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**Specifications of materials approved by SSC reference to indent No. 933 dated 26/04/2010**

- 1) **Oligonucleotide primer synthesis.** Primer length 17-80 bases long at **50nmol scale** and a minimum **OD of 20**. Provided as Desalted/Salt free pellet in screw cap 2 ml polypropylene tube supplemented with the synthesis data sheet. The requirement per demand will be raised for synthesis of single or multiple primers over a span of three years period. Total requirement for the three years period would be greater than 1000 bases with primer lengths ranging between 17-80 nucleotides per primer.
- 2) **High purity oligonucleotide primer synthesis.** Primer length 17-80 bases long at **100 nmol scale** and a minimum **OD of 50**. Provided as Desalted/Salt free pellet in screw cap 2 ml polypropylene tube supplemented with the synthesis data sheet. The requirement per demand will be raised for synthesis of single or multiple primers over a span of three years period. Total requirement for the three years period would be greater than 1000 bases with primer lengths ranging between 17-80 nucleotides per primer.
- 3) **Low electroendosmosis agarose (LE Agarose)** with following specifications  
*Chemical Analysis:*  
EEO (mr)  $\leq 0.12$   
Gelling Temp (1.5%)  $35^{\circ}\text{C} \pm 1.5^{\circ}$   
Melting Point  $89^{\circ}\text{C} \pm 1.5^{\circ}$   
Gel Strength (1%)  $\geq 1800 \text{ gm/cm}^2$   
Moisture  $< 6\%$   
Sulfate  $< 0.2\%$   
Ash  $< 0.2\%$   
*Quality Control:*  
Should not have nonspecific Endonuclease, Exonuclease or RNase activity. Must be suitable for **separation, elution and ligation of 100 bp to 30Kbp** DNA fragments.  
*Storage Conditions:*  
Powder stable at room temperature.  
Quantity 500 gram
- 4) **Gel elution Kit (250 preps);** Quantity; 1 Kit  
**Silica membrane based minispin cartridges** and associated solutions (gel melting, column wash, DNA elution) capable of **95% recoveries** in the range of **100 bp – 10 kb DNA** fragments and supplied with binding buffer, concentrated wash buffer, elution buffer, purification columns, collection tubes and detailed protocol. **The eluted DNA should be pure enough for restriction digestion, DNA sequencing, DNA ligation and labeling.**
- 5) **PCR Purification Kit (250 preps);** Quantity; 1Kit  
PCR Purification Kit for rapid and efficient purification of DNA from PCR and other enzymatic reaction mixtures. **Silica membrane based spin columns** capable of **90-100% recoveries in the range of 100 bp – 10 kb** and supplied with binding buffer, concentrated wash buffer, elution buffer, purification columns, collection tubes and detailed protocol. **The eluted DNA should be pure enough for restriction digestion, DNA sequencing, DNA ligation and labeling.**

**6) Miniprep Plasmid Isolation kit (250 preps); Quantity 1 Kit**

Efficient **silica membrane based spin columns** system for the isolation of high quality plasmid DNA from recombinant *E.coli* cultures. The **yield should be up to 20 µg** of high quality plasmid DNA **per 3 ml culture**. The kit should be provided with Resuspension solution, Lysis solution, Neutralization solution, Wash solution, RNase solution, Elution buffer, Silica membrane based spin columns, Collection tubes and detailed protocol. **The isolated plasmid DNA should be pure enough for restriction digestion, DNA sequencing, DNA ligation and labeling.**

**7) Restriction Enzymes**

The enzyme should be provided with specific buffers and any other required supplementary materials like BSA, Triton, Tween or DTT solutions. One unit of the restriction enzyme should completely digest 1 µg of lambda DNA in one hour (Weiss unit). The enzyme should preferably utilize a universal buffer. The prices should be quoted for only required number of units but not bulk packs. The specifications should be easily accessible as a brochure with the quote/supply or internet search using catalogue number, which should be clearly provided.

	<b>Restriction Enzymes</b>	<b>Quantity</b>	<b>Strength</b>
i)	Pfo1	200 units	10 units/µl
ii)	SSe83871 (SdaI)	300 units	10 units/µl
iii)	SgrD1	200 units	5 units/µl
iv)	Mss1	250 units	5 units/µl
v)	Swal (SmiI)	1000 units	10 units/µl
vi)	Asc1 (SgsI)	300 units	5 units/µl
vii)	Eco911	1000 units	10 units/µl
viii)	Eco811	500 units	10 units/µl
ix)	Cpo1	200 units	10 units/µl
x)	Not1	300 units	10 units/µl
xi)	Sgf1 (SfaAI)	1000 units	10 units/µl
xii)	Pac1	250 units	10 units/µl
xiii)	AdeI	500 units	10 units/µl
xiv)	SfiI	1000 units	10 units/µl
xv)	Sac1	1200 units	10 units/µl
xvi)	SacII (Cfr421)	1200 units	10 units/µl
xvii)	Xho1	2000 units	10 units/µl
xviii)	Xba1	1500 units	10 units/µl
xix)	HindIII	5000 units	10 units/µl
xx)	BamH1	4000 units	10 units/µl
xxi)	EcoR1	5000 units	10 units/µl
xxii)	Hpa1 (KspAI)	300 units	10 units/µl
xxiii)	Kpn1	4000 units	10 units/µl
xxiv)	Cla1 (Bsu151)	600 units	10 units/µl
xxv)	Sph1 (PaeI)	500 units	10 units/µl

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- 8) Pfu DNA Polymerase,** Quantity; 100 units (2.5 units / $\mu$ l)  
Highly thermostable (retaining 95% activity after 2 hours incubation at 95°C). Should generate blunt ended PCR products and capable of incorporating normal as well as modified nucleotides (e.g. biotin, digoxigenin, fluorescently-labeled nucleotides). The enzyme should catalyze the template-dependent polymerization of nucleotides into duplex DNA in the 5' to 3' direction. *Pfu* DNA Polymerase should also exhibit 3' to 5' exonuclease (proofreading) activity, that could enable the polymerase to correct nucleotide incorporation errors. It should have no 5' to 3' exonuclease activity. One unit of the enzyme could be able to catalyze the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 min at 72°C. The error rate of *Pfu* DNA Polymerase should not be higher than  $2.6 \times 10^{-6}$  errors per nt per cycle. The accuracy of PCR should be  $3.8 \times 10^5$ . The enzyme should be supplied with the following two buffers as the normal components of enzyme/buffer system. i) 10X *Pfu* Buffer with 20 mM MgSO<sub>4</sub> and ii) 10X *Pfu* Buffer without MgSO<sub>4</sub>. The specifications should be easily accessible as a brochure with the quote/supply or internet search using catalogue number.
- 9) T4 DNA Ligase** 1000 Weiss Units (5 Weiss units/ $\mu$ l)  
The enzyme should catalyze the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA. The enzyme must be capable of repairing single-strand nicks in duplex DNA, RNA or DNA/RNA hybrids. It should efficiently join DNA fragments with either cohesive or blunt termini, but should not have such activity on single-stranded nucleic acids. The enzyme should remain 100% active in restriction enzyme, PCR and RT buffers, supplemented with ATP. One unit (Weiss unit) of the enzyme should catalyze the conversion of 1 nmol of [<sup>32</sup>PP<sub>i</sub>] into Norit-adsorbable form in 20 min at 37°C. There should be no endo-, exodeoxyribonucleases, phosphatases and ribonucleases activities associated with the enzyme and the quality tests on the enzyme must be there on the product insert. The enzyme should be supplied with 10X T4 DNA Ligase Buffer. The specifications should be easily accessible as a brochure with the quote/supply or internet search using catalogue number.
- 10) T4 DNA Polymerase;** Quantity 100 units (5 units / $\mu$ l)  
The enzyme should catalyze 5' to 3' DNA synthesis from primed single-stranded DNA. The enzyme should have a 3' to 5' exonuclease activity, but lack 5' to 3' exonuclease activity. Should have stronger 3' to 5' exonuclease activity on single-stranded than on double-stranded DNA. The exonuclease activity should be more than 200 times stronger than *E. coli* DNA polymerase I and Klenow fragment for successful blunting of DNA ends through filling 5'-overhangs or/and removal of 3'-overhangs. Should remain active in restriction enzyme, PCR, RT and T4 DNA Ligase buffers. One unit of the enzyme should be able to catalyze the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 min at 37°C. The enzyme should be provided with 5X reaction buffer. The specifications should be easily accessible as a brochure with the quote/supply or internet search using catalogue number.
- 11) Taq DNA Polymerase (recombinant),** Quantity; 500 Units (5 units / $\mu$ l)  
Thermostable with half life more than 40 min at 95°C. Should generate PCR products with 3'-dA overhangs and supplied with two buffers – 10X Taq Buffer with KCl and 10X Taq Buffer with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> for testing at wide range of magnesium concentrations and decreases unspecific priming. The 25mM MgCl<sub>2</sub> should also be supplied. The enzyme

should be capable of incorporating modified nucleotides (e.g., biotin-, digoxigenin-, fluorescently-labeled nucleotides). For routine PCR the amplification of DNA fragments up to 5 kb should be possible. The enzyme should catalyze 5' to 3' synthesis of DNA with no detectable 3' to 5' exonuclease (proofreading) activity. It should possess negligible or low 5' to 3' exonuclease activity. In addition, Taq DNA Polymerase should exhibit deoxynucleotidyl transferase activity to add extra adenines at the 3'-end of PCR products. One unit of the enzyme should catalyze the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 min at 70°C. The error rate of Taq DNA Polymerase should not be more than  $2.2 \times 10^{-5}$  errors per nt per cycle, and the accuracy of PCR should be  $4.5 \times 10^4$ . The enzyme must be devoid of *E.coli* DNA and non-specific amplifications must not be obtained in a template free PCR. The specifications should be easily accessible as a brochure with the quote/supply or internet search using catalogue number.

**12) dNTPs;** 100mM each (not mixed) Quantity; one set

One vial each for dATP, dCTP, dTTP and dGTP. The set should comprise of 4 x 0.25 ml (4 x 25 µmol of 100 mM solution). Greater than 99% pure and confirmed by HPLC. Free of human and *E.coli* DNA. Should be highly stable at neutral pH. The stability should meet a minimum of 100 freeze-thaw cycles. 90-95% of dNTPs should remain in triphosphate form even after 7 weeks at room temperature. 85-90% of dNTPs should remain in triphosphate form after 30 cycles of PCR (1 min at 94°C; 3 min at 72°C). Designed especially for high efficiencies in long range PCR (40 kb), cDNA synthesis, RT-PCR, real-time PCR, standard PCR, and high fidelity PCR. The specifications should be easily accessible as a brochure with the quote/supply or internet search using catalogue number.

**13) Taq Master Mix (2x);** Quantity; 1000 reactions

20 x 1.25 ml (for 1000 reactions of 50 µl each). The mix should have high sensitivity Taq polymerase for efficient amplification of at least 6 Kb DNA fragments from genomic DNA and up to 20 Kb from viral DNA. The amplified DNA fragments should have 3'-dA overhangs for efficient ligation in TA vector. Capable of incorporating modified nucleotides and supplied with nuclease free water. The specifications should be easily accessible as a brochure with the quote/supply or internet search using catalogue number.

**14) SYBR Green/Fluorescein qPCR Master Mix (2X);** Quantity 200 reactions

2 x 1.25 ml (for 200 react. of 25 µl each). Ready-to-use solution optimized for quantitative real-time PCR and two-step real-time RT-PCR with the Bio-Rad iQ machine. The master mix should include Hot Start Taq DNA Polymerase and dNTPs in an optimized PCR buffer. The reaction mix should contain SYBR Green I dye supplemented with fluorescein passive reference dye. Only template and primers should be required. The fluorescein included in the master mix should allow for the collection of Dynamic Well Factors with the Bio-Rad iQ5 machine, but should not affect qPCR efficiency. The dUTP should be included in the mix for optional carryover contamination control using uracil DNA glycosylase (UDG). The nuclease free water should also be a component of the kit for volume adjustments.

**15) TA cloning kit for PCR products;** Quantity 1 kit (30 reactions per kit)

The TA kit should be specifically designed for one-step cloning of PCR products with 3'-dA overhangs generated by Taq DNA polymerase and other thermostable DNA polymerases (e.g. Tth, Tfl) which lack proofreading activity. The 3'-ddT overhangs at both ends of the

cloning site should prevent recircularization of the vector during ligation and should be stable enough to withstand several rounds of repeated freezing and thawing cycles. The TA vector should be capable of yielding atleast 90% recombinant clones upon ligation. The TA vector backbone must contain a wide variety of restriction sites (MCS) around the cloning site for efficient mapping and manipulations of the cloned insert. The backbone vector should also have M13F and M13R primer sites (one on each side of the cloning site) for direct sequencing of the cloned fragment. The T7 promoter should be available in close proximity to the cloning site for in vitro transcription studies.

## Plasticware

- 16) Disposable and sterile plastic petriplates for plant tissue culture, size 90-100mm (dia) and 15-16mm deep, ; 20 per pack. Quantity 2,000 plates
- 17) 1.6 ml Eppendorf tubes. Clear polypropylene, autoclavable. Unbreakable at 14,000 rpm/16,000 rcf. The tubes should withstand liquid nitrogen. The tubes should not break when spun after liquid nitrogen treatment. The lid/cap should provide enough grip surface for easy opening/closing of tubes at ultra low temperature. Frosted lid surface for writing. Calibrations for volume on the tube surface should be engraved or printed.  
Required in Bulk Pack. Quantity: 5000 tubes
- 18) 0.2 ml thin walled autoclavable and clear polypropylene PCR tubes, **optically transparent cap/lid**, required in bulk pack. Quantity: 4,000 tubes
- 19) 200  $\mu$ l **ridged** tips, clear polypropylene and autoclavable, should accurately fit Gilson, Eppendorf and BioHit pipettman. Required in bulk pack. Quantity: 5,000 tips
- 20) **Ridged** 10  $\mu$ l tips, clear polypropylene and autoclavable,, should accurately fit Gilson, Eppendorf and BioHit pipettman. Required in bulk pack. Quantity: 5,000 tips
- 21) **Ridged** 1 ml pipette tips, light blue or clear polypropylene and autoclavable, should accurately fit Gilson, Eppendorf and BioHit pipettman. Required in bulk pack. Quantity; 5000 tips

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