**Chronocoulometric biosensor for K-ras point mutation
detection based on E.coli DNA ligase and AuNPs
amplification effects**

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25 **Abstract**

26 We report a novel method for the amplified detection of K-ras point mutation by
27 using a chronocoulometric DNA sensor based on *E.coli* DNA ligase and AuNPs
28 amplification effects. Our method is based on a ‘sandwich’ detection strategy,
29 involving capture probe immobilized on a gold electrode (GE) and reporter probe
30 loaded on Au nanoparticles (AuNPs probe). Each probe flanks one of two fragments
31 of the target DNA sequence. Successive hybridization event brings AuNPs, along
32 with hundreds of reporter probes to form the ‘sandwich’ structure. After high-fidelity
33 *E.coli* DNA ligase reaction and denaturing at an elevated temperature, We then
34 employ chronocoulometry to detect $[\text{Ru}(\text{NH}_3)_6]^{3+}$ electrostatically bounding to the
35 DNA probe strands. If there is an allele mismatch, no ligation takes place. In this way,
36 the novel approach had successfully distinguish the K-ras mutant DNA and the K-ras
37 wild DNA. The charge variable (ΔQ) is proportional to the logarithmic K-ras mutant
38 DNA concentration from 1.0 nM to 0.1pM, with the detection limit of 0.01 pM.
39 Owing to its ease of operation and low detection limit, it is expected that the proposed
40 approach may hold great promise in both research-based and clinical genomic assays.
41 *Keywords:* K-ras point mutation; *E.coli* DNA ligase; AuNPs report;
42 Chronocoulometry; RuHex

43 **1. Introduction**

44 Pancreatic cancer is particularly tragic because up to 10% patients with
45 pancreatic cancer have a family history of the disease and individuals with a family
46 history of pancreatic have an increased risk of developing pancreatic cancer
47 themselves [1,2]. Even patients treated with current state-of-the-art therapies have a
48 5-year relative survival rate of only 3% [3], and in advanced pancreatic cancer 5-year
49 survival is zero [4]. It’s greatly difficult to treat the disease because clinical symptom
50 is relatively late; most patients are diagnosed with metastasis disease and few shows a
51 sustained response to chemotherapy or radiation therapy [5]. Hence, considering the
52 seriousness of pancreatic cancer, early diagnosis is extremely key to successfully treat

53 the disease and lessen the mortality rate of pancreatic cancer, especially for the people
54 with a family pancreatic cancer predisposition, for the curative ration of surgical
55 removal of the tumor is considerable at the early cancer.

56 Excitedly, among a variety of genomic alterations in precursor lesions of
57 pancreatic intraepithelial neoplasia (PanIN), mutation of the gene encoding the small
58 GTPase, K-ras mutation, is most frequent. Codon 12 of the wild type K-ras is GGT,
59 but the mutant type mutates into GAT. What's more, in pancreatic cancers,
60 approximately 70-90% of cases indicated K-ras mutations [6] and frequently detected
61 in the most common precursor lesion of pancreatic cancer, indicating the K-ras
62 mutant can act as marker gene in pancreatic cancer presence [7]. Thus, detection of
63 K-ras point mutation gene will afford an early diagnosis and monitor of the disease. It
64 is very essential to develop a sensitive, time-saving, inexpensive approach to detect
65 DNA point mutation.

66 Recently, there has been significant progress in developing biosensors for the
67 rapid, cheap and accurate detections of specific DNA sequence [8-10]. Various
68 sensing strategies have been developed for DNA point mutation detection, aiming at
69 the improvement of sensitivity and selectivity of electrochemical detection [11]. In
70 our present work, we developed a novel electrochemical DNA biosensor for the
71 amplified detection of K-ras point mutation by using a chronocoulometric DNA
72 sensor based on *E.coli* DNA ligase and AuNPs amplification effects. The fabrication
73 method is shown in Scheme 1. Briefly, the capture probe was immobilized on the GE
74 surface. The successive hybridization of the capture probe with the target DNA and
75 the AuNPs probe leaded to the formation of 'sandwich' structure. *E.coli* DNA ligase
76 was used to close the nick between the capture probe and the AuNPs probe, which
77 would not take place even in the case of a single-base mismatch. After denaturing at
78 an elevated temperature, the AuNPs probe linking with capture probe would remain
79 on the GE surface only for the case of perfect match K-ras mutant target DNA. When
80 K-ras wild target DNA did not match perfectly with the capture probe and AuNPs
81 probe, the nick between the capture probe and AuNPs probe would not be closed by
82 the *E.coli* DNA ligase. After denaturing, the capture probe was only remained on GE

without AuNPs probe. So, after *E.coli* DNA ligase reacted and ‘sandwich’ structure denatured, we applied the chronocoulometry (CC) response change to discriminate the K-ras mutant target DNA and wild target DNA. The proposed method requires only a small sample volume, involves harmless reagents and possesses some merits. Firstly, RuHex, served as the electrochemical signal molecule, electrostatically binds to the phosphate backbone of DNA immobilized on the GE surface and comes into being an electrochemical signal whose intensity is proportional to the amount of DNA [12]. Secondly, the Au nanoparticles prepared by one-step direct chemical reduction were used to link with the huge amount of report DNA to absorb more RuHex to amplify the later electrochemical detection signal. Thirdly, the high-fidelity *E.coli* DNA ligase provides high accuracy for discriminating single-base mismatch[13]. Because the *E.coli* DNA ligase only catalyzes the joining of two juxtaposed probes perfectly matched to the target DNA at the site of their join. At this site even a single nucleotide mismatch between the probes and target would prevent the ligation of these two probes[14-15]. Therefore, such an allele-specific ligation reaction provides the possibility of designing highly specific assay protocols with single-base mismatch discrimination capability. The *E.coli* DNA ligase possesses commendable high-fidelity in the identification of single-base mismatches. The developed method has been successfully applied to the authentication of point mutation in codon 12 of K-ras oncogene target. The satisfactory results obtained distinctly show that the approach holds more potential biomedical applications in the future.

Scheme 1

2. Experimental

2.1. Reagents and apparatus

All the DNA sequences and *E.coli* DNA ligase were purchased from Takara Biotechnology Co., Ltd (Dalian, China). The sequences of the oligonucleotides are listed in Table S1.

Chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), trisodium citrate dihydrate,

111 Tris(hydroxymethyl)aminomethane, ethylenediamine-tetraacetic acid (EDTA),
112 potassium hexacyanoferrate, potassium hexacyanoferrate trihydrate, NaCl, HCl,
113 H_2SO_4 , $\text{Na}_2\text{HPO}_4 \cdot 10\text{H}_2\text{O}$ and $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ were purchased from Sinopharm
114 Chemical Reagent Co., Ltd. These reagents were of analytical reagent grade.
115 6-mercapto-1-hexanol (MCH) and hexaammineruthenium ($[\text{Ru}(\text{NH}_3)_6]\text{Cl}_3$, RuHex)
116 were obtained from Sigma. The ultrapure water with a specific resistance of 18.2
117 $\text{M}\Omega\cdot\text{cm}$ was prepared with Milli-Q-purified system. Gold electrode (GE) ($\Phi=2\text{ mm}$)
118 was obtained from Shanghai ChenHua Instruments Co (China).

119 Phosphate buffer solution (PBS, $\text{pH}=7.0$) acted as the hybridization buffer
120 containing 0.1 M $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$ and 0.1 M NaCl and adjusting its pH with
121 50mM H_3PO_4 and NaOH. TE buffer ($\text{pH}=8.0$) was used to dilute the capture DNA,
122 containing 10 mM Tris, 1.0 mM EDTA and 1 M NaCl and pH of the solution was
123 adjusted with 10 mmol/L HCl. All the solution was stored in a refrigerator (4°C) when
124 not used.

125 2.2. Preparation of AuNPs and AuNPs probe

126 AuNPs was prepared according to the previous literature [16, 17]. All the
127 glassware used in the following procedure was soaked in the fresh aqua regia with 3
128 parts of HCl and 1 part of HNO_3 and rinsed thoroughly in ultrapure water. In a 500mL
129 round bottomed flask, 4.0 mL of 1% sodium citrate was quickly added to 250mL of
130 boiling 0.01% HAuCl_4 solution with simultaneously vigorous stirring. The color of
131 solution gradually changed from yellow to red violet. The mixture was boiled
132 continually for an additional 10min under continuous stirring to perfectly red violet.

133 The preparation of AuNPs probe was performed following the published
134 procedure [18, 19]. The report probe was immobilized on the surface of AuNPs by
135 3'-terminal mercapto of oligonucleotide. Firstly, 1 OD of the report probe was added
136 into 2 mL of the 13 nm Au colloid. After standing for about 16 h at 25°C , the mixed
137 solution finally contained 10 mM $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ by adding 100 mM PBS
138 ($\text{pH}=7.2$). Then, the colloid solution was brought to 100 mM NaCl and placed 40 h
139 aging the mixed solution. To remove excess free report probe, the solution was

centrifuged for 30 min at 15000 rpm. After washed with PBS (pH=7.2) three times, the solution was redispersed in 1 mL of 0.3 M KCl and PBS (pH =7.2) and stored at 4°C when not used. Transmission electron microscopy (TEM, philips company) and UV-Vis were used to characterize AuNPs and AuNPs probe.

2.3. Pretreatment of GE and fabrication of electrochemical DNA biosensor

Prior to modification, the bare GE was polished in sequential order with 0.3 µm and 0.05µm alumina powder (AlfaAesar, USA) suspension on microcloth pad and then thoroughly sonicated in HNO₃ solution (volume ratio of water to HNO₃ is 1:1), ethanol and H₂O for 3 min, respectively. After drying with nitrogen, the polished GE was electrochemically cleaned to remove any remaining impurities by voltammetrically cycling in a fresh 0.5M H₂SO₄ solution [20].

The 3µL of capture probe was immediately dropped on the prepared GE to immobilize the capture probe for 2 h at room temperature. Electrodes with different surface density were interrogated. Low-density, medium-density and high density surfaces were prepared by incubation of electrodes with 0.5µmol/L, 1.0µmol/L, 5.0µmol/L different concentration of capture probes, respectively (Fig. S1). After thoroughly rinsed with PBS and deionized water, capture probe/GE was immersed in a 100µL of 1 mM MCH for 1 h at room temperature to remove the nonspecific DNA adsorption and optimize the orientation of capture probe on the electrode to make subsequent hybridizing easier. The electrode was adequately rinsed with PBS and ultrapure water to obtain the modified electrode denoted as capture probe/MCH/GE.

Then, the capture probe/MCH/GE was immersed in a 100µL of different concentration of target DNA for 1 h at 48°C. After the target DNA hybridized with the capture probe, the electrode was cleaned with the PBS. Next, the modified electrode was immersed in a 100µL of AuNPs probe for 1 h at 48°C to form a ‘sandwich’ structure. The 10µL 0.6U/µL of *E.coli* DNA ligase was added on the above electrode for 0.5 h at 37°C. After washing with PBS and ultrapure water, the electrode was placed in deionized water at 75°C to denature the double helix structure to form a single stranded DNA.

2.4. Electrochemical measurements

A 10 mM Tris-HCl solution (pH=7.4) containing 50 μ M RuHex was defined as the RuHex solution. A 10 mM Tris-HCl solution (pH=7.4) acted as the detection solution. The main CC performance was carried out on a CHI 760d electrochemical workstation (Shanghai ChenHua Instruments Co., China) in the detection solution. The detection solution and the RuHex solution were thoroughly deoxygenated by bubbling high-purity nitrogen through the solution for the 10 min before the measurements began. Electrochemical impedance spectroscopy (EIS) was performed on an Autolab PGSTAT302F system (Eco Chemie, The Netherlands) in the fresh test solution containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and 1 M KNO_3 . A conventional three-electrode system was used in this experiment, where modified GE acted as the working electrode, Ag/AgCl electrode (saturated with KCl) as reference electrode, and platinum wire as auxiliary electrode, respectively. The UV spectrum was detected by ultraviolet and visible spectrophotometer (UV-2450, Shimadzu, Japan). The TEM images were obtained from transmission electron microscopy. The pH measurements were made with a PHS-3E meter (Shanghai electronics scientific instrument co., China).

3. Results and discussion

3.1. Characterization of the AuNPs, AuNPs probe

AuNPs and AuNPs probe were characterized by UV-Vis spectrophotometer. Fig. 1 shows that the UV-vis spectra of the report probe only had a maximum absorption peak at 260 nm (curve a) and the AuNPs had a maximum absorption peak located at 520 nm (curve b), After AuNPs reacted with report probe, two obvious absorption peaks of AuNPs probe were observed at 260 nm and 524 nm (curve c), respectively. Moreover, the absorption peak of the AuNPs linking with report probe happened red shift about 4nm. These observations were in good agreement with a previous report [21].

TEM was used to observe the AuNPs and AuNPs probe. The results were shown

197 in inset of Fig.1, the pure AuNPs diameter was $13 \text{ nm} \pm 1.7 \text{ nm}$, but dispersion of
198 AuNPs linking with report probe was significantly better than the unitary AuNPs,
199 that's because strong intermolecular repulsions among the negatively charged
200 phosphodiester backbones of report probe increase the stability of report
201 probe-AuNPs. [22]. The results adequately demonstrated that the report probe was
202 successfully loaded on the surface of AuNPs.

203 Fig.1.

204 3.2. Electrochemical characterization of different modified electrodes

205 EIS has been usually used to characterize the stepwise reaction on the surface of
206 the modified electrode, so EIS was used to evaluate the interfacial electron-transfer
207 resistance (R_{et}) at different stages of biosensor preparation. As shown in Fig. 2,
208 distinct differences in the R_{et} were observed upon the stepwise modified electrode
209 within the range from 100 Hz to 100MHz. There was few impedance observed on
210 the bare GE (curve a), implying a very fast electron transfer process of $[\text{Fe}(\text{CN})_6]^{3-/4-}$.
211 After the electrode surface immobilized the capture probe, the interfacial electron
212 resistance evidently increased (curve b), indicating the thiolated capture probe
213 successfully self-assembled on the electrode and the negatively charged DNA
214 immobilized on the electrode restricted the redox probes $[\text{Fe}(\text{CN})_6]^{3-/4-}$ toward
215 electrode surface [23]. After hybridizing with K-ras mutant target DNA, the R_{et}
216 further increase (curve c), suggesting the double-stranded DNA with more negatively
217 charged phosphate backbone greatly prevented the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ near the electrode
218 surface [24,25]. A larger R_{et} increase was observed after the hybridization with AuNPs
219 probe (curve d), because the AuNPs probe with a large mass of negative charges
220 greatly blocked the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ anions. After the fabricated electrochemical sensor
221 denaturing, a R_{et} (curve e) smaller than curve e was obtained, indicating the
222 'sandwich' structure on the electrode surface melted to form a single stranded
223 oligonucleotides which consisted of capture probe and AuNPs probe. The results well
224 showed that DNA sensor had been successfully fabricated.

225 Fig. 2.

226 3.3. Optimization of experimental conditions

227 Both the concentration of capture probe and the reaction time of *E.coli* DNA
228 ligase are very significant to obtain a perfect experimental result, so the two
229 conditions were all sidedly investigated in the work. As shown in Fig. S3, the value of
230 CC increased with increasing the concentration of capture probe from 0.1 μM to 0.5
231 μM . However, when the high concentration exceeded 0.5 μM , the value of CC
232 decreased. The reason might be that high density of capture probe on the electrode
233 would impact the hybridization rate with target DNA. Therefore, the 0.5 μM of
234 concentration was chosen as the optimal concentration of capture.

235 Fig. S4 showed the influence of the reaction time of *E.coli* DNA ligase on the
236 value of CC. As can be seen, the value of CC gradually increased with increasing
237 the reaction time of ligase from 10 min to 40 min, but the value began to stabilize
238 with the reaction time more over 40 min. Thus, the reaction time of *E.coli* DNA ligase
239 was optimized to be 40 min.

240 3.4. *E.coli* DNA ligase reaction and specificity of the DNA sensor

241 In order to improve the signal-to-noise ratio and obtain high analytical
242 performance, the *E.coli* DNA ligase was proposed and employed to improve the
243 specificity. AuNPs probe was employed to improve the sensitivity. The experiment
244 without or with the *E.coli* DNA ligase was performed with the capture probe
245 hybridization with 1.0 pM K-ras mutant target DNA and AuNPs probe to evaluate the
246 function of *E.coli* DNA ligase. As shown in Fig. 3, in the absence of *E.coli* DNA
247 ligase, after denaturing, the modified GE was immersed in the RuHex solution for 15
248 min. After rinsed with the detection solution, the modified electrode was put into the
249 detection solution for chronocoulometry measurement. It is observed that the CC
250 response (curve d) is almost equal to the blank value (curve b). When *E.coli* DNA
251 ligase was used for ligation function, the CC signal was significantly increased (curve
252 e). The results demonstrated that the ligation function of *E.coli* DNA ligase was very
253 effective.

254 The specificity of biosensor was a significant feature for the practical application.

255 The *E.coli* DNA ligase possesses commendable high-fidelity in the identification of
256 single-base mismatches [26]. In this work, the selectivity of the proposed sensor was
257 evaluated by distinguishing the K-ras mutant-type and the wild-type. Fig. 3 shows the
258 CC signals for the K-ras mutant-type and the wild-type with the same concentration of
259 1.0 pM. The chronocoulometric response of K-ras wild-type (curve c) was far less
260 than K-ras mutant-type (curve e) and very closed to the blank value (curve b). The
261 chronocoulometric response of K-ras mutant-type was significantly increased. From
262 the SEM image (Fig. S2). We can see that plenty of gold nanoparticles remain on the
263 GE surface when capture probe hybridisation with K-ras mutant-type target DNA and
264 AuNPs probe to form the ‘sandwich’ structure. After high-fidelity *E.coli* DNA ligase
265 reaction and denaturing at an elevated temperature. Since K-ras mutant target DNA
266 matched perfectly with the capture probe and AuNPs probe, the nick between the two
267 probes would be closed in the presence of *E.coli* DNA ligase. After denaturing,
268 AuNPs probe would remain on the surface of the GE, the huge amount of report DNA
269 loaded on AuNPs to absorb more RuHex to amplify the electrochemical detection
270 signal. On the contrary, K-ras wild target DNA did not match perfectly with the
271 capture probe and AuNPs probe, so the nick between the capture probe and the
272 AuNPs probe would not be closed by the *E.coli* DNA ligase. After denaturing, the
273 capture probe was only immobilized on GE. The results fully showed that the *E.coli*
274 DNA ligase had perfect fidelity and the proposed sensor possessed commendable
275 specificity and successfully made a distinction between the K-ras mutant and K-ras
276 wild target DNA. This serves as the basis of the proposed method for distinguishing
277 K-ras one point mutation.

278 Fig. 3.

279 3.5. Quantification detection of K-ras mutant DNA

280 In the present study, we had further employed the accurate chronocoulometry to
281 sensitively detect K-ras mutant target DNA under optimized conditions. Prior to the
282 electrochemical measurements, the modified GE was immersed in the RuHex solution
283 for 15 min. After rinsed with the detection solution, the modified electrode was put

284 into the detection solution for chronocoulometry measurement. Because the CC
285 signals generated by RuHex corresponded to the number of DNA on the GE, a
286 succession of CC curves were obtained in different concentration conditions (Fig. 4).
287 The charges of RuHex correspondingly increased with the increase of target DNA
288 concentration and the charge variable (ΔQ) is proportional to the logarithmic K-ras
289 mutant DNA concentration from 1.0 nM to 0.1 pM (Fig. 4 inset). The linear
290 regression equation is $\Delta Q (\mu C) = 0.1575x - 0.0252$, $R=0.9916$. The limit of detection
291 (LOD) for the mutant DNA is estimated to be 0.01 pM using the three times of
292 standard deviation over the blank value. The proposed approach in the present study
293 may be another alternative for the detection of K-ras mutant DNA besides the
294 previous method [11, 27]. The enhanced analytical performance can be attributed to
295 AuNPs probe. AuNPs were used to link with the huge amount of report DNA to
296 absorb more RuHex to amplify the electrochemical detection signal.

297 A series of repetitive measurements ($n \geq 3$) with various concentration of mutant
298 DNA can all have a relative standard deviation with 7.6%, so the reproducibility of
299 the recommended biosensor can be satisfactory.

300 Fig. 4.

301 One major challenge of the method pertains to the adaptivity of the procedure to
302 detect selectively the respective mutations in real clinical genomic samples. Because
303 most clinical samples are the heterozygotic gene, we decided to design the simulation
304 test for quantitatively detect K-ras mutant DNA in the mixed hybridization solution
305 containing K-ras wild-type DNA as interference background. The capacity of the
306 prepared sensor was evaluated by detecting these mixtures prepared by mixing 1.0 nM
307 K-ras wild-type DNA with K-ras mutant DNA of concentration varying from 1.0 pM
308 to 1.0 nM. As shown in Fig. S4, K-ras mutant DNA concentration as low as 1.0 pM
309 can be detected in the presence of wild-type DNA as high as 1.0 nM. According to the
310 results, the ΔQ is also proportional to the logarithmic K-ras mutant DNA
311 concentration from 1.0 pM to 1.0 nM, and the linear regression equation is $\Delta Q (\mu C) =$
312 $0.1362x + 0.0658$, $R=0.9970$ (Fig. S4 inset). [The reproducibility of the proposed](#)
313 [biosensor was investigated by successive detection of 0.1 nM K-ras mutant DNA for](#)

314 five times in the presence of 1.0 nM K-ras wild-type DNA as interference background.
315 The relative standard deviation (RSD) was 9.2%, showing a satisfactory
316 reproducibility. The results fully indicated that the proposed method could
317 quantitatively determine K-ras mutant DNA in heterozygous sample.

318 **4. Conclusions**

319 In the present paper, we report a novel method for identification of the K-ras
320 mutant gene and wild gene. The proposed method successfully combined the
321 high-fidelity of the *E.coli* DNA ligase with the amplification function of AuNPs for
322 the detection of K-ras point mutation. The use of *E.coli* DNA ligase and AuNPs well
323 heightened the sensitivity and specificity, and offered the assay system with
324 portability and low-costing. And the new method can have LOD with 0.01 pM for
325 K-ras mutant DNA, implying the method may theoretically be used to directly detect
326 genomic DNA samples and study the genotyping of single nucleotide polymorphism
327 (SNP) in genetics. This method has been demonstrated to be capable of detecting 1.0
328 pM K-ras mutant target in the presence of 1.0 nM K-ras wild type DNA. The research
329 results demonstrate that the novel approach may promisingly be applied to clinical
330 diagnosis of genetic diseases and pharmacokinetics in the future.

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347 **References**

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399 **Figure Captions:**

400 Scheme. 1. Schematic diagram of the proposed method for K-ras point mutation
401 detection.

402 Fig. 1. UV-absorption spectrum of report DNA (a), AuNPs (b) and AuNPs report (c);
403 TEM images of AuNPs (inset A) and AuNPs report (inset B).

404 Fig. 2. Electrochemical impedance spectra (Nyquist plots) of bare GE (a), capture
405 probe/GE (b), K-ras mutant target DNA/capture probe/GE (c), 'sandwich' structure
406 modified GE (d), After denaturing of 'sandwich' structure modified GE (a single
407 stranded DNA consisting of capture probe and AuNPs probe) (e) in the presence of in
408 the solution containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and 1 M KNO_3 with frequency from 100
409 Hz to 100MHz and a bias potential of 0.20V (vs. Ag/AgCl).

410 Fig. 3. Chronocoulometric response of capture probe immobilized on the electrode (a),
411 and after hybridizing with blank buffer (blank test) (b), K-ras wild DNA with *E.coli*
412 DNA ligase (c), K-ras mutant DNA without *E.coli* DNA ligase (d) and K-ras mutant
413 DNA with *E.coli* DNA ligase (e) in the detection solution after incubating in the
414 RuHex solution for 15 min. Inset shows the bar grip of increase of surface charge
415 density (ΔCharge).

416 Fig. 4. Chronocoulometric curves for obtained in the presence of different
417 concentrations of K-ras mutant target DNA (from a to h: 0, 0.1 pM, 0.5 pM, 1pM, 5
418 pM, 10 pM, 100 pM, 1nM) in the detection solution after incubating in the RuHex
419 solution for 15 min. Pulse with: 0.25s. Inset shows relationship between the
420 logarithmic plot of concentration of K-ras mutant DNA (blank diamonds) and
421 wild-type DNA (red rounds) and increase of surface charge density (ΔQ). The ΔQ are
422 averages for three electrodes.

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Scheme. 1.

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Fig. 1.

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Fig. 2.

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Fig. 3.

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Fig. 4.

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