High throughput screening of *Toxoplasma gondii* clones by PCR after limitation cloning

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Toxoplasma gondii is a kind of obligate intracellular parasites that are capable of infecting virtually all warm-blooded animals. It is one of the most common parasites in human. Serological studies estimated that up to a third of the global population has been chronically infected with the parsites. *Toxoplasma gondii* is also used as a model orgnisim of Apicomplexans that includes *Plasmodium* – the parasites that cause malaria. Gene knockout is a very important way to study gene function in all organisms. When it comes to Toxoplasma, it's very difficult as this haploid parasite has strong adaptability to circumvent the condition of gene defects by gene duplication. High throughput screening at very early stage of transfection is very important for generating a true knockout of this parasite. For some genes that are required for*Toxoplasma gondii*, the high throughput screening is necessary. Different labs use different protocols. After reading and practicing protocols from different papers or different labs, here I established an efficient pipeline for this purpose.

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High throughput clone screening after limitation cloning

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For some genes that are required for *Toxoplasma gondii*, the high throughput screening of clones after gene modification is necessary. Different labs use different protocols. After reading protocols from different papers or different labs, I established an efficient pipeline for this purpose.

I suggest that at least four 96-well plates should be used to clone parasites from four T25 flasks in order to get at least two independent clones at limitation cloning step.

Preparations before screening

Prepare sufficient 24-well plates confluent with HFF or Hter cells, eg. 8 plates.

Make PCR lysis buffer: eg. For 100 ul buffer:

10 ul	10x PCR buffer or equivalent
0.5 ul	1% SDS solution
1 ul	20mg/ml Proteinase K
88.5 ul	H_2O

Sample preparation protocol:

1) Check plaques in the 96-wells plates, and mark the wells with single plaques.
2) Change the media of 24-well plaques.
3) Scrape circularly as well a pipet up and down of the marked wells in 96-well plates
4) Transfer all the media from the well in 96-well plate to a well of 24-well plate. Do until sufficient clones are picked. Bubbles are good for preventing mistakes. No label is necessary in this step.
5) Put 24-well plates in incubator for parasites growing.
6) Check the plates routinely to find out a time point when monolayers of most wells are about 50%-90% lysed.
7) Take out new confluent 24-well plates, and aspirate all the media (NOT replacing media).
8) Transfer ALL supernatant of the infected 24-well plates to the new plates well by well carefully trying not to disturb the monolayer. Please do remember to label plates properly.
9) Put the new plates back to incubator.
10) Apply 100 ul PCR lysis buffer to each well the infected plates shake back and forth.
11) Fill the space among wells with water to keep humidity in the next steps
12) Wrap the plates tightly and incubate at 50C O/N. Additional water in last step is critical, or your PCR lysis buffer will dry out very quickly.
13) Add 400ul of dH_2O to each well, pipet up and down to mix, and take 150 ul to a 96-well PCR plate. Use one 200-ul tip for each sample should be fine.
14) Cap the PCR plates tightly and inactivate the proteinase K at 89C for 30min or 95 for 10 min.
15) Spin the PCR plates for 5 min using eppendorf centrifuge 5810 Model.
16) Place the PCR plates on ice for use, or store them in 4C.

PCR Protocol:

- For each reaction:
  10 ul 2xGoTaq Mix,
  1ul of each 10mM primer,
  adjust water to 15ul.
  This should be scaled to the volume you need for all your reactions.
- Aliquot the PCR mix to each well of a new 96-well PCR plate. I suggest you do this well by well if you don't have more than two plates of samples.
- Add 5ul of each sample to the PCR plates prepared in last step by using multi-channel pipets. It's important to use multi-channel pipet, which greatly reduces the likelihood of making mistakes.
- Run your PCR according to the protocol of reagent manufacture.
- Check your PCR by running agarose gels, and inoculate your positive clones you need to T25.

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