CHAPTER 1

INTRODUCTION

1.1 Background

Lung cancer is one of the most common and lethal types of cancer with the ratio of incidence to mortality at 0.87 with 1.8 million new cases in 2012 (Ferlay et al., 2012). Lung cancer develops spontaneously through the accumulation of impairments and changes in genetic and epigenetic profiles in response to environmental factors such as tobacco smoke, carcinogens, and air pollution. However, underlying genetic factors are key determinants of disease development and progression (C. Lovly, Horn, & Pao, 2018). Among others, EGFR (Epidermal Growth Factor Receptor) mutations in lung cancer, which span from exon 18 to exon 21, has captured interests due to its significance in lung cancer progression and treatment (El-Telbany & Ma, 2012).

The type of EGFR mutation is vital for predicting the treatment of patients with either Tyrosine Kinase Inhibitors (TKIs) or chemotherapeutic agents. Different types of EGFR mutation will also result in different median progression-free survival (Lee et al., 2013). Therefore, it is vital to detect the presence and type of EGFR mutation as early as possible to administer the correct medication, palliative therapies, and predict the patients' prognosis.

Reverse dot-blot, also known as reverse allele-specific oligonucleotide assay, is an assay that has been developed to detect and molecularly characterize high-mutation spectrum disorders (Gold, 2003). It relies heavily on the hybridization between spotted oligonucleotide probes and a denatured PCR sample. The detection processes utilize colorimetric and non-radioactive substances such as the use of streptavidin-horseradish peroxidase incubation followed by color development using tetramethylbenzidine and hydrogen peroxide. This assay is well known for its efficiency – in time and cost – and robustness in detecting mutations (Derakhshandeh, 2015; Gold, 2003). There are other methods that can be used to detect EGFR mutations such as sequencing, High Resolution Melting (HRM), and even other diagnostic

platforms. However, other methods are usually less cost-efficient, more difficult to interpret, or require longer time when compared to RDB.

The current project will provide validation on whether the RDB kit developed by Stem Cell and Cancer Institute (SCI) is reliable to be used in detecting EGFR mutations, specifically for Exon 19 Deletion and 21 L858R (21R) mutations. The project will be used to support the kit for commercial launch and use in real life lung cancer patients as well as preparing the kit for future improvements, such as the inclusion of a multiplex PCR kit.

1.2 Research Objectives

- To evaluate the reliability of the EGFR Mutation Kit to detect the different subtypes of Exon 19 Deletion mutations specifically.
- To develop and optimize the multiplex PCR formula for the amplification of exon 20 T790M, Exon 21L858R, and Exon 19 Deletion mutations.

1.3 Hypothesis

- The EGFR Mutation Kit is able to detect the different subtypes of Exon 19 Deletion mutations specifically.
- The multiplex PCR formula is able to amplify exon 19, 20, and 21 of EGFR effectively.