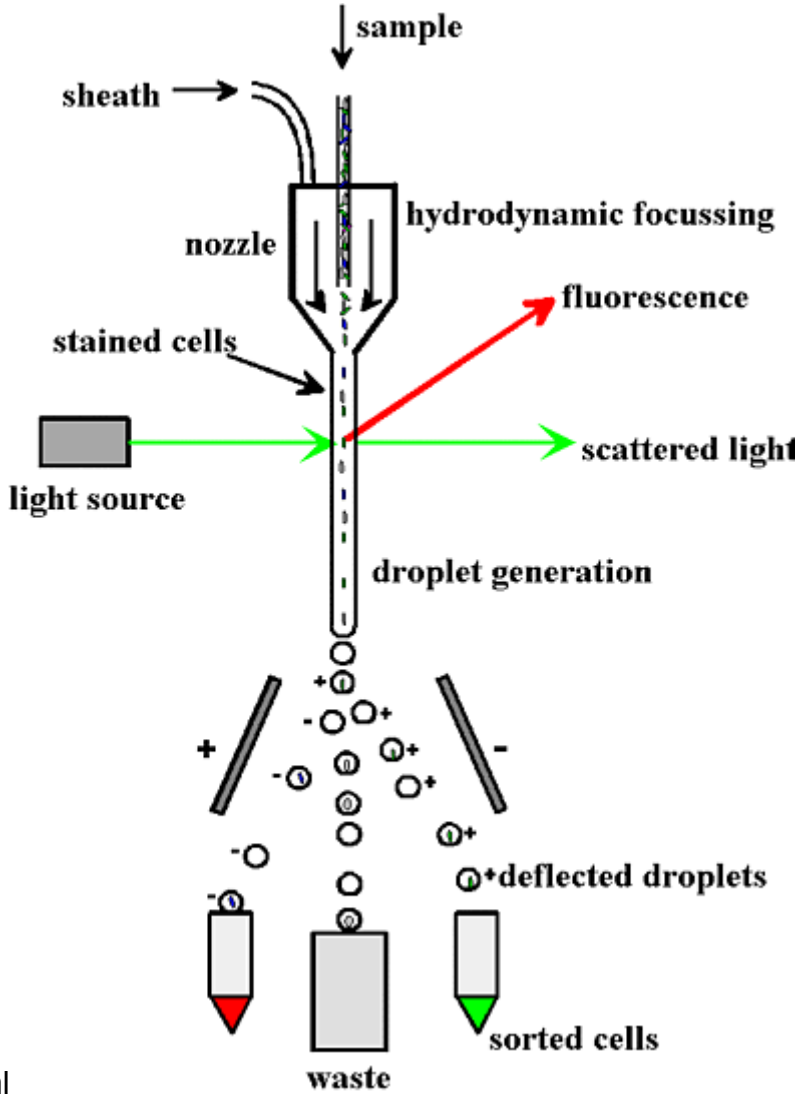
FACS

- Fluorescence Activated Cell Sorter
- Late 60’s early 70’s
- Medical applications

Flow Cytometry : principle



Emission (laser)

Detection

Data

http://www.flow-cytometry.de/start.html

FACS

FACSAria

FACSCalibur

FACS

FACSAria

http://www.bdbiosciences.com/video/BD_FACSAriaTM_high.mov

- Emission : 3 lasers : 488nm, 633nm, 407nm
- Detection :
    - 5 wavelengths from 488nm laser
    - 2 wavelengths from 633nm laser
    - 2 wavelengths from 407nm laser

Flow Cytometry : applications

- Direct or indirect fluorescence (antibody conjugated to a fluorescent dye)
- DNA staining (propidium iodide) (sorting dead cells, tracking the cell division cycle)
- Cell division counts (CFSE binding to membrane and dividing equally at the division)
- Gene expression (reporter gene or labeling the product)

Selecting FPs

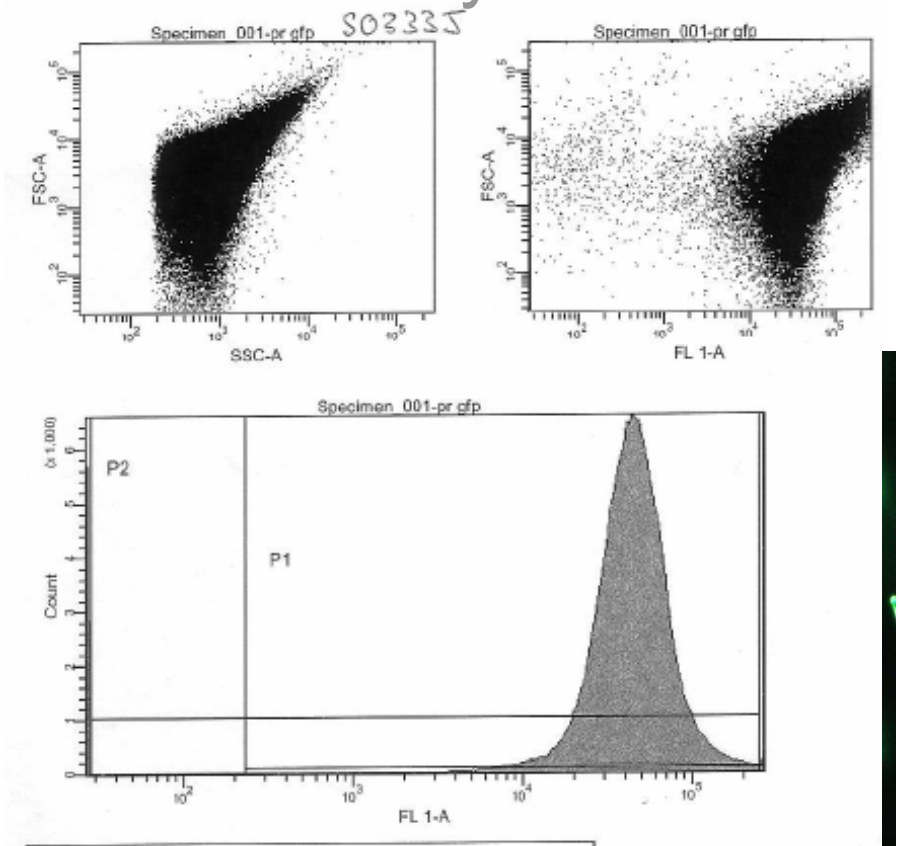
Table 1 | Properties of the best FP variants[a,b]

Class	Protein	Source laboratory (references)	Excitation[c] (nm)	Emission[d] (nm)	Brightness[e]	Photostability[f]	pKa	Oligomerization
Far-red	mPlum[g]	Tsien (5)	590	649	4.1	53	<4.5	Monomer
Red	mCherry[g]	Tsien (4)	587	610	16	96	<4.5	Monomer
	tdTomato[g]	Tsien (4)	554	581	95	98	4.7	Tandem dimer
	mStrawberry[g]	Tsien (4)	574	596	26	15	<4.5	Monomer
	J-Red[h]	Evrogen	584	610	8.8*	13	5.0	Dimer
	DsRed-monomer[h]	Clontech	556	586	3.5	16	4.5	Monomer
Orange	mOrange[g]	Tsien (4)	548	562	49	9.0	6.5	Monomer
	mKO	MBL Intl. (10)	548	559	31*	122	5.0	Monomer
Yellow-green	mCitrine[i]	Tsien (16,23)	516	529	59	49	5.7	Monomer
	Venus	Miyawaki (1)	515	528	53*	15	6.0	Weak dimer[j]
	YPet[g]	Daugherty (2)	517	530	80*	49	5.6	Weak dimer[j]
	EYFP	Invitrogen (18)	514	527	51	60	6.9	Weak dimer[j]
Green	Emerald[g]	Invitrogen (18)	487	509	39	0.69[k]	6.0	Weak dimer[j]
	EGFP	Clontech[l]	488	507	34	174	6.0	Weak dimer[j]
Cyan	CyPet	Daugherty (2)	435	477	18*	59	5.0	Weak dimer[j]
	mCFPm[m]	Tsien (23)	433	475	13	64	4.7	Monomer
	Cerulean[g]	Piston (3)	433	475	27*	36	4.7	Weak dimer[j]
UV-excitable green	T-Sapphire[g]	Griesbeck (6)	399	511	26*	25	4.9	Weak dimer[j]

[a]An expanded version of this table, including a list of other commercially available FPs, is available as Supplementary Table 1. [b]The mutations of all common AFPs relative to the wild-type protein are available in Supplementary Table 3. [c]Major excitation peak. [d]Major emission peak. [e]Product of extinction coefficient and quantum yield at pH 7.4 measured or confirmed (indicated by *) in our laboratory under ideal maturation conditions, in (mM · cm)–1 (for comparison, free fluorescein at pH 7.4 has a brightness of about 69 (mM · cm)–1). [f]Time for bleaching from an initial emission rate of 1,000 photons/s down to 500 photons/s ($t_{1/2}$; for comparison, fluorescein at pH 8.4 has $t_{1/2}$ of 5.2 s); data are not indicative of photostability under focused laser illumination. [g]Brightest in spectral class. [h]Not recommended (dim with poor folding at 37 °C). [i]Citrine YFP with A206K mutation; spectroscopic properties equivalent to Citrine. [j]Can be made monomeric with A206K mutation. [k]Emerald has a pronounced fast bleaching component that leads to a very short time to 50% bleach. Its photostability after the initial few seconds, however, is comparable to that of EGFP. [l]Formerly sold by Clontech, no longer commercially available. [m]ECFP with A206K mutation; spectroscopic properties equivalent to ECFP.

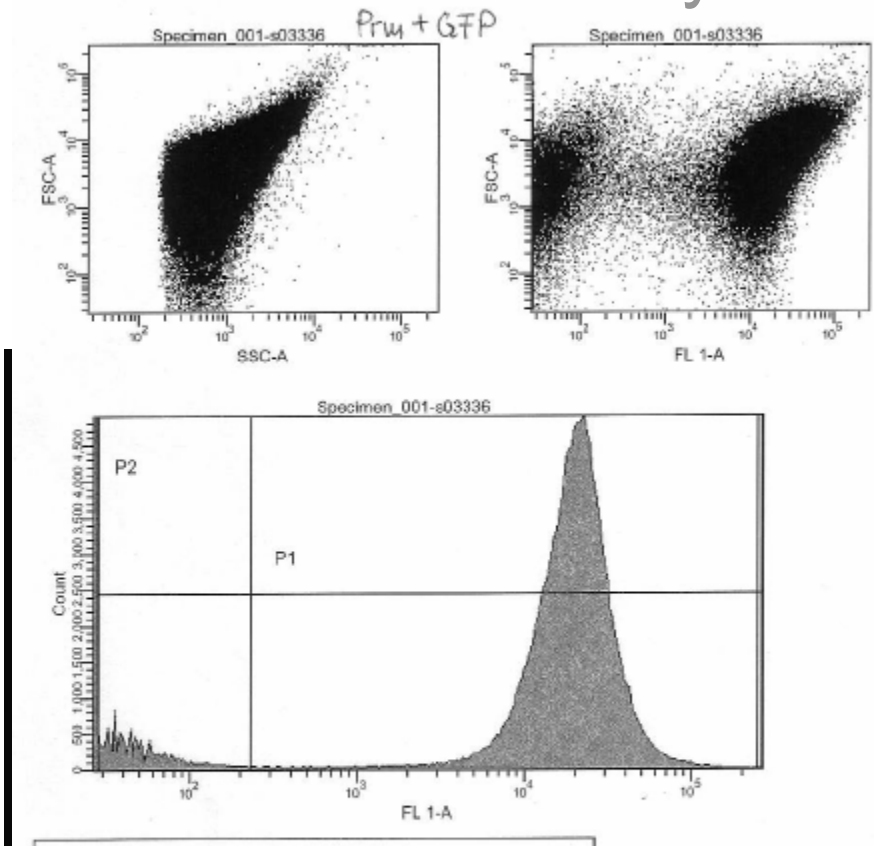
Promoter activity

Pr activity vs **Prm** activity



Experiment Name:	gv lll1 gfp
Specimen Name:	Specimen_001
Tube Name:	pr gfp
Record Date:	Oct 31, 2005 10:03:27 AM
GUID:	723e53dd-895d-4751-a845-46292b8a3ca1

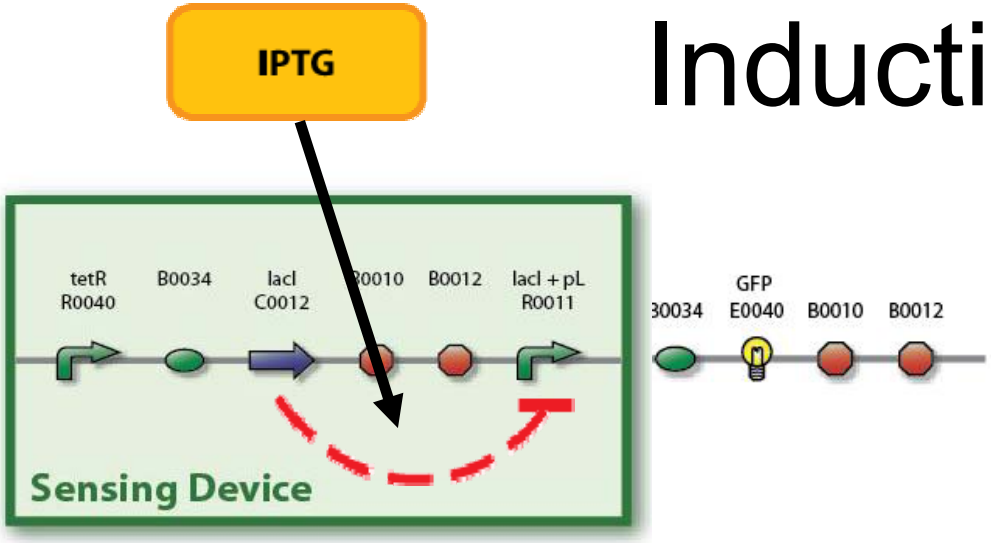
Population	#Events	%Parent	FL 1-A Mean
All Events	200,000	####	49,283
P1	193,913	97.0	50,832
P2	193,549	96.8	49,893

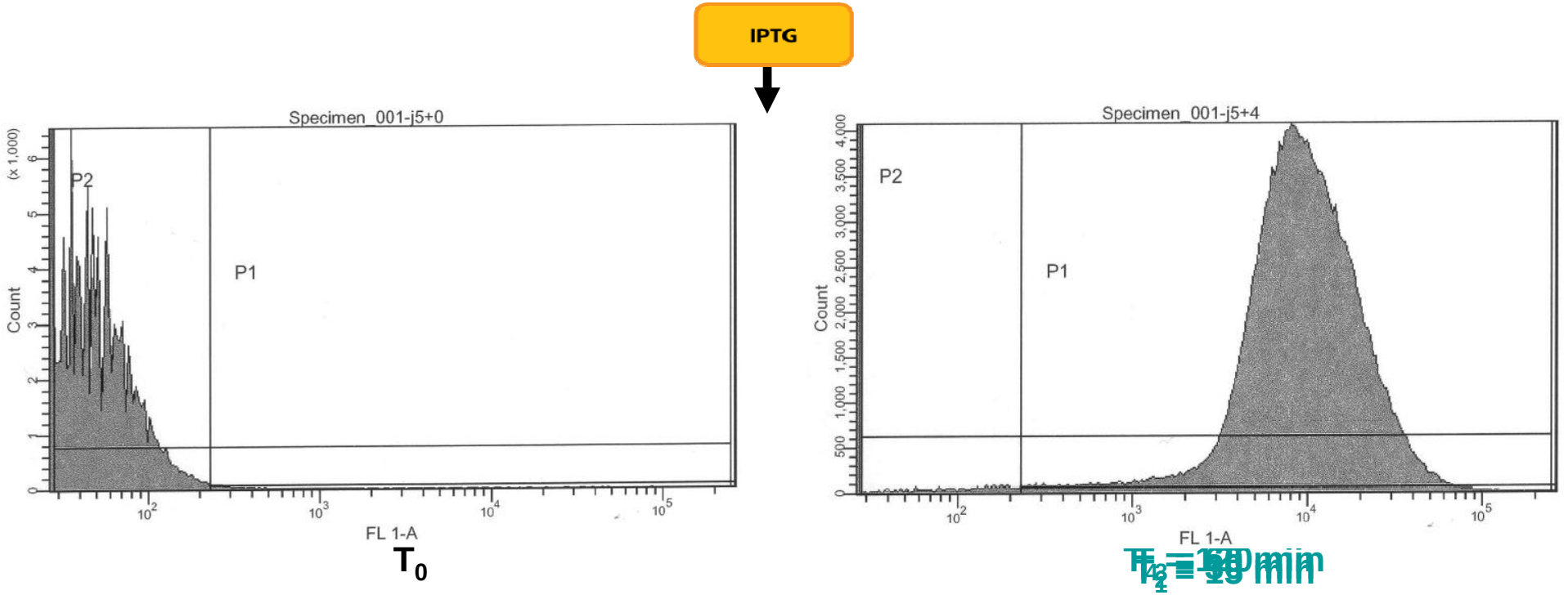


Experiment Name:	gv lll1 gfp
Specimen Name:	Specimen_001
Tube Name:	s03336
Record Date:	Oct 31, 2005 10:05:37 AM
GUID:	972e9eb0-7e14-4150-8c72-40294fa8ce0a

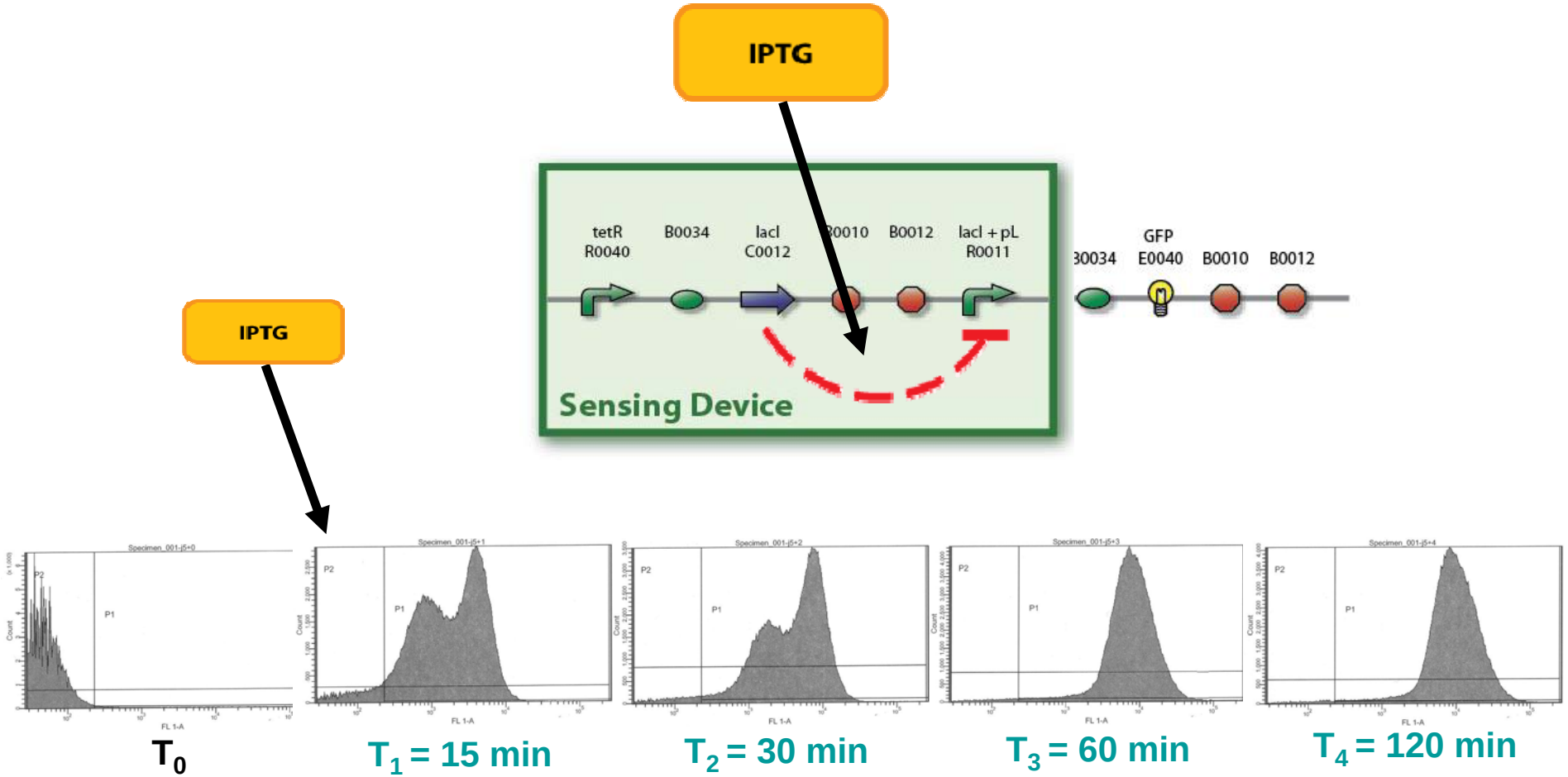
Population	#Events	%Parent	FL 1-A Mean
All Events	200,000	####	16,483
P1	147,362	73.7	22,366
P2	159,329	79.7	20,672

Induction


IPTG
tetR
R0040
B0034
lacI
C0012
B0010
B0012
lacI + pL
R0011
B0034
GFP
E0040
B0010
B0012
Sensing Device

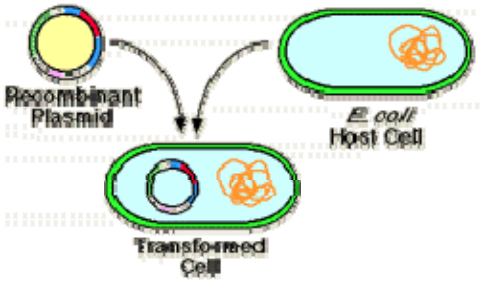

IPTG
Specimen_001-j5+0
P2
P1
Count
(x 1,000)
FL 1-A
T_0
Specimen_001-j5+4
P2
P1
Count
FL 1-A
$T_{1/2}$ = 29 min

Induction


IPTG
tetR
R0040
B0034
lacI
C0012
B0010
B0012
lacI + pL
R0011
Sensing Device
IPTG
B0034
GFP
E0040
B0010
B0012
T0
T1 = 15 min
T2 = 30 min
T3 = 60 min
T4 = 120 min

How does a FACS experiment look like…

1. Transformation



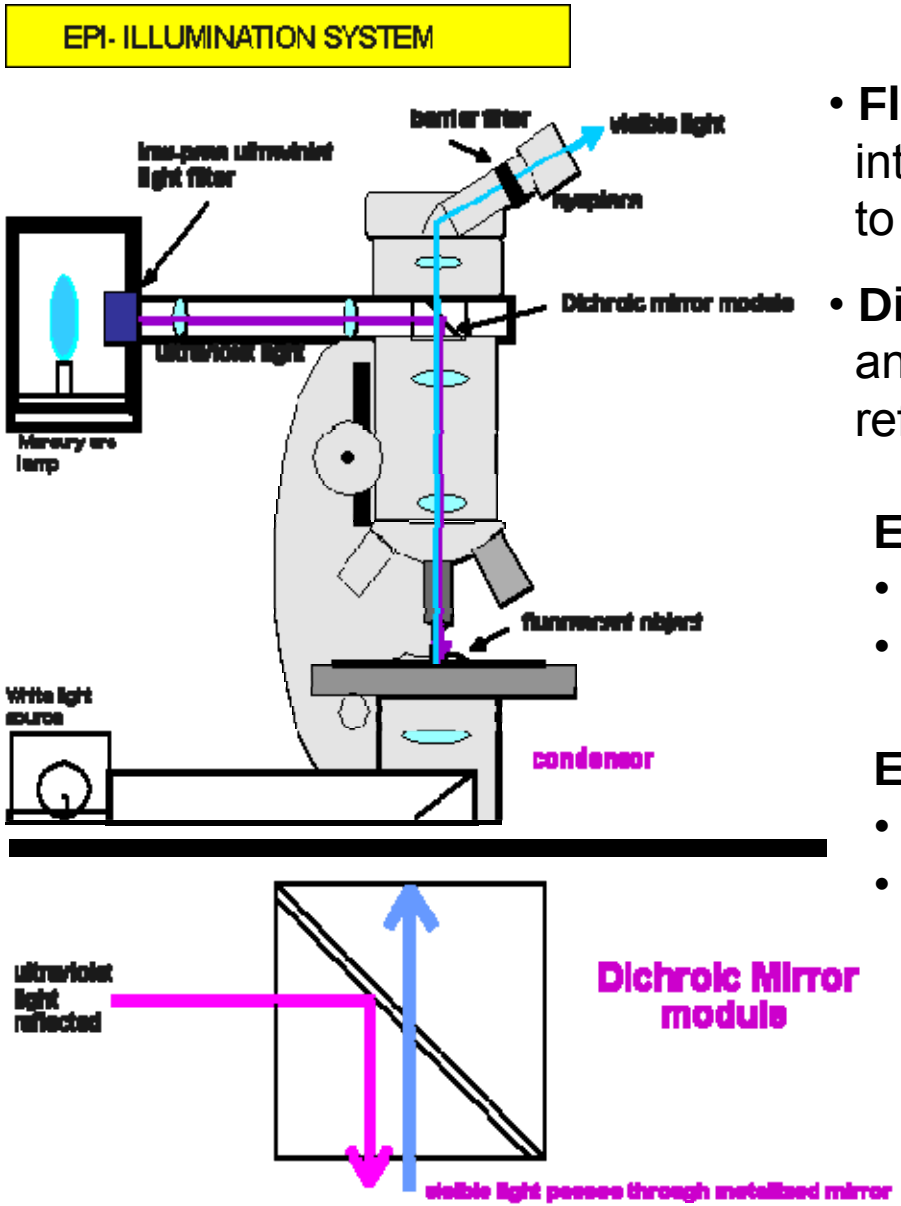
2. Inoculation of preculture from single colony

3. Growth of the culture (ev. induction)

4. Sample preparation (spin, wash, filter)

5. Go for FACS!!!

Fluorescence microscope



- **Fluorescence microscope** uses high intensity light to illuminate the sample and to excite fluorescence.
- **Dichroic mirror** used to separate excitation and emission light paths (excitation light is reflected; emission passes through)

Excitation filter:
- placed in excitation path prior to mirror
- to select the excitation wavelength

Emission filter:
- placed in emission path prior to mirror
- to specifically select the emission λ of light emitted from the sample