PlateTrak™ Application Note

Automation of DNA Purification Using the PlateTrak Automated Microplate Processing System

Advances in throughput, reproducibility, and reagent conservation

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With the recent increase in genomic research, laboratories are pressed for increased throughput and reliability along with the need to reduce cost. The enormous scale of the Human Genome Project has created a demand for automated systems aimed at high throughput and reproducibility. PerkinElmer Life Sciences, Inc. has completed a collaboration with the Center for Genome Research, at the Whitehead Institute for Biomedical Research, Cambridge, MA to develop expanded capabilities on the PlateTrak conveyor based microplate processing instruments to automate the DNA purification process. The Whitehead Institute is one of the largest contributors to the Human Genome Project. This collaboration led to the development of many exciting new microplate processing solutions on the PlateTrak system that greatly enhance the capabilities for the automation of genomic research. In addition, the ability to reduce the scale of sequencing reactions through advanced pipetting systems produced significant cost savings.

The standard procedures developed for isolating DNA typically employ centrifugation and solvent extraction methods that are very difficult to automate. To overcome this, the scientists at the Whitehead Institute developed a new procedure using SPRI (Solid Phase Reversible immobilization) chemistry (Hawkins et al). The SPRI procedure is based on DNA binding to the surface of coated paramagnetic particles. These beads display magnetic properties when placed in a magnetic field, but retain no residual magnetism when removed from the magnetic field. This enables rapid and reproducible automation of buffer exchanges and extensive washes required for DNA preparations. The procedure has been developed and optimized for single stranded DNA isolation, such as M13 phage utilizing iron oxide magnetic particles and double stranded plasmid DNA utilizing carboxyl coated magnetic particles. The SPRI procedure allowed the development of an automated procedure in a microplate format with a throughput of five hundred 384-well plates, or 200,000 separate DNA preparations per day. This is the highest throughput achieved in any lab today for genomics. The procedure is rapid, low cost, and provides high quality DNA sequencing results.
PerkinElmer paired newly engineered technologies with standard ones to develop several automated systems. The following is a list, with a brief description, of the separate modules utilized in these systems:

MultiPosition Dispense Module: A 96- or 384-channel dispensing head capable of movements in the X, Y, and Z directions. The dispense head moves across a multiposition grid deck, extending the accessible stations on the conveyor path. There are 3 model sizes available for varying capacity for plates, reagents, tip boxes or plug and play cartridge accessories.

- **10 inch MPD** – 3 position deck, 1 plate stop, 2 cartridge slots
- **20 inch MPD** – 9 position deck, 2 plate stops, 6 cartridge slots
- **30 inch MPD** – 15 position deck, 3 plate stops, 10 cartridge slots

Gantry Gripper: Pick and place robotic arm capable of moving plates from and to the conveyor for additional processing behind the conveyor. The 30 inch module has been integrated with 4 position orbital shakers, 8 position magnetic plate deck, and an 8 position magnetic plate shelf.

SideTrak*: Pick and place robotic arm for the integration of peripheral microplate devices to the PlateTrak system. The SideTrak arm moves plates from diving board to external ancillary equipment. Peripheral devices include thermal heat sealers, readers, microplate handlers, microplate hotels and carousels.

Plate Sealer: Thermal microplate sealer.

Lift and Transfer Station: Mechanism that enables microplates to be lifted off and returned to conveyor after aspirating or dispensing. An example would be compression or expansion of plate libraries.

Plate Piercing Module: Module that pierces sealed microplates at an adjustable height for 96- or 384-well plates.

Stacker Module: Stacker Module with the capacity to hold a maximum of 50 SBS standard microplates. The cassette can accommodate a variety of plate types including standard plates, deepwell plates, PerkinElmer Tip Racks, and microplates with carriers.

Dispense Module: A 96- or 384-tip dispense head that moves only in the Z direction. Reagents or Tip Wash are kept below the conveyor so they can be accessed when a plate is not in position. The heads have a wide volume range depending on tip size.

- 96-channel head, P235 Disposable Tip (5 µL to 235 µL)
- 96-channel head, P50 Disposable Tip (0.5 µL to 50 µL)
- 96-channel head, P20 Disposable Tip (0.5 µL to 20 µL)
- 384-channel head, P30 Disposable Tip (0.5 µL to 30 µL)

Connecting Diving Boards: Multifunctional diving boards used for accessing the conveyor by another robotic system. These may be connected to join two separate PlateTrak systems.

Wash Module: A Wash Module with a 96 probe manifold with fused aspirate and dispense probes. There is a 96-well magnetic plate lift positioned beneath the deck for magnetic bead washing.

Recirculating Chillers: Chilling mechanisms with single or quad positions that keep reagents chilled to 4° C. Quad chillers are ideally suited for the setup of dye primers and terminators for sequencing reactions.

Plate to Waste Station: Shuttle mechanism at the end of the conveyor to allow plates to be lifted from the conveyor and discarded to a waste station.

Mixing Bottle and Reservoir: Adjustable speed control reagent bottle and reservoir cartridge for keeping magnetic beads in suspension.

Magnetic Plate Shelf: Programmable sliding magnetic shelf to increase deck capacity for plate handling for separation steps.
The instruments for the Whitehead Institute can be broken down into five phases:

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Resuspension and Lysis procedure</td>
</tr>
<tr>
<td>2 &amp; 3</td>
<td>DNA purification, which is handled in two steps;</td>
</tr>
<tr>
<td>4</td>
<td>Sequencing set up and if needed</td>
</tr>
<tr>
<td>5</td>
<td>Reaction Pooling or Cleanup</td>
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Following are detailed drawings of each instrument in the system, along with a quick summary of the process during each phase.

In operation, the modules are optimally accessed in parallel and sequentially — all are working at the same time on different plates and the plates do not move backward. The PlateTrak systems are utilizing an assembly-line approach to plate processing. Keeping the line moving in one direction offers optimal throughput. However, since the conveyor moves in both the forward and reverse directions, modules can be utilized in any order.

These procedures are currently run in a 96-well format. There will be a conversion to a 384-well format in the near future.

These procedures are currently run in a 384-well format.

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**30 inch MultiPosition Gripper Tool integrated with an orbital shaker and an 8 position magnetic plate deck.**

**Close-up of orbital shaker.**
**Transfer Deck**

**Purpose:** The purpose of this instrument is two fold:

1) **M13 Lysis**
   Single M13 clones grown in deepwell plates that have been centrifuged or filtered to pellet host cells are placed into the system. These supernatants are pipetted to new shallow well microplates and SDS is added to lyse the recombinant M13 phage. Once the M13 plates are lysed, they move directly to the Purification #2 instrument. Since M13 is a filamentagous virus, it is genetically programmed to expel itself from the host bacterial *E. Coli* cell. Simple centrifugation separates the *E. Coli* cells and its DNA from the growth media containing the recombinant viral DNA.

2) **Plasmid Cell Pellet Resuspension**
   Growth plates are again centrifuged and cells pelleted. The supernatant is discarded, and the plates are placed onto this device. This robot then adds resuspension buffer to the cell pellets and vortexes the plates to resuspend the cell pellets. Once the cells are resuspended, they are transferred from the growth plates to Microtiter® plates that are delivered to the downstream Purification #1 instrument.

**Output:** One Microtiter plate for each deepwell plate. Each Microtiter Plate contains M13 Phage or *E. Coli* cells containing Plasmids which have been resuspended into solution. The M13 plates move directly to the Purification #2 instrument. The plasmid plates move directly to the Purification #1 instrument.

This PlateTrak instrument is not currently connected to any other PlateTrak system.

**Throughput:** One 96-well plate per minute.
**DNA Purification Deck #1**

**Purpose:** The purification decks are where the SPRI protocol is performed. The purification is based on size selection of the DNA. Once the host cell has become lysed, the host *E. Coli* DNA is in solution with the plasmid DNA. Both molecules share identical biochemical properties but can be separated based on size. The *E. Coli* DNA is 4.2 Million base pairs (Mb), and most plasmids are usually about 3-10 thousand base pairs (Kb). The function of the Purification #1 deck is to perform a differential PEG precipitation with paramagnetic particles. The protocol is designed to selectively precipitate large DNA fragments of the *E. Coli* cells and proteins to paramagnetic microspheres without precipitating the plasmid DNA. The beads are separated leaving plasmids in solution. This “plasmid enriched supernatant” is then collected and deposited into a new Microtiter plate for further purification on the Purification #2 system. The magnetic beads containing the *E. Coli* DNA are discarded at a “Plate to Waste” station.

**General Protocol:** Initially, the magnetic beads are added to lysed phage or lysed *E. Coli* cells containing plasmids in the shallow well Microtiter plates together with a binding buffer of PEG and salt. The PEG and salt concentrations have been optimized for both the single stranded M13 and double stranded DNA binding protocols.

**Input Source:** 96-well plates of *E. Coli* bacteria containing plasmids.

**Output Product:** 96-well Microtiter plates containing plasmid enriched supernatant. The bacterial DNA is bound to the magnetic beads and is discarded with the source plates. This is now analogous to the M13 preparations after resuspension and lysis.

There is no *E. Coli* DNA to compete for magnetic beads in the second half of the purification protocol on Purification Deck #2. These output plates move onto the Purification #2 instrument.

This PlateTrak system is connected to DNA Purification Deck #2 by a conveyor to conveyor link.

**Throughput:** One 96-well plate every 100 seconds.

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<table>
<thead>
<tr>
<th>Modules</th>
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<tr>
<td>1</td>
<td>Stacker for Microtiter plates (source plates)</td>
</tr>
<tr>
<td>2-3</td>
<td>Dispenses NaOH (caustic) and SDS (detergent) into source plates for bacterial cell lysis</td>
</tr>
<tr>
<td>4-6</td>
<td>MultiPosition Gripper Tool moves plates to orbital shakers to improve lysis</td>
</tr>
<tr>
<td>7</td>
<td>Open Module</td>
</tr>
<tr>
<td>8-9</td>
<td>96-channel dispenser adds PEG (binding) and magnetic bead solution</td>
</tr>
<tr>
<td>10-12</td>
<td>MultiPosition Gripper Tool moves plates to the orbital shaker and magnetic plate deck for incubation</td>
</tr>
<tr>
<td>13</td>
<td>Stacker for Microtiter plates (destination plates)</td>
</tr>
<tr>
<td>14-15</td>
<td>Reads barcodes and aspirates supernatant from source plates and dispenses it to destination plates</td>
</tr>
<tr>
<td>16</td>
<td>Stacker for destination plates</td>
</tr>
<tr>
<td>17</td>
<td>For future robotic integration</td>
</tr>
<tr>
<td>18</td>
<td>Moves source plates to trash and destination plates to attached PlateTrak</td>
</tr>
</tbody>
</table>
MultiPosition (MPD) module with 96-tip dispense and bottle mixer to keep beads in suspension.

Magnetic Plate Shelf extended behind the PlateTrak to expand capacity for an additional 8 magnetic plates.

96 probe plate washer with magnetic plate lift.
DNA Purification Deck #2

**Purpose:** The Purification Deck #2 performs the entire purification process for the M13 DNA and the second half of the plasmid DNA preparation, which is the separation of the plasmid DNA from the RNA in solution, utilizing the magnetic beads. This system is also utilized for the purification of PCR products with the same procedures.

**Input Source:** 96-well plates containing lysed M13 phage DNA or plasmid DNA in solution. Since this system is connected to DNA Purification Deck #1, the plasmid DNA plates can be automatically transferred to this system.

**Output Product:** Dry Microtiter plates with DNA bound to magnetic beads.

The plates are manually moved to the Sequencing Deck (#5).

**Throughput:** One 96-well plate every minute.

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<tr>
<td>1</td>
<td>Stack for Microtiter plates (source plates)</td>
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<tr>
<td>2-3</td>
<td>Adds PEG and magnetic bead solution</td>
</tr>
<tr>
<td>4-6</td>
<td>MultiPosition Gripper Tool moves plates to orbital shakers and magnetic plate deck for incubation</td>
</tr>
</tbody>
</table>
| 7       | Wash Module with magnetic plate lift  
• 1X 70\% EtOH wash with final aspirate to remove EtOH |
| 8       | Wash Module with magnetic plate lift  
• 2X 70\% EtOH wash with final aspirate to remove EtOH |
| 9       | Wash Module with magnetic plate lift  
• 1X 70\% EtOH wash with final aspirate to remove EtOH |
| 10      | Stack for Microtiter plates (destination plates) |
| 11-12   | Receives plates from DNA Purification Deck #1 |
| 13      | 2 position diving board - for future robotic integration |

Plate Lift and Transfer mechanism incorporated into a 20 inch MultiPosition Dispense Module to securely transport a 384-well master plate behind the conveyor.

Close-up of the plate lift mechanism.
**Sequencing Deck**

**Purpose:** The sequencing system can set up sequencing reactions utilizing both Dye Primer and Dye Terminator chemistries in the forward and reverse directions. The magnetic beads are separated and the supernatants removed for testing and sequencing. The magnetic beads are re-suspended in a water elution buffer with a three minute incubation at room temperature. The three minute incubation can be avoided with a simple 20 second shake utilizing an in-line shaker mechanism on the conveyor. For dye primers, the system will add the sequence mixtures (Dye Primer and – A, T, C, and G) which are chilled to 4°C in a color-coded four quad chilling station. These plates are then reformatted into 384-well plates, and the forward or reverse mixtures are added to the plate. For dye terminator chemistries, the dye terminator solution chilled to 4°C is added to the DNA. Purified DNA products from two 96-well plates are added to a 384-well plate with two copies positioned in diagonal quadrants of the 384-well plate. The forward reaction mixture is added to one copy and the reverse mixture is added to the second. This ensures that the data will be accurate even if the plate becomes flipped in orientation throughout the process. The DNA will be ready for the thermocycling operation and subsequent identification on the PerkinElmer 3700 series sequencer.

**Input Source:** Purified DNA in 96-well Microtiter plates. If appropriate, 384-well plates will be added for the reformatting.

**Output Product:** Sealed plates ready for the thermocycler. These plates are put on a thermocycler and then moved to the PE 3700 sequencer. This instrument is integrated to a SideTrak robotic arm, which moves plates into a thermocycling. The plates are loaded to a PlateStak™ instrument on the SideTrak following sealing. This maintains unidirectional processing for optimum throughput.

**Throughput:** One 384-well plate every two minutes.
**Pooling Deck**

**Purpose:** The function of Pooling Decks is two fold. First a Pooling Deck can take a 384-well cycle plate and compress or “pool” to a single 96-well purification plate for bead based cleanup. This is required when utilizing the dye primer chemistries for sequencing. This can also function for the second half of the purification steps, and is upgradable to a 384-well bead prep clean-up protocol.

**Input Source:** The source can be 384-well plates or 96-well Microtiter plates.

**Output Products:** There can be two products: 96-well plates ready after bead prep clean-up or dry Microtiter plates with DNA bound to magnetic beads. This system is not currently connected to another system.

**Throughput:** One 384-well plate every minute.
The SPRI technology coupled with the PlateTrak systems developed for the Whitehead Institute have greatly enhanced processing capabilities. One of the primary advantages of this procedure for DNA purification is the reagent savings, in particular Taq FS polymerase, through the ability of the PlateTrak instruments to reproducibly set up small volume reactions. For the sequencing reactions, only 1 mL Taq additions and 3 µL DNA additions are necessary. The yield for the M13 purification is 4-5 µg DNA, and the yield for the plasmid purification is 2-4 µg DNA. There is a total of 2-4 µg in 40 µL from the resuspension. Only 3 µL of this is utilized for the sequencing. Also, 85% of the DNA preparations work with sequencing reads of greater than two-hundred 99% accurate (phred 20) bases. Utilizing 1/16th of the recommended amount of BigDye reagent from PerkinElmer, we attain five-hundred-fifty 99% accurate (phred 20) bases. In addition to generating DNA suitable for sequencing, there are also great results with PCR product purification as assessed by concordance levels with microarrays. In regards to purity, the DNA is suitable for mammalian cell transfections, suggesting very low endotoxin levels.

This SPRI-based procedure is rapid, economical (pennies per well), and an excellent means of obtaining DNA sequencing results. It allows the Whitehead Institute to be the largest contributor toward the Human Genome Project by obtaining the highest throughput in any lab with this application.

The SPRI protocols can be performed for the purification of plasmid DNA, M13 DNA, and PCR products, as well as, the clean-up of fluorescent sanger sequencing reactions. The combination of PerkinElmer’s automation equipment and SPRI chemistry delivers a fully automated purification platform that provides a high yield of high quality DNA suitable for a variety of applications.