Drug selection for transgenesis in *Caenorhabditis* species

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Outline

- Why drug selection?
- Available drug selection systems
- Which drug is best?
- Dual drug selection for bombardment
  & its use in non-elegans species

Why drug selection?

Drug vs. unc-119

Ferguson et al. 2009

Rapid and easy (for bombardment)

- unc-119 worms tricky to grow in large numbers
- Selection by starvation (several weeks) vs 4 day drug selection

Universal (dominant marker)

- No requirement for specific genetic background
- Can be used for **any species** and **any strain**

Why drug selection?

Other dominant markers (rol-6, fluorescent)

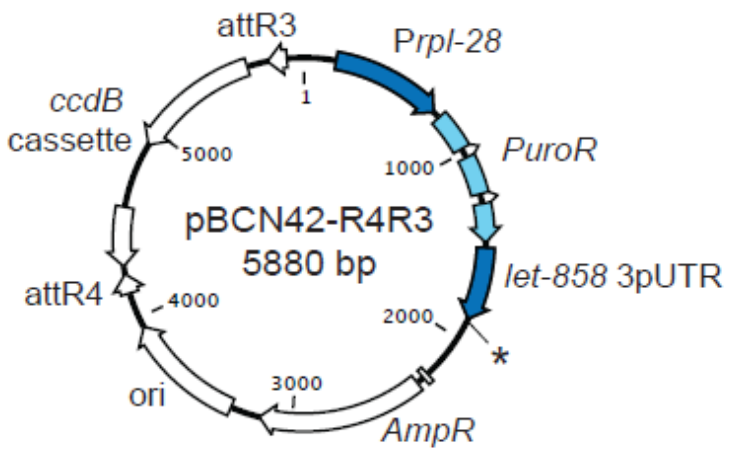
- Easy visual tracking of transgene for crosses ✓
- Difficult to select ✗

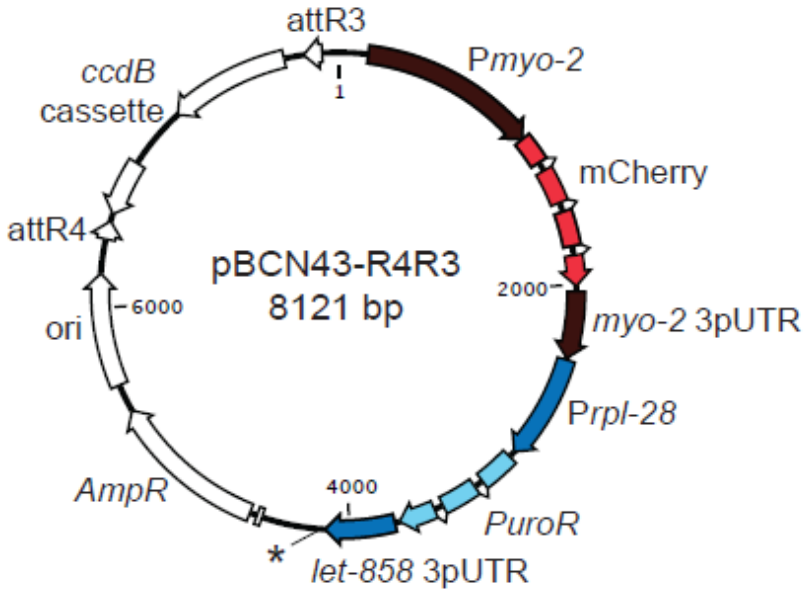
Drug selection

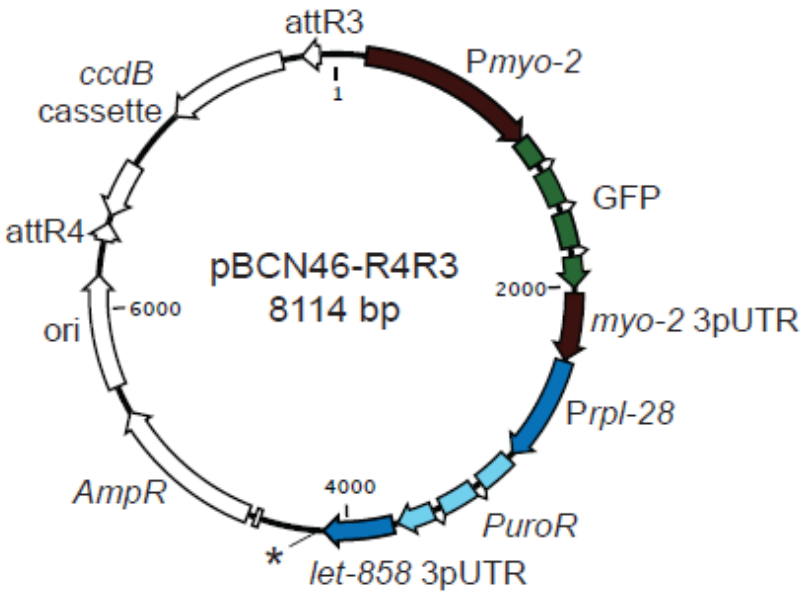
- Can easily select for rare events without specialist equipment

Drug & fluorescence combo

Puromycin selection







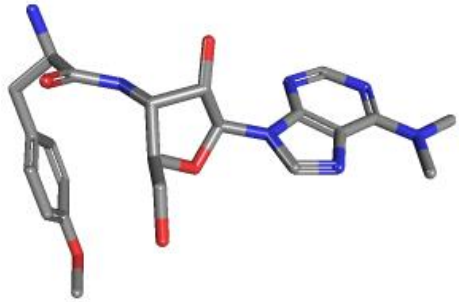
* SpeI-BglII-XcmI-AvrII-SacI

PuroR = puromycin *N*-acetyl-transferase gene from *Streptomyces* bacteria + synthetic introns

Drug selection systems available

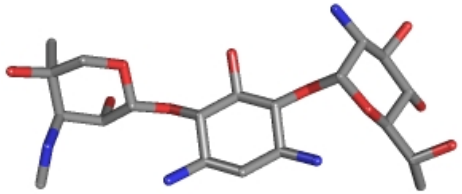
Puromycin (Ben Lehner Lab)

- Nat Methods, 2010. 7(9):725-7
- Nat Methods, 2012. 9(2):118-9



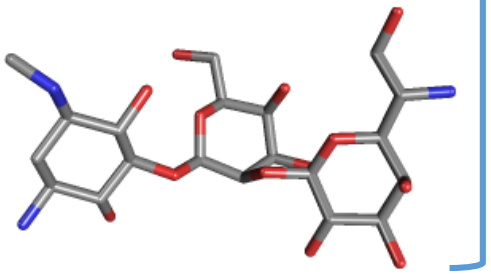
Neomycin/G418 (Denis Dupuy Lab)

- Nat Methods, 2010. 7(9):721-3



Hygromycin B (Jason Chin Lab)

- J Am Chem Soc, 2011. 133(36):14196-9
  Expanding the genetic code of an animal.



Aminoglycoside antibiotics from Streptomyces species

Inhibit ribosome activity

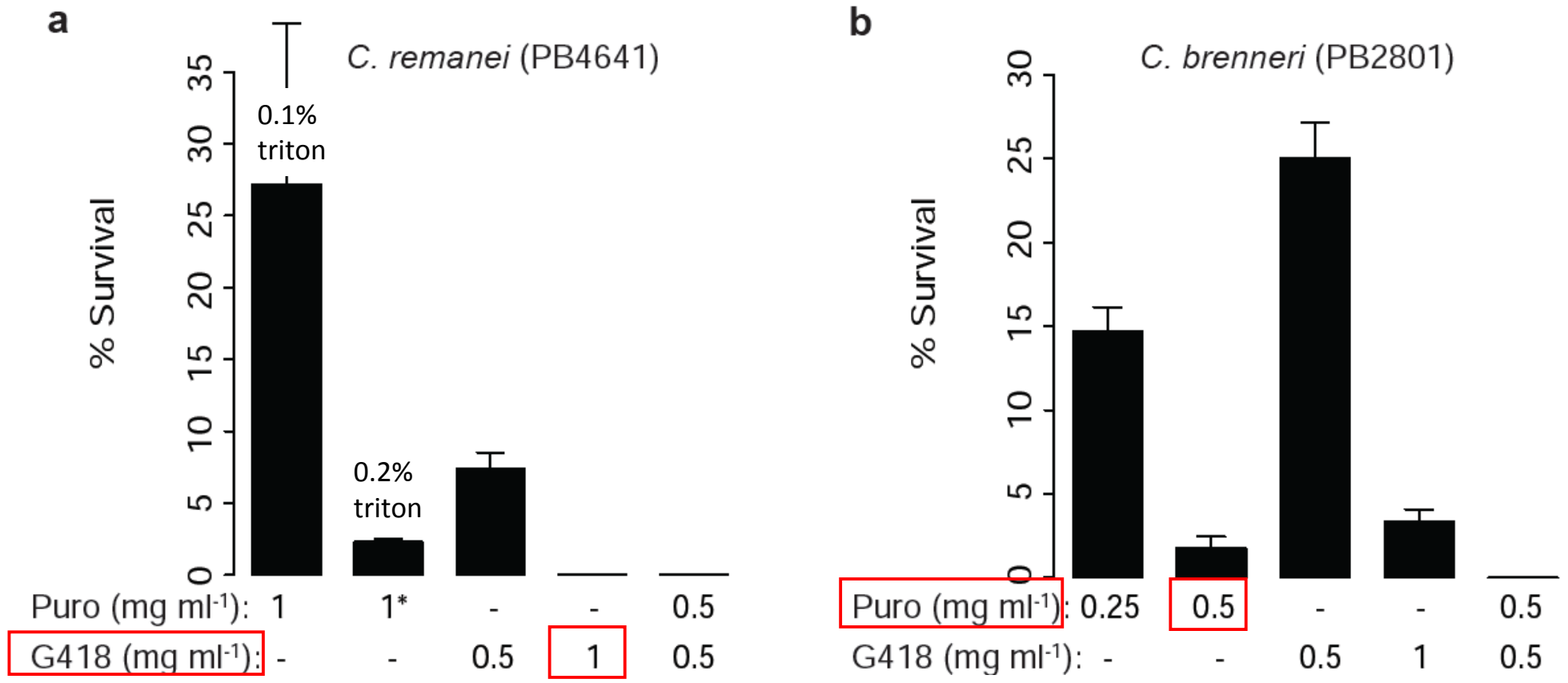
Which drug is best?

Depends on the application:

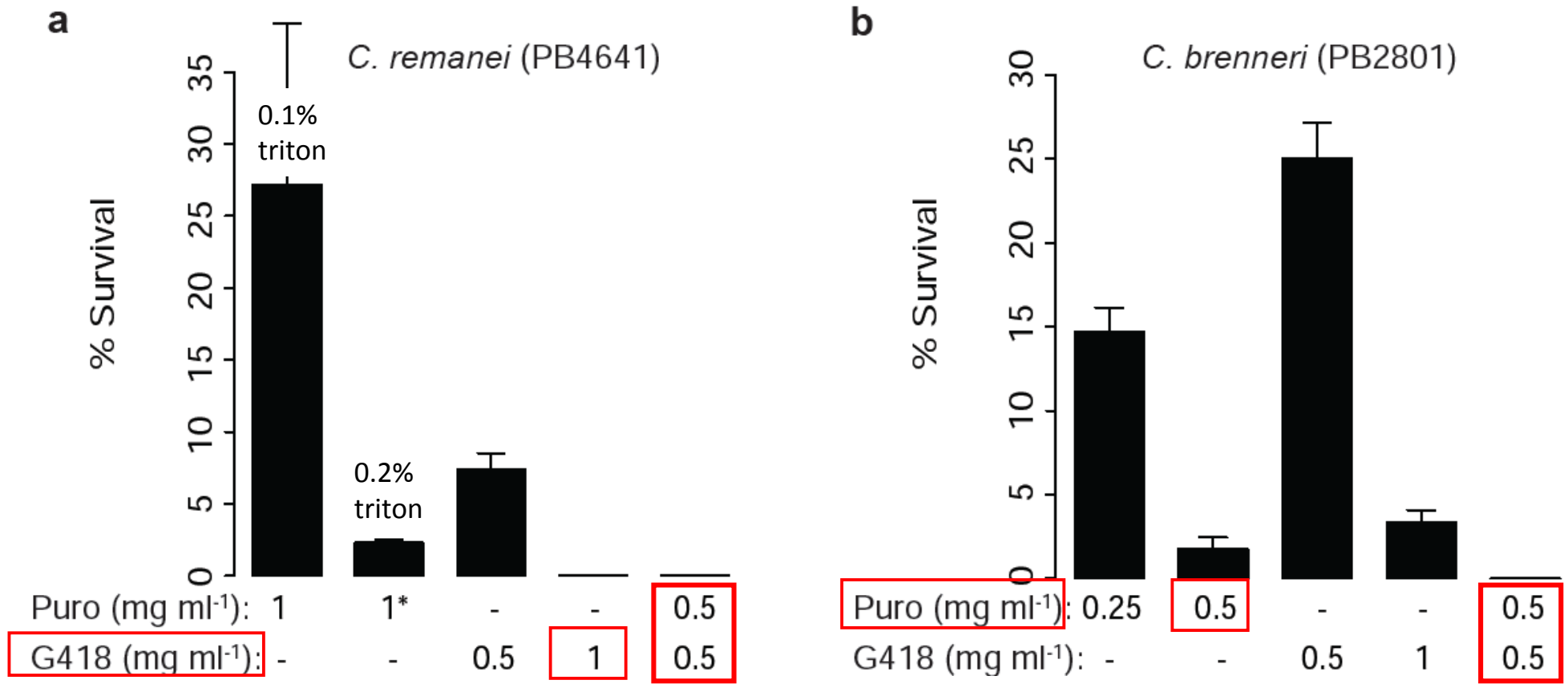
Drug	Price per Bombardment	Selection on plates	Liquid selection	Bombardment *C. elegans*	Bombardment other species
Puromycin (+triton)	7.6-26 USD	* (because of price)	***	***	Depends on species
G418	0.42-1 USD	***	**	***	Depends on species
Hygromycin B	0.68-3 USD	***	?	***	?
Puromycin + G418	**4-13.5 USD**	**NA**	***	***	***

Suppliers: Sigma, ForMedia, InvivoGen (?)

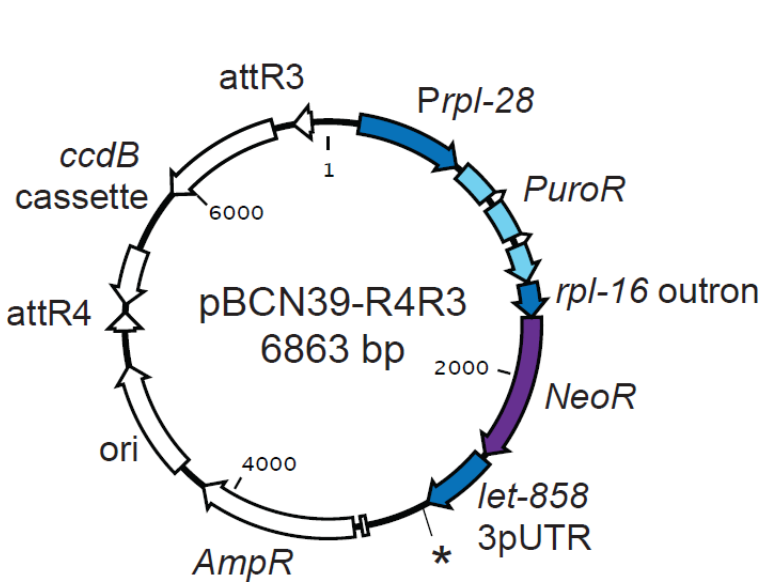
Species variation in drug resistance

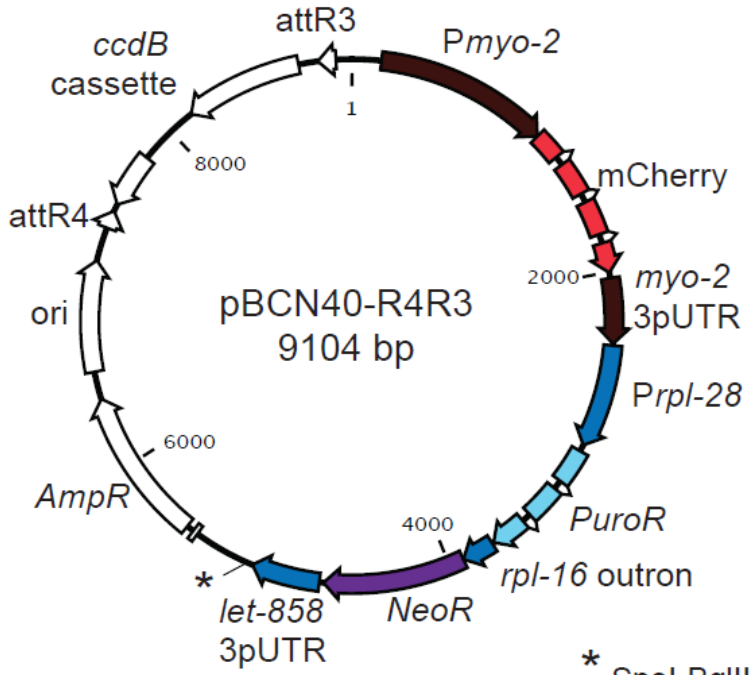


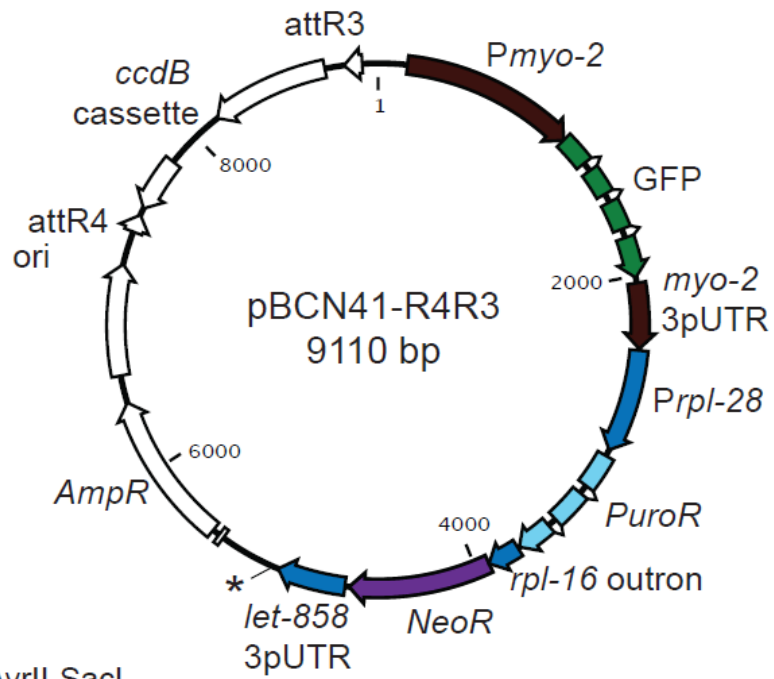
Species variation in drug resistance



Dual drug selection for bombardment







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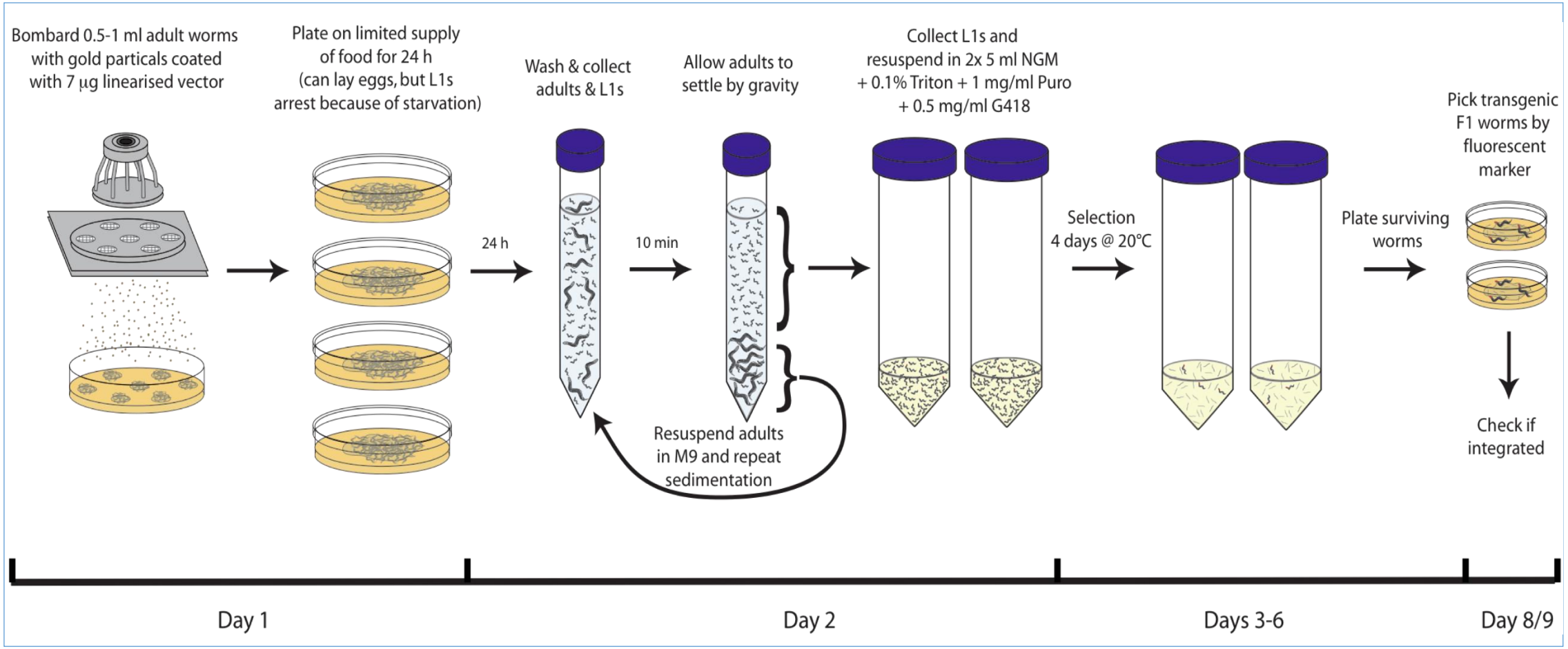
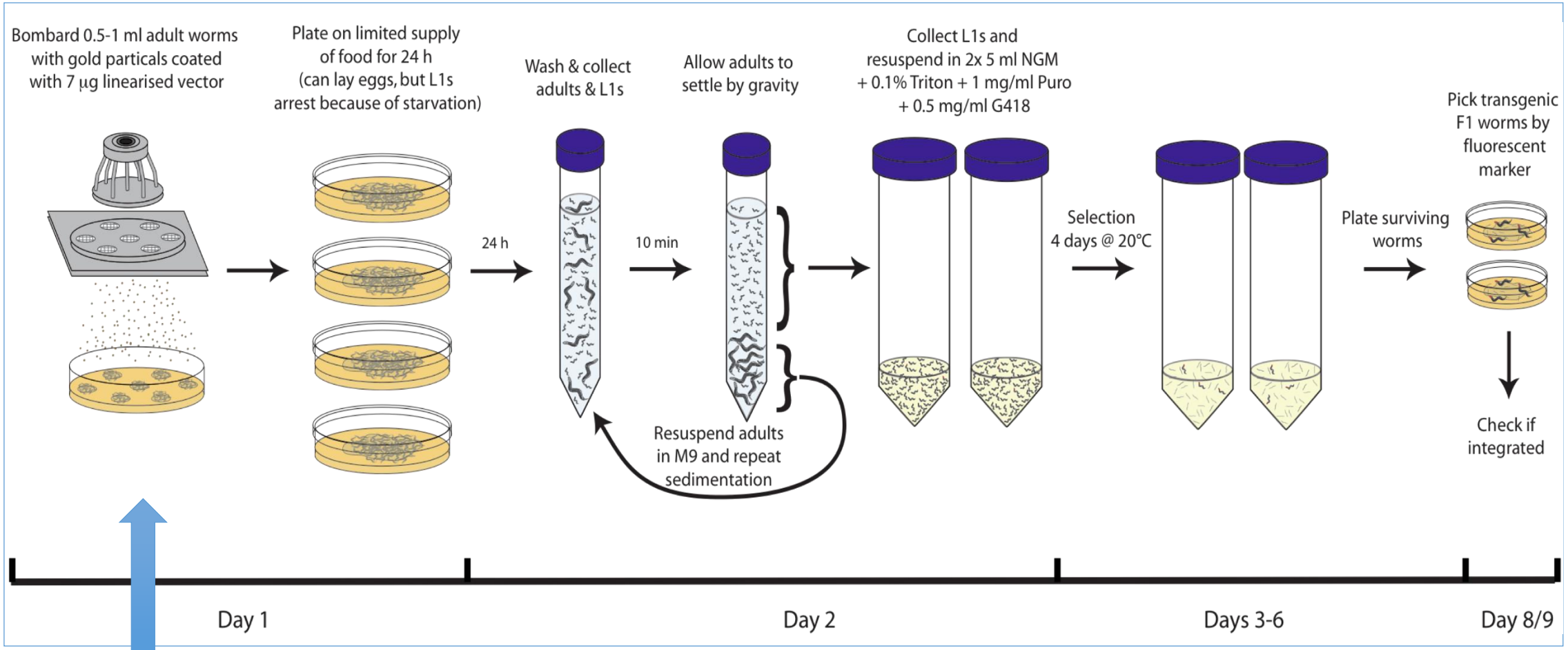
Integrated transgenes with bombardment



Integrated transgenes with bombardment



Bombard wild-type worms

Bombardment tips

Use >1 ml of worms (~100,000 worms for N2):

- C. *elegans*:

  5 egg yolk plates with 20,000 worms/plate

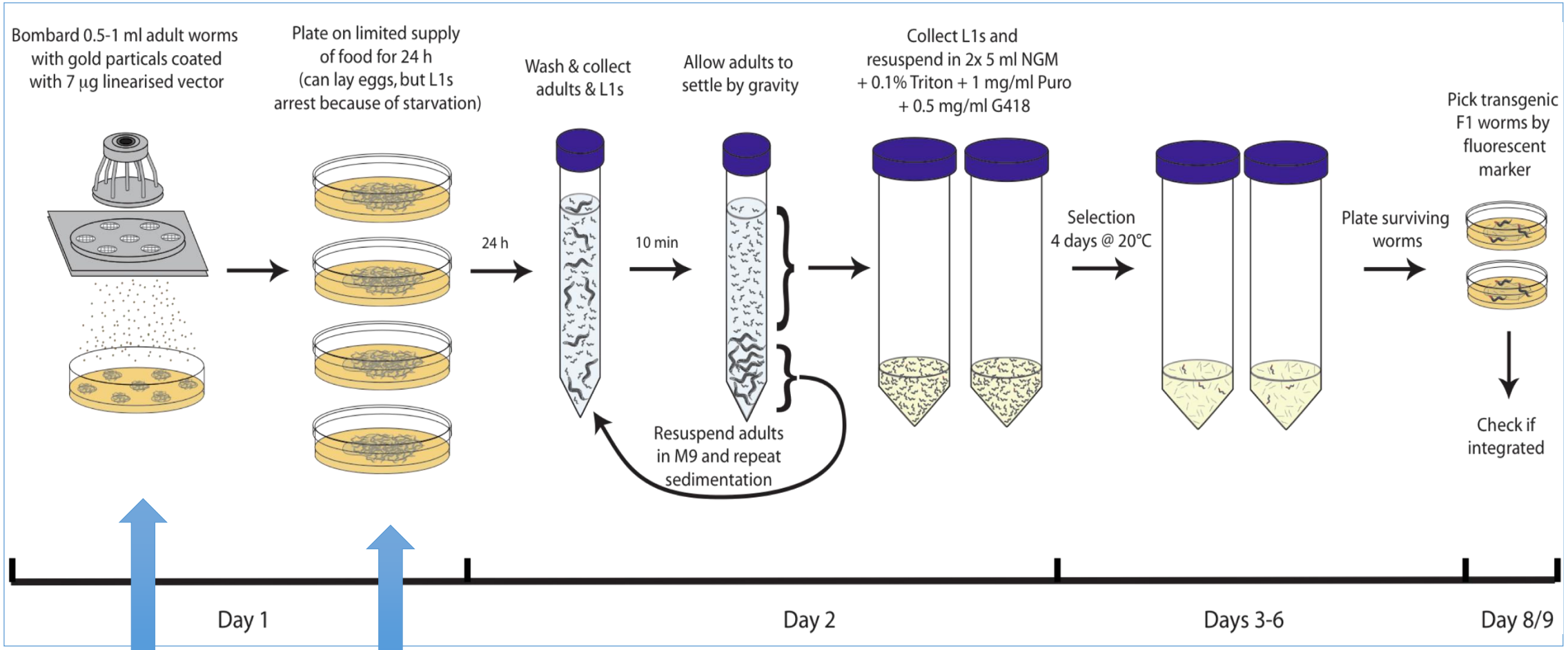
- C. *briggsae* (smaller), C. *remanei* and C. *brenneri* (only 50% will contain eggs):

  7-8 egg yolk plates with 20,000 worms/plate

C. *remanei* and C. *brenneri* tend to burrow into plates :

2.5-3% NGM agar plates to make egg yolk plates

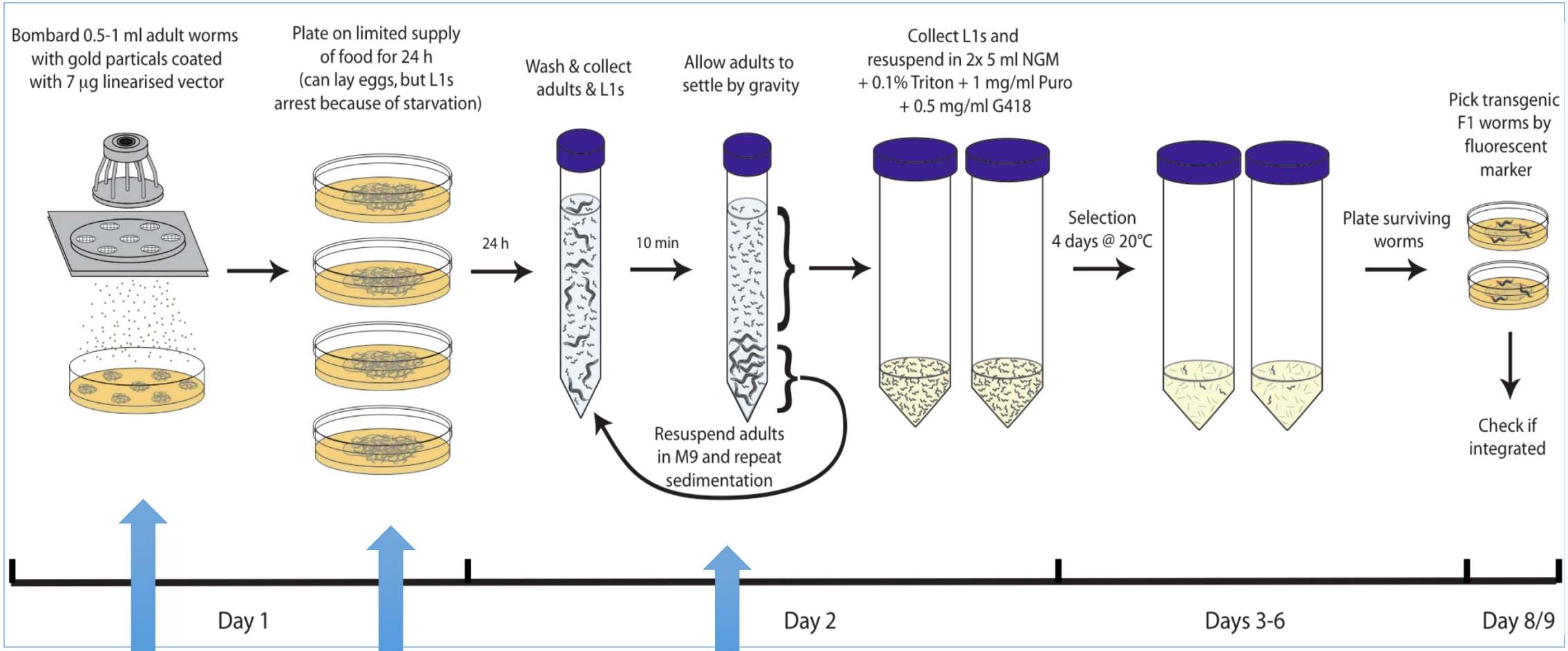
Integrated transgenes with bombardment



Bombard wild-type worms

Plate on small amount of food

Integrated transgenes with bombardment



Bombard wild-type worms

Plate on small amount of food

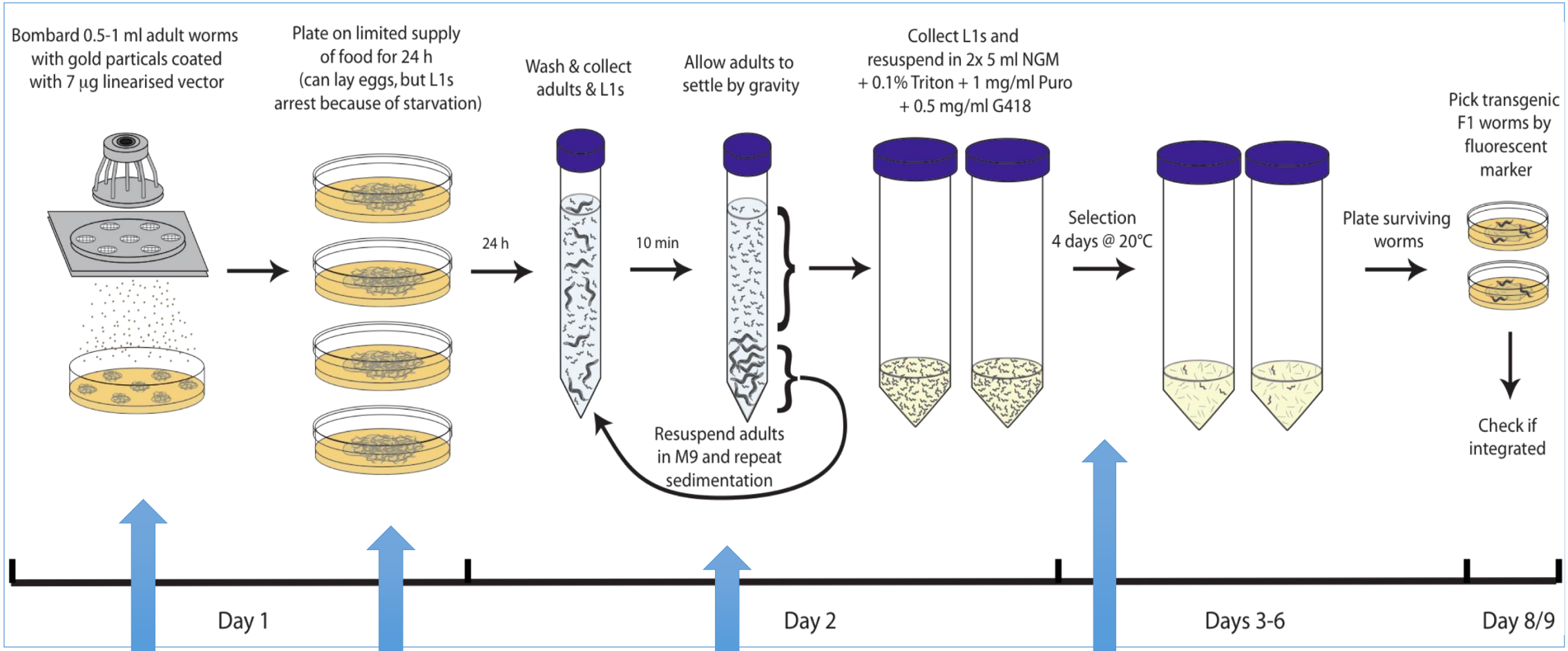
Separate out F1 generation

Washing off worms and separating by gravity 2x

Very few L1s after 24h (slower development of some species):

After separation of L1s, bleach adults and allow embryos to hatch overnight before selection.

Integrated transgenes with bombardment



Bombard wild-type worms

Plate on small amount of food

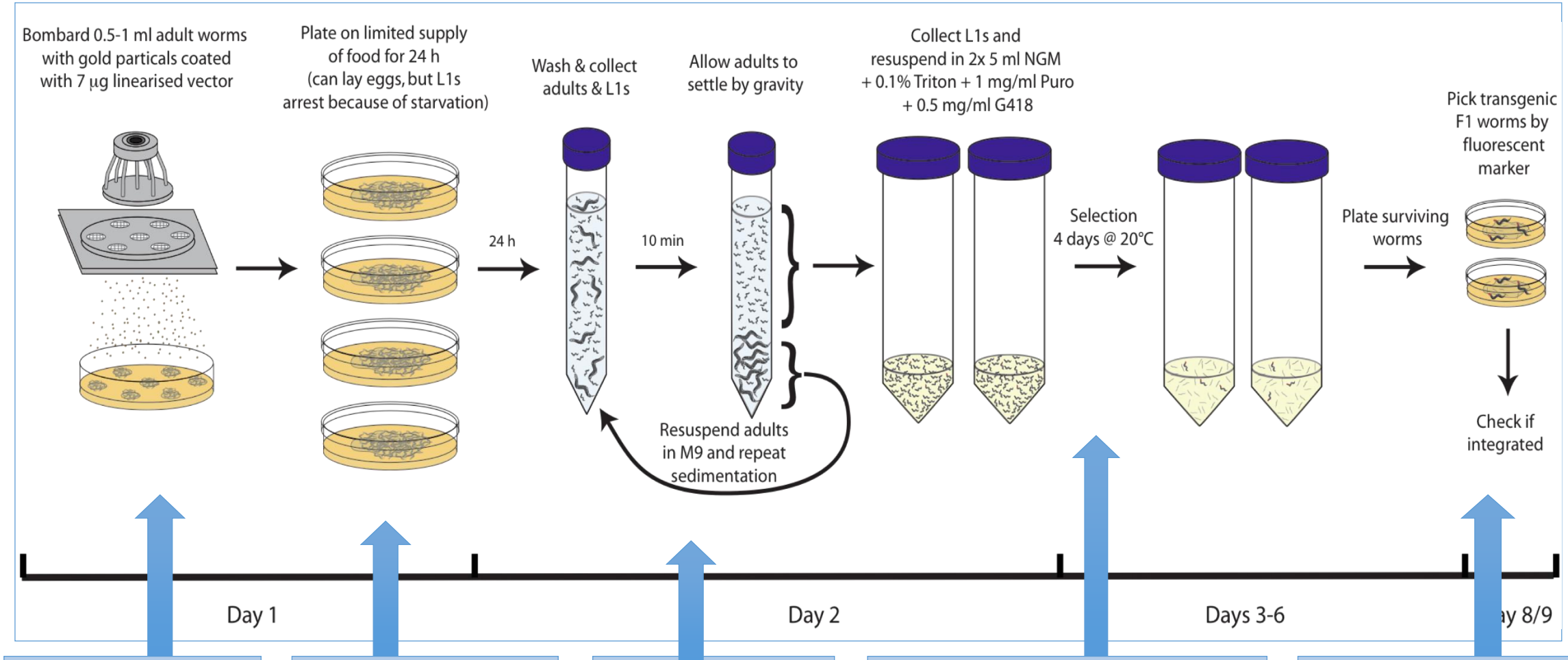
Separate out F1 generation

Select with combination of puromycin and G418

Selection with puromycin + G418

- Total volume 10-15 ml (~100 worms/µl)
- Good aeration: divide between 2x 50 ml conical tubes laid on their side (shaking incubator)

Integrated transgenes with bombardment



Bombard wild-type worms

Plate on small amount of food

Separate out F1 generation

Select with combination of puromycin and G418

Pick transgenic F1 worms after 8-9 days

Post bombardment

- Plate L1s on 2x 60mm NGM plates
- Integrated lines may have weak expression!! (low copy number)
- Usually get 10-1000 worms surviving, several extrachromosomal lines and 1-2 integrated
- Use fluorescent marker to pick transgenic worms
  - uniform expression in whole pharynx

Most common mistakes

- Not enough worms for bombardment
- Not enough perseverance scanning for weakly expressing transgenic worms

Summary

- Drug selection is independent of genetic background
- Can be easily used with a variety of *Caenorhabditis* species and strains
- Single drug selection CAN be used for bombardment (1 mg/ml)
- Dual drug selection more robust to differences in resistance between species
- Vectors available from Addgene

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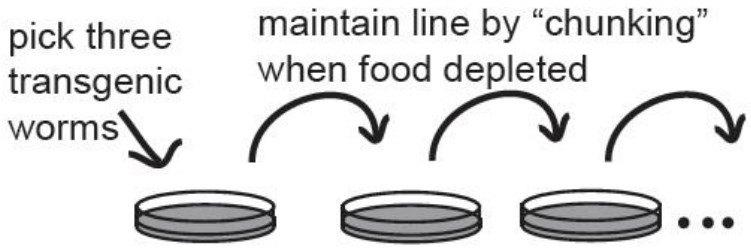
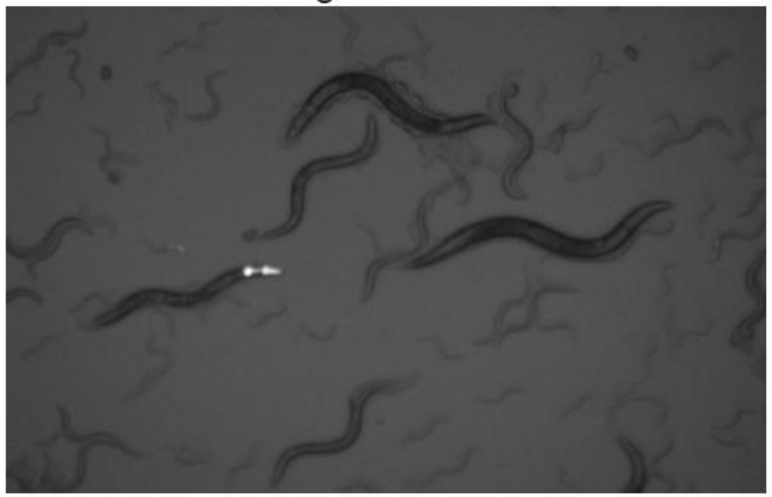
What applications is it good for?

Maintaining non-integrated strains on plates

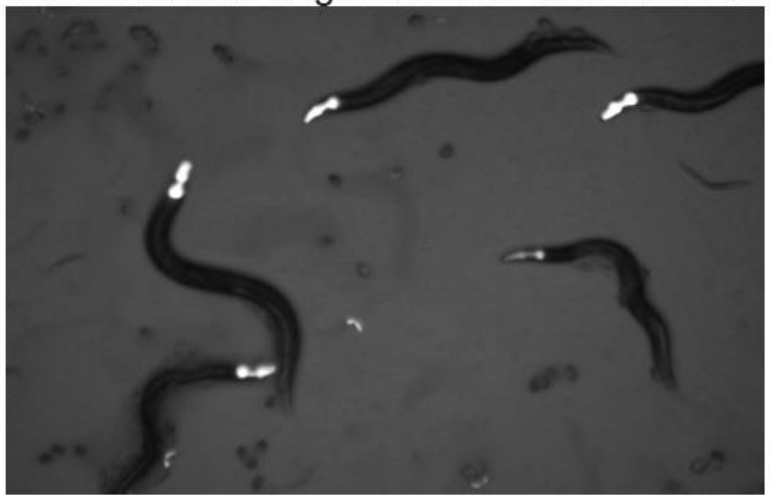


BCN6005 after 3 “generations” without selection



NGM agar

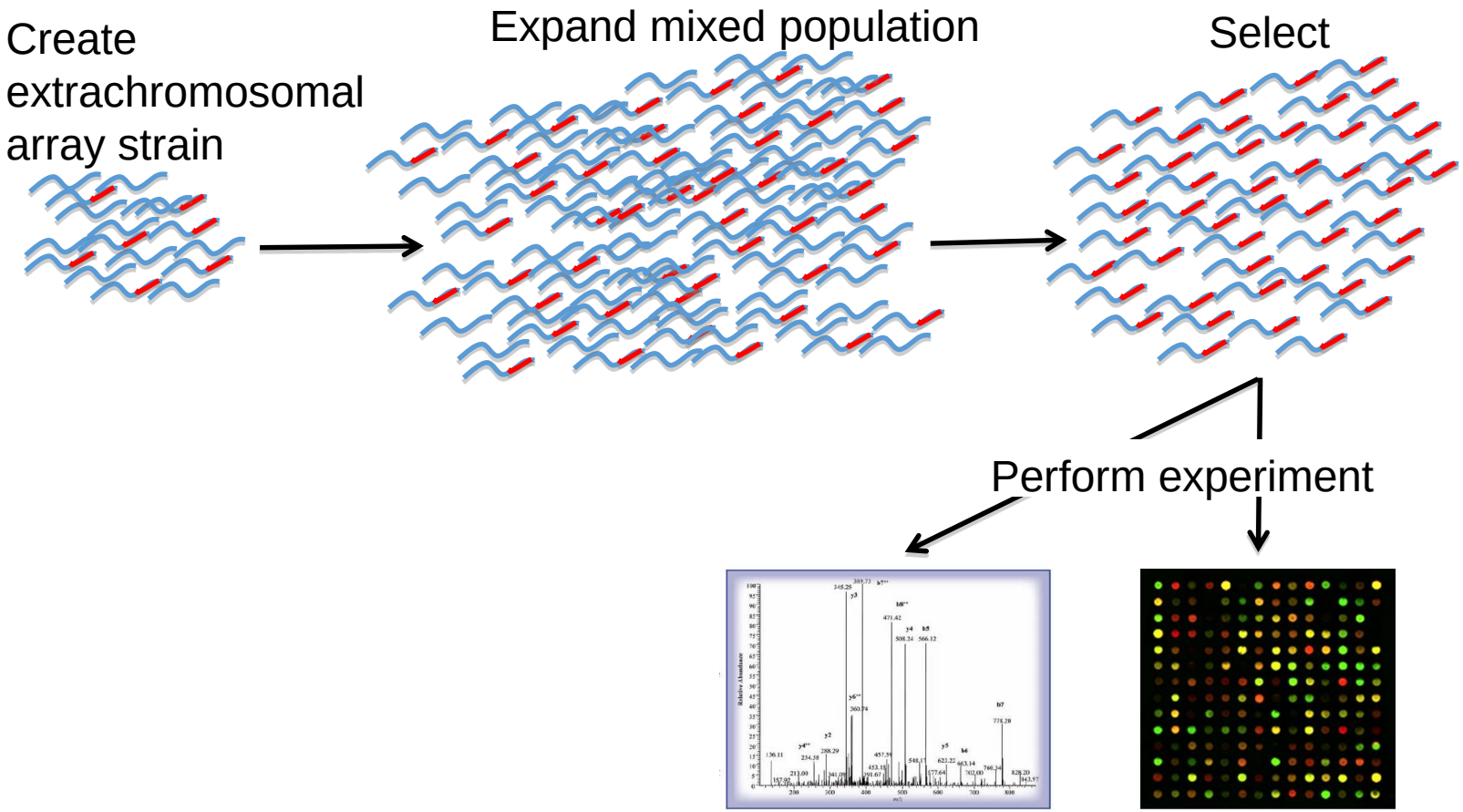
BCN6005 after 8 “generations” with selection



NGM agar + 0.5 mg ml^{-1} puromycin

What applications is it good for?

Obtaining large populations of transgenic worms from non-integrated strains



What applications is it good for?

Generating integrated lines:

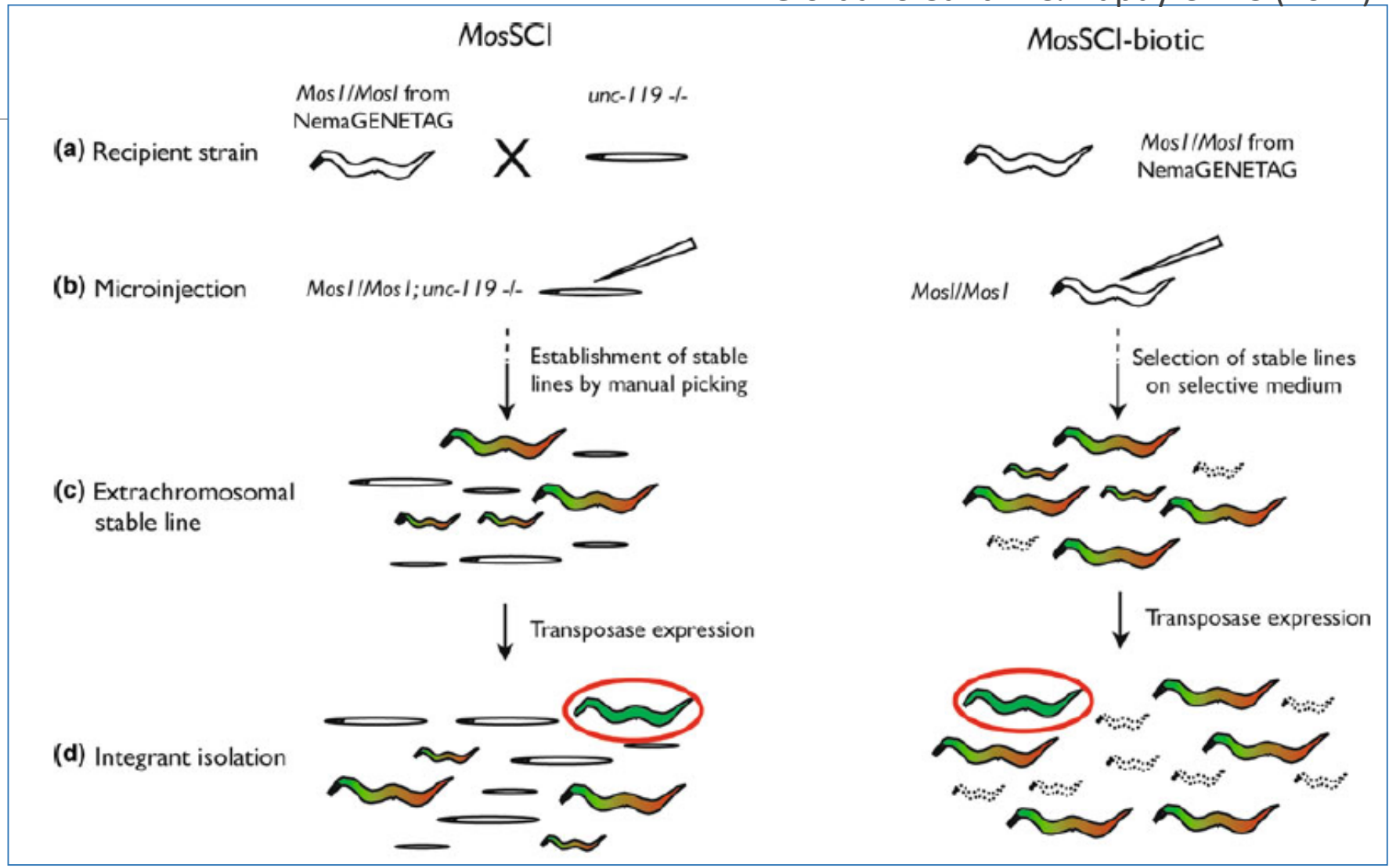
- MosSCI (Dupuy Lab)
- Biolistic Bombardment (Lehner Lab)

What applications is it good for?

MosSCI-biotic
(Dupuy Lab)

Giordano-Santini & Dupuy CMLS (2011)

- No need to cross the unc-119 mutation into new Mos tagged strains
- Especially useful for Mos-Del



What applications is it good for?

Biolistic bombardment
(Lehner Lab)