

PCR Supplies used by Dr. Kerry Kriger.

savethefrogs.com/chytrid

Supplies I keep in the 2 crates for PCR lab use:

- Foam Cooler for ice, and a hot water heater with baking pan for boiling water
- PCR plates
- V-bottom plates
- Vortexer (IKA LabDancer)
- Bunsen burner/23 gauge needles/4 needle holders, wire cutters, lighter
- Ziploc bags
- PCR plate holder (this keeps the bottom clean so the PCR's laser can shine through)
- DEPC H₂O (this is essentially very clean water. DNA/RNA free)
- A credit card or id card for sealing plates
- 4 floating microtube holders
- Beads and little plastic dishes to hold beads in
- Calculator
- 1.5 mL and 2.0mL microtubes
- A vbottom and a PCR plate that are half full and sealed, to use as balances in centrifuge
- Permanent markers (big one and a fine tip one)
- PrepMan Ultra
- Pipetman P20 (does 2 to 20 mikes), P200 (does 20 to 200 mikes) and P1000 pipettes
- 20 mike, 200mike and 1000 mike barrier pipette tips
- Vbottom and PCR plate sealer film (for covering them)
- A few 96-microtube holders

Supplies I keep in the freezer:

- IPC
- ITS
- MGB
- 5.8S
- Standards
- QPCR Rox mix. (Keep in the refrigerator once it's thawed).

Supplies I keep in the box in lab:

- TE buffer (don't need in Panama)
- Qiagen dneasy plant mini kit (don't need in Panama)
- MoBio PowerClean (don't need in Panama)

Random other supplies:

- A large centrifuge that can hold PCR plates

Supplies and Suppliers

Note: All prices in AU\$ unless otherwise noted. While virtually everything I use for PCR is on this list, I may not use every product on this list.

--1.5 mL centrifuge tubes: Quality Scientific Plastics Cat # 509-GRD-Q get from stores at \$13.44 per 500 (that's 2.6 cents each)

(originally used Treff 96.7246.9.01 \$38/1000 from Medos Company ph: 03 9562 6888 info@medos.com.au www.medos.com.au) depending on the beadbeater. And tube racks! Cat# T96.3793.9.01 \$18 holds 40 1.5mL tubes have 3 in stock shipping \$19.50)

- **Standards from Donna (5mikes of her stuff is 1000zoospores) free.**
- **Swabs: Biomerieux 02 8852 4700 or 1800 333 421 fine tip MW100 swabs \$46.33/100 excluding GST fax: 02 9899 1232 Gary: 07 3902 6416 "Special Price, please refer to Philippe." \$Phillipe gives me a price of \$43/box if I get 10 boxes.**
- 2x Taqman Master Mix (Applied Biosystems - Catalogue #4304437) **user id: kerrykriger** \$650/5mL and should be stored at 2C-8C. They also sell it as a 10-pack for \$5,690.
Jenny McShane Molecular Biology Products
1800 801 644
Applied Biosystems
phone: 1800 801 644
Tel 07-3369-2589
Fax 07-3511-6588
<http://www.appliedbiosystems.com.au/>
- PrepMan Ultra (Applied Biosystems #4318930) store at room temp! \$210- 20mL (should do 400 samples I think=\$0.53 each) their current stock expire Jan25 2007.
- Probe (Applied Biosystems) **Takes 3 weeks! Order online**
ChytrMGB2 5' - 6FAM CGAGTCGAACAAAAT MGBNFQ - 3'
6,000picomL --\$520 (Kevin says we get it for \$457.60!) (cat.#4316034); (at \$457.60: enough for 274 samples = \$1.66 each)
20,000picomole \$830 (cat#:4316033); (enough for 1066 samples = \$0.91 each in triplicate, or about \$0.24 each on the first singlicate run, plus \$22/plate of re-analyses)
50,0000 picomole \$1,200 (cat#:4316032). (enough for 2666 samples = \$0.53 each)
- TaqMan Exogenous Internal Positive Control Reagents (VIC Probe) # 4308323 \$350 (delivery \$30)

- **PRIMERS:** (GeneWorks) username is 'kmk5g@yahoo.com'.
<http://www.geneworks.com.au/> 1800 882 555
 Scale: 40 nmoles
 Purity: Sequencing/PCR Scale of 40:
 Oligo name: ITS1-3 Chytr 5'- CCTTGATATAATACAGTGTGCCATATGTC-3' (\$24.65)
 and Oligo name: 5.8S Chytr 5'- AGCCAAGAGATCCGTTGTCAAA -3' (\$18.70)
 Each of these should last for roughly 375 triplicates.

Better is Scale of 200. Comes to about \$137 combined for both primers. You get about 5 times as much

- PowerClean DNA Clean-Up: 50 preps (catalogue no. MB-12877-50) and is \$240.00 (exc GST). give quotation #MB GU 28 Feb to receive a 5 % discount. \$20 shipping.
- DEPC water made by Ambion. Geneworks sells it: \$29 for 200mL cat#MB-17011-200
 - **Mini Beadbeater 8 (Biospec Products #3110BXEUR) and 0.5mm zirconia/silica beads (Biospec Products #11079105z) US\$38**
 if from USA, advise shipper to put on customs declaration clearly stating that it is a purchase for research purposes at a university (to avoid duty). USD\$1,450 + \$242 UPS (plus 10% GST) (this shipping price is with the beads inside as well)
 - Finnpiquette multistep pipettor 200mikes if possible and a multichannel pipettor that does 5mike volumes if possible (but not necessary).
 - I got a 20 mike (P20), 200mike (P200), and 1mL (P1000) Gilson Pipetman Pipettors from stores for about AU\$300 each)
- **96 well V-bottom plate Linbro/Titertek Cat # 76-321-05 from MP Biomedicals**
www.mpbio.com.au
 in boxes of 100 non-sterile 02 9838 7422
 We are based in Sydney.
 The price for a box of 100 plates is \$189.10 Delivery is \$20, prices are gst exclusive. We have 6 boxes in the Sydney warehouse.
 Our fax number is 02 9838 7390. Pam Sadler pisadler@mpbio.com
 ph 02 9838 7422
- **Linbro MicroPlate Sealer with Adhesive Back #76-402-05**
 (for sealing v bottom plates if necessary)
 Pam Sadler
pisadler@mpbio.com
 MP Biomedicals
 PO Box 187
 Seven Hills NSW 1730
 \$60.05 AUD
- floating trays to hold the eppendorfs: VWR in New Jersey sells them for US\$19.85 1-800-932-5000 www.vwrsp.com
 catalogue #18876-0000 (If I need it in Australia, Crown Scientific, catalogue #18876-0000.

<http://www.crownsoci.com.au/> 1300 727 696 from US so takes 6 weeks 4-pack is \$65.88 www.bel-art.com makes them) **I Ordered from VWR!**

tech@integratedsciences.com.au 02 9417 7866 I think

- 25 Thermofast 96 PCR detection plates (\$158 Integrated Sciences AB-1100 ph:1800 252 204) <http://www.integratedsci.com.au/> order 5-9packs:\$148
 - 100 Adhesive PCR film (seals for PCR plate) (\$185 Integrated Sciences AB-0558)
 - ABSolute QPCR ROX 500nM Mix: made by Abgene. Sold by Integrated Sciences <http://www.abgene.com/productDetails.asp?prodID=30>
 - AB-1138a: 2.5mL (comes in 2 1.25mL vials) is \$229 til end of year.
 - AB-1138b: 20mL (comes in 16 1.25mL vials) is \$1691 til end of year
 - AB-1139a: 5mL (comes in a 5mL vial) is \$420 **quote CS372 to get that price!**
- Storage: it arrives frozen. Keep it frozen until use, then just refrigerate the bottle in use (i.e. once thawed, do not re-freeze!). Lasts a year in the freezer, a month in the frig) \$25 shipping + \$15 for cooler.

- Inoculating Needle Holder: part # 2420-150 shipping will be \$10. Company is Met -App Pty Ltd 29 Stafford St Huntingdale, VIC 3166 \$19.20 each *I got 4 of them.* 03 95626582 bsb 063209 acct 10037139 acct name: met app pty ltd commonwealth bank metapp@bigpond.com.au amy

- 22 or 23 gauge needles I get from stores.

- Kevin charges me \$30/plate for PCR machine use.

- Pipette tips are made by Neptune and are Barrier Tips. I believe stores has them:

BT1000	Barrier Tips, Aerosol 100-1000ul (8/pk)	\$77.00
BT20	Barrier Tips, Aerosol 2-20ul (10/pk)	\$93.50
BT200	Barrier Tips, Aerosol 50-200ul (10/pk)	\$93.50

They come out to 9.7 cents per tip.

- Edwards Instruments Qik Spin \$340 (usually 365) + \$20shipping (mini-microfuge) 1800 024 407.
- IKA lab dancer \$199 + \$15 (vortexer)
- **Qiagen DNeasy Plant mini kit will theoretically extract and purify DNA:** packet of 50 costs \$285. #69104 +\$35 shipping. packet of 250 costs \$1265 #69106. Ordering: 1-800-243-800 Still beadbeat it when I add the lysis solution.

Possible total costs per sample for triplicates (IPC is not included in these calculation! May add a dollar or two per sample):

- Labor (96 samples per 24hrs (4samples per hour) *\$20/hr) = \$5/sample
- Tubes \$0.10
- Taqman master: \$5.80
- Prepman Ultra: \$0.53
- Probe \$0.91
- Primers \$0.10
- DEPC water: \$0.10
- Pipette tips: ~\$0.60 how many tips does it take ???
- Plates/sealers: \$0.60
- PCR machine fee: \$1.25
- Totals...~**\$10/sample** (plus \$5labor = \$15 per sample)

To run a plate in triplicate costs:

- ~\$240 (24 samples per plate.)

Possible total costs per sample for a singlicate plate of 93 samples:

- Tubes \$0.10
- Taqman master: \$1.50 (with ABgene: \$1.00)
- Prepman Ultra: \$0.51
- Probe \$0.24
- Primers \$0.03
- DEPC water: \$0.08
- Pipette tips: ~\$0.50 how many tips does it take ???
- Plates/sealers: \$0.20
- PCR machine fee: \$0.33
- Totals...~**\$3.49/sample** (with ABgene: \$2.99/sample)

To run a plate in singlicate costs:

- ~\$325 (93 samples per plate.) (\$278 with ABGene)

Possible total costs per sample for the triplicate “follow-up plate” plate of 24 samples:

- Tubes \$0.04
- Taqman master: \$5.80 (with ABgene: 3.88))
- Prepman Ultra: \$0.00
- Probe \$0.91
- Primers \$0.10
- DEPC water: \$0.03
- Pipette tips: ~\$0.50 how many tips does it take ???
- Plates/sealers: \$0.50
- PCR machine fee: \$1.25
- Totals...~**\$9.13/sample** (with ABgene: costs \$7.21)

To run a followup plate in triplicate costs:

- ~\$219 (24 samples per plate.) (\$173 with ABGene)

If 25.8% of singlicates come out positive, you will then have 24 samples for which you must run triplicates, which at \$9.13 each is \$219 dollars more...\$325 plus \$219 is **\$544** total to run the 93 samples (**\$5.89/sample**). If you had run triplicates of the 93 samples from the start, it would have cost \$930! (And taken 4 hrs longer or so.)