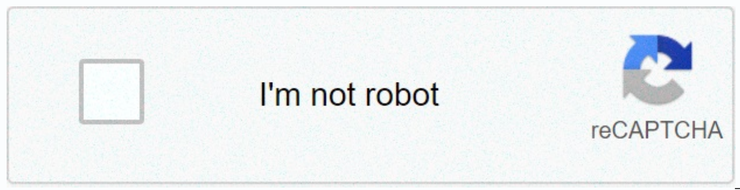

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Competent cells transformation method

Bacterial transformation is a key part of the cloning process and has been widely used in many studies (Swords, 2003; Gigova and co., 2006). The mechanism is marked by two phases, the first phase involves the uptake of DNA through the cell envelope, and the second phase involves the formation of DNA in the cell as stable genetic material (Hanahan, 1983). The transformation procedure is physico-chemical in nature rather than strictly a chemical or physical procedure, since cells are manipulated by cations (or a combination of cation) and temperature imbalance to make them competent to capture foreign DNA. It is generally understood that chemical manipulation is associated with induction of abilities, while physical manipulation is associated with the absorption of foreign DNA. The two methods act together to make an act of artificial DNA internalization. Various methods have been used over time to make cells competent, for example by using dimethylsulfoxide (DMSO), divalent cations or polyethylene glycol (PEG) (Klebe and Col., 1983; Chan and co., 2013). In addition to these chemical methods, electroporation was also tested, which is used to induce capabilities (Dower et al., 1988; Yoshida and Sato, 2009; Liu a., 2013). The use of divalent cations was the most effective chemical treatment that brought about transformation (Day, 2004). Among the various cations, divalent calcium quotations (Ca2+) were found to be the most effective (Weston a.k.a., 1981) alone (Dagert and Ehrlich, 1979) and in different combinations. A combination of divalent and monovalent ions such as calcium and magnesium (Taketo, 1974; Wensink and co., 1974), calcium and manganese (Enea and co., 1975), calcium, rubidium and dimethylsulfoxide (Kushner, 1978) and other alkaline metals with prolonged incubation at 0 °C (Taketo, 1972; Dagert and Ehrlich, 1979) were also reported as effective (Roychoudhury et al., 2009). In general, all divalent cations improve the transformation process. Hanahan (1983) found that the presence of magnesium in bacterial culture media increases transformation efficiency by 15- to 20 times compared to cells grown in the absence of magnesium. He also noted that the addition of magnesium in the media 30 minutes before the time of cell collection also increases the transformation to ~ 60%. However, the addition of magnesium as cells are harvested and incubated on ice increases the transformation to ~40%. The addition of calcium or manganese ions also showed almost the same stimulating effect as magnesium ions (Hanahan, 1983). However, the incubation period with calcium chloride or any other divalent forations should be optimised. A period of 24 hours of incubation in cold calcium chloride has been observed to make bacterial cells 20-30 times more competent and 4-6 times more effective for transformation compared to cells obtained immediately after Treatment with CaCl2. et al., 1977; Dagert and Ehrlich, 1979). Curtiss et al. (1977) experimented on the E. coli X1776 strain and observed the effect of different conditions on transformation efficiency. He treated bacterial culture with a combination of manganese, calcium, rubidia and potassium ions together with DMSO and sucrose at 0 °C, followed by a thermal pulse at 42 °C. However, these conditions did not produce successful results when other Strains of E. coli were used (Hanahan, 1983). Meselson and Yuan (1968) found these conditions promising for a successful transformation in the case of E. coli MM294 as standard calcium chloride protocol. Bolivar et al. (1977) reported 106 transformers when cells were treated with calcium alone, while Kushner (1978) reported that he acquired 107 transformers after treatment of rubidium cells along with calcium chloride. Norgard et al. (1978) was also able to obtain 107 transformants using the method followed by Kushner (1978) in the case of the K-12 X1776 e. coli tribe. However, transformers yield varied from specie to specie and suspense to suspense (Mercer and Loutit, 1979). Sjöström et al. (1972) stated that the optimal concentration of calcium chloride for DNA absorption of S. aureus is 0,1 M CaCl2. Resistance between foreign DNA and bacterial cells due to negative fillings on both, these divalent cations are overcome. This applies to linear DNA fragments as well as to circular DNA molecules such as plasmids (Mandel and Higa, 1970; Tsen a., 2002). Divalent cations are thought to bind to both cell and DNA, completely neutralising the charge. DNA-bound calcium further helps dna adsorbed into the cell (Panja et al., 2008b). In addition, DNA-binding proteins present in the cell membrane could also assist in this interaction. Anchoring DNA to the membrane eliminates the risk of DNA detachment or expulsion (Clark, etc., 2002). In addition, the low temperatures used in the transformation protocols converge the lipid part and consequently limit the fluidity of the cell membrane, which strengthens the interaction of the surface of calcium cells. In this way, calcium ions, bound to the surface of cells as well as foreign DNA, brings DNA into the cell. Clark et al. (2002) showed that the relative association of divalent cations (e.g. Ca2+) is more with the cell membrane compared to its association with foreign DNA, while certain trivalent cations (e.g. spermidine) interact more easily with DNA (Li et al., 2004). This study also reported that Ca2+ plays a more significant role in the development of capabilities compared to spermidine or trivalent cations (Clark and co., 2002). Membranes absorb calcium very easily, and once inside the cell, calcium ions are neutralized with membrane phosphates present on the cytosolic side (Melcrová a., col., 2016). Binding of calcium ions to the membrane also cause changes in the membrane (Li a., 2004). Treatment with divalents or trivalents on ice is followed by treatment with an elevated temperature as a heat shock, which creates a temperature imbalance. Molecules with increased Brownian movement outside the cell are likely to push the DNA molecule inside the cell. However, it is unclear whether this kinetic force is sufficient to push adsorbed DNA molecules inside. Panja et al. (2008a) examined the effectiveness of cooling and heating cycles by increasing the number of cycles until maximum transformation efficiency was reached. It was derived that the decrease in temperature actually contributes to the loss of protein, while heating contributes to the loss of lipids, and so together these cycles increase the efficiency of transformation (Panja et al., 2008a) because it increases the size of pores on the cell surface. In addition, due to the loss of lipids and proteins, the membrane is depolarized, further reducing the repellent between the DNA molecule and the membrane. In addition, cell density may also affect transformation efficiency and it has been reported that maximum competence is observed at cell density between 107 and 108 ml cells in the diary phase (Taketo, 1974; Norgard a., 1978). However, the question remains; whether the pores (through which foreign DNA enters the cell) are formed by calcium treatment or are naturally present. There are natural channels, often called Bayer bridges, in the membrane that can serve as a potential route for DNA intake (Dreiseikelmann, 1994; Sperandeo a.k., 2007; Srivastava, 2013). Hanahan (1983) stated that the cells concerned have many sites or channels and all these sites and channels have an independent chance to participate in DNA tweaks towards the transformation process. All cells, whether competent or not, compete for plasmid absorption, but if only the cells concerned are used for transformation, the efficiency increases by up to 50-fold, as Hanahan said (1983). The DNA uptake factor is the sum of all the probabilities of DNA uptake through each channel. It has been reported that the chance of transformation is increased not by an increase in DNA concentration, but by an increase in the number of channels through which DNA capture takes place (Hanahan, 1983; Nikaido and Vaara, 1985). In addition, calcium has a dual role in this process; not only neutralizes the charge, but also weakens the cell membrane for the production of invaginations (Stein, 1990; Thomas and Rice, 2014). Although divalent cations have been known to help neutralize the charge, complex ions can also serve to create a static force of attraction in the DNA molecule. This leads to the folding of DNA into a compact sphere-like structure that facilitates its entry into the cell (Clark et al., 2002). A sub-cooled sphere as a plasmid structure will have a better chance of entering the appropriate cell for transformation than the enlarged open ring form of plasmid. However, if the size of the DNA is close to pore size, the probability of transformation decreases sharply. With the use of spermidine or other trivalents, the size of the ball structure of DNA may exceed the size of the pores in the cell membrane, which can only be solved by changing the physical parameters used in the protocol, especially heating and cooling cycles. Regardless of whether they use divalents or trivalents, their concentrations must be optimised so that all DNA phosphates are not inaccessible, as some parts of DNA have to be adsorbed on the cell surface and free phosphates are needed for this, as infers Panja et al. (2008b). The effectiveness of transformation is greatly influenced by the type of host cell, because they have different structures of the cell surface, especially in relation to O-polysaccharides that protrude from the cell surface. These surface structures interact with divalent forations and DNA, making the cell competent for transformation. Various strains of E. coli have been reported, as mentioned above, which showed a variation in transformation efficiency due to differences in the chemical properties of their cell envelope (Taketo, 1972). A very dense O-polysaccharide will become a deterrent to DNA thym. However, it was also claimed that the widespread elimination of LPS by excessive pre-treatment of ethanol reduces the efficiency of transformation (Roychoudhury a.c., 2009). This can be explained by the above hypothesis that DNA first binds to some external component of the cell membrane, which then helps its movement inside the cell. Together with density, the composition of O-polysaccahride also plays a role in the acceptance of the incoming DNA molecule (Deficiency, 1977). In addition, calcium ions interact with the membrane, and at a concentration of 100 mM CaCl2, almost all calcium is absorbed by phosphatidylcholine and phosphatidylserine cell membrane (Melcrová a col., 2016). Therefore, the properties of the membrane play a major role in dna adsorption. Evidence clearly indicates that the physical and chemical treatments used during transformation, i.e. temperature imbalance and CaCl2 treatment, help to cope with obstacles to DNA uptake, such as charge repellent and pore size (Figure 1). Combinations of magnesium and calcium are rarely used in transformation protocols, the importance of which should be considered. The combination of divalent and trivalent quotes with prolonged incubation times may be designed to improve transformation efficiency; as in addition to stabilization of the charge, trivalent formations can compact DNA, further aiding its internalization. Bacterial cells could also be grown in the presence of CaCl2 and MgCl2 before inducing abilities. Heating and cooling cycles used only once in transformation protocols could also be increased to three times for greater transformation efficiency. These conditions need to be adapted and optimised for bacterial species and strains due to differences in their surface properties. However, it is necessary to obtain concrete evidence based on experiments designed solely to develop this phenomenon. Figure 1 shows the barriers/limitations in the uptake of DNA by a bacterial cell, which are; repellent due to negative charges on the cell membrane and DNA and porosity of the membrane. These are manipulated by chemical treatments such as calcium ions that neutralize negative charges. Physical parameters can be used to improve porosity and permeability. The AA and HM worked out the manuscript. YR put forward the idea of handwriting and edited the manuscript into the final account. RT helped write the manuscript. Conflict of Interest Statement Authors declare that the research was conducted in the absence of any business or financial relationships that could be interpreted as a potential conflict of interest. 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