

Protocols: PCR amplification and visualization on agarose gels.

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Each group will have dilutions of DNA extracted from 46-48 pools from the LA 407 TAC library. We will need to discuss appropriate controls. Using these pools we expect a 92.25% chance that any given clone will be present in the library at least once. The expected average representation of a given gene will be 3 times.

Genome size of tomato 950 Mb
Average clone size for the library 29.5 Kb
Sub-library I ~1000 clones per pool
Sub-library II ~2,700 clones per pool

Procedures:

Make up PCR mix leaving out the DNA. Add 45 ul of the mix to tubes; then, using fresh pipette tips, add DNA from the 48 pools to individual reaction tubes. Cover each reaction with a drop of mineral oil and amplify.

Mix for 1 50 ul reaction:

Scale up for ____ PCR reactions

water	33.6
DNA	5.0 (1 ng/ul)*
buffer	5.0
dNTP	2.0
Forward Primer	2.0
Reverse Primer	2.0
Taq polymerase	0.4

Amplification protocol for primers:

step 1 94 C for 2 min
step 2 94 C for 1 min
step 3 ___ C for 1 min (55 C-62 C depending on primers)
step 4 72 C for 2 min
step 5 go to "step 2" (30-40 X)
step 6 72 C for 5 min
step 7 4 C for 00 00 00
step 8 end

Agarose gels

Procedure:

Dilute 10X TBE stock 1/10 with dd water (working solution = 0.089 M Tris base, 0.089 M boric acid, 0.002 M EDTA)

Add 1 X buffer to boiling flask

Weigh agarose and add to buffer (1 gm/100 ml = 1% solution; agarose gels range from 0.7% - 4.0% depending on application and size range for separation)

Boil in a microwave until agarose is completely dissolved

Let cool while gently shaking or stirring. Agarose gels can be pored when 65 C.

Gels are run from the (-) electrode to the (+) electrode. Remember that DNA has a (-) charge.

Visualize the DNA by staining in dilute EtBr (10-20 ul / 250 ml water or 1x TBE) and placing gel on a UV transilluminator.

Reagents:**dNTP stock:**

5 mM dNTP (1.25 mM ea)

988 ul water

13 ul each of four 100 mM dNTPs (Pharmacia Ultrapure set #27-2035-01)

PCR buffer (10X stock):

100 mM Tris pH 8.3

500 mM KCl

15 mM MgCl₂

0.01% w/v gelatin

Primers:

Stocks should be 25X (25 uM)

Chromosome	Marker	Primer	Annealing Temp (°C)	Cycles
2	TG091	F 5'TGCAGAGCTGTAATATTTAGAC3' R 5'CFFTCTCAGTTGCAACTCAA3'	60	36
5	CD031	F 5'ATCTCGGGATCATGGTTGAC3' R 5'ATFFCCAFAGAAATTCCAAA3'	57	33

T1/10E Stock:

10 mM Tris (7.5-8.0)

0.1 mM EDTA

This stock contains 1/10 the amount of EDTA found in "TE" and is used for diluting DNA templates for use in PCR. Water can be used to dilute templates that will not be stored for any length of time.

10X stock TBE electrophoresis buffer

108 g Tris Base

55 g boric acid

7.44 g EDTA (or 40 ml 0.5 M EDTA pH 8.0)

dissolve in dd water and adjust to 1 liter

Gibco BRL ultraPure Agarose #15510-027

Ethidium bromide (EtBr) Stock

10 mg/ml