

# Digestion of agarose-embedded DNA

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Restriction digestion of DNA embedded in agarose is a technique used for analysis of extremely large DNA fragments. Complete digestion of substrate DNA is affected by substrate purity, units of enzyme, and incubation time. The following protocol describes a general procedure for the digestion of 1 µg of agarose-embedded DNA in 16 hours.

## Digest agarose-embedded DNA with restriction enzyme

1. Embed the substrate DNA in 1% low melting temperature agarose at a concentration of 1 µg DNA / 30 µL agarose.
2. Prepare ~30 µL agarose plugs with a gel syringe dispenser or similar agarose dispensing system.
3. Equilibrate the plug in 100 µL of the appropriate 1X restriction enzyme buffer for 15 minutes.
4. Place the plug in 100 µL of fresh 1X buffer containing the restriction enzyme (see Table 1 for recommended number of units).
5. Incubate at 37°C for 16 hours.

**Note:** Certain enzymes may require incubation at higher or lower temperatures. Refer to Table 1 for details on specific enzymes.

**Table 1. Recommended units of enzyme for digestion of agarose-embedded DNA**

Enzyme	Cat. no .	Units of enzyme required for digestion in 16 hours <sup>[1]</sup>
AanI (PstI)	ER2061	5
AarI	ER1581	5
AatII	ER0991	5
Acc65I (Asp718I)	ER0901	5
Adel (DraIII)	ER1231	10
AjiI (BmgBI)	ER1941	5
AjuI	ER1951	5
Alw44I	ER0041	5
ApaI	ER1411	5
BamHI	ER0051	5
BauI (BssSI)	ER1841	5
BcuI (SpeI)	ER1251	10
BfuI (BciVI)	ER1501	5
BglI	ER0071	5
BglII	ER0081	5
BoxI (PshAI)	ER1431	10
BpiI (BbsI)	ER1011	5
BplI	ER1311	10

<b>Enzyme</b>	<b>Cat. no .</b>	<b>Units of enzyme required for digestion in 16 hours<sup>[1]</sup></b>
Bpu1102I (B pl)	ER0091	5
BseSI (Bme1580I)	ER1441	5
Bsh1285I (BsiEI)	ER0891	5
BshNI (BanI)	ER1001	5
BshTI (AgeI)	ER1461	20
Bsp68I (NruI)	ER0111	5
Bsp119I (BstBI)	ER0121	5
Bsp120I (PspOMI)	ER0131	5
Bsp1407I (BsrGI)	ER0931	10
BspOI (BmtI)	ER2041	20
BspTI (AflII)	ER0831	5
Bst1107I (BstZ17I)	ER0701	5
BstXI	ER1021	5
Bsu15I (ClaI)	ER0141	5
CaiI (AlwNI)	ER1391	5
Cfr9I (XmaI)	ER0171	5
Cfr10I (BsrFI)	ER0181	5
Cfr42I (SacII)	ER0201	20
DraI	ER0221	5
Eam1104I (EarI)	ER0231	5
Eam1105I (AhdI)	ER0241	5
Ecl136II (EcoICRI)	ER0251	5
Eco24I (BanII)	ER0281	5
Eco31I (BsaI)	ER0291	5
Eco32I (EcoRV)	ER0301	5
Eco47III (AfeI)	ER0321	5
Eco52I (EagI)	ER0331	5
Eco72I (PmlI)	ER0361	5
Eco81I (Bsu36I)	ER0371	5
Eco88I (AvaI)	ER0381	5
Eco91I (BstEII)	ER0391	5
Eco105I (SnaBI)	ER0401	5
Eco130I (StyI)	ER0411	5
Eco147I (StuI)	ER0421	5
EcoO109I (DraII)	ER0261	5
EcoRI	ER0271	5
Ehel (SfoI)	ER0441	20

<b>Enzyme</b>	<b>Cat. no .</b>	<b>Units of enzyme required for digestion in 16 hours<sup>[1]</sup></b>
Esp3I (BsmBI)	ER0451	5
FspBI (Bfal)	ER1761	10
Hin1I (BsaHI)	ER0471	5
HindIII	ER0501	5
KpnI	ER0521	5
Kpn2I (BspEI)	ER0531	5 (55°C)
KspAI (HpaI)	ER1031	5
Lgul (SapI)	ER1931	5
MauBI	ER2081	5
Mbil (BsrBI)	ER1271	5
MlsI (MscI)	ER1211	20
MluI	ER0561	5
MssI (PmeI)	ER1341	5
MunI (MfeI)	ER0751	5
Mva1269I (Bsml)	ER0961	5
NcoI	ER0571	5
NdeI	ER0581	5
NheI	ER0971	5
NotI	ER0591	5
Nsbl (FspI)	ER1221	5
OliI (AleI)	ER1631	5
PaeI (SphI)	ER0601	5
Pacl	ER2201	5
PagI (BspHI)	ER1281	5
PasI	ER1861	10 (55°C)
Paul (BssHII)	ER1091	10
PdiI (NaeI)	ER1521	20
Pdml (XmnI)	ER1531	10
PfI23II (BsiWI)	ER0851	5
PfoI	ER1751	10
Ppu21I (BsaAI)	ER1971	5
PscI (PciI)	ER1871	5
Psp5II (PpuMI)	ER0761	5
Psp1406I (AclI)	ER0941	10
PstI	ER0611	5
PsyI (Tth111I)	ER1331	5
PvuI	ER0621	5

Enzyme	Cat. no .	Units of enzyme required for digestion in 16 hours <sup>[1]</sup>
PvuII	ER0631	5
RseI (MstII)	ER2001	5
SacI	ER1131	5
SalI	ER0641	5
Scal	ER0431	20
SdaI (SbfI)	ER1191	5
SfaAI (AsiSI)	ER2091	5
SfiI	ER1821	5 (50°C)
SgeI	ER2211	3
SgrDI	ER2031	5
SgsI (AscI)	ER1891	5
SmaI	ER0661	5 (30°C)
SmiI (SwaI)	ER1241	10 (30°C)
SmoI (SmlI)	ER1981	5 (55°C)
SspI	ER0771	5
SspDI (KasI)	ER2191	5
Van91I (PflMI)	ER0711	5
VspI (AseI)	ER0911	5
XagI (EcoNI)	ER1301	5
XapI (ApoI)	ER1381	5
XbaI	ER0681	5
XhoI	ER0691	5
XmaJI (AvrII)	ER1561	10
XmiI (AclI)	ER1481	5
I-SceI	ER1771	20

<sup>[1]</sup> Unless otherwise noted in parentheses, incubation temperature is 37°C.

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