Supplementary Materials For:

Automatic Genomics: a user-friendly program for the automatic designing and plate loading of medium-throughput qPCR experiments

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Materials and methods

Samples

Eighty-eight samples from different frozen breast cancer tumors and cultured human breast cancer cell lines (BT474, HCC1937, MCF7, MDAMB157, MDAMB231, MDAMB436, T47D, UACC3199) were used in this study.

RNA isolation

Total RNA was isolated using TRIzol Reagent (Cat. no. 15596-026; Invitrogen, Carlsbad, CA, USA). Contaminating DNA was removed by on-column treatment with DNase (RNase-free DNase Set, Cat. no. 79254; Qiagen, Hilden, Germany).

Reverse transcription

Total RNA from tumors (500 ng) and cell lines (1000 ng) were reverse-transcribed using the High Capacity cDNA RT kit (Cat. no. 4374966; Applied Biosystems, Foster City, CA, USA). Five 1:10 RNA dilutions from cell line MDAMB157 were reverse-transcribed to build standard curves.

Quantitative PCR

Assays were designed using the Roche Applied Science Universal Probe Library web site (www.roche-applied-science.com; Roche Diagnostics, Basel, Switzerland). The following assays were used:

PPIA. Peptidyl-prolyl *cis-trans* isomerase A (RefSeq ENST00000355968.3; ENSG00000196262.3). Forward primer, 5′-CCTAAAGCATACGGGTCCTG-3′; reverse primer, 5′-TTTCACTTTGCCAAACACCA-3′; Probe #48 (Cat. no. 04688082001; Roche).

MRPL19. Mitochondrial ribosomal protein L19 (RefSeq NM_014763.3). Forward primer, 5′-GGAATGTTATCGAAGGACAAGG-3′; reverse primer, 5′-CAGGAAGGGCATCTCGTAAG-3′; Probe #42 (Cat. no. 04688015001; Roche).

ERCC5/XPG. DNA-repair protein complementing XP-G cells (RefSeq ENST00000355739.3; ENSG00000134899.7). Forward primer, 5′-CCAAGCGCAGAAGAACATTA-3′; reverse primer, 5′-TTAAGCAAGCCTTTGAGTTGG-3′; Probe #82 (Cat. no. 04689054001; Roche).

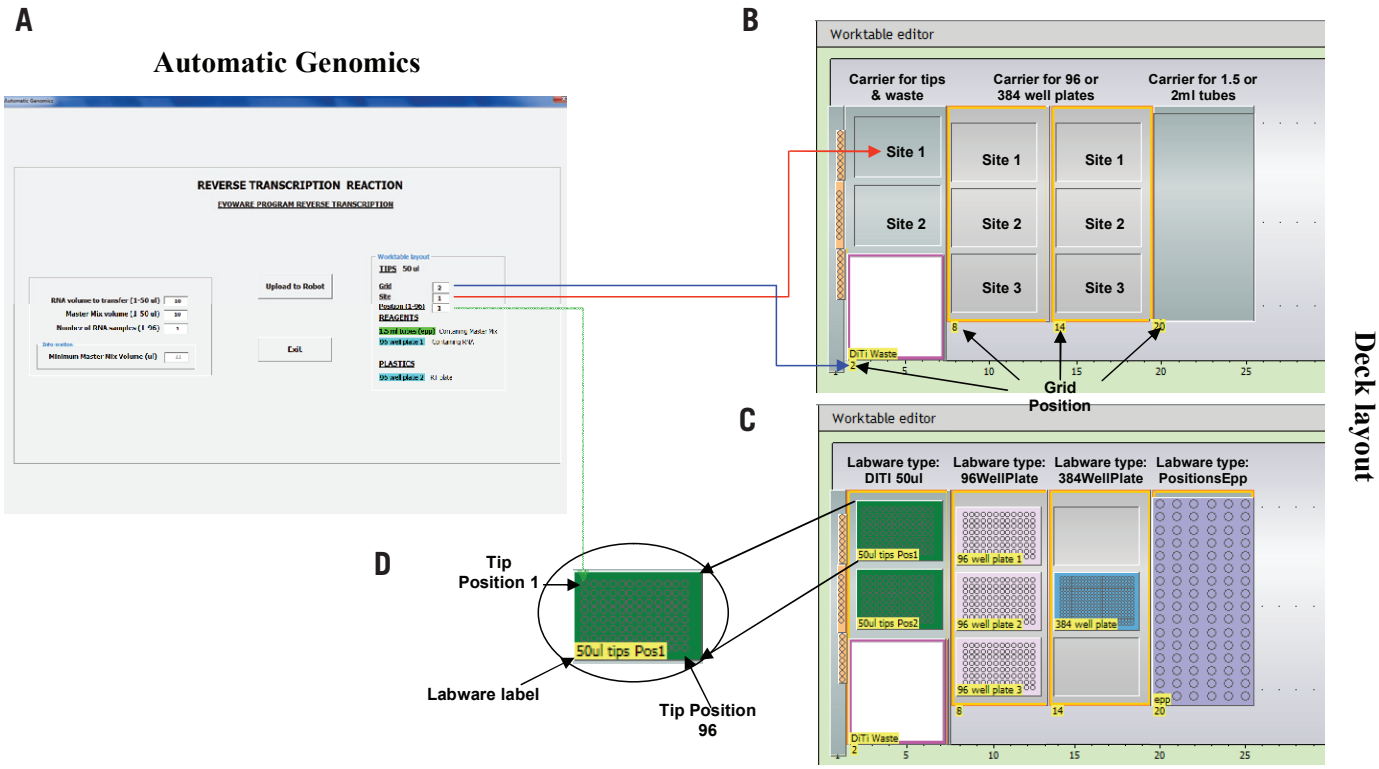
Each qPCR was set up in final volume of 10 µL as follows: 5 µL TaqMan Universal PCR Master Mix (Cat. no. 4304437; Applied Biosystems), forward and reverse primers at a final concentration of 500 nM each, FAM-labeled probe at a final concentration of 100 nM, and RNase-free water up to 8 µL.

Template was 2 µL of a 1:5 dilution of the cDNA obtained by reverse transcription. For standards, the template was 2 µL of the cDNA obtained from dilutions of RNA from the cell line MDAMB157.

qPCRs were conducted in 384-well plates (Cat. no. 4344345; Applied Biosystems) using an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). The cycling protocol was 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 60 s at 60°C.

qPCR analysis

Baseline and threshold were automatically set in the SDS 2.3 software (Applied Biosystems) to calculate Cq values. Cq values were then imported into qBasePlus for further analysis.

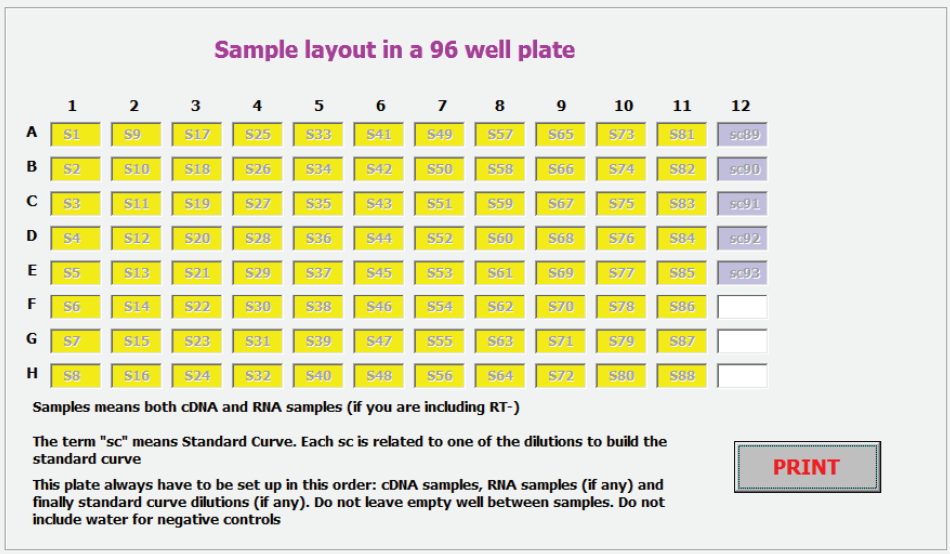


Supplementary Figure S1. Exemplary deck layout. Grid position for tips, carriers, and labware will be different for each robot, and there is no need to keep the configuration shown in this figure. Tips, labware, and carriers can be located at any position on the deck. (A) An exemplary AG interface in which users must define the position of the first available tip by entering the grid, site, and tip position. (B) A possible distribution of different carriers: one carrier with two sites for tips and one site for DiTi waste; two carriers with three sites each for microplates; and one carrier with 96-well positions for 1.5- or 2-mL tubes. Grid positions are indicated by yellow labels. (C) A possible distribution of labware on the different carriers indicating labware type (in black) and labware label (in yellow). Only labware type and labware label have to be named exactly as shown (see tutorial videos for more details). Blue arrow indicates where to find the grid position that should be typed in the Grid text box in the AG interface. Red arrow indicates the site number that should be typed in the Site text box in the AG interface to indicate where the tips are located. Green arrow indicates the position of the first available tip in the tip rack; this should be typed in the Position text box in the AG interface. (D) Note that in the Evoware software, the positions of tips and wells within tip racks and microplates are always numbered by columns and not by rows (see the Evoware help section for more information).

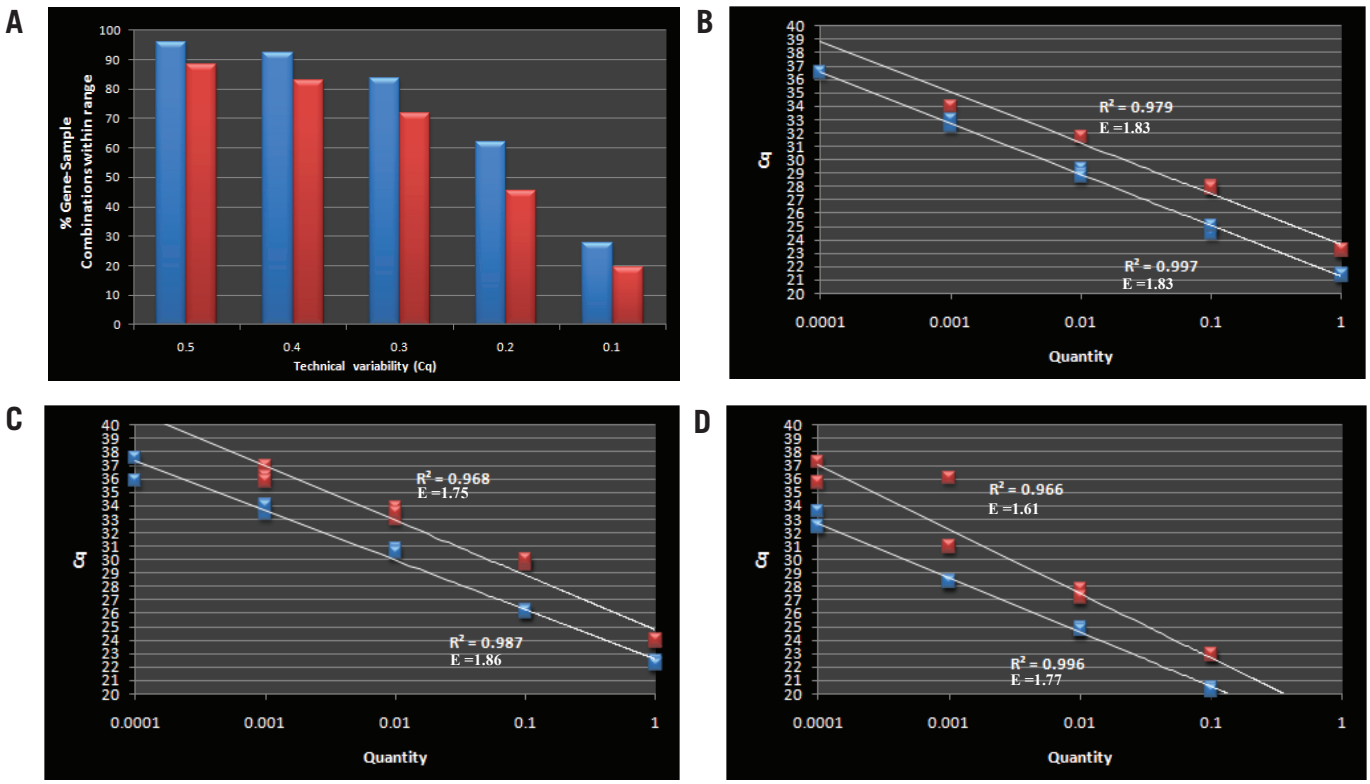
Supplementary Table S1. Liquid classes used by AG

Tecan protocol	Action	Liquid class
SampleTransfer	Transfers samples from 1.5-mL tubes to 96-well plate	Genomica01
SampleTransfer	Transfers samples from 96-well plate to 1.5-mL tubes	Genomica01_transfer
ReverseTranscription	Distributes RT MasterMix (before sample transfer)	Genomica03
ReverseTranscription	Transfers samples to 96-well plate	Genomica01
ReverseTranscription	Distributes RT MasterMix (used when samples are already in the reverse transcription plate)	Genomica03_Notouch
qPCR	Distributes qPCR MasterMix	Genomica300
qPCR	Transfers samples to 384-well plate	Genomica01_custom

Each aspiration-dispensing action is ruled by a liquid class. This table lists the liquid classes used in different steps of the various protocols. If a specific aspiration or dispensing step is not performed accurately, the corresponding liquid class should be adjusted. The adjustment will depend on laboratory-specific conditions (humidity, temperature, etc.). Information on how to adjust a liquid class can be found in the Evoware help section or at the Tecan helpdesk.



Supplementary Figure S2. Example sample layout in the 96-well plate for transfer to the 384-well plate for qPCR. This example includes 88 cDNA samples and five dilutions of a cDNA standard for building the standard curve. Samples are sorted by columns leaving no empty wells (in yellow). Dilutions for standard curves must be located just after the samples, from the most concentrated to the most dilute (in light purple).



Supplementary Figure S3. Comparison of technical variability obtained by robotic and manual loading. All gene-sample combinations were loaded in triplicate in 384-well qPCR plates in order to perform a quality control. Robotic loading data are in blue, and manual loading data are in red. (A) Percentage of gene-sample combinations that pass different technical reproducibility cutoffs. This figure is usually 10% higher when using robotic loading, and the time invested was approximately 50% less compared with manual loading. (B–D) Comparison of standard curves for the three genes (*ECRR5*, *MRPL19,* and *PPIA*) included in the validation generated by robotic and manual loading. Standard curves were built using five 1:10 cDNA dilutions. The x-axis is the dilution factor for the cDNA template used in the qPCR. Technical variation was higher when plates were manually loaded, as shown by the R^2 values. Note that even the efficiency (E) can vary slightly depending on how samples are loaded. The six original text files containing Cq values can be found in the Supplementary Material.