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Protocol status: Working
We use this protocol and it's working

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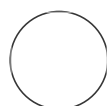
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Prozedere Bacterial stabs in Kultur nehmen bis Übernachtkultur ernten V.2

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ABSTRACT

Das Protokoll beschreibt die In-Kulturnahme von Bacterial stabs zur Anreicherung einzelner Klone und zur Produktion von ausreichend Bakterien zur Gewinnung der gewünschten Plasmide mittels Maxi Prep.

GUIDELINES

Addgene ships plasmids as transformed bacteria in stab culture format. A stab culture is a type of Luria Broth (LB) Agar media, similar to a standard LB Agar plate. Unlike an LB Agar plate, a stab culture is created by piercing the LB agar with the bacteria instead of spreading it on the surface. The bacteria in a stab culture grow from the puncture site to spread across the surface of the stab culture.

Short Term Storage of Bacterial Stab

Stab cultures should be **stored at 4°C** upon receipt. The bacteria in the stab is guaranteed to live for at least 2 weeks when stored at 4°C.

Long Term Storage - Isolating and Verifying the Plasmid and Creating a Glycerol Stock

Within **2 weeks** of receiving your new Addgene plasmid you should verify that the plasmid is correct and create a glycerol stock. Please follow Addgene's recommended protocols below.

1. [Streak Bacteria for Single Colonies](#) - use your bacterial stab to streak bacteria onto a plate, grow overnight, and isolate single colonies.

2. Select an isolated, single colony to [inoculate an overnight culture](#). Using a liquid culture will allow you to grow enough bacteria for plasmid DNA purification and for [creating glycerol stocks](#).
1. Addgene recommends selecting and inoculating 2-4 separate colonies for plasmid screening and verification.
3. [Isolate your Plasmid DNA](#) - many companies sell miniprep or maxiprep kits for easy plasmid isolation. Addgene also provides a protocol for plasmid purification without a kit.
4. After isolating plasmid DNA, Addgene recommends verifying the plasmid by diagnostic digest or sequencing before beginning any experiments.
 1. [Perform a Diagnostic Digest](#) - verify backbone and insert sizes
 2. [Sequence your Plasmid](#) - verify key regions of the plasmid using DNA sequencing and compare these to sequences Addgene obtained from the plasmid, found on the plasmid page under "Sequences"
 3. After screening 2-4 colonies, if you find that your plasmid DNA is not correct, please view the instructions on how to [report a problem with a plasmid](#).
5. Once you have verified that the plasmid DNA is correct, keep the glycerol stocks corresponding to the colony that was verified- most plasmids are stable for years when stored as bacterial glycerol stocks.



MATERIALS

Mikrowelle
Schüttelinkubator/-wasserbad 37°C + 50 °C
LB-Medium/ mit AB
sterile Spitzen
15 ml Falcontubes
sterile Erlenmeyerkolben
10 cm Greiner-Schalen
50 % Glycerol
Trockeneis/EtOH-Bad



Before Start

- 1 **Ampicillin:**
100 mg/ml in sterilem H₂O lösen; sterilfiltrieren, aliq.; - 20 °C
1:1000 zu dem Platten bzw. Medium => 100 µg/ml
- 2 **Agarplatten gießen:**
10 cm Greiner-Schalen
1,5 g Agar auf 100 ml LB-Broth-Medium o. 3,5 g LB Agar auf 100 ml ddH₂O
aufkochen, abkühlen auf 50 °C im Wasserbad
unter der Bench + AB; gießen (3-4 mm hoch)
reicht für ca. 4-5 Platten
gegossene Platten im Kühlschrank in Plastiktüte über Kopf lagern
- 3 **LB-Medium ansetzen:**
20 g in 1 L ddH₂O lösen
121 °C 15 min autoklavieren





Bacterial stabs austreichen



- 4 Bacterial stab mit steriler Pipettespitze auf den Agarplatten austreichen; trocknen lassen
- 5  Overnight im Inkubator bei  37 °C über Kopf inkubieren

Vorkultur und Übernachtkultur


- 6 mit einer sterilen Pipettenspitze Klone picken und im 15 ml Falcontube in ca.  2 mL Medium/
mit AB einbringen
- 7 bei  37 °C im Schüttelinkubator für einige Stunden anziehen (Deckel leicht geöffnet)

8 restliche Platte mit Parafilm abdichten; als Backup im Kühlschrank in Plastiktüte über Kopf lagern

9  250 µL auf  100 mL Medium/ mit AB in 250 ml Erlenmeyerkolben mit leicht geöffneter Alufolie als Deckel;  Overnight bei  37 °C im Wasserbad schütteln

10 Zellen abzentrifugieren  4000 x g, Room temperature  00:10:00 und zur 10m
Plasmidisolierung weiter mit Protokoll:

Protocol



NAME
E.Z.N.A.® Plasmid DNA Maxi Kit Centrifugation Protocol (modified)


CREATED BY
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PREVIEW

Glycerolstocks

5m

11  700 µL Übernacht-Kultur +  300 µL 50 % Glycerol

12 für  00:05:00 in Trockeneis/EtOH-Bad einfrieren 5m

13 aufbewahren bei -70 °C