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Development of Midi-Plasmid Isolation Kit and Cost Optimization of DNA Molecular Weight Ladder: Economy of Laboratory vs. Commercial Products

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ABSTRACT

Major impediments to biotechnology research are the excessive operational expenses involved in each set of experimental procedures not only due to use of high purity chemicals and enzymes but also various molecular biology Kits and other commercial products. This is a work which envisages to establish primary molecular biology protocols in lab to produce economical kits and products which will not only bring down the operational expenses, but also aid in the laboratory courses of the college. Preparation of competent cells of various E. coli strains is a mandatory requirement for transformation and cloning. The plasmid isolation protocol used for this purpose was midi preparation. Consequently, Plasmid Transformation, Restriction and a low cost molecular weight composite ladder (pBR322/ Hinf I+BamH I) has been prepared. The cost of ladder production could be reduced further by lowering the concentration of Restriction Enzyme and/ or increasing the incubation time for restriction digestion. Finally the cost of commercially available kits and ladder are compared to obtain the effective economy achieved in laboratory. These attempts will be beneficial as these standardized, economically efficient protocols/ products for competent cell preparation, plasmid isolation and molecular weight DNA ladder generation could be used for routine Laboratory experiments and embedded, even into critical experimental procedures.

Key Words: Plasmid, pBR322, DNA Ladder, Restriction Digestion, Agarose Gel Electrophoresis

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INTRODUCTION

Major impediments to biotechnology research are the excessive operational expenses involved in each set of experimental procedures not only due to use of high purity chemicals and enzymes but also various molecular biology Kits and other commercial products (e.g. DNA molecular

weight ladders, bacterial strains, cloning vectors, etc.) [6, 13, 14]. Establishment of efficient research laboratory requires generation and conservation of resources along with reduction in the operational cost of experiments. The first step in this direction is to analyze operational cost of each protocol (Chemicals, Glassware's, plastic wares and other misc. items) [18, 19, 20], thereafter planning experiments judiciously so that the wastage, repetitions etc. could be minimized. Subsequently, accessing what requirements could be avoided, supplemented or produced indigenously at much affordable rate than that available commercially. Also, it is very important to follow various aspects of Good Laboratory practices viz. Quality control (water, consumables and equipments), lab record maintenance, and general management and hygiene of the laboratory and accurate functioning of equipments.

This work envisages establishing primary molecular biology protocols in lab to produce economical kits and products which will not only bring down the operational expenses, but also aid in the laboratory courses [23]. The products could also be commercialized by transfer of technology or with a private partnership [12]. A recent example in this regard is collaboration between Anna University, Chennai and company Medox Biotechnology, Chennai India. They have launched new product profile at a significant lower cost then that available in the market. The advantage of such an effort are enormous in terms of sensitizing researchers of the cost involved in a protocol and also the most critical and expensive steps. The actual production cost and the commercial rates could be compared, which may indicate whether the rates offered by the companies are justified. It may even initiate some young minds for any possibility of entrepreneurship opportunity. Several such procedures and protocols were studied and few of the protocols which are very frequently employed in biotechnological research are- Genetic Transformation [26], Cloning [4, 5, 16] and Agarose Gel Electrophoresis. Preparation of competent cells of various *E. coli* strains is a mandatory requirement for transformation [8] and cloning. Similarly, plasmid isolation is a readily implemented procedure [1, 2, 9, 22, 27, and 28]. Hence, attempts were made to reduce the operational costs for both these processes, by modifying the standard protocols for economy and efficiency. This work has been undertaken with the primary objective to further reduce the operational expenses of the plasmid isolation and consequently [7, 25], DNA ladder production, by producing plasmid on larger scale using Midi Prep [10, 17]. The Midi plasmid isolation protocol was designed especially suited for using "Oakridge Tube", which will help in obtaining plasmid in larger quantity and also eliminating the expenses of many other plastic wares (which are not reusable) [24,21]. The cost of ladder production could be reduced further by lowering the concentration of Restriction Enzyme [15, 11, 12] and/ or increasing the incubation time for restriction digestion [26, 4]. Finally the cost of commercially available kits and ladder was compared to obtain the effective economy achieved in laboratory [6, 9]. These attempts will be beneficial as these standardized, economically efficient protocols/products for competent cell preparation, plasmid isolation and molecular weight DNA ladder generation could be used for routine Laboratory experiments and embedded, even into critical experimental procedures.

MATERIALS AND METHODS

Bacterial Strain

Escherichia *coli* (k12) JM101 *(F TraD36, Δ (lac-proAB), LacIqΔ (lacZ) M15, Pro* A^+B^+*)*

Media
LB Broth, LB Agar, Nutrient agar

Reagents/Chemicals
Plasmid isolation
GTE, STE, Lysis solution, neutralizing solution, TE Buffer, Glucose solution, Isopropanol, Ethanol, Lysozyme, RNaseA, Phenol: Chloroform: Isoamyl alcohol.

Transformation
Calcium chloride (ice cold), LBplate + Amp, LB medium, single colony culture of E.*coli*.

Restriction analysis
Hind III digested λ DNA, Isolated Plasmid, Restriction buffers, Agarose, TAE/TBE, EtBr, Bromophenol blue

Plasmid used
pBR322 is an *E. coli* plasmid cloning vector 4361 bp in length containing the origin of replication from pMB1(a plasmid in the ColE1 compatibility group). It is also a high copy number plasmid with copy no.~15-20 [3], >25 [4].

Procedure
As an initial step the bacteria was Cultured for overnight in LB medium.

- ***E. coli* competent cell preparation**
  The competent cells of *E. coli* strain JM101 were prepared by $CaCl_2$ method [24].
- **Transformation of plasmid to *E. coli***
- **Plasmid isolation**
  Preparation of Plasmid DNA by Alkaline Lysis with SDS [1, 2] using **Specialized Midi Protocol (for 50 ml Oakridge Tube)**
- **Restriction Digestion**

Table 1: Composition of respective buffers used *(Final conc. in mM (1X))*

R.Enzyme	Buffer(pH)	TrisHCl	NaCl	MgCl2	DTT
BamHI*	B (pH=8.0)	10	100	10	1
HinfI, HindIII	C (pH=7.8)	10	50	10	1

**Requires BSA (100µg/ml) for optimum activity.*

RESULTS AND DISCUSSION

Primarily all the media & reagents were prepared in accordance to the prescribed standard compositions using triple distilled water. The methodology specified in the section Material & methods was undertaken stringently. The results and discussion in this section are being summarized in following sub sections; **1.** Competent Cell Preparation, Plasmid Transformation, along with cost analysis and estimation of achieved economy. **2.** Plasmid Isolation using Mini Prep and Various Midi Prep

Protocols, along with cost analysis and estimation of achieved economy. **3.** Restriction Digestion Analysis of Plasmid pBR322, along with cost analysis and estimation of achieved economy. **4.** Estimation of Achieved Economy in Development of DNA Molecular Weight Ladder and Plasmid Isolation Midi Kit.

1. Competent Cell Preparation and Plasmid Transformation

The competent cells of *E. coli* strain JM101 (Figure 1) is prepared for transformation with plasmid pBR322 (Figure 2).

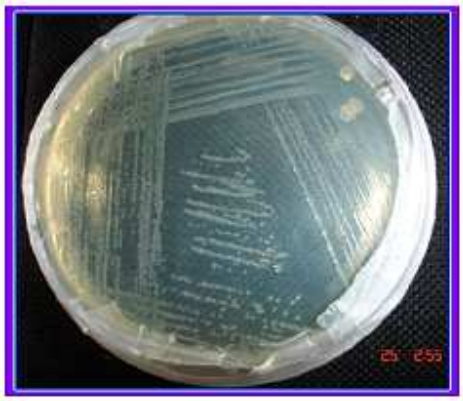

Figure 1: Native strain *E. coli* (K12) JM101 Streaked plate LB agar (from our laboratory).
(Genotype JM101: *F TraD36, Δ (lac-proAB), LacIq Δ (lacZ) M15, Pro A+B+, glnV, thi*

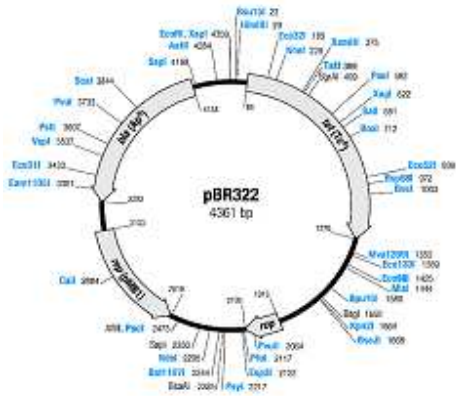

Figure 2: Restriction map of pBR322

Enzymes which cut pBR322 DNA once:

AatII 4284, AflIII 2473, BamHI 375, BoxI 712, Bpu10I 1580, BsaAI 2225, BseJI 1668, BsgI 1650, Bsp68I 972, Bst1107I 2244, Bsu15I 23, BveI 1063, CaiI 2884, Eam1105I 3361, Eco31I 3433, Eco32I 185, Eco52I 939, Eco88I 1425, Eco130I 1369, EcoRI 4359, Esp3I 2122, HindIII 29, Kpn2I 1664, MlsI 1444, Mva1269I 1353, NdeI 2295, NheI 229, PaeI 562, PfoI 2117, PscI 2473, PstI 3607, PsyI 2217, PvuI 3733, PvuII 2064, SalI 651, SapI 2350, ScaI 3844, SgrAI 409, SspI 4168, TstI 389, VspI 3537, XagI 622, XapI 4359

A single colony bacterial culture of *E. coli* strain JM101 was maintained in conical flasks & Mc-cartney bottles. As soon as the OD at (600nm) reached a value 0.375 the cells were imposed to $CaCl_2$ competent cell preparation protocol. The standard protocol prescribed the storage of

prepared competent cells at -70°C, but we stored the cells at 0°C. In brief the following modifications were made in the standard protocol for its optimization, as shown below.

Table 2: Comparison of Standard and Modified Protocol

Parameter	Standard Protocol	Used/ Modified
Incubation (shaking)	250rpm	200rpm
Volume cultured	25ml	-do-
Optical Density	0.40	0.375
Rest steps	-	-do-
Aliquot storage	-70°C	0°C

The competence of prepared cells was checked by transformation with plasmid pBR322 plated on selection medium LB Ampicillin (50 mg/L). These competent cells were stored as 200 µL aliquots. The transformation efficiency was found to be sufficiently high (1.3×10^6 cfu/ µg pBR322 DNA, on LB-ampicillin plates at 50 mg/L concentration, as shown below) and was comparable to the cells preparation as recommended for commercial kit.

Table 3: Transformation efficiency of competent cell

1. 200 µL competent cells/ aliquot + 10 ng pBR322 was taken for transformation		
2. The culture was made up to 1.0 ml with LB		
3. different amount of culture was spread on LB-ampicillin plates 50 mg/L concentration		
Amount of culture	**No of colonies**	**Transformation efficiency**
25 µL	-	-
75 µL	~ 1000 no	1.3×10^6 cfu/ µg pBR322 DNA
150 µL	-	-

The transformation efficiency of the prepared competent cells was further checked a month later and was found that the efficiency was unperturbed as checked by other transformations (data not shown). The transformed bacteria obtained by spread plating are shown in figure 3. Subsequently, the cost analysis was made for competent cell preparation and transformation protocol and comparison was made with the kits available commercially.

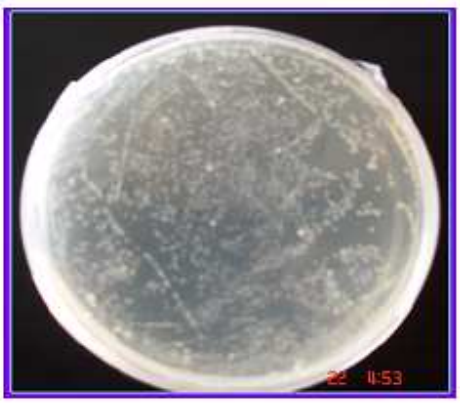

Figure 3: Transformants of *E.coli* JM101 with pBR322, Spread plated on LB-ampicillin (50 mg/L). (From our laboratory)

1.1. Cost Analysis of stock for Competent Cell prep. & Transformation

The cost of chemicals, reagents/ stock solution was initially calculated. Thereafter, the operational cost of the protocol is calculated, depending upon the quantity of each stock required in various steps. Subsequently, the cost of indigenous protocol was calculated according to the amount of reagents provided in the commercially available Kits.

Table 4: Cost Analysis of Stock Component

S. No.	Chemical	Rate (Rs.)	Amount	Cost(Rs)
1.	**Calcium chloride (100 ml)**			
	Calcium chloride	384/100gm	14.75gm	**11.328**
2.	**LB Broth (1000 ml)**			
	Tryptone	1555/500gm	10gm	31.1
	Yeast extract	970/500gm	5gm	9.70
	NaCl	95/500gm	5gm	0.95
	NaOH	1.84/100ml	1ml(1M)	0.0184
				41.7684
3.	**LB Agar (1000 ml)**			
	Tryptone	1555/500gm	10gm	31.1
	Yeast extract	970/500gm	5gm	9.70
	NaCl	95/500gm	5gm	0.95
	NaOH	1.84/100ml	1ml(1M)	0.0184
	Agar	1875/500gm	15gm	56.25
				98.0184
4.	**Ampicillin**	278/gm	1.4mg	**0.417**

The cost analysis of stock components used for preparation of competent cells and transformation indicates that the major effect is due to LB media and the cost of agar significantly enhances the cost. Therefore use of solid media must be made judiciously.

1.2. Cost Analysis: Competent cell preparation & Transformation reaction

The cost analysis of all the requirements for preparation of 2000 µL is provided in the following table. The major cost effecting requirements are LB agar and LB broth, hence the wastage and optimization of media use is important. The cost of 2000 µL competent preparation required Rs. 4.166, hence 5000 µL of competent cells costs Rs. 10.42, as compared to the cost of commercially available Kit for same amount of competent cell production at Rs. 4250.00. Thus it is obvious that the company is charging enormously for a comparatively simple procedure or in other words there is good profit margin in commercial production of competent cell preparation and transformation Kit.

Table 5: Cost Analysis of Reagents

S. No.	Chemical	Rate(Rs.)	Amount	Cost(Rs.)
01.	**Calcium chloride (1M)**	11.328/ 100ml	2.2ml	0.249
02.	**Ampicillin**	278/ gm	1.4mg	0.417
03.	**LB Agar**	10.8/ 100ml	25ml	2.45
04.	**LB Broth**	4.2/ 100ml	25ml	1.05
Cost Per reaction (2000 µl) competent cell preparation				**Rs. 4.166**

1.3. Estimation of achieved economy

Table 6: Cost analysis and profitability

Cost Per reaction (5000 µl)Competent Cell preparation and Transformation	
Commercial Preparation	**Rs. 4250.00**
Laboratory Preparation	**Rs. 0010.42**
No of Kits produced for Rs. 4250.00	**~400 no**
Estimated sales value of 400 Kits(@ Rs. 4250.00)	**17,00,000.00 (17 Lakhs)**

2. Plasmid Isolation using Mini Prep and Various Midi Prep Protocols

The selected transformants (Single colony of bacteria grown on LB ampicillin plates 50 mg/L) were used for plasmid isolation using alkaline lysis miniprep & the exact protocol as given below was followed. For plasmid isolation the Optical Density of transformed bacterial cells was 0.58 (miniprep and midiprep) at 600 nm. The modifications made in the standard protocol (Material and Method), is presented comparatively in the next sub section.

Table 7: Modifications made in Standard Protocol (Minipreparation)

Parameter	Standard	Used
Incubation (shaking)	250 rpm	200 rpm
Volume culture/ tube	1.5ml	2.0ml
Optical Density	0.40	0.58 (0.5-0.6)
STE	Optional	Added
Lysis soln. I, II, III added	-	-do-
Storage/ Pl checking	-	Yes
Phenol Extraction	Optional	Once
Incubation at RT	5min.	5min-RT & Overnight- 0^oC
Rest steps	-	-do-
Stored in	TE	TE/ H_2O
Aliquot storage	-70^oC	$0\text{-}4^oC$

Table 8: Modifications made in Standard Protocol (Midipreparation)

Parameter	Standard	Used
Incubation (shaking)	250 rpm	200 rpm
Volume culture/ tube	10ml	35ml
Optical Density	0.40	0.58 (0.5-0.6)
STE	Optional	Added
Lysis solution I,	200 µl	750µl, 937µl (25%*), 1125µl (50%*)
Lysis solution II	400 µl	1500µl, 1875µl (25%*), 2250µl (50%*)
Lysis solutionIII	300 µl	1100µl, 1875µl (25%*), 1650µl (50%*)
Storage/ Pl checking	-	Yes
Phenol Extraction	Optional	Once
Incubation at RT	5min.	5min-RT & Overnight- 0^oC
Rest steps	-	-do-
Stored in	TE	TE/ H_2O
Aliquot storage	-70^oC	$0\text{-}4^oC$

** Extra amount added as compared to normal (Material and Methods)*

The incubation step in standard protocol prescribes 5 min of incubation at RT but the vials were left for overnight incubation at 0^{o}C. It was observed that the plasmid isolated from the overnight incubated vials gave better yield as compared to the sample isolated on same day (previous results from our lab). The appearance of plasmid bands in the gel indicates the conformations it exists in the host cell, which in turn depends on the specific combination of host genotype and type of plasmid. The higher molecular weight of plasmid is due to multimers and nicked conformation. The multimers are created during plasmid replication and also due to homologous recombination as the strain (JM101) is a $recA^{+}$ host.

In previous reports (standard protocols), there were no evidence whether the plasmid isolation protocol could be discontinued and supernatant stored after addition of solution III followed by centrifugation. It was also observed that the supernatant could be tested for presence of plasmid at this step itself in addition to supernatant storage. This fact is evident from figure 4. Lane no 3, 7 and 8 confirmed presence of plasmid from mini-prep protocol and lane no 4, 5 and 6 from midi-prep (25%, 50% and normal respectively). Thus the plasmid confirmation could be done at a much earlier stage and further successful plasmid isolation confirmed that the protocol could be discontinued at this stage. The supernatant was stored at 0^{o}C for further use.

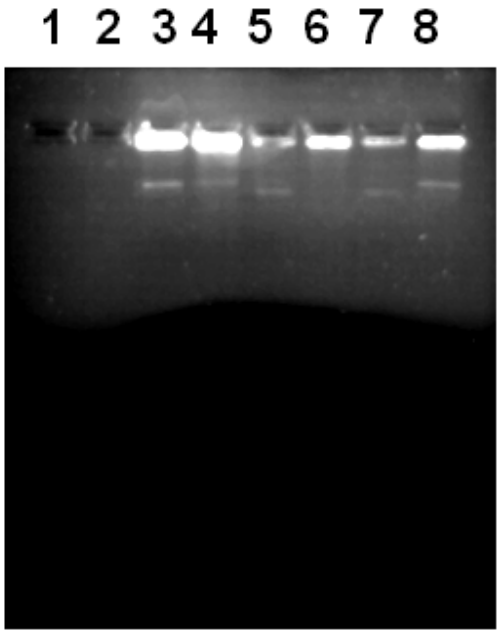

Figure 4: Isolation of Plasmid (pBR-322/ JM-101)

Lane 1&2: *Blank*
Lane 3: *Plasmid (Miniprep**)***
Lane 4: *Plasmid (Midiprep, 25% extra solution)*
Lane 5: *Plasmid (Midiprep, 50% extra solution)*
Lane 6: *Plasmid (Midiprep, Normal)*
Lane 7: *Plasmid (Miniprep)*
Lane 8: *Plasmid (Midiprep)*
(All samples immediately after addition of solution III)
Agarose Gel (0.8%, TBE)

The figure 5 provides comparative results for various variations of midi-prep protocol (lane 2-7 no) in comparison to mini-prep protocol (lane no 8). The best results for plasmid isolation were obtained when 50% extra solutions (Lane 2 and 3) in comparison to 25% extra (Lane 4 and 5) and normal (Lane 6 and 7) solutions (alkaline lysis solution I. II and III) were used. However, it is not sure whether the poor result for normal solutions were because of handling error or protocol itself.

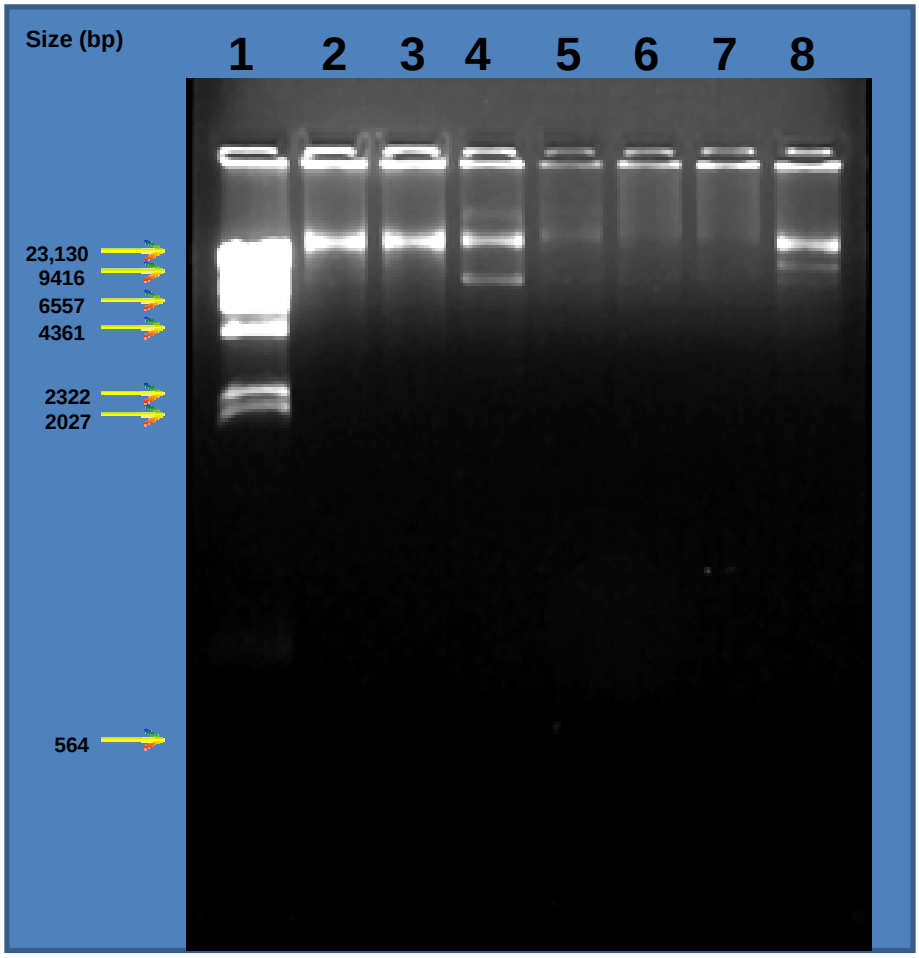

Figure 5: Standardization of Plasmid pBR-322 isolation using various Midi Prep Protocols.

Lane 1: *Marker- λ DNA/ Hind-III*
Lane 2: *Plasmid (50% extra solution, 0.32 µg)*
Lane 3: *Plasmid (50% extra solution)*
Lane 4: *Plasmid (25% extra solution, 0.25 µg)*
Lane 5: *Plasmid (25% extra solution)*
Lane 6: *Plasmid (normal solution)*
Lane 7: *Plasmid (normal solution)*
Lane 8: *Plasmid (miniprep, 0.25 µg)*
Agarose Gel (0.8%, TBE, sample 7µl+5µl Dye)

Table 9: Approximate quantification of the amount of plasmid (µg) in various protocols

Sample	Concentration (7.0µl)	Used	Total amount
Midi-prep			
50% extra Solution	0.32 µg	500 µl	**22.86 µg**
25% extra Solution	0.25 µg	500 µl	**17.85 µg**
Normal	Not specified	500 µl	-
Mini prep			
	0.25 µg	25 µl	**0.89 µg**

Subsequently, the cost analysis of the components for plasmid isolation and preparation of midi-prep kit and DNA molecular weight ladder is in next section.

2.2 Cost Analysis of stock components for Plasmid Isolation

The cost of chemicals, reagents/ stock solution was initially calculated. Thereafter, the operational cost of the protocol is calculated, depending upon the quantity of each stock required in various steps. Subsequently, the cost of indigenous protocol was calculated according to the amount of reagents provided in the commercially available Kits.

Table 10: Cost Analysis of stock components

S. No.	Chemical	Rate (Rs.)	Amount	Cost(Rs.)
01.	**Tris.HCl buffer (1M, pH 8.0), 100 ml**			
	Tris HCl	572/100gm	7.16gm	**40.95**
	Tris Base	1780/500gm	6.62gm	**23.56**
				64.51
02.	**EDTA (500mM, pH 8.0), 100 ml**			
	EDTA	119/100gm	18.61gm	**22.14**
	NaOH	148/500gm	2gm	**0.592**
				22.732
03.	**Potassium acetate (5M, pH 4.8), 100ml**			
	Potassium acetate	190/500gm	25.95gm	**9.861**
	Acetic acid	167/500ml	13.476ml	**4.5**
				14.361
04	**Glucose (1%w/v), 50 ml**			
	Glucose	124/500gm	5.0gm	**1.24**
05.	**Isopropanol (abs.), 50ml**			
	Isopropanol	215/500ml	50ml	**21.5**
06.	**Ethyl Alcohol (70%), 100ml**			
	Ethyl Alcohol	675/500ml	100ml (70%)	**94.5**
07.	**Ethyl Alcohol (Abs.), 100ml**			
	Ethyl Alcohol	675/500ml	100ml	**135.0**
08.	**Lysozyme (10mg/ml), 1ml**			
	Lysozyme	1095/gm	10mg	**10.95**
	Tris-Cl	40.95/100ml	1ml	**0.4095**
				11.3595
09.	**RNaseA (10mg/ml), 1ml**			
	RNaseA	1870/100mg	10mg	**187.00**
10.	**Tris.HCL saturated Phenol 40ml**			
	Phenol	290/500gm	50ml	**31.05**
	Tris Base	1780/500gm	10gm	**35.6**

				66.65
11.	**Phenol: Chloroform: Isoamyl alcohol 80ml**			
	Phenol	66.65/40ml	40ml	**66.65**
	Chloroform	270/500ml	38.4ml	**20.736**
	Isoamyl alcohol	290/500ml	1.6ml	**0.928**
				88.314
12.	**Sodium Chloride (1M), 100ml**			
	NaCl	95/500gm	5.85gm	**1.12**
13.	**Sodium hydroxide (1M), 100ml**			
	Sodium hydroxide	148/500gm	4.0gm	**1.184**
14.	**GTE, 100ml**			
	Glucose	1.24/50ml	9ml (1%w/v)	**0.223**
	Tris. HCl	64.51/100ml	2.5ml (1M)	**1.6128**
	EDTA	22.732/10ml	2ml (500mM)	**0.4546**
				2.2904
14.	**STE, 50ml**			
	NaCl	1.12/100ml	5ml (1M)	**0.056**
	Tris.HCl	64.51/100ml	0.5ml (1M)	**0.323**
	EDTA	22.732/10ml	100µl (500mM)	**0.023**
				0.402
15.	**NaOH-SDS, 100ml**			
	NaOH	1.184/100ml	20ml (1M)	**0.249**
	SDS	737/100gm	10ml (1%)	**0.737**
				0.986
16.	**TE Buffer, 100ml**			
	TrisHCl	64.51/100ml	1ml (1M)	**0.645**
	EDTA	22.732/100ml	200µl (500mM)	**0.046**
				0.691

The cost of Tris.Hcl buffer, Ethanol and RNAse is the major cost effecting factors and must be used judiciously. It is observed that the Tris.Hcl buffer must be formed strictly following Henderson-Hasselbatch equation and not by adjusting the pH of Tris base with HCL. This is because once the pH plateau in the buffering range is reached the changes in it cannot be accurately determined by pH-meter. Also there are chances of accidental addition of excess amount of HCL and the solution is of no use now. Another use of Tris.HCL buffer is for saturation of phenol, where in order to quickly reach the desired pH large amount of buffer may be wasted. In such case first use Tris base to adjust the pH and in the second step use Tris.HCL to finely adjust the pH to desired level. Ethanol must be economically used in quantity and isopopanol may be used for precipitation. RNAse use should be optimized and the incubation time should be instead increased. One way is to add RNAse with Solution I and thereby most of enzyme could be removed during Phenol/ chloroform/ isoamyl alcohol extraction.

The cost analysis of components has been provided below calculated for 10 preparations for comparison with commercially available Kits.

2.3 Cost Analysis of components for Plasmid Isolation (10 preparation)

Table 11: Mini-prep Plasmid Isolation (10 preparation)

S. No.	Chemical	Rate(Rs.)	Amount	Cost(Rs.)
01.	**STE**	0.402/50ml	6.0ml	**0.048**
02.	**GTE**	2.290/100ml	1.0ml	**0.023**
03.	**RNase**	187.0/ml	30µl	**5.610**
04.	**Lysozyme**	11.3595/ml	20µl	**0.226**
05.	**Solution II (NaOH SDS)**	0.986/100ml	2.0ml	**0.020**
06.	**Solution III (K-acetate)**	14.33/100ml	1.5ml	**0.215**
07.	**Phenol/Chloroform/ Isoamyl alc**	88.314/80ml	4.5ml	**4.968**
08.	**Ethyl alcohol**	135/100ml	6.0ml	**8.11**
09.	**Ethyl alcohol (70%)**	94.5/100ml	3.6ml	**3.402**
		Gross		**Rs.22.622**

Table 12: Midi-prep Plasmid Isolation (10 preparation)

i. Using solutions in normal calculated amount

S. No.	Chemical	Rate(Rs.)	Amount	Cost (Rs.)
01.	**STE**	0.402/50ml	90ml	**0.7236**
02.	**GTE**	2.290/100ml	7.5ml	**0.1717**
03.	**RNase**	187.0/ml	20µl	**3.74**
04.	**Lysozyme**	11.3595/ml	70µl	**0.795**
05.	**Solution II (NaOH SDS)**	0.986/100ml	15ml	**0.1479**
06.	**Solution III (K-acetate)**	14.33/100ml	11ml	**1.5763**
07.	**Phenol/Chloroform/ Isoamyl alc**	88.314/80ml	40 ml	**44.157**
08.	**Isopropanol**	21.5/50ml	19.8 ml	**8.514**
09.	**Ethyl alcohol (70%)**	94.5/100ml	10ml	**9.45**
10.	**LB broth**	4.2/ 100ml	350 ml	**14.70**
		Gross		**Rs.83.98**

ii. Using solution 25% extra than calculated amount

S. No.	Chemical	Rate(Rs.)	Amount	Cost(Rs.)
01.	**STE**	0.402/50ml	90ml	**0.7236**
02.	**GTE**	2.290/100ml	9.3ml	**0.2130**
03.	**RNase**	187.0/ml	20µl	**3.74**
04.	**Lysozyme**	11.3595/ml	70µl	**0.795**
05.	**Solution II (NaOH SDS)**	0.986/100ml	18.75ml	**0.1849**
06.	**Solution III (K-acetate)**	14.33/100ml	13.75ml	**1.97**
07.	**Phenol/Chloroform/ Isoamyl alc**	88.314/80ml	40 ml	**44.157**
08.	**Isopropanol**	21.5/50ml	25 ml	**10.75**
09.	**Ethyl alcohol (70%)**	94.5/100ml	10ml	**9.45**
10.	**LB broth**	4.2/ 100ml	350 ml	**14.70**
		Gross		**Rs.86.68**

iii. **Using solutions 50% extra than calculated amount**

S. No.	Chemical	Rate(Rs.)	Amount	Cost(Rs.)
01.	**STE**	0.402/50ml	90ml	**0.7236**
02.	**GTE**	2.290/100ml	11.25ml	**0.2576**
03.	**RNase**	187.0/ml	20µl	**3.74**
04.	**Lysozyme**	11.3595/ml	70µl	**0.7951**
05.	**Solution II (NaOH SDS)**	0.986/100ml	22.5ml	**0.22185**

06.	**Solution III (K-acetate)**	14.33/100ml	16.5ml	**2.3645**
07.	**Phenol/Chloroform/ Isoamyl alc**	88.314/80ml	40ml	**44.157**
08.	**Isopropanol**	21.5/50ml	30ml	**12.9**
09.	**Ethyl alcohol (70%)**	94.5/100ml	10ml	**9.45**
10.	**LB broth**	4.2/ 100ml	350 ml	**14.70**
		Gross		**Rs.89.40**

The cost benefit analysis indicates that 50% extra solution produces significantly higher yield of plasmid Section 2.1b and Figure 5 at no significant cost increase (84, 87 and 89 Rs. Respectively, as calculated above). The major cost impediments use of RNase, Phenol/ chloroform/ isoamyl alcohol purification and use of ethanol. Care must be taken in using these ingredients and must be substituted with other cheaper alternative available.

The work is still open for plasmid isolation through maxi protocol. The estimated cost for plasmid isolation using maxi protocol (for 10 preparations) has been calculated, which will give a rough idea of the expenses involved in this.

Table 13: Estimated cost for plasmid isolation using maxi protocol

S. No.	Chemical	Rate(Rs.)	Amount	Cost(Rs.)
01.	**STE**	0.402/50ml	2000ml	**16.08**
02.	**GTE**	2.290/100ml	100ml	**2.290**
03.	**RNase**	187.0/ml	3µl	**0.561**
04.	**Lysozyme**	11.3595/ml	10ml	**113.595**
05.	**Solution II (NaOH SDS)**	0.986/100ml	200ml	**1.479**
06.	**Solution III (K-acetate)**	14.33/100ml	150ml	**21.495**
07.	**Isopropanol**	21.5/50ml	210ml	**90.3**
09.	**Ethyl alcohol (70%)**	94.5/100ml	20ml	**18.9**
10.	**LB broth**	4.2/ 100ml	5000 ml	**210.00**
		Gross		**Rs.474.70**

2.4 Estimation of achieved economy in Plasmid Isolation

Table 14: The cost analysis and profitability of Mini-prep Plasmid Isolation

	Cost per Mini Prep	Cost (25) Mini Prep
Commercial Preparation	**Rs. 228.00**	**Rs. 5700.00**
Laboratory Preparation	**Rs. 002.26**	**Rs. 0056.50**
No of Kits produced for Rs. 5700.00	**~100 no**	
Estimated sales value of 100 Kits (@ Rs. 5700.00)	**5,70,000.00 (5.7 Lakhs)**	

Table 15: The cost analysis and profitability of Midi-prep Plasmid Isolation

	Cost per Midi Prep	Cost (10) Midi Prep
Commercial Preparation	**Rs. 485.00**	**Rs. 4850.00**
Laboratory Preparation	**Rs. 8.94**	**Rs. 0089.40**
No of Kits produced for Rs. 4850.00	**~54 no**	
Estimated sales value of 54 Kits (@ Rs. 4850.00)	**Rs. 2.63 Lakhs (Cost Price Rs. 4828.00)**	

3. Restriction Digestion Analysis of Plasmid pBR322

The plasmid isolated from the secondary culture of transformed *E. coli* (JM101-pBR322) was digested using three restriction enzymes HindIII, BamHI & HinfI. BamH-I & HindIII, both these enzymes being single cutter to pBR322 produced a single band. Hinf being a multiple cutter to pBR322 produce 8 distinct bands of transcending sizes (Figure 6). The observed fragments were compared to the standard ladder and the fragment sizes were found to be in accordance to the following table and the result could also be confirmed in the hypothetical banding profile generated using the bioinformatics software DNAMAN (figure 7; lane 1 and 2) :

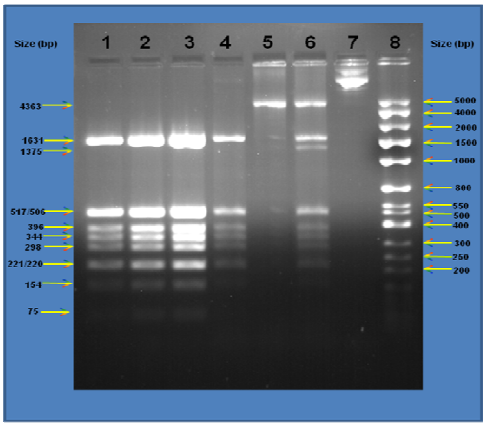

Figure 6: Restriction Digestion profiles of pBR322 created

Bioinformatics Software DNAMAN.

Lane 1: *pBR-322/Hinf-I (0.25µg)*
Lane 2: *pBR-322/Hinf-I (0.50µg)*
Lane 3: *pBR-322/Hinf-I (0.75µg)*
Lane 4: *pBR-322/Hinf-I (Lab; 0.1µg)*
Lane 5: *Bam H I*
Lane 6: *Lane 4+ Lane 5*
Lane 7: *Control Plasmid*
Lane 8: *Marker*

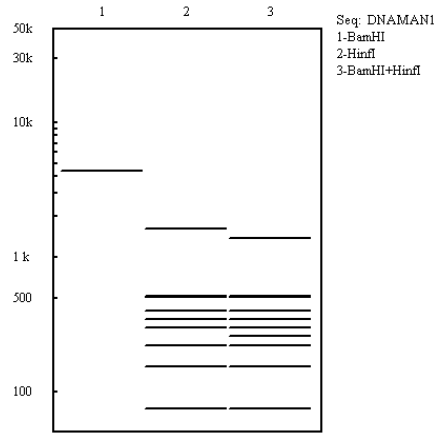

Figure 7: Restriction Digestion profiles of pBR322 using

Lane 1: *pBR-322/ BamHI*
Lane 2: *pBR-322/ HinfI*
Lane 3: *pBR-322/ BamHI+HinfI*

Table 16: Observed fragment sizes

S. No.	Fragment	Size(bp)
01.	Frag.1	**1613**
02.	Frag.2	**517/506**
03.	Frag.3	**396**
04.	Frag.4	**344**
05.	Frag.5	**296**
06.	Frag.6	**221**
07.	Frag.7	**154**
08.	Frag.8	**75**

The quality of indigenously isolated plasmid restriction profile was tested for a comparative banding pattern with the commercially available ladder. The isolated Plasmid was digested using restriction enzyme Hinf I and the results were analyzed by Agarose gel electrophoresis (data not shown).

Table 17: Cost Analysis of Restriction enzyme used per reaction

S. No.	Restriction Enzyme	Rate(Rs.)	Amount used per 10 µg pBR322	Cost(Rs.)
01.	Hinf I	1750/2000units	20units	17.50
02.	Hind III	1000/4000 units	20units	5.00
03.	Bam HI	140/400units	20units	7.00

Table 18: Estimation of Achieved Economy in DNA molecular weight Ladder formation

Cost for Laboratory preparation of pBR322/ Hinf I ladder	
Cost of Plasmid (10µg pBR322) (Mini-prep)	Rs.150.60*
Cost of Plasmid (10µg pBR322) (Mini-prep)	Rs.25.417
Cost of plasmid (10µg pBR322) (Midi-prep)	Rs.3.92
Cost of restriction enzyme (for 10µg pBR322)	Rs.017.50
Gross (Midi-prep)	Rs.21.42
Gross (Mini-prep)	Rs.42.917

**Cost obtained previously in our Lab*

Table 19: Estimation of Achieved Economy in DNA molecular weight Ladder formation for 10 µg

Cost Per (10µg pBR322/ Hinf I DNA Molecular Weight ladder)	
Commercial Preparation	Rs. 1400.00
Laboratory Preparation (Midi-prep)	Rs.21.42
Laboratory Preparation (Mini-prep)	Rs.168.10*
Laboratory Preparation (Mini-prep)	Rs.42.917
Amount of Ladder for Rs. 1400.00	~65 units

**Cost obtained previously in our Lab*

4. Estimation of Achieved Economy in Development of DNA Molecular Weight Ladder and Plasmid Isolation Midi Kit.

From the above cost analysis it was concluded that:

- DNA Molecular Weight ladder is 2 -7.8 times economical when plasmid was isolated using Midi-prep as compared to Mini-prep, so the cost is much more economical.
- Additional cost of plastic ware is minimized to a great extent.

The restriction digestion analysis also provided an opportunity for development of restriction digestion and restriction mapping teaching kit with significant cost reduction as described below.

Table 20: Significant Cost Reduction of Teaching Kits

5 Experiments	Cost of Restriction digestion teaching kit	Cost of Restriction mapping teaching kit
Commercial Preparation	Rs. 3650.00	Rs. 3750.00
Lab Preparation (single; 0.25 µg)	Rs. 0004.20	Rs. 0004.20
Lab Preparation (5 exp 1.25 µg)	Rs. 0021.00*	Rs. 0021.00*
Lab Preparation (5 exp 1.25 µg) Midi-prep	Rs.0000.40*	Rs.0000.40*

No of Kits produced for Rs. 3750.00	**~178 no**	
Estimated sales value of 178 Kits	**~6.5 Lakhs**	**~6.67 Lakhs**

** cost of restriction enzymes extra as applicable*

Finally, analyzing all the procedures and results in the perspective of achieved economy, we have come to draw the following prominent conclusions that, we have successfully prepared:

- ✓ Competent cell preparation & transformation kit.
- ✓ Plasmid isolation kit. (Mini and Midi)
- ✓ Composite DNA molecular weight ladder.
- ✓ Restriction digestion teaching kit.
- ✓ Restriction mapping teaching kit.

CONCLUSION

The work was undertaken to reduce the operational expenses involved in transformation and cloning techniques and sensitize researchers about cost involved in each set of procedures so that undesired expenses may be minimized. The preparation of competent cells of various *E.coli* strains is a mandatory requirement & also a routine process for transformants determination and various other purposes. Similarly, plasmid isolation is a readily implemented procedure. The operational cost for both these processes was tried to be minimized using slightly modified forms of standard protocols. Finally, using the same isolated plasmid a low cost molecular weight ladder was generated by restriction digestion. Such ladders are a frequent component of molecular biology experiments. The cost of commercially available kits and ladder were later compared to the effective laboratory cost. Thus, the work standardized economically efficient protocols for competent cell preparation, transformation, plasmid isolation and molecular weight DNA ladder generation, which could be embedded even into critical experimental procedures. Ultimately, analyzing all the procedures and results in the perspective of achieved economy, thus, the following prominent conclusions that have successfully prepared are:

- ✓ Competent cell preparation & transformation kit.
- ✓ Plasmid isolation kit using mini and midi preparation.
- ✓ Composite molecular weight DNA ladder.
- ✓ Restriction digestion teaching kit.
- ✓ Restriction mapping teaching kit.

Also, the comparative cost analysis reveals that:

- ✓ The competent cell preparation & transformation kit prepared by us costs Rs.10.42, which is nearly **400 times cheaper** as compared to the kit, supplied by Bangalore Genei @ Rs.4250 (per reaction 5000µl).
- ✓ Similarly, the Plasmid Isolation kit (mini-preparation) prepared in the laboratory costs Rs.56.50 which is nearly **100 times lesser** than that supplied by Bangalore Genei Rs.5700 (for 25 preparations).
- ✓ Plasmid Isolation kit (midi-preparation) prepared in the laboratory costs Rs.89.40, which is nearly **54 times lesser** than that supplied by Bangalore Genei Rs.4850 (for 10 preparations).

- ✓ We produced a HinfI/ HindIII digest composite DNA ladder in an effective cost of Rs.168.10 (Rs.42.917 in present studies) which is nearly **8 - 32 times cheaper** than the ladder being provided by Bangalore GeneI @ 1400(for 10µg pBR322 HinfI digest ladder).
- ✓ Ultimately we produced a HinfI/ HindIII digest (Midi-prep plasmid isolation) composite DNA ladder in an effective cost of **Rs.21.42** which is nearly **65 times cheaper** than the ladder being provided by Bangalore GeneI @ 1400(for 10µg pBR322 HinfI digest ladder).

Although, the companies provide a plasmid purification column in the kit, which could improve the results, but if even this cost is summed up to the effective cost, it creates very minor hike in the final cost. Also, all these analysis were corresponding to the subsequent steps through alkaline lysis method. The analyzed effective cost may be reduced to a higher extent if the miniprep and midiprep method is substituted by Maxiprep methodology; this work is still open for further improvement.

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