

**Protocols for Digestion of BamHI-KillerRed-KpnI**  
**iGEM Grenoble-EMSE-LSU 2013**  
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June 2013

**Restriction enzyme digestion of plasmid DNA:**

- 1) Prepare buffer IV, DNA, BamHI, KpnI
- 2) Digest 800 ng KillerRed with 1 U of the different restriction enzymes. Test the digest with 0, 1 or 2 enzymes that are active in the same buffer.

Volumes ( $\mu$ L)	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
React buffer 4	2	2	2	2	2
DNA	800 ng	800 ng	200 ng	200 ng	200 ng
BamH1	1	1	1	0	0
Kpn1	1	1	0	1	0
H <sub>2</sub> O	complete to 20 $\mu$ l	complete to 20 $\mu$ L	complete to 20 $\mu$ l	complete to 20 $\mu$ l	complete to 20 $\mu$ l

- 3) Incubate 2h at 37°C.
- 4) Perform a gel electrophoresis, followed by DNA gel extraction to purify the BamHI-KillerRed-KpnI insert.