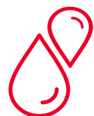

Automating In-Fusion® Snap Assembly Cloning on the Beckman Coulter Biomek i7 Hybrid Workstation

Platform: Beckman Coulter Life Sciences Biomek i7 Dual Hybrid Workstation

Kit: Takara Bio In-Fusion Snap Assembly Master Mix

Application: Automated cloning reaction using a 96-well plate

Processing time: Processing 96 samples of 10 µl and 5 µl volumes takes 42 min on a 96-well plate and uses 96 x 40 µl-sterile filtered tips (Beckman Coulter Life Sciences, Cat. # B85771).

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Introduction

Liquid handling systems reduce human errors and hands-on time, and increase throughput and reproducibility. Molecular cloning is one of the many genetic engineering techniques that greatly benefit from these platforms, which are widely used for high-throughput gene assembly for applications such as antibody discovery, proteomics, and oligo/gene synthesis.

PCR-based seamless cloning has many benefits for high-throughput platforms over traditional cloning approaches such as restriction enzyme-based ligation or TA cloning due to its simplicity, accuracy, and flexibility. However, the compatibility of cloning reagents with a specific liquid handling instrument is often overlooked, and a protocol is not always available.

In this application note, we developed an automated cloning workflow using In-Fusion Snap Assembly on the Biomek i7 Dual Hybrid Workstation. In-Fusion cloning is simple, scalable, directional, and highly efficient (>95%), making it ideal for high-throughput workflows. Furthermore, this automated In-Fusion Snap Assembly protocol showcases the ability of the Biomek i7 Dual Hybrid Workstation to perform 1 µl transfers using the 1,200 µl Multichannel head.

Results and Discussion

Figure 1 shows the overall experimental workflow of the In-Fusion cloning reaction using the Biomek i7 Dual Hybrid Workstation. For this study, we focused on the capability of the liquid handler to automate the cloning step only; other steps, including PCR set-up and purification, were performed manually. For information on automating the upstream PCR purification steps, please visit **https://www.takarabio.com/learning-centers/nucleic-acid-purification/automated-dna-and-rna-purification**.

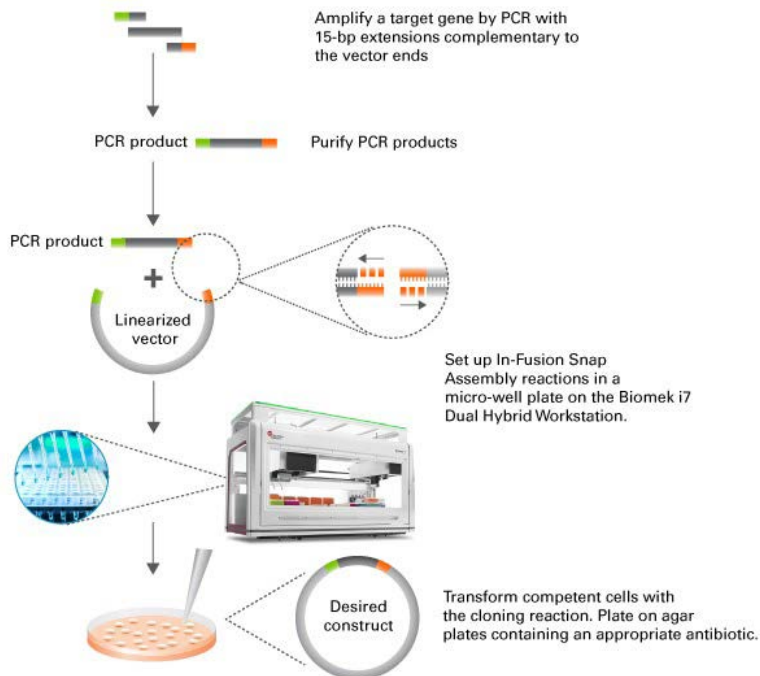

Figure 1. Schematic of the automated In-Fusion cloning workflow on the Biomek i7 Dual Hybrid Workstation. Target sequence is amplified by PCR using a forward and reverse primer with 5' ends homologous to the respective 5' and 3' ends of the linearized vector. After purification, the In-Fusion Snap Assembly reaction is dispensed into a 96-well plate using a Biomek i7 Dual Hybrid Workstation. Following incubation, the cloning reaction is transformed into competent cells and plated on selective plates for further analysis.

To simplify automated pipetting steps, the master mix plus vector solution (5X In-Fusion Snap Assembly Master Mix with linearized vector and water) was pipetted into a 96-well plate first, followed by the addition of inserts. All pipetting using the Biomek i7 Dual Hybrid Workstation was carried out at room temperature. An advantage of using In-Fusion Snap Assembly on a liquid handler is that the pre-mixed solution (the master mix plus vector) is stable at room temperature for 2 hours (data not shown). Therefore, the preparation for the cloning reaction using a liquid handler is not dictated by the timeline or sample number.

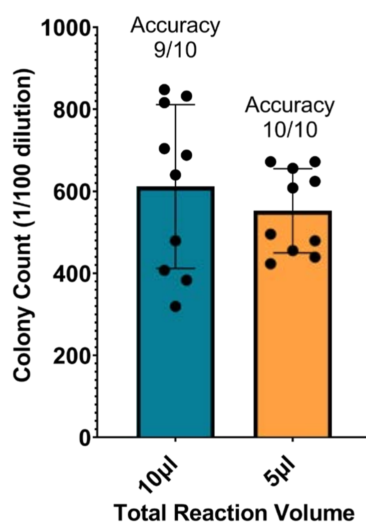

Figure 2. Colony counts and cloning accuracy of In-Fusion cloning reactions using the Biomek i7 Dual Hybrid Workstation. Two reaction volumes, 10 µl and 5 µl, were tested. Graphed values are the mean colony counts of three independent cloning reactions. Error bars show ± the standard deviation. Accuracy was determined by Sanger sequencing.

For the cloning reaction, we assembled a 3.7 kb insert into a 2.6 kb plasmid and tested two reaction volumes: 10 µl (the manufacturer's recommended reaction volume) and 5 µl (a scaled-down volume). To ensure that setting up the In-Fusion Snap Assembly reaction using the Biomek i7 Dual Hybrid Workstation does not affect cloning efficiency and accuracy, we transformed Stellar™ Competent Cells with cloning reactions that were set up manually as positive controls and counted the resulting colonies. As shown in Figure 2, both reaction volumes demonstrated the expected efficiency (i.e., colony numbers).

To determine the accuracy of the cloning reaction, we sequenced 10 clones obtained from each reaction volume. A clone was considered accurate if the insert exactly matched the expected in silico sequence with no point mutations. Both reaction volumes demonstrated sequence accuracy of 90–100%. There was one inaccurate clone in the 10 µl reaction group that was identified as an empty vector; however, we consider 0–10% of clones containing empty vectors as a normal level of background as this is what is observed with manual pipetting protocols.

The colony counts and sequence accuracy of gene assemblies in both reaction volumes tested are comparable to what is observed when using the manual pipetting procedure, suggesting that automating the In-Fusion Snap Assembly cloning reaction on the Biomek i7 Dual Hybrid Workstation can be successfully carried out without compromising cloning efficiency and accuracy.

Conclusion

We demonstrate that PCR-based In-Fusion seamless cloning can be easily implemented on the Biomek i7 Dual Hybrid Workstation without compromising the high cloning accuracy and efficiency that we expect from this technology. We showed that using the Biomek i7 automated workstation not only allows scientists to increase their cloning throughput but also reduces the upfront cost of cloning reagents by scaling down the cloning reaction volumes.

Materials and Methods

Vector and insert preparation

pUC19 vector (2.7 kb) was linearized with Hind III restriction enzyme. An insert DNA fragment (3.7 kb) was prepared by PCR amplification with a primer set including 15 bp sequences complementary to the ends of the linearized pUC19 vector. Primers were designed using the **In-Fusion Cloning Primer Design Tool**.

Cloning reaction setup on Biomek i7 Dual Hybrid Workstation

1 µl transfers were performed with a 1,200 µl Multichannel Head as a proof of principle to showcase the ability of low-volume range liquid handling. The method requires only 4–5 deck positions and can be easily mapped to a Biomek i5 Multichannel Workstation. The Guided Labware Setup was used to reduce setup errors and provide a more interactive user experience. A graphic of the method workflow is shown in Figure 3.

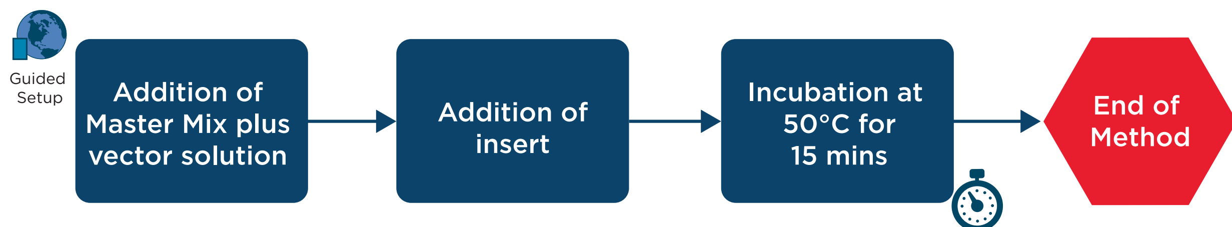

Figure 3. Worktable setup of the In-Fusion cloning automation workflow.

The 5X In-Fusion Snap Assembly Master Mix was first manually mixed with linearized plasmid and water to create the master mix plus vector solution. The Biomek i7 Dual Hybrid Workstation was used to first dispense the master mix plus vector solution, followed by dispensing of the inserts, into the corresponding wells of a 96-well plate **(Table 1)**. Two reaction volumes were tested: 10 µl and 5 µl. For each reaction volume group, 32 PCR cloning reactions were performed along with 16 negative control (no insert) reactions. 9 µl and 4 µl of master mix plus vector solution were used in the 10 µl reaction group and 5 µl reaction group, respectively. For all reaction volume groups, 1 µl of insert DNA fragment was used **(Table 1)**.

	1	2	3	4	5	6	7	8	9	10	11	12
A	9+1 µl	9+1 µl	no insert	9+1 µl	9+1 µl	no insert	4+1 µl	4+1 µl	no insert	4+1 µl	4+1 µl	no insert
B	9+1 µl	9+1 µl	no insert	9+1 µl	9+1 µl	no insert	4+1 µl	4+1 µl	no insert	4+1 µl	4+1 µl	no insert
C	9+1 µl	9+1 µl	no insert	9+1 µl	9+1 µl	no insert	4+1 µl	4+1 µl	no insert	4+1 µl	4+1 µl	no insert
D	9+1 µl	9+1 µl	no insert	9+1 µl	9+1 µl	no insert	4+1 µl	4+1 µl	no insert	4+1 µl	4+1 µl	no insert
E	9+1 µl	9+1 µl	no insert	9+1 µl	9+1 µl	no insert	4+1 µl	4+1 µl	no insert	4+1 µl	4+1 µl	no insert
F	9+1 µl	9+1 µl	no insert	9+1 µl	9+1 µl	no insert	4+1 µl	4+1 µl	no insert	4+1 µl	4+1 µl	no insert
G	9+1 µl	9+1 µl	no insert	9+1 µl	9+1 µl	no insert	4+1 µl	4+1 µl	no insert	4+1 µl	4+1 µl	no insert
H	9+1 µl	9+1 µl	no insert	9+1 µl	9+1 µl	no insert	4+1 µl	4+1 µl	no insert	4+1 µl	4+1 µl	no insert

Table 1. Plate setup using a 96-well plate.

After all the reagents for In-Fusion Snap Assembly cloning were dispensed, the PCR plate was incubated at 50°C for 15 min using the on-deck thermal cycler. At the end of the method, the PCR plate was removed from the Biomek i7 Dual Hybrid Workstation, sealed with a PCR film, and stored at –20°C until further analysis.

Reactions from 10 wells containing 10 µl total reaction volume and 10 wells containing 5 µl total reaction volume were randomly picked from the plate and transformed into competent cells using a standard transformation procedure. Two negative control (no insert) samples from each reaction volume were also transformed into competent cells. Ten random colonies were chosen from the array of plates corresponding to each reaction volume and analyzed by Sanger sequencing to determine sequence accuracy. Sequences were required to be 100% identical to the expected reference sequence to be counted as accurate.

Spotlight

The Biomek i7 Dual Hybrid (Multichannel 96, Span-8) Workstation delivers reliability and efficiency to increase user confidence and walk-away time as compared to manual library preparation. Features include:

- 1,200 µl Multichannel Head with 1–1,000 µl pipetting capability
- Span-8 pod with fixed and disposable tips
- Enhanced selective tip multichannel pipetting to transfer a custom array of samples
- Independent 360˚ rotating gripper with offset fingers
- High deck capacity with up to 45 positions
- Shaking, heating/cooling, and tip washing for controlling sample processing
- Spacious, open platform design to integrate on-deck and off-deck elements (e.g., Automated Thermal Cycler (ATC))

Figure 4. Biomek i7 Dual Hybrid Workstation with optional enclosure on a Biomek Mobile Workstation.

Product use limitations and safety information

Please read the In-Fusion Snap Assembly manual before performing the method for the first time.

Ordering information

Product	Size	Cat #
In-Fusion Snap Assembly Master Mix	10 Rxns	638947
In-Fusion Snap Assembly Master Mix	50 Rxns	638948
In-Fusion Snap Assembly Master Mix	250 Rxns	638949
In-Fusion Snap Assembly Master Mix	500 Rxns	638943
In-Fusion Snap Assembly Master Mix	1,000 Rxns	638944
In-Fusion Snap Assembly EcoDry™ Master Mix	8 Rxns	638954
In-Fusion Snap Assembly EcoDry Master Mix	32 Rxns	638955
In-Fusion Snap Assembly EcoDry Master Mix	96 Rxns	638956

The Takara Bio In-Fusion Snap Assembly products are for research use only and sold by Takara Bio. Beckman Coulter makes no warranties of any kind whatsoever express or implied, with respect to this protocol, including but not limited to warranties of fitness for a particular purpose or merchantability or that the protocol is non-infringing. All warranties are expressly disclaimed. Your use of the method is solely at your own risk, without recourse to Beckman Coulter. This protocol is for demonstration only and is not validated by Beckman Coulter.

Biomek Automated Workstations are not intended or validated for use in the diagnosis of disease or other conditions.

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