

## Multiple displacement amplification products are compatible with recombination-based cloning

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Multiple displacement amplification (MDA) is a method that utilizes  $\phi$ 29 DNA polymerase and random hexamer primers to amplify DNA fragments (1–3).  $\phi$ 29 DNA polymerase is a highly processive enzyme that incorporates at least 70,000 nucleotides per binding event (4). The starting material for amplification can be a small amount of bacterial cells containing a plasmid, an isolated plasmid, an M13 phage, or any circular DNA sample. Bacterial colonies can be picked from agar plates and added directly to an amplification reaction. MDA products are high-molecular weight, concatenated repeats of the input plasmid DNA that can be used for a variety of molecular biology techniques, including sequencing, without additional purification steps (1,3). Here we demonstrate that MDA products can also be used directly in recombination-based cloning strategies, thus eliminating the need to purify DNA from liquid cultures of *Escherichia coli*.

The Gateway® cloning technology (Invitrogen, Carlsbad, CA, USA) is a highly efficient system for cloning DNA fragments into any kind of vector while maintaining reading frame and orientation. The basis for this in vitro system is the  $\lambda$  recombination system. Target sequences are first inserted into

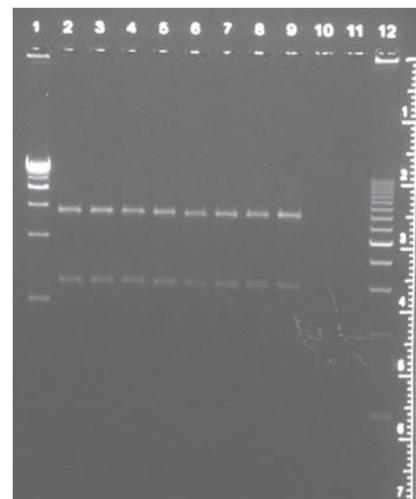
a commercially available entry vector and are then recombined into specific destination vectors as required for expression in different experimental systems (5,6). The LR reaction is based on the  $\lambda$  phage recombination system (6) and is represented as follows:

$$\begin{aligned} &attL1\text{-gene-}attL2 \text{ (entry clone)} \times \\ &attR1\text{-}ccdB\text{-}attR2 \text{ (destination vector)} \\ &>>>> attB1\text{-gene-}attB2 \text{ (expression} \\ &\text{clone)} \times attP1\text{-}ccdB\text{-}attP2 \text{ (by product)}. \end{aligned}$$

The Gateway-compatible destination vector pLHC-GW [pLHCX from Clontech (Palo Alto, CA, USA) converted with a Gateway cassette] and entry clone pENTR-GOI (in this example the gene of interest is 3.5 kb), were prepared using QIAprep® miniprep kits (Qiagen, Valencia, CA, USA). The resulting plasmid was 150–200 ng/ $\mu$ L.  $\phi$ 29 amplification was performed with the TempliPhi™ kit (GE Healthcare, Piscataway, NJ, USA). Single colonies were mixed with 50  $\mu$ L water, and 0.5  $\mu$ L of this mixture was used in a reaction with 5  $\mu$ L sample buffer. The sample was denatured at 95°C for 3 min, then 5  $\mu$ L reaction buffer and 0.2  $\mu$ L enzyme mixture were added. Samples were incubated for 18 h at 30°C, then enzyme was inactivated at 65°C for 10 min. All samples were diluted with 40  $\mu$ L water prior to further use.

To demonstrate that products of MDA can be used successfully in the Gateway cloning system, we have run four sets of LR reactions, each in replicates of eight as described in Table 1. Sample 1, both destination vector and entry clone were plasmid DNA purified from *E. coli*; sample 2, destination vector was purified plasmid and entry clone was a product of MDA; sample 3, destination vector was a MDA product and entry clone was purified plasmid; and sample 4, both entry clone and destination vector were MDA products.

The LR reactions were briefly vortex mixed and incubated at 25°C for 1 h, followed by addition of 2  $\mu$ L Proteinase K solution and incubation at 37°C for 10 min. One microliter of the LR reaction was transformed into



**Figure 1. LR recombination cloning using DNA from MDA reactions results in correct plasmids.** Image of agarose gel analysis of eight clones resulting from the LR reaction. Both entry clone and destination vector were multiple displacement amplification (MDA) products. Restriction digest was performed with *Bgl*III on all eight samples (lanes 2–9). Lane 1 is the 2.5-kb molecular ruler, and lane 12 is the 1-kb molecular ruler (Bio-Rad Laboratories, Hercules CA).

**Table 1. Samples Used to Test Multiple Displacement Amplification (MDA)-Generated DNA**

Reaction Components	Sample 1	Sample 2	Sample 3	Sample 4
pENTR-GOI Entry Clone	3 $\mu$ L (plasmid)	1 $\mu$ L (MDA)	3 $\mu$ L (plasmid)	1 $\mu$ L (MDA)
pLHC-GW Destination Vector	2 $\mu$ L (plasmid)	2 $\mu$ L (plasmid)	1.7 $\mu$ L (MDA)	1.7 $\mu$ L (MDA)
5 $\times$ LR Clonase™ Reaction Buffer	4 $\mu$ L	4 $\mu$ L	4 $\mu$ L	4 $\mu$ L
Tris-EDTA buffer, pH 8.0	7 $\mu$ L	9 $\mu$ L	7 $\mu$ L	9 $\mu$ L

Four different conditions were used to test MDA-generated DNA in different combinations in the LR reaction. The LR Clonase system is described by Invitrogen. Approximately 300 ng DNA were used in each reaction.

**Table 2. Cloning Accuracy for Samples Tested in the LR Reaction**

	Sample 1	Sample 2	Sample 3	Sample 4
Entry Vector	Plasmid	MDA Products	Plasmid	MDA Products
Destination Vector	Plasmid	Plasmid	MDA Products	MDA Products
Experiment 1	6/8	6/8	7/8	7/8
Experiment 2	8/8	7/8	8/8	8/8
Experiment 3	8/8	8/8	8/8	8/8
Transformation Efficiency (cfu/ $\mu$ g DNA)	$2.2 \times 10^4$	$1.8 \times 10^4$	$4.5 \times 10^4$	$5.6 \times 10^4$

The four combinations tested in the LR reaction are shown as the number of correct clones/number of clones tested by restriction digest. The transformation efficiency [colony-forming units (cfu) per microgram DNA] from a representative experiment is also presented. MDA, multiple displacement amplification.

TOP10 chemically competent cells (Invitrogen), and 100  $\mu$ L transformation mixture were plated on LB plates containing 50  $\mu$ g/mL ampicillin. To determine if the proper products were formed during recombination, miniprep DNA was prepared from liquid cultures that had been inoculated with individual colonies. DNA was digested with diagnostic restriction endonucleases (New England Biolabs, Ipswich, MA, USA). Restriction digest of the correct product plasmid with *Bgl*III enzyme resulted in fragments of 7 kb and 3.1 kb. As shown in Figure 1, all eight colonies selected from the MDA-based reactions yielded the proper fragments upon digestion. Results from three independent experiments demonstrated that neither the cloning accuracy nor the transformation efficiency was affected by the source of the DNA used in the LR recombination reaction (Table 2).

Restriction digest is a rough view of whether the resultant products are correct. To determine whether recombined plasmids from reactions containing MDA products contain a greater number of mutations, the resulting plasmids were sequenced. The 3.5-kb insert transferred from the entry vector, as well as flanking region surrounding each of the *att* recombination sites, were sequenced on both strands in eight clones from each combination. Sequencing was performed on an ABI PRISM® 3100 Genetic Analyzer using BigDye® version 3.1 chemistry (both from Applied Biosystems, Foster City, CA, USA). Primers for sequencing were designed flanking inserts every 500 bp within the gene of interest in both directions. Sequencing data was loaded into Lasergene SeqMan Pro (DNASTAR,

Madison, WI, USA) for contig assembly and alignment. No mutations were identified in any of the samples that were correct by restriction digest (data not shown). Recombination cloning using MDA products has been repeated with multiple entry and multiple destination vectors (2.5–6.9 kb) with gene inserts ranging from 567 bp to the 3.5 kb presented here with essentially the same results (data not shown).

Our results clearly demonstrate that MDA-generated DNA is compatible with Gateway recombination-based cloning strategies. While the precise mechanism is unknown, it is clear that the system has the ability to utilize relatively long stretches of double-stranded DNA (dsDNA) present in the concatenated MDA product or to decatenate the product prior to recombination. Cloning accuracy was very high, the cloning efficiency was no different compared with that using purified plasmid, and the mutation rate was extremely low. In agreement with earlier studies of the fidelity of the MDA reaction (7), no mutations were detected in the more than 80 kb sequenced. A single in vitro amplification will generate enough material for use in many reactions. Destination vectors can be in vitro amplified, rather than propagated in special bacterial strains. Also, because entry clones can be amplified directly from colonies using MDA prior to recombination-based cloning, time and effort will be saved without sacrificing quality or efficiency.

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## COMPETING INTERESTS STATEMENT

*The authors declare no competing interests.*

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