Depletion of repetitive sequences – WGS libraries

(modified from Shagina et al. 2010, BioTechniques 48:455-459;
Matvienko et al. 2013, PLoS ONE 8(2): e55913)

If you still have to make your library, see "A few considerations about adapters and primers" in the lab blog post.

Starting material: at least 240 ng (**ideally 480 ng**) of your library in a **3 μl volume**, in a 0,2 ml PCR tube. Efficiency of depletion decreases with much higher or much lower concentrations.

Timing is quite important during the enzymatic step, so it is best to do no more than 8 samples at a time. It is important to have your samples in separated tubes, so you can work with a sample at the time without taking the others out of the thermal cycler.
I normally cut two tubes out of a PCR strip, put the sample in one of them, and keep the other empty. Individual tubes sometimes fly away in the small table tabletop centrifuge next to the thermal cycler, and that's really annoying (Really. Annoying. You'll see why in a bit). The second tube increases stability during centrifugation.

- Add **1 μl** of **4X hybridization buffer**. Mix by tapping the tube with your finger (keep the lid of the tube in place with your other hand, since they don't always close very well), spin down.

- Overlay with **10 μl of mineral oil**. Spin down

- In a thermal cycler:

	98°C	2 minutes
	78°C	forever

- Leave at **78°C** for about **44 hours**.

- Start a different thermal cycler at 70°C constant. Pre-warm at **70°C 5 μl of DSN (Duplex Specific Nuclease) buffer** for each sample (plus some).

- Add **5 μl of DSN** buffer to your samples while they are still in the 78°C thermal cycler. Close the tube, tap it with your finger to mix, spin briefly, **move to the 70°C thermal cycler**. Do one sample at the time; since all the steps are "add liquid - mix - spin", if you start your timer when you are done with the first sample the timing will be right also for all the other samples in the following step. It is important that you are as quick as possible while doing this and the following steps, as you want your reaction to be outside the thermal cycler for the shortest time possible. Incubate at **70°C for 5 minutes**.

- Add **0.2 μl of a 1:2 dilution of DSN enzyme**. Mix, spin, but back at **70°C**. That's usually when one of your samples fly around the centrifuge if you use individual tubes. After about 2 days of waiting.

- Incubate at **70°C for 15 minutes.**

- Stop the reaction by adding **10 μl of Stop solution** (a glorified name for 10 mM EDTA). Mix, spin, put on ice.

- Leave on ice for a few minutes, add **20 μl of water**. Mix, spin, put back on ice

- Re-amplify your library. I normally make a 25 μl reaction using the Kapa HiFi HotStart MasterMix (which for some reason gives much better yield

than either Phusion or the NEBNext polymerase), 1 μl of each primer (10 μM), and 4 μl of template, with the following program:

98°C	2 min	
98°C	15 sec	
62°C	30 sec	8 cycler
72°C	30 sec	
72°C	5 min	

- Purify your PCR reaction using **1.6 volumes of SPRI beads**, resuspend in 25 μl. A little bit of primer dimers might be left after one round of purification. If that is a concern, purify another time with 1.6 volumes of SPRI beads.

At the bioanalyzer, you will see that your libraries are now slightly shifted towards smaller fragments. I am not sure why that is the case (possibly larger fragments are more likely to anneal to something and get degraded?), but it's normal.

While you added the Stop solution and all, the enzyme is supposedly still partially active. You want to do therefore the PCR after the DSN treatments as soon as possible, put the DSN reaction at -20 immediately after you are done, and ideally not keep it in the freezer for longer than a couple of weeks.

4X hybridization buffer

- 200 mM HEPES pH7.5
- 2M NaCl
- 0.8 mMEDTA

2X DSN buffer

- 0.1 M Tris pH8.0
- 10 mM MgCl2
- 2 mM DTT

2X Stop solution

- 10 mM EDTA