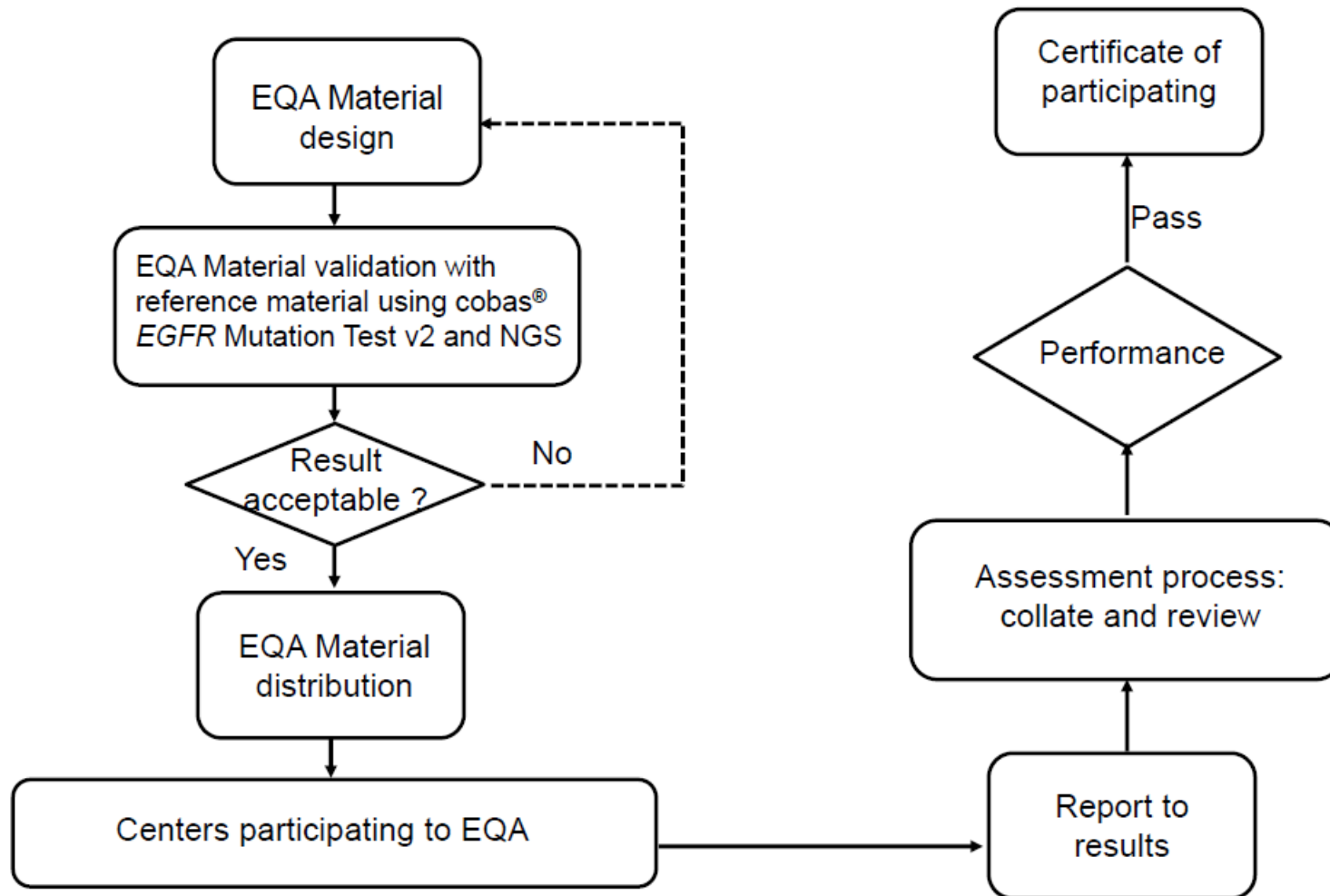


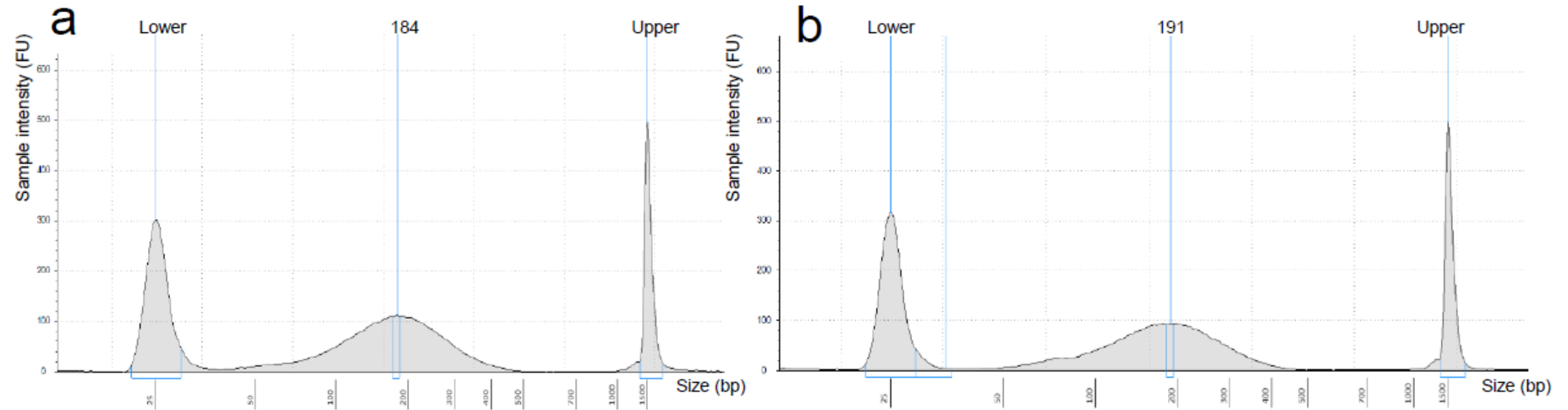
## Supplementary materials

Supplementary Figure S1. Workflow of the pilot external quality assurance scheme.



**Supplementary Figure S2.** Electropherograms of DNA sheared by ultrasonication and run on an Agilent D1000 ScreenTape System.

Sheared genomic DNA from wild-type and (a) the *EGFR* p.T790M reference standard and (b) the *EGFR* p.L858R reference standard.



**Supplementary Table S1.** External quality assurance material for evaluation of limit of detection

EQA materials	Reference Materials	Gene	Variant	Allele frequency (%) <sup>a</sup>	Copies of wild type DNA per $\mu\text{L}$ <sup>a</sup>	Copies of mutant DNA per $\mu\text{L}$ <sup>a</sup>	Spiked NHP (2 mL) <sup>b</sup> of		
							Expected copies of wild type DNA <sup>c</sup>	Expected copies of mutant DNA	The total fragmented DNA (ng) <sup>d</sup>
Level 1	5% Multiplex I cfDNA Reference Standard (HD777)	<i>EGFR</i>	p.L858R	4.59	7904	380	16970	760	80.18
			p.E746_A750del	5.77	4896	300	10954	600	
			p.T790M	4.52	4440	210	10042	420	
			p.A767_V769dup	4.39	3944	181	9050	362	
Level 2	2.5% Spiked Standard (HD777: WT100% = 1 : 1)	<i>EGFR</i>	p.L858R	2.29	8140	190	17442	380	60.42
			p.E746_A750del	2.89	4936	150	11034	300	
			p.T790M	2.26	4428	105	10018	210	
			p.A767_V769dup	2.19	3988	91	9138	181	
Level 3	1% Multiplex I cfDNA Reference Standard (HD778)	<i>EGFR</i>	p.L858R	0.85	8144	70	17450	140	68.02
			p.E746_A750del	1.24	4296	54	9754	108	
			p.T790M	0.93	3848	36	8858	72	
			p.A767_V769dup	0.84	3672	31	8506	62	
Level 4	0.1% Multiplex I cfDNA Reference Standard (HD779)	<i>EGFR</i>	p.L858R	0.13	7824	10	16810	20	65.74
			p.E746_A750del	0.11	4408	5	9978	10	
			p.T790M	0.13	3896	5	8954	10	
			p.A767_V769dup	0.06	3616	2	8394	4	

<sup>a</sup>Mutant allele frequency, copy number values of wild type and mutant DNA measured using digital droplet PCR were provided from manufacturer.

<sup>b</sup>1  $\mu\text{L}$  of standard DNA were mixed with 1 mL of NHP.

<sup>c</sup>1162 wide type copies per 2 mL NHP were added.

<sup>d</sup>cfDNA was extracted from 2 mL of spiked NHP using MagMAX™ Cell-Free DNA Isolation Kit (Thermo Fisher Scientific) and then the cfDNA concentration and the fragment size distribution assessed using a 2200 TapeStation Instrument (Agilent Technologies).

\* Abbreviation: EQA, external quality assurance; NHP, normal human plasma.

**Supplementary Table S2.** External quality assurance material for evaluation of precision

EQA materials	Reference Materials	Expected allele frequency <sup>a</sup>	The spiked DNA <sup>b</sup> were consists of					The spiked NHP <sup>e</sup> (1 mL) of				
			gDNA from reference standard DNA (50ng/ $\mu$ L)	Wild type DNA ( $\mu$ L) 50ng/ $\mu$ L	Total DNA (ng)	Measured concentration of sheared DNA (ng/ $\mu$ L) <sup>c</sup>	Expected copies of wild type DNA per $\mu$ L <sup>d</sup>	Expected copies of Mutant DNA per $\mu$ L <sup>d</sup>	Expected copies of mutant DNA	Expected copies of wild type DNA <sup>f</sup>	Allele frequency (%)	Extracted total DNA (ng/mL) <sup>g</sup>
P-1	<i>EGFR</i> p.T790M Reference Standard (HD258)	50%	10 $\mu$ L	90 $\mu$ L	5000	33.1	480.0	9119.1	1295.9	25202.4	4.9	74.5
P-2	<i>EGFR</i> p.L858R Reference Standard (HD254)	50%	10 $\mu$ L	90 $\mu$ L	5000	31.2	452.4	8595.6	1221.5	23789.1	4.9	80.2
P-3	<i>EGFR</i> p.E746_A750del Reference Standard (HD251)	50%	10 $\mu$ L	90 $\mu$ L	5000	38.4	556.8	10579.2	1503.4	29144.8	4.9	79.5

<sup>a</sup>Expected mutant allelic frequency were measured using digital droplet PCR were provided from manufacturer.

<sup>b</sup>Spiked DNA was fragmented to about 180 ~190 bp by sonication using a Covaris M220 (Covaris Inc.).

<sup>c</sup>The concentration of sheared DNA which was distributed between 100 bp and 300 bp was assessed using a 2200 TapeStation Instrument (Agilent Technologies).

<sup>d</sup>Copies per ng were 290.

<sup>e</sup>2.7  $\mu$ L of spiked DNA were mixed with 1 mL of NHP.

<sup>f</sup>581 wild type copies per 1 mL NHP were added.

<sup>g</sup>cfDNA was extracted from 1 mL of spiked NHP using MagMAX™ Cell-Free DNA Isolation Kit (Thermo Fisher Scientific) and then the concentration of sheared DNA which was distributed between 100 bp and 300 bp was assessed using a 2200 TapeStation Instrument (Agilent Technologies).

\* Abbreviation: EQA, external quality assurance; NHP, normal human plasma.

**Supplementary Table S3.** Preparation material for performance evaluation

Materials	Reference Materials	Gene	Variant	Allele frequency (%) <sup>a</sup>	Copies of wild type DNA per $\mu\text{L}^a$	Copies of mutant DNA per $\mu\text{L}^a$	Spiked NHP (2 mL) <sup>b</sup> of		
							Expected copies of wild type DNA <sup>c</sup>	Expected copies of mutant DNA	Expected mutant allele frequency (%)
Test 1	5% Multiplex I cfDNA Reference Standard (HD777)	<i>EGFR</i>	p.L858R	4.59	7904	380	16970	760	4.29
			p.E746_A750del	5.77	4896	300	10954	600	5.19
			p.T790M	4.52	4440	210	10042	420	4.01
			p.A767_V769dup	4.39	3944	181	9050	362	3.85
Test 2	2.5% Spiked Standard (HD777: WT100%=1:1)	<i>EGFR</i>	p.L858R	2.29	8140	190	17442	380	2.13
			p.E746_A750del	2.89	4936	150	11034	300	2.65
			p.T790M	2.26	4428	105	10018	210	2.05
			p.A767_V769dup	2.19	3988	91	9138	181	1.94
Test 3	1% Multiplex I cfDNA Reference Standard (HD778)	<i>EGFR</i>	p.L858R	0.85	8144	70	17450	140	0.80
			p.E746_A750del	1.24	4296	54	9754	108	1.10
			p.T790M	0.93	3848	36	8858	72	0.81
			p.A767_V769dup	0.84	3672	31	8506	62	0.72
Test 4	0.5% Spiked Standard (HD778: WT100%=1:1)	<i>EGFR</i>	p.L858R	0.43	8260	35	17682	70	0.39
			p.E746_A750del	0.62	4636	27	10434	54	0.51
			p.T790M	0.46	4132	18	9426	36	0.38
			p.A767_V769dup	0.42	3852	15	8866	30	0.34
Test 5	0.1% Multiplex I cfDNA Reference Standard (HD779)	<i>EGFR</i>	p.L858R	0.13	7824	10	16810	20	0.12
			p.E746_A750del	0.11	4408	5	9978	10	0.10
			p.T790M	0.13	3896	5	8954	10	0.11
			p.A767_V769dup	0.06	3616	2	8394	4	0.05

<sup>a</sup>Mutant allele frequency, copy number values of wild type and mutant DNA measured using digital droplet PCR were provided from manufacturer.

<sup>b</sup>1  $\mu$ L of standard DNA were mixed with 1 mL of NHP.

<sup>c</sup>1162 wild type copies per 2 mL NHP were added.