Haplobank

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Barcode PCR: PCR to Confirm the Identity of Individual Clones

Each gene trap vector was initially designed in a way that it contains a unique barcode stretch of 32 bps just downstream of each terminal repeat which can be readily amplified by PCR and Sanger sequenced. The obtained barcode sequence is aligned to the one published for each clone in our collection, thereby confirming the correct identity of the clone at hand. Any deviations from the published barcode sequence are indications that the clone (i) is not the right one, or (ii) carries more than one gene trap.

barcode PCR primer sequences

barcodePCR-F	GGTTGATCTGAGCTACTCATCAACGGT
barcodePCR-R	CAAGTTCCTTCTGGTTCTGGCTCTGCT

barcode PCR reaction

crute ES cell lysate	5 ul
primer barcodePCR-F 5 uM	2 ul
primer barcodePCR-R 5 uM	2 ul
10 mM dNTP mix	1 ul
10x Klentaq buffer	5 ul
20x Klentaq polymerase	3 ul
dH2O	32 ul
	50 ul

Biorad C1000 cycle parameters

95deg, 3'
95deg, 10"|
58deg, 20"| 35x
72deg, 30"|
72deg, 5'

analyze 20 ul on an agarose gel, and purify PCR products for Sanger sequencing, e.g. by using illustra ExoStar 1-step kit (GE Healthcare)

use barcodePCR-R primer for Sanger sequencing