

DNA purification and concentration by Ampure XP

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This protocol is written for the purification of DNA from a relatively clean sample. For example this would not be appropriate to use with a sample with high SDS concentration. As a general rule, only use this protocol for the purification from enzymatic reaction like ligations or PCR.

MATERIALS

Buffers and Solutions:

5M NaCl (500ml)

146.1075g	NaCl	5M
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Bring up to 500ml.

2M MgCl₂

19g	MgCl ₂	2M MgCl
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Bring up to 100ml. Autoclave 20 min at 121 C.

40% PEG 8000 Stock Solution (50ml)

20g	PEG 8000	40%
1ml	2M MgCl ₂	40mM

Bring to 50ml, .22µm filter and store in fridge.

1M Tris pH 8.5 Stock Solution (50ml)

4.35g	Tris Base(121.14g/m)	0.7177M
2.22g	Tris HCl(157.6g/m)	0.2823M

Bring up to 50ml. Check that pH is 8.5. Store in fridge for up to 6 months.

Ampure XP (Beckman cat. A63880)

70% EtOH

TE (10ml)

100ul	1M Tris pH 8	10mM
200ul	500mM EDTA	10mM

Bring up to 10ml. Use day of.

EB (10ml)

100ul	1M Tris pH 8.5	10mM
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Bring up to 10ml. Use day of.

Procedure:

1. Add 1.8 µl Ampure per 1 µl DNA solution.

2. Mix solution with pipette and let solution incubate for 5 min.
3. Collect the beads with magnet, allow 4 min for collection. (Leave the samples on the magnet for the next 2 steps. Remove sample from magnet for drying step.)
4. Aspirate supernatant and wash with 70% EtOH 2X (1 min incubation)
5. Aspirate EtOH and dry in thermomixer (60C for 10 min at 1200 RPM [ependorf thermomixer])
6. Add 40 μ l TE or reagent grade water and mix with pipette. Incubate in thermomixer(60C for 10 min at 0 RPM). Change volume as needed.
7. Collect with magnet. (5 min)
8. Transfer supernatant to new tube.