyrA96, relA1, lac, recB, recJ, sbcC, umuC::Tn5(kari), umvC::Tn5(kari), lacZΔM15 [Tr10(ter1)]</td>
<td>Increased stability of DNA containing inverted repeats or Z-DNA (Stratagene); F’ episome-able to make ssDNA from phagemid DNA.</td>
</tr>
<tr>
<td>Y1088</td>
<td>Δ(lacU169), supE, supF, hisdR (rK- m+), metB, trpR, tonA21, [proC::Tn5] (pMC9)</td>
<td>Host for amplification of cDNA libraries. pMC9 confers ampr, tet’.</td>
</tr>
<tr>
<td>Y1089</td>
<td>Δ(lacU169), proA-, Δ(lacZ2Y)6, araD139, strA, hisA150, (chr::Tn10(ter)), (pMC9)</td>
<td>cDNA cloning- lon(-), hisA150 (enhances lysisogeny), pMC9- maintain on ampic/tet plates: Used primarily for generation of preparative amounts of recombinant fusion protein. pMC9 confers ampr, tet’.</td>
</tr>
<tr>
<td>Y1090</td>
<td>Δ(lacU169), proA-, Δ(lacZ2Y)6, araD139, strA, supF, [trpC22::Tn10(ter)], (pMC9), hisdR (rK- m+)</td>
<td>cDNA cloning. Useful for screening expression cDNA libraries. pMC9 confers ampr, tet’.</td>
</tr>
</tbody>
</table>

Strains listed in boldface are available from Promega.

* Indicates strains available as competent cells.
Table 14. Applications of Promega’s Competent Cells.

<table>
<thead>
<tr>
<th>Applications</th>
<th>Cells</th>
<th>Genotype</th>
<th>&gt;1 x 10⁶ cfu/µg</th>
<th>&gt;1 x 10⁷ cfu/µg</th>
<th>&gt;1 x 10⁸ cfu/µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloning</td>
<td>JM109</td>
<td>endA1, recA1, gyrA96, thi hsdR17 (r_C-, m_B+), relA1, supE44 Δ(lac-proAB), [F’ traD36, proAB lacF2ΔM15].</td>
<td>✔</td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>T-Vector Cloning</td>
<td>HB101</td>
<td>F’, thi-1, hsdS20 (r_B-, m_B+), supE44, recA13, ara-14, leuB6, proA2, lacY1, galK2 rpsL20, (str), xyl-5, mtl-1</td>
<td>✔</td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>Blue/White Screening</td>
<td>BMH 71–18 mutS</td>
<td>thi, supE, Δ(lac-proAB), [mutS::Tn10], [F’ proAB, lacF2ΔM15]. Mutagenesis</td>
<td></td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>Site-Directed Mutagenesis</td>
<td>ES1301 mutS</td>
<td>lacZ53, mutS201::Tn5, thyA36, rha-5, metB1, decC1, IN(rrnD-rrnE).</td>
<td></td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>Protein Expression</td>
<td>BL21 (DE3)pLysS(a)</td>
<td>F’, ompT, hsdR604(r_B-, m_B+), dcm, gal, (DE3), pLysS, Cm²</td>
<td></td>
<td>✔</td>
<td>✔</td>
</tr>
</tbody>
</table>

Table 15. Cloning Enzyme 10X Reaction Buffer Formulations.

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

* 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 MgCl2, 20mM DTT, 2mM ATP, 10% polyethylene glycol (MW 8000).

The Rapid Ligation Buffer enables performance of ligation reactions in as little as 1 hour.
ENZYME RESOURCE GUIDE

CLONING ENZYMES

General References

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 Cell 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 colormetric and chemiluminescent detection reagents.
Alpha Complementation: The process by which a functional β-galactosidase (lacZ) gene is generated when the lacZ α-peptide (N-terminus) complements the lacZ α-peptide (C-terminus). In general, a cloning vector contributes the lacZ α-peptide and the host bacterial strain provides the C-terminal complementation. 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 lacZ 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.
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 macromolecule 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: Dithiothreitol.
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 deoxynucleotidyl 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.
kₚ: Maximum number of substrate molecules converted to products per active site per unit time.
kDa: KiloDalton.
Kₘ: 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: 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.
s: 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 (kₚ): 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 bacteriophages.