

# cDNA PCR Amplification and Clone Preparation (V1.0.1)

cDNA clone inserts are amplified using the following protocol:

1. Human clones (Reaserch Genetics) are inoculated into tubes containing 3 ml of LB/Ampicillin (50 µg/ml) and incubated for 16 hours at 37°C and 200 rpm in a shaking incubator. In addition, three separate 75 µl aliquots are archived for future use in microtiter plates containing 25 µl of 50% glycerol at -80°C. Mouse clones (NIA 15K set) are inoculated into tubes containing 3 ml of 2X YT/Ampicillin.
2. Following overnight growth, clones are prepped using Autogen Plasmid Isolation system.
3. M13 primers have been designed to allow amplification of clone inserts from various vectors encountered in the cDNA library. The universal amplification primers are:
  - M13 F: CTGCAAGGCGATTAAGTTGGGTAAC
  - M13 R: GTGAGCGGATAACAATTCACACAGGAAACAGC
4. cDNA clone inserts are amplified in 75 µl PCR reactions in 96 well reaction plates. A reaction master mix is prepared for each plate:

	Volume	Final Concentration
ddH <sub>2</sub> O	5.9 ml	/
10X PCR buffer	750 µl	1X
50 mM MgCl <sub>2</sub>	375 µl	2.5 mM
M13 Forward primer (10 µM)	150 µl	0.2 µM
M13 Reverse primer (10 µM)	150 µl	0.2 µM
dNTPs (15 µl per 100mM dNTP)	60 µl	0.2 mM
Platinum Taq (5 U/µl)	26 µl	1.3 u
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Total volume:	7.4 ml	

5. For each clone, add 74 µl of master mix to 96 well plate. DNA is stamped into plate using 96 pin tool, delivering 1 µl of prepped Autogen DNA.

6. Reactions are amplified in a MJ Research PTC225 thermal cycler using the following cycling protocol (File# 52-30 in thermocycler):
  1. 95°C X 3min
  2. 95°C X 30sec, 52°C X 30sec, 72°C X 2min (30 cycles)
  3. 4°C forever
7. 5 µl of 1:5 diluted PCR products are separated and visualized on a 1% agarose gel. A 250 bp ladder is loaded and used as a size standard for estimating product size. Gel images are stored electronically, and product sizes are estimated by the AlphaEase software.
8. PCR reaction plates are stored at 4°C until purification. **Note: DO NOT freeze PCR products.**