Inhibition of EGFR kinase activities by EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib results in effective treatment for patients with NSCLC. However, not every patient has benefit for receiving target therapy. In addition, the efficacy of target therapy will be reduced in NSCLC patients due to drug resistance. EGFR-activating mutations in exon 19 and exon 21 that correlated with a high TKIs treatment response rate and prolonged progression-free survival have been identified. Therefore, establishment of the platform for gene testing to select patients with EGFR mutations is necessary in worldwide clinical lung cancer management especially in East Asia and Taiwan with the EGFR mutation rate as high as 50%. To achieve this aim, we utilized nucleotide mass spectrometry to set up EGFR mutation detection platform with high sensitivity and high specificity. This platform not only has high detection limitation to identify mutation frequency as low as 1% among wild-type background but also selects patients with benefit for target therapy 30% more than traditional EGFR mutation detection system. Furthermore, we also find that T790M resistant mutation may occur before drug administration. This leads us to pay more attention on patients with pretreatment T790M mutation in following clinical care. This study provides a gene test method not only for lung cancer but also for other disease required translational personalized medicine.