#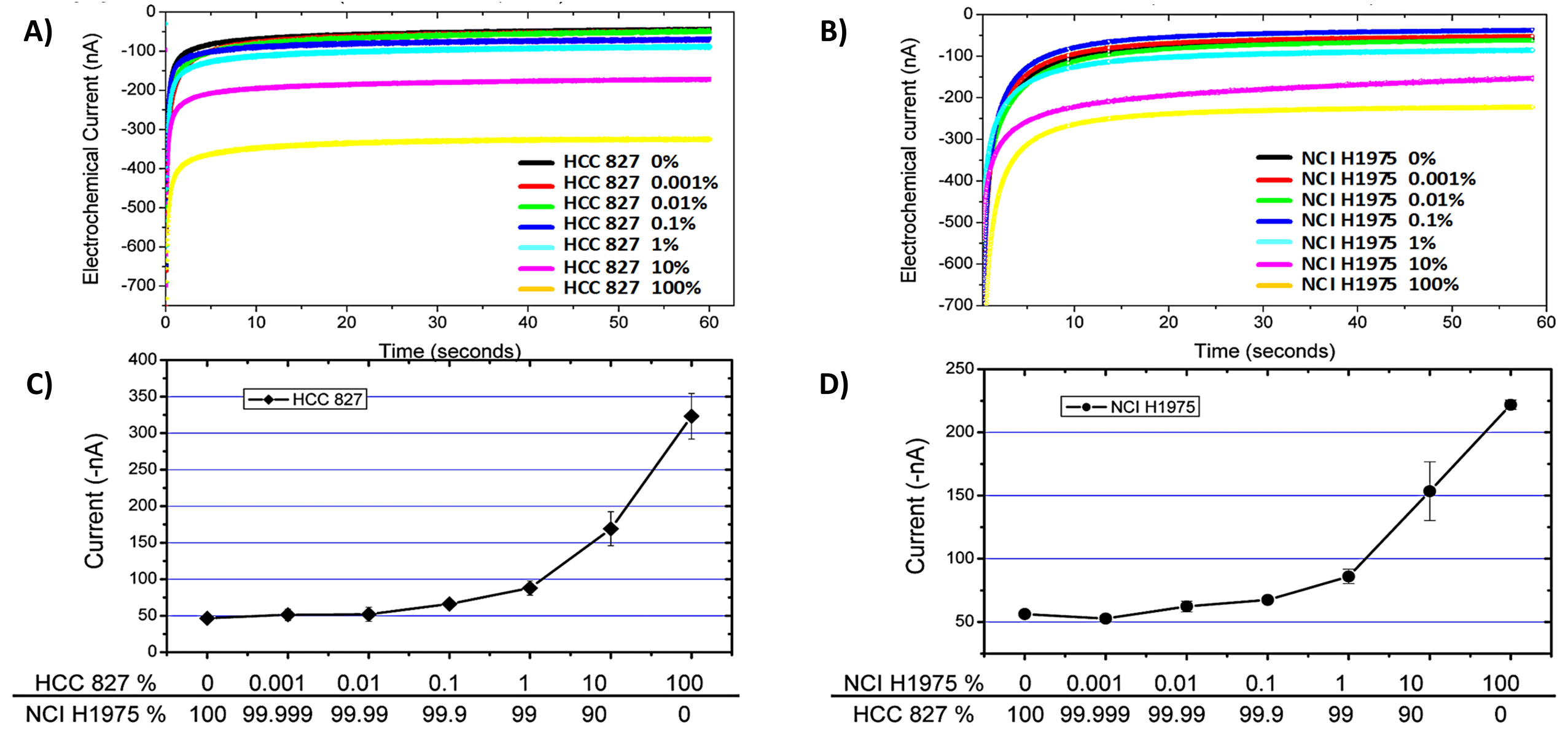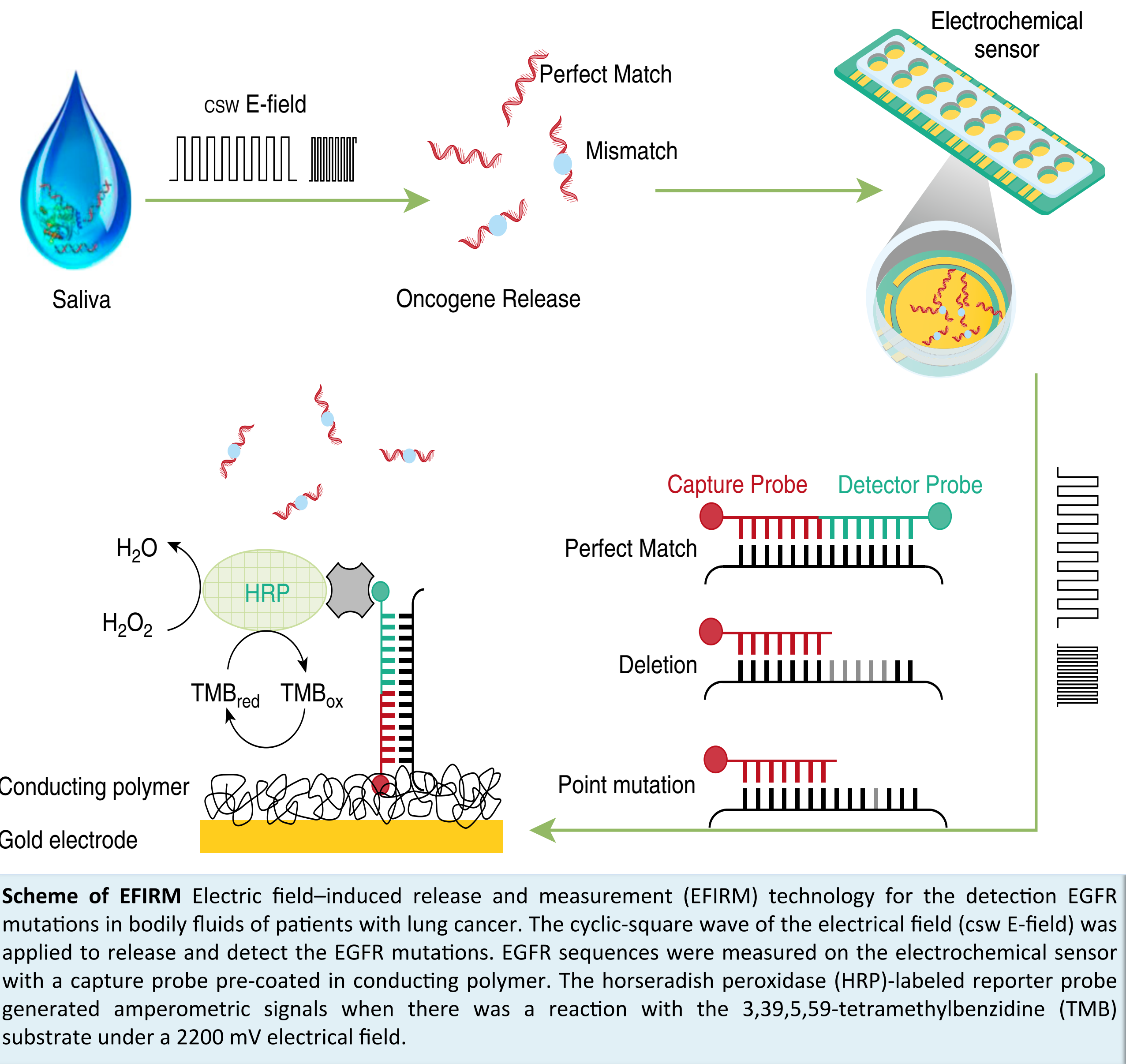 Electric Field-Induced Release and Measurement (EFIRM) can detect EGFR mutations directly from body fluids of lung cancer patients

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Scheme of EFIRM Electric field–induced release and measurement (EFIRM) technology for the detection EGFR mutations in bodily fluids of patients with lung cancer. The cyclic-square wave of the electrical field (csw E-field) was applied to release and detect the EGFR mutations. EGFR sequences were measured on the electrochemical sensor with a capture probe pre-coated in conducting polymer. The horseradish peroxidase (HRP)-labeled reporter probe generated amperometric signals when there was a reaction with the 3,39,5,59-tetramethylbenzidine (TMB) substrate under a 2200 mV electrical field.
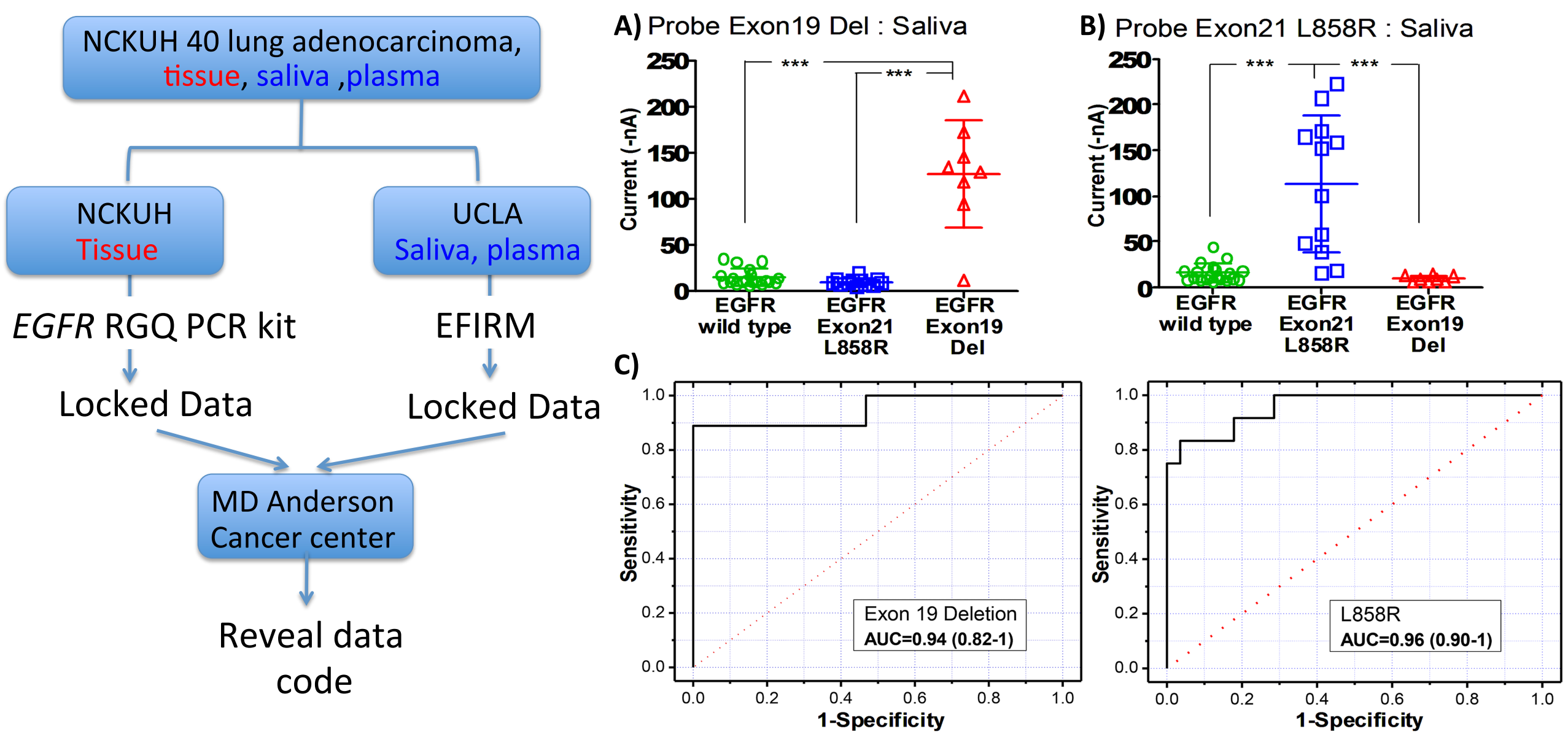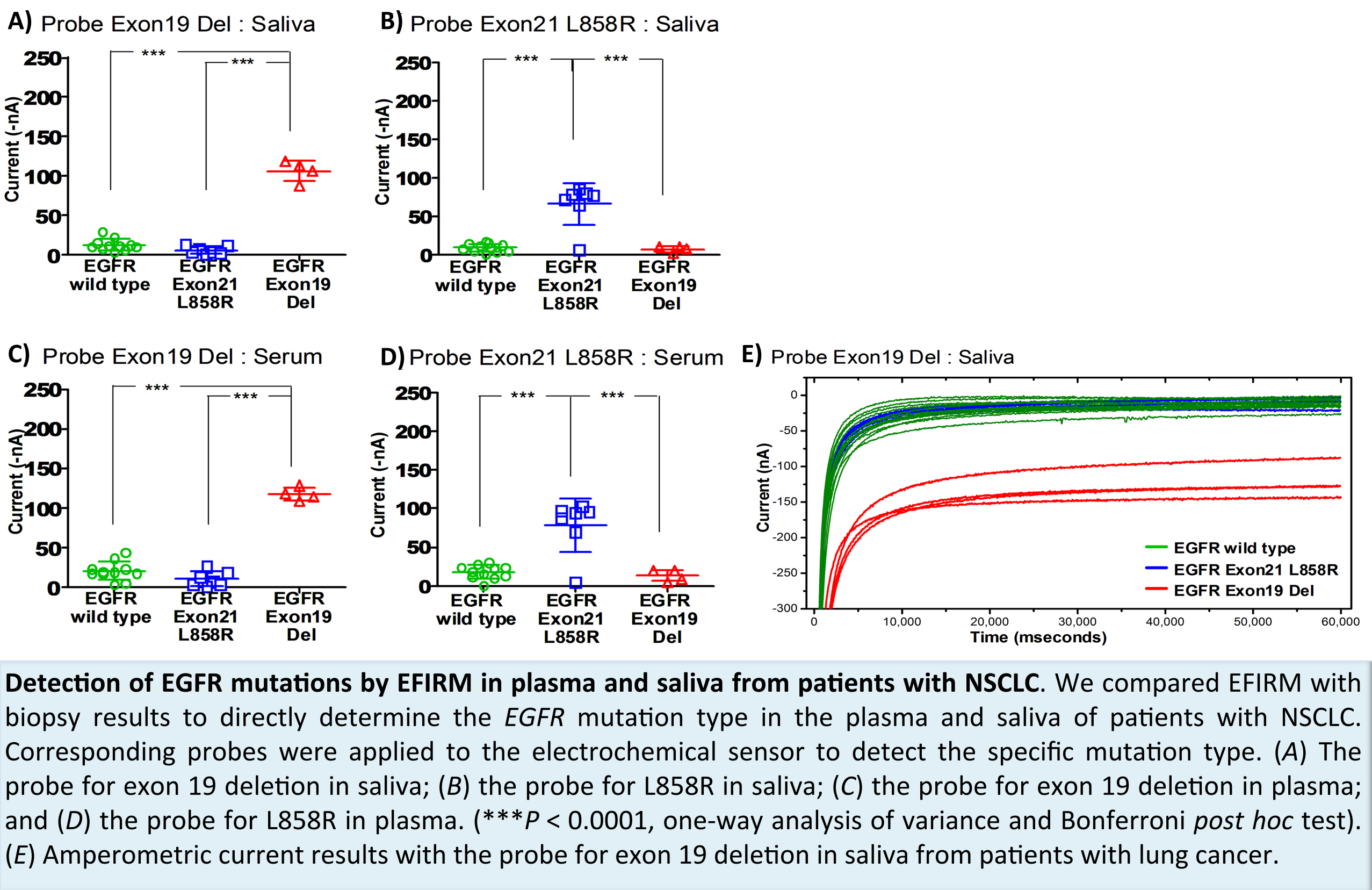

In vitro optimization of EFIRM for EGFR The tyrosine kinase inhibitor–sensitive EGFR mutations, including the exon 19 deletion (from HCC827 cells) and L858R (from NCI-H1975 cells), were assayed by decreasing the ratio of targeted oncogene sequence to other sequences. Electrochemical current readouts are listed in amperometric signals for (A) the exon 19 deletion and (B) L858R. Reactions were performed in triplicate using 20 ng of input DNA. Means and SDs from triplicate experiments are provided for (C) the exon 19 deletion and (D) L858R.
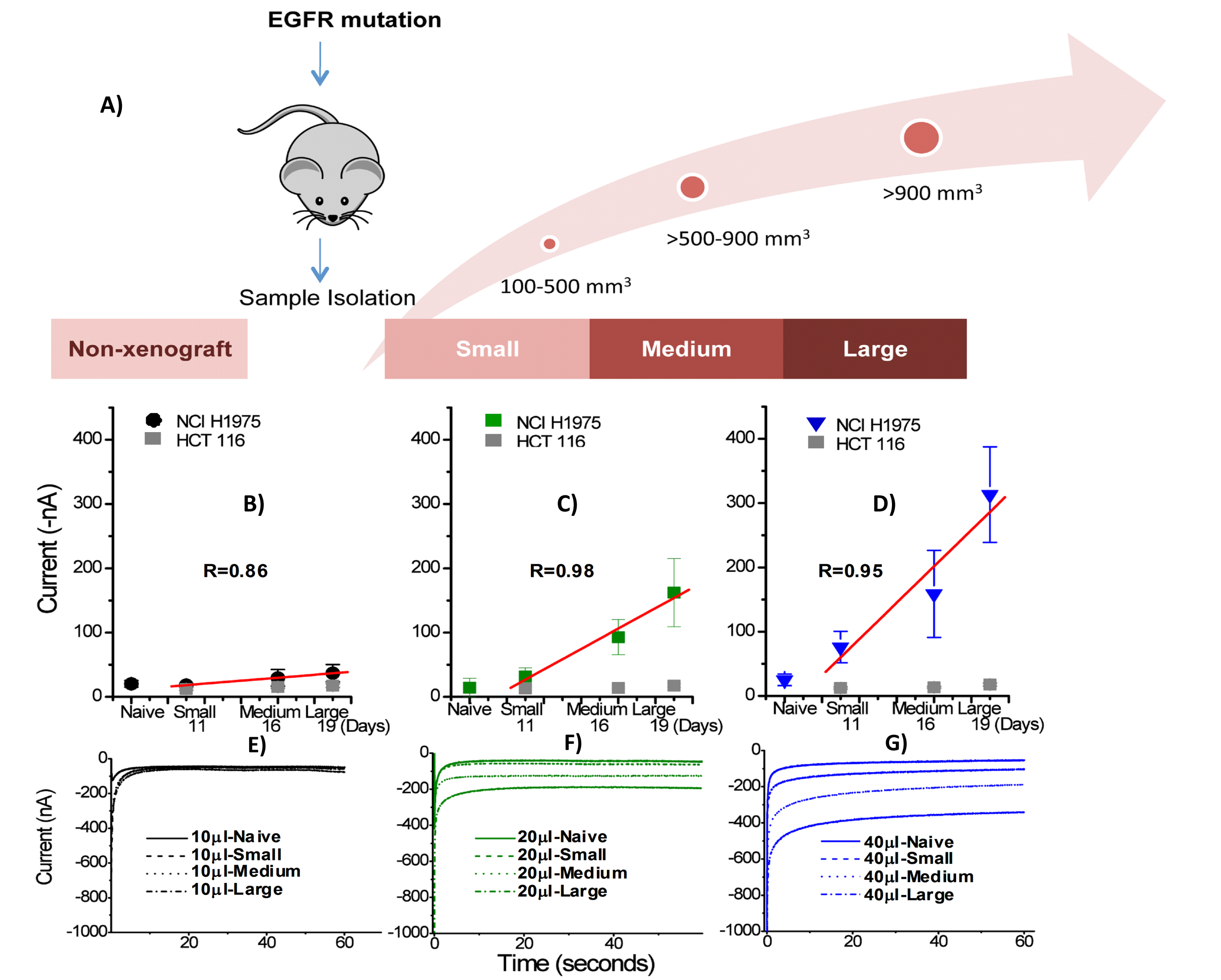
Detection of EGFR L858R in xenografted lung cancer mice via EFIRM. (A) Design of the tumor burden study using EGFR L858R xenografted mice. EFRIM of the four groups of mice (three mice per group) with (B) 10 μl of plasma (R = 0.86), (C) 20 μl of plasma (R = 0.98), and (D) 40 μl of plasma (R = 0.95). The reactions were performed in triplicate with both the means and SDs provided. Linear fits to the data appear in red and the correlation coefficient R is provided. Data from the control group with wild-type EGFR are illustrated in gray squares. The amperometric current readout is listed with (E) 10 μl of plasma, (F) 20 μl of plasma, and (G) 40 μl of plasma.

Background:
In non-small cell lung cancer (NSCLC), epidermal growth factor receptor (EGFR) mutations have emerged as important biomarkers in predicting the response to the EGFR tyrosine kinase inhibitors. The identification of these mutations is based on invasively obtained biopsy samples, which is not always acceptable in a clinical setting. The analysis of circulating tumor DNA or circulating tumor cells in the blood is an alternative approach but is often complicated, technique dependent, and time consuming. A noninvasive, readily available, diagnostic procedure with minimal preparation that provides immediate information on EGFR mutation status is desirable.

Result:
We developed a novel core technology, Electric Field-Induced Release and Measurement (EFIRM) that can detect EGFR mutations directly from body fluids. We first demonstrated that EFIRM can detect EGFR mutations (exon 19 deletion and L858R) from saliva and serum of 22 NSCLC patients. And a blinded test on saliva samples from 40 NSCLC patient showed that EFIRM detected the exon 19 deletion and L858R with an area under the curve (AUC) of 0.94 and 0.96 respectively. For total 62 patients, the sensitivity of EFIRM in detecting L858R and exon 19 deletion in saliva is 84.2% and 91.7% respectively. And the specificity of detecting L858R is 95.3% which is the same as in exon 19 deletion. The amperometric currents EFIRM recorded was not affected by tumor burden, metastatic organ and staging. Finally, we investigate if digital PCR can be used to detect EGFR mutant DNA in saliva. The detection rate was 14.2% in exon 19 Del and 12.5% in L858R respectively. Our data indicated that EFIRM is accurate and user-friendly for the detection of EGFR mutations in the saliva and serum of NSCLC patients.

Detection of EGFR mutations by EFIRM in plasma and saliva from patients with NSCLC. We compared EFIRM with biopsy results to directly determine the EGFR mutation type in the plasma and saliva of patients with NSCLC. Corresponding probes were applied to the electrochemical sensor to detect the specific mutation type. (A) The probe for exon 19 deletion in saliva; (B) the probe for L858R in saliva; (C) the probe for exon 19 deletion in plasma; and (D) the probe for L858R in plasma. (***P < 0.0001, one-way analysis of variance and Bonferroni post hoc test). (E) Amperometric current results with the probe for exon 19 deletion in saliva from patients with lung cancer.

Blinded and randomized clinical EFIRM-based detection of EGFR mutations in saliva. Protocol of blinded randomizing 40 NCLC patients and the absolute values of the signal associated with (A) the exon 19 deletion and (B) the L858R point mutation according to patient subgroup are presented (***P < 0.0001, one-way analysis of variance and Bonferroni post hoc test). (C) The receiver operating characteristic curves for detecting (left to right) the exon 19 deletion (area under the curve [AUC] = 0.94, 95% confidence interval [CI], 0.82–1) and the L858R mutation, respectively (AUC = 0.96, 95% CI, 0.90–1).

|	Nonblinded Cohort	Blinded Validation Cohort	P Value
Age, yr	62.1 ± 12.7	58.8 ± 10.4	0.28*
Sex			1.00†
Total	22	40	
Male	12 (54.5)	22 (55.0)	
Female	10 (45.5)	18 (45.0)	
Smoker	7 (31.8)	11 (27.5)	0.95†
Stage			1.00‡
III	5 (22.7)	8 (20.0)	
IV	17 (77.3)	32 (80.0)	
EGFR mutant type			0.98†
Wild	11 (50.0)	20 (50.0)	
L858R	7 (31.8)	12 (30.0)	
Exon 19del	4 (18.2)	8 (20.0)	

Definition of abbreviation: EGFR = epidermal growth factor receptor.
Data are presented as mean ± SD or n (%).
*t test.
†Chi-square test.
‡Fisher test.

Patient Characteristics of the Testing and Blinded Validation Groups The EGFR mutation rate was comparable to other studies that detected EGFR mutations in Asian lung adenocarcinoma, ranging from 38% (29) to 55%. The amperometric currents recorded by EFIRM was not affected by tumor burden, metastatic organ and staging.
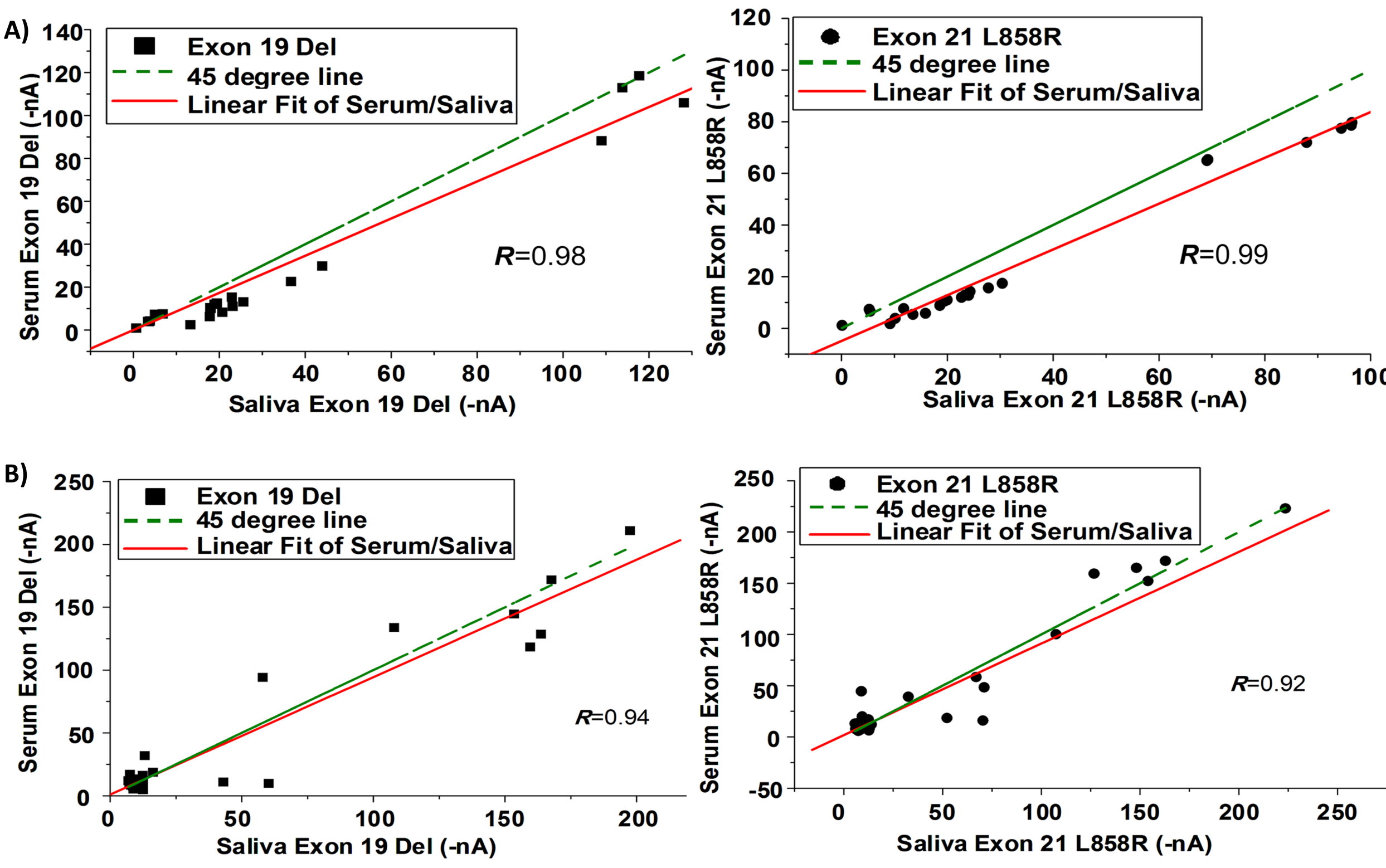
Correlation of EGFR mutation statuses between plasma and saliva using EFIRM The scattergram shows the correlation and linear regression between amperometric currents recorded using plasma and saliva. Each *dot* represents data for one patient in (A) the testing group and (B) the blinded group.
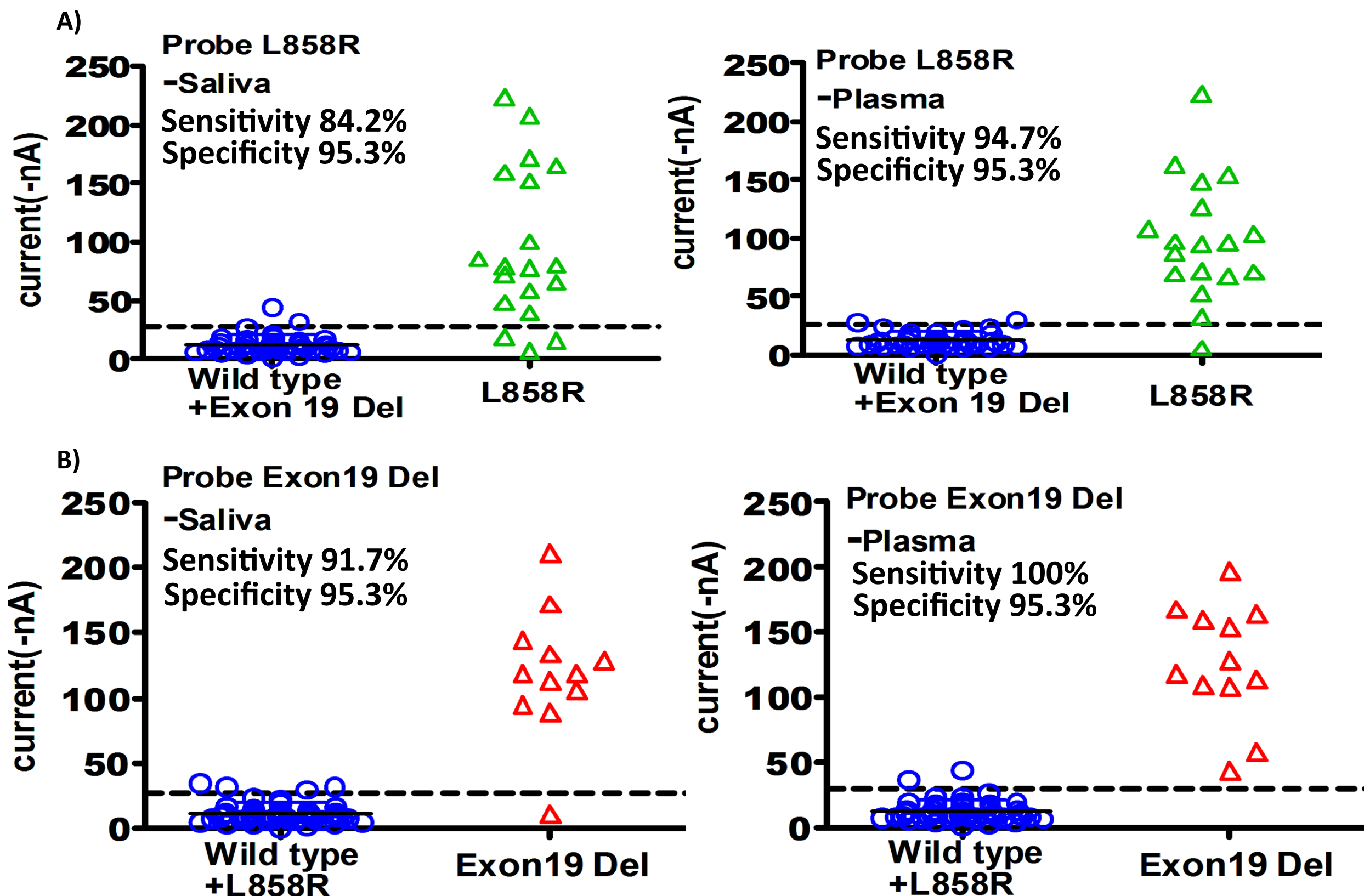
The sensitivity and specificity of EFIRM For total 62 patients, we use a cutoff at 2 standard deviations above the mean value from the control group and Exon 19 deletion to determine the threshold of current value of L858R(A). The threshold of current value of Exon 19del was determined by the same method (B).
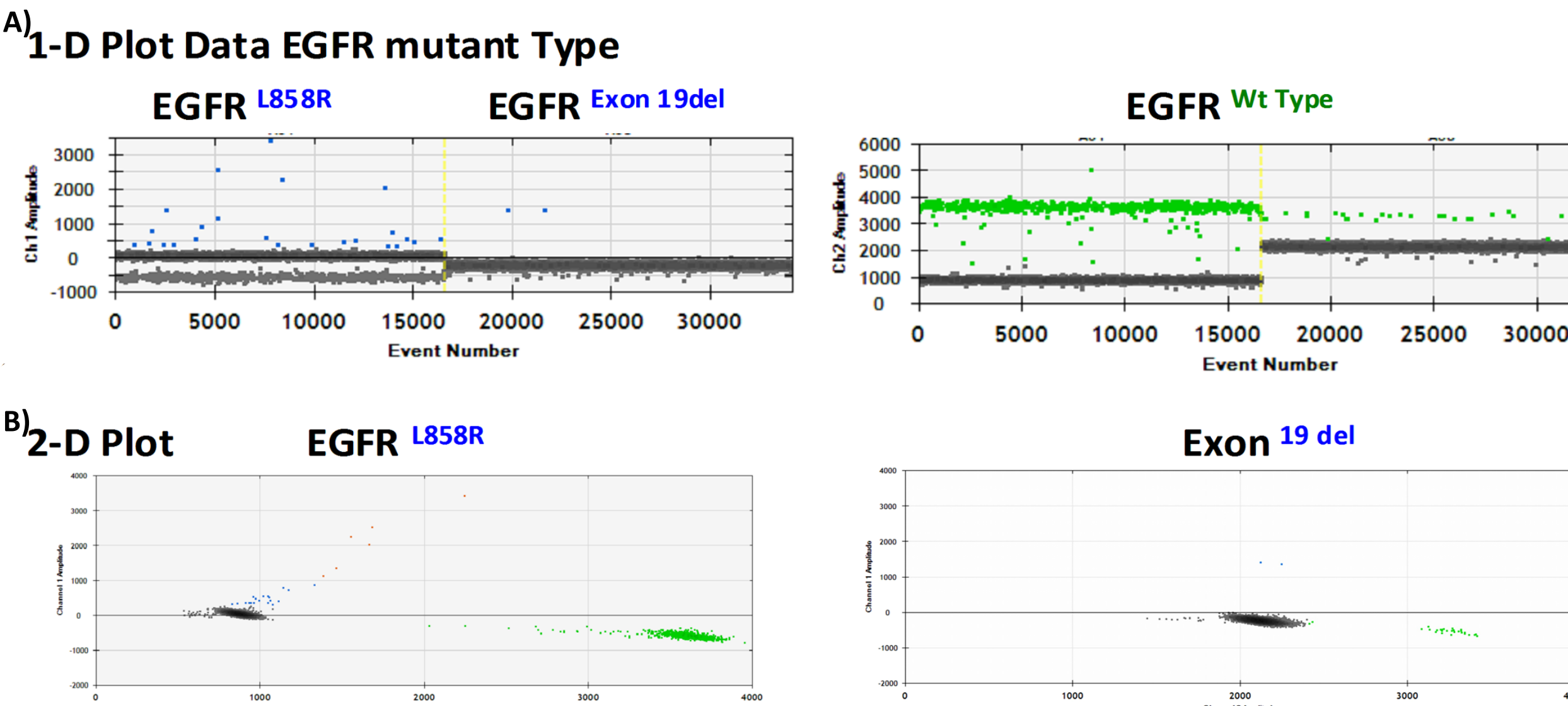
Detection of EGFR DNA from saliva using digital PCR. Saliva DNA was extracted by using QIAmp circulating nucleic acid kit (Qiagen) and subjected to digital PCR(bio rad) analysis. The 1-D plot (A), 2-D plot (B) were displayed.