	INPUT / OUTPUT		Less stringent	PARAMETERS Suggested	More stringent
1. EXTRACT DATA	SFF file				
	No: Multiplexed sample — Yes: **Split by multiplex identifier (MID)**			**sfffile -s file.sff**	
	Extract sequence data (with ssfinfo) *		-s -n file.sff >file.fasta	**-s file.sff >file.fasta**	
	Extract quality data (with sffinfo) *		-q -n file.sff >file.qual	**-q file.sff >file.qual**	
	FASTA and QUAL file				
2.	**Convert FASTA + QUAL to FASTQ file**				
	FASTQ file				
3. TRIM ENDS	No: Sequence has tag — Yes: **Trim tag from 5' (and 3') end**		3+ mismatches	**2 mismatches**	No mismatch
	Trim low quality ends **		Mean ≥ 15, W:1, S:1	**Mean ≥ 15, W:2, S:1**	Mean ≥ 20, W:2, S:1
	No: mRNA / cDNA data — Yes: **Trim poly-A/T tails *****		At least 5 bp long	**At least 20 bp long**	At least 50 bp long
4. FILTER SEQUENCES	**Filter too short and/or too long reads**		60 bp minimum	**Mode ±2 SD**	Mode ±1 SD
	Filter low quality reads		Mean ≥ 15	**Mean ≥ 20**	Mean ≥ 25
	Filter reads with ambiguous base N		≤ 5% (5 out of 100)	**≤ 1% (1 out of 100)**	No Ns
	Filter low complexity reads ***		Entropy ≥ 40	**Entropy ≥ 50**	Entropy ≥ 70
	Filter read duplicates		5' only	**5' and rev. compl.**	5', 3' and rev. compl.
5.	**Remove sequence contaminants *****		Cov ≥ 80, Ident ≥ 80	**Cov ≥ 90, Ident ≥ 90**	Cov ≥ 95, Ident ≥ 94
	FASTQ file				
6.	**Convert preprocessed FASTQ to FASTA file**				
	FASTQ and FASTA file		Less stringent	**Suggested**	More stringent

* Option -n: output untrimmed reads with key, MID tag (if multiplexed) and low quality regions
** W = Window size, S = Step size
*** Optional

PROGRAM: SFF Tools; PRINSEQ; TagCleaner; DeconSeq