

## 1 **Data S1. Supplementary materials and methods**

2 *DNA and oligo pool preparation.* Genomic DNA or cDNA samples were diluted to 25 to 50 ng/ $\mu$ l  
3 concentration in TE buffer (for all samples with UV A260/A280 ratio between 1.7 and 2.0). DNA  
4 was amplified by polymerase chain reaction (PCR) prior to performing an extension reaction of the  
5 SNP of interest. DNA-containing plates or tubes were kept at 4°C.

6 The Agena Assay Design Suite Version 2.2 (Agena Bioscience) automatically designed PCR and  
7 extension primers (probes) for each SNP to be investigated. All oligonucleotides for PCR and  
8 iPLEX reactions were ordered unmodified, with standard purification. Forward and reverse PCR  
9 primers were reconstituted at equimolar concentrations of 100  $\mu$ M and underwent further dilution  
10 after the pooling to a working concentration of 0.5  $\mu$ M each. Probes (for iPLEX extension) were  
11 reconstituted at equimolar 500  $\mu$ M concentrations. The PCR assay pool plexes consisted of the  
12 multiplexed forward and reverse PCR oligonucleotide primers for each reaction present together in  
13 one multiplexed assay pool. The Single Base Extension (SBE) pool plexes consisted of the  
14 multiplexed oligonucleotide primers, which anneal adjacent to the polymorphic site for each  
15 reaction present together in the multiplexed assay pool. By pooling locus-specific primers, many  
16 individual loci of DNA with their corresponding SNP sites were analyzed in a one-well reaction.  
17 Due to the inverse relationship between peak intensity and analyte mass, extension primers in  
18 iPLEX assays were adjusted by concentration to ensure that the extension primers were as equal in  
19 intensity as possible. The primers were sorted into four groups based on mass, the first group of  
20 low mass primers mixed at 7.0  $\mu$ M, the following group at 9  $\mu$ M, the third group at 11.3  $\mu$ M, and  
21 the higher mass group at 14.0  $\mu$ M. Extension oligo pools were spotted on SpectroCHIPs and run  
22 on detectors to verify uniformity of intensities and accuracy of the manual pooling procedure. Any  
23 oligos of low-peak intensity were spiked in by adding volume to equalize the probe peak intensities.  
24 For example, if a given peak was about half the intensity of the other peaks, an equal volume was  
25 spiked in to double the peak intensity. Diluted working oligos were stored at 4°C and concentrated  
26 stocks at -20°C.

27  
28 *PCR.* The PCR cocktail was prepared following the protocol described in Table SI. Cocktails (4  
29  $\mu$ l) were transferred to each well of the 96-well microtiter plate and 1  $\mu$ l DNA (25-50 ng) added to  
30 each well. The plate was centrifuged at 123 x g and PCR performed with the following cycling  
31 conditions: Initial denaturation at 94°C for 2 min, 45 cycles of denaturation (94°C for 30 sec),  
32 annealing (56°C for 30 sec) and extension (72°C for 60 sec). A final extension step was performed at  
33 72°C for 5 min and the samples kept on a 4°C hold.

34  
35 *Shrimp alkaline phosphatase (SAP) clean-up.* Treatment with SAP was performed to remove  
36 remaining, non-incorporated dNTPs from amplification products. The SAP mixture was pulse

1 vortexed five times and centrifuged briefly at room temperature, and 2  $\mu$ l of the SAP solution added  
2 to each well containing the PCR product. The plate was incubated at 37°C for 40 min, then 85°C  
3 for 5 min and kept on hold at 4°C until the next step.

4  
5 *iPLEX SBE extension.* The SBE or iPLEX reaction was used to detect the single base  
6 polymorphisms or small insertion/deletion polymorphisms in the amplified DNA. After PCR clean-  
7 up, a primer extension reaction cocktail (containing extend primer, buffer, enzyme, and mass-  
8 modified ddNTPs) was added to the amplification products. During the iPLEX reaction, the primer  
9 was extended by one mass-modified nucleotide depending on the allele and the design of the assay.  
10 For this, 2  $\mu$ l of the iPLEX-SBE master mix was added to each well of the SAP treated plate (for a  
11 total volume of 9  $\mu$ l). The mixture was briefly vortexed, centrifuged and amplified using the follows  
12 conditions: Initial denaturation at 94°C for 30 sec, 60 cycles consisting of denaturation at 95°C for 5  
13 sec, annealing at 52°C for 3 sec and extension at 80°C for 3 sec. A final extension step at 94°C for 180  
14 sec and the samples kept on hold at 15°C until the next step.

15  
16 *Primer extension reaction resin clean-up.* A slurry of the resin was added directly to primer  
17 extension reaction products to remove salts such as Na<sup>+</sup>, K<sup>+</sup>, and Mg<sup>2+</sup> ions. The SBE plate was  
18 pulse vortexed five times, then centrifuged at 3,200 x g for 5 sec at room temperature and 41  $\mu$ l  
19 nuclease free water added to each well in the SBE plate, covered with an adhesive sealing sheet  
20 and centrifuged at 1,422 x g for 1 min. The dimple plate was spread with 15 mg resin and left to  
21 dry for 10-60 min. The SBE plate was turned upside down and aligned to the dimple plate. Both  
22 plates were held and turned over, so resin dropped into the wells. The resin was tapped into the  
23 wells. The reaction plate was sealed with film, centrifuged at 1,422 x g, after which it was placed  
24 on the rotator for 30 min. The reaction plate was then centrifuged at 1,422 x g for 5 min to pellet  
25 the resin. The dimple plate was cleaned with NanoPure water, dried and stored.

26  
27 *Spotting primer extension products on SpectroCHIPs.* To incorporate oligonucleotides with the  
28 appropriate matrix for MALDI-TOF (3-hydroxypicolinic acid), a small volume (~12 nl) was arrayed  
29 onto existing matrix spots on the silica chip. This process used the capillary action of slotted pins  
30 and contact dispensing for Nanovolumes. The Nanodispenser was conditioned with 1M NaOH using  
31 the "Weekly Condition" program (10 min) and cleaned with 100% ethanol using the "Daily Clean"  
32 program (30 min). Subsequently, the sonicator was drained and the ETOH bottle filled with a 50%  
33 ethanol solution. Using the "Maintenance Menu", the Nanodispenser insert was switched to 6-pins  
34 (following on-screen prompts). The plate and new SpectroCHIP (note the chip barcode ID) were  
35 placed on the Nanodispenser and the transfer set up to run by selecting VOLUME CHECK in the

1 method and using the following options: Aspirate offset = 9.5 mm, Aspirate time = 3 sec, Aspirate  
2 Speed = 50 mm/sec, Dispense offset = 1 mm, Dispense Speed = 140 mm/sec and Dispense time =  
3 1 sec. Subsequently, 'Auto-tuning' and 'Analyte and Calibrant' in the method were selected. A total  
4 of 80  $\mu$ l 3pt calibrant was loaded in the white reservoir and run ensuring that the volumes dispensed  
5 were close to the target volume 12  $\mu$ l. When done, the SBE plate was sealed and stored at -20°C.  
6 The remaining calibrant from the white reservoir was pipetted back into the tube and stored at 4°C.  
7

8 *Mass spectrometry.* For mass spectrometry, the Agena MassARRAY System (Agena Bioscience),  
9 and a positive ionization mode was used, where the positively charged DNA ions were accelerated  
10 and migrated through the vacuum tube towards a highly sensitive detector at different speeds  
11 dependent on the mass of the ions, leading to different arrival times (time of flight). The "Use  
12 Calibrant" option was turned on, followed by the "High Voltage" button, and a switch to the  
13 "Autorun" tab conducted. "Start Autorun" was pressed, after which the instrument read the chip  
14 barcode and began collecting data. Once the data had been collected, the "Typer Analyzer"  
15 application was used to examine the data.