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. PEG solution has been added to make large volume samples feasible. Lower PEG concentrations will exclude small nucleotides from precipitation.

MATERIALS

Buffers and Solutions:

5M NaCl (500ml)

146.1075g	NaCl	5M

Bring up to 500ml.

2M $MgCl_2$ (100ml)

19g	$MgCl_2$	2M MgCl

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

Bring up to 10ml. Use day of.

Procedure:

1. Add 0.5 µl Ampure, 0.225µl PEG (0.2 for 8%), and 1/3 µl 5M NaCl per 1 µl DNA solution.
    a. Never add more than 10ul Ampure beads unless solution has a high concentration of DNA.

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 2X by adding 1ml 70% EtOH, incubating for 1 minute, and then aspirating the solution.

5. Dry sample in vacufuge at room remprature for 5 minutes. If liquid remains after 5 minutes repeat vacufuge.

6. Add 40 µl TE or reagent grade water and mix with pipette. Incubate in thermomixer(37C for 10 min at 1200 RPM). Change volume as needed.

7. Collect with magnet. (5 min)

8. Transfer supernatant to new tube.