DNA strand exchange reaction

- solutions: - 5x strand exchange buffer:

for 1 ml 5x SEB mix	150 µl	1 M Tris Ac pH 7.5 [150 mM]
	12.5 µl	0.4 M DTT [5 mM]
	25 µl	10 µg/µl BSA (from NEB) [250 µg/ml]
	125 µl	0.1 M ATP [12.5 mM]
	100 µl	1 M MgOAc [100 mM]
	100 µl	1 M phosphocreatine [100 mM]
	487.5 µl	H_2O

- stop buffer: 60 µl 10% SDS
600 µl 0.5 M EDTA
180 µl 20 mg/ml Proteinase K

- 10x loading buffer: bromphenolblue in 50% glycerol (no xylencyanol)

- DNA ΦX174 ssDNA 130 ng/µl (412 µM [ntd]) in TE
ΦX174 dsDNA digested with *Pst*I 260 ng/µl (412 µM [bp]) in TE

Preparation of linear ΦX174 dsDNA:
- digest 26 µg of RFI dsDNA in 100 µl of NEB buffer #3 with *Pst*I
- remove restriction enzyme with phenol/isoamyl alcohol extraction (2x), precipitate with ethanol/NH_4Ac, dissolve in 50 µl TE
- measure A260 and determine concentration using absorption coefficient ε_{260}=6500/cm
- adjust concentration to 412 µM [bp] with TE

Reactions:
- mix:
2.2 µl 0.1 M ATP
2.5 µl 5x SEB
0.296 µl 0.1 M spermidine
1.2 µl 1 µg/µl creatine kinase
1 µl ssDNA
x µl Rad51 [10.3 µM]
add H_2O to 10.5 µl
- incubate 15 min at 30°C
- add RPA [1.8 µM]
- incubate 30 min at 30°C
- add 0.5 µl dsDNA, Rad54/storage buffer [0.2 µM] and 0.296 µl 0.1 M spermidine
- incubate at 30°C for 1-6 h

- stop reaction by adding 2 µl stop buffer
- incubate 20 min at 30°C
- add 2 µl 10x loading buffer
- separate samples on 0.8% agarose gel (without ethidium bromide) at 25-30 V over night (100 ml gel)
- stain gel in 1 µg/ml ethidium bromide for 1 h, destain in H_2O for 1-2 h,
- take photograph on UV transilluminator