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Last modified 4/1/08

DNA Clean-up Protocol for Crude Lysates

One might use a variety of methods for cleaning up crude lysates, including simple precipitation, passing through a size-exclusion column, &/or passing through a DNA-affinity column. I tried a combined approach of an affinity column first and then a size-exclusion columns. The order of these two steps was simplest because it also allowed final concentration of the DNA.

The DNA affinity columns used were Qiagen DNeasy columns, and the size-exclusions columns were Millipore Microcon columns.

I spoke with Qiagen tech support and the standard DNA extraction protocol is NOT appropriate for crude lysates – it results in very high loss from pre-extracted DNAs. They recommend instead using the *QIAamp DNA Micro Protocol for Genomic DNA Clean-up*, (from the DNeasy 96 kit), and scaling up – the buffer and column material are identical to those used in the standard single-column DNeasy kit.

Side notes about using DNeasy columns:
- ***yields from DNeasy columns rarely exceed 60%.***
- the max. recommended binding capacity is 50ug.
- beware using Ambion water in this protocol, as it's acidic and pH really matters for column binding.
- make sure all buffers, etc., are used at RT.
- **the protocol below can be used with single DNeasy columns or in high-throughput with the DNeasy 96 kit, followed by the ExcelaPure 96-well vacuum-based size-exclusion plate.**

Protocol used:
1. Make all volumes of DNA up to 200ul (in e.g. water).
   Add 20ul Buffer AW1
   Add 500ul Buffer AW2 and mix by pulse-vortexing 10 sec.

2. Pipette onto middle of DNeasy columns without touching
   Spin 6000 x g for 1"

3. Transfer column to new collection tube
   Add 600ul Buffer AW2
   Spin 6000 x g for 1"

4. Transfer column to new collection tube
   Spin 20000 x g for 3" to dry the membrane

5. Place column in new collection tube

Apply buffer AE to center of membrane, add 200ul let sit at RT for 1", then spin max speed for 5".

You can repeat the elution with two more 200ul volumes to increase the yield significantly.

6. To concentrate and further clean eluted DNA, pass through Microcon column, e.g. Microcon-100, nucleotide cutoff of 125-nt for dsDNA, when spun 500 x g. Wash e.g. once with 600ul TE, concentrate to desired volume.

Usage of this protocol:

1. Initial use was on **phenol-extracted Prochlorococcus DNA** from a variety of strains.

DNA was quantified by Picogreen and Nanodrop, before and after each step. Overall, yields were higher using both methods in samples that had more DNA to start with.

Yields through the DNeasy step: By Picogreen, yields ranged from 65% to 395%, with the higher total DNA in (30ug) generally showing yields just over 100%, while the lowest DNA in (100 – 200ng) showed the largest yields of 250 – 400%.

By Nanodrop the yields ranged from 5% for the lowest DNA in to 50 – 120% for the larger amounts of DNA in.

Sample Loss through DNeasy step

	Total in by Picogreen, (ug)	Total Out by Picogreen	**% yield by Picogreen**	ng/ul by Nanodrop, before	volume in	Total in by Nanodrop (ug)	Total Out by Nanodrop	**% yield, by Nanodrop**
SS120	30	41	**137%**	1688.7	26.98	46	30	**65%**
9312	30	31	**104%**	863.56	58.37	50	25	**49%**
MED4	30	39	**130%**	530.64	70.42	37	35	**95%**
9211	30	38	**125%**	362.39	95.54	35	31	**88%**
NATL2A	30	20	**65%**	1613.86	24.41	39	19	**49%**
9313	30	32	**107%**	724.77	44.31	32	27	**84%**
9515	15	47	**311%**	243.28	178.57	43	51	**118%**
new 9211	0.2	1	**395%**	54	100	5	1	**18%**
Syn	0.1	0.2	**248%**	163.4	100	16	1	**5%**

Yields through the Microcon step: Through this second step only, Picogreen-based yields ranged from 63% to 102%, and Nanodrop-based yields ranged from 34% to 89%.

Sample Loss through Microcon step

	Total in by Picogreen, ug	Total Out by Picogreen	% yield	Total In by Nanodrop	Total Out by Nanodrop	% yield
SS120	41	26	63%	30	26	89%
9312	31	28	89%	25	20	82%
MED4	39	33	84%	35	31	87%
9211	38	30	80%	31	27	89%
NATL2A	20	19	98%	19	16	85%
9313	32	33	102%	27	24	89%
9515	47	34	74%	51	40	78%
new 9211	0.8	0.8	101%	0.9	0.5	54%
Syn	0.2	0.2	96%	0.7	0.3	34%

For the Microcon step, there was fairly good agreement in % yields between the two quantification methods, except for at the lowest end of the [DNA].

Yield of overall two-step process: Yields ranged from 64% to 400% by Picogreen, and 2% to 91% by Nanodrop.

Sample Loss through 2-step clean-up process

	Total in by Picogreen, ug	Total Out by Picogreen	% yield	Total In by Nanodrop	Total Out by Nanodrop	% yield
SS120	30	26	86%	46	26	58%
9312	30	28	93%	50	20	40%
MED4	30	33	110%	37	31	83%
9211	30	30	100%	35	27	79%
NATL2A	30	19	64%	39	16	42%
9313	30	33	109%	32	24	74%
9515	15	34	229%	43	40	91%
new 9211	0.2	0.8	399%	5	0.5	9%
Syn	0.1	0.2	238%	16	0.3	2%

2. This protocol was also used to clean up **eight environmental samples, crude lysates from Monterey Bay** from 1997 which had been showing inhibitation of amplification.

There are only Nanodrop-based yield-estimates for these, which ranged from <1% to 27%. Inhibition of amplification was removed.

3/15/06 Env DNA clean-up, loss estimates:

	Before				After				
Sample ID	**ng/ul**	**260/ 280**	**volume (ul)**	**total DNA IN (ng)**	**ng/ul**	**260/ 280**	**volume (ul)**	**total DNA OUT (ng)**	**% recovery**
JD274_97_M1_0m	276.31	1.74	45	12434	40.2	2.08	83	3336.6	27%
JD274_97_M1_20m	192.19	1.66	45	8648.55	19.5	1.88	52	1014	12%
JD274_97_M1_100m	136.51	1.59	42	5733.42	9.88	2.04	47	464.36	8%
JD274_97_M1_150m	788.89	1.68	61	48122.3	4.95	3.99	30	148.5	0%
JD295_97_M1_0m	234.77	1.67	35	8216.95	15.81	2.09	66	1043.46	13%
JD295_97_M1_20m	90.5	1.61	21	1900.5	7.51	2.23	31	232.81	12%
JD295_97_M1_40m	99.43	1.59	45	4474.35	6.03	2.49	47	283.41	6%
JD295_97_M1_100m	69.94	1.6	57	3986.58	6.56	1.71	46	301.76	8%
JD295_97_M1_200m	153.15	1.6	39	5972.85	14.08	2.11	35	492.8	8%

3. The 96-well version of this two-step approach is what was used by Tracy Mincer, to clean up environmental crude lysates for Q-PCR in his 2007 EM paper. **Excerpt from methods of Mincer *et al*., 2007. EM:** "Subsequent clean-up of crude nucleic acid preparations was performed using a modification of the DNA extraction protocol supplied with the 96 well DNeasy columns from Qiagen (Chatsworth, CA) as follows: crude nucleic acids (~5–10 μg) brought up to 200 μl volume in water were added to 20 μl Buffer AW1 and 500 μl Buffer AW2, mixed completely and loaded onto the DNeasy columns. The 96 well columns were spun in a swinging bucket rotor at 6000 g for 10 min, put in new collection tubes, washed with another 600 μl Buffer AW2 and spun again at 6000 g for 10 min. The washed columns were dried at 70°C for 15 min, put in new collection tubes and nucleic acids eluted three times with 200 μl aliquots of Buffer AE, spinning after each addition of buffer for 2 min at 6000 g. DNA eluates were concentrated using a 96 well size-exclusion-based ExcelaPure 96 well UF plate vacuum filtration system from EdgeBioSystems (Gaithersburg, MD). DNA was filtered to dryness, resuspended in 200 μl dilute TE buffer (1 mM Tris pH 8, 0.1 mM EDTA), quantified using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE) and stored at -20°C."