Making 5' Probe for Conditional Knock-Out Mice

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

Total volume: 200 μL		DNA conc.: 10μg / 50μl		Enzyme: 200 Units	
Subclone Conc. (μg/μl)	Enzyme	Enzyme (μl)	DNA (μL)	ddH2O (μl)	10x Buffer (μl)
	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 μl Ethidium Bromide / 100 μl agarose gel.
   b) Use mini-gel apparatus (~50 ml total gel volume).
   c) Prepare samples by mixing together the following:
      - 2 μl DNA sample
      - 1 μl Orange G
      - 7 μl ddH2O
   d) Use 2 μl 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 μl Ethidium Bromide / 100 μl agarose gel.
   b) Add 1 μl Orange G / 10 μl digested sample.
   c) Use 5 μl 1 Kb Plus DNA ladder.
   d) Run gel for ~5 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 μl Ethidium Bromide / 100 μl agarose gel.
   b) Use mini-gel apparatus (~50 ml total gel volume).
   c) Prepare samples by mixing together the following:
      - 5 μl DNA sample
      - 2 μl Orange G
      - 3 μl ddH2O
   d) Prepare λ ladder by mixing together the following and incubating for 10 minutes at 65°C:
      - 1 μl λ marker
      - 2 μl Orange G
      - 7 μl ddH2O
   e) Use 5 μl λ ladder.
7. Compare sample band with λ ladder bands to determine concentration.