DNA isolation of shRNA constructs from SUPER RNAi™ library

DNA from shRNA constructs can be recovered in different formats. Every well of the library was cloned by mixing oligonucleotides for three different shRNA constructs in one well. After annealing, the three combined oligo-sets were ligated in one ligation reaction and transformed in DH5α bacteria. As a result every well should contain a mixture of three different shRNA constructs targeting one gene.

A. DNA isolation polyclonal from one well (3 shRNA constructs against one gene)

5µl of a thawed glycerol culture is transferred to 1 ml LB/AMP (100µg/ml) in a deep well block, sealed with an airpore filter and grown O/N at 37C with vigorous shaking (>250 rpm). After culture DNA can be isolated with standard mini-prep procedures. The presence of the insert can be checked by digestion with EcoRI and XhoI. This should give a 300bp band.

B. DNA isolation of individual shRNA constructs for one gene

A scrape is taken from the frozen bacterial glycerol stock and plated onto LB/AMP (100 µg/ml) agar plates and grown O/N at 37C. Individual colonies are picked and transferred to 1ml LB/AMP (100µg/ml) in a deep well block, sealed with an airpore filter and grown O/N at 37C with vigorous shaking (>250 rpm). After culture, DNA can be isolated with standard mini-prep procedures. The identity of the shRNA construct is determined by sequencing. To recover all three different shRNA constructs from each well (one gene), on average 15 colonies have to be analyzed by sequencing.

C. Large scale DNA isolation from individual wells (mix of three different shRNA vectors)

5µl of a thawed glycerol culture is transferred to 4 ml LB/AMP (100µg/ml) and grown O/N at 37C with vigorous shaking (>250 rpm). From this culture 20 µl is plated on to LB/AMP (100 µg/ml) agar plates and grown O/N at 37C. The lawn of bacteria form this plate is scraped in 5 ml LB and transferred to a maxi-prep culture (approx 200-300 ml LB/AMP (100µg/ml) and grown O/N at 37C with vigorous shaking (>250 rpm). After culture, DNA can be isolated with standard plasmid DNA isolation protocols.

D. Large scale DNA isolation from 96 well plates (mix of 288 different shRNA vectors against 96 genes).

5µl of each thawed glycerol culture of one 96 well plate is transferred to 1 ml LB/AMP (100µg/ml) in a deep well block, sealed with an airpore filter and grown O/N at 37C with vigorous shaking (>250 rpm). These 96 1 ml cultures are combined and added to 100-200 ml LB/AMP (100µg/ml). This bacterial culture is grown for another 2-3 hrs until it reaches a late log phase (OD~ 0.6). From this culture, DNA can be isolated with standard plasmid DNA isolation procedures.