

Schistosoma RNAi

Jim Collins 12/07/2015

PCR reagents/reaction

0.5 µL pJC53.2-based plasmid
2.5 µL 10x PCR Buffer
0.75 µL 50mM MgCl₂
0.5 µL dNTP's
0.2 µL 100 µM EX_highmelt_T7 primer
0.25 µL Platinum Taq
to 25 ul with H₂O.

PCR cycling conditions

Step 1: 4 min @ 95
Step 2: 30 sec @95
Step 3: 30 sec @ 55
Step 4: 1 min @ 72
Step 5: Go to 2 39 times
Hold at 4°C

Run on 1% agarose gel to confirm PCR was successful.

High yield *In vitro* Transcription

Reagents/Reaction

5 uL of PCR product
10uL of 10x High-Yield transcription buffer
20uL of 25mM rNTPs (ea)
5uL of T7 RNA polymerase (homebrew)
1uL of IPP (inorganic pyrophosphatase 100U/mL, NEB)
To 100ul with RNase-free water

Incubate at 37°C for 4hrs to overnight. Sample should become viscous as the RNA is synthesized.
Treat with 5ul DNase for 30min at 37°C.
Precipitate at RT for 10min by adding 50ul 7.5M ammonium acetate and 750ul of EtOH
Spin 10,000g for 5 min
Aspirate EtOH and allow to dry for ~1-2 minutes
Resuspend pellet in 300ul RNase-Free water
Anneal dsRNA by successive 3min incubations at 95°C, 75°C, and 55°C. Allow to cool at RT for 5 min.
Dilute 1:10 and determine concentration on Denovix. Expect ~0.5mg of dsRNA per 100ul prep.
Run remainder of sample dilution on 1% agarose gel.
Dilute dsRNA sample to 1 µg/µL with DEPC water

RNAi in *S. mansoni*

Euthanize mice with pentobarbital plus heparin. Perfuse parasites from mice using 37°C DMEM + 5%FBS and collecting parasites in 15cm sterile Petri dishes. In my experience perfusion with DMEM and serum keeps parasites happier than sodium citrate-based perfusion solution.
In hood, pool parasites in 50ml conical tubes and allow them to settle. Replace bloody media with fresh DMEM until blood is removed.
Replace DMEM with warmed Basch Media 169.
Put 5-10 healthy male/female worm pairs in 3 ml of media in a 6cm Petri dish. This is day 0.
Add dsRNA to a final concentration of 20-30ug/ml.
Replace media and dsRNA on days 1 and 2.
Change media every other day (5-6 ml) and provide new dsRNA every 5-8 days.

10x High-Yield transcription buffer

0.4M TRIS pH 8.0
0.1M MgCl₂
20mM Spermidine
0.1M DTT