Bench sheet · EnGeL-MOLB-EXOSAP

PCR product clean-up with ExoSAP and preparation of samples for Sanger sequencing · version 1.1.1 (2026-11-20). Full protocol: EnGeL-MOLB-EXOSAP. Tick each step when done; write deviations in Notes.

Date	
Operator	
Plate ID / samples	
ExoSAP reagent lot	
Sequencing order number	

Before starting

☐	No.	Step
☐	•	Clean the working space and material with bleach and water and leave the UV light on for at least 15 min.
☐	•	Use filter tips at all steps.
☐	•	Clean forceps and spatulas with 3% sodium hypochlorite and 96% ethanol between samples.

1. Judge the PCR products

☐	No.	Step
☐	1	Look at the gel image of each PCR product. Mark it as **strong** (clear, bright band) or **faint** (visible but weak band). Products without a single band of the expected size are not sent.

2. Clean up the PCR products

☐	No.	Step
☐	2	Mix 5 µL of PCR product with 2 µL of ExoSAP-IT Express on ice.
☐	3	In the thermocycler: 37 °C for 4 min, 80 °C for 1 min, then hold at 4 °C.
☐	4	Mix 5 µL of PCR product with 2 µL of ExoSAP-IT on ice.
☐	5	Run the ExoSAP-IT programme (about 30 min in total) [TO CONFIRM: temperatures and times from the ExoSAP-IT user guide].
☐	6	Per sample, mix 1 µL SAP (1 U/µL), 0.25 µL Exonuclease I (20 U/µL) and 3.75 µL nuclease-free water, and add 15 µL of PCR product (20 µL in total).
☐	7	In the thermocycler, with the heated lid switched off: 37 °C for 40–45 min, 80 °C for 15 min, then hold at 4 °C.

3. Prepare the sequencing samples

☐	No.	Step
☐	8	For each product and each primer, label a tube (or plate well) with the sample name of the order, following the provider's instructions for tube type and labelling.
☐	9	Pipette the cleaned product and water:

Band	Cleaned product	Nuclease-free water
Strong	1 µL	4 µL
Faint	2.5 µL	2.5 µL

☐	No.	Step
☐	10	Add 5 µL of diluted primer (10 pmol). Total volume: 10 µL.
☐	11	If more volume is needed, scale all three volumes by 1.5 (total 15 µL) [TO CONFIRM: when the 1.5× volumes are used].
☐	12	Close, spin briefly and keep at 4 °C (fridge) until shipping.

4. Record and send

☐	No.	Step
☐	13	Complete your sheet in the Sanger recording file (see Data and metadata) before the samples leave the lab.
☐	14	Send the samples to the provider, following its current instructions for volumes, tubes and labels:

Provider	How samples are sent
Eurofins	Drop-off box at UC-Biotech; samples in a closed plastic zip-lock bag
StabVida	Drop-off box at UC-Biotech; samples in a closed plastic zip-lock bag
Macrogen	Pick-up service; a shipping label is generated for the shipment when needed

After finishing

☐	No.	Step
☐	•	Clean the bench with 70% ethanol.
☐	•	Empty the small bench bin into the right waste bin.
☐	•	Wash your hands.
☐	•	Turn off equipment and lights.
☐	•	Lock doors, fridge and freezer.

Notes / deviations
