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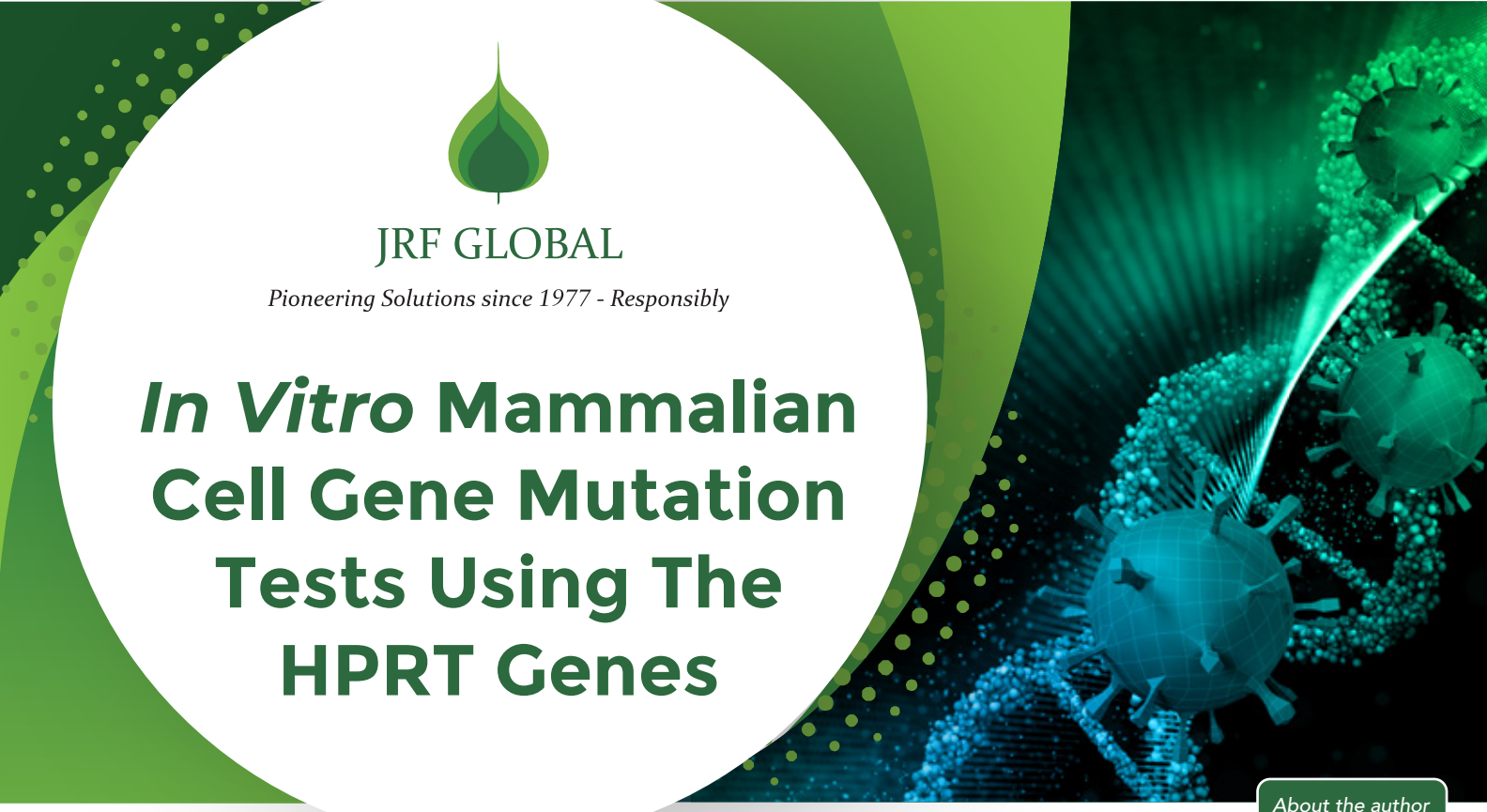
In Vitro Mammalian Cell Gene Mutation Tests Using The HPRT Genes



The OECD guidelines for testing of chemicals has originally adopted the test guideline 476 in 1984. It is the part of test guideline on genetic toxicology. TG476 is further revised and adopted as a new guideline dedicated to in vitro mammalian cell gene mutation tests using the thymidine kinase gene (TG490). This test analyses the forward mutations of reporter genes viz. hypoxanthine-guanine phosphoribosyl transferase (HPRT) and xanthine-guanine phosphoribosyl transferase transgene (Xprt). However, HPRT is more common test for assessing gene mutation as compared to XPRT test in regulatory point of view.

About the author

Karishma Desai
Sr. Research Officer
Genotoxicity

Karishma is a Group Leader (Senior Research Officer) in the Mutagenicity Section. She has substantial experience in both *in vitro* and *in vivo* genotoxicity testing as well as *in vitro* alternative toxicity assays. She played a key role in creating a GLP-compliant in vitro skin sensitization setup at JRF. She has more than 10 years of professional experience.

PRINCIPLE OF THE TEST

Cells deficient in Hypoxanthine-guanine Phosphoribosyl Transferase enzyme, due to forward mutation at HPRT gene, are resistant to the cytotoxic effects of the purine analogue (6-thioguanine). HPRT enzyme proficient cells are sensitive to 6-thioguanine which causes the inhibition of cellular metabolism and halts further cell division. HPRT enzyme deficient cells are presumed to arise through mutation at the HPRT locus; they cannot metabolise 6-thioguanine and thus survive and grow in its presence. In this test, the cells are treated with the test chemicals for 3-6 hours, sub-cultured and the cytotoxicity is assessed.

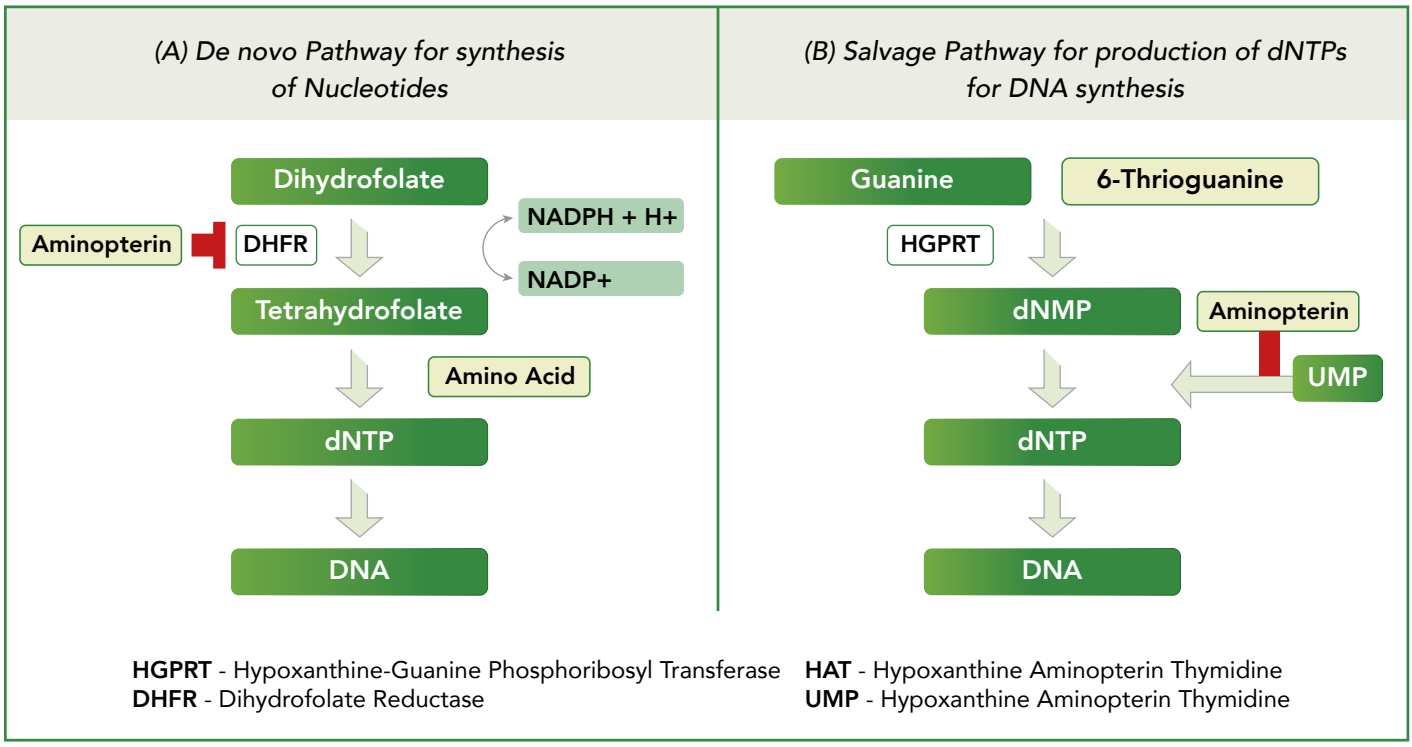


Figure 1: Pathways for DNA Synthesis

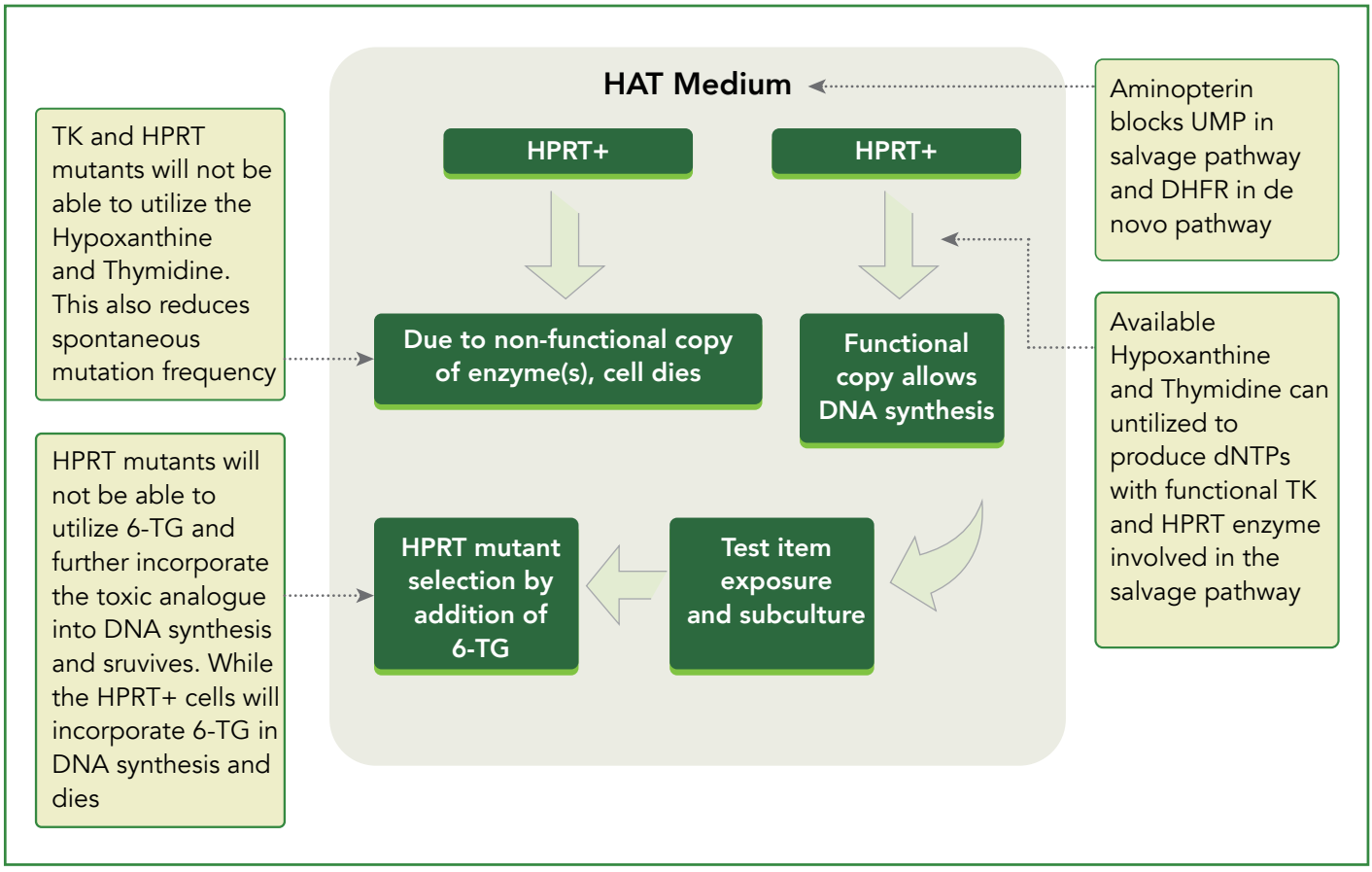


Figure 2: Schematic representation of HPRT Assay showing the effect of aminopterin (HAT medium) in cleansing and 6-Thioguonine in mutant selection.

PROCEDURE

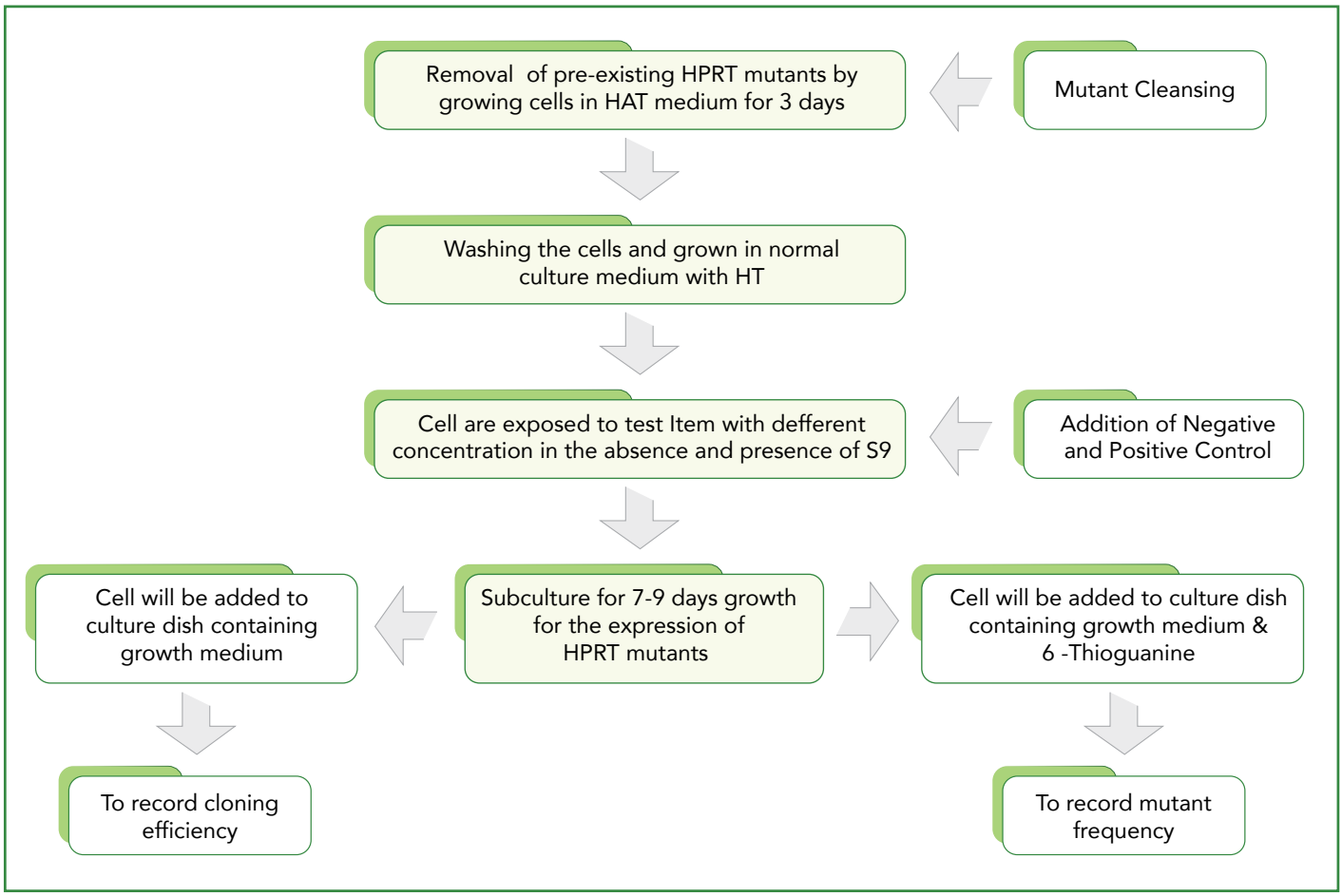
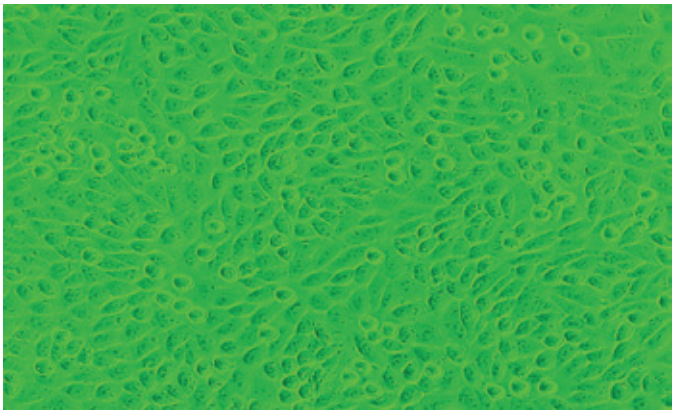


Figure 3: Experimental Procedure

Most commonly, CHO-K1 cells are used for HPRT test while other cells such as CHL and V79 lines of Chinese hamster cells, L5178Y mouse lymphoma cells, and TK6 human lymphoblastoid cells can also be used with different protocols. We use CHO-K1 cell line for conducting this assay. The proliferating cells are exposed to the test items for 3-6 hours and cytotoxicity is evaluated. Following treatment, the treated cells are maintained for a minimum 7-9 days in growth medium and the phenotypic expression for the induced mutation is allowed. A known number of cells are seeded in the medium with selective agent, 6-thioguanine to detect mutant colonies. Whereas the cloning efficiency or cell viability is determined in medium without selective agent. The mutant frequency is evaluated based on the calculated mutant colonies and cloning efficiency.



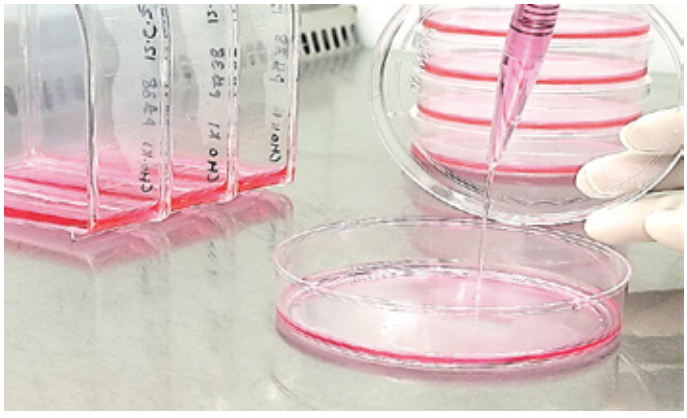


Figure 4: Test System-CHOK1 Cell line and it's use

Figure 5: Experimental Procedure

Figure 6: Mutation Selection Plates

Cloning Efficiency (CE) = Number of colonies / Number of cells plated

$$\textbf{Mutant frequency} = \frac{\text{Cloning efficiency of mutant colonies in selective medium}}{\text{Cloning efficiency in non-selective medium}}$$

OUTCOMES

This assay helps to detect the the mutagenic potential of Test Item in the presence and absence of metabolic activation, at various concentrations, that can potentially lead to gene mutation at HPRT (gene that is responsible for Hypoxanthine-guanine Phosphoribosyl Transferase; also known as HPRT) locus.

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Jai Research Foundation (JRF) located at Vapi in the southern region of Gujarat is well equipped with in vitro test facility and trained staff to perform the **"*In Vitro* Mammalian Cell Gene Mutation Tests using the HPRT gene"** and provides the service to the customer throughout the year.

References:

1. Li, A.P., Carver, J.H., Choy, W.N., Hsie, A.W., Gupta, R.S., Loveday, K.S., O'Neill, J.P., Riddle, J.C., Stankowski, L.F. and Yang, L.L.,1987: A Guide for the Performance of Chinese Hamster Ovary Cell/Hypoxanthine-Guanine Phosphoribosyl Transferase Gene Mutation Assay, Mutation Research, 189, pp. 135-141.
2. Nestmann, E.R., Brillinger, R.L., Gilman, J.P.W., Rudd, C.J. and Swierenga, S.H.H. 1991: Recommended protocols based on a survey of current practice in genotoxicity testing laboratories: II. Mutation in Chinese hamster ovary, V79 Chinese hamster lung and L5178Y mouse lymphoma cells, Mutation Research, 246, pp. 255-284.
3. OECD 2016: The Organization for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals, OECD 476, In Vitro Mammalian Cell Gene Mutation Tests using the HPRT and xprt genes, adopted by the Council on July 29, 2016.
4. OECD 2014: Draft Introduction to the OECD guidelines on genetic toxicology testing and guidance on the selection and application of assays.