I am Doctor Sunita Borkar from PESRN College of Arts and Science Farmagudi, Ponda Goa. In this module we are going to study about PBR and PUC series of plasmids as important vectors. At the end of this presentation we will be able to understand and describe PBR and PUC series as vectors.

In genetic engineering, PBR series of vectors are artificial plasmids that were constructed by Bolivar and. Rodriguez. It's Francisco Bolivar Zapata and Raymond L. Rodriguez. In PBR series. the alphabet P stands for plasmid and BR for Bolivar and Rodriguez scientists who constructed them. We have different examples under the series being PBR 322, PBR 324, PBR 325. PBR 326, PBR 328 etc. The numbers to the series are given in order to distinguish them from one another.

PBR 322 is a workhouse of gene cloning laboratory and is an artificial plasmid that was first widely used in *E. coli* as cloning vector. Created in 1977 in the laboratory of Herbert Boyer at the University of California, San Francisco. The genetic material of PBR 322 It's an artificial plasmid derived from 3 naturally occurring plasmids, the Col E1 plasmid that provides the replication elements of PMB 1 ampicillin resistant gene of RSF, 2124 and tetracycline resistant gene of PSC 101.

This illustration describes the origin of PBR 322 plasmid having ampicillin resistance gene from RSF 2124. Tetracycline resistant gene from PSC 101. and the origin of replication from PMB 1, Ampicillin resistance gene and Tetracycline resistance gene act as markers .PBR 322 is 4361, base base having two sets of antibiotic resistant genes, tetracycline and ampicillin used as selectable marker genes. There are unique restriction sites for more than 20 restriction endonucleases which lie within the antibiotic resistant gene. For example, in ampicillin resistant gene we have restriction sites for three different restriction endonucleases obtained from *Providentia stuartii, Proteus vulgaris* and *Streptomyces caespitosus* Tetracycline resistant

gene has recognition sequences for BamHI, Bsp MI, Eco RV, Nhe I, Nru I, Sal I, Sph I which are all restriction endo nuclease sites. The other unique sites. for different types of restriction endonucleases being Aval 1 , Hind III from *Proteus vulgaris, , Nitobacter denitrificans* and so on. This illustration explains the presence of recognition sequence in the ampicillin resistant gene of *Providentia stewarti*, in tetracycline resistance gene. we can see the restriction endonucleases site for Bam HI from *Bacillus amyloliquifaciens* Sal I from *Streptomyces. albus* Nde I from *Neisseria Denitrificans* and with the origin of replication. The advantages of using PBR 322 as a vector is that it's most versatile and most popularly used. It's smaller than a natural plasmid and DNA of six kilobase length can be inserted and has two antibiotic resistant genes for ampicillin and tetracycline where one can also be used for insertional inactivation. This plasmid has relaxed origin of replication. And has single restriction sites for various restriction endonucleases and it's a plasmid that is used to construct several other plasmids as cloning vectors. However, PBR 322 plasmid has also certain disadvantages such as instability because it is lost on continuous culture in the same Organism, the copy number being high, although is useful for getting the product in large amount but it is not suitable when expression signals are studied using this plasmid. Availability of cloning sites. Although there are 21 restriction sites but only a few can be used since 11 are present on antibiotic resistant containing genes, the detection scheme for ampicillin resistance and tetracycline resistance are not very prominent and there is host range limitation. As this plasmid can be used only in. *E coli* .PUC plasmids are a series of plasmid cloning vectors which were created by Joachim Messing and co-workers where P stands for plasmid and UC represents the University of California as these plasmids were constructed first here . example of plasmids we have are pUC 18 and pUC 19 .PUC 18 and 19 are circular double stranded DNA made up of 2686 base pairs.They are small, having high copy number vectors for

replication in *E. coli* These plasmids are constructed using ampicillin resistant gene and the PMB 1 origin of replication from PBR 322 plasmid. They have multiple cloning sites within the lac Z gene the lac Z gene is inserted in this plasmid. Foreign DNA inserted within the lac Z Gene disrupts the beta galactosidase activity. and gives rise to white colonies on X gal Agar plates, helping in the distinguishing of recombinants. A non recombinant will show white colored colonies. where as the non-recombinants will be blue.

This illustration shows puC.19 plasmid with origin of replication resistance gene for ampicillin.

and lac Z gene, which is also used as a marker gene for insertional inactivation. and with a lack repressor protein pUC 18 and pUC 19 have a lot of resemblance with one another.

The difference is that the cloning sites for restriction endonucleases are in a reverse direction.

Cloning a gene in the plasmid vector using pUC 19 is that the DNA or the gene of interest.

after cleaving the plasmid vector with the same enzyme when mixed together. results in the formation of a recombinant DNA. This recombinant vector would carry the gene of interest, which when introduced into a host cell like *E. coli,* would express the gene of interest.These are the references for the module.

Thank you.