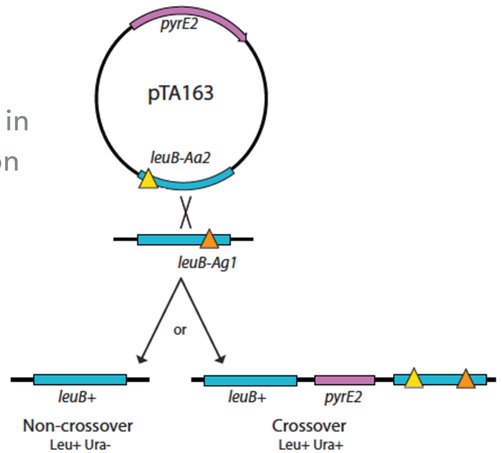
RECOMBINATION ASSAY

RECOMBINATION ASSAY – PRINCIPLE

- The assay is based on scoring recombination between a mutant chromosomal *leuB-Ag1* allele in a Haloferax strain (derivatives of H195 or H164 strains) and a mutant *leuB-Aa2* allele present on non-replicative pTA163 plasmid. Recombination between these alleles results in a wild-type *leuB+*, which is scored by the ability to grow on Hv-Min media lacking leucine.



HALOFERAX TRANSFORMATION

Day 1

- Use the general protocol for Haloferax transformation.

For each strain being assayed for recombination (including a WT control), two transformations are needed:

1. Transform with 1 µg of **non-replicative** plasmid pTA168 (*pyrE2+, leuB-Aa2*) (dam- version of pTA163).
   A. Plate transformants on **Hv-Min+Ura** (+Trp, +Thy, if relevant) at **10^{-1} - 10^{-2}** dilutions to select for cells that have undergone recombination between the plasmid *leuB-Aa2* allele and the chromosomal *leuB-Ag1* allele, generating a wild-type *leuB+* allele.
   B. To determine the total viable count, plate transformants on non-selective media (e.g., **Hv-YPC** or Hv-Ca-Ura (+Trp, +thy, if relevant) at **10^{-4} - 10^{-6}** dilutions.

2. Transform with 1 µg of **replicative** plasmid pTA357 (pyrE2+) (dam- version of p354).
   A. Plate on Hv-Ca plates (+Trp, +Thy, if relevant) at **10^{-1} - 10^{-2}** dilutions to select for cells that had taken up the replicative plasmid and thereby determine the transformation efficiency of the strain.
   B. Plate on **Hv-YPC** at **10^{-4} - 10^{-6}** dilutions to determine the total viable count.

The recombination frequency will be normalised to the transformation efficiency for each strain.

Lestini et al (2010); Wardell et al (2017); Lever et al (2023)

RECOMBINATION ASSAY CONTINUED

Strain	WT	*Δhel308*	*hel308-F316A*	*hel308-D145N*	*hel308-D145N-F316A*
Recombination Frequency (RF)	$1.92x10^{-5}$ ($+/- 6.71x10^{-6}$)	$4.13x10^{-4}$ ($+/- 2.73x10^{-4}$)	$1.81x10^{1}$ ($+/- 4.59x10^{0}$)	$3.07x10^{-5}$ ($+/- 2.05x10^{-5}$)	$9.35x10^{-4}$ ($+/- 3.36x10^{-4}$)
Transformation Efficiency (TE)	$1.34x10^{-3}$	$4.85x10^{-3}$	$7.63x10^{-3}$	$4.23x10^{-3}$	$1.28x10^{-1}$
Relative RF normalised by TE	$1.43x10^{-2}$	$7.85x10^{-2}$	$2.37x10^{3}$	$7.26x10^{-3}$	$7.32x10^{-3}$
	1	**5.5x**	**166000x**	**0.51x**	**0.51x**
CO fraction	16.5%	21.9%	<0.5%	35.7%	36.8%
NCO fraction	83.5%	78.1%	>99.5%	64.3%	63.2%

Day 5.

- Count colonies on plates and calculate recombination frequency (*RF*). This should be normalised to transformation efficiency (*TE*):
  - $RF = (Leu^{+}_{pTA168} / viable_{pTA168}) \times TE$
    - Where $TE = (Ura^{+}_{pTA357} / viable_{pTA357})^{WT} / (Ura^{+}_{pTA357} / viable_{pTA357})^{Test}$
- Normalise recombination frequencies to WT, i.e. RF^{Test} relative to RF^{WT} (e.g. above, values in **bold**).
- Determine fraction of crossover (CO) and non-crossover (NCO) recombination events by patching Leu^{+}_{pTA168} colonies (from Hv-Min+Ura plates obtained in 1A) on:
  - **Hv-Min** (+Trp, +Thy if relevant) to select for CO recombination events in cells that integrate pTA168, becoming *pyrE2+ leu+*
  - and then on **Hv-Min+Ura** (+Trp, +Thy if relevant), to ensure that all colonies patched are *leu+*
- CO events are *leuB+ pyrE2+*, the remaining recombination events (*leuB+, pyrE2-*) are NCO

PRACTICAL TIPS

- Over dry plates to avoid lawning. Dry plates for ~40 minutes as opposed to the standard 20 minutes.
- Always use freshly streaked cells from the -80°C and use within 1 week.