GENIE BLOTTER INSTRUCTIONS

(Original 1984, 1995, 2000, revised August 2013)

THEORY: GENIE'S magic lies in its plate electrodes. The large surface area gives the highest, most uniform field possible. Ordinary blotters waste most of the field pushing current through the shroud of bubbles surrounding the electrode wire.

DANGER: THE GENIE should not be connected to high voltage power supplies (greater than 50 volts), as the GENIE does not have a safety lockout to prevent it from being opened while it is energized. The power supplies that are modified by Idea Scientific for use with the GENIE are the safest and least expensive power supplies for the GENIE. The voltage table elsewhere in the instructions was calculated using these power supplies.

NOTE: The **GENIE** is supplied with an alloy anode (economy models) or a platinized titanium anode (premium models) ,and an alloy cathode (- electrode). The alloy anode in economy models may show slight surface corrosion and should be wiped occasionally with SCOTCH-BRITE to remove this surface corrosion. The platinized titanium anode in premium models requires no maintenance.

CAUTION: Never use the platinized electrode as a cathode, and never abrade the platinized surface.

CAUTION: Chloride ion damages all anodes. Never adjust the pH of an electroblotting buffer with HCl. When running SDS-PAGE gels, run the Bromphenol blue to the bottom or off of the gel, as the separating gel contains chloride ion.

CAUTION: Lengthy blots (more than two hours) with more conductive buffers (low methanol Westerns, nucleic acid blotting) may overheat and damage alloy anodes. A platinized titanium anode is recommended for use with these buffers.

WESTERN BLOTTING

The most common Western blotting buffer is 25mM Tris-glycine (pH 8.3)/20% methanol (Towbin buffer)[1]. (See blotting buffers, #1) SDS gels are loaded directly into the blotter without presoaking, and blotted onto nitrocellulose or PVDF, (or rarely nylon), at 12 volts for one hour. NOTE: PVDF is very hydrophobic and must first be wetted in 100% methanol, then equilibrated in the blotting buffer for at least 15 minutes before use.

Large proteins (greater than 200kD), or thick gels (thicker than 1mm) may require extending the blotting time. Large proteins may be precipitated by the methanol in the blotting buffer. One can add 0.01 to 0.05% SDS to the blotting buffer, or reduce the methanol to maintain protein mobility. A favorite method for blotting large proteins is to use 1/2x the Tris-Glycine buffer and set the power supply at 24 volts for two hours.

Small proteins (less than 20 kD) may pass through the membrane without binding. One can reduce the voltage to 6 volts or use a smaller pore membrane. 0.2u membrane will capture smaller proteins better than the usual 0.45u membrane, but cannot be used for larger proteins, as the bands will diffuse upon blotting.

Acetic acid (see blotting buffers #3) is used for blotting acid-urea and isoelectric focusing gels. Blotting is toward the cathode (-), the opposite direction of other Western blotting. The membrane must be placed on the cathode (-) side of the gel. Do not reverse the power leads. A platinized titanium anode will be damaged if used as a cathode.

Proteins and peptides can be blotted onto PVDF for sequencing using the CAPS/10% methanol buffer (see electroblotting buffer #3).

NUCLEIC ACID BLOTTING

Nucleic acid electroblotting with the GENIE blotters is a rapid, high-resolution replacement of capillary, vacuum and pressure blotting.

NORTHERN BLOTTING:

RNA gels run in 2.2 M formaldehyde , 1X MOPS can be blotted directly to + charged nylon membrane in 1/2X MOPS without the formaldehyde (see electrophoretic blotting buffer #5), in 2 hours at 6 volts, or one hour at 12 volts. NOTE: Gels can be stained with Acridine orange (15ug/ml), photographed, then destained one hour in electrophoretic blotting buffer #5 before blotting. RNA gels run in TBE can be blotted in TBE.[4]

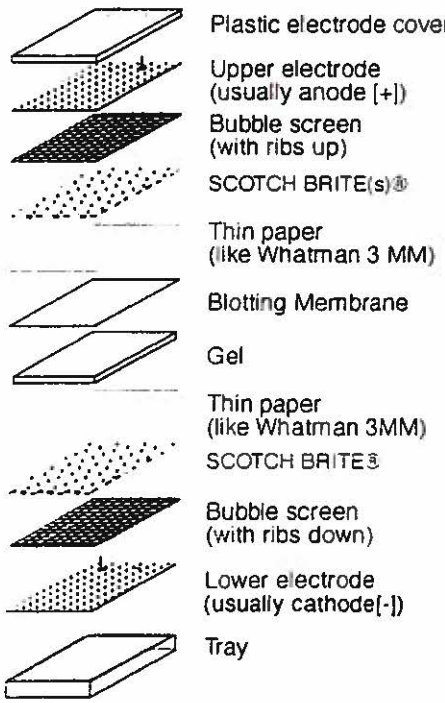
SOUTHERN BLOTTING:

DNA gels electrophoresed in TBE can be blotted directly to + charged nylon in TBE (or1/2X TBE.[11]). (see electrophoretic blotting buffer #4) Even 1 million base pair DNA from CHEF gels can be blotted without fragmenting the DNA.[9,10] The DNA is blotted in the native(double-stranded) state and must be denatured after transfer by soaking the membrane in 0.4 N NaOH for 10 minutes. Then rinse the membrane in 2X SSC for ten minutes and probe as usual. NOTE: Gels run in TAE must be blotted in TAE, no details available.[5]

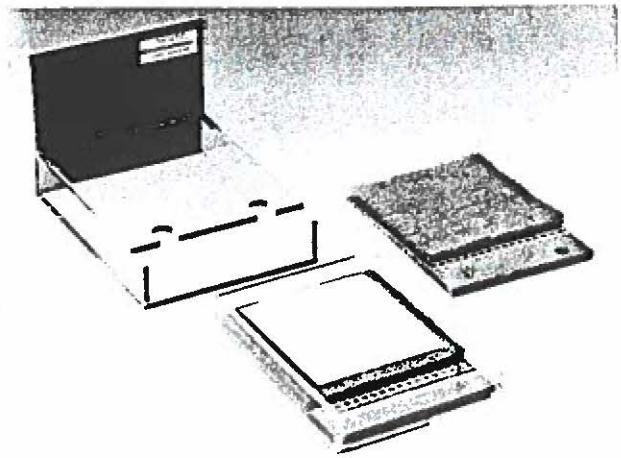
LOADING THE GENIE:

1. Place the tray on a relatively level surface.
2. Place two plastic bubble screens in the bottom of the tray. (Optional.)
3. Place the cathode in the bottom of the tray. The banana connector should be in the upper left hand side. (Note: Do not use a platinized cathode)
4. Place a plastic bubble screen, ribbed side down, on the cathode.
5. Place SCOTCH-BRITE pad(s) in the tray to half-depth of tray and add transfer buffer to submerge pad(s). Press pads to expel all bubbles.
6. Place a sheet of filter or chromatography paper,cut to the size of the SCOTCH-BRITE pad, on the SCOTCH-BRITE pad. Be sure not to trap bubbles under the blotting paper. It is important that the paper be thin (like Whatman 3MM). Some labs use a coffee filter for this. Do not use so-called "blotting papers" as they are thick and a barrier to current.
7. Position the gel on the filter paper. Gels also can be easily transferred to the apparatus by adhesion to a wet or dry piece of thin filter or blotting paper which becomes the pad under the gel. Gels can also be transferred by adhesion to prewetted transfer membrane. Note: When botting IEF or acid urea gels, the membrane must be placed first.

8. Place the pre-wetted transfer membrane on the gel. This is easily performed by holding a sheet of transfer membrane at opposite ends and allowing the sagging center to touch the gel first. Slowly lower the ends. If done slightly below the level of the transfer buffer, no bubbles should be trapped between the membrane and the gel.
9. Rub the transfer membrane to expel all excess buffer from between the gel and the membrane. Failure to do this will result in fuzzy transfers. One can even roll a glass rod over the gel to do this. Do not have too much buffer in the tray when doing this or the membrane will tend to float off the gel.
10. Place a piece of filter paper over the membrane being careful not to trap any bubbles.
11. Place SCOTCH-BRITE pads over the filter paper (usually two pads when the SCOTCH-BRITE is new, increasing to five as they flatten with usage), and add buffer until tray is almost full. A second blot can be put between these pads.
12. Place a plastic bubble screen, ribbed side up, on the pad and place the anode (banana connector should be in the upper right corner) on it.
13. Position the two-holed plastic anode cover over the anode.
14. The sandwich should need to be compressed about 2 mm. to slide into the tray holder. If not, the gel may slide during the electroblotting and another SCOTCH-BRITE pad should be added to the sandwich. Note flattening in #11 above.
15. Keeping the tray level, slide the tray into the tray holder. Be careful not to pinch the electrode cover between the tray and the tray holder as the tray will jam and be difficult to remove.
16. Slowly tip the GENIE to its vertical running position. Add buffer if it appears the final buffer level will not cover the blotting area.
17. Connect the GENIE to the power supply and begin blotting. Left-hand connection is (-), cathode: Right-hand connection is (+), anode.
18. Monitor the temperature during blotting.



LOADING THE GENIE BLOTTER



LOADING THE GENIE BLOTTER

ELECTROPHORETIC BLOTTING BUFFERS
NOTE: Never adjust the pH of an electroblotting buffer with HCl. The chlorine that will be evolved will severely corrode the electrode connections and is very toxic. Do not use commercial "pre-mixed" buffers as they often contain HCl.

1. 25mM Tris-Glycine/20% methanol (pH 8.3). This is the standard buffer that is used for most "Western" blotting.[1]

Tris Base	3.03 g.
Glycine	14.4 g.

Dilute to one liter with 20% V/V methanol; this buffer does not need pH adjustment. See TROUBLESHOOTING if you have trouble getting complete transfer with this buffer.

2. 0.7% Acetic Acid (7 ml. glacial acetic acid diluted to 1l.) is used for the transfer of isoelectric focusing gels and acid-urea gels. Proteins are blotted toward the cathode (-) in this buffer

3. 10mM CAPS /10% methanol (pH 11.0)

CAPS (3-(Cyclohexylamino)-1-propanesulfonic acid)	2.213g.

Dissolve in 900 ml. distilled water
Adjust pH to 11.0 with NaOH
Add 100 ml. methanol
Transfers proteins to PVDF for direct sequencing.

4. 89mM Tris-Borate, 2mM EDTA (pH approx 8.3)

Tris Base	10.8 g.
Boric Acid	5.5 g.
EDTA	0.76 g.

Dilute to one liter with distilled water. pH adjustment with Boric Acid is unnecessary.

5. MOPS buffer for Northern blots (pH 7.0)

Morpholinopropanesulfonic acid (MOPS)	4.626 g.	(20 mM)
Sodium Acetate	.6805 g.	(5 mM)
EDTA	.1861 g.	(0.5mM)

Dilute to one liter with distilled water; adjust pH with acetic acid

Common blotting conditions with the GENIE

Gel System	Membrane	Buffer	Time@voltage	Comments
SDS-PAGE or Native gels (20kD to 200kD)	Nitrocellulose or PVDF	25mM Tris-Glycine /20% MeOH (buffer #1)	60 minutes@12 volts or 30 minutes @ 24 volts or 40 minues @ 18 volts or 30 min. @ 6 volts, then 90 minutes @ 24 volts	PVDF must be soaked in blotting buffer for at least 15 minutes
Small proteins (5kD to 20 kD)	0.2u Nitrocellulose or small pore PVDF	"	30 minutes @ 6 volts	"
Peptides (less than 5kD)	0.05u Nitrocellulose	"	10 minutes @ 6 volts	Nitrocellulose is product #BA75 from Schleicher and Schluell
Large proteins (larger than 200 kD)	Nitrocellulose or PVDF	12.5mM Tris-Glycine / 10% MeOH (1/2X buffer #1) (modify buffer if poor transfer)	1 to 2 hours @24 volts	Add 0.01 to 0.05% SDS to blotting buffer, or eliminate the MeOH.
Acid-Urea Gels, IEF or Native gels	Nitrocellulose	0.7% Acetic acid (buffer # 2)	1 hour @ 12 volts	Membrane must be on cathode (-) side of gel
Proteins for direct sequencing	small pore PVDF	10 mM CAPS /10% MeOH (buffer #3)	30 minutes @ 12 volts 1 hour @ 6 volts	CAPS precipitates some proteins (see "Troubleshooting")
RNA acrylamide gel	+ charged nylon	89 mM TBE (buffer #4)	30 minutes @ 12 volts	uncharged nylon will not work
RNA agarose gel	"	"	1.5 hours @ 12 volts[4]	"
RNA agarose gel	"	MOPS-Acetate (buffer #5)	2 hours @ 6 volts	"
DNA acryamide gel	"	89mM TBE (buffer#4)	30 minutes @ 12 volts	"
DNA agarose gel	"	"	1 hour @ 12 volts	1/2 X TBE often used[11]

Some brand names of nylon membranes

Positively charged nylon membranes (should work for electroblotting): HyBond N+ (Amersham), Zeta-Probe, Zeta-Probe GT (Bio-Rad), BioBlot N+ (Costar), ZetaBind (AMF-Cuno), GeneScreen Plus (DuPont/NEN), Bio Trace HP (Gelman), OptiBLOT (IBI), BIOTRANS+ (ICN), MAGNACHARGE (Micron Separations), Immobilon-NY+ (Millipore), Biodyne+, Biodyne B (Pall), Maximum Strength Nytran + (Schleicher and Schuell), Flash (Stratagene).

Neutral charged nylon membranes (will not work for electroblotting): Hybond N (Amersham), Immun-lite (Bio-Rad), BioBlot N (Costar), Gene Screen (DuPont/NEN), BIOTRANS (ICN), MAGNA (Micron Separations), Biodyne A (Pall), Maximum Strength Nytran (Schleicher and Schuell), Duralon (Stratagene).

REFERENCES:

[1]Proc Nat Acad Sci. 76, 4350-4354, (1979). (The classic Western blot publication).

[2]Biotechniques 12, 650-654, (1992). (30 minute Western blots with the GENIE)

[3]J. Cell Biology 110, 1623-1633, (1990). (30 minute Western blots with the GENIE).

[4]Biochem. Biophys. Res. Comm., 238, 277-279, (1997). (A unique1.5 hour Northern blot with a very low background that does not use formaldehyde)

[5]EMBO Journal 13, 3368-3377, (1994). (Northerns from urea sequencing gels transfered with TAE)

[6]J. Bact. 176, 3820-3823,(1994). (tRNA northerns)

[7]Gene 151, 209-214, (1994). (tRNA northerns)

[8]J. Biol. Chem. 268, 14045-14053 (1993) (Northern blots of yeast RNase P RNAs from acrylamide gels).

[9]The Plant Cell 10, 1349-1359, (1998). (Blotting of native 200 kB DNA from CHEF gels with the GENIE).

[10]Dr. Robert Haire, personal communication. (Blotting of one million base pair DNA from CHEF gels)

[11]Science 279, 349-352, (1998). (Electroblot with GENIE to measure telomere length)

TROUBLESHOOTING THE GENIE ELECTROPHORETIC BLOTTER:

Problem	Poor transfer: staining gel shows molecules have not left gel.
Possible Reasons	Too low field; too short transfer time; methanol pre-soak is fixing proteins in the gel; pH of the transfer buffer is the pI of the protein to be blotted; blotting paper too thick or too many sheets; gradient gel run until proteins are forced into an area of high % acrylamide in which molecules will not move; power supply inoperative, anode surface oxidized (alloy anode) or depleted of platinum (platinized anode); Insufficient equilibration of PVDF membrane with the blotting buffer; too much methanol in the blotting buffer,

Problem	Poor binding to membrane.
Possible Reasons	Pore size of membrane too large; MeOH needed if "Western" to nitrocellulose; poor grade of nitrocellulose; voltage too high for small proteins; too much SDS in the blotting buffer: not enough methanol in the blotting buffer; proteins incompatible with methano requiring a switch to ethanol (rare).

Problem	Proteins washed off media after transfer.
Possible Reasons	Detergents in media wash are removing proteins, requiring a change to Tween, NP-40, or elimination of detergent.

Problem	Apparatus overheats.
Possible Reasons	Buffer is too concentrated for the voltage being applied. Use 1/2 X buffer or lower the voltage. (1/2 X Tris-Glycine with 10 or 20% methanol is commonly used for westerns).

Problem	Smudgy or fuzzy blots.
Possible Reasons	Too much liquid between gel and media; SCOTCH-BRITE too old and flattened; gel sliding during blot; gel improperly equilibrated and shrinking during blot; improper probing procedures; over-loaded gel blotted onto 0.2 micron nitrocellulose smears as pores clog. Insufficient equilibration of PVDF membrane with the blotting buffer.

Problem	Alloy anode (+) corrosion.
Possible Reasons	Blotting too lengthy (maximum 2 hours at 24 volts, 4 hours at 6 or 12 volts).
Solution	Use a platinized titanium anode.

Problem	Extreme alloy anode (+) corrosion.
Possible Reasons	Chloride ion present (sources: Buffer pH'd with HCl; use of Tris-Cl rather than Tris base, EDTA stock pH'd with HCl; gel not electrophoresed long enough so that separating gel chloride ahead of the marker front is blotted into the blotting buffer, recent cleaning of building distilled water system has left traces of Cl in laboratory distilled water supply).

Problem	Proteins will not blot in CAPS buffer
Possible Reasons	The CAPS buffer is quite popular for blotting proteins to PVDF for sequencing. Some proteins are precipitated in the gel by the CAPS buffer. Sometimes a higher concentration of CAPS will solve the problem, but the blotter can get quite hot. Alternatively, use a borate buffer or the Tris-Glycine buffer and rinse the blot free of glycine.

NEED HELP? PHONE OR E-MAIL US.

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