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# Primer Designing and PCR Amplification of Glutelin Complete Promoter Region in Sri Lankan Rice Variety (Japonica Group)

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## ABSTRACT

**The long-term goal of the project in progress is to develop a metabolically engineered rice variety possessing flour qualities that would be suitable for making dough-in effect, 'wheat-like' rice. The objective of the present study is to amplify the complete promoter region of glutelin of rice. In this study, rice DNA was extracted by using a modified CTAB procedure and designed the new primers for the complete promoter region (glutelin) of rice. An attempt was taken to isolate glutelin complete promoter region of rice with several PCR optimizations.**

**KEYWORDS:** Glutelin complete promoter, Japonica group, *O. sativa.*

## INTRODUCTION

Rice (*Oryza sativa L.)* which belongs to family Poaceae, is the single most important crop occupying 34 per cent (0.77/ million ha) of the total cultivated area in Sri Lanka. On average 560,000 ha are cultivated during *maha* and 310,000 ha during *yala* making the average annual extent sown with rice to about 870,000 ha.

Rice provides 45% of the total calorie and 40% of the total protein requirement of an average Sri Lankan. Sri Lanka currently produces 2.7 million tones of raw rice annually and satisfies around 95 percent of the domestic requirement. The per capita consumption of rice fluctuates around 100 kg per year depending on the price of rice, bread and wheat flour (Anon, 2007).

Glutelin is the major seed storage protein of rice and it accounts for about 80% of the total endosperm proteins. It is an oligomeric protein (Quddus and Ma, 2003). A multigene family encodes this protein. About six known genes encoding these proteins are classified into two subfamilies, known as GluA and GluB. Members within each subfamily share more than 80% homology and homology between the two subfamilies share about 65% homology based on their DNA sequences. The subfamily GluA contains *GluA* -1, *GluA* -2 (Gt-1), *GluA* -3 (Gt-3) and *GluA* -4 (Chuan – Yin *et al.*, 1998).

Gluten proteins are the major storage proteins that accumulate in wheat endosperm cells and are important for the unique suitability of wheat flour for bread making. Gluten consists mainly of two types of seed storage proteins, namely, glutenins and gliadins. These glutenins are polymerized through intermolecular disulfide bonds, which are important to the properties of wheat flour dough (Gupta and Sheperd, 1990). The glutenins could be divided into two main groups. These two groups are high molecular weight glutenin (HMW glutenin) and low molecular weight glutenin (LMW glutenin), based on the mobilities in SDS – polyacrylamide gel electrophoresis. The variations on bread – making quality among different varieties are explained by the variation in HMW glutenin composition and the LMW glutenin with a smaller proportion of gliadins. The interactions of these gluten proteins play an important role in the determination of gluten strength and bread making quality.

Wheat flour has its unique property of being able to form dough, a property that is required for making bread and other related food products. Quality of rice proteins is comparatively low since they lack some of the essential amino acids such as lysine. The protein content of rice is also lower than is some cereals such as wheat. In spite of being a major rice consuming country, Sri Lanka has a relatively high wheat consumption of around 900,000 kg /year [Grain report number CE 1001 (2001)]. This is attributed to the convenience in production and versatility of wheat flour based food products. As wheat cannot be grown successfully in Sri Lanka, increasing wheat consumption means increase in foreign exchange expenditure and heavy dependence on imports.

The long-term goal of the project in progress is to develop a metabolically

# * Identification of *Mycobacterium tuberculosis* in PCR products of clinical samples by using an oligonucleotide based ELISA detection method

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**Abstract**

The present study was focused on the early definitive laboratory identification of *Mycobacterium tuberculosis*. The research design was based on the specific detection of biotinylated PCR products, which were captured in a microtiter plate coated with streptavidin, with a digoxigenin labeled oligonucleotide probe.

*M. tuberculosis* DNA was PCR amplified using the forward primer Pt18 and the reverse primer InS2 biotinylated at the 5'end. PCR was based on the amplification of a 249 base pair fragment of the *M. tuberculosis* complex-specific insertion element IS6110. The sensitivity of PCR-ELISA was found to be10 times higher than PCR alone, where the amplified product was detected by agarose gel electrophoresis (AGE). 10 fg of *M.* tuberculosis DNA could be detected in the ELISA test in comparison to a detection level of 100 fg in PCR-AGE.

67 clinical samples were tested for acid-fast bacilli by direct microscopy and culture and for the presence of *M. tuberculosis* complex DNA by PCR-AGE and PCR-ELISA. All 67 clinical samples were negative for acid-fast bacilli by Ziehl-Neelsen staining. Out of the 67 clinical samples, 63 were negative while 4 were positive for *M. tuberculosis* complex DNA in both the PCR-AGE and PCR-ELISA tests. PCR-ELISA can detect as little as 2 bacterial cells in comparison to the detection limit of 20 bacterial cells by PCR alone. Nevertheless, since the results obtained in the ELISA test for the clinical samples were in complete agreement with those of PCR-AGE, further studies are needed to confirm whether this test could be used to detect *M. tuberculosis* in clinical samples that give an ambiguous or negative result with PCR-AGE.

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# Paper 9

**Title:** Designing and implementing learning support to address increasing student diversity needs

**Theme:** Examining the links between research, evaluation, policy and changing practice, including teaching, assessment and supporting learning

**Presenter(s):** W.S. Sulochana Wijesundera, Kusum De Abrew and Wasantha Gunatunge
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## Abstract:

University education, both in developed and developing countries, is expanding to bring in non-traditional student groups having diverse learning needs. Universities worldwide have therefore become concerned with diversity initiatives addressing and supporting learning needs of diverse student groups. In Sri Lanka, a South Asian developing country, there is immense pressure for university places that far exceed the numbers admissible. Social pressures have made governments to implement a quota-based university admission policy where the limited places are allocated to include underprivileged schools, bringing in students with diverse backgrounds and learning experiences. Such student diversity is also a reality in developed countries when financially pressed universities admit higher fee-paying overseas students who challenge universities to develop in them abilities and competencies that they seriously lacked at entry level.

We discuss here a process where a group of academic staff in a medical school in Sri Lanka succeeded in setting in motion a voluntary student support structure whereby the ill effects stemming from the diversity of students' learning backgrounds was addressed. Making use of our experience, seminar participants will explore how poor student learning that frustrate university teachers can be isolated to their causative factors for identifying distressing student needs, how different theoretical options can be found and evaluated as ameliorating interventions, how support structures and motivational mechanisms to network interested teachers can be activated, how responses from students and other staff can be reflectively evaluated, how structures to overcome learner impediments can be designed, and how sustainability of the evolved support programme can be ensured.

## Summary

This portfolio is a reflection of my professional development during the past years. It also demonstrates how I have applied the reflective practice that I learned through the ASTHE course to further developed my teaching to become an effective academic.

*Reading through the portfolio, it will be evident that I am a caring person, able to work collaboratively and open to criticism.*

I have structured my portfolio as follows. The preamble gives a brief introduction to myself, my duties and responsibilities as a lecture and about the Staff Development Course. The first chapter is on reflective practice. In chapter 2 I have discussed learning theories, and how I have used these learning theories to improve my teaching strategies. In chapter 3, I have addressed the problems encountered by the freshmen in the university and the initiation of a programme in the Medical Faculty to assist students to manage the change. Chapter four is on developing transferable skills in students through active learning. In chapter five I discuss assessment methods currently used in the Faculty / Department and I have proposed ideas on how to change assessments in order to make students better learners. Critical appraisal of my teaching practices is discussed in chapter six. In the final chapter I identify my values and goals in teaching and research and how I will work towards achieving my goals to excel in my profession.