1. Plasmid DNA Extraction and Agarose Gel Electrophoresis

A. *Plasmid DNA Extraction*

Plasmids have been found to be wide distribution in bacteria. They are autonomously replicating extrachromosomal elements which are not essential for the growth of their host cells. However, they may encode a wide range of genetic products which may permit their host to adapt better to adverse conditions, for example, in the presence of antibiotics.

In cloning work, very often the recombinant plasmids have to be isolated from their transformed hosts in order to characterize by restriction analysis and sequencing. The information from these analyses provides a basis for the mode of their presentation to the transformants and the planning of future experiment for the recombinant molecules.

Among the various methods available for the preparation of plasmid DNA for rapid screening, a protocol involving the use of an alkaline solution to lyse the cells, salt precipitation to remove cell debris and chromosomal DNA and application to hibind DNA column to eliminate proteins and other contaminants has been widely employed.

Gel electrophoresis, which is easily performed, rapid, inexpensive and reproducible, has become the most popular resolution technique in nucleic acid research. Gel electrophoresis using agarose, a highly purified linear polysaccharide derived from agar, has been widely used in the detection and characterization of plasmids, also the linear DNA fragments. Plasmids of sizes ranging from less than one kilo-base (kb) to over a few hundred kb can resolved by conventional agarose gel electrophoresis.

Since all the DNA molecules have the same charge to mass ration, electrostatic charge is not a factor in electrophoretic mobility. Different DNA molecules will nevertheless move through a gel at different rates on the basis of size and conformation. The electrophoretic mobility of a DNA species through a gel is described in the following equation:

$$\text{Electrophoretic mobility} = d/Et$$

where d is the distance travelled in cm. E is the electric field strength in V/cm and t is the time in seconds. The mobility of a DNA band is subject to alterations resulting

from variation in voltage supply, gel concentration, ionic strength, pH of the electrophoresis buffer and temperature.

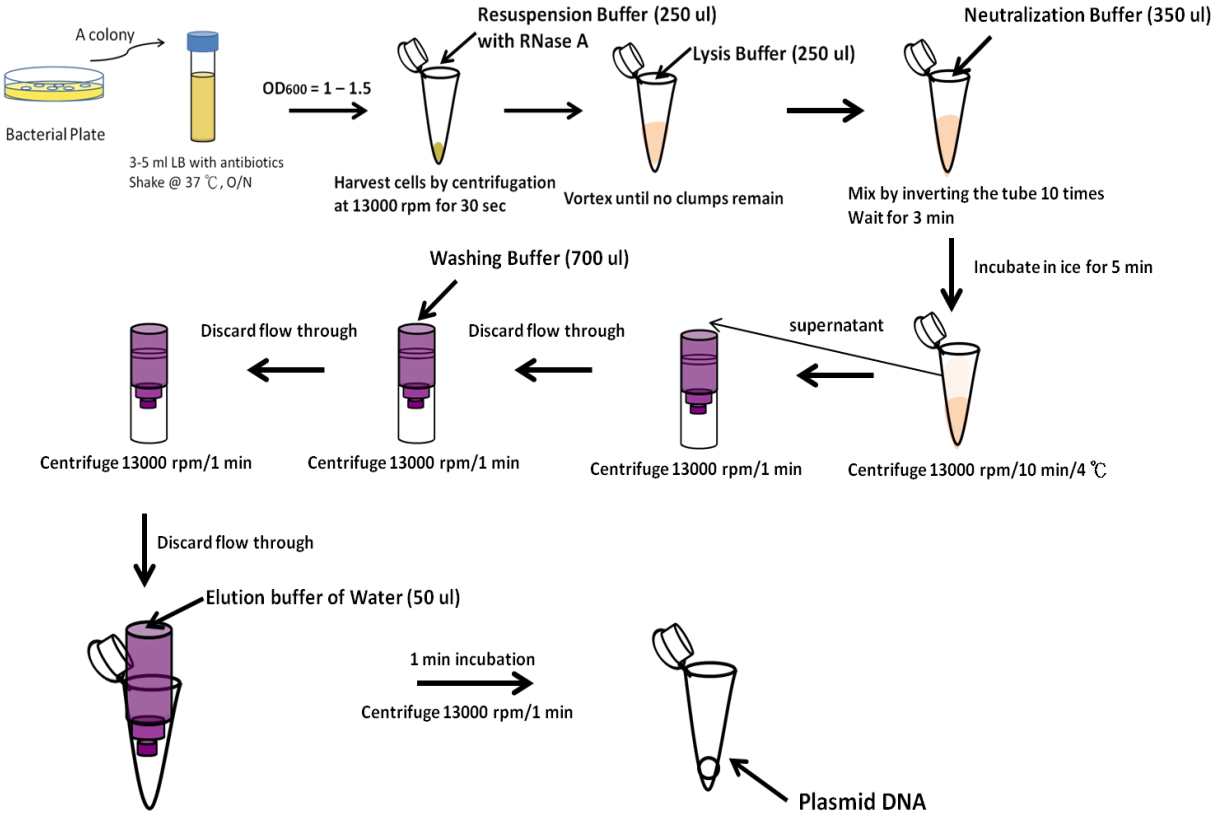
Under the influence of an electric field, the motilities of different DNA species through a gel are inversely related to their respective molecular sizes. Therefore, those with larger sized will move more slowly. If it so happens that two DNA species are of the same size, but different conformation, for example a covalently closed circular (CCC) species versus its open circular (OC) counterpart, agarose gel electrophoresis can still be used to separate them as CCC molecules are more compact, so less retarded than those of OC form.

Materials

Mini-prep plasmid DNA extraction kit
ddH_2O (deionized, sterile, DNase-free)
DNA ladder (25bp/ 100bp/ 1kb)
6×/10× DNA loading dye
DNA staining dye (SYBR Green/ Red Safe/ Gel Red…DO NOT use Ethidium Bromide)
Agarose gel (use DNA grade agarose)
TAE buffer (Tris-Acetate-EDTA buffer)
Gel imager: UV trans-illuminator
(Beware of UV damaging your skin, eye and your DNA samples)

Procedures

Plasmid DNA extraction



(This video shows the procedures of plasmid DNA extraction)

B. Agarose gel electrophoresis

1. Setting up an agarose gel:

1. For a small gel (the one used in our lab), add 20 ml 1×TAE buffer to a conical flask. (If there is none, dilute the 50×TAE buffer by 50 times.)
2. Then, add 0.2 g agarose (1%) to the conical flask and heat it by microwave oven by 30-45 s to dissolve it until it becomes a clear and transparent liquid.
3. Cool it down a little bit by running water for around 15 s.
4. Add about 1 µl (for 20 ml TAE) of DNA staining dye, red safe (20000×).
5. Pour the solution to the white tightened tank with gates to allow it to solidify. Add the gel comb so as to create wells for the gel. Wait >15-30 min until it is gel-like and ready to use.

2. Running agarose gel:

1. Orient the gel with wells (comb removed) facing the BLACK negative electrode. Check if the gel is covered by TAE buffer in the tank.
2. Add 6×/10× loading dye to the DNA to a total volume of <25 µl (depended on the well) before adding to the wells. Mix loading dye to DNA to make the solution colored.
3. Load the sample to the wells (<25 µl/ well)
4. Add 3-5 µl DNA ladder to a separate well.
5. Connect the electrodes to the power supply with correct color, black to black, red to red. Apply power supply with 120 V. Check if there are bubbles on the negative electrodes.
6. Allow it to run for about ~30 min (the time is variable based on the gel concentration and the size of interested DNA. Be aware the samples run into the gel by checking if the blue band stays on the gel.
7. After electrophoresis for 30 min, disconnect power, take the gel to imager, and turn UV on to observe bands.

**REMINDER:* Never run a gel with >200V, as the heat so generated can melt the gel and also easier to cause electric leakage. Range from 80 – 160 V is acceptable.

Usually it needs at least 100 ng DNA for a band to be seen and visualized on the UV trans-illuminator.

(This video shows the procedures of agarose gel electrophoresis)

Reference:

eshop.intronbio.com (2012). *DNA Prep :: Plasmid & Probe DNA*. Retrieved January 15, 2016 from http://eshop.intronbio.com/Product/View.asp?pIdx=1&pageno=1&MainItem=A&SubItem=A