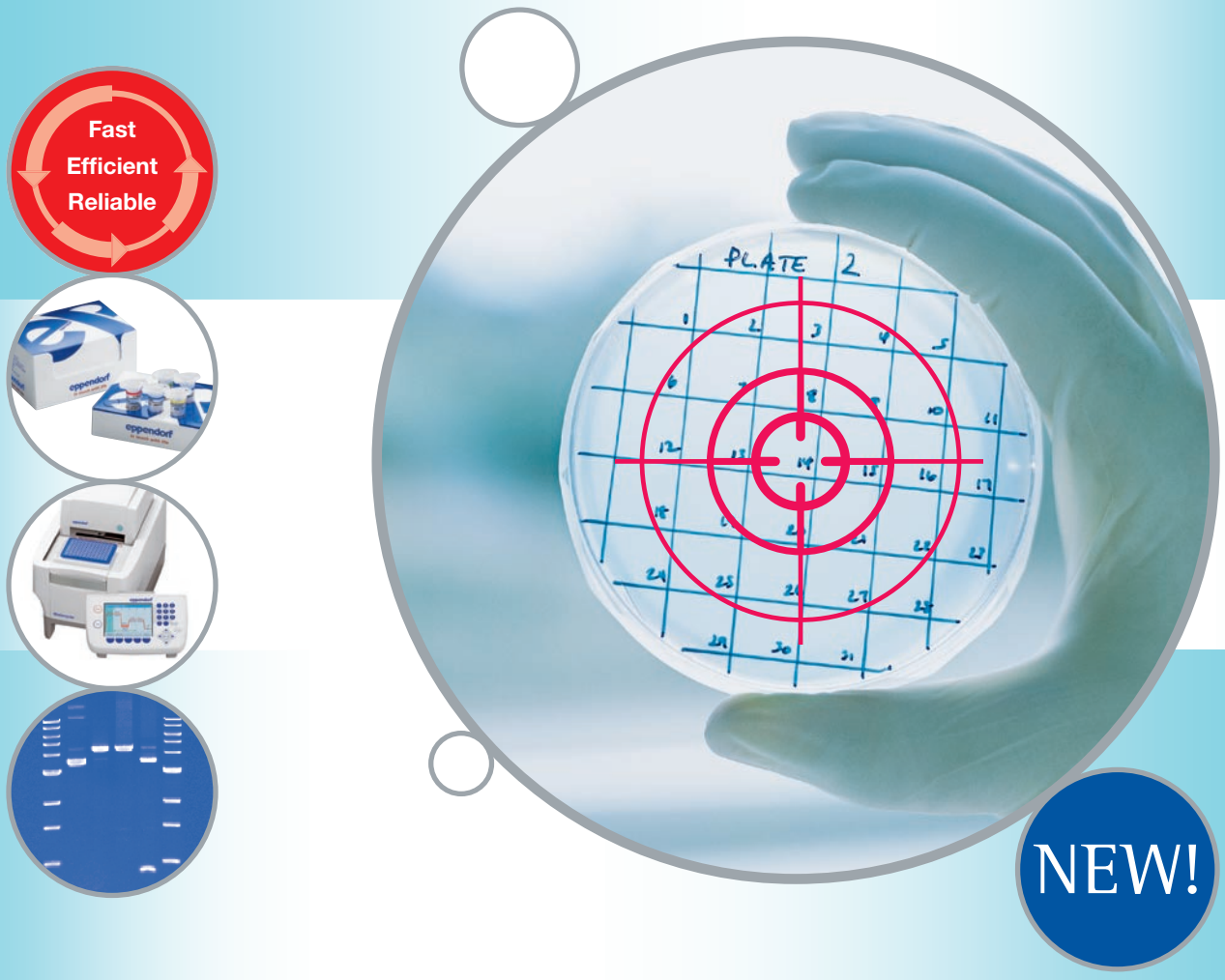


On Target!

Quick and easy screening of plasmid DNA with OriMaster®

eppendorf

Good news for researchers

OriMaster *in vitro* plasmid amplification system

Screening of bacterial clones often is a tedious and time consuming procedure. Overnight culture growth, manual reaction set-up and laborious restriction digests require several hours before visible results are available.

With the new **OriMaster** *in vitro* plasmid amplification system, Eppendorf introduces a simple and fast solution for preparing plasmid DNA for cycle sequencing, size determination, restriction site mapping and other enzymatic reactions.

Offering cost-effective and reliable screening of plasmid DNA, OriMaster uses an amplification method that starts from a single bacterial colony.

In contrast to the traditional plasmid purification, no overnight cultures are needed. OriMaster produces linear, double-stranded copies of plasmids up to 9 kb. It provides consistent and high yields of product independent of the copy number of the plasmids.

No cleanup is required for the downstream applications due to very low levels of RNA and enzymatic inhibitors. The reactions are scalable – the reaction volume can be adapted to the needs, leading to a highly flexible system.

Compared to most other products available, OriMaster saves time, saves money and will increase the efficiency of your lab.

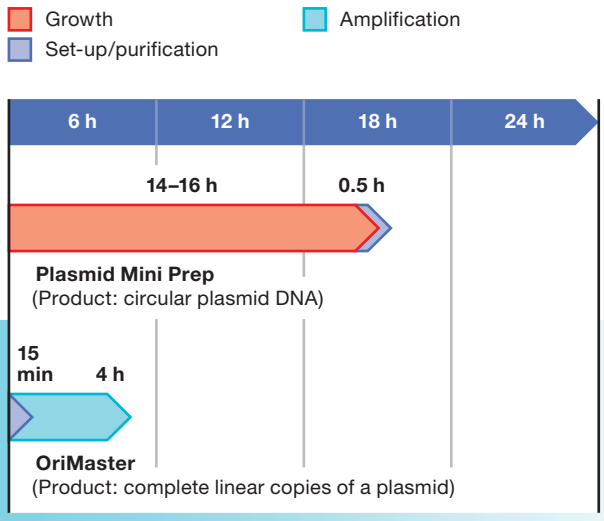
OriMaster advantages

over Plasmid Mini Prep:

- On average 3 to 4 times faster processing time:
  – no liquid cultures to grow, spin down
  – Plasmid is amplified rather than purified
- Linear templates are better substrates for enzymatic reactions than supercoiled templates

over Colony PCR:

- No primer design or optimization required
- No chemistry optimization required
- No post-PCR cleanup prior to DNA sequencing



Plasmid Applications with Eppendorf products

Application	Recommended product
Plasmid size determination, <9 kb	OriMaster
Sequencing	
● <9 kb, high copy	● OriMaster, FastPlasmid
● <9 kb, low copy	● OriMaster, Perfectprep Plasmid Mini
● >9 kb, high copy	● FastPlasmid
● >9 kb, low copy	● Perfectprep Plasmid Mini
Restriction mapping	OriMaster FastPlasmid Perfectprep Plasmid Mini
***In vitro* transcription**	OriMaster FastPlasmid Perfectprep Plasmid Mini
PCR	OriMaster FastPlasmid Perfectprep Plasmid Mini
Transformation	FastPlasmid Perfectprep Plasmid Mini
Cloning	FastPlasmid Perfectprep Plasmid Mini
Transfection	Perfectprep Plasmid Mini
96 well format	
● Screening	● OriMaster
● All applications	● Perfectprep Plasmid 96 Vac DB

Screening of plasmid DNA with Eppendorf's OriMaster

Application

Introduction

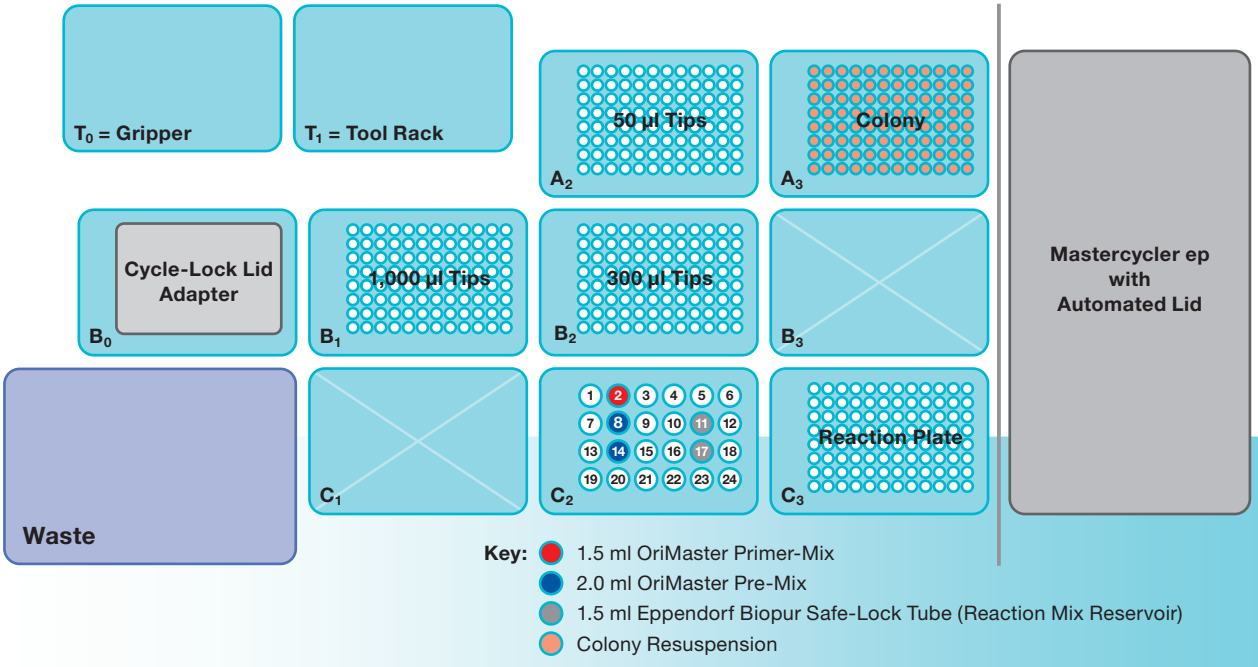
Screening of bacterial colonies is routine work in every molecular biology lab. Bacterial cultures are grown overnight, followed the next day by the isolation of the plasmid DNA. The success of this methodology is dependent on culturing conditions, bacterial host strains and the copy number of the plasmids.

The OriMaster system uses a different approach, which is not influenced by these factors. A blend of long-range, high fidelity enzymes amplifies plasmids of different sizes (up to 9 kb) directly from a single colony in less than four hours. The specially developed primers of the OriMaster kit anneals in the ColE1 origin of replication (e.g. pUC, pBR322), making it suitable for most of the common plasmids used in molecular biology labs. The method does not require time-consuming primer design or chemistry optimization nor further cleanup for the downstream applications like size screening, restriction digestion or sequencing. Because the OriMaster reactions are scalable, the reaction volumes can be optimized for the amount of DNA needed for these analyses.

For applications needing a higher throughput like the screening of cDNA libraries, the OriMaster reaction set-up can be easily automated. For this purpose the **Eppendorf epMotion 5075** is an ideal solution. This compact, fully automated workstation provides a user-friendly operation and a high degree of programming flexibility.

● Fig. 1: Layout of the ep*Motion* 5075 MC deck for OriMaster

- **T_0:** Gripper
- **T_1:** Tool rack (100–1,000 µl / 20–300 µl single; 1–50 µl 8-channel)
- **A_2:** Eppendorf epT.I.P.S Motion 1–50 µl Filtertips
- **A_3:** Eppendorf fully-skirted twin.tec 96-well PCR plate (10 µl colony resuspensions)
- **B_0:** Eppendorf Cycle-Lock Lid and Adapter
- **B_1:** Eppendorf epT.I.P.S. Motion 20–300 µl Filtertips
- **B_2:** Eppendorf epT.I.P.S. Motion 100–1,000 µl Filtertips
- **B_3:** Empty
- **C_1:** Empty
- **C_2:** 1.5 ml Tube Rack (OriMaster Pre-Mix, OriMaster Primer Mix, empty tubes for reaction mix)
- **C_3:** Eppendorf fully-skirted twin.tec 96-well PCR plate (reaction plate)



Application (continued)

Material and Methods

Creation of the cDNA Library
Total RNA from the venom gland of the Monocled Cobra (Naja kaouthia), was isolated by the method of Chomczynski and Sacchi[1]. The protocol was modified for the use of the Eppendorf Phase Lock Gel (2 ml tubes, heavy) for the optimal separation of the phases. The Eppendorf cMaster RTplus PCR kit was used to reverse transcribe and amplify the cDNA with universal three-finger toxin primers that anneal in the 5'- and 3'-untranslated regions. The resulting product was inserted into pGEM-T (Invitrogen, USA) and transformed into competent *E.coli*.

Colony growth
The transformants were streaked from frozen culture stored at –80 °C onto LB Amp 100 plates (Teknova, USA) using a sterile loop. The plates were grown overnight at 37 °C until colonies were 1 mm in diameter (~18 h).

***in vitro* plasmid amplification**
192 single colonies were picked with sterile loops and resuspended in 10 µl Molecular Biology Grade water (MBGW) by swirling for 10 seconds. Once all colonies were resusupended, the plate was sealed and vortexed for ten seconds. The OriMaster Pre-Mix and Primer Mix were thawed and vortexed, and all components were arranged on the deck of the epMotion 5075 MC (**Fig. 1**, method file can be obtained at **www.epmotion.com**). Using the epMotion, OriMaster Pre-Mix and Primer Mix were combined to a 1x mastermix and this was multi-dispensed into each well of the reaction plate. 2.5 µl of each colony resuspension was dispensed column-wise into the well containing the 1x mastermix and thoroughly mixed.

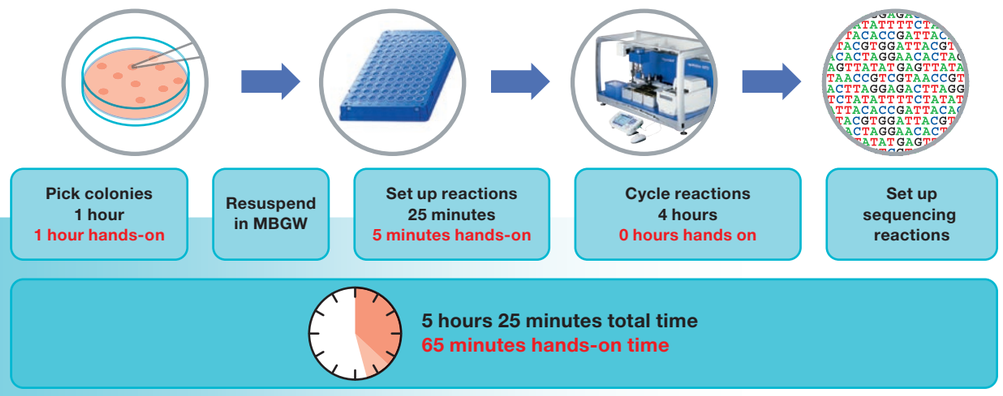
The reaction plate was then transferred to the preheated thermal cycler, sealed with a Cycle-Lock Lid, and cycled according to the protocol in the product manual. After amplification, the products were confirmed and size-screened on a 1% 1x TAE agarose gel. 2 µl of the samples were loaded and run on the gel at 170 V for 90 minutes and then stained with ethidium bromide.

Sequencing
For sequencing, only 1 µl of the OriMaster sample is needed. The samples are used directly without cleanup. They were sent to the DNA sequencing facility at the Bioresource Center of the Cornell University (Ithaca, NY) and their PHRED sequencing quality score (Qscore, Codoncode, USA) was determined. A quality score is an algorithm-determined read length that takes into account several factors that affect overall sequence quality, and reflects 99% accurate sequence data.

Results

Eppendorf OriMaster offers time savings and easier handling in comparison to the traditional plasmid purification technologies. Especially when working in a higher throughput, the reaction set-up can be easily done automatically using the epMotion 5075 using the OriMaster Pre-Mix and Primer Mix tubes directly out of the box. With this combination, it takes less than six hours with approximately one hour hands-on time to amplify complete linear copies of plasmids (with a length of up to 9 kb) from 192 independent bacteria clones (**Fig. 2**). Samples can be sequenced that same day, and sequencing results are ready for further analysis by the next morning.

● **Fig. 2: OriMaster procedure using the automated reaction set-up with the Smart Workstation epMotion 5075 MC**



[1] *Chomczynski, P., and N. Sacchi.* Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162: 156–159, 1987.

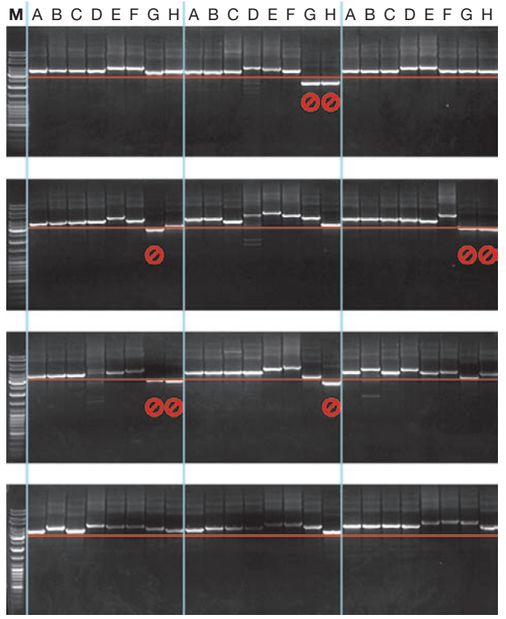
Size screening
As an initial check the OriMaster products were analyzed directly in an agarose gel confirming that all samples yielded sufficient product. Because the product is linear plasmid DNA, empty vectors could be easily identified and excluded from the sequencing reactions (**Fig. 3**).

Sequencing
All insert-containing plasmids were further analyzed by determining the sequence of the cloned cDNA. Excellent sequencing results were obtained by using only 1 µl of the unpurified OriMaster samples as templates in the sequencing reactions. The average Q200 Pass Rate (Qscore>200) was 99 %, the average Q500 Pass Rate (Qscore>500) was 87 %, comparable to the expected pass rates for purified circular plasmids (**Fig. 4**). All sequences with a Qscore>500 were aligned to the corresponding sequences of purified plasmids. There were no deviations found over a range of more than 66,000 bases, showing the high sequence fidelity of the OriMaster system due to the high fidelity enzyme mix used in the kit.The complete analysis of the cDNA library is described in the application note “The efficiency of Eppendorf OriMaster in screening a cDNA library of the venom gland from the monocled cobra, Naja kaouthia, using the epMotion 5075”. Please visit **www.eppendorf.com/orimaster** for more information.

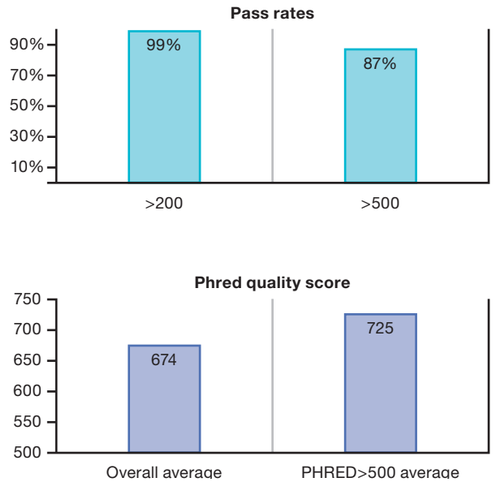
Discussion
The Eppendorf OriMaster kit for the *in vitro* amplification of plasmid DNA provides excellent results in the screening of bacterial clones. It leads to reliable results for the typical screening applications like gel electrophoresis for size determination and in sequencing reactions without prior plasmid DNA cleanup.
The OriMaster reactions require significantly less overall time: It does not require an overnight culture growth step. Starting with a single colony, it takes less than six hours to generate templates ready for the sequencing reaction set-up. A high number of samples can be easily handled using the epMotion 5075 MC for a simple automated reaction set-up, needing minimal hands-on efforts. The determination of the plasmid sizes can be done immediately – the complete copies of the plasmids are already linear. Tedious restriction digests can be avoided. Sequencing can even be done the same day; set-up is simplified as the OriMaster reactions can be used without cleanup and without determination of the DNA concentration. Using 1 µl of the reactions, the quality of the sequences is comparable to the results from purified plasmid DNA with regard to quality scores and sequence fidelity.

With the combination of OriMaster and the epMotion 5075 workstation, Eppendorf offers a perfect system for quick, efficient and easy screening for a high number of clones, like a cDNA library.

● **Fig. 3: Determination of the plasmid sizes**
Immediately after the cycling reaction, the OriMaster samples can be run on an agarose gel. Due to the linearity of the products a direct size screening is possible. Vectors without insert (Ø) can be identified easily and excluded from further analysis.



● **Fig. 4: Sequencing statistics for Eppendorf OriMaster plasmid amplification kit**
Pass rates and the average PHRED scores for 192 OriMaster reactions were determined (Qscore, Codoncode, USA).



Plasmid amplification system components

Ordering information

Description				Order no.
OriMaster[*1]				
100 reactions				0032 002.486
500 reactions				0032 002.488
ep*Motion* Smart Workstations				
ep*Motion* 5070, basic device includes control panel, software, optical sensor, waste container, MMC and reader, operating instructions, 200–240 V, 50–60 Hz				5070 000.000
ep*Motion* 5075 LH, 200–240 V, 50–60 Hz (liquid handling)				5075 000.008
ep*Motion* 5075 VAC, 200–240 V, 50–60 Hz (includes vacuum station)				5075 000.016
ep*Motion* 5075 MC, 200–240 V, 50–60 Hz (Mastercycler® ep not included)				5075 000.032
Mastercycler ep thermal cyclers				
Mastercycler® ep gradient[*2]				
with manual lid, 230 V, 50–60 Hz				5341 000.019
with motorized lid, 230 V, 50–60 Hz				5341 000.108
Mastercycler® ep gradient S[*2]				
with manual lid, 230 V, 50–60 Hz				5345 000.013
with motorized lid, 230 V, 50–60 Hz				5345 000.102
twin.tec PCR Plate 96, skirted (clear wells), 25 pcs.				
Clear				0030 128.648
Yellow				0030 128.656
Green				0030 128.664
Blue				0030 128.672
Red				0030 128.680
Phase Lock Gel Light[*3]	**Tube size**	**Sample size**	**Number of tubes**	
1.5 ml	1.5 ml	100–500 µl	200	0032 007.961
2.0 ml	2.0 ml	100–750 µl	200	0032 005.101
15 ml	15 ml	1–6 ml	100	0032 005.209
50 ml	50 ml	5–20 ml	25	0032 005.306
Phase Lock Gel Heavy[*3]				
1.5 ml	1.5 ml	100–500 µl	200	0032 007.953
2.0 ml	2.0 ml	100–750 µl	200	0032 005.152
15 ml	15 ml	1–6 ml	100	0032 005.250
50 ml	50 ml	5–20 ml	25	0032 005.330
cMaster RT System				
50 reactions				0032 002.323
200 reactions				0032 002.331
cMaster RT$_{plus}$ PCR System				
20 reactions				0032 002.358
100 reactions				0032 002.366

[*1] This product is sold under licensing arrangements with F. Hoffmann-La Roche Ltd., Roche Molecular Systems, Inc. and Applied Biosystems.
[*2] A Control Panel or epCycleManager software (both sold separately) is required for operation; CAN_Bus connection cables are required to link cyclers together as a network.
[*3] Note: 15 ml and 50 ml conical tubes can be centrifuged up to 3,500 x g.

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