

Making 5' Probe for Knock-In Mice

1. Digest Eco B/E subclone with Pst I and Xba I overnight at 37°C.

Total volume: 200 μ L		DNA conc.: 10 μ g / 50 μ l		Enzyme: 200 Units	
Subclone Conc. (μ g/ μ l)	Enzyme	Enzyme (μ l)	DNA (μ L)	ddH ₂ O (μ l)	10x Buffer (μ l)
	Pst I, H.C.	5			20
--	Xba I, H.C.	5	--	--	--

2. Run analytical gel to check digest.
 - a) Use 1% agarose gel with 5 μ l Ethidium Bromide / 100 μ l agarose gel.
 - b) Use mini-gel apparatus (~50 ml total gel volume).
 - c) Prepare samples by mixing together the following:
 - 2 μ l DNA sample
 - 1 μ l Orange G
 - 7 μ l ddH₂O
 - d) Use 2 μ l 1 Kb Plus DNA ladder.
3. If digested completely, run samples on medium-sized gel (~100 mL total gel volume).
 - a) Use 1% agarose gel with 5 μ l Ethidium Bromide / 100 μ l agarose gel.
 - b) Add 1 μ l Orange G / 10 μ l digested sample.
 - c) Use 5 μ l 1 Kb Plus DNA ladder.
 - d) Run gel for ~4 hr at 100 V.
4. Excise 0.6 Kb band from gel (___ band from bottom). Take pictures of before & after.
5. Extract DNA from Gel using Qiagen QIAEX II Agarose Gel Extraction Protocol.
6. Quantify sample using lambda ladder.
 - a) Use 1% agarose gel with 5 μ l Ethidium Bromide / 100 μ l agarose gel.
 - b) Use mini-gel apparatus (~50 ml total gel volume).
 - c) Prepare samples by mixing together the following:
 - 5 μ l DNA sample
 - 2 μ l Orange G
 - 3 μ l ddH₂O
 - d) Prepare λ ladder by mixing together the following and incubating for 10 minutes at 65°C:
 - 1 μ l λ marker
 - 2 μ l Orange G
 - 7 μ l ddH₂O
 - e) Use 5 μ l λ ladder.
7. Compare sample band with λ ladder bands to determine concentration.