Fully Automated Genomics BioCel: PCR Prep and unattended PCR for high-throughput Genotyping

Technical Overview

Authors

Daniel McDonough and Marc Valer
Agilent Technologies, Inc.
Santa Clara, CA, USA

Introduction

Sequencing biological samples is a well established approach to uniquely identifying a particular strain of pathogen, detecting genetic susceptibility to disease, documenting DNA sequence similarity or disparity between individuals, as well as myriad other applications. Sequencing also involves a time and labor intensive process that is susceptible to variations in technique and timing between individuals, as well as user errors due to the repetitive but lengthy nature of the process. When sequencing known organisms for genotyping purposes, well characterized small regions of the genome (300 to 1000 bases) can be cloned *in vitro* to overcome limited sensitivity and read length of traditional sequencing techniques like the Sanger method. Agilent Automation Solutions has designed an integrated preparative PCR system capable of making sequencer-ready sample plates from purified RNA or DNA with the absolute minimum of user intervention. The Genomics BioCel expands upon Agilent's expertise building integrated systems and benchtop workstations to automate many genomics processes. The Genomics BioCel is a base system that can be fully customized to each researcher's needs to offer a truly turn-key solution.

Agilent Technologies

Application fields of interest

Genotyping is used across different fields. Many of them have a need to process large numbers of samples and/or running unattended such as:

- Clinical healthcare/research:
  - Individualized medicine: customized treatments by tumor cell genotyping
  - Preventive healthcare with disease risk prediction based on genetic profile

- Epidemiology/bio-defense
  - Rapid genotyping of new and existing pathogens to assert its identity, variances and origin, and predict pandemic risks as well as immunization effectiveness

- Agricultural/biotechnology
  - Food testing (species, varietal, purity of vegetables, meats, and fish)
  - Plant variance testing and selection of Genetically Modified Organisms (GMOs)

- Forensics
  - Individual identity and genetic familiarity

In addition, many PCR-based assays can be adapted to the presented system. The addition of a sample extraction cell or the addition of fluorescence or UV read-out after or during PCR can permit many types of fully unattended confirmation assays. Some advantages of the standardized, complete workflow solution are as follows:

- User input limited to a single loading of sample and reagent plates, tips per run, and the maintenance of washing solutions and plate sealing/pealing rolls

- Enclosed clean system maintains a clean constant environment for better consistency

- Transforms a labor intensive process into a simple routine procedure, minimizing labor input, and eliminating user-to-user variation in technique or timing

- Facilitates deployment of identical processes to multiple sites and immediate sharing of updated protocols

- Ensures optimal use of reagents and samples, volume tracking, and maintains them in sealed plates at appropriate temperature at all times

Quality assurance: Each step or processing is recorded with barcode tracking of liquid transfers for verification of successful assay completion. Incubation times and time constraints as well as environmental conditions are logged and flagged as needed.

Modular design offers flexibility and customization options

The Agilent BioCel platform is a fully customizable solution that is tested, delivered, and supported to user specifications. The genotyping system described here is one of multiple possible configurations, and has been thoroughly evaluated. Designed around a widely used reagent set, the Agencourt AMPure and CleanSEQ kits, it utilizes single step RT-PCR to minimize plate processing times and deliver sequencing ready plates. **Figure 1** shows two possible configurations of this highly integrated solution, but the scalability of the platform permits the adaption of the platform to multiple workflows, throughputs, and capacity needs.

Integrated BioCel systems with Agilent VWorks Automation Control software are engineered to be extremely flexible, permitting the design of new assays or variants around the existing platform at any time as well as running simultaneous unrelated protocols. **Figure 2** highlights some of the assays that can be easily addressed by adapting a genotyping system.

The following list summarizes some of the key features that make the genotyping BioCel platform ideal for automated high-throughput genomics workflows:

- Capable for both liquid handling and thermal cycling, BioCel cells are scalable to meet varied throughput demands

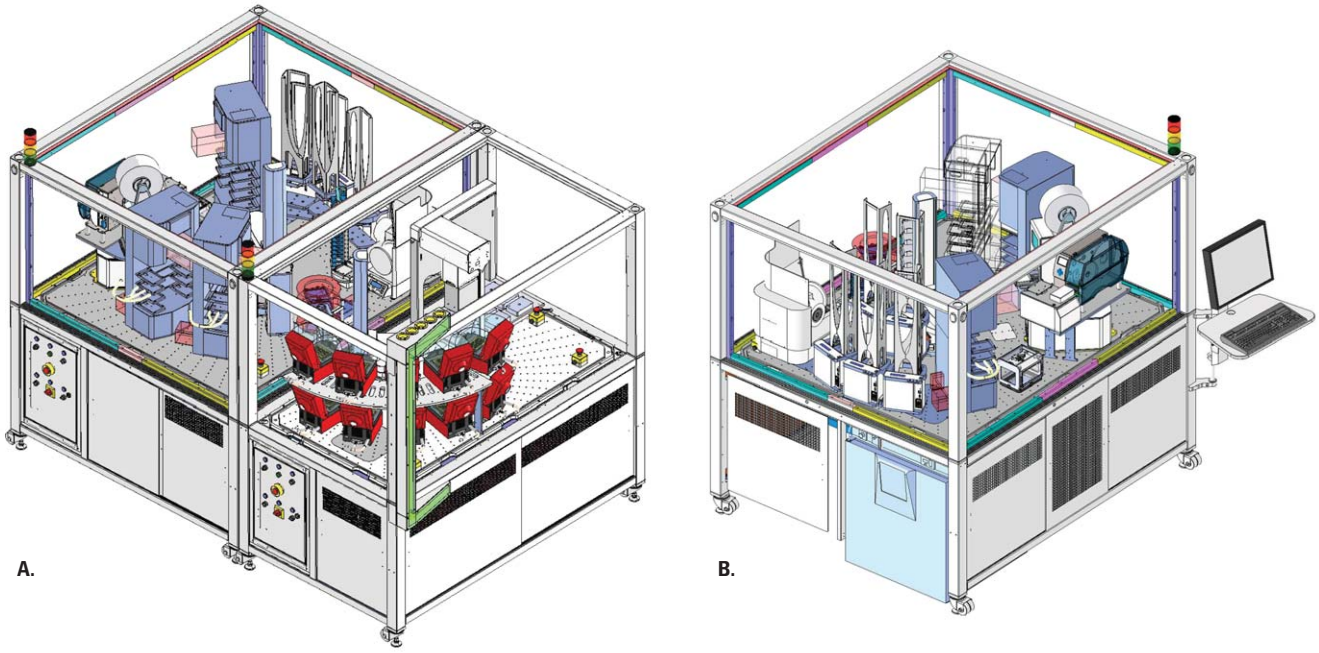


Figure 1: automated genotyping system – RNA/DNA purification, plate reformatting, reverse transcription, reaction clean-up, PCR and sequencing plate preparation, and a 2nd optional cell for automated thermal cycling. (A) 1800/1200 Dual BioCel system integrates liquid handling operations and thermal cycling. Labs with sufficient thermal cycling capabilities can select the preparative BioCel alone (B) and for offline thermal cycling processing of plates.

- Spatial separation of liquid handling and plate storage from thermal cycling, together with the use of a high-capacity automated incubator and an on-deck chilled hotel, keep temperature sensitive samples and reagents safe from heat and evaporation

- Nucleic acid extraction and cherry picking for confirmation can be automated and integrated to the overall workflow on the optional Agilent Bravo Automated Liquid Handling Platform.

- Sample throughput is scalable by adding liquid handling capacity and increasing the number of thermal cyclers. Throughput estimates are based on the protocols developed by Agilent Automation Solutions for genotyping influenza RNA samples in collaboration with UCLA and Los Alamos National Laboratory.

  - Standard throughput (10 thermal cyclers) is expected to allow runs of up to 20 samples per run with 192 clones amplified for forward and reverse sequencing in 384-well plates in about 12 hours. Up to 18 thermal cyclers can be placed on the optional cell to significantly reduce run time.
  - Reducing the gene number increases the sample throughput proportionally (i.e. 80 samples could be processed with a 48 gene primer set on the same system and time).
  - True device pooling provided by Agilent VWorks permits the addition of new devices to help solve bottle necks. In the occurrence of a device going offline, the system does not stop, but rather spreads the task ahead to the still available devices.

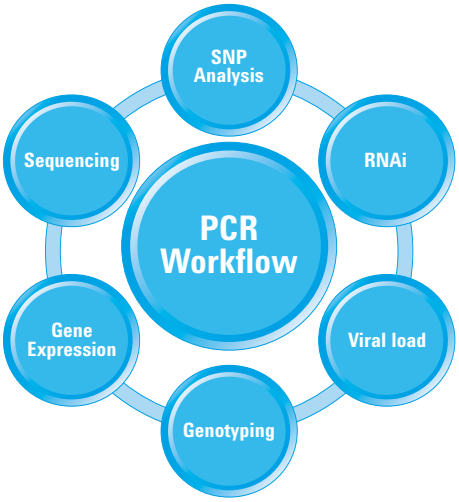


Figure 2: Many genomics application use PCR or similar sample treatment protocols. A system like the Agilent-based Genotyping Biocel system can be configured to perform related genomics assays.

Technical Details

Protocol overview

The retroviral genotyping system is designed around a robust, proven genotyping protocol, based on a single-step reverse transcription PCR reaction and multiple parallel *in vitro* cloning reactions that are then interrogated on a Sanger sequencer (ideally a 48- or 96-capillary DNA analyzer). **Figure 3** shows the different samples, reagents, and key steps of the functional protocol. For more detailed protocols, visit the BioCel Genotyping page online (http://www.chem.agilent.com/en-US/Products/Instruments/automation/Pages/ViralRNAGenotyping.aspx).

A top view layout of the system, including the optional thermal cycling cell and devices, is shown in **Figure 4**.

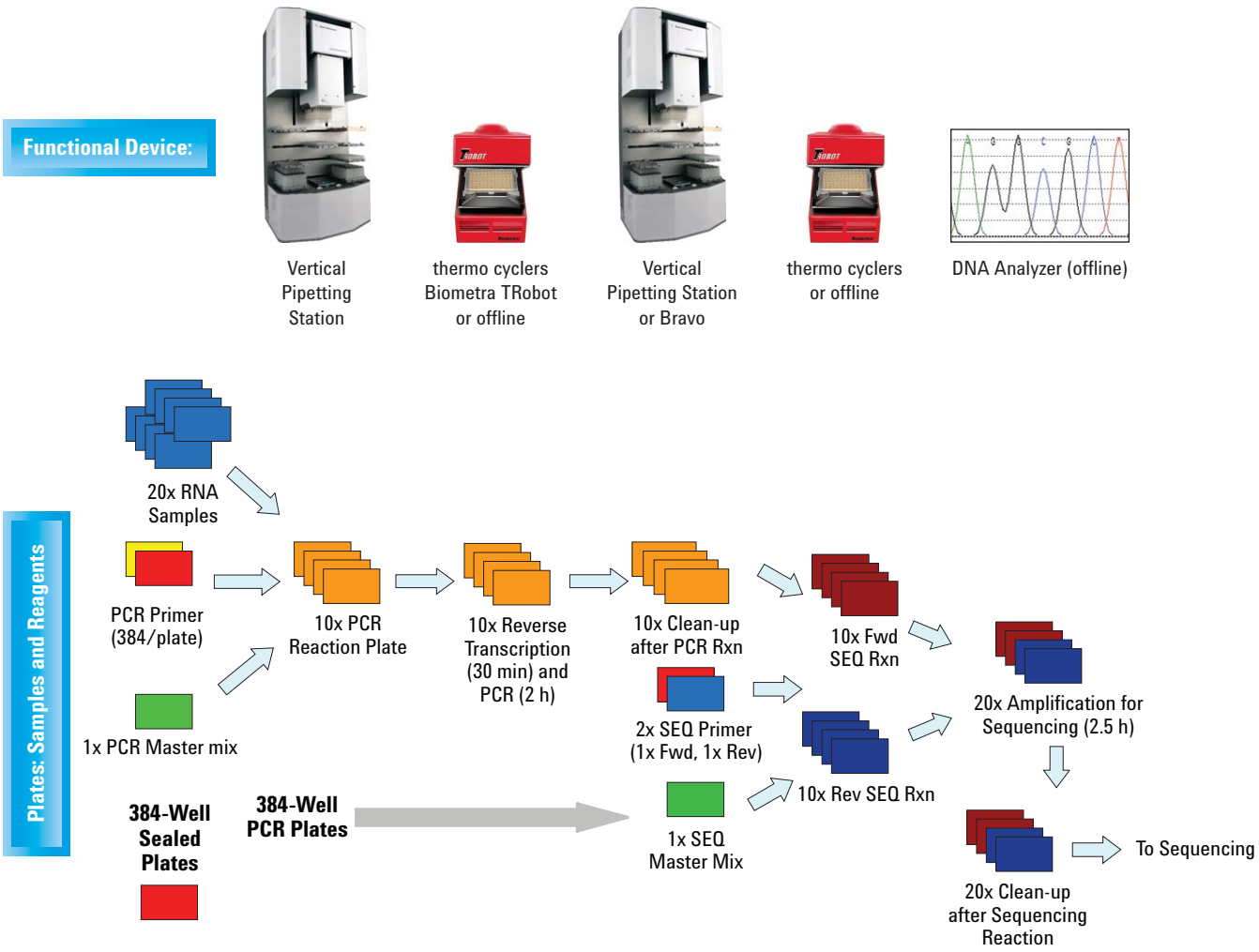
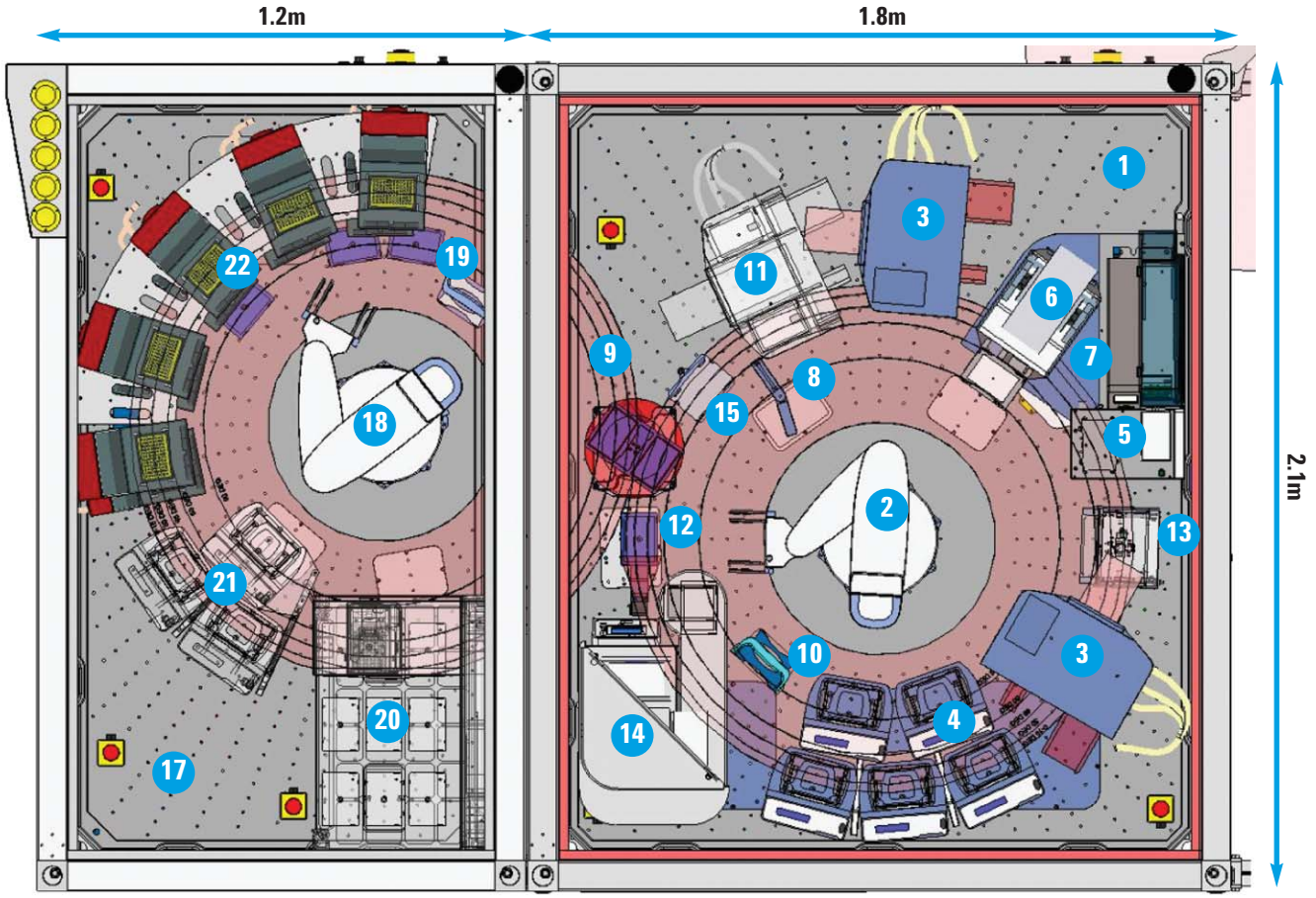


Figure 3: Genotyping by sequencing workflow: 20 RNA samples/12 hour run in 384 format. The diagram highlights the different stages a plate traverses in the system and the devices where the key operation take place.



Optional: Thermal Cycling and additional processing

Core: Sample/PCR preparation, purification and storage

Agilent Components

- (1) Filtered BioCel 1800 Cell
- (2) Direct Drive Robot
- (3) Vertical Pipetting Station (Qty 2)
- (4) Labware Stacking (Qty 5)
- (5) Plate Labeler
- (6) PlateLoc Plate Sealer
- (7) Centrifuge
- (8) Vacuum Delid Station
- (9) Double Stage Rotator
- (10) 16-Pos Plate Hotel
- (11) Optional 3rd Vertical Pipetting Station
- (17) Filtered plus, cold 1200
- (18) Direct Drive Robot
- (19) Plate Hotel
- (20) Optional Bravo (cherry picking, vacuum filtration)
- (21) Optional Labware Stackers (Qty 3-6)

3rd Party Components

- (12) Keyence Barcode Reader
- (13) ThermoCytomat 6001-C4 (under deck)
- (14) Nexus XPeel
- (15) Mecour Chilled Tower 6P
- (3) V&P Scientific – Magnetic Post Separation Block 384 on pipetting station (Qty 4)
- (3) Acorn Autofill Reservoirs on pipetting station (Qty 4)
- (22) Biometra TRobot (Qty 5-16)
- Optional Fluorescence/UV Reader (not shown)

Figure 4: Genotyping system layout: top view representation. Translucent devices indicate the location of optional components. The core PCR sample preparation cell is represented on the right side, on the BioCel 1800 unit with the central Direct Drive Robot and under-deck incubator. The optional BioCel 1200 sub-cell (left) features 10 Biometra TRobot thermocyclers for fully unattended operation. Plate transfer between cells is operated by a new double deck electronic rotator station.

Liquid handling

Agilent Vertical Pipetting Station (up to 3) for high speed sample reformatting, RT-PCR preparation, and post PCR clean-up:

#1: PCR sample preparation and RT-PCR DNA clean-up

#2: Sequencing ready DNA amplicon clean-up

#3: (Optional) Used to separate plate preparation from DNA clean-up and allow automated tip washing (i.e. dilute bleach and water) to significantly reduce tip waste.

Magnetic separation pads for Agencourt AMPure and CleanSEQ magnetic bead preparations are mounted on the Vertical Pipetting Station for automated DNA clean-up.

Agilent Bravo (Optional): sample preparation, RNA purification, and vacuum filtration, if required. Capable of accessing single well or tube, or rows/columns for cherry picking, or 96 to 384 plate re-formatting.

Thermal Cycling

Automated thermal cycling can be achieved with the addition of a sub-cell with integrated BioMetra TRobot thermocyclers under the control of Agilent VWorks. Throughput estimations are based on the following assumptions for cycling programs.

RT-PCR: Total ~2.5 hours
RT step: 30′ at 50°C, 15′ at 95°C
PCR step: 30 cycles (30 sec at 94°C, 30 sec at 58°C, 1′ at 72°C)
Extension for 10′ at 72°C after cycling

Sequencing PCR (~2.5 hours):
2′ at 95°C,
25 cycles (30 sec at 95°C, 20 sec at 50°C, 4′ at 60°C)

BioCel Environmental Control

In order to maintain a clean environment for sample processing and PCR, the Agilent BioCel 1800 unit is filtered. The 1200 sub-cell is refrigerated to remove excess heat generated by the thermal cyclers.

Plates are sealed and unsealed on demand during operation, helping to maintain samples and reagents as clean as possible.

Storage and incubation

Incubator: all PCR enzymes, primers, and RNA/DNA are kept at 4°C until they are required for processing. PCR products are returned to 4°C storage after cycling until ready for clean-up.

Chilled plate hotel: valuable samples and enzymes are stored on positions maintained at 4°C in between processing steps while in the system.

Stackers: rapidly supply fresh labware on demand. Additional stackers can add to the unattended operation time but will not affect throughput.

Plate processing

Agilent Direct Drive Robots (DDRs) provide fastest plate transfer time (typically below 4 sec) while providing top reliability, ease-of-use and a safer work environment for operators.

Automated plate labeling and tracking permits plate flagging of any incidence as well as LIMS integration.

The integrated plate centrifuge helps eliminate the risk of cross-contamination with the automated sealer/de-sealer and pipetting of plates.

Fast and secure plate sealing with the Agilent PlateLoc Thermal Microplate Sealer and de-sealing with the Nexus Xpeel ensure sample integrity at all times by minimizing the times samples and reagents are exposed.

VWorks

Agilent VWorks Automation Control software ensures maximum throughput and optimal resource utilization. An intuitive graphical user interface makes it easier for users to create new protocols, connect and configure devices, run protocols, and monitor progress. VWorks is 21 CFR part 11 compliant and features a unique error handling library that permits an automated response to any incidence during the run for maximum walk away time.

Like all BioCel systems, the Genomic solution is pre-packaged and tested in house with the turn key protocols for PCR and sequencing. Protocols as supplied are fully accessible to the end user for modification based on individual assay needs and optimizations.

Additional scripting or a full LIMS integrated can be added, for instance, to make automated smart process choices based on barcode and database information, or trigger clean-up/wash processes at the adequate times.

To read more about the Agilent fully automated genotyping BioCel and other genomics applications, visit the applications page at Agilent Automation Solutions: http://www.chem.agilent.com/en-US/Products/Instruments/automation/Pages/automationsolutionapplications.aspx#Genomics

www.agilent.com/lifesciences/automation

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