Washing and detection

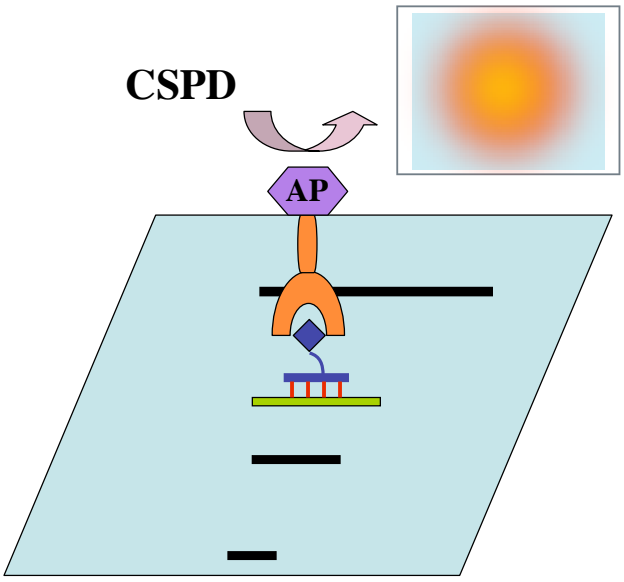
- 1. Wash away unbound and non-specifically bound probes;
- 2. Incubate with an anti-DIG antibody which is AP conjugated after a blocking wash;
- 3. Detect AP with a chemiluminescent substrate of AP called CSPD and expose to a film.

Stringency and washing

- Stringency= homology
- High stringency= high specificity= few mismatches
- To increase stringency: ↑**temperature,** ↓**[salt]**

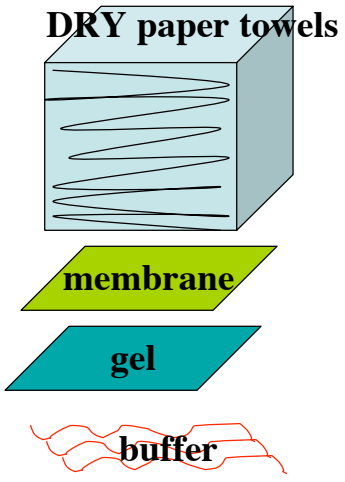
- 1st wash: RT, 2×SSC -- low stringency, unbound probes
- 2nd wash: 68°C, 0.5×SSC -- high stringency, non-specifically bound probes

Detection

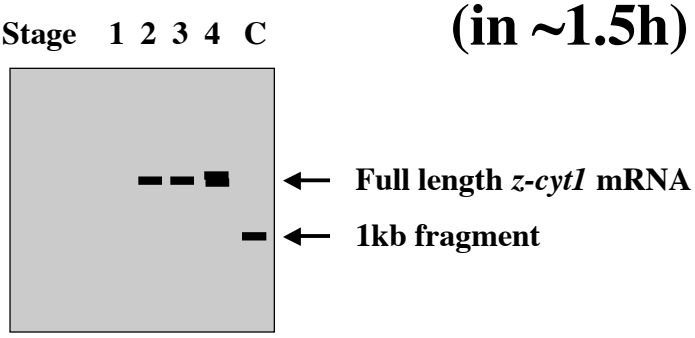


Incubate in CSPD solution for 15 minutes! (sign up for this)

- Label your blot (on saran wrap) on the side with NO RNA, i.e. **the side with pencil marks**!



When you get your film back…. (in ~1.5h)



Q: Can you decide from the intensity of these bands how much *z-cyt1* mRNA is present at different stages? A: NO! You need a loading control, rRNA in this case (the gel picture you took before transfer.)

Northern vs Western

Gel	Denaturing agarose gel	SDS-PAGE
Transfer	Capillary action	Electroblot
Pre-hyb	Casein + SDS	blotto
Probe	DIG labeled DNA	Specific 1° antibody
Hyb T (°C)	50	4
Wash	Stringency considerations	no
Detection	AP-conjugated anti-DIG Ab CSPD substrate	AP-conjugated 2° Ab NBT/BCIP substrate

Zebrafish development and teratogenesis

LiCl treatment time (post fertilization)	Typical phenotype
1h	Lethel due to severe pattern defects
3h	Higher survival rate; anterior defects (no eyes)
6h	Normal development

LiCl @ 1h post fertilization (picture taken @26h)

Figures removed due to copyright reasons.

Stachel et al. (1993)

LiCl @ 3h post fertilization (picture taken @26h)

Figures removed due to copyright reasons.

Stachel et al. (1993)

Day 5 interpretations due today!

Clean up the lab!

Course evaluation!