PrimeSTAR PCR

pcr
scan for the full protocol

PROTOCOL

1. For each reaction you set up, **top-label a PCR tube** with the name(s) indicated on the labsheet.
2. Retrieve your **oligos** (10 µM) and **template** from the freezer and thaw them at room temperature.
3. Set up the reaction, in order:

32	ddH_2O
10	5× PrimeSTAR GXL buffer
4	dNTP mix (2.5 mM each)
1	primer 1 (10 µM)
1	primer 2 (10 µM)
1	template
1	PrimeSTAR GXL polymerase
50	**µL total**

4. Retrieve the **enzyme cooler**, and add **1 µL polymerase** to each sample, last.
5. **Cap**, **slam on the bench** to mix, **quick spin**.
6. Run the program from your labsheet — it depends on insert length and annealing temperature.

MASTER MIX — 4 OR MORE REACTIONS

Scale **everything except the template** by the number of reactions, plus **10%** so you do not run short on the last tube.

Each tube is then **49 µL master mix + 1 µL template**, and the template is the only thing you pipette per-tube.

Analytical Gel

gel
scan for the full protocol

PROTOCOL

1. For each sample, **top-label a PCR tube** with the name indicated on the labsheet.
2. Set up each sample, in order:
   - **8** loading dye (tube marked **LOAD**)
   - **3** PCR product
   - **LOAD has to be fresh — made within 2 days.** The stain does not keep. If it is older, make a new aliquot: **100 µL blue juice + 1 µL dye** in an Eppendorf tube, labelled **LOAD**.
   - **Marker, one per section:** 8 µL loading dye into a tube of marker (yellow).
3. **Mix, quick spin.**
4. Cut a **1% gel slab** with enough wells for all samples, the marker, and a few spare.
5. Place it in the rig, fill with **1× TAE** to just cover it, and brace it with the plastic wedge.
6. Load **9 µL** per well with a P20, in labsheet order.
7. Lid and leads on: **run to red** — load at the black end.
8. Run at **175 V** for about **10 min**. **Do not set a timer and walk away** — check every minute or so and stop when the blue front is **2/3–3/4 down the gel**.
9. Image it on the imager **through the orange filter**, with a label strip above the gel. Name the file by date and time, and upload it.

Zymo Cleanup

zymo
scan for the full protocol

PROTOCOL

1. **Side-label one Zymo column per sample** with an **ethanol-resistant pen** — **not a Sharpie**. Put each column in a collection tube in a rack.
2. Pipette **180 µL ADB** into the column.
3. Transfer all **~50 µL** of the reaction into the ADB **in the column**, and pipette up and down to mix.
   - **Alternatively**, premix ADB and sample in an Eppendorf tube, vortex, spin, then transfer to the column. Several ways work — what matters is that it ends up **well mixed and in the column**.
4. **Spin 15 s** at full speed. Discard the flow-through.
5. Add **200 µL PE**. **Spin 15 s.** Discard the flow-through.
6. Add **200 µL PE**. **Spin 15 s.** Discard the flow-through.
7. **Spin 90 s** to dry the column. PE is 70% ethanol and carryover inhibits downstream enzymes.
8. **While the drying spin runs, clean up.** Check the bench for drips of salt or ADB. Use **70% ethanol** if you suspect any.
9. **Top- and side-label new 1.5 mL Eppendorf tubes**, one per column, as indicated on your labsheet.
10. Insert the **dry column** into its elution tube.
11. Add **25 µL EB** slowly to the **centre of the membrane**. Do not let it run down the walls.
12. **Spin 45 s** to elute.
13. **Discard the column.** The DNA is in the tube.

Golden Gate Assembly

goldengate
scan for the full protocol

PROTOCOL

1. For each reaction you set up, **top-label a PCR tube** with the name(s) indicated on the labsheet.
2. Retrieve **water** (white **W** tubes) and **T4 ligase buffer** aliquots (**red** tubes) from the **enzyme freezer** and thaw them at room temperature.
3. Set up the reaction, in order:

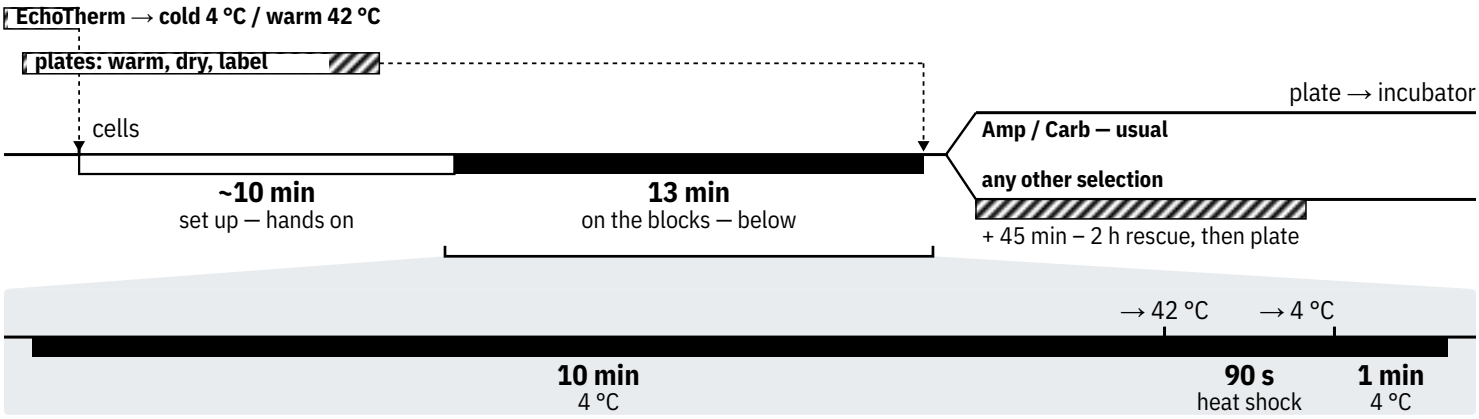
6	water
1	10× T4 DNA ligase buffer
2	DNA
0.5	T4 DNA ligase
0.5	restriction enzyme
10	**µL total**

   – If you have **more than 2 fragments** to join, premix **equal volumes of each DNA** in a tube, then use **2 µL of that mix** for the reaction.
   – Be sure you are using the **right one** of BsaI, BsmBI, BseRI, AarI, SapI, BbsI, as indicated in your **construction file and labsheets**.
4. Retrieve the **enzyme cooler**, and add **0.5 µL each** of the ligase and the restriction enzyme to each sample.
5. **Cap, mix, spin.**
6. Run the **GG1** program on the thermocycler.

KCM Heat-Shock Transformation

transformation
scan for the full protocol

TIMING



PROTOCOL

1. **Plates in the incubator first** — they are the slow thing. Check they carry the antibiotic(s) on your labsheet. Cold plates carry condensation and **you cannot write on a wet plate**.
2. Turn on the **EchoTherm**: **cold block 4 °C**, **warm block 42 °C**. Leave both there throughout.
3. Once warm and dry, **label plate bottoms**: date, initials, strain, plasmid, selection.
4. Put a **100 µL** cell aliquot and the DNA tube on the **cold block**. Thaw ~30 s, add **25 µL KCM** to the cells, pipette gently. One aliquot does **3 reactions**.
5. Add **40 µL** of the cell/KCM mix to the DNA. Mix gently.
   – Assumes DNA is ~20% of the total; **too much dilutes the salts**. Retransforming a miniprep: **0.5 µL** into 10 µL cells.
6. **Run the block sequence on the timeline above.**
7. **Rescue only if not Amp/Carb:** 200 µL 2YT, 1.5 mL tube, shake 37 °C **45 min – 2 h**. **Not less, not more.**
8. Plate everything, spreading with **beads**. Incubate **inverted** at 37 °C overnight, and **cancel the temperature programs**.

Picking Colonies

picking
scan for the full protocol

PROTOCOL

1. **Photograph the plates twice** — **blue-light transillumination** and **ambient light**. Save both; once you have picked, these are the only record.
2. **Tubes or a block.** Up to **4 cultures**: snap-cap tubes, each labelled with its clone ID. More than that: a **24-well block**, labels on the **airpore sheet** — the wells are identified by position.
3. Fill each tube or well with **4 mL 2YT + antibiotic** — the one on your labsheet. **It is not always carb.**
4. Pick **4 colonies from each plate**: touch a sterile toothpick to a single colony and drop it in. It stays there.
   - **Pick ones that are easy to pick and look healthy** — well separated, round and smooth. Big and well isolated is usually best. Contorted or smudgy means water seepage, or two colonies grown into one.
   - **Which colonies to choose is set by the experiment** — brightest, smallest, largest, red, or deliberately naive. **Your labsheet says which.**
   - **Into a block, fix the order before you start and write it down.** Wells go by position, so if your record and the block disagree the results are silently scrambled — nothing errors. Use your labsheet grid if it has one; **if not, draw one yourself** and keep it with the block. Tubes do not have this problem.
5. **Cover** — tubes: caps on loosely so they breathe; block: the airpore sheet — and grow in the **shaking incubator**, **16–36 h**. Fridge by **36 h** or they overgrow.
6. **Parafilm the plates**, store them **upside-down in the fridge** — you may need them again.

Qiagen Miniprep

miniprep
scan for the full protocol

PROTOCOL

1. For each clone, **top-label a 1.5 mL tube** with the clone ID from the labsheet. This is the tube you will elute into.
2. **Pellet 2 mL** of the 4 mL saturated culture — fill a 2 mL tube. Spin 1 min, pour off the supernatant — **bleach it** before it goes down the drain. The buffer volumes are the same for anything from 1 to 5 mL.
3. **Resuspend** in **250 µL P1**. No clumps. The RNase A must already be in the P1.
4. **Lyse** with **250 µL P2**. Mix gently — **do not vortex**. It goes clear and viscous.
5. **Neutralise** with **350 µL N3**. Invert thoroughly. A white precipitate forms.
6. **Spin 5 min** at max speed to pellet the debris.
7. Transfer the **supernatant** to a blue QIAprep column. Spin **15 s**.
8. Add **500 µL PB**, spin. Add **750 µL PE**, spin.
9. Discard the flow-through and **spin 90 s to dry**. PE is 70% ethanol and carryover ruins everything downstream.
10. Move the column to your labelled tube. Add **50 µL EB** to the **centre of the membrane** and spin **45 s**.

– Label **the top and the side** of each tube — a tube marked only on the lid goes anonymous the moment someone puts the lids down.
– **A clone that yields no DNA was probably a satellite colony.** Note it and exclude it.

Cycle Sequencing

sequencing
scan for the full protocol

PROTOCOL

1. For each read, **top-label a 1.5 mL tube** with **the exact name you put on the submission form**. **Not PCR tubes.** Not an abbreviation, and not a name that only makes sense to you — the facility matches tube to form by that string.
2. Set up each reaction:

3	G00101 sequencing oligo (2.66 µM)
10	miniprep DNA
13	**µL total**

   - Our sequencing oligos are standardised to **2.66 µM**, so **3 µL is exactly one reaction's worth**.
   - **Medium copy** — pBR322, pAC, and **pP6**: **10 µL** miniprep.
   - **High copy** — pUC: **4 µL** miniprep + 6 µL water.
   - **Low copy** — pSC101, BACs: **PCR the region first** and sequence the product.
3. Specify the **dGTP protocol** on the submission form. Standard chemistry **dies inside a hairpin** and will lose the read.
4. Submit. Results come back in **1–2 days**.

NOTES

- Pick a primer **upstream** of what you want to see — the read starts **20–50 bp downstream** and gives **400–1000 bp**.
- **Sequence everything you picked**, including the boring ones. The middle of the range is the informative part.

Tecan M Nano: Fluorescence + OD600

platereader
scan for the full protocol

PROTOCOL

1. **Fill the plate.** P200 multichannel, **100 µL** per well into a **black-walled, clear-bottom 96-well plate**. Each culture **2×**. Skip any culture that did not grow.
   – Tips all seated, pipette up and down to resuspend, no air gaps. **Volume sets the signal** — pipette accurately.
2. **Start up, in this order, from everything off:** the **computer** (PIN from a supervisor), then the **instrument** (toggle **on the back**), then **iControl**.
3. **Connect:** choose **Infinite 200 Pro**, OK. It always takes a while.
   – **Will not connect?** End iControl (Task Manager if it hangs), switch everything off, and start again **in the same order**.
4. **Default script.** Select only the **wells you filled**.
5. Add **Absorbance**: **600 nm**. Add **Fluorescence Intensity**: ex **483 nm**, em **525 nm**, gain **40**. **Leave everything else at its default.**
6. Open the tray (iControl or the corner button). Plate **in the slot**, **A1 top left**. Close the same way. **Never push or pull the door by hand.**
7. **Start.** Results open in **Excel**, absorbance and fluorescence on separate sheets. **File** → **Save As** to the desktop with a real name, then USB stick or email.
8. **Plate out first**, close the tray. Quit Excel; quit iControl — **yes** disconnect, **no** save script. Shut down the computer, **then** the instrument.
9. Bleach and rinse the plate.

> **Reads OVER?** Out of range — lower the gain and read again. Numbers weak — raise it.