LABORATORY OF MOLECULAR PATHOLOGY

Amplicon purification

Using AMPure beads to purify and size-select PCR amplicons

Prep:

- ☐ 01 Bring AMPure beads to **RT** before starting the purification (30 minutes).
- ☐ 02 **Prepare fresh 80% EtOH.** You will need approximately 0.5 mL per each sample.
- ☐ 03 **Vortex** the **AMPure** beads for at least **60 seconds**.
- ☐ 04 Combine the PCR reaction and the AMPure beads. Per 25 µL of PCR reaction you need to use **20 µL** of **AMPure** beads. **Mix properly** by pipetting or vortexing.
- ☐ 05 Incubate at RT for **5 minutes**. *Off the magnet.*
- ☐ 06 Put the samples on the **magnet**. Incubate for **2 minutes**.
- ☐ 07 Keep the samples on the magnet and remove the supernatant. Be careful not to damage the pellet.
- ☐ 08 Add **200 µL** of **80% EtOH**. Incubate for **30 seconds**. **Remove** the ethanol/supernatant.
- ☐ 09 **Repeat the previous step**: *Add 200 µL of 80% EtOH, wait 30 seconds, remove the EtOH.*
- ☐ 10 Close the tubes/put foil on the plate. Do quick spin to collect the remaining ethanol.
- ☐ 11 Put the samples back on the magnet. Wait a minute. Remove the residual ethanol with a 10 µL pipette. Be careful not to damage the pellet.
  *The goal is to remove as much ethanol as possible – but **the pellet cannot dry up**!*
- ☐ 12 Remove the samples from the magnet.
- ☐ 13 Add **32 µL** of **Elution** buffer**.**
- ☐ 14 Close the tubes/put foil on the plate. Mix the samples properly. Vortex. Quick spin.
- ☐ 15 Incubate at RT for **5 minutes**. *Off the magnet.*
- ☐ 16 Put the samples on the **magnet**. Incubate for **2 minutes**.
- ☐ 17 Transfer **30 µL** of the supernatant into a clean tube/plate. Be careful not to damage the pellet. *You can use the 10 µL three times to minimise the bead contamination.*