Scientific Institute of Public Health

Division of Biosafety and Biotechnology

Cloning of promoters found in human tumors into *Escherichia coli*

1. Aim of the project

It is known that anomalies (hypermethylation) among promoters of specific genes can be related to the presence of cancers and/or potential metastatic tumors. The aim of the present project is to analyze various promoters found in human tumors, using the "methylation specific polymerase chain reaction" (MSP) technique combined with the "molecular beacon technology". Those manipulations are not related to the contained use legislation, but to provide effective quantitative assessments, cloning of the promoters into *Escherichia coli* are required in order to establish standard curves. The approach used for the setting up of those standard curves is the following:

- DNA extraction from cell lines commercially available or from human tumors (prostate, lung and breast tissues)*;
- Methylation-specific PCR assays on the extracted DNA in order to amplify non-coding regions corresponding to the designated promoters;
- Cloning of the amplified fragments into a bacterial vector (type pCR-TOPO);
- Transformation of the resulting vector into *E. coli* K-12 MG1655.

2. Vector/plasmid

The bacterial vector used in this example is a well-characterized and commercially available vector: type pCR-TOPO.

3. Insert

The inserts correspond to various non-coding regions of promoters amplified by PCR from commercial cell lines or from DNA extracted from human tissues (prostate, lung, breast). Those inserts are unlikely to alter the pathogenicity of the recipient bacterial host.

4. Localization of inserted genetic material

Episomal.

5. Receptor organism

The *E. coli* K-12 strain was isolated in 1922 from human faeces of a patient suffering from diphtheria and was kept as a stock strain (called EMG2) in the Bacteriology Department at Stanford University. Since then, K-12 has been widely distributed to laboratories across the world and rapidly became a primary model organism in research. K-12 and derivatives (mutants) have been grown in the laboratories for many generations and are now considered as weakened organisms that are unable to colonize the human intestine anymore and almost unable to survive under environmental conditions.

K-12 MG1655 strain, which is genetically very close to the original strain, was considered as the representative strain and was chosen for the K-12 genome sequencing (published in 1997 and updated in 2004). MG1655 (F- λ- *ilvG*- *rfb*-50 *rph*-1) have only been cured of the temperate bacteriophage lambda and F plasmid by ultraviolet light and acridine orange, respectively. These treatments actually resulted in a mutation at the end of *rph* gene, causing a pyrimidine starvation phenotype, and a mutation in *ilvG* gene disrupting one of the isoleucine-valine biosynthesis pathways. The *rfb-50* mutation, which consists in an IS5 insertion at the downstream end of *rfb*, is present in the original strain EMG2 as well as in most K12 derivatives. This mutation results in the absence of O-antigen synthesis in the lipopolysaccharide, explaining the low capacity of those strains to colonize the intestinal gut.

K-12 strains in use today are not recent environmental isolates but are from standard culture collections, such as the American Type Culture Collection and are well characterized. Furthermore, no case of laboratory-acquired infection has been described so far. *E. coli* K-12 strains can therefore be considered as a non-pathogenic strains belonging to the **class of risk 1**.

IPH/15/Dr. C. Verheust/07-1228
2007

6. Biological hazards and/or considerations related to the manipulation of the resulting genetically modified micro-organism (GMM)

Transformation of the *E. coli* strain with the given bacterial vector does not confer the host organism with any potential harmful effect to humans, animals, plant or environment. The resulting GMM remains non pathogenic and belongs to the **class of risk 1**.

7. Class of risk of the resulting GMM

The genetically modified *E. coli* used in this project is assigned to class of risk 1.

8. Class of risk of the activity

Molecular manipulations such as cloning techniques are of class of risk 1.

9. Recommended containment measures

All bacterial genetic manipulations should be performed in a standard biosafety level 1 facility **(BSL1)**.

10. Reference and further reading

Riley M, Abe T, Arnaud MB, Berlyn MK, Blattner FR, Chaudhuri RR, Glasner JD, Horiuchi T, Keseler IM, Kosuge T, Mori H,. Perna NT, Plunkett G III,. Rudd KE, Serres MH, Thomas GH, Thomson NR, Wishart D, Wanner BL. (2006). Escherichia coli K-12: a cooperatively developed annotation snapshot—2005. Nucleic Acids Res. 34(1), 1–9.

*Note: Despite the fact that the given animal cell cultures of human origin - **which are not intentionally infected with pathogens nor genetically modified** - falls beyond the scope of the regulatory provisions relative to contained use, it is recommended to perform cell culture manipulations in a biosafety level 2 facility (BSL2) with the additional use of a biosafety cabinet type II and implementation of adequate work practices. For more information on the risk assessment of animal cell cultures, see: http://www.biosafety.be/CU/animalcellcultures/mainpage.html